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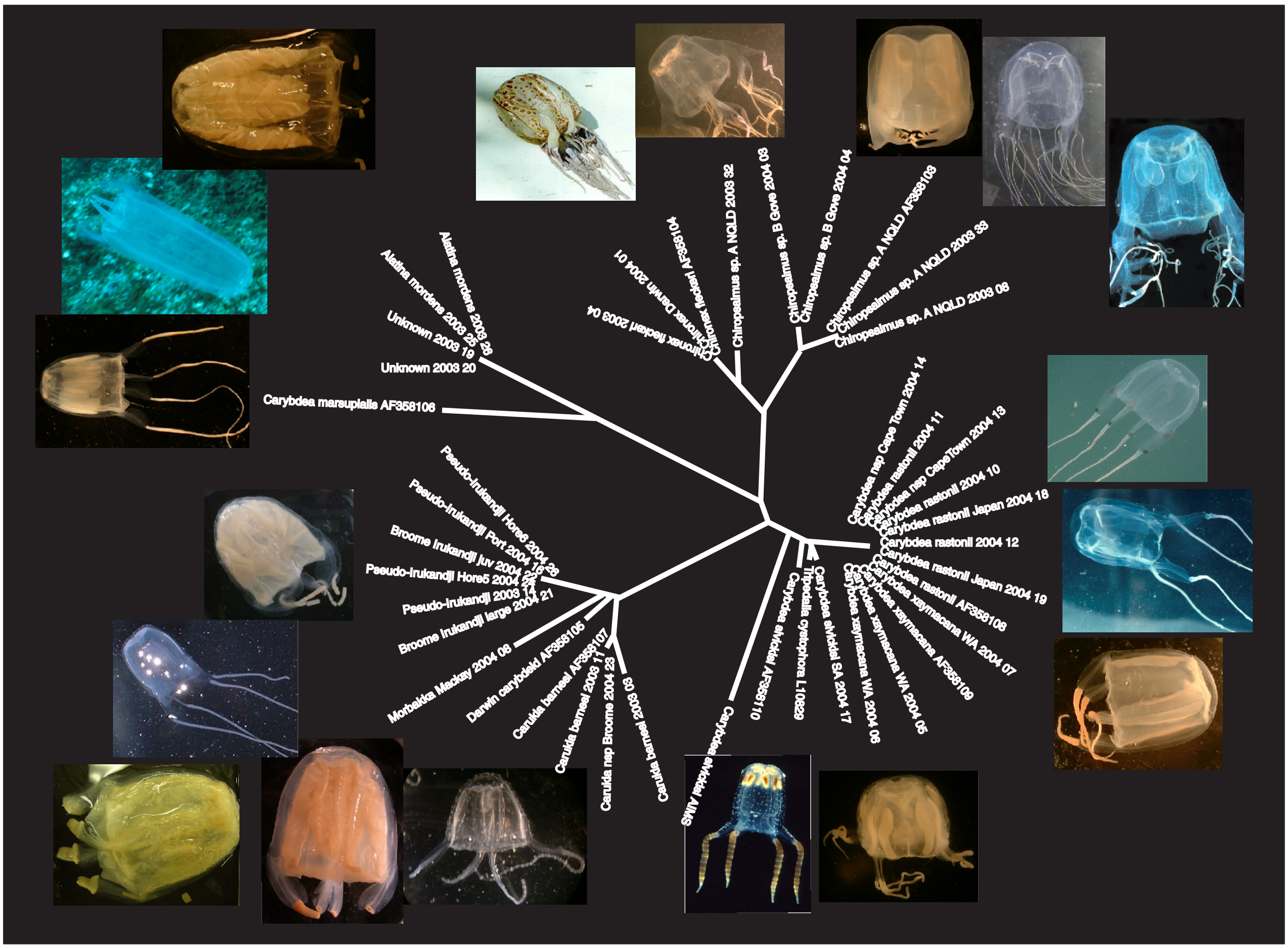
TAXONOMY AND PHYLOGENY OF AUSTRALIAN CUBOZOA



Thesis submitted by
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James Cook University

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Lisa-ann Gershwin

ABSTRACT

Jellyfishes in the class Cubozoa are species rich and often abundant in Australian waters. They are geographically widespread in tropical and temperate waters and they have global significance both economically and recreationally as dangerous marine stingers. They are interesting evolutionarily and with respect to ecology and life history. Despite this, the taxonomy of cubozoans is too coarse to allow discrimination of closely related species, hindering further advances in all aspects of cubozoan biology. The objectives of this thesis were to revise the taxonomy of the Cubozoa based on structural characters, and to elucidate the evolutionary relationships of cubozoan species based on qualitative comparison of morphological and molecular phylogenetic analyses.

I present a detailed historical and contemporary review of 85 morphological characters, many of which have not been previously used. These include (where possible) nematocysts and statoliths (balance stones), that allow the identification of ethanol-preserved specimens, frozen-defrosted material, fragmented or badly damaged samples, and possibly even fossil species. Additional characters that give increased taxonomic resolution include apical decorations, pedalial keel ratios and armament, pedalial canal shape and branching, tentacle decorations and banding forms, phacellae branching and cirri length, rhopalial horns and windows, number of eyes, frenulae, perradial lappets, velarial armament, lips shape, and a new approach to interpreting mesenteries. Accurate identification of cubozoans is based on many morphological characters. There is no small set of characters that can be universally compared to identify taxa with high reliability, but rather, different sets of characters are reliable for different groups and at different levels. For example, the historical split of the chirodropids (with gastric saccules) from the carybdeids (without gastric saccules) is no longer accurate; the undescribed spotted chirodropid (*Chirodropus* sp. A) lacks gastric saccules. Similarly, rhopaliar niche ostium shape and direction of phacellae work well for separating many (but not all) families of carybdeids, but are uniform in the chirodropids. The synoptic identification tools presented in this thesis will allow for reasonably reliable identification for the species herein, being mindful of preservational distortions, ontogenetic character changes, biological variation, and unrealized species. I recommend the use of the full range of characters presented in this study for identification and recognition of new species and species outside Australian waters.

Phylogenetic relationships within the Cubozoa were inferred by comparing parsimony analysis of 31 species scored for 85 morphological characters against Bayesian maximum likelihood analysis of partial 18S rDNA sequences from 42 individuals representing 13-16 species. Numerous patterns are congruent and well supported in both data sets as follows:

separation of the "*Carybdea alata*" species complex from the other *Carybdea* spp., a grouping of *Carybdea sivickisi* with *Tripedalia* spp., and monophyly of the Chirodropida. Furthermore, there were three distinct groups of highly toxic jellyfish whose stings result in Irukandji syndrome; although differences exist between the morphological and molecular tree topologies, there was nonetheless strong support for a clade herein referred to by the non-taxonomic common designation "Irukandjiidae".

Based on the combination of morphological and molecular phylogenetic analyses, numerous changes to the existing taxonomic framework were indicated. A revised classification is proposed, along with synopses of the species and a dichotomous key to taxa collected in Australian waters. Furthermore, a new family is proposed, the Alatinidae, with detailed descriptions of a new genus, *Alatina*, and two new species, *A. mordens* and *A. rainensis*. Other new taxa are indicated throughout the text, but will be fully treated in a monographic revision of the Cubozoa generated from this work.

Practical application of these results has already begun. The Irukandji clade identified in this study contains at least two assemblages of medical interest, the *Carukia* spp. and the "Pseudo-Irukandji" group. Species from each of these sub-clades have been associated with Irukandji syndrome, the latter linked with a fatal sting event. These two groups are further sorted on numerous macro-morphological features, cnidomes, statoliths, behavioural patterns, and spatio-temporal distribution, and there is some indication that syndrome severity may sort along phylogenetic lines. The link between these species and symptoms remains to be conclusively shown, but the correlative evidence suggests it should be an active area of research.

This study covers new ground in many respects, including detailed examination of a wide range of morphological characters and production of comparable robust phylogenies from molecular and morphological data sets. A sound taxonomy is required as the basis for communication and comparison in all other types of cubozoan studies, such as ecology, toxinology, and basic biology, all of which will, in turn, be necessary for the successful management of Australia's jellyfish problem.

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STATEMENT OF SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Molecular work in Chapter 3 was done collaboratively with M. van Oppen and L. Peplow; 2003-series DNA sequences were derived by them and remain their intellectual property. Chapter 3 will be published jointly as part of this collaboration.

Statolith work in Chapter 2 was done collaboratively with M. Kingsford, and a larger study stemming from this work will be published jointly. The original idea to explore statoliths as a taxonomic character was conceived by M. Kingsford.

____________________________________ ____________________

Lisa-ann Gershwin

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DEDICATION

This thesis is dedicated to four people who have had the greatest positive influence on my life:

1) Judy Nelson, whom I consider one of the finest humans I have ever had the privilege of knowing, thank you Mom

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I love you all very much.

Life is not measured by the number of breaths we take, but rather, by the moments that take our breath away

CHAPTER 1: GENERAL INTRODUCTION

Following the tragic sting-related deaths of two tourists in early 2002, box jellyfishes, and "Irukandji" in particular, became listed as a high priority research area in North Queensland. Focus was placed on all aspects of scientific and medical enquiry that related to predicting, preventing, and treating Irukandjis or Irukandji syndrome. However, other than a single described species and a report linking one of the fatalities to a new species (Huynh et al., 2003), it was unclear how many species of Irukandjis there were, what their spatial and temporal distribution might be, and how the syndromes of different species might compare. Some medical reports had speculated that Irukandji species diversity might be higher than previously recognized, based on documented variations in sting symptoms, some of which were regional, as well as indications that some forms of the syndrome were much more severe than others (Fenner et al., 1985; Fenner et al., 1988; Martin and Audley, 1990; Fenner and Heazlewood, 1997; Cheng et al., 1999; Fenner and Carney, 1999; Mulcahy, 1999; Currie, 2000a; Little et al., 2001; Taylor et al., 2002). But the root of the problem was far removed from North Queensland in space and time; cubozoan systematics still reflected the 19th century views under which they were established, based on scant sampling from other regions.

Cubozoan systematics are badly in need of revision at all levels. As detailed below and in Chapters 3 and 5, the higher taxa have not grown with our understanding. In most cases these taxa are too broadly defined, obscuring the natural biodiversity. At the species level, it has been long and widely recognized that the Australian cubozoan diversity is understated (Southcott, 1985; Kinsey, 1986; Kinsey, 1988; Williamson et al., 1996; Fenner, 2000; Currie et al., 2002; Fenner and Hadok, 2002; Huynh et al., 2003), and yet only five Australian species are currently recognized. Worldwide, the number of valid species only numbers approximately 17, based on Kramp's Synopsis (1961) plus later-named species (Southcott, 1967; Moore, 1988). The number of species is disputatious: Werner (1984) recognized 16 (but did not list specifically which ones), whereas Franc (1995) enumerated only 13. Whichever number one chooses to use, many of these "species" comprise exceedingly disparate forms when compared side by side (Gershwin, unpublished).

Describing a few new species is an inadequate solution; what is needed is to update cubozoan systematics to the level of comprehensive and clearly delineated species descriptions and to be able to harness the predictive power of a well supported phylogeny. The task ahead is one of redefining taxon boundaries and establishing usable species recognition criteria throughout the class. Furthermore, in order to adequately assess and express the true species richness of the group, a range of analytical methods should be applied and combined, such as molecular systematics, cladistics, population genetics, morphometrics, behavioural and distributional ecology, and toxinological comparisons. This thesis does not address all these issues, but seeks to clarify the systematics as a basis for further study.

While previous authors have used very few characters to separate cubozoan species, I believe that a more exhaustive approach will allow us to discern species richness to an extent that has not been feasible based on the historical limited data set; specifically, my predecessors used a total of eight carybdeid characters and three chirodropid characters to distinguish species, whereas I have used a total of 85 (Plate 1.1). Extensive illustrative tools are employed throughout this thesis, in order to better understand the overall and detailed morphology of cubozoan species. Specifically, diagrams of major external structures (Plate 1.2), internal structures (Plate 1.3), and micro-morphology (Plate 1.4) are provided at the end of this chapter to aid in better understanding the overall morphology discussed throughout this thesis; and a glossary of cubozoan terminology (Appendix 1) and figures of cubozoan species (Chapter 4 plates) and characters (Chapter 2 plates) are included to further aid in clarity.

1.1 HISTORICAL BACKGROUND

The cubomedusae have received little attention because they were always regarded as a minor group. Furthermore, most species are regionally and temporally rare or uncommon, and as such, comparative material is often wanting. As a result, the intermittent attempts by occasional workers on sporadic specimens have amounted to enormous taxonomic confusion.

The first described species of the group was *Carybdea marsupialis* (Linnaeus, 1758), with the name *Medusa marsupialis*, although this had earlier been recognized by Plancus (1739) as "*Urtica soluta marsupium referens*". Nearly a century later, Lesson (1829; 1843) added three more species, none of which are today recognizable: *Beroe gargantua*, *Bursarius cythereae*, and *Marsupialis flagellata*. In Lesson's Centurie Zoologique, Reynaud (1830) added the species *Carybdea alata.* Müller (1859) added two more species, *Tamoya haplonema* and *T. quadrumana*; the latter was the first described chirodropid, and was subsequently transferred to a new genus, *Chiropsalmus* (Agassiz, 1862). Up to this point, cubomedusae were artificially grouped with some species of coronate scyphozoans and some narcomedusan hydrozoans (Agassiz, 1862).

It was not until the work of Ernst Haeckel (1880) that cubozoology really began to take form, with a usable classification and the addition of 15 new species, many of which, however, are no longer recognized: *Carybdea obeliscus, Carybdea philippina, Carybdea pyramis, Carybdea murrayana, Procharagma aurea, Procharagma prototypus, Procharybdis cuboides,* *Procharybdis securigera, Procharybdis tetraptera, Procharybdis turricula, Tamoya prismatica, Chirodropus gorilla, Chirodropus palmatus, Chiropsalmus zygonema, and Chiropsalmus quadrigatus*. An additional 14 species were described in the next three decades, only four of which are still recognized: *Tamoya punctata* Fewkes, 1883; *Carybdea rastonii* Haacke, 1886, 1887; *Carybdea brevipedalia* Kishinouye, 1891; *Carybdea latigenitalia* Kishinouye, 1891; *Carybdea arborifera* Maas, 1897; *Carybdea xaymacana* Conant, 1897; *Tripedalia cystophora* Conant, 1897; *Carybdea aurifera* Mayer, 1900; *Carybdea grandis* Agassiz and Mayer, 1902; *Carybdea verrucosa* Hargitt, 1902; *Carybdea moseri* Mayer, 1906; *Chiropsalmus buitendijki*, Horst, 1907; *Carybdea mora* Kishinouye, 1910; *Tamoya virulenta* Kishinouye, 1910.

The next landmark work was that of Mayer (1910), the first widely available, English classification; unfortunately, some of Mayer's conclusions were so general that many clearly different forms were encompassed within a single name (e.g., *Carybdea alata*, *Chiropsalmus quadrigatus*). Only two species were added over the next three decades, *Carybdea sivickisi* Stiasny, 1926, which was so distinctive that it simply did not fit with any of the existing species, and *Carybdea madraspatana* Menon, 1930, which was quickly lost in synonymy. In 1938, Bigelow published what is still the clearest narrative of the major characters that separate the different forms, but the study was limited to American carybdeids. Bigelow (1938) also named a species which had been previously described but not named by Stiasny (1930), *Carybdea stiasnyi*. The rest of the 20th century saw only three more species added, all Australian: *Chironex fleckeri* Southcott, 1956; *Carukia barnesi* Southcott, 1967; and *Tripedalia binata* Moore, 1988. At the close of the 20th century, some 17 species were recognized as valid, less than half the total number historically described (Kramp, 1961; Southcott, 1967; Moore, 1988).

1.2 CURRENT CLASSIFICATION

The current taxonomy is still based on Haeckel's original system (Haeckel, 1880). Most authors since that time have either added species without much systematic clarification or have broadened species definitions to the point of overlap between clearly disparate forms; some have attempted to find order in the chaos, but examined too few species to be comprehensively useful. Either way, the classification scheme established by Haeckel has never been challenged, but, as I hope to show, clearly fails to accommodate and communicate the biodiversity of the group. Throughout the Cubozoa, it is difficult, if not impossible, to accurately identify many of the species, because the older descriptions and figures, where available, vaguely apply to numerous exceedingly different forms. Furthermore, much of the historical type material is no longer extant, making clarification a subjective task. A corollary to this ambiguity is a "trash-bin" phenomenon at higher levels, with dissimilar taxa grouped together simply because they do not fit elsewhere, resulting in polyphyletic groupings.

Cubozoan taxonomy suffers from two major impediments as a result of being governed by a nineteenth-century paradigm: first, it emphasizes key character similarity rather than a wholistic approach of analysis of character suites, which often differ among isolated populations, and second, by obscuring these subtle (and often not so subtle) differences, taxon definitions at all levels of the classification often differ widely by region and by worker. A natural consequence of utilizing too few characters, or in some cases uninformative characters, along with inconsistent interpretation of taxon definitions and boundaries, has led to a third problem, a gross underestimation of species diversity.

PROBLEM 1. KEY CHARACTER SIMILARITY AND SPECIES CONCEPTS

The problem of key character similarity is an old and complex one. Historically, and even still to some extent, it was handy for a naturalist to know the one or two defining (key) characters for identification of different species. Pre-Darwin, the emphasis was on species "essences" or the idealized concept of a species, and variants (or "sports") were seen as accidents of birth or experiments by God (Bateson, 1894; Futuyma, 1998). In this paradigm, the focus was on differences between species, and many species were described based on color differences, size differences, ontogenetic differences, mutations, amputations, or collection damage. Often, a single minor difference was adequate justification to erect a new species. There was no concept of variation; the essences were perfect, and a specimen falling outside "the norm" of one species was simply regarded as a different species with a different essence. Most of the cubozoan species described from this period are unrecognizable today due to the inadequate descriptions and lack of type specimens.

In the hundred years Post-Darwin, the emphasis was on the relationships between and among species, and variants were seen as evolutionary intermediates or species in the making (Haeckel, 1880; Bateson, 1894; Futuyma, 1998). There was a tendency among cubozoologists of this time toward a reductionistic species concept (Mayer, 1910; Uchida, 1929; Stiasny, 1937a; Bigelow, 1938). In this paradigm, focus was on a given "key character", and all forms possessing it were deemed to belong to the same species, and the other characters were then seen as mere population variation. The focus was on similarity between species, and forms of different sizes and morphologies from different areas were thought to be different growth stages of the same species (Mayer, 1910). New species were described if a form was found that did not have the "key" characteristics of any known species. There was no concept yet of biogeography or the role that spatial or temporal isolation could play in defining species; thus, many of the species described from this period are often said to have extremely wide distributions, with many “local varieties”. Most of the cubozoan species recognized today were described from this period, or by later workers who still held this philosophy.

Under modern systematic philosophy, species are delineated based on some delicate mix of morphological, genetic, or biochemical difference, that can generally be tracked along spatial, temporal, or behavioural boundaries (Mayr and Ashlock, 1991; Ridley, 1996; Futuyma, 1998). Ideally, a species is defined somewhere between the two earlier paradigms, i.e., the species should include individuals that form a cohesive natural group in space and time, and exclude individuals that unify more naturally with other such groups. Thus, the range of variation should reflect biological reality rather than systematic convenience. Most often, species are recognized in nature by their shared morphological features, but the underlying basis for grouping must be based on common ancestry. Two divergent philosophical approaches exist to species identification. The first has an *a priori* assumption that the species is known, and seeks to match it up from among the choices. The second is a more *a posteriori* approach, with no assumption about whether the species is known, but simply compares it character by character to those of known species.

Species concepts and species recognition criteria vary widely among groups, and are thus refined alongside our understanding of species and their diversity in a given group through a process of reciprocal illumination. Specifically, as we better understand a group of species, we are better able to circumscribe each member and the relationship that each bears to the others. The first step in erecting a taxonomy is to define morphospecies, providing working hypotheses from which a more meaningful delimitation of species or relationships within species can be developed with genetic, ecological, and physiological approaches. In the Cubozoa, we are still in the initial descriptive phase, rapidly adding new species and expanding the classification to accommodate new forms.

Species concepts in the Cubozoa have not kept pace with developments in evolutionary and molecular biology, because most cubozoan species are poorly defined, rarely collected, difficult to preserve, and not easily cultured in the laboratory. Existing approaches to systematics in this group (Haeckel, 1880; Mayer, 1910; Bigelow, 1938; Kramp, 1961) have been entirely morphological, typically reductionistic, and often misleading. Phylogenetic inference in the Cubozoa is beginning to benefit from modern methodology (Collins, 2002; Collins et al., in review), but is currently outrunning baseline taxonomy in terms of the number of species awaiting description or adequate redescription (Gershwin, unpublished). The questions of species boundaries and species recognition criteria in the Cubozoa have not been examined in any modern context; thus all studies assuming a stable taxonomy and adequately circumscribed species are largely vulnerable to confusion through changes that seem inevitable as the taxonomy is refined. Modern methods can suggest phylogenetic relationships based on statistical analysis among samples, but more information is required about the fundamental units that those samples are meant to represent before informed decisions can be made about their applicability to species relationships in the natural world.

Currently, a fierce battle rages on in the scientific literature as to what, exactly, is a species. There exists no lack of species concepts available for consideration, nor lack of debate and speculation about what a species concept should be and do (Lloyd, 2001; Wilkins, 2002). In general, species concepts fall into one of two categories: mechanistic, i.e., species as participants in the process of speciation, or historical, i.e., species as the end results of processes (Luckow, 1995). Furthermore, species concepts have a functional duality, the two aspects of which may not be usefully compatible, similar conceptually to trying to look at both sides of a coin at the same time. On the one hand, there is the philosophical species, which is the grist for evolutionary diversification and evolutionary studies. On the other hand, there is the operational species, which is necessary for basic biological communication; we need a form that we can illustrate in field guides, refer to in materials and methods, and classify in museum collections. Countless other authors have noted this repellent relationship of the philosophical species with the operational species, perhaps none more cogently than Adams (2001), who highlighted the problem in terms of the Heisenberg Uncertainty Principle; Adams observed that the most philosophically satisfying species concepts are the least operational, and that as they become more operational, they lose their philosophical integrity.

At this point in time, we do not have enough information about reproductive isolation, phylogeny, gene flow, ecological niche, and genetic identity of the various cubozoan species to evaluate which of the competing species concepts best fits the operational and philosophical needs of this group. Until such an assessment can be adequately made, it seems most prudent to follow a conservative, multi-disciplinary approach of describing or redescribing putative species under the traditional system as they are discovered, and developing phylogenetic hypotheses about their relationships to one another by molecular means, the results of which feed back into the nomenclature. Like all scientific hypotheses, each must be tested, and may be refuted. In this way, we can begin moving forward with our understanding of this group. The luxury of awaiting the conclusions of philosophical debate cannot presently be afforded to the Cubozoa; this group contains “the deadliest creature on Earth” (Cropp and Cropp, 1984; Endean, 1988), as well as other highly toxic species with extremely serious medical and financial implications.

My species concept falls somewhere in the middle of the two earlier extremes, and comes from an understanding that a species is a stage in a lineage that is readily identifiable from other such stages and lineages. Thus, species are here inferred based on the idea that morphology is the observable result of evolutionary history, and that qualitatively diagnosable units are evolutionarily independent. An assumption of this concept is that these units possess a reproductive cohesiveness which underlies the morphological cohesiveness; however, no such studies have been conducted to support or refute this assumption. This species concept is a hybrid of the morphological species concept (MSC), under which organisms are classified as the same species if they appear identical by anatomical criteria, and the phylogenetic species concept (PSC), which postulates that a species is "a diagnosable cluster of individuals within which there is a parental pattern of ancestry and descent, beyond which there is not, and which exhibits a pattern of phylogenetic ancestry and descent among units of like kind" (Eldredge and Cracraft 1980:92). In contrast to the widely applied biological species concept (BSC), no inferences are made about the inability of these species to interbreed, or the role that any interbreeding might play in species recognition; reports abound on the ability of cnidarians and other "lower" animals to interbreed (Hamel and Mercier, 1994; Benzie et al., 1995; Marquez et al., 2002a; Marquez et al., 2002b; Miller and van Oppen, 2003; Beaumont et al., 2004). Almost without fail, the species recognized herein are believed to be geographically, temporally, or behaviourally isolated; however, most are based on small numbers of samples, such that the actual boundaries are unclear. Thus, a real problem exists in how to decide case by case where to draw the line between intraspecific variation and interspecific diagnosis. I have tended toward a conservative approach in my species determinations; thus, I expect that eventually some of the putative species identified by me will prove to be species complexes or higher taxa.

While many animal taxa differ from their congeners on fine-scale characters such as the number of hairs on a leg segment (copepods), the number of beads on the shell margin (snails), or the relative length of body parts (lizards and fishes), most cubozoan species differ from each other in multiple structural characters. Furthermore, the nearshore cubozoans have yet to be reexamined throughout most of South America, Africa, southeast Asia and the Indian subcontinent, four out of the five regions where the Cubozoa are the most prevalent. Thus, it seems exceedingly likely that, with morphological examination of more material and DNA studies of populations, the number of cubozoan species will increase dramatically, I would estimate by an order of magnitude.

The issue of "what is a species" will continue to be a hot topic in the systematic literature; without doubt, different groups operate under different criteria, not only in the interpretational taxonomic sense, but also in the evolutionary mode and tempo that drives speciation. However, two non-concordant methods of expressing this biological reality are currently employed, while the needs of modern taxonomy straddle two different paradigms.

On the one hand, one must be able to quickly and accurately identify different species, and this is most easily accomplished by comparison of autapomorphies, such as was common practice historically. On the other hand, one must also be able to infer phylogenetic relationships, and this can only be accomplished through identification of synapomorphies, such as is common practice today. This dichotomy in approaches splits the very essence of Darwin's "descent with modification", focusing differentially on the shared features that demonstrate descent or the unique features that manifest the modification. This dichotomy furthermore splits down philosophical lines, with those workers more focused on the descent part preferring the straightforward approach of cladistic taxonomy and the clarity of monophyly; in contrast, those workers more focused on the modification part prefer the more traditional approach of evolutionary phylogenetics and the more biologically realistic use of paraphyly. In this thesis, and in papers derived from it, I have tried to integrate these two systems, using the "what it looks like" to develop a means of identification of the forms that we observe in the natural world, and the "where it is on the tree" to understand the evolutionary relationships of these natural forms to each other.

PROBLEM 2. INCONSISTENT TAXON DEFINITIONS

Up to the 1970's, the Cubomedusae were a defined order within the class Scyphozoa. All of the taxa were grouped into two families, the Carybdeidae (those taxa with simple pedalia, and lacking gastric saccules) and the Chirodropidae (taxa with complex pedalia, and with gastric saccules). These groupings were insufficient to express the true biodiversity of the cubomedusae, resulting in artificial relationships of dissimilar taxa in both families and most genera.

Werner (1973b) elevated the Order Cubomedusae to the Class Cubozoa, based on polyp characters of absolute radial symmetry (i.e., lacking any trace of tetramerous symmetry) and total metamorphosis into the medusa stage. It has variously been noted, but should be regarded as no less important, that the Cubozoa are also unique in the following features: the planula larvae have a transverse band of pigment spots; the polyps have a single large nematocyst or ring of nematocysts in the end of each solid tentacle; and the medusae have a strongly imposed tetramerous cuboid symmetry, wing-like pedalia as the tentacular bases, rhopaliar niches in the body wall, complex eyes, and a velarium of subumbrellar origin. Many other features are either scyphozoan or hydrozoan, or both hydrozoan and scyphozoan, serving to place the Cubozoa firmly outside both classes in the traditional Linnaean system. Under this scheme, the Class Cubozoa and the Order Cubomedusae are redundant, referring to precisely the same set of taxa, i.e., all box jellies *sensu lato*. A decade later, Werner (1984) recognized the families at the ordinal level, i.e., Carybdeida and Chirodropida. This leaves us with an equally perplexing redundancy at the next level down, i.e., the Order Carybdeida and Family Carybdeidae are identical, as are the Order Chirodropida and Family Chirodropidae. However, Werner's later publication is not often cited, and the Cubozoa are still widely regarded as grouping into two families, resulting in a bottom-heavy taxonomy.

There is not even agreement on whether the Class Cubozoa should be recognized as separate from the Class Scyphozoa. Compelling developmental and anatomical evidence that the Cubozoa form a unique group has been given by Werner (1971; 1973a; 1973b; 1975; 1976). Later work on nematocysts (Calder and Peters, 1975), microanatomy (Chapman, 1978), behaviour (Stewart, 1996; Stewart, 1997), and genetics (Collins, 2002; Collins et al., in review) supported Werner's conclusions. However, Calder and Peters (1975) argued that although the cubomedusae were clearly distinct from the other orders of the Scyphozoa, a more conservative approach than class elevation would be to recognize two subclasses. Satterlie (1979) and Satterlie and Spencer (1979; 1980) argued that there was no fundamental difference in neurophysiology between the two groups, and that "this similarity is so convincing as to make it unnecessary to create a new class, the Cubozoa, to accommodate any unique features" (Satterlie and Spencer, 1980: p. 377). More recently, Dawson (2003) misinterpreted Collins (2002), regarding the Scyphozoa as paraphyletic; based on this, Dawson regarded the Cubozoa as within the Scyphozoa.

A corollary to the Cubozoa-Scyphozoa argument postulates that the Cubozoa is most closely related to the Hydrozoa, based on radial symmetry of the polyp (Werner, 1973b; Bouillon, 1981; Cornelius, 1991) and total metamorphosis of the polyp into just one medusa, as in the hydrozoan Narcomedusae (Petersen, 1979; Bouillon, 1987). Contemporary support (morphological or molecular) for this view is lacking (Collins, 2002; Chapter 3, herein). Furthermore, Salvini-Plawen (1978) used the same criteria (i.e., unique metamorphosis and cycloradial polyps) to recognize the Cubozoa as a class.

While the higher taxa continue to be debated, the generic delimitations and recognition criteria inherited from Haeckel (1880) are no doubt at the crux of the problem. For example, the genus *Carybdea* is typically thought to include the traditional forms such as *C. marsupialis* and *C. rastonii*, with bush-like gastric phacellae and heart-shaped rhopaliar niche ostia, plus the various problematical forms of *C. alata* with crescentic phacellae and T-shaped rhopaliar niche ostia, and the strange *C. sivickisi* with vertical rhopaliar niche ostia and exumbrellar adhesive pads (Mayer, 1910; Bigelow, 1938; Kramp, 1961). The larger, more robust forms are often all grouped into *Tamoya*, regardless of whether they have vertical clusters of cirri, or whether they even have cirri at all (Brooks, 1882; Kishinouye, 1910; Uchida, 1929; Menon, 1930; Rao, 1931; Menon, 1936; Bigelow, 1938; Uchida, 1947a; Ranson, 1949; Pope, 1951; Kramp, 1955a; Kramp, 1956b; Pope, 1957; Kramp, 1958; Kramp, 1959; Payne, 1960; Kramp, 1962; Kramp, 1968a; Uchida, 1970; Yamasu and Yoshida, 1976; Calder, 1977; Chakrapani, 1984; Fenner et al., 1985; Exton et al., 1989; Pagès et al., 1992; Holmes, 1996; Williamson et al., 1996; Kubota, 1998; Currie, 2000b; Pastorino, 2001). Assumptions and inferences are reciprocally cross-pollinated among species grouped in a genus, whether the groupings are natural or artificial (Uchida, 1929; Uchida, 1970; Williamson et al., 1996; Morandini and Marques, 1997).

The chirodropids have not escaped the confusion, with numerous different forms all being grouped into the genus *Chiropsalmus*, regardless of the form of the gastric saccules, the arrangement of pedalial fingers and tentacles, or the presence or absence of exumbrellar warts (Brooks, 1882; Horst, 1907; Light, 1914; Beebe, 1928; Menon, 1936; Nair, 1951; Searle, 1957; Guest, 1959; Kramp, 1959; Barnes, 1965; Barnes, 1966; Mohan, 1971; Calder and Peters, 1975; Burke, 1976; Chakrapani, 1984; Yamaguchi, 1985; Ming et al., 1990; Bengston et al., 1991; Cortés, 1997; Marques et al., 1997; Gordon, 1998; Carrette et al., 2002; Currie et al., 2002; Sun et al., 2002; Segura-Puertas et al., 2003). To a lesser extent the same problem has happened with the genera *Chironex* and *Chirodropus*, resulting in erroneous risk assessment (Thiel, 1936; Kramp, 1955a; Southcott, 1956; Cleland and Southcott, 1965; Williamson et al., 1996).

While much of the problem has resulted from lumping dissimilar forms, splitting has also led to confusion. *Chironex fleckeri* was given generic recognition (Southcott, 1956), yet it hardly differs from several forms still typically (erroneously!) identified as *Chiropsalmus quadrigatus*. *Carukia barnesi* was defined on the basis of lacking gastric cirri (Southcott, 1967), yet no less than nine other species also lack gastric cirri (Gershwin, unpublished). Furthermore, numerous juvenile forms have been given formal nomenclatural status (Haeckel, 1880; Fewkes, 1883; Hargitt, 1902), although they cannot be reliably assigned to, nor separated from, known adult species.

PROBLEM 3. UNDERESTIMATION OF BIODIVERSITY

The use of too few characters, or in some cases uninformative characters, along with inconsistent interpretation of taxon definitions and boundaries, may lead to an underestimation of species diversity. Of the 42 cubozoan species described to date, only 17 are currently recognized (Kramp, 1961; Southcott, 1967; Moore, 1988). However, preliminary studies include revalidation of at least seven historical species, no less than 30 new species from around the world awaiting formal description (Gershwin, unpublished), and an estimated hundred more yet undiscovered.

Ecology, toxinology, and phylogeny all rely on accurate assessment of species boundaries. Furthermore, the ability to discern new species relies on accurate sorting of existing species; some of the taxonomic implications of fuzzy species boundaries are elaborated in discussions about "*Chiropsalmus quadrigatus*" (Section 1.3; Section 2.1.1, Haeckel) and "*Carybdea alata*" (see Chapter 5). Finally, when the health risks associated with cubozoans are juxtaposed against the large number of unknown forms, it becomes evident that risky mistakes can too easily be made (e.g., "this species is too big to be *Carukia barnesi*; therefore, it must not be dangerous").

1.3 IMPLICATIONS OF UNCLEAR TAXONOMY

The issues enumerated above are not just philosophical problems, but rather, they have serious practical consequences concerning the identification of dangerous taxa. The need for a precise and understandable taxonomy of the Cubozoa can be illustrated in several recent examples, all suffering from lack of clear communication.

First, in northeastern Australia, two morphologically similar chirodropid forms occur together, one that is known to kill humans (*Chironex fleckeri*) and one that can not (*Chiropsalmus* n. sp. A, Chapters 2, 3, 4) (Barnes, 1965; Kinsey, 1986; Carrette et al., 2002). When recognized, the latter is usually erroneously identified as *Chiropsalmus quadrigatus*, a species which is reported to be lethal and widespread throughout the Indo-Pacific (Mayer, 1910; Light, 1914; Dawydoff, 1936; Stiasny, 1937a; Searle, 1957; Kramp, 1961; Russell and Nagabhushanam, 1996; Koyama et al., 2000; Nagai et al., 2002; Sakanashi et al., 2002; Sun et al., 2002). However, "*Chiropsalmus quadrigatus*" in Australia and throughout the Indo-Pacific actually comprises several quite different species, some of which are probably lethal and some that are not (Gershwin, unpublished). Depending on which description is at hand, "*C. quadrigatus*" may have heavy tentacles with lavender bands, or flat, ribbon-like tentacles, or fine round ones; it may have large, digitated gastric saccules, or sessile knob-like round ones; and it may reach a body size of over 15cm, or only 10. The confusion stems, in part, from the descriptions of *C. quadrigatus* by Haeckel (1880), Mayer (1910; 1915; 1917), and Barnes (1965). Preliminary examination of the specimens of Mayer and Barnes, as well as Haeckel's holotype, suggests that Mayer's redescription was based on at least two quite different forms, neither of which matches the holotype, and the specimens of Barnes were in turn different from those of Mayer and from the holotype (Gershwin, unpublished). Taxonomically, this argument stands on its own as compelling reason for a revision; however, in dealing with lethal species where management and treatment rely on proper diagnosis, a compromised taxonomy is just simply unacceptable.

In a second example concerning another major dangerous Australian jellyfish, *Carukia barnesi* has long been known to cause “Irukandji syndrome” (Barnes, 1964; Southcott, 1967), but errors have been made on estimating its distribution by sting reports rather than by specimens. This tautology has affected the accuracy of field guides, museum records, and medical documents. The species is reported to occur all across northern Australia (Edmonds, 1975; Sutherland, 1981; Sutherland, 1983; Marsh and Slack-Smith, 1986; Sutherland and Sutherland, 1999; Sutherland, 2001), and yet no confirmed specimens have ever been caught north or west of Port Douglas, QLD, or south of the Whitsundays, a range of only about 600km. Worse yet, based on an unclear understanding of what physical characters define *Carukia barnesi*, attempts at development of an antivenom have been hampered by wasting precious time and resources on not only the wrong species, but several unique forms that were assumed to be identical because they were of a similar size (K. Winkel, pers. comm., 1999). Furthermore, it has recently become clear that the Irukandji syndrome can be attributed to many species, not just one, and yet there still exists no clear definition of what characters diagnose the forms, and how the forms are related. Finally, about 40-50 tourists are hospitalized each year with Irukandji syndrome in northeastern Australia, most often stung while swimming in “stinger-resistant enclosures” (Fenner, 1988; Little and Mulcahy, 1998; Mulcahy, 1999), believing that they are safe and thus not taking additional precautions. In fact, the term “stinger”, by local convention, refers only to the larger deadly *Chironex fleckeri*, which cannot get through the stinger enclosures, not to the smaller Irukandji, *Carukia barnesi*, which can. Thus, tourists naively assume they are being protected, when, in fact, their health is at risk due to the vagaries of jargon.

A third example does not relate to human health, but clearly illustrates some of the taxonomic confusion and also has its own grist for systematic and ecological intrigue. Numerous quite distinct forms have been thought to be conspecific with the common Mediterranean cubozoan, *Carybdea marsupialis* (Thiel, 1936; Kramp, 1961; Studebaker, 1972; Larson and Arneson, 1990). Specifically, two geographically and morphologically distant forms, namely the Caribbean *C. xaymacana* and an undescribed California species, were lumped in with *C. marsupialis* based on comparisons of too few characters. Based on these assumptions, strange patterns of distribution have been hypothesized and our accurate understanding of the ecology and biology of these species has been misguided. Furthermore, insight has been obscured by confusion over a totally different form from southern Australia, *C. rastonii*, and the relationship that it bears to *C. marsupialis*. Some authors have thought that

C. rastonii should be regarded as identical to *C. marsupialis* (Mayer, 1910; Thiel, 1936); perhaps these authors had never actually seen *C. rastonii*, which differs in many conspicuous characters from *C. marsupialis* (Gershwin, unpublished). The undescribed species mentioned above from California has also variously been assumed to be *C. rastonii* for unclear reasons (Gladfelter, 1973; Satterlie, 1979; Matsumoto, 1995), but in fact, is more similar (but not identical) to the Mediterranean *C. marsupialis*; distribution was used preferentially over morphology in species recognition. Oddly enough, several workers have misidentified a peculiar southwestern Australian population of *C. xaymacana* as *C. rastonii*, presumably because of distribution, but certainly not based on morphology (Marsh and Slack-Smith, 1986; Fenner and Williamson, 1987; Ingram et al., 1992; Coleman, 1999; Sutherland and Nolch, 2000). An identical form occasionally occurs coastally in the Cairns region, and is typically (erroneously!) identified as the common Irukandji *Carukia barnesi* (Williamson et al., 1996; Sutherland and Sutherland, 1999; Sutherland and Nolch, 2000). A fascinating and ironic pattern has emerged in this story, namely that the morphological "*C. xaymacana*" comprises three distinct populations, one in the western Atlantic, one in the southern Pacific, and one in the eastern Indian Ocean. Only through accurate species identifications and robust phylogenetic comparisons will we ever know whether these forms represent exotic introductions, or some unelucidated evolutionary story of stasis or convergence.

Finally, the so-called widespread species "*Carybdea alata*" has become a trash-bin for any form with crescentic gastric cirri, currently comprising about 7-10 quite different forms based on preliminary comparison of other structural characters. At least one of these forms is apparently capable of causing life-threatening Irukandji syndrome (Mulcahy, 1999), but it appears that the rest cannot, based on known distributions and sting records (Mayer, 1910; Kramp, 1961; Williamson et al., 1996; Thomas et al., 2001). However, a few cases of Irukandji syndrome have been reported from Hawaii (Yoshimoto and Yanagihara, 2002), where one of the *alata*-species is common (Thomas et al., 2001; Yanagihara et al., 2002); further investigation may show that this species is the cause of the sickness. Whatever medical effects of the "*Carybdea alata*" group are eventually elucidated will depend in part on a clear taxonomy of its members; the taxonomy of this group is revised in Chapter 5.

1.4 DIFFERENT APPROACHES TO CUBOZOAN SYSTEMATICS

1.4.1 Macro- and micro-morphology: The need for numerous characters

Much of the taxonomic confusion lies in the characters used historically to differentiate the groups, and their interpretation. Previously, only eight characters have typically been used for differentiation of the carybdeids (i.e., phacellae shape or orientation, velarial canal number and complexity, pedalia number, presence of body warts, tentacle complexity, stomach size, and presence of mesenteries; Figures 1.1, 1.2, 1.3, 1.4), and three for the chirodropids (i.e., tentacle number, pedalial branching pattern, and gastric saccule form; Figures 1.1, 1.2). In contrast, in the present study I have examined and scored 85 continuous and discontinuous characters for every species, many of which have never before been used in cubozoan taxonomy in any meaningful way (Chapters 2, 3, 5).

In practice, focusing on a small number of key characters may make it quite simple to determine which named species a given specimen is similar to, but quite difficult to determine whether they are identical or separate evolutionary species. In contrast, taxonomy based on a large number of characters allows for more sensitive species recognition, both in the philosophical sense of identifying greater species richness and diversity, and in the practical sense of being able to recognize new species when they are at hand.

1.4.2 Hard and soft characters: The need for statoliths

Even though I examine far more characters than did my predecessors, I am nonetheless constrained by the literal and figurative “floppiness” of these characters – almost all the characters are gelatinous, and as such are easily distorted by types and concentrations of fixative, condition of the specimen at the time of fixing, and the contractions of the specimen in the throes of death.

However, one character in particular shows much promise for identification, namely the statolith (balance stone), a small, gypsum structure within the balance organ, which provides a unique opportunity for quantification of a hard part in a soft-bodied organism. It would be widely valuable to be able to easily identify ethanol distorted specimens, fragmentary or decomposing specimens, or thawed specimens, and statolith identification may even prove useful in recognizing fossil species or cubozoan remains in the stomachs of predators. Similar approaches have been used for stock differentiation and species recognition with the otoliths of fishes, the beaks and pens of cephalopods, and the statoliths of worms and squids (Castonguay et al., 1991; Smith, 1992; Campana and Casselman, 1993; L'Abee-Lund and Jensen, 1993; Friedland and Reddin, 1994; Envall, 1996; Bizikov and Arkhipkin, 1997; Clarke, 1998; Arkhipkin and Bjorke, 2000; Roeleveld, 2000; Begg et al., 2001). In Chapter 2, I present the results of a preliminary study on the statoliths of several species; a full morphometric analysis of statoliths is underway, but is beyond the scope of this thesis.

1.4.3 Molecules and morphology: The need for comparative datasets

While much of my work on the systematic problem is based on qualitative reassessment of numerous morphological characters, I also here use comparative phylogenetic datasets from morphology and DNA sequences. This approach has been successful in countless other groups, often providing supportive evidence of morphological patterns (Moritz et al., 1992; Garey et al., 1999; Krajewski et al., 2000; Janies, 2001; Cameron et al., 2002; Marques and Collins, 2004), and in some cases, providing novel insights that were not possible with morphology alone (Ammerman and Hillis, 1992; Costas et al., 1995; Siddall et al., 1995; Aguinaldo et al., 1997; van Oppen et al., 2001), but has not yet been comprehensively used with the Cubozoa. Previously, the 18S rDNA gene was sequenced from each of nine species of cubozoans, including six from Australia (Collins, 2002). The two main revelations that came from that analysis that are applicable herein were that A) the Cubozoa is a monophyletic group, and B) the genus *Carybdea* is not; both of these conclusions are supported by morphology and are further discussed in Chapter 3.

1.5 PROJECT OBJECTIVES

The primary objectives of this thesis were to develop a solid foundation for the taxonomy (i.e., identification and classification) and phylogeny (i.e., evolutionary relationships) of the Cubozoa, using the Australian taxa subset as a working model. Of the approximately 10 major morphological groups of cubozoans, 8 are represented in Australian waters. Many of these are not yet formally described. Specifically, among the 17 currently recognized species of cubozoans, only 5 occur in Australian waters, but at least 16 additional undescribed species are known to me from specimens or photographs, two of which will be described in detail as part of this thesis (Chapter 5). Based on existing species plus new Australian species, I will revise the characters that are meaningful for diagnosis and identification, and develop and compare molecular and morphological phylogenies to determine the how the species are related to one another. The specific questions I address are as follows:

1) Does the current classification scheme accurately represent evolutionary patterns and biodiversity of the group? [Chapters 1, 3, 5].
2) What are the molecular relationships of species within the Cubozoa? [Chapter 3].
3) Is there congruence between the morphological and molecular datasets, and if so, can we combine them for a stronger dataset? [Chapter 3].
4) What are the morphological characters useful for operational taxonomy? [Chapters 2, 3, 4].

5) What is the species diversity of known or suspected Irukandji causing jellyfish, and do they form a monophyletic group within the Cubozoa? [Chapter 3].

This thesis is organized as follows: An historical review and contemporary reassessment of cubozoan characters is presented in Chapter 2, in order to provide the foundation for the subsequent chapters. Chapter 3 presents molecular and morphological phylogenies, based on partial 18S nuclear ribosomal gene sequences and on the 85 morphological characters treated in Chapter 2, respectively. In Chapter 4, I present a key to the Australian species, plus a synoptic treatment of the existing and new Australian taxa. A full taxonomic treatment of the Cubozoa was not possible due to thesis space limitations, but was generated from the work herein and is forthcoming; a representative section is presented in Chapter 5, comprising a revision of the "*Carybdea alata*" group. Therein, a new family (Alatinidae fam. nov.) is proposed along with the descriptions of a genus (*Alatina* gen. nov.) and two new species (*Alatina mordens* sp. nov., and *Alatina rainensis* sp. nov.). Finally, a general discussion is presented in Chapter 6.

1.6 SIGNIFICANCE OF THIS THESIS

Contained within the Cubozoa are some of the world's most dangerous animals. Indeed, "the most venomous animal on Earth" (Endean, 1988) is a cubozoan, the Australian "deadly box jellyfish" *Chironex fleckeri*. But another cluster of species that has more recently come to the attention of medical and zoological researchers, "the Irukandji jellyfishes", comprising *Carukia barnesi* and several other undescribed species, inflict a potentially lethal sting with an horrific set of systemic symptoms known as "Irukandji syndrome". The initial sting is often mild, but after a 5-30 minute delay, severe symptoms begin, including unbearable lower back pain, waves of abdominal cramps, shooting spasms, nausea and vomiting, difficulty breathing, profuse sweating, dry coughing, and a feeling of impending doom (Williamson et al., 1996). Those unlucky enough to be stung by one of the more dangerous species also get runaway high blood pressure that can lead to heart failure or brain hemorrhage and death. Irukandji toxin is thought to be far more potent than that of *Chironex fleckeri*. It is still unclear precisely how many species are capable of giving Irukandji syndrome, or precisely which species were responsible for the two recent fatalities mentioned above.

It is generally acknowledged that numerous unidentified Irukandji species exist across northern Australia (Southcott, 1985; Kinsey, 1986; Kinsey, 1988; Williamson et al., 1996; Fenner, 2000; Currie et al., 2002; Fenner and Hadok, 2002; Huynh et al., 2003), and there is every reason to expect that these forms will have species-specific behaviours, bloom cycles, life spans, inshore-offshore preferences, and toxicities. Management strategies such as decisions to close certain beaches at high risk times, which reefs to avoid during certain parts of lunar cycles, and stinger net mesh sizes, will depend on species-specific ecology and biology. Similarly, treatments may be tailored to certain aspects of envenomation which may be species-specific, and the long term goal of developing a rapid diagnostic test will likely depend on species-specific surface proteins or DNA. Finally, an antivenom to treat Irukandji stings has not yet been developed (Winkel et al., 2003); while this may prove species-specific, as in those for snakebites and spiderbites, even its development cannot proceed without accurate identification of the specimens being processed. Thus, attempts at managing these different species as a single unit will certainly prove problematical, while such delays in advancements may result in additional human suffering.

The significance of the work in this thesis is that a sound taxonomy and phylogeny will provide a solid foundation for communication and comparison in all types of study, and will enable experts and non-experts to easily identify known and unknown species as a means of proper risk assessment. As explained above, there is currently no common agreement on species boundaries and species recognition criteria in the Cubozoa; this situation is unacceptable in northern Australia, where the stakes are extremely high because the species can be deadly. In most geographic and taxonomic areas, if one makes an error in identification, at the very worst, one suffers embarrassment (however, in practice, most mistakes probably go unnoticed); with northern Australian cubozoans, an error in identification leading to an erroneous risk assessment could cost a human life.

Having a means of accurate species identification is necessary but not sufficient to managing the cubozoan problem. A well supported phylogeny further allows for predictions to be made about lesser known species, based on their hypothesized relationships to well known species. For example, potential public health risks associated with poorly known carybdeid species could be predicted based on their phylogenetic position relative to known Irukandjis. Venoms of common species can be analyzed for similarity to venoms of rare, closely related species, perhaps hastening antivenom development or understanding how different groups of venoms affect different types of tissues. Similarly, bioprospecting studies may benefit from this work by searching for useful compounds in common relatives of chemically interesting rarer species. Ecological hypotheses can be developed and tested on common species, and extrapolated as the basis for predictions on the arrival and departure of dangerous species from certain areas. Less applied forms of enquiry such as evolutionary and developmental work may also benefit from this study by utilizing phylogenetic relationships as the cornerstone of basic comparison. Finally, non-scientific stakeholders may benefit from these results as well. For example, doctors assessing potential complications of stings, by using the predictive power of phylogenetic relationships; state and local management agencies, by having accurate identification and distribution information for different species with which to develop and monitor safety policy guidelines; tourism interests such as charter boats and resorts, by having the means to identify when harmful species are present and when they are not; and Surf Life Saving, by being able to accurately identify harmful from harmless species when they are found in swimming areas.

In summary, any type of study that relies on accurately identified specimens, or benefits from the comparative value of a phylogeny, will build on this work. The issues of scientific accuracy are compelling reasons to revise any taxonomic group. However, as illustrated above, in the present case of the Cubozoa, the lack of a reliable system of identification and classification has many urgent medical, economic, and ecological implications as well.

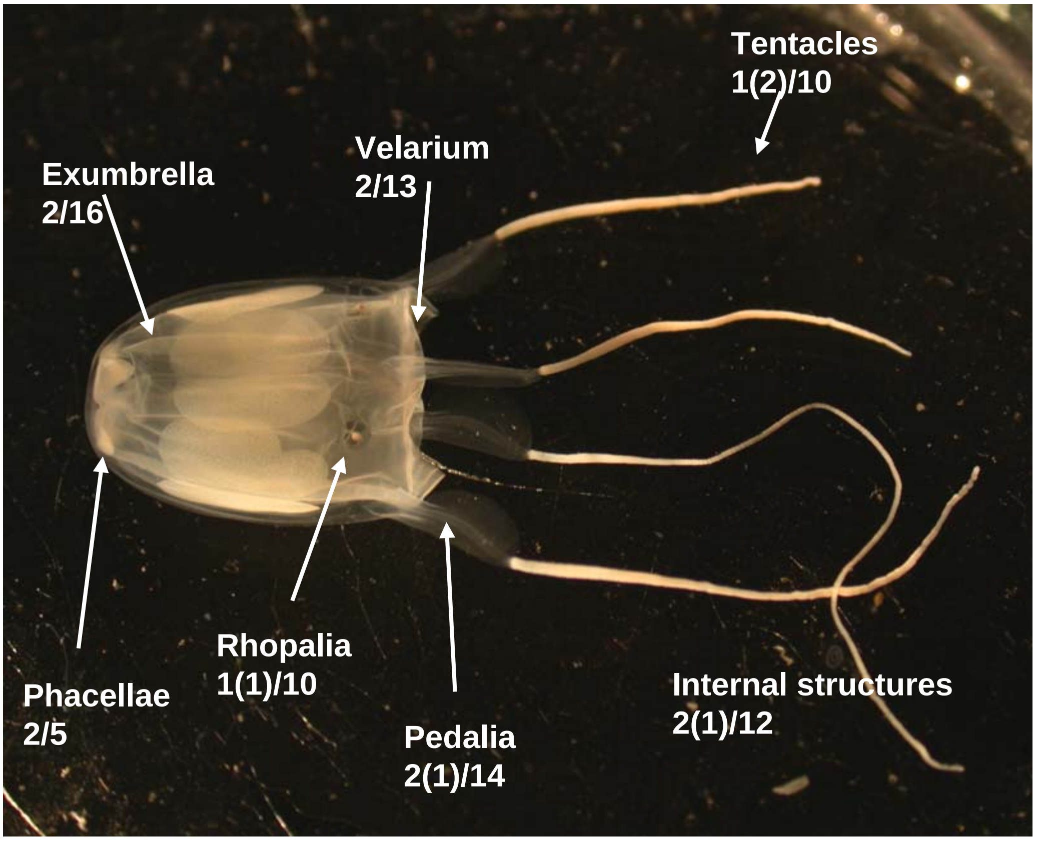

Plate 1.1. Historical/revised characters used for identification of cubozoan species. Historical characters used are listed below; those used only once are designated in parentheses. Revised characters used are listed and explained in the text.

Exumbrella: size, presence/absence of warts
Velarium: number and branching of canals
Tentacles: number (cross section shape, banding)
Phacellae: shape, location
Rhopalia: niche shape (number of eyes)
Pedalia: carybdeid shape, chirodropid # branches (canal bend structure)
Internal structures: saccule shape, mesenteries pres/abs, manubrium length (gonad location)

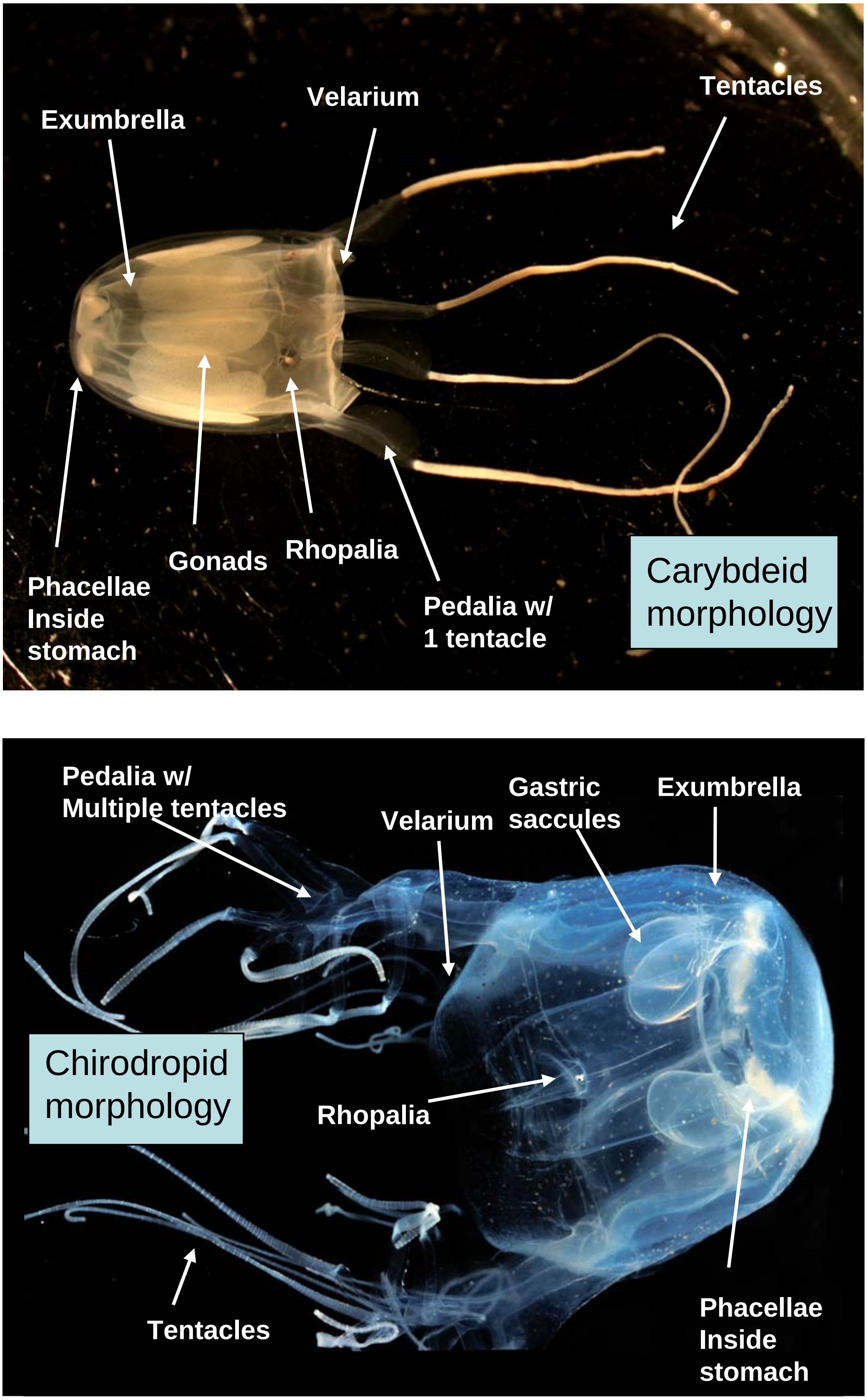

Plate 1.2. Overview of cubozoan external and internal morphology.

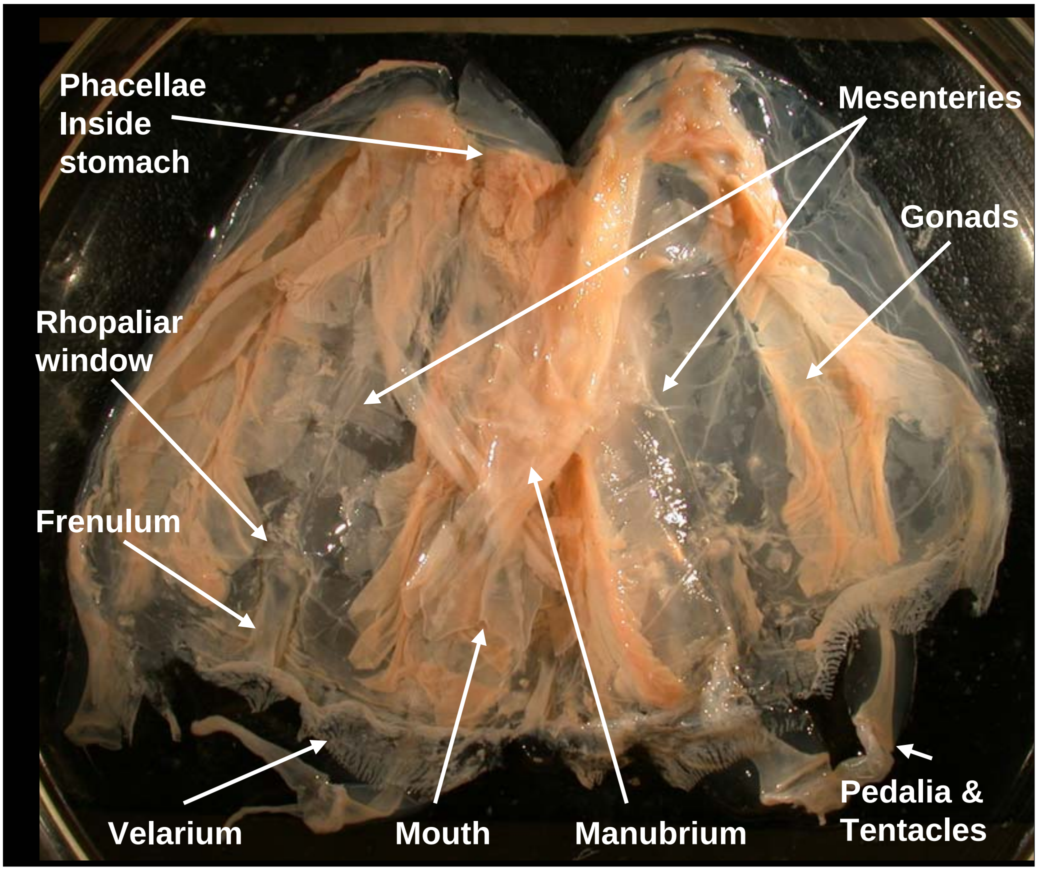

Plate 1.3. Overview of carybdeid subumbrellar morphology; chirodropid subumbrellar morphology is similar, but with the addition of gastric saccules. Specimen is dissected open and splayed out flat to facilitate ease of observation of important structures.

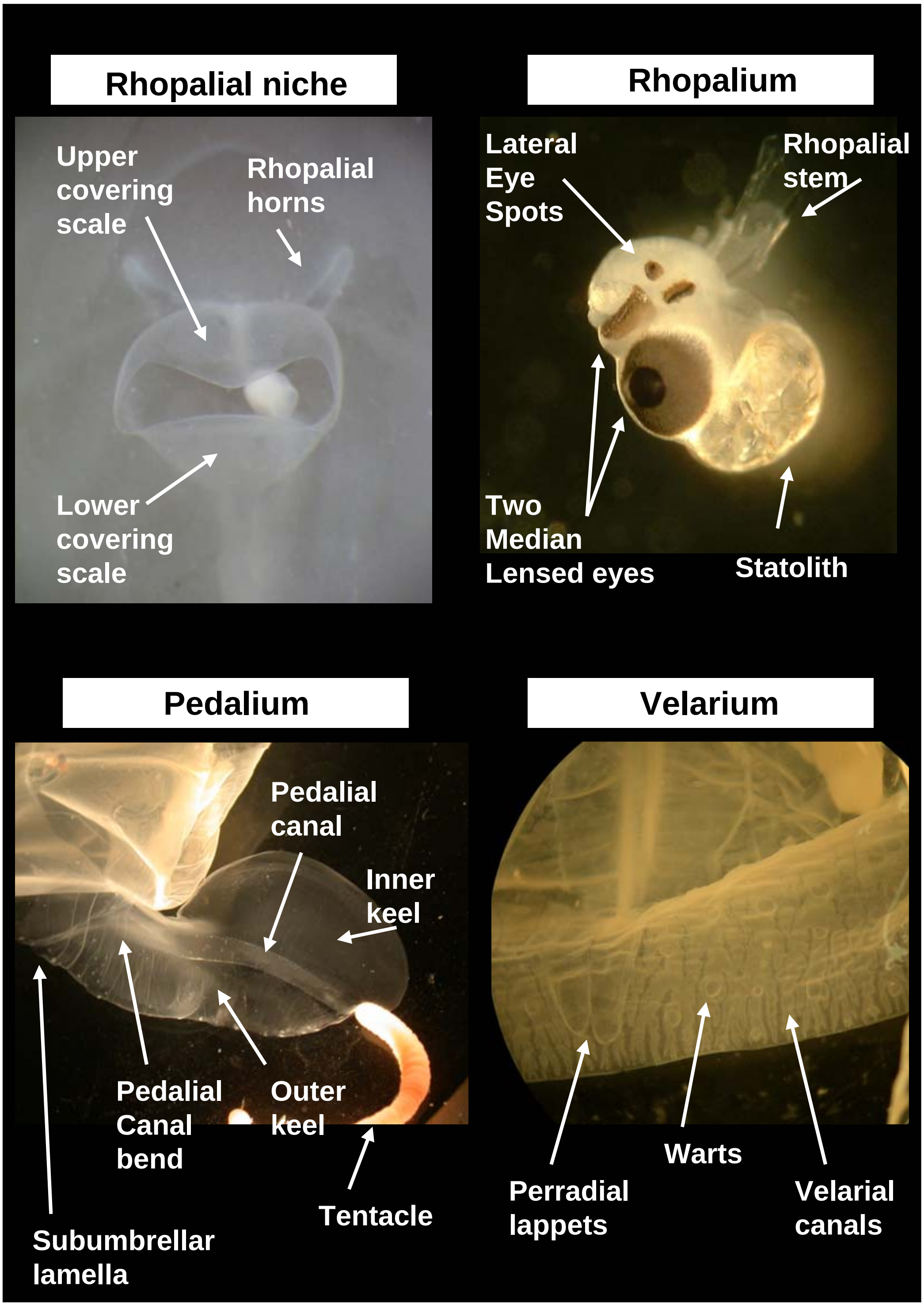

Plate 1.4. Overview of cubozoan micro-morphology; all structures are plainly visible with a dissecting microscope.

CHAPTER 2: A REVIEW OF, AND NEW PERSPECTIVES ON, CUBOZOAN CHARACTERS USEFUL FOR IDENTIFICATION AND CLASSIFICATION

2.1 INTRODUCTION

The taxonomy of the Cubozoa has not been comprehensively reexamined since the original classification of Ernst Haeckel (1880). Later workers have used Haeckel's classification and have made synonymies or additions, but comparative character reassessments have been lacking or misleading. The few who have carefully studied characters and character states have tended to interpret them so broadly that it has become nearly impossible to distinguish biologically meaningful species. Currently we recognize some 17 valid species, about half the total number historically described, but this number is a gross underestimation of the morphological biodiversity of the group (Gershwin, unpublished).

Indeed, many of the cubozoan "species" valid today differ from one another in almost all structural characters, thus creating wide morphological gaps between taxa, so it is not surprising that "intermediates" exist (see, for example, Kramp, 1961). Historically, these intermediates were typically assigned to one or the other endpoint, rather than being evaluated in their own context (e.g., Mayer, 1910). I am not suggesting that all intermediates should be considered different species, but I am suggesting that if a creature is found which appears to be intermediate between, say for example, the dogs and the horses, then the range of hypotheses considered should include that it is neither a dog nor a horse, but rather something unique from both. This approach is not just a historical problem, but is still often used, especially by non-specialists who seek to quickly label a specimen and move on to the next, rather than to really assess whether it is the same as or merely similar to something in a field guide.

As explained in Chapter 1, the classification scheme established by Haeckel and followed up to the present day relies on a series of "key characters" to diagnose different species. One of the main tradeoffs to this *a priori* approach to evaluating of one or two shared characters is that it typically provides a good estimate of common ancestry (i.e., closely related species often resemble each other in some features), but not necessarily of shared identity (i.e., one or two similarities gives limited predictive value regarding similarity of other characters). Of course, character similarity can also be due to homoplasy, which might not be evident in such a restricted analysis. I assert that species identification is a reciprocal process of evaluating all the available characters against those of known species. Condensing many different forms into one identity may simplify the task of identification, and it may even be satisfying to eliminate oddballs and unknowns, but in the final analysis it can give an inaccurate estimation of the patterns and processes that we seek to understand in the natural world.

A common misperception about cubozoans, as with all jellyfishes, is that they are too simple to tell apart, i.e., they do not have enough characters. While it is true that taxa higher on the evolutionary tree often have many more physical features, the Cubozoa have many features with which to diagnose many different morphological forms. All characters used herein are observable either by the naked eye or with the aid of a hand lens or dissecting microscope. Most do not require dissection, though incising the body wall to expose the phacellae is often helpful. The only characters requiring a compound microscope are nematocysts, detailed below.

The aim of this chapter is to reexamine the morphological characters and character states of the Cubozoa, and in doing so, to correct the misinterpretations of the past and to establish an understandable system for the present and future. Herein, I review a total of 23 major categories of characters, including four that have not been used previously and two that have been used in a different sense. From these, a total of 85 (74 structural and 11 morphometric) characters are routinely scored (Plate 1.1; Appendix 2). Most characters have discrete gaps among the states representing different taxa, as indicated in Chapter 3. Detailed explanations are given of historical and modern interpretations of the characters, as well as for identifying and understanding the various character states. Comparative tables of many of the character states are provided, along with summary tables integrating the main characters (Table 2.14a, b). Comparison is made among the characters of 20 carybdeids and 8 chirodropids, including many Australian forms which represent new and undescribed species. A comprehensive revision of the Cubozoa based on these criteria herein will follow shortly; one section from this revision is presented in Chapter 5.

2.1.1 Historical character emphases

For most of the history of cubozoology, it was common practice to focus on certain characters and separate the taxa according to whether or not they possessed these key characters. This approach has often led to gross misunderstandings in species boundaries and species recognition criteria, and to similar misunderstandings at higher taxonomic levels. This chapter is dedicated to understanding the wholistic Cubozoa, in terms of using all the characters and their nuances to better circumscribe the species. However, the current state of taxonomic disarray is the manifestation of many layers of misunderstanding, and I believe that sorting it out is not just a matter of understanding that there is a problem, but also understanding how the problem came to exist. Thus, a brief synopsis of the views of the cubozoologists will help put the study of characters into perspective. Following is a synopsis of the main bodies of thought on characters of the Cubozoa, arranged by worker in chronological order.

Early history

The first described cubozoan was *Carybdea marsupialis* (Linnaeus, 1758), differentiated from other jellyfishes on the four marginal tentacles as illustrated by Plancus (1739). Taxonomic confusion in the Cubozoa started early, with Linnaeus listing the Mediterranean as the type locality for Plancus's *C. marsupialis* from Rimini in the northern Adriatic, leading no less than three other authors to publish the "first record" of this species in the Adriatic (Boero and Minelli, 1986; Mizzan, 1993; Bettoso, 2002). To date, no studies have been conducted to determine whether the Adriatic and Mediterranean forms are the same, although there is every biogeographical reason to question this assumption, based on the distributions of other cubozoan species in other regions (e.g., see Chapter 4).

A few other species were added during the first half of the 19th century based on extremely brief descriptions and vague or misleading figures; these are largely unrecognizable today. For the first hundred years after Linnaeus, the carybdeids were classified with the hydrozoan medusae. It wasn't until 1859 that the first chirodropid was discovered.

Louis Agassiz

Agassiz (1862) separated out the cubozoan species from other medusae, but only at the familial level. He distinguished his new genus *Chiropsalmus* from the others based on the forked pedalia (Plate 1.1; Section 2.3.4), but he did not state what criteria he used to separate the carybdeid genera and species.

Prior to Agassiz, the genus *Carybdea* was used to group the taxa having 8 radiating stomach pouches with internal gonads, and therefore brought together several disparate taxa, including the narcomedusan hydrozoan now known as *Solmundella bitentaculata*, at least two varieties of the coronate scyphozoan now known as *Periphylla periphylla*, and, of course, the cubozoans. Agassiz was the first to separate these, erecting four different families, one for the narcomedusa and its relatives, one for each of the coronates, and a fourth for all the cubozoans. Curiously, he kept the cubozoan name *Carybdea* with one of the coronates, and instead adopted Lesson's (1837; 1843) name *Marsupialis planci* for the cubozoan species we now know as *Carybdea marsupialis*, and put all the cubozoans in Lesson's unpublished family, the Marsupialidae. The family name Charybdeidae was given to *Charybdea periphylla* (a scyphozoan), and was reinstated to the Cubozoa by Haeckel some years later.

Ernst Haeckel

Haeckel (1880) separated the carybdeid genera based on presence/absence of velarial canals and frenulae, size of stomach, development of mesenteries, and direction of phacellae (Plate 1.1, 1.2; Sections 2.3.7, 2.3.15, 2.3.16, 2.3.19, and 2.3.22). The chirodropid genera were separated on whether the gastric saccules were simple or branched (Plate 1.1, 1.2; Section 2.3.21). Unlike later workers, Haeckel didn't regard particular characters as important in separating species, but rather, he separated them by their unique features.

Most of the species described by Haeckel (1880) have not been seen again, and most of his material has been lost. Furthermore, his descriptions were often too vague for proper recognition, or focused on characters since found to not be useful for adequate diagnosis. Therefore, many of the species he described are not generally regarded as valid. However, despite the unrecognizability of many of his taxa, he is still properly considered the Father of Cubozoology for many reasons. He erected the taxonomic system that is still in use today; despite its lack of fine resolution, it has nonetheless served to distinguish the major forms over the last 120 years. Haeckel was the first to *really look* at the wholistic morphology of the different forms, and few have done so since. Furthermore, Haeckel was the only worker who examined enough material from around the world to understand the species in a pan-global biogeographical context; all subsequent workers have, at best, worked on the species of a given region, often making conclusions based on assumptions and misinterpretations of unseen taxa. In fact, as Haeckel established, the species comprising the Cubozoa are quite distinct from region to region.

Haeckel had a reputation for being somewhat too artistic with his species and character interpretations throughout the Medusozoa. Some of his critics accused him of fabricating characters for the sake of aesthetics (Browne, 1916), and this may have led to many of his species with unexpected features subsequently being disregarded. However, some of these invalidated species have again been found, and the features are exactly as originally described (Stiasny, 1926a; Bigelow, 1938; Goy, 1979; Pagès et al., 1992).

It is ironic that at least two of the species he described appear to have been described as new by later German-speaking workers, who apparently ignored his earlier findings. In the first case, although most of Haeckel's material is no longer extant, I have been able to study the badly damaged juvenile holotype of *Chiropsalmus quadrigatus*. Any juvenile specimen in poor condition would normally be considered impossible to identify. However, one character in particular makes this species immediately distinctive from almost all other chirodropids, namely, the pedalial branching form (Section 2.3.4, Char. 13). The pedalia were originally described as flat-form and laterally compressed, and despite the absence of a figure, this character would seem difficult to misinterpret; plain and simply, instead of being three-dimensionally hand-like (when the hand is curled into a claw-like form), they are branched only in a single plane. However, the holotype was been repeatedly misinterpreted (Stiasny, 1922; Kramp, 1955b), and its distinctive pedalia were not recognized as similar to the later-named *Chiropsoides buitendijki* (Horst, 1907). As a result, the species was redescribed based on material dissimilar to the true *Chiropsalmus quadrigatus* (1910; 1915; 1917); the inaccurate redescriptions of Mayer have been widely adopted.

As for the other of Haeckel's species subsequently described, I have been unable to find any extant material, but the description is so accurate for *Procharagma aurea* that it may as well be the description of the later-named *Carybdea sivickisi* (Stiasny, 1926a). The similarity of the size at maturity, colouration, and overall body form, along with the type locality, make this difficult to dispute.

The only other of Haeckel's cubozoan types that I have been able to find are the two syntype specimens of *Carybdea murrayana*, which have not aged well and are quite fragile, but nonetheless most of the important characters can be confirmed. It is clear to me from study of the types and the descriptions (Haeckel, 1880; Haeckel, 1881), that this species is closely related to, but distinct from, *Carybdea marsupialis*, and should be regarded as valid. It was considered valid by Mayer (1910), but Bigelow (1938) regarded it as an overgrown version of *C. marsupialis*, and presumably on that basis it was reduced to a junior synonym of *C. marsupialis* by Kramp (1961).

Alfred Goldsborough Mayer

Mayer (1910) put emphasis for species distinction on the shape and size of the pedalia, and on the number and degree of branching of the velarial canals (Plate 1.1; Sections 2.3.4, 2.3.15). He stated that for the genus *Carybdea* "owing to the slight differences between them, it is exceedingly difficult to separate the species one from another" (p. 506). As such, he interpreted all the carybdeids as varieties of only 4 species, namely, *Carybdea marsupialis*, *C. alata*, *Tamoya haplonema*, and *Tripedalia cystophora*. In fact, these species as defined by Mayer represent morphological groupings of groupings concordant with DNA sequence analysis (Chapter 3), and are assigned familial status in Chapters 3-5 of this thesis in accordance with the Linnaean taxonomic system.

Similarly, Mayer believed that the large stomach, well developed mesenteries, and vertical gastric phacellae of *Tamoya* were insufficient for separating the genus from *Carybdea*, "for the differences between them are merely of an intergrading character" (p. 512). However, he failed to notice that the two groups also differ in nearly every other major and minor structural character. He further believed *Tripedalia* to be closely related to *Chiropsalmus*, differing on the basis of the pedalia themselves branching in the latter but not in the former, and in the latter having gastric saccules but lacking in the former. In fact, *Tripedalia* is a carybdeid in every nuance of its morphology, and genetically it is well nested within the Carybdeida clade (Chapter 3).

As detailed above (Section 1.3; Haeckel), one of the most perpetuated errors in cubozoology was initiated by Mayer (1910; 1915; 1917), namely, the nomenclatural tangle surrounding the name *Chiropsalmus quadrigatus*. Without studying the holotype from Rangoon housed in the Copenhagen Museum, or apparently even its original description, Mayer redescribed the species based on specimens from the Philippines, some 3500 kilometers and a few bioprovinces away. The species he redescribed does not match the distinctive characters of the holotype. Furthermore, the specimens he used in his description comprise more than one species (Gershwin, unpublished). Since that time, the name *Chiropsalmus quadrigatus* has become a catch-all for just about any Indo-Pacific chirodropid, regardless of its morphology.

Gustav Stiasny

Stiasny was among the most observant of cubozoologists, not only in the gross morphology of the specimens, but also in thinking critically about what the morphology meant in terms of the relationships. Early in his career, he was a strong influence on the separation of the genera *Carybdea* and *Tamoya* (Stiasny, 1919; Stiasny, 1930), influencing Uchida (1929; 1970), Bigelow (1938), and Kramp (1961), against others who preferred to merge them, including Claus (1877), Fewkes (1883), and Mayer (1910). Stiasny blamed the disagreement on the poor state of preservation of specimens, variable characters such as form and size of the pedalia, and important differences not being detectable in young stages. He concluded that certain characters could be used reliably in separating the genera *Carybdea* and *Tamoya*, namely, the body size and shape, the stomach size, presence of mesenteries, direction of phacellae, and folding of the gonad margins (Plate 1.1; Sections 2.3.1, 2.3.7, 2.3.8, and 2.3.19). He regarded as somewhat variable characters such as the thickness of the apical mesoglea, presence or absence of corner pillars, and smoothness of the sensory niche.

A few years later, Stiasny (1934) changed his opinion on the importance of the folding of the gonad margin, believing the grade of pleating of the gonads to be dependent on age, and thus not of specific importance. Unfortunately, Stiasny also got caught up in Uchida's (1929) confusion regarding "*Tamoya alata*", and, like Uchida, concluded that the direction of gastric phacellae is of specific rather than generic importance. In fact, *Tamoya haplonema* sensu Müller differs from "*Tamoya*" *alata* sensu Reynaud (= "*Carybdea alata*") in nearly every structural character (see below, and Chapters 3, 4, 5), the direction of gastric cirri being merely one of the more obvious. The two species would not be considered closely related today by morphological or genetic standards.

Stiasny (1937a) made one of the most misguided statements in the history of cubozoan taxonomy, "In spite of differences in the sculpture of the ex-umbrella all these specimens belong, I believe, to one very variable species, because they all agree in the form of the dumb-bell-shaped opening of the sensory-pit" (p. 211). Stiasny's view was characteristic of many authors' views of the time, i.e., focus on a single defining character rather than a broad assessment of overall similarity and difference. However, there are, in fact, at least 11 different species spanning four natural groups with the dumb-bell-shaped rhopalial niche ostia, but otherwise differing in numerous structural characters.

Henry Bryant Bigelow

Bigelow (1909) provided important insight on the significance of constancy and isolation of geographic races, stating, in part, "in the case of such a geographically restricted and local genus as *Charybdea* (sic) equally small variations, when not representing mere developmental differences, are of much greater systematic importance, and, if they prove to be constant for different localities, may well be regarded as the basis for specific distinctions" (p. 16). He went on to state that the characters of greatest specific importance include size and general form of the adult, phacellae structure, and velarial canal number and complexity (Plate 1.1; Sections 2.3.1, 2.3.7, and 2.3.14). However, he doubted that any adult species in the genus would have simple canals.

Bigelow (1938) gave the most cogent analysis of cubozoan characters, though limited to only the carybdeids. His descriptions and illustrations of the phacellae, pedalia, and rhopalial niche ostia make these characters and their various states utterly unmistakable. While Bigelow's analysis was a substantial contribution, he was nonetheless focused too narrowly on a few characters, which he perceived as species level differences. He thus failed to notice additional differences that would have better expressed cubozoan diversity.

Bigelow misunderstood two major differences between *Carybdea marsupialis* and *C. xaymacana*, leading him to interpret the latter as a paedomorphic variety of the former. First, he misinterpreted the phacellae (Section 2.3.7), thinking that the "8-10 major branches" in *C. marsupialis* correspond with the "several primary branches" of the main trunk that he observed in *C. xaymacana*. But this conclusion was erroneous; the single main trunk of each of the four phacellae in *C. xaymacana* corresponds to the 8-10 main trunks in *C. marsupialis*. In both species, the main trunks each connect to the floor of the stomach, and each has several primary branches. Regardless of what one chooses to call them, the four phacellae of *C. marsupialis* each have numerous stalks connecting to the floor of the stomach, whereas the four phacellae of *C. xaymacana* each have only a single stalk connecting to the floor of the stomach. Second, Bigelow somewhat misinterpreted the velarial canals (Section 2.3.14). While he correctly noted that *C. marsupialis* typically has 3-4 canals per octant and *C. xaymacana* only has 2, he interpreted these as representing an ontogenetic series. In fact, the number of canals is constant throughout ontogeny in the two species, and thus more accurately representative of different lineages.

Tohru Uchida

Uchida (1929) gave a good account of the characters and development, but seriously misunderstood the species and their relationships. Throughout his career, he seems to have staunchly followed Haeckel's principle of ontogeny recapitulating phylogeny, often linking different described forms from different areas as mere stages in development. He wrote about the genus *Carybdea*, "About 10 species have been reported as belonging to the genus, but in my opinion they should be reduced to three or four…. Specimens of the same species from different localities and in different stages are sometimes described as different species. Moreover, characters generally regarded as specific, such as shape of the pedalia and velar canals, are not enough for the purpose" (Uchida, 1929, p.156; see Plate 1.1, Sections 2.3.4, 2.3.14 herein).

He went on to misidentify *Carybdea sivickisi* (as the juvenile of *Tamoya alata*, an error corrected in 1970). However, he also erred on the adult form; his specimen clearly had no features in common with *Carybdea alata*, with whom it was supposed to be identical. There was a big mix-up with the identities of *Tamoya alata* sensu Agassiz, 1862 and *Carybdea alata* Reynaud, 1830, which was finally resolved some years later (Bigelow, 1938; Kramp, 1956b). Uchida appears to have mixed this up even further, by discussing the various forms typically associated with the true *Carybdea alata* in the context of *Tamoya*. In all, Uchida created a nomenclatural mess that took decades to resolve.

Uchida (1947a) identified a form from Arnhem Land as *Tamoya bursaria*, based on the overall bell shape and the presence of mesenteries (Plate 1.1; Sections 2.3.1, 2.3.22). Although he noted the small size at maturity and the absence of gastric phacellae, he ignored these in his identification. Most likely, based on morphology and known species distributions, Uchida had found the so called (undescribed) "Darwin carybdeid" (see below; Chapters 3, 4).

Uchida (1970) corrected some major errors that were made in his 1929 paper. However, the corrections were on species identification, and no attention was given to the interpretations of the characters that led to the inaccurate conclusions. Furthermore, he made several more major identification mistakes in his later paper! First, it is unclear why he referred a specimen from Cape Town to the species *Carybdea alata*. There are literally no characters described or illustrated that would indicate that the form he studied was referable to *C. alata*, whereas every character indicated that it was an undescribed relative of *C. marsupialis*. Second, his observations of the growth series of "*Tamoya*", which led to his conclusion that *Tamoya* and *Carukia* lose their gastric cirri as they grow, were utterly erroneous. The specimens in the growth series possessed characters typically associated with *Carybdea rastonii*, namely, linear horizontal phacellae, heart-shaped rhopalial niche ostia, and a flat stomach. A group of large, robust medusae with a *Tamoya*-like appearance are known along the eastern coast of Australia collectively as "Morbakka"; these differ from *Tamoya* in several structural respects, the most obvious of which is the lack of gastric phacellae. Although it is unclear whether Uchida's Japanese specimens were identical with one of the Australian forms, it does seem clear that ontogenetic traits of one form of Pacific medusae cannot necessarily be extrapolated to a different form of Atlantic medusae; sometimes taxa really are different.

In general, Uchida's observations tended to be incredibly meticulous and his descriptions incredibly articulate; it is therefore incredibly regrettable that he misunderstood the cubozoan taxa and their characters as seriously as he did. Perhaps in trying so hard to find phylogenetic similarity, he focused less on biological reality.

Paul Lassenius Kramp

Kramp wrote numerous papers that included cubozoans to some extent, but his main area of expertise was in the Hydromedusae. His cubozoan conclusions were often lacking in depth. In his landmark Synopsis (Kramp, 1961), he focused primarily on the bell size, wartiness of the bell, rhopalial niche ostium shape, and phacellus shape in differentiating the carybdeids (Plates 1.1, 1.2; Sections 2.3.1, 2.3.3, 2.3.7, and 2.3.9). For the chirodropids, he focused mainly on the bell height, number and arrangement of the tentacles, and size and shape of the gastric saccules (Plates 1.1, 1.2; Sections 2.3.1, 2.3.5, 2.3.6, and 2.3.21). I believe it is important to note that these characters are necessary but not sufficient for proper identification.

In writing the Synopsis, he listed only the most prominent characters that separated one form from the next, without mention of other distinguishing characters. Thus, all too often, new forms have been erroneously attributed to one of the species listed in Kramp's Synopsis, based on general similarity to the given characters. However, many of these most prominent characters are now proving indicative of higher taxa. The characters listed are not incorrect; they are just incomplete, and thus often misleading.

Ronald Vernon Southcott

Southcott's two main papers were the descriptions of the chirodropid *Chironex fleckeri* and the carybdeid *Carukia barnesi* (Southcott, 1956 and 1967, respectively). In his description of *Chironex*, Southcott focused primarily on the structural differences of the gonads (Plate 1.1; Section 2.3.8). In *Chironex*, the lateral gonads, i.e., the paired leaf-like structures arising along the interradial septa, are greatly reduced, and the superior gonads, i.e., the gonadal tissues extending out onto the gastric saccules, are the primary gonadal structures. In contrast, Southcott assumed that most other chirodropids have well developed lateral gonads and reduced superior gonads. While Southcott was accurate in his description of *Chironex*, the gonadal differences are not the only feature that characterizes *Chironex*, and in fact, probably not the best. Superior gonads are also well developed in *Chiropsalmus quadrumanus*, *Chirodropus gorilla*, *Chiropsoides buitendijki*, one of the two Philippine chirodropids often collectively referred to as *Chiropsalmus quadrigatus*, and even in larger individuals of *Chiropsalmus* sp. A of Australia. I have no doubt that any fully mature chirodropid would have well developed superior gonads, and therefore, it would be misleading to focus on this character when trying to identify a specimen.

Southcott's Irukandji paper (1967), also gave undue focus to a single character, i.e., the absence of gastric cirri (Plate 1.1; Section 2.3.7). I have identified a number of forms that lack gastric cirri, yet differ substantially from *Carukia barnesi* in numerous other respects. Like all species, *Carukia barnesi* is best identified by its unique combination of characters.

Bernhard Werner

Werner's contribution to the world of cubozoology was simultaneously minor and massive. Minor in a taxonomic context, in that he described no new species, studied little comparative material, and published only a few papers, but paradigm-shifting, in that his meticulous work led him to recognize the group as distinct from the Scyphozoa, based on the total metamorphosis of the polyp to the medusa (Werner et al., 1971; Werner, 1973b; Werner, 1975; Werner, 1976; Werner, 1983). This was disputatious at the time (Calder and Peters, 1975; Satterlie and Spencer, 1979; Leonard, 1980; Satterlie and Spencer, 1980), and has recently become so again. While the original argument was based on philosophical conservatism along with the neurophysiological similarities between the Cubozoa and Scyphozoa, the current arguments are based on phylogeny (Dawson, 2003). These arguments are difficult to settle because they are firmly rooted in opinion and interpretation rather than empirical facts.

The nervous system argument of Satterlie and Spencer (1979; 1980) holds that the neurophysiology is nearly identical to that of the scyphozoans, and thus the Cubozoa does not warrant recognition as a separate class. However, the two groups otherwise differ dramatically in life history, planula larvae, polyp and medusa anatomy, metamorphosis, toxins, genetics, cnidomes, and behavior. While I do not intend to diminish the importance of the nervous system in any way, one might expect closely related groups to share some characters and not others, but the possession of synapomorphies does not constitute sameness. In fact, a recent molecular phylogeny (Collins, 2002) suggested that the Scyphozoa and Cubozoa are close relatives, so one might expect numerous features in common. The decision of where to draw the line at what deserves class-level status and what does not is a difficult question, but the answer seems clearer when viewed through a broader lens.

The phylogeny argument of Dawson is based on the cladistic protocol of not accepting paraphyletic groups. In other words, because the ancestor to the Cubozoa was probably a scyphozoan, therefore, recognizing the Cubozoa as a class would leave the class Scyphozoa paraphyletic, which would be outside the allowable parameters of cladistic philosophy and practice. However, this is actually an inconsistent argument, which seeks to join fragments of two incompatible paradigms. Specifically, the reasons that the Cubozoa is considered a group separate from the Scyphozoa under the Linnaean system are based on the differences (= the “modification” of Darwin’s “descent with modification”), whereas the reasons it is considered to be part of the Scyphozoa under the cladistic system are based on the similarities (= the “descent” part). At the present time, unfortunately, we have no system which embodies both halves of Darwin’s “descent with modification”; thus, one’s conclusion as to whether the Cubozoa is a class or not depends on which half of Darwin’s theory one chooses to focus.

2.2 MATERIALS AND METHODS

While the focus of this thesis is the Australian cubozoan species, the taxa currently regarded as valid by Kramp (1961) and later authors are included as the basis of organization and comparison. The data in this chapter are synthesized from original descriptions, published redescriptions by other authors, and my own examinations of type material or specimens designated to become types, as well as non-type material and new material from reasonably near type localities. There is a natural and unavoidable tradeoff in basing these studies on type specimens only, i.e., we gain a deeper comparative understanding of all the species, but we lose perspective on the range of variability. However, a well described holotype provides a tool for testing variability among paratypes and non-type material. My aim herein is to set a foundation on which secondary questions such as variability can build; thus, replication of this study based on additional material from multiple localities is of the utmost importance in truly understanding species boundaries.

Because of the confusion over species boundaries that I am trying to unravel, I have restricted some information where I have doubted that it might apply to the taxon of concern (e.g., for *Carybdea marsupialis*, I have restricted data to specimens and reports from the Mediterranean and adjacent seas; for *Chiropsalmus quadrumanus*, I have restricted data to specimens and reports from the western Atlantic; for *Chiropsalmus quadrigatus*, I have restricted data to the holotype specimen). One might argue that such restrictions are subjective; however, it would be circular to define a species based on *a priori* assumptions of its variability across geography and morpho-space. I have taken the more conservative approach of defining a species based on its type morphology, then subsequently determining its variability according to the type standard. I have used the reverse reasoning for *Carybdea alata* (transferred to a new genus, *Alatina*, in Chapter 5), in broadening the data to include all members of the "*Carybdea alata* species complex", because the form in the original description of *C. alata* cannot be determined; usage of the names "*Carybdea alata*" and "*Alatina* spp." are more or less interchangeable, with *C. alata* referring to my predecessors' collective forms of the species and *Alatina* spp. referring collectively to the species as I herein recognize them. Finally, I have applied the same logic to the "Morbakka" group as for the *C. alata* group. It is clear that "Morbakka" comprises multiple species, but they are not yet satisfactorily resolved; thus, "Morbakka" is used to refer to any or all of the collective species in the "Morbakka" group.

The systematics follows the International Code of Zoological Nomenclature (1999). In higher taxon names (e.g., Sections 3.4.11, 4.3.2), I have followed the Principle of Coordination, including, where applicable, above the family level, i.e., the taxon author is he who first published the stem of the name, and he who first used it at the current rank is listed in parentheses following the author.

Institutional abbreviations are as follows: Australian Institute of Marine Science (AIMS); Australian Museum, Sydney (AM); Institut Royal des Sciences Naturelles de Belgique, Brussels (IRSNB); Jack Barnes collection (Barnes); James Cook University (JCU); Museum and Art Galleries of the Northern Territory, Darwin (NTM); Museum of Comparative Zoology, Harvard (MCZ); Museum of Tropical Queensland, Townsville (MTQ); Museum of Zoology, University of Sao Paulo, Brazil (MZUSP); Natural History Museum, London (NHM); Naturalis (Leiden); Queensland Museum, Brisbane (QM); Robert Hartwick collection (Hartwick); Scripps Institution of Oceanography, Univ. Calif. San Diego (Scripps); South African Museum, Cape Town (SAMA); South Australian Museum, Adelaide (SAM); and United States National Museum, Washington DC (USNM); University of California Museum of Paleontology, Berkeley (UCMP); Western Australian Museum, Perth (WAM); Zoological Museum of the University of Copenhagen (ZMUC). Standard state and locality abbreviations are used, e.g., Queensland (QLD), Northern Territory (NT), South Australia (SA), Western Australia (WA), New South Wales (NSW), Great Barrier Reef (GBR). All other abbreviations are explained in the text. Translations of German and French descriptions were made electronically with Power Translator 6.0 for Windows.

Most of this chapter is arranged by functional groups of characters, and the discussions are broken up accordingly. Each section begins with a list of the characters and character states correlating with the character matrix for the morphological phylogeny (Chapter 3; Appendix 2). The remainder of the section then details the different perspectives dealing with those characters and character states. The historical perspectives are provided for two reasons, first, to compare the different interpretations of different workers, and second, to provide a basis for understanding the modern interpretations relative to the historical. In juxtaposing the historical with the modern, and in specifying my current perspectives on how to locate the characters, how to score the character states, and what they mean in terms of the different species, it is my goal that future workers and curious naturalists will be able to build on my foundation, rather than just having another unclear perspective to try to navigate through. The names used throughout this chapter are synopsized and keyed in Chapter 4, with a figure of each species provided.

2.2.1 How to identify cubozoans

All structures except nematocysts can be identified under a dissecting microscope or with the naked eye. Probably the single most important tool is a good strong side light; I use a double-arm fibre-optic light, with an arm shining into the specimen from each side. The key is to get the light coming into the side, very low, shining through the glass dish, not over it. The refractive nature of mesoglea will catch the side light in such a way as to make the structures glow brightly; if lighted from above or below, the structures wash out and are difficult to discern. The specimen should be examined in liquid, both to keep the specimen in good condition and also to take advantage of the refractive index.

All structures except the phacellae are observable without dissection; a small incision near the phacellae is typically sufficient to peel back the mesoglea for study of the root and cirri structures. From there, a small piece of gonad can also be removed for biopsy, although the sex of the specimen can usually be determined through the body wall, especially in specimens preserved in formalin. If a clean view of the subumbrellar structures is desired, a single cut can be made up the entire height of the bell along one of the non-structural radii, i.e., between the rhopaliar and pedalial radii.

Nematocysts can be prepared for study by placing a bit of tentacle, bell snip, or cluster of cirri onto a glass slide, covering with one or two drops of Glycergel (Dako Corp., California), and finishing with a cover slip; gently pressing or tapping with a blunt object will help squash the sample for easier study. Fresh samples can be made to discharge with the addition of a drop of freshwater, saliva, or ethanol prior to adding the Glycergel; preserved nematocysts cannot be discharged. Since nematocyst identification is ultimately based on the shaft and tubule structures, fresh samples are preferable. For long-preserved samples, especially those in ethanol, it is sometimes impossible to get a tentacle squash because the tentacle has become too hard and brittle; in a droplet of water on a coverslip, the tentacle can be crushed repeatedly between forceps to free some nematocysts – this is a last resort, as the full cnidome will not typically be recovered this way. Nematocysts are best examined under a compound microscope with a 40x objective.

The various structures are detailed below, along with where on the animal to look for them, how to identify the different character states, and references to earlier philosophies about them. A summary of the main diagnostic characters is presented in Table 2.14a, b, as well as in Chapter 4.

2.3 CHARACTER RESULTS AND DISCUSSION

2.3.1 Bell measurements

1. *Bell height - mature max* (Table 2.1): **(0)** minute (<5 mm); **(1)** very small (<1 cm); **(2)** small (1-2 cm); **(3)** about 2.5 cm; **(4)** medium (3-6 cm); **(5)** large (7-15 cm); **(6)** very large (>>15 cm).
2. *Bell shape i.e., height:width ratio* (Plate 2.1): **(0)** shorter than 1:1; **(1)** 1:1; **(2)** 1.25 tall to 1 wide; **(3)** 1.5 tall to 1 wide; **(4)** about 1.75x tall to 1 wide; **(5)** about 2x tall to 1x wide; **(6)** more than 2x tall to 1x wide.

While most structural characteristics of the bell are probably worthy of consideration, it is less clear whether relative dimensions can be similarly used. Bigelow (1909) argued that relative proportions of different parts of the bell are unstable characters, and that older species founded on these differences are today unrecognizable. Although I routinely take a standardized series of measurements on each specimen I examine, I agree with Bigelow that one must be cautious in using them diagnostically. Differential preservation methods result in different amounts of shrinkage and distortion for different parts of the body; therefore, it would be preferable to restrict morphometric studies to fresh specimens. Furthermore, one must be vigilant about comparing similar growth stages. When used with care, I do think that relative measurements can, in combination with numerous structural characters, be quite useful; however, I would not advocate defining species on bell proportions alone.

In general terms, the relative body proportions are probably less subject to differential distortion than literal measurements. Cubozoans in lateral view have either a very square body (all chirodropids), a somewhat taller than wide rectangular body (most carybdeids), a very much taller than wide body (most forms in the *Carybdea alata* species complex), a slightly wider than tall body (tripedaliids), or a rounded pyramidal body (*Carukia barnesi*).

For those cases where one feels that bell measurements must be used, such as for specimen descriptions or for relative general comparisons, I have found the following measurements to be useful as standards: bell height (BH), diagonal bell width (DBW), inter-rhopalial width (IRW), and tentacle-base width (TBW). I typically measure BH with a set of digital calipers from the apex of the bell to the turnover of the bell below the rhopalium. An alternative method I have used is to place a mm-ruler underneath the specimen, and measure it at the same points described above. The DBW is measured with the specimen laying flat, i.e., with two pedalia splayed out to the left and right sides, and the other two facing up and down in the center. The measurement is taken at the lamella where the top of the pedalium meets the bell. Note that this gives a measurement of the width of two facets of the bell, not an actual bell diameter. I found that measuring one facet between pedalia was subject to too much error, because it largely depended on how the specimen was laying and the orientation of the pedalia to one another (e.g., facing out, facing up); only really perfect specimens which retained their boxy shape in water could be accurately measured in this way. The IRW seems a better measure of a true bell width (i.e., single facet width), and is subject to much less variability than across pedalia, by taking the widths of half of two adjacent facets simultaneously. With the specimen still lying flat from the DBW, the width is measured between the two exposed rhopaliar stems, or if the rhopalia themselves are not visible, between the center-points of the rhopalial niches. The bell height-to-width ratio is the BH divided by the IRW. The TBW is measured simply across the widest points of the tentacle, as close to the junction with the pedalium as possible.

The summary of comparative measurements of species in Table 2.1 is based on holotypes, neotypes, or syntypes, or specimens designated to become them. While the measurements of a single individual cannot give statistically meaningful data, I do believe these measurements nonetheless provide a starting point for a relative size comparison among species.

2.3.2 Bell morphology

3. *Apex shape* (Plate 2.1): **(0)** with depression; **(1)** domed; **(2)** flat; **(3)** pyramidal.
4. *Apical decorations* (Plate 2.2): **(0)** lacking decorations; **(1)** with sub-apical coronal furrow; **(2)** with 4 apical adhesive pads; **(3)** with reticulations.
5. *Bell consistency*: **(0)** rigid; **(1)** flimsy.

6. *Bell thickness*: **(0)** thickened apically; **(1)** evenly thin; **(2)** evenly thick; **(3)** thickened on interradii.
7. *Interradial furrows* (Plate 2.3A, B): **(0)** absent; **(1)** shallow; **(2)** deep.
8. *Adradial furrows upper half* (Plate 2.3C, D): **(0)** absent; **(1)** shallow; **(2)** well defined, i.e., defining the interradial pillars.
9. *Adradial furrows lower half* (Plate 2.3C, D): **(0)** absent; **(1)** shallow; **(2)** well defined, i.e., defining "smile lines" around the rhopaliar region; **(3)** defining only pillars but not smile lines; **(4)** defining only smile lines but not pillars; **(5)** defining both pillars and smile lines.

Bell morphology has often been used diagnostically, though Stiasny (1930) argued against the usefulness of bell sculpture. Lesson (1829) described a form, *Beroe gargantua*, which has remained unrecognizable due to the remarkable vertical rods that comprise the body wall; it would be extraordinary if this species were found again, matching its original morphology. An apical concavity has variously been regarded as a good character or mere distortion or contraction (Menon, 1930; Stiasny, 1930; Bigelow, 1938). *Carybdea alata* auct. has been typically held as distinct based on its lack of bell sculpturing (see Bigelow, 1938); however, there are many differences that separate the species in the *C. alata* species complex collectively from other taxa (see Chapter 6).

As observed by Bigelow (1909), the carybdeid genera have characteristic sculpturing of the body. In fact, within the traditional genus *Carybdea*, at least 3 extremely distinct sculpture groups can be identified (*Carybdea marsupialis* clade, *Carybdea alata* clade, and *Carybdea sivickisi*), which are also separable on numerous other structural and molecular characters. The chirodropids tend to be more constant in their body sculpture, although the undescribed spotted chirodropid from the GBR (*Chirodropus* n. sp. A) is quite sculptured.

- Apical morphology

The apex may be flat, domed, or concave. A concave apex is fairly unusual, but was used as a distinguishing character by Menon (1930) and Stiasny (1930) in defining *Carybdea madraspatana* and *Manokia stiasnyi*, respectively. Curiously, Bigelow (1938) rejected Menon's use of the apical depression in *C. madraspatana*, regarding it as distortion or contraction, but made no comment on Stiasny's use of the same character in *M. stiasnyi*, given specific recognition by Bigelow in that very paper; an apical depression is also conspicuous in *Tripedalia*, though not noted by early authors. Most species have a domed apex, even if only somewhat so. This is particularly obvious in most of the chirodropids and in the *Carybdea* spp., where the apical jelly is thickened. In a few species the apex is quite flat, such as in the truncate pyramidal body of some *Alatina* spp., and in the stouter *Tamoya haplonema* and “Morbakka”, and in the somewhat smaller “Broome Irukandji”. The apical morphology has been noted in most species descriptions, but has not been typically regarded as diagnostic.

Perhaps the most unusual apical morphology is that of *Carybdea sivickisi*, which has exumbrellar adhesive patches overlying the four phacellae (Hartwick, 1991); these were typically noted by past authors (Stiasny, 1926a; Uchida, 1929; Uchida, 1970; Hoverd, 1985), but not realized for their uniqueness (Plate 2.2C).

- Exumbrellar furrows (Plate 2.3)

The vertical body walls may be smooth, as is characteristic of the *Carybdea alata* species complex, or, more often, decorated with patterned interradial or adradial vertical furrows. The interradial furrows typically run from the level of the stomach down to just above the pedalia. In some species, the mesoglea is considerably thickened on both sides of a deep interradial furrow into “pillars”.

Adradial furrows are present in some species, further accentuating the interradial pillars. In some species, the adradial furrows broaden at about the level of the rhopaliar niche, with one edge curving toward the perradii. In these taxa, conspicuously thinner triangular regions are bordered by the pillars on the outside, the rhopalial region on the inside, and the velarium below; as a consequence of the shallower triangular regions, the rhopalial niche regions appear raised up off the bell wall in the lower half. I have termed these triangular furrow expansions “smile lines” because of the vague resemblance to the pattern of creases around the human mouth created during a smile.

In some species a circumferential furrow demarcates the apical dome from the vertical body walls, at about the height of the gastric phacellae (Plate 2.2B). This is common in the “true carybdeas”, i.e., *C. marsupialis*, *C. xaymacana*, and *C. rastonii*, and is always absent in the members of the *C. alata* species complex. It is also found in some chirodropid species.

2.3.3 Nematocyst warts

10. *Exumbrella texture* (Table 2.2; Plate 2.4): **(0)** lacking warts and freckles; **(1)** lacking warts, but with flush nematocyst freckles; **(2)** warts or freckles concentrated on interradii; **(3)** warts or freckles concentrated apically; **(4)** scattered warts; **(5)** very warty; **(6)** mammillated.
11. *Bell nematocyst types*: **(0)** lacking bell nematocysts; **(1)** spherical isorhizas; **(2)** ovoid isorhizas; **(3)** euryteles; **(4)** mastigophores.

Southcott (1967) regarded the prominent warts on the bell and velarium of *Carukia barnesi* as one of the most highly diagnostic characters of the genus. However, there are numerous taxa in different clades with highly warted bells and velaria.

The term "warts", as applied to exumbrellar nomenclature, has had various meanings and is often confusing. By common usage, a wart is a lump or a growth, but it is inconsistently used in the Cubozoa to refer to raised or unraised nematocyst clusters, as well as to raised parts with or without nematocysts. Herein, I specifically use it only to refer to raised gelatinous protuberances, whether or not they underlie nematocysts, and I refer to nematocyst clusters which lack such a protuberance as "freckles". Thus, nematocysts and warts may be independent. For example, *Tamoya haplonema* and *Carukia barnesi* both have prominent gelatinous exumbrellar warts studded by nematocysts, whereas some forms in the *Alatina* species complex have conspicuous nematocyst freckles but completely lack gelatinous warts.

The cubozoan exumbrellar surface may be armed with gelatinous warts, nematocyst freckles, both freckles and warts, or neither (Table 2.2; Plate 2.4). In general, chirodropids do not have exumbrellar nematocysts, the known exceptions being *Chiropsalmus quadrumanus* and a new form from the Pacific coast of Mexico. The two better-known Australian chirodropids, *Chironex fleckeri* and *Chiropsalmus* sp. A (N. QLD, often erroneously identified as *Chiropsalmus quadrigatus*) have unarmed bodies.

Bell nematocysts in the Cubozoa are almost always of the non-penetrant isorhiza type. Tentacular nematocysts are treated below (Section 2.3.6), and a more thorough explanation of nematocysts is given in Section 2.4 and Appendix 1, below. Cubozoan species cnidomes are summarized in Table 2.13, and nematocyst types are illustrated in Plates 2.25-2.27.

2.3.4 Pedalia

12. *Number of pedalia per corner*: **(0)** one; **(1)** two; **(2)** three.
13. *Pedalia complexity*: **(0)** simple and unbranched; **(1)** branched biserially; **(2)** branched linearly.
14. *Pedalia length, if not branched*: **(0)** rudimentary; **(1)** less than 0.25x bell height; **(2)** about 0.25x to 0.33x bell height; **(3)** about 0.33x bell height; **(4)** between 0.33x and 0.5x bell height; **(5)** approximately 0.5x bell height; **(6)** greater than 0.5x bell height.
15. *Pedalial inner wing shape, if simple* (Plate 2.5): **(0)** narrow (barely flared); **(1)** moderate (scalpel shaped); **(2)** widely rounded (hemispherical).
16. *Pedalia length to width ratio*: **(0)** 1 to 1; **(1)** 1.25 to 1; **(2)** 1.50 to 1; **(3)** 1.75 to 1; **(4)** 2 to 1; **(5)** 3 to 1; **(6)** 5 to 1.

17. *Pedalial outer keel width*: **(0)** 0.5x canal; **(1)** 1x canal; **(2)** 2x canal.
18. *Pedalial inner keel width*: **(0)** 0.5x canal; **(1)** 1x canal; **(2)** 1.5x canal; **(3)** 2x canal; **(4)** 3x canal; **(5)** 4x canal; **(6)** 5x canal; **(7)** 6-8x canal; **(8)** more than 8x canal width.
19. *Pedalial inner keel overhang* (Plate 2.5): **(0)** overhang absent, i.e., inner pedalial wing above tentacle insertion; **(1)** overhang present, i.e., inner pedalial wing extends below tentacle insertion.
20. *Pedalial armament* (Plate 2.6): **(0)** lacking nematocysts; **(1)** 1 row of nematocysts on outer keel; **(2)** scattered nematocysts on outer keel; **(3)** a row of lateral nematocyst bars on outer keel; **(4)** scattered nematocysts on both keels.

The tentacles of cubozoans arise from tough, gelatinous extensions called pedalia, one at each of the four corners of the body. In carybdeids, each tentacle arises from a single blade-like or oar-like extension, whereas in chirodropids the tentacles arise singly from the "fingers" of hand-like or claw-like extensions. This ordinal division between oar-like pedalia and hand-like pedalia is constant, except for an unusual new species of *Chiropsalmus* from the Mexican Pacific which is characterized, in part, by having oar-like pedalia with multiple tentacles arranged at the very tip. Mayer (1910) thought that the carybdeid pedalia are probably used as keels to steer the animal through the water.

Stiasny (1930) argued against the reliability of using pedalia shape diagnostically, stating that the form and size change in different developmental stages and also seem very variable in adult specimens. While there certainly is variability, reliable generalities can still be made in light of other characters examined (see below).

Historically, the pedalia have been variously called "randblättern" (Gegenbaur, 1857), "fortzatze der eckwülste" (Müller, 1859), "tentacular lobes" (Agassiz, 1862), "schirmlappen" (Claus, 1878), and "gelatinous sockels" (Haeckel, 1881). The term pedalia is also used elsewhere in the Cnidaria to refer to the radial thickenings in the coronate scyphozoans. They appear to be similar in name only, with no apparent homology in the actual structures. Agassiz (1862) and Claus (1878; Haeckel, 1881) believed them to be homologous with the "marginal lobes" of the coronates; Agassiz separated the cubozoans from the coronates based on the two kinds of marginal lobes in the latter, compared to only one kind in the former. Haeckel (1880) was apparently the first to call them "pedalia" (or "pedalien"). He later (1881) showed that they are merely analogous with the coronate pedalia, which are adradial, whereas cubomedusan pedalia are interradial. In addition, unlike the coronate pedalia, which are exumbrellar, Conant (1898) showed that cubozoan pedalia are actually structures of the subumbrella, separated from the exumbrella by the vascular lamella. Furthermore, in coronates, the internal septa connect the two main body walls on the radii between the pedalia, whereas in the cubozoans, the internal septa connect the body walls on the pedalial radii. Finally, the four cubozoan pedalia are vascularized with a single large canal through each leading to a hollow tentacle, whereas the coronate pedalia lack canals, with some pedalia each leading to a solid tentacle, while others each lead to a rhopalium. In some of the species in the *Carybdea alata* species complex, the pedalial stalk is particularly long.

- Carybdeid pedalia (Plates 2.5, 2.6)

The carybdeid pedalia may be single, double, or triple, but they are all of the same form, i.e., blade-like, with a median canal, and with abaxial and adaxial "keels". The adaxial keel (Plate 2.5) may be narrow (as in *Carybdea sivickisi*), moderate and scalpel shaped (as in *Carybdea marsupialis* and *Carybdea rastonii*), or wide and broadly rounded (as in the *Carybdea alata* species complex). In all species, the abaxial keel is fairly narrow, and may or may not be studded with nematocysts (Plate 2.6). If present, the nematocysts may be in a single row of freckles (as in *Carybdea marsupialis* and *Carybdea rastonii*), scattered freckles (as in some members of the *Carybdea alata* species complex and an undescribed *Carybdea* from South Africa), or a row of horizontal bars (as in *Carybdea sivickisi*). The pedalial nematocyst clusters are typically paired, i.e., mirror-imaged on both facets of the abaxial keel.

In *Tripedalia* spp., the pedalia are particularly curious. *Tripedalia cystophora*, the type species of the genus, as the name suggests, has three pedalia per corner. They are arranged in a definite pattern, with the centermost being precisely on the interradius, and slightly larger and raised above the insertion point of the flanking two. *Tripedalia binata*, which was described nearly a hundred years after *T. cystophora*, is clearly closely related to *T. cystophora* but has only two pedalia per corner. All specimens share a previously unnoted peculiar pattern. Instead of the two pedalia equally flanking the axis, as one might expect, it is actually always the one on the animal's left that is absent. The larger, central pedalium is still in its axial, slightly raised position, and the pedalium to the animal's right is relatively smaller and lower. It is interesting to note that this same pattern is occasionally found in laboratory raised specimens of *Tripedalia cystophora* originally from Puerto Rico (Gershwin, unpublished observations), but the character does appear to have fixed in the Australian form known as *T. binata* (Moore, 1988). For this reason, I think the species *T. binata* should be retained.

- Chirodropid pedalia (Plates 2.7, 2.8)

The chirodropids all have only a single pedalium to each corner, but it is always branched with multiple "fingers" and tentacles. The chirodropid pedalia are either unilaterally branched, i.e., branched in a linear series along the abaxial edge of the pedalium only (as in *Chiropsoides buitendijki*), or bilaterally branched, i.e., branched to the right and left of the abaxis (all other taxa).

In the taxa with bilaterally branched pedalia, the "fingers" may be arranged alternate or opposite. Whether this character is taxonomically consistent is ambiguous; study of more specimens and more species would be required to determine this conclusively. In most species, the "fingers" are distributed fairly evenly throughout the lower half of the pedalium, whereas in the new species from Gove, they are clumped toward the distal end, giving the pedalium a long, lean appearance. This is even more exaggerated in a new species from the Mexican Pacific, in which the pedalium is actually more similar in appearance to the carybdeid oar-like form than the typical hand-like form of the chirodropids.

2.3.5 Pedalial canals

21. *Pedalial canals single or divided* (Table 2.3; Plate 2.7A, B): **(0)** divided, with lateral branches arising from two main lateral branches; **(1)** single, with all lateral branches arising from a single main canal.
22. *Pedalial canals - opposite or alternate* (Plate 2.8A, B): **(0)** opposite; **(1)** alternate.
23. *Pedalial canals cross section shape* (Table 2.3; Plate 2.7C, D): **(0)** oval or flattened throughout; **(1)** round throughout; **(2)** quadratic proximally and flattened distally; **(3)** quadratic throughout.
24. *Pedalial canals bend form* (Table 2.3; Plate 2.9): **(0)** simple, smooth, just goes down; **(1)** knee-like (smoothly rounded); **(2)** 90º corner; **(3)** with small upward-pointing nub; **(4)** with lateral pointing nub; **(5)** with slight upward pointing volcano; **(6)** with substantial upward pointing volcano; **(7)** with prominent upward pointing thorn; **(8)** with very narrow upward pointing thorn; **(9)** with raised rounded knob.
25. *Pedalial canal shape at tentacle insertion*: **(0)** straight; **(1)** flared; **(2)** truncate bulbous.

Pedalial canals typically leave the body in a perpendicular orientation, and then bend downward after a short distance; often times this bend is simple and knee-shaped, but sometimes it is marked by an extension between the vascular lamella and the outer wall of the pedalium. This extension may take the form of an upward-pointing spike, an outward-pointing spike, an upward rounded bulge, a broad upward-pointing volcano, or a right-angle bend.

In carybdeids, the cross section of the pedalial canals may be flat (as in the *Carybdea alata* species complex), quadrate (as in *Tamoya haplonema* and a new group containing the "Darwin carybdeid"), or somewhat quadrate proximally and flattened distally (as in *Carybdea* *rastonii* and *Carybdea marsupialis*). The distal-most end of the pedalial canals is typically straight where it meets the tentacle base, but may be conspicuously flared (as in *Tamoya haplonema* and the "Darwin carybdeid")

In chirodropids, there are three branching patterns of the pedalial canals. First, in *Chiropsoides buitendijki*, the canals, like the pedalia themselves, are unilaterally branched; i.e., there is only a single canal and it is only branched on one side, always in the abaxial direction. There may or may not be small protrusions along the main canal or the branches. The remaining chirodropids have bilaterally branching pedalia, but not necessarily bilaterally branching canals. In some species the canal is single throughout its length, and the canal branches correspond with the "fingers" on both sides of the canal. In other species, the median canal bifurcates about midway, and the branches arise only from their corresponding side. The right and left rows of "fingers" on the bifurcated canal species are broadly expanded out from each other, whereas the right and left rows of "fingers" on the single canal species are more narrowly cupped inward toward each other.

2.3.6 Tentacles

26. *Number of tentacles per pedalium*: **(0)** 1; **(1)** 2; **(2)** 3-4; **(3)** approx. 5; **(4)** 7-9; **(5)** 9-11; **(6)** 12-15; **(7)** 21.
27. *Tentacle complexity*: **(0)** rudimentary; **(1)** unbranched; **(2)** with lateral branches.
28. *Tentacle base shape* (Plate 2.10A-C): **(0)** straight; **(1)** flared slightly; **(2)** flared greatly.
29. *Tentacle base width*: **(0)** about 0.5 mm; **(1)** about 0.66 to 0.75 mm; **(2)** less than 1 mm; **(3)** about 1 mm; **(4)** about 2 mm; **(5)** 3-4 mm; **(6)** more than 5 mm.
30. *Tentacle decorations* (Table 2.4; Plate 2.10D-F): **(0)** lacking decorations; **(1)** with handkerchiefs; **(2)** with halos; **(3)** with lateral branches; **(4)** with repeating hourglasses or segments.
31. *Tentacle shape in cross section* (Table 2.4; Plate 2.11): **(0)** wide and ribbon-like; **(1)** laterally flattened a bit; **(2)** round.
32. *Tentacle banding pattern* (Table 2.4; Plate 2.11): **(0)** evenly banded; **(1)** alternate major/minor, i.e., 1-2-1-2-1-2; **(2)** in 1-2-3-2-1 pattern; **(3)** in pattern of major-1-2-3-minor-1-2-3-major; **(4)** in pattern of Major-1-2-1-minor-1-2-1-minor-1-2-1-minor-1-2-1-major; **(5)** major bands separated by 7 alternating thicker and thinner bands (i.e., 1-3-2-3-2-3-2-3-1).
33. *Tentacle "segmenting"* (Table 2.4; Plate 2.11D): **(0)** not appearing segmented; **(1)** every 10th or 11th band constricted to appear segmented; **(2)** bands not constricted, but overall shape repeating hourglass.

34. *Number of tentacular nematocyst types* (see also Section 2.3.24; Table 2.13): **(0)** 1 type and size class only; **(1)** 2 different types or size classes; **(2)** 3 types or size classes; **(3)** 4 types or size classes; **(4)** 5 types or size classes; **(5)** 6 types or size classes.
35. *Principal tentacular nematocyst type* (see also Section 2.3.24; Table 2.13; Plates 2.25-2.27; Appendix 1): **(0)** microbasic p-mastigophores type 1 - spines orientated at right angle to capsule; **(1)** microbasic p-mastigophores type 2 - spines orientated toward capsule; **(2)** microbasic p-mastigophores type 3 - small spines orientated toward capsule, large spines away from capsule; **(3)** microbasic p-mastigophores type 4 - spines orientated away from capsule; **(4)** trirhopaloids; **(5)** cylindrical isorhizas; **(6)** ovate isorhizas; **(7)** lemon-shaped tumiteles; **(8)** stenoteles; **(9)** microbasic euryteles.
36. *Microbasic p-mastigophores* (see also Section 2.3.24; Table 2.13): **(0)** absent; **(1)** type 1, i.e., spines orientated as right angle to capsule; **(2)** type 2, i.e., spines orientated toward capsule; **(3)** type 3, i.e., small spines orientated toward capsule and large spines orientated away from capsule; **(4)** type 4, i.e., spines orientated away from capsule.
37. *Microbasic trirhopaloids or tumiteles or euryteles* (see also Section 2.3.24; Table 2.13): **(0)** absent; **(1)** large and football shaped with heavy tubule; **(2)** lemon-shaped with fine tubule; **(3)** nearly spherical, with thick capsule wall; **(4)** small football shaped euryteles.
38. *Oval isorhizas* (see also Section 2.3.24; Table 2.13): **(0)** absent; **(1)** large; **(2)** small.
39. *Cylindrical or rod-shaped isorhizas* (see also Section 2.3.24; Table 2.13): **(0)** absent; **(1)** present.
40. *Stenoteles* (see also Section 2.3.24; Table 2.13): **(0)** absent; **(1)** present.

The tentacles are typically simple or, rarely, branched. I have observed branched tentacles as anomalies in *Chironex fleckeri* and *Carybdea xaymacana* (WA form). In most occurrences the branches do not seem to be taxonomically important, but they were the basis for distinguishing the species *Manokia stiasnyi*. However, in the latter, the tentacles do appear to be branched in a conspicuous pattern, which is different from the haphazard branching that I have observed in other taxa.

The tentacular nematocysts are always arranged in raised rings, and these banding patterns may be highly diagnostic. In most species the rings are of an even, smooth shape and similar size (i.e., 1-1-1-1-1-1). In some species the rings are of a smooth shape but different sizes, and arranged in a certain pattern (e.g., 1-2-1-2-1-2-1-2, or 1-2-3-2-1-2-3-2-1). While sometimes this may vary in a single individual from the proximal to the distal regions of the tentacle, some generalizations can nonetheless be made. I find it best to examine the banding pattern when the specimen is both alive and fixed, in several different regions of the tentacle.

The hardest state to have confidence in is the 1-2-1-2-1-2, whereas the 1-1-1-1-1-1 and the 1-2-3-2-1 are typically very easy to identify and quite consistent. A few taxa have highly modified tentacular banding. *Carukia barnesi*, for example, has widely spaced bands that are drawn out into little "tails" in one direction, i.e., like the neckerchief worn by John Wayne in his Western movies. The undescribed species "Halo-Irukandji" has very closely spaced bands but each has a completely-encircling perpendicular projection of tissue, like little Saturn rings, with the nematocysts arrayed in a single plane along the distal edge, projecting outward.

The tentacles of most cubozoans are round or slightly oblong in cross section; however, in a few taxa they are notably flattened such that they resemble tape-worms. This difference is one of the easiest ways to distinguish *Chironex fleckeri* (with wide flattened tentacles) from *Chiropsalmus* sp. A (with fine round tentacles). In some taxa, the top of the tentacle where it connects to the pedalium may be conspicuously flared; in these taxa, the pedalial canal is also typically flared.

Identification of nematocysts can be difficult, and their terminology can be bewildering. Some species, such as members of the Irukandji clade (see Chapter 3), have only a single nematocyst type on the tentacles, typically of the mastigophore penetrating form. In contrast, the chirodropids typically have 4-7 nematocyst types on the tentacles, including both penetrant and non-penetrant forms. A more thorough explanation of cubozoan nematocysts is given in Section 2.3.24 below and in Appendix 1. The nematocyst types are illustrated in Plates 2.25-2.27, and species cnidomes are summarized in Table 2.13; not all nematocyst types easily dissociate from preserved tentacles, so one must keep an open mind for additional nematocyst types in the cnidomes presented. Bell nematocysts are treated in Section 2.3.3 above.

2.3.7 Phacellae

41. *Phacellae* (Table 2.5; Plate 2.12a, b): **(0)** absent; **(1)** present.

42. *Phacellae form* (Table 2.5; Plate 2.12b, b): **(0)** in brush-like bundle on single trunk; **(1)** in tight cluster of numerous trunks; **(2)** in horizontal arrangement of small bundles; **(3)** in vertical arrangement of numerous bundles; **(4)** large and broad in crescent form; **(5)** inverted crescent; **(6)** singly-rooted V-shaped corner mass; **(7)** forming a continuous ring around stomach.

43. *Phacellae location* (Table 2.5; Plate 2.12a, b): **(0)** corner tufts; **(1)** horizontal across corners; **(2)** crescentic across corners; **(3)** vertical along stomach wall; **(4)** V-shaped corner mass; **(5)** forming continuous ring around stomach.

44. *Phacellae branching* (Table 2.5; Plate 2.12a, b): **(0)** unbranched, singly rooted cirri; **(1)** cirri rooted in pairs; **(2)** tufted, i.e., branched once some distance above root; **(3)**

dendritic.

45. *Gastric cirri length* (Table 2.5; Plate 2.12a, b): **(0)** short; **(1)** long.

The bundles of gastric cirri in each of the 4 corners of the stomach are collectively called phacellae, i.e., a cubozoan has 4 phacellae, unless they are lacking altogether. They are typically found in the uppermost corners of the stomach, and thus easily visible through the top of the bell. In chirodropids they are all of the same form, i.e., many hundreds of short cirri attached directly along the corners of the stomach wall in large V-shaped corner masses, whereas in carybdeids, the form of the phacellae tends to differ markedly between different major groups.

Uchida (1929) and Stiasny (1934) regarded the arrangement of the phacellae to be of specific rather than generic importance; however, most authors have regarded the phacellae arrangement to be a generic character separating *Tamoya* from *Carybdea* (e.g., Haeckel, 1880; Mayer, 1910; Kramp, 1961). In my analysis of characters and taxa, I have come to the conclusion that the form of the phacellae is an extremely useful character for separating some of the major carybdeid clades, including species, genera, and families.

The carybdeid phacellae can form either a single tuft or tightly-packed group of tufts in each corner, each tuft being comprised of a trunk and numerous cirri, sometimes arranged dendritically into smaller tufts along the trunk. In the *Carybdea alata* species complex, the cirri are quite long (up to 10 mm) and lay more or less parallel to one another in a large, crescentic-shaped bundle. In most other taxa the cirri themselves are short (about 1-3 mm). In *Carybdea marsupialis* and its closest relatives, the tufts of the phacellae form a tight bundle in the very corner of the stomach; in *C. xaymacana* there is but a single tuft, *C. marsupialis* typically has 8-10, whereas *C. murrayana* has about 12-15, and an undescribed form from the Cape region of South Africa has about 20. In *Carybdea rastonii*, the tufts are in a linear arrangement, obliquely across each stomach corner. In *Tamoya haplonema*, the tufts are extremely numerous and arranged in a vertical band down the interradial sides of the stomach wall (rather than in the upper corners). In *Carukia barnesi* and several undescribed taxa (the “Darwin carybdeid”, “Morbakkas”, and “Pseudo-Irukandjis”), the phacellae are lacking altogether. *Carybdea sivickisi* is the only carybdeid with singly-rooted cirri, similar to the state found in the chirodropids.

2.3.8 Gonads

46. *Lateral gonads*: **(0)** absent; **(1)** present.

47. *Lateral gonad length*: **(0)** reach both stomach and pedalium; **(1)** reach neither stomach nor pedalium; **(2)** reach stomach but not pedalium; **(3)** reach pedalium but not stomach.

48. *Gonad attachment* (Plate 2.13A-C): **(0)** in center (like butterfly); **(1)** along entire length (leaf-like); **(2)** attached at top, i.e., pendant; **(3)** a series of hundreds of little bundles of filaments.

49. *Interradial suture* (Plate 2.13D): **(0)** simple, i.e., lacking perforations; **(1)** perforated.

The gonads appear to be homogeneous among the majority of carybdeids, the exception being the separation of the tripedaliids from the remaining taxa (see below). The chirodropid gonads tend to be similar too, but this feature, as discussed above, was used by Southcott (1956) as the primary character to distinguish *Chironex fleckeri* from all other cubozoans.

Fully mature carybdeid gonads expand laterally towards the perradii, and in fact, often overlap, whereas chirodropid gonads tend to expand upward onto the gastric saccules.

Cubozoans sex is easy to determine (Plate 2.13E, F). Female gonads have an unmistakable granular appearance to the naked eye (when preserved or very gravid), and thousands of spherical eggs can be easily observed under a dissecting microscope in any stage of development in which gonads are present. Males are a bit more challenging to discern, but under a dissecting microscope a conspicuous “fingerprint” pattern can be observed; to the naked eye, very ripe male gonads look smoother than female gonads.

- Lateral gonads

All cubozoans have lateral gonads, i.e., paired leaf-like structures arising along the interradial septa and projecting out into the coelenteric cavity toward the perradii. Sometimes they overlap, and sometimes they are highly pleated. The pleating of the gonads was used by Uchida (1929) to separate the genus *Tamoya* from *Carybdea*. However, I agree with Stiasny (1930; 1934) that the pleating is indicative of degree of maturity, and is not of taxonomic value, though some species are more inclined toward pleating than others (e.g., *Tamoya* and *Alatina* spp.).

In the carybdeids, the gonads clearly separate three different groups. The gonads of most carybdeids are attached along more or less the whole length of the septum. Immature or unripe gonads are observable as narrow slits along most of the length of the four septa. However, in the tripedaliids, the gonads grow from the center-point of the septum and at maturity, have a butterfly appearance, attached only at the mid-septal latitudes. In *Carybdea sivickisi*, the gonads have a somewhat lesser butterfly appearance, but are attached only at the uppermost parts of the interradial septum. Most chirodropid lateral gonads are broader and more well developed in the upper half, but nonetheless span the full height of the interradii.

- Superior gonads

Superior gonads are only found in the chirodropids, but, despite Southcott's assertion that they are peculiar to *Chironex*, are actually found in most species. They are essentially just extensions of the gonadal tissue over toward, and often enveloping, the gastric saccules.

2.3.9 Rhopaliar niche ostia

50. *Rhopalial niche contour*: **(0)** flush with bell; **(1)** upper scale raised, but lower scale unraised; **(2)** shallowly raised from body wall; **(3)** prominently raised from body wall; **(4)** rhopalial region is raised, but niche itself is not.
51. *Rhopalial niche ostium shape* (Table 2.6; Plate 2.14): **(0)** lacking covering scales; **(1)** frown (1 upper and 1 lower covering scales); **(2)** heart (1 upper and 2 poorly developed lower); **(3)** T (1 upper and 2 well developed lower); **(4)** dome-shaped (1 upper, but a flat-shelf below); **(5)** vertical key-hole shaped; **(6)** dome shaped lacking lower scale or shelf.
52. *Upper covering scale* (Table 2.6; Plate 2.14): **(0)** straight ; **(1)** narrowly concave; **(2)** broadly concave; **(3)** shallowly convex; **(4)** broadly convex; **(5)** narrowly convex; **(6)** with pronounced center flap; **(7)** pointed; **(8)** W-shaped; **(9)** M-shaped.

The cubozoan rhopalia are set within a cavity located on the 4 flat sides of the body, midway across, some distance up from the bell margin. The shape of the opening is highly diagnostic in supra-specific identification.

The rhopaliar niches have been called a variety of names: Müller (1859) called them "grunde der nische"; Haeckel (1880), Stiasny (1919), and Thiel (1928) called them "sinnes nichen"; Fewkes (1883) and Hargitt (1902) called them "pockets"; Kishinouye (1910) called them "cryptae rhopalares"; Stiasny (1926a) called the covering scales collectively "squama rhopalaris"; Southcott (1967) called them "rhopalar cavities"; Mianzan and Cornelius (1999) called them "rhopalial niches"; and Bigelow (1938) interchangeably called them "sensory nitches", "rhopalar nitches", and "sensory pits". Most other authors have called them "sensory niches" (Conant, 1898; Horst, 1907; Uchida, 1929; Kramp, 1961; Uchida, 1970; Moore, 1988), "sensory nitches" (Arneson, 1976), or "rhopalar niches" (Southcott, 1956; Hartwick, 1991), while Mayer (1910) called them simply "niches" and Pagès et al. (1992) called them simply "cavities".

The shape of the rhopalial niche ostium and the depth of the cavity have been noted by many authors. Uchida (1929) regarded the depth of the rhopalial niches to be of generic value, distinguishing the genus *Carybdea*, with shallow niches with triangular roofs, from *Tamoya*, with deep, pit-like niches. Stiasny (1930) believed the rhopalial niche depth to be of specific rather than generic value, citing that *Carybdea rastonii* and *C. sivickisi* have deep niches, whereas in *C. xaymacana* they are more flattened. The shape of the ostium has been widely regarded to be of specific rather than generic value (Stiasny, 1919; Stiasny, 1937a; Bigelow, 1938; Kramp, 1961). However, Williamson et al. (1996) and Cornelius (1997) used the shape of the ostium to separate the genus *Tamoya* with "a roughly horizontal slit" from *Carybdea* with the ostium "Y-shaped or a vertical slit like a keyhole". This is somewhat simplistic; in fact, the ostium of *Carybdea* spp. under the current classification ranges from T-shaped to Y-shaped to heart-shaped to vertical, and many species besides *Tamoya* have a similar shaped "roughly horizontal" frown-shaped ostium.

There are two basic ways of thinking about carybdeid ostia, namely, the number and development of the covering scales, or the entirety of the margin. Chirodropid rhopaliar niches are all of the same form, i.e., with a dome-shaped upper scale and a thickened, flat, shelf-like surface in place of the lower scale.

Carybdeids with three scales and a discontinuous ostial margin. In the *Carybdea marsupialis* group and *Carybdea rastonii*, the ostia are heart-shaped, i.e., with one covering scale above, and two rudimentary covering scales below. The ostia of the *Carybdea alata* species complex are T-shaped, i.e., with one covering scale above, and two well developed scales below. *Manokia stiasnyi* has a peculiar variation of the T-shaped ostium, in which the upper scale has a median flap, giving the ostium a distinctively Y-shaped appearance in a different way from those of other carybdeids. In all these discontinuous-margin forms, the upper scale is separated from the lower scales at the sides. These two character states could be considered gradational, the T-shaped niches having the more well developed lower scales than the heart-shaped niches.

Carybdeids with two scales or an entire ostial margin. In *Tamoya haplonema*, the "Darwin carybdeid", the "Morbakkas", the "Pseudo-Irukandji", and *Carukia barnesi*, the ostia are frown-shaped, kidney bean-shaped, or dumb-bell-shaped, i.e., with one covering scale above, and one below, and the two are connected at the sides. The *Tripedalia* species have a similar frown-shaped structure, except that it is extremely shallow. In *Carybdea sivickisi*, the ostia are small and vertical, i.e., the oblong ostium is in the oral-aboral axis, and covering scales are lacking in the traditional sense. Furthermore, the ostium of *C. sivickisi* has a beveled edge, whereas that of the other groups is simple.

2.3.10 Rhopalial "horns"

53. *Rhopalial horns length* (Plate 2.15): **(0)** absent; **(1)** present; **(2)** short and thick; **(3)** short

and narrow; **(4)** long and relatively narrow; **(5)** long and very narrow.

54. *Rhopalial horns shape* (Plate 2.15): **(0)** straight; **(1)** curved inward; **(2)** curved outward.

55. *Rhopalial horns angle*: **(0)** more or less vertical; **(1)** about 45° oblique; **(2)** more horizontal than vertical.

This curious character has not been previously noted, but is worthy of mention, being found in the Australian species comprising the Irukandji clade. At the uppermost region of the rhopalial niche, lateral to where the rhopalial stalk meets the body wall, lies a pair of blind canals, projecting upward or at about a 45° angle. In some species they are straight, long, and narrow, whereas in others they are curved, short, and somewhat broader. In the group of taxa with frown-shaped rhopalial niche ostia, rhopalial horns have been confirmed in all species of the Irukandji clade, but are absent in *Tamoya haplonema*. Curiously, they are also present in *Carybdea sivickisi*, which is outside this group both morphologically and genetically (Chapter 3). The function of these structures is unknown.

2.3.11 Rhopalial windows

56. *Rhopalial window shape* (Plate 2.16A-C): **(0)** flat; **(1)** shallowly convex; **(2)** considerably convex; **(3)** concave; **(4)** with a small concavity at the rhopalial stalk.

Another character that has not been previously noted is the subumbrellar rhopalial window, or the thin sheet of mesoglea that separates the rhopalial niche from the subumbrellar space. This window is typically thinner and more transparent than the surrounding bell wall, lacking muscles and not overgrown by gonads. Indeed, the gonads often grow in a key-hole sort of shape around the windows, leaving the window area unobstructed. In the *Carybdea alata* species complex, the windows are typically broadly convex, like little helmets, allowing the eyes to swing up into the subumbrellar space. In the species with frown-shaped rhopalial niche ostia (e.g., *Carukia*, “Morbakka”, “Darwin carybdeid”), the window is flat, with a slight indentation at the upper edge. In the true *Carybdea* (e.g., *C. marsupialis*, *C. rastonii*, *C. xaymacana*), it is shallowly convex with a slight indentation marking the junction of the rhopaliar stem.

2.3.12 Rhopalial structures

57. *Eyes* (Table 2.7; Plate 2.17): **(0)** 6 (2 major with lenses + 4 lateral pigment spots); **(1)** 4 (2 major with lenses + 2 lateral pigment spots); **(2)** 2 (2 major with lenses only, lacking lateral pigment spots); **(3)** 1 (median lensed eye only); **(4)** 1 median lensed eye, plus one

median eye spot and 4 lateral eye spots.

58. *Rhopalial warts* (Plate 2.16D): **(0)** lacking; **(1)** 1 on rhopalial stem.

The rhopalium itself comprises typically two median eyes with lenses, 2 pairs of lateral eye spots, and a large terminal statocyst. The statocyst contains a single, large statolith, which is visible in fresh material as a large sparkly granule in the bottom half of the rhopalium. The larger, lower lensed eye is orientated to look straight into the bell cavity, whereas the smaller, upper lensed eye looks up toward the mouth. In some cases, the smaller, upper lensed eye is difficult to observe due to distortion during preservation. In at least one member of the *Carybdea alata* species complex, the lower, larger lensed eye is surrounded by a very large, darkened ring of pigment, and the upper lensed eye is absent. The lateral eye spots occur in pairs, i.e., on each side there may be 2 lateral eye spots, 1 lateral eye spot, or no lateral eye spots.

Mayer (1917) believed that the lateral eye spots probably fuse with the median eyes in the tropical Pacific form of *Carybdea alata*. Apparently, he based this on his observation of small specimens with lateral eyes and large specimens lacking lateral eyes. I have no data to support or refute his hypothesis, but it seems equally logical that there could be more than one species involved.

The forms which comprise the *Carybdea alata* group have the most variable eyes, and no doubt the arrangement of eyes will prove diagnostic for the different forms once their taxonomy is resolved. The Irukandjis are also characterized by variable eyes, with the several undescribed forms from Australia lacking the lateral eye spots.

2.3.13 Statoliths

59. *Statolith shape* (Table 2.8; Plate 2.18): **(0)** sausage-shaped, i.e., long and narrow; **(1)** short thick sausage; **(2)** chiton-shell-shaped, i.e., long and broad; **(3)** triangular biscuit, i.e., roughly pyramidal; **(4)** truncate teardrop; **(5)** V-shaped.

The statoliths are small concretions believed to be used by the jellyfish for balance, and are made of the gypsum form of calcium sulfate (Chapman, 1985). Berger (1900) reported that their removal did not influence the animal's pulsation rhythm or swimming balance; he speculated that they might function as weights for keeping the rhopalia properly suspended, rather than as balance organs. They have not been previously used taxonomically, but provide a unique opportunity for study of a hard part in a soft-bodied organism, such as might be helpful in identification of fragmentary, thawed, or otherwise unidentifiable material, including fossil specimens. Morphometric analysis of statolith shape is beyond the scope of this thesis, but overall shapes are summarized below.

In general, the overall shapes are diagnostic of most genera and many species defined on other morphological criteria, and especially appear quite distinct among species within the genus *Carybdea*. In addition to overall shape, characters such as apical projections, particular crystal formations, and plate arrangements have been noted as useful.

A couple of words of caution in working with cubozoan statoliths are in order. First, they tend to disintegrate rapidly once they are removed from the rhopalium. I have found that storing them intact on the rhopalium in 90-100% EtOH seems to work well, whereas storing them in seawater definitely does not. Second, orientating them the same way for comparative analysis can be tricky. For convenience, I have orientated the images on Plate 2.18 with the “inside” surface toward the lower edge of the photograph, i.e., the side that abuts the nerve tissue, facing “upward” in the rhopalium behind the main eye. However, many of these cannot be critically shape-analyzed this way because their hemispherical “back” makes them rest at irreproducible angles.

2.3.14 Velarium

Absence of the velarium, along with absence of the pedalia, was used by Haeckel (1880) to define his genus *Procharagma*. This form has never been seen again, and neither of the two described species was adopted by any subsequent author. However, one of the species, namely *P. aurea*, remarkably matches the description for the later-named *Carybdea sivickisi*. I think that Haeckel had the same form, but simply misinterpreted the velarium and the pedalia, for both structures are present (though small) in *C. sivickisi*. The velarium was also used to diagnose the genus *Procharybdis*, but this time based on the lack of velarial structures (Haeckel, 1880); the 5 species described by Haeckel are generally taken to be juveniles or otherwise unrecognizable.

The velarium is a shelf-like ring of tissue extending inward from the lower edge of the body wall, forming a narrowing diaphragm at the oral opening of the body. It is thought to be useful in jet propulsion. It is superficially similar to the hydromedusan velum, but differs in at least one important respect. Whereas the hydromedusan velum consists only of a simple mesogleal layer surrounded by ectoderm, the cubozoan velarium is vascularized, and thus contains an inner layer of endoderm continuous with the internal cavities of the body. This was explained more completely by Mayer (1910, p. 504).

The velarium itself is fairly uniform, and is not currently used diagnostically. However, the velarium is home to several structures which are highly important in species recognition, namely, the velarial canals, perradial lappets, frenulae, and in some taxa, nematocyst warts.

2.3.15 Velarial canals

60. *Velarial canals number per octant* (Table 2.9): **(0)** 1; **(1)** 2; **(2)** 3; **(3)** 4; **(4)** more than 4; **(5)** more than 10.
61. *Velarial canals - number of roots per octant leaving stomach*: **(0)** 1 root; **(1)** 2 roots; **(2)** 3 roots.
62. *Velarial canal # per octant leaving velarial turnover*: **(0)** 1; **(1)** 2; **(2)** 3; **(3)** 4; **(4)** 6; **(5)** 7; **(6)** about 10 or more.
63. *Velarial canal # tips per octant*: **(0)** 1; **(1)** 2; **(2)** 3; **(3)** 4; **(4)** 5-9; **(5)** more than 10.
64. *Velarial canals branching* (Table 2.9; Plate 2.19A-D): **(0)** simple; **(1)** biforked; **(2)** triforked; **(3)** paw-like, i.e., with numerous forks at base; **(4)** umbelled, i.e., forked only at end; **(5)** dendritic; **(6)** extremely complexly branched, hard to tell number.
65. *Velarial canals lobations* (Plate 2.19E-F): **(0)** lacking lateral lobations; **(1)** with lateral lobations.
66. *Velarial canals similarity*: **(0)** all the same form; **(1)** of two different forms.

Some authors have believed that the form and number of the canals change through the course of development (e.g., Bigelow, 1909; Stiasny, 1930). In general, the carybdeid velarial canals do tend to increase in complexity through ontogeny, so they are best studied in mature individuals. But in mature specimens, there are definite differences between species. The number of canals, on the other hand, remains constant as the animal grows. In the chirodropids, however, the velarial canals appear to be meaningless for species identification; I have yet to find any appreciable difference among species.

The velarial canals are simply extensions of the gastric cavity into the substance of the velarium. The number and degree of branching of the velarial canals can be highly diagnostic in the carybdeids, whereas the chirodropid canals tend to be fairly uniform among species. Because the number and form can occasionally vary in the same individual, the velarial canals are typically studied per octant, with the 8 octants then being used to average the number and form. The number of velarial canals per octant (= between adjacent perradii and interradii) may be easy to identify, or the canals may be so tightly packed that they are impossible to count. The canals may be simple and unbranched, bifurcated (= Y-shaped), trifurcated or paw-like (= multiple fingers arising from a common origin), dendritic (= complexly branched so as to be without a definite pattern), or feather-like (with many lateral diverticula from a central canal). In most species the velarial canals are not decorated with nematocyst warts, but *Carukia barnesi* is distinctive in always having a single large wart on the canal nearest the perradius, and some of its closest relatives have one or more warts on the canals as well.

The degree of branching of the velarial canals formed the basis of Haeckel's (1880) separation of the subgenera *Charybdella* (species today grouped under "*Carybdea alata*") and *Charybdusa* (*Carybdea marsupialis*, etc.). However, most subsequent authors agreed that this was erroneous. In my analyses, I have come to the conclusion that this is partly right, and partly not. While I agree with Haeckel that the velarial canals are a useful character, I disagree on his separation of subgenera on this character alone. These two groups which he distinguished can be partitioned on nearly every scorable character (see Chapter 5).

2.3.16 Frenulae

67. *Frenulum development*: **(0)** well developed; **(1)** narrow.
68. *Frenulum # of sheets* (Plate 2.20): **(0)** absent; **(1)** comprising a single simple sheet; **(2)** comprising a single solid gelatinous substance; **(3)** comprising two sheets; **(4)** single hollow structure; **(5)** comprising numerous vertical folds.
69. *Frenulum distance to margin*: **(0)** barely extending onto velarium; **(1)** only about half-way to margin; **(2)** reaching about three-fourths to margin; **(3)** very nearly reaching margin; **(4)** reaching velarial margin.

The absence of frenulae was one of the characters used by Haeckel (1880) to differentiate his genus *Procharybdis* from others (Section 2.3.14). I have never studied a specimen that truly lacked frenulae, though I have studied some in which they were relatively narrow. Most descriptions mention that the taxa have frenulae, but the finer details of various forms have never been used for taxonomic differentiation.

The frenulae are bracket-like membranes that help maintain the right-angle arrangement of the velarium to the body, presumably assisting in jet propulsion. There are four frenulae, one on each perradius. The vertical portion of the frenulum typically tapers from the velarium up to the level of the rhopalium. The velarial portion of the frenulum may extend only a short distance or the entire distance toward the velarial margin.

Some specimens of *Tamoya haplonema* have a double walled, hollow structure; in *Carybdea rastonii* it is a single thickened wedge of rigid mesoglea, whereas in most species it is merely a single, thin sheet of flexible tissue.

2.3.17 Perradial Lappets

70. *Perradial lappet* (Table 2.10; Plate 2.21): **(0)** absent; **(1)** present.

71. *Perradial lappet shape* (Table 2.10; Plate 2.21): **(0)** narrowly triangular; **(1)** moderately triangular; **(2)** broadly triangular; **(3)** nearly straight; **(4)** broadly rounded.

72. *Perradial lappets branching* (Table 2.10; Plate 2.21): **(0)** simple and unbranched; **(1)** simple sided, with "nipple tips"; **(2)** with canals branching off sides; **(3)** turning into many parallel canals.

The perradius is marked by the line of junction of the frenulum on the velarium. In some taxa, this region is further marked by a thickened triangular patch, which is referred to as the perradial lappet. The perradial lappets have been infrequently discussed; Southcott (1956) and Kramp (1955a) referred to them in name only, but did not ascribe to them any taxonomic significance. They have been illustrated (often without comment) by Southcott (1956: figs. 1, 8; 1967: fig. 3), Stiasny (1937a: fig. 5), Thiel (1928: abb. 3; 1936b: abb. 116), While the term lappet is frequently used with reference to scyphozoan structures (scalloping of the adult bell margin, or arm-like appendages in the ephyrae), there is no obvious homology between the scyphozoan and cubozoan lappets.

Mayer (1910) and Kramp (1955a) wrote about the tendency among 19th century authors to regard the velarium as being composed of a series of fused lappets; however, this is fundamentally different from the perradial lappets discussed herein. The perradial lappets are actual structures, appearing as a raised patch of tissue over the perradial region of the velarium, whereas whether the velarium is composed of fused lappets is a philosophical question of homology with the Scyphozoa or Hydrozoa.

The perradial lappets, when present, often tend to be almost perfect equilateral triangles, although they may be quite narrowly triangular in a few species. As a general rule, the lappets are absent in the majority of the Carybdeida, but conspicuously present in the Irukandji group and the chirodropids. In the Irukandji group, they are sometimes decorated with prominent nematocyst warts in one or more rows (Section 2.3.18). In the chirodropids, they are undecorated. In some taxa of both groups, velarial canals may emanate from the sides or tips of the lappets.

2.3.18 Velarial nematocyst warts

73. *Velarial armament* (Table 2.10; Plate 2.21): **(0)** lacking warts or nematocyst freckles; **(1)** with single freckle on perradial lappets; **(2)** with a row of freckles on each lappet; **(3)** with a single wart on perradial lappets; **(4)** with a row of warts on each lappet;

(5) with a single large wart on canals; **(6)** with a row of warts on canals; **(7)** with scattered nematocyst freckles or warts.

Nematocyst warts on the velarium have not been previously used diagnostically, but are indeed diagnostic of several species. While nematocyst warts may occur on the velarial canals in some species (e.g. *Tamoya*, "Morbakka", *Carukia* spp., and some *Alatina* spp.), they consistently occur in rows on the perradial lappets in most of the Irukandji clade.

2.3.19 Stomach

74. *Stomach size*: **(0)** shallow or flat; **(1)** large and bag-like.
75. *Manubrium length*: **(0)** very short; **(1)** about one half bell height; **(2)** nearly reaching bell margin; **(3)** protruding from bell margin.

Most authors considered the large, balloon-like stomach to be characteristic of *Tamoya*, compared with the short, flat stomach characteristic of *Carybdea* (Bigelow, 1909; Mayer, 1910; Kramp, 1961). While this generality is true, it is also true that these features are characteristic of other groups. The carybdeid species with frown-shaped rhopaliar niche ostia tend to have larger stomachs, whereas other carybdeids and all chirodropids tend to have flatter stomachs.

It is important to note that the stomach depth was typically associated by most authors with the presence or absence of mesenteries in separating the genus *Carybdea* (flat stomach, no mesenteries) from *Tamoya* (deep stomach, mesenteries present). However, these two characters are not always in association (Section 2.3.22).

2.3.20 Mouth shape

76. *Lips shape* (Plate 2.22): **(0)** narrow pointy; **(1)** broadly pointy; **(2)** narrow rounded; **(3)** broadly rounded.
77. *Lips - frizziness*: **(0)** smooth-edged; **(1)** a bit wrinkled; **(2)** quite frizzy.

The corners of the mouth are drawn out into four recurved lips, typically folded at the midline. In most taxa they appear as fairly narrow triangular projections, arranged in a cruciform manner, but in some taxa such as *Chirodropus gorilla* and the "Darwin carybdeid", they are quite broad, giving a more quadrate appearance. The lips also may be quite short (e.g., *Tripedalia*), or very long (e.g., some chirodropids). The mouth shape has not been previously used diagnostically.

The lips are generally smooth-edged, except in Haeckel's *Carybdea pyramis*, which was defined, in part, based on having frizzy lips. In most species, the lips have nematocysts that can be detected microscopically, but in *Tamoya haplonema*, numerous warts are scattered about the outer portion of the lips, and in a new species of *Tamoya* from South Carolina, very large warts are present along the lips and manubrium.

2.3.21 Gastric saccules

78. *Gastric saccules*: **(0)** absent; **(1)** present.
79. *Gastric saccule shape* (Table 2.11; Plate 2.23): **(0)** round and hemispherical; **(1)** coalesced heart-shaped; **(2)** laterally flattened and cock's-comb-shaped; **(3)** finger-shaped; **(4)** highly branched, like clumps of grapes; **(5)** feather-like, with numerous filaments.
80. *Gastric saccules length*: **(0)** very short, knob-like, not pendant; **(1)** pendant a little bit; **(2)** about 1/2 the length of the bell cavity; **(3)** nearly the entire bell cavity in length.
81. *Gastric saccules hollow or solid*: **(0)** hollow; **(1)** solid; **(2)** semi-filled.

This character is exclusive to the Chirodropidae, and is the main structure used historically to distinguish the species, but has been the subject of much misunderstanding. These structures are interchangeably termed "Taschen-Armen" by Haeckel (1880); "pocket arms" by Horst (1907); "hernia-like pouches" by Mayer (1910); "diverticula (Umbralsäcke)" by Uchida (1929); "gastric pouches" by Bigelow (1938); "superior gonad" by Southcott (1956); "diverticula", "projections", "gastric saccules", or "hernia-like pouches" by Kramp (1961); "perradial nuclei" by Barnes (1965); "divertikel der gastraltasche" by Werner (1984); and "diverticula" by Mianzan and Cornelius (1999).

These structures are typically interpreted as gastric pouch extensions (e.g., Bigelow, 1938); however, they are typically not hollow and there is no evidence that they perform a gastric function. Mind you, there is also no evidence that they don't.

The overall shape of the saccules may be short and fingerlike, as in *Chiropsalmus quadrumanus*; long and fingerlike, as in *Chiropsoides buitendijki*; long and tapered with numerous filaments, as in *Chirodropus gorilla*; cock's-comb-like as in *Chironex fleckeri*; or simple, solid, hemispherical knobs, as in the undescribed species often erroneously identified as the Australian form of *Chiropsalmus quadrigatus*. In the undescribed spotted Chirodropid (*Chirodropus* sp. A), the saccules are entirely lacking.

2.3.22 Mesenteries

82. *Perradial mesenteries* (Table 2.12; Plate 2.24): **(0)** absent; **(1)** cord-like only in stomach region; **(2)** cord-like band of tissue extending from stomach to rhopalium; **(3)** flap-like only in stomach region; **(4)** flap-like in stomach region, with a cord-like extension to mid-body; **(5)** flap-like to saccules, with thickened cord-like extension to frenulum; **(6)** flap-like halfway to rhopalium, without extensions; **(7)** flap-like halfway to rhopalium, with cord-like extension to rhopalium; **(8)** flap-like all the way to rhopalium; **(9)** flap-like portion split; **(10)** extension to rhopalium is clear but not raised.

As stated in Section 2.3.19, the stomach may be short and flat, or large and bag-like. When large, it is typically fastened to the subumbrellar surface with perradial bracket-like mesenteries. Stiasny (1919; 1934) and Uchida (1970) erroneously thought that the mesenteries were interradial, i.e., on the pedalial radii. The mesenteries have also been called “suspensoria” (Conant, 1898; Uchida, 1929; Southcott, 1956; Southcott, 1967) or “mesogonia” (Conant, 1898; Southcott, 1956), and the hollowed regions between them are typically called “funnels” (Conant, 1898; Uchida, 1929) or “blind pockets” (Bigelow, 1938). The presence of mesenteries has been used by most authors as the primary basis to separate *Tamoya* from *Carybdea* (e.g., Bigelow, 1909, 1938; Mayer, 1910; Uchida, 1929; Kramp, 1961). However, Mayer (1910) argued that “the so-called mesenteries of Haeckel are merely the flattened, perradial sides of the cruciform stomach.” On this basis, he suggested that the genus *Tamoya* is very closely related to, if not identical with, *Carybdea*, and that the differences between them are merely that of an intergrading character. In fact, Mayer could not have been more wrong. Although many identification errors have been made with species assigned variously to each group, the genera themselves (as defined by their type species) differ widely in nearly every character that can be compared. If they were insects or mammals, they would likely be placed in different orders!

In *Tamoya haplonema* the mesenteries are indeed well developed, as they are in several undescribed taxa lacking phacellae, including the “Morbakkas” and the “Darwin carybdeid”. The members of the *Carybdea alata* species complex entirely lack all traces of mesenteries. In the *C. marsupialis* clade and *C. rastonii*, the mesenteries are reduced and thus non-functional, but they are still present as a thickened strand of tissue which runs some distance from the stomach toward the rhopalium. As in some *Carybdea* spp., in the two described species of *Tripedalia* the mesenteries are weakly developed, extending about halfway to the rhopalium, whereas they are absent in a third, undescribed form.

Thus, there is a spectrum ranging from completely absent, to present but poorly developed as sessile cord-like thickenings, to well-developed flap-like or web-like structures with crescentic free margins. Furthermore, the mesenteries may be differentially developed at different locations, e.g., web-like or cord-like proximally and cord-like or absent distally. This spectrum has typically been overlooked by previous authors. Herein, I define well developed to mean those forms with a web-like crescentic free margin, and weakly developed to mean those forms with various degrees of thickened tissue, but not a web-like crescentic free margin.

2.3.23 Colour patterns

83. *Body color*: **(0)** transparent and colorless; **(1)** quite yellow; **(2)** with red pigment spots over pedalia and stomach; **(3)** with brownish mottling and spots; **(4)** translucent whitish; **(5)** translucent brownish.
84. *Nematocyst wart or freckle color*: **(0)** very red; **(1)** pink; **(2)** violet; **(3)** whitish.
85. *Tentacle color*: **(0)** all dark purplish; **(1)** outer tentacles bright purple; **(2)** pink; **(3)** yellowish; **(4)** banded purple; **(5)** banded orange and white; **(6)** whitish; **(7)** colorless.

Most cubozoans' bodies are remarkably transparent and colourless, or may have a slightly translucent, ghostly appearance. Of the species with nematocyst warts or freckles on the bell, they are typically whitish, but may be distinctly red (e.g., *Carukia barnesi, Carukia* sp. B (from Broome), and *Chiropsalmus quadrumanus*), faintly purple ("Pseudo-Irukandji", "Halo-Irukandji", and "Broome Irukandji"), or pinkish ("Morbakka"). The tentacles of most species are pale pinkish, brownish, or whitish, but may appear dark purplish in water. Several species possess very pink tentacles (e.g., *Carybdea rastonii*, Outer Reef Irukandji, and "Morbakka").

Notable exceptions to these norms include some well known and also some not-so-well known species. Some of the larger Chirodropida, e.g., *Chironex fleckeri*, may have a brownish tint to the body mesoglea, and most of the tentacles are pale "dirty brownish" except the outer unpaired tentacle on each pedalium, which may be blue or purple. *Carybdea sivickisi* is remarkable in having bright orange gonads filling the upper part of the body, and thus giving the medusa a distinctly orange appearance, and in having tentacles that are banded orange and brown. An undescribed species of *Carybdea* from South Africa has distinctive brownish red blotches over the phacellae and at the "shoulder" of each pedalium. An undescribed species of Chirodropida from the Great Barrier Reef has numerous large and small brown spots over the entire body and distinctly purple tentacles. An undescribed carybdeid from Jamaica and an undescribed chirodropid from NW Australia are both characterized by black tentacles. Finally, an unidentified species of Chirodropida figured by Beebe (1928) had conspicuous dark lines running the entire height of the bell at each adradius, plus a similar line encircling the base of each pedalium.

Many taxonomists have traditionally argued against the use of colour as a diagnostic character, because A) colours are lost in preservation, and B) colours can be derived from variable sources such as food type or symbionts. For this reason, some practicality needs to be exercised in the use of colour as a taxonomic character. The difference of bright pink tentacles versus pale pink tentacles might be suspect, whereas the differences between pale pink tentacles, blue tentacles, and brown-and-yellow alternate-banded tentacles should not be overlooked.

2.3.24 A note on Nematocysts

I have included nematocysts for reference (Table 2.13; Plates 2.25-2.27; see glossary, Appendix 1), but with some reluctance for two reasons. First, although I believe they are interesting and that it is important to be thorough, nematocysts are nonetheless peripheral to taxonomic diagnosis in the Cubozoa, i.e., accurate identification can be obtained from structural characters alone. While it is theoretically possible that eventually additional cryptic taxa will be distinguished on the basis of their cnidomes (e.g., the Japanese "*Carybdea rastonii*" and the Australian "*Carybdea xaymacana*"), the philosophical issue of cryptic taxa is beyond the scope of this thesis. It should be noted, however, that cnidomes are not equally unique among cubozoan taxa; while most carybdeid genera can easily be diagnosed with nematocysts, and even some species, the same is not true for the chirodropids.

Second, most of the specimens I have had available for examination, and no doubt most of the specimens that others will seek to identify, are preserved. Different preserving methods have different effects on the shape and size of the nematocysts, leading to inconsistent results; furthermore, many preservation methods inactivate the firing mechanism, resulting in unidentifiable shaft structure, on which proper identification relies. Accurate nematocyst taxonomy has always been based entirely on shaft morphology of discharged capsules, i.e., length of the shaft relative to the capsule, number and position of the shaft swellings, and position of spines (Weill, 1934; Halstead, 1965; Mariscal, 1971; Calder, 1974; Williamson et al., 1996; Östman, 2000). While the nematocyst type is determined by the discharged anatomy, the nematocyst size is measured on undischarged capsules for comparison between species. Although clearly different capsule shapes and tubule winding patterns can be found in different taxa, these are often grouped under the same named nematocyst type, because there is currently no system for recognizing undischarged differences. Therefore, one must be vigilant about describing these features so that they can eventually be incorporated into an accurate and comprehensive system based on discharged and undischarged morphology.

Nonetheless, I think there is value in knowing as much about a species as possible, i.e., having more tools in our proverbial toolbox. The synopsis of nematocyst types and measurements given in Table 2.13 is only a clumsy first step toward a thorough study, but does serve to illustrate differences consistent with the phylogenetic results in Chapter 3. A thorough treatment will include parallel measurements of nematocysts from different locations on the tentacles and bell, and from numerous specimens of different sizes and states of maturity.

In cases of fatality, syndrome-species linkages, or simple curiosity, it is sometimes desirable to attempt to identify species based on nematocysts left behind during sting events. However, skin scrapings or sticky-tape samples must be interpreted with caution; while the recovered nematocysts may be able to rule in or out certain species, conclusive identification at the species level is often unrealistic. Many closely related species have similar cnidomes, and not all nematocyst types that characterize a species are necessarily left behind in a sting event. Furthermore, cnidomes often change through ontogeny, and most species have different ratios and sizes, and often different nematocyst types, on different parts of the body and even at different locations on the tentacles. Finally, confusing artifacts may be produced in the skin-scraping and preparation process, as elucidated by Rifkin (in Williamson et al., 1996); if forensic identification is going to be attempted, the best chance appears to be from a sticky-tape sample taken as soon after the sting as possible to avoid nematocyst loss, ideally while still at the beach (Currie and Wood, 1995; Williamson et al., 1996).

Several recent attempts at forensic identification illustrate this problem. First, Little and Seymour (2003) linked the nematocysts from a severe Irukandji envenomation to a new species of Irukandji originally identified by me and held in the Queensland Museum collection awaiting publication (*Carukia* n. sp. A). However, the recovered nematocysts could have been left by any of several species in the North QLD region; they are characteristic of three different species of *Carukia* (one named, and two undescribed; see Plate 2.25B, Plate 2.26C), and two different species of *Alatina* gen. nov. (described in Chapter 5; see Plate 2.25C), and similar to one species of *Carybdea*. *Carybdea* spp. are not known to give Irukandji syndrome. The syndrome characteristics (Mulcahy, 1999; Little et al., 2001; Little and Seymour, 2003) would seem to preclude this from having been *Carukia*, in which the syndromes are typically slow to onset, typically characterized by nausea and vomiting, and typically do not cause severe hypertension. Of the two remaining species, both in my new genus *Alatina*, one is suspected of causing Irukandji syndrome (see Chapter 5) and the other does not (H. Taylor, pers. comm. 2004). Furthermore, I have studied three specimens caught at the time of the sting in question, and they are all my new species *Alatina mordens* (described in Chapter 5). Thus, while we do not have conclusive proof that *Alatina mordens* was the stinger, there is sufficient evidence to question whether the new species of *Carukia* was, as determined by Little and Seymour (2003).

Second, Huynh et al. (2003) provided cnidome evidence that the stinger that killed an American tourist in 2002 could not have been *Carukia barnesi*; however, they did not assign the nematocysts to any particular known or unknown species. The nematocyst figured is unlikely to have come from any species other than those in my "Pseudo-Irukandji" group (see Chapters 3, 4), which has a tentacular monocnidome of extremely characteristic nematocysts (Plate 2.25A, Plate 2.26A, B). Unfortunately, the recovered nematocyst is incompletely discharged, and thus cannot be identified to species.

Third, Wiltshire and her colleagues (2000) identified their subject as *Carukia barnesi*, but even a casual perusal of the literature pertaining to cubozoan nematocysts would have indicated that this could not have been accurate. The nematocysts they presented were elongate and club-shaped, as in the "Pseudo-Irukandji" group, rather than short and lemon-shaped, as in *Carukia*. Furthermore, the specimen in their photographs is far too large and robust to be *Carukia*, the tentacles are too thick, and the bell is too evenly rounded; these characters are all consistent with species in the "Pseudo-Irukandji" group.

Nematocyst terminology can be a bit bewildering to the non-expert; those wishing a more thorough explanation are directed to Östman (2000). Nematocysts are comprised of three parts, the capsule, the shaft (= basis, or butt; the thickened section first out of the capsule), and the tubule (the long narrow section that extends beyond the shaft). Macrobasic and microbasic (derived from the word "basis") simply refer to the length of the shaft compared to the length of the capsule. Older classifications referred to shafts about 3-4 times the length of the capsule as macrobasic, whereas microbasic was used for nematocysts with a shorter shaft, whereas recent workers (N. Boero and J. Bouillon, lecture notes, 2000) regard 1x the capsule length as the cut-off point (i.e., microbasic is any nematocyst with a shaft up to 1x the capsule length, whereas macrobasic is any nematocyst with a longer shaft). This makes a lot more sense because the shaft must twist or fold if it is longer than the capsule, whereas how many times it twists or folds is simply a matter of scale. Macrobasic nematocysts are not known within the Cubozoa. In cubozoans, three main categories of nematocysts are common: euryteles (also called tumiteles), mastigophores, and isorhizas. These names also make a bit more sense when one understands their origins. Eurytele translates to "distally widened shaft" (eury = wide; telum = weapon, spear, dart, sword, dagger). Mastigophore translates to "whip-bearing". Isorhiza translates to "equal root", in reference to nematocysts in which the shaft is lacking and the tubule is of equal diameter throughout. Names with "trich" refer to the spination, i.e., atrichous (without spines), holotrichous (completely covered with same-sized spines), heterotrichous (spines of different sizes), basitrichous (spines only at the base), merotrichous (spines only in the middle), and apotrichous (spines only at the end).

Other confusing names that one might encounter with cubozoan nematocysts include: haplonemes (without a well defined shaft; referring to a higher grouping including isorhizas), rhabdoids (tubule of constant diameter; referring to a higher grouping including mastigophores), and rhopaloids (tubule of unequal diameter; referring to a higher grouping including euryteles, tumiteles, stenoteles, and trirhopaloids). Euryteles have shafts with a distal swelling bearing spines; tumiteles have the swelling and spines on the middle of the shaft; stenoteles have large spines at the constriction on the shaft; and trirhopaloids have spines on the largest, middle swelling of the shaft. In many species of Cubozoa, the dominant penetrant-type nematocysts are p-mastigophores (with an abrupt demarcation between the shaft and tubule), with three types currently recognized: Type 1 (spines orientated at right angles to capsule), Type 2 (spines orientated towards capsule), and Type 3 (shorter spines orientated towards capsule, longer spines orientated away). To these, I would add Type 4 (spines orientated away from the capsule), which are characteristic of the non-*Carukia* species of the Irukandji clade, e.g., “Broome Irukandji”, “Pseudo-Irukandji”, and “Morbakka” (Plates 2.26A, B).

Not all authors have used the same terminology for the same nematocyst types, although for the most part it has been more consistent than the nomenclature concerning medusa morphology. For example, not all authors accept Southcott’s name “tumitele” for the tentacular nematocysts of many carybdeids (Southcott, 1967; Matsumoto, 1995; Hartwick, Unpublished), and only recently have the heterotrichous microbasic euryteles of *Chironex fleckeri* been reclassified as trirhopaloids (Rifkin and Endean, 1983; Williamson et al., 1996; Carrette et al., 2002). A synopsis of the history of nematocyst nomenclature is given by Östman (2000), and a synopsis of the cnidomes of some cubozoan species is given by Shostak (1996).

Cubozoan bell nematocysts tend to be non-penetrant isorhizas, whereas tentacular nematocysts are either entirely penetrant-form mastigophores or euryteles, or a mixture of penetrant and non-penetrant types. This, of course, makes sense when viewed in terms of functional biology: prey must be killed quickly and held tightly (typically by the tentacle nematocysts), but a yucky mouth taste and quick release are more desirable in defensive maneuvers against predators (presumably the function of the bell nematocysts). However, this leaves some questions regarding Irukandji stings attributed to *Carukia barnesi*, the “Darwin carybdeid”, and “Morbakka”, in which the few cases with a visible mark tend to show an amorphous or bell-shaped blotch rather than a tentacular streak (Kinsey, 1988; Williamson et al., 1996; Fenner, 1997). It is possible that something other than penetrant-form nematocysts is responsible for Irukandji syndrome; whether this might be the non-penetrant isorhizas or possibly some secreted substance of non-cnidocystic origin is mere conjecture at this point.

For further information on cubozoan nematocysts, the following literature should be consulted: Southcott (1967), Calder and Peters (1975), Endean and Rifkin (1975), Williamson et al. (1996), Avian et al. (1997), and Marques et al. (1997).

A summary of cnidomes of cubomedusae is given in Table 2.13. Cubozoan nematocyst types are illustrated in Plates 2.25-2.27. For cnidome information on cubopolyps, refer to the following: *Carybdea marsupialis* (Stangl et al., 2002); *Carybdea xaymacana* (as *C. marsupialis*) (Studebaker, 1972; Cutress and Studebaker, 1973); *Tripedalia cystophora* (Werner, 1975; Chapman, 1978; Werner, 1984); *Carybdea sivickisi* (Hartwick, 1991); *Chironex fleckeri* (Yamaguchi and Hartwick, 1980); "*Carybdea alata*" (Arneson, 1976; Arneson and Cutress, 1976), and the Japanese "*Carybdea rastonii*" (Okada, 1927).

2.3.25 A note on Juvenile forms

Numerous species have been described through the years based on juvenile forms, e.g., *Carybdea verrucosa*, *Carybdea aurifera*, *Carybdea obeliscus*, and *Chiropsalmus quadrigatus*.

I have not yet examined enough newly metamorphosed and young medusae of known identity to be able to taxonomize the juveniles, but my initial observations suggest that many taxa could be identified. I have observed (but not classified) carybdeid juveniles that are clearly only a few hours to a few days old, some with many scattered nematocyst clusters, some with a definite pattern, and others almost lacking nematocysts entirely; some juveniles are golden yellow, while others are quite clear or reddish; some have one or two gastric cirri at each interradius, while others lack them completely. In other words, they do not all look the same.

Juveniles caught during a *Carukia barnesi* bloom, which were subsequently reared to maturity (and proved to be *C. barnesi*), had very clear bodies, numerous red nematocyst warts haphazardly arranged, and no gastric cirri. Juveniles identified as *Carybdea sivickisi*, based on a continuous growth series with that species, were faintly yellowish with 8 red spots above the margin marking the main radii, lacked any trace of bell warts, and had a few gastric cirri in the stomach. Juveniles that grew up to be *Chiropsalmus* n. sp. A (N. QLD) were pale yellowish compared to the red-studded *Carukia barnesi* of the same size.

Arneson and Cutress (1976) concluded that *Carybdea verrucosa*, *C. aurifera*, and "*C. alata*" were one and the same, based on their culturing results with their form of "*C. alata*". It seems unlikely that *C. verrucosa* and *C. aurifera* are identical, given that even at their young age, they are readily identifiable from one another; however, which one is, or even if either one is, identical to the South Atlantic "*C. alata*" is currently unclear.

In the Chirodropidae, the widely reported but poorly characterized *Chiropsalmus quadrigatus* was originally described based on a badly damaged juvenile. In general, as noted by Barnes (1966), it is exceedingly difficult to identify chirodropids under about 4 cm bell height (though this is not the case for the undescribed species from Gove, which reaches sexual maturity at that size).

From my experience, I can often identify very small specimens to family, or even in some cases to genus, based on some characters that develop quite early; however, other than reasonable hunches based on correlating these specimens with known geographical ranges, I cannot yet confidently identify juveniles to the species level. Some of the more useful characters in juveniles are as follows. In North Queensland, young chirodropids have nematocyst-free bells that are yellowish in colour, whereas carybdeids have nematocyst-warted bells that are more whitish or pinkish. *Chironex* and *Chiropsalmus* can be distinguished down to about 1 cm bell height based on the pedalial canal bend (Char. 24), with the bend of *Chironex* having a sharp upward-pointing spike, but lacking in *Chiropsalmus*; the tentacles are also indicative (Char. 31), being fine and round in *Chiropsalmus* but flat and more robust in *Chironex*, but some young *Chironex* have fine tentacles, so this is not 100% reliable. In the carybdeids, the rhopaliar niche ostium (Char. 2.3.9) develops at an extremely young age, allowing specimens just a few mm tall to be identified to family or genus. Irukandjis have a frown-shaped rhopaliar niche ostium, whereas this structure is T-shaped in the *Alatina* spp. and heart-shaped in *Carybdea* spp., but vertical oval in *Carybdea sivickisi*. Furthermore, Irukandji spp. do not have gastric cirri in the stomach (Char. 2.3.7), whereas the species from other groups do. The tentacle bands of *Carukia* spp. are formed with the characteristic “tails” from about 3-4 mm bell height, whereas all other species do not have these bands at any age. These “quick-and-dirty” identification methods are specific to North Queensland taxa; the extent to which these characters apply to other species in these genera from other localities is unknown. Furthermore, as specimens approach the post-release phase from the polyp, they become increasingly more difficult to identify even to family or order. There currently exists no identification scheme for newly released juvenile cubozoans.

It should be added that life cycle observations can be an exceedingly valuable tool for taxonomy, in providing additional characters with which to support or question the current paradigm (see, for example, Gershwin & Collins, 2002).

Table 2.1. Comparison of relative measurements of cubozoan species (Section 2.3.1; Plate 2.1). All measurements were made on preserved material unless otherwise noted, and are rounded to the nearest millimeter, except in the case of very fine tentacles, which are rounded to the nearest 0.5 mm. These measurements are provided as a general guide only, and should not be interpreted to encompass the range of variation in a species. Holotype is designated by (H) and neotype is designated by (N); for syntype material (S), the largest individual was measured.

Species	**BH (mm)**	**DBW (mm)**	**IRW (mm)**	**TBW (mm)**	**Max. BH known (mm)**
Alatina mordens n. sp.	81 (H)	64	30	2	96
Alatina rainensis n. sp.	18 (H)	16	8	0.5	--
Manokia stiasnyi	24 (H)	20	10	1	--
Carybdea alata species complex	Various				Ca. 250
Carybdea rastonii	31 (N)	37	19	1	
Carybdea marsupialis	41 (N)	54	26	3	--
Carybdea xaymacana	34	37	19	2	--
Carybdea sivickisi	10-12 (S)	12-14	--	--	Ca. 12
Tripedalia binata	8 (H)	13	8	0.5	8
Tripedalia cystophora	9 (N)	14	8	0.5	13
Carukia barnesi	13 (H)	12	6	0.5	14
Carukia sp. A (Russell's)	13 (H)	14	8	0.5	--
Carukia sp. B (Broome)	17 (H)	16	7	1	--
"Broome Irukandji"	43 (H)	37	18	1	48 (live)
"Dampier Irukandji"	19 (H)	17	8	1	--
"Halo Irukandji"	31 (H)	30	16	1	
"Pseudo-Irukandji"	24 (H)	22	11	1	
"Darwin carybdeid"	46 (H)	48	24	4	61
"Morbakka" (Port Douglas)	118 (H)	104	43	16	Ca. 150
Tamoya haplonema	~130 (N)	~100	~50	~5	
Chirodropus gorilla	~150 (Lit)	~120			
Chirodropus n. sp. A (spotted)	150 (H)	160		8	--
Chironex fleckeri	118 (H)			6	380 (Goggin et al., 2004)
Chiropsalmus quadrumanus	~50 (N)	~80		~1.5	
Chiropsalmus quadrigatus	52 (H)	61	29	2	--
Chiropsalmus n. sp. A (N. Qld)	68 (H)	89	43	1	105 (Carrette et al., 2002)
Chiropsalmus n. sp. B (Gove)	45 (H)	63	34	2	51
Chiropsoides buitendijki	65 (S)	89	49	3	

Table 2.2. Comparison of exumbrellar armament among cubozoan species (Section 2.3.3.; Plate 2.4; see also Section 2.3.24, Table 2.13, and Plate 2.25D).

Species	**Gelatinous warts**	**Exumbrellar nematocysts**
Alatina mordens n. sp.	Absent	Present
Alatina rainensis n. sp.	Absent	Present
Manokia stiasnyi	Present	Present
Carybdea alata species complex	Absent	Present/Absent
Carybdea rastonii	Absent	Present
Carybdea marsupialis	Absent	Present
Carybdea xaymacana	Absent	Present
Carybdea sivickisi	Absent	Present
Tripedalia binata	Absent	Present
Tripedalia cystophora	Absent	Present
Carukia barnesi	Present	Present
Carukia sp. A (Russell’s)	Present	Unknown
Carukia sp. B (Broome)	Present	Present
“Broome Irukandji”	Present	Present
“Dampier Irukandji”	Unknown	Unknown
“Halo Irukandji”	Present	Present
“Pseudo-Irukandji”	Absent	Present
“Darwin carybdeid”	Absent	Present
“Morbakka” (Port Douglas)	Present	Present
Tamoya haplonema	Present	Present
Chirodropus gorilla	Absent	Absent
Chirodropus n. sp. A (spotted)	Absent	Absent
Chironex fleckeri	Absent	Absent
Chiropsalmus quadrumanus	Present	Present
Chiropsalmus quadrigatus	Absent	Absent
Chiropsalmus n. sp. A (N. Qld)	Absent	Absent
Chiropsalmus n. sp. B (Gove)	Absent	Absent
Chiropsoides buitendijki	Absent	Absent

Table 2.3. Comparison of pedalial canals of cubozoan species (Section 2.3.5; Plates 2.7-2.9).

Species	**Cross section**	**Bend form**
Alatina mordens n. sp.	Flat, bowed toward abaxial keel	Round to slightly angular
Alatina rainensis n. sp.	Quadrate in stalk portion, flattened through the rest	90º bend
Manokia stiasnyi	Flat	With small upward nub
Carybdea alata species complex	Flat	More or less knee-like
Carybdea rastonii	Somewhat quadrate proximally, flat distally	Knee-shaped
Carybdea marsupialis	Round throughout	90º angle
Carybdea xaymacana	Quadrate proximally, flat distally	Knee-shaped
Carybdea sivickisi	Flat	Simple
Tripedalia binata	Flat	Simple
Tripedalia cystophora	Flat	With small upward nub
Carukia barnesi	Oval to flat	Simple
Carukia sp. A (Russell’s)	Oval	Knee-shaped
Carukia sp. B (Broome)	Flat	Rounded, knee-like
“Broome Irukandji”	Strongly quadrate	With flat, oblique extension
“Dampier Irukandji”	Flat	N/A (obscured by poor preservation)
“Halo Irukandji”	Strongly quadrate	Straight or with small hump
“Pseudo-Irukandji”	Quadrate proximally, flat distally	With upward nub
“Darwin carybdeid”	Strongly quadrate	With prominent thorn
“Morbakka” (Port Douglas)	Strongly quadrate	With prominent thorn
Tamoya haplonema	Strongly quadrate	With prominent thorn
Chirodropus gorilla	Flat, divided	With prominent thorn
Chirodropus sp. A (spotted)	Flat, undivided	With prominent thorn
Chironex fleckeri	Flat, divided	With prominent thorn
Chiropsalmus quadrumanus	Flat, undivided	With volcano-shaped diverticulum
Chiropsalmus quadrigatus	Flat, unilateral	With prominent thorn
Chiropsalmus sp. A (N. Qld)	Flat, divided	Knee-shaped
Chiropsalmus sp. B (Gove)	Flat, divided at terminal end	Volcano-shaped
Chiropsoides buitendijki	Flat, unilateral	With prominent thorn

Table 2.4. Comparison of tentacle morphology among cubozoan species (Section 2.3.6; Plates 2.10, 2.11).

Species	**Tentacle cross section and banding form**
Alatina mordens n. sp.	Round, alternating 1-2-1-2, or “segmented” every 10 or so bands
Alatina rainensis n. sp.	Round, “segmented” every 10-11 bands, or alternating 1-2-1-2
Manokia stiasnyi	Round, evenly banded, each with a projection
Carybdea alata species complex	Round, evenly banded
Carybdea rastonii	Round, evenly banded
Carybdea marsupialis	Round, evenly banded
Carybdea xaymacana	Round, evenly banded
Carybdea sivickisi	Round, 1-2-1-2-1-2
Tripedalia binata	Round, evenly banded
Tripedalia cystophora	Round, 1-1-1-1- to 1-2-1-2-1-2
Carukia barnesi	Round, with neckerchief-like bands
Carukia sp. A (Russell’s)	Round, with neckerchief-like bands
Carukia sp. B (Broome)	Round, with neckerchief-like bands
“Broome Irukandji”	Round, primarily 1-2-1-2-1-2, with “segmenting”
“Dampier Irukandji”	Round, with halo-like bands
“Halo Irukandji”	Round, with halo-like bands, with “segmenting”
“Pseudo-Irukandji”	Round, evenly banded, with “segmenting”
“Darwin carybdeid”	Round to slightly flattened, evenly banded
“Morbakka” (Port Douglas)	Flat, wide and ribbon-like
Tamoya haplonema	Round to flattened a bit, evenly banded
Chirodropus gorilla	Flat, wide and ribbon-like; banding not noted
Chirodropus sp. A (spotted)	Flat, wide, and heavy, banding complex
Chironex fleckeri	Flat, wide and ribbon-like, with highly complex banding (e.g., 1-4-3-4-2-4-3-4-1)
Chiropsalmus quadrumanus	Flattish and fine, 1-2-2-2-1 to 1-3-2-3-1
Chiropsalmus quadrigatus	Flat, wide and ribbon-like, complexity unknown
Chiropsalmus sp. A (N. Qld)	Round and fine, 1-3-2-3-1 or more complex
Chiropsalmus sp. B (Gove)	Round and fine, could not interpret banding
Chiropsoides buitendijki	Flat, wide and ribbon-like, 1-2-1-2-1-2

Table 2.5. Comparison of phacellae characters among cubozoan species (Section 2.3.7; Plate 2.12).

Species	**Phacellae form**
Alatina mordens n. sp.	Crescentic, broad, with multiple roots of long cirri
Alatina rainensis n. sp.	Crescentic, singly rooted or in pairs of long cirri
Manokia stiasnyi	Crescentic, long, with apparently a single trunk
Carybdea alata species complex	Crescentic, with 1 or more trunks of long cirri
Carybdea rastonii	Linear oblique with many trunks
Carybdea marsupialis	Corner bundle with 8-10 trunks
Carybdea xaymacana	Corner bundle with 1 trunk
Carybdea sivickisi	Crescentic with numerous singly rooted short cirri
Tripedalia binata	Corner bundle with 1 trunk
Tripedalia cystophora	Corner bundle with 1 trunk
Carukia barnesi	Lacking
Carukia sp. A (Russell's)	Lacking
Carukia sp. B (Broome)	Lacking
"Broome Irukandji"	Lacking
"Dampier Irukandji"	Lacking
"Halo Irukandji"	Lacking
"Pseudo-Irukandji"	Lacking
"Darwin carybdeid"	Lacking
"Morbakka" (Port Douglas)	Lacking
Tamoya haplonema	Along vertical sides of stomach wall with many trunks
Chirodropus gorilla	V-shaped with many short, singly attached cirri
Chirodropus sp. A (spotted)	In 10 horizontal rows per corner, arranged vertically along stomach wall
Chironex fleckeri	V-shaped with many short, singly attached cirri
Chiropsalmus quadrumanus	V-shaped with many short, singly attached cirri
Chiropsalmus quadrigatus	Unknown, holotype damaged
Chiropsalmus sp. A (N. Qld)	V-shaped with many short, singly attached cirri
Chiropsalmus sp. B (Gove)	V-shaped with many short, singly attached cirri
Chiropsoides buitendijki	V-shaped with many long, singly attached cirri

Table 2.6. Comparison of rhopalial niche ostium shape among cubozoan species (Section 2.3.9; Plate 2.14).

Species	**Rhopalial niche ostium shape**
Alatina mordens n. sp.	Strongly T-shaped, with broadly convex upper scale
Alatina rainensis n. sp.	Strongly T-shaped, with W-shaped upper scale
Manokia stiasnyi	Y-shaped (with a central flap)
Carybdea alata species complex	Strongly T-shaped
Carybdea rastonii	Heart-shaped
Carybdea marsupialis	Heart-shaped
Carybdea xaymacana	Heart-shaped
Carybdea sivickisi	Vertical keyhole-shaped
Tripedalia binata	Frown-shaped, with very shallow lower scale
Tripedalia cystophora	Frown-shaped, with an indented shallow lower scale
Carukia barnesi	Frown-shaped
Carukia sp. A (Russell’s)	Wide open smile-shaped
Carukia sp. B (Broome)	Frown-shaped
“Broome Irukandji”	Frown-shaped
“Dampier Irukandji”	Frown-shaped
“Halo Irukandji”	Frown-shaped
“Pseudo-Irukandji”	Frown-shaped
“Darwin carybdeid”	Frown-shaped, with well developed upper and lower scales
“Morbakka” (Port Douglas)	Frown-shaped
Tamoya haplonema	Frown-shaped
Chirodropus gorilla	Dome-shaped
Chirodropus sp. A “spotted”	Dome-shaped
Chironex fleckeri	Dome-shaped
Chiropsalmus quadrumanus	Dome-shaped
Chiropsalmus quadrigatus	Dome-shaped
Chiropsalmus sp. A (N. Qld)	Dome-shaped
Chiropsalmus sp. B (Gove)	Dome-shaped, with long flat median flap on upper scale
Chiropsoides buitendijki	Dome-shaped

Table 2.7. Comparison of eyes among cubozoan species (Section 2.3.12; Plate 2.17).

Species	**Number of eyes**
Alatina mordens n. sp.	2 median eyes, lacking lateral eye spots
Alatina rainensis n. sp.	2 median lensed eyes, plus at least 1 pair of lateral spots, possibly 2 pairs
Manokia stiasnyi	2 median lensed, plus 4 lateral
Carybdea alata species complex	Variable: 1-2 median, 0-2-4 lateral
Carybdea rastonii	2 median lensed, plus 4 lateral
Carybdea marsupialis	2 median lensed, plus 4 lateral
Carybdea xaymacana	2 median lensed, plus 4 lateral
Carybdea sivickisi	2 median lensed, plus 2 lateral
Tripedalia binata	Unknown
Tripedalia cystophora	2 median lensed, plus 4 lateral
Carukia barnesi	2 median lensed, plus 4 lateral
Carukia sp. A (Russell's)	Unknown
Carukia sp. B (Broome)	2 median lensed, plus 4 lateral
"Broome Irukandji"	2 median lensed eyes only
"Dampier Irukandji"	Unknown
"Halo Irukandji"	Unknown
"Pseudo-Irukandji"	2 median lensed eyes only
"Darwin carybdeid"	Unknown
"Morbakka" (Port Douglas)	2 median lensed eyes only
Tamoya haplonema	2 median lensed, plus 4 lateral
Chirodropus gorilla	Unknown
Chirodropus sp. A (spotted)	Unknown
Chironex fleckeri	2 median lensed, plus 4 lateral
Chiropsalmus quadrumanus	2 median lensed, plus 4 lateral
Chiropsalmus quadrigatus	Unknown, holotype too old
Chiropsalmus sp. A (N. Qld)	2 median lensed, plus 4 lateral
Chiropsalmus sp. B (Gove)	2 median lensed, plus 4 lateral
Chiropsoides buitendijki	2 median lensed, plus 4 lateral

Table 2.8. Comparison of statolith shape among cubozoan species (Section 2.3.13; Plate 2.18).

Species	**Statolith outline shape**
Alatina mordens n. sp.	Tall truncate teardrop-shaped & garnet red
Alatina rainensis n. sp.	Unknown
Manokia stiasnyi	Unknown
Carybdea alata species complex	Unknown
Carybdea rastonii	Broad kidney-bean shaped, 5 plates
Carybdea marsupialis	Unknown
Carybdea xaymacana (Aus)	Broad kidney-bean shaped, 5 plates, with center crystals
Carybdea sivickisi	Long and narrow, arched sausage shape
Tripedalia binata	V-shaped
Tripedalia cystophora	Unknown
Carukia barnesi	Sub-circular to slightly squarish-circular, without basal concavity
Carukia sp. A (Russell's)	Sub-circular, without basal concavity
Carukia sp. B (Broome)	Unknown
"Broome Irukandji"	Rounded pyramidal, with basal concavity
"Dampier Irukandji"	Unknown
"Halo Irukandji"	Unknown
"Pseudo-Irukandji"	Rounded pyramidal, with apical tooth, with basal concavity
"Darwin carybdeid"	Unknown
"Morbakka" (Port Douglas)	Unknown
Tamoya haplonema	Unknown
Chirodropus gorilla	Unknown
Chirodropus sp. A (spotted)	Unknown
Chironex fleckeri	Short sausage; straight rod with perfectly rounded ends
Chiropsalmus quadrumanus	Unknown
Chiropsalmus quadrigatus	Unknown
Chiropsalmus sp. A (N. Qld)	Unknown
Chiropsalmus sp. B (Gove)	Unknown
Chiropsoides buitendijki	Unknown

Table 2.9. Comparison of velarial canals of cubozoan species (Section 2.3.15; Plate 2.19). See text for explanation of forms. AI = adinterradial, AP = adperradial.

Species	**Number per octant**	**Branching form**
Alatina mordens n. sp.	3	Simple to biforked or triforked only at the tip
Alatina rainensis n. sp.	3	Simple
Manokia stiasnyi	4	Simple, undulating
Carybdea alata species complex	Ca. 3	Typically simple or only slightly branched distally
Carybdea rastonii	2	Dendritic, complex
Carybdea marsupialis	3-4	Dendritic, complex
Carybdea xaymacana	2	AP bifurcated, AI dendritic
Carybdea sivickisi	2	Paw-like
Tripedalia binata	3-4	Simple
Tripedalia cystophora	3	Simple
Carukia barnesi	2	Unbranched triangular
Carukia sp. A (Russell's)	1	Tri-forked
Carukia sp. B (Broome)	1	Twice biforked
"Broome Irukandji"	Ca. 4, from one root	Simple to end-bifurcated
"Dampier Irukandji"	2	AP widely bifurcated, AI narrow and simple; with lateral diverticula
"Halo Irukandji"	4	Complexly branched
"Pseudo-Irukandji"	1	Paw-like
"Darwin carybdeid"	7	With laminar branching, without lateral diverticula
"Morbakka" (Port Douglas)	Too many to count	Feather-like with lateral diverticula
Tamoya haplonema	Numerous, broad	Dendritic and diverticulated
Chirodropus gorilla	Too numerous to count	Feather-like
Chirodropus sp. A (spotted)	Too numerous to count	Long and parallel, without lateral diverticula
Chironex fleckeri	Too numerous to count	Extremely complex
Chiropsalmus quadrumanus	Too numerous to count	Extremely complex
Chiropsalmus quadrigatus	[Not observed]	[Not observed]
Chiropsalmus sp. A (N. Qld)	Too numerous to count	Extremely complex
Chiropsalmus sp. B (Gove)	Too numerous to count	Extremely complex
Chiropsoides buitendijki	Too numerous to count	Extremely complex

Table 2.10. Comparison of perradial lappets among cubozoan species (Sections 2.3.17, 2.3.18; Plate 2.21).

Species	**Perradial lappet form**
Alatina mordens n. sp.	Absent
Alatina rainensis n. sp.	Absent
Manokia stiasnyi	Absent
Carybdea alata species complex	Absent
Carybdea rastonii	Absent
Carybdea marsupialis	Absent
Carybdea xaymacana	Absent
Carybdea sivickisi	Absent
Tripedalia binata	Absent
Tripedalia cystophora	Absent
Carukia barnesi	Narrow, slightly raised, reaching about 4/5 to margin, typically lacking nematocyst warts
Carukia sp. A (Russell's)	Narrowly triangular, reaching velarial margin, without nematocyst warts
Carukia sp. B (Broome)	Narrowly triangular to nearly rectangular, with single large wart on right side
"Broome Irukandji"	Narrow, with 2 rows of 1-4 (typically 2) nematocyst warts, often with terminal and lateral canals
"Dampier Irukandji"	Broadly rounded with large diverticula, with single large wart
"Halo Irukandji"	With finger-like extensions reaching margin; with 2 rows of 3-4 nematocyst warts
"Pseudo-Irukandji"	Present, with 2 rows of 2-3 nematocyst warts
"Darwin carybdeid"	Broadly triangular, with two rows of approximately 5 warts
"Morbakka" (Port Douglas)	Present, thick and very well developed, with 2 rows of large warts plus smaller scattered warts
Tamoya haplonema	Absent
Chirodropus gorilla	Present, narrow but well developed, reaching margin
Chirodropus sp. A (spotted)	Present, broadly triangular, turning into parallel canals
Chironex fleckeri	Present, broadly triangular with lateral and distal canals
Chiropsalmus quadrumanus	Present, quite broad, with scattered nematocyst warts
Chiropsalmus quadrigatus	Unobservable, specimen too damaged
Chiropsalmus sp. A (N. Qld)	Present, broad and well developed
Chiropsalmus sp. B (Gove)	Present, broad with lateral and distal canals
Chiropsoides buitendijki	Present, only reaching about halfway to margin

Table 2.11. Comparison of gastric saccule morphology among cubozoan species (Section 2.3.21; Plate 2.23).

Species	**Gastric saccule shape**
Alatina mordens n. sp.	Lacking
Alatina rainensis n. sp.	Lacking
Carybdea alata species complex	Lacking
Manokia stiasnyi	Lacking
Carybdea rastonii	Lacking
Carybdea marsupialis	Lacking
Carybdea xaymacana	Lacking
Carybdea sivickisi	Lacking
Tripedalia binata	Lacking
Tripedalia cystophora	Lacking
Carukia barnesi	Lacking
Carukia sp. A (Russell's)	Lacking
Carukia sp. B (Broome)	Lacking
"Broome Irukandji"	Lacking
"Dampier Irukandji"	Lacking
"Halo Irukandji"	Lacking
"Pseudo-Irukandji"	Lacking
"Darwin carybdeid"	Lacking
"Morbakka" (Port Douglas)	Lacking
Tamoya haplonema	Lacking
Chirodropus gorilla	Long with many grape-like clusters of diverticula, hollow
Chirodropus sp. A (spotted)	Absent
Chironex fleckeri	Cock's-comb-shaped, solid
Chiropsalmus quadrumanus	Short and finger-shaped, curled, said to be hollow, but solid in life
Chiropsalmus quadrigatus	Too young for determination
Chiropsalmus sp. A (N. Qld)	Knob-like hemispheres, solid
Chiropsalmus sp. B (Gove)	Kidney-bean shape, solid, sessile
Chiropsoides buitendijki	Long and finger-shaped, hollow

Table 2.12. Comparison of mesenteries among cubozoan species (Section 2.3.22; Plate 2.24).

Species	**Form of the mesenteries**
Alatina mordens n. sp.	Short and weakly developed, or completely absent
Alatina rainensis n. sp.	Completely lacking
Manokia stiasnyi	Lacking
Carybdea alata species complex	Typically lacking completely
Carybdea rastonii	Flap-like to edge of stomach, with cord-like extension to mid-body
Carybdea marsupialis	[Not noted]
Carybdea xaymacana	Cord-like to upper quarter of bell, lacking extensions to rhopalium
Carybdea sivickisi (QLD)	Cord-like band of tissue from stomach to rhopalium
Tripedalia binata	Flap-like halfway to rhopalium, without extensions
Tripedalia cystophora	Flaps extending halfway to rhopalium, cord-like to rhopalium
Carukia barnesi	Flap-like halfway to rhopalium, cord-like extending all the way to the rhopalium
Carukia sp. A (Russell’s)	Cord-like to rhopalium; no flaps present
Carukia sp. B (Broome)	Flap-like half way; cord-like to rhopalium
“Broome Irukandji”	Flap-like one-third way to rhopalium; cord-like to rhopalium
“Dampier Irukandji”	Flaps three-fourths to rhopalium; with transparent cord reaching rhopalium
“Halo Irukandji”	Flap-like half way; cord-like to rhopalium
“Pseudo-Irukandji”	Flap-like halfway to rhopalium; transparent band extending to rhopalium
“Darwin carybdeid”	Robust, flap-like halfway to rhopalium, without cord-like extension
“Morbakka” (Port Douglas)	Robust, flap-like extending about halfway to the rhopalium, with fine cord-like extension to the rhopalium
Tamoya haplonema	Robust, flap-like extending about halfway to the rhopalium, cords extending to the rhopalium
Chirodropus gorilla	Robust, with flap-like extensions all the way to the rhopalium
Chirodropus sp. A (spotted)	Robust, flap-like all the way to rhopalium, continuous with frenulum
Chironex fleckeri	Cord-like from stomach to rhopalium
Chiropsalmus quadrumanus	No trace of flaps or cords
Chiropsalmus quadrigatus	Unable to determine from damaged immature holotype
Chiropsalmus sp. A (N. Qld)	Narrow and flap-like in the stomach region, with wide, unraised transparent band extending to rhopalia
Chiropsalmus sp. B (Gove)	Flap-like halfway to rhopalium, with clear flush extension to rhopalium
Chiropsoides buitendijki	Cord-like only from stomach to rhopalium

Table 2.13. Comparison of medusa cnidomes of cubozoan species (Section 2.3.24; Plates 2.25-2.27); polyp cnidomes are not given here but may be found in the literature cited in the text. Where nematocysts were reported previously by other workers and again by me (herein), new reports are indicated by my initials (LG). Terminology follows Williamson et al. (1996). All measurements are in microns (μm), given as length x width.

	Tentacles	**Exumbrellar warts**	**Gastric cirri**	**Manubrium**	**Citations**
Alatina mordens n. sp.	Lemon-shaped euryteles, 19.30-27.25 x 11.10-14.85, n=57	Spherical isorhizas, 28.25-31.71, n=24	Nematocysts not found	Nematocysts not found	Herein
Alatina rainensis n. sp.	1) Lemon-shaped microbasic euryteles, 14.99-19.70 x 10.43-13.11, n=37 2) Sub-spherical isorhizas, 16.42-23.78 x 13.50-18.34, n=42; 6.19-8.24 x 5.58-6.78, n=13 3) Tiny nearly spherical microbasic amastigophores, 6.19-7.16 x 6.02-6.80, n=6	Spherical isorhizas, 9.22-10.47, n=22	Small euryteles, 6.53-8.96 x 4.61-6.55, n=24	Nematocysts not found	Herein
Manokia stiasnyi	Sub-spherical euryteles with a thick capsule wall, 13.42-16.53 x 11.54-13.63, n=12	Not examined	Not examined	Not examined	Herein
Carybdea marsupialis	1) Atrichous isorhiza haplonemes, 8.99-18.05 x 4.29-9.88 2) Heterotrichous microbasic euryteles, 17.02-42.26 x 12.11-23.99 3) Holotrichous isorhizas, 15.11-24.94 x 13.79-22.86	Not reported	Not reported	Not reported	Avian et al. 1997
Carybdea n. sp., South Africa	1) Small oval isorhizas, 13.39-17.62 x 6.06-8.20, n=15 Heterotrichous microbasic euryteles, 19.33-31.12 x 11.98-16.39, n=33	Spherical isorhizas, 15.53-20.64, n=40 Oval ?amastigophores, 15.42-16.30 x 9.54-11.05, n=28	Not examined	Not examined	Herein
Carybdea rastonii	Southcott, Matsumoto: Tumiteles Cleland & Southcott: Microbasic mastigophores (Cl & S: pl 2) LG: 1) Football-shaped microbasic euryteles, 19.58-29.75 x 12.55-18.43, n=34 2) Egg-shaped isorhizas, 8.88-12.85 x 6.64-7.88, n=12	Tumiteles; holotrichous haplonemes LG: Spherical isorhizas, 18.81-21.89, n=44	LG: Sub-spherical euryteles, 10.09-12.48 x 8.41-9.21, n=9	LG: Nematocysts not found	Cleland & Southcott, 1965 (Pl. 2); Southcott, 1967; Matsumoto, 1995; Herein

	Tentacles	**Exumbrellar warts**	**Gastric cirri**	**Manubrium**	**Citations**
Carybdea sivickisi , N. QLD specimen	1) Football-shaped euryteles, 13.43-19.40 x 9.60-12.40, n=21 2) Football-shaped isorhizas with full tubules, 15.82-18.77 x 9.60-11.52, n=16 3) Round euryteles, 10.58-11.94 x 9.60-10.60, n=6 4) Small isorhizas, 9.02-11.41 x 4.38-5.96, n=4	Not examined	Not examined	Not examined	Herein
Carybdea xaymacana	Berger (Caribbean form): Two kinds, larger and smaller, similar to *Tripedalia cystophora* LG (SW Australian form): 1) Large club-shaped microbasic euryteles, 26.27-37.02 x 11.67-15.40, n=43 2) Small oval isorhizas, 9.17-11.93 x 5.41-6.95, n=8 (Caribbean form): club-shaped microbasic euryteles, 28.73-32.67 x 12.20-15.31, n=17	LG, SW Australian form: Spherical isorhizas, 12.48-15.96, n=23	Nematocysts not found	Nematocysts not found	Berger, 1900; Herein
Tripedalia cystophora Puerto Rico specimen	Werner: 1) Holotrichous haplonemes, 9-14 x 6-7, 12-15 x 11-14 2) Heterotrichous microbasic euryteles, 16-23 x 12-16 LG: 1) Small oval isorhizas, 10.86-12.29 x 5.65 x 6.24, n=6 2) Heterotrichous microbasic euryteles, 14.79-18.07 x 9.35-11.29, n=61	Holotrichous haplonemes, 11-12 x 6-7, 14-15 x 12-14 LG: Not examined	Heterotrichous microbasic euryteles, 7-9 x 6-7 LG: Heterotrichous microbasic euryteles, 5.44-6.57 x 3.88-4.97, n=14	Heterotrichous microbasic euryteles, 9-11 x 7-9 LG: Nematocysts no found	Werner, 1975; Herein
Tripedalia binata Non-type specimen, Darwin, NT	1) Spherical atrichous isorhizas, 12 2) Stenoteles, 18-20 x 10-15 10:1 ratio of stenoteles to isorhizas LG: 1) Large heterotrichous microbasic euryteles, 20.13-24.61 x 13.04-15.35, n=25 2) Small heterotrichous microbasic euryteles, 14.66-16.55 x 9.94-11.31, n=12 3) Small oval isorhizas, 9.72-11.68 x 5.84-6.86, n=14	Spherical atrichous isorhizas, 12 LG: Spherical isorhizas, 11.67-15.99, n=37	Not reported; LG: Heterotrichous microbasic euryteles, width 6.08-6.66, n=7	Not reported LG: Heterotrichous microbasic euryteles, width 5.58-9.42, n=21	Moore, 1988; Herein
Carukia barnesi Paratype	Southcott: Egg-shaped tumiteles, 25-26 x 15-18 Hartwick: Microbasic p-mastigophores, 25-45 long LG: Egg-shaped tumiteles, 22.88-26.93 x 14.59-16.46, n=8	Southcott: Spherical anisorhizas, 18-21 LG: Spherical isorhizas, 17.30-19.15, n=21	N/A	Southcott: Not reported LG: Not found	Southcott, 1967; Hartwick (ID poster); Herein
Carukia n. sp. A (Russell's)	Lemon-shaped tumiteles, with distal-facing spines only at distal end, 17.70-24.76 x 12.75-14.70, n=13	Not examined	N/A	Not examined	Herein

	Tentacles	**Exumbrellar warts**	**Gastric cirri**	**Manubrium**	**Citations**
Carukia n. sp. B (Broome)	Lemon-shaped tumiteles: 18.73-27.78 x 13.21-18.44, n=17	Spherical isorhizas, 17.56-24.27, n=27	N/A	Not examined	Herein
"Broome Irukandji"	Club-shaped sub-ovate microbasic p-mastigophores (Type 4); spines full length: 34.55-49.32 x 14.59-19.65, n=58	Spherical isorhizas, 23.59-29.82, n=49	N/A	Not examined	Herein
"Dampier Irukandji"	Rice-shaped sub-ovate microbasic p-mastigophores: 31.67-40.47 x 14.01-16.50, n=19	Not examined	N/A	Not examined	Herein
"Halo Irukandji"	Club-shaped sub-ovate microbasic p-mastigophores, 32.98-37.56 x 11.65-16.36, n=30	Not examined	N/A	Not examined	Herein
"Pseudo-Irukandji"	Club-shaped sub-ovate microbasic p-mastigophores (Type 4); spines terminal: 30.27-36.68 x 13.02-16.04, n=44	Spherical isorhizas, 20.10-24.87, n=45	N/A	Not examined	Herein
"Darwin carybdeid" Holotype	O'Reilly: Lemon-shaped LG: Club-shaped sub-ovate microbasic p-mastigophores (Type 4); spines full length: 43.32-59.39 x 14.62-17.25, n=27	O'Reilly: Spherical LG: Spherical isorhizas, 21.15-24.77, n=21	N/A	Not examined	O'Reilly et al., 2001; Herein
"Morbakka"	Cleland & Southcott: Holotrichous isorhizas, un-id haplonemes. Hartwick: Microbasic p-mastigophores, 45-75; 2 types of football shaped isorhizas, 45 LG: 1) Club-shaped microbasic p-mastigophores, 60.99-69.97 x 13.72-18.62, n=28 2) Oval isorhizas of two types, one with loose tubule, one with tight tubule, 49.07-56.61 x 28.31-34.02, n=8	Hartwick: Sub-spherical isorhizas of two types, similar to those on tentacles; LG: 1) Spherical isorhizas, 27.41-30.41, n=28 2) Oval, poorly defined, with papillated outer surface and loosely wound tubule, as wide as type 1, but 1.5x as long	N/A	Not examined	Cleland & Southcott, 1965 (Pl. 2); Hartwick (ID poster); Herein, from Port Douglas, QLD
Tamoya haplonema (South Carolina spec.)	1) Club-shaped, discharged, 56.42-59.47 x 15.67-17.38, n=6 2) Sub-spherical, discharged, 29.85-36.19 x 19.73-28.44, n=2	Not examined	Nematocysts not found	Spherical isorhizas, 23.85-28.80, n=48	Herein

	Tentacles	**Exumbrellar warts**	**Gastric cirri**	**Manubrium**	**Citations**
Chirodropus gorilla (specimen from Ghana)	Similar to *Chironex* but smaller; size difference of nematocysts may be due to specimen size	N/A	Not reported	Not reported	Kingston & Southcott, 1960 [Note: dubious ID]
Chirodropus n. sp. A (spotted)	Banana-form microbasic mastigophores, 42.68-61.39 x 6.18-12.30, n=27 2) Tiny spherical isorhizas, ca. 12x10, n=1	N/A	Not examined	Not examined	Herein
Chiropsalmus quadrumanus	Marques: 1) Microbasic mastigophores, 55.0-187.2 x 11.0-42.0, n=2 2) Ellipsoid isorhizas, 10.8-14.4 x 3.6-5.4, n=14 3) Ovoid isorhizas, 6.0-8.4 x 3.0-3.6, n=5 4) Medium microbasic euryteles, 16.2 x 12.0, n=1 5) Small microbasic euryteles, 7.2 x 6.0, n=1	Marques: Ovoid isorhizas, 5.4-7.8 x 3.0-5.4, n=12	Marques: Microbasic euryteles: Large, 21.0-22.8 x 13.2, n=2; medium, 13.0-16.0 x 8.9-14.4, n=20; and small, 7.8-10.2 x 4.8-7.2, n=20	Marques: Medium microbasic euryteles, 12.6-17.4 x 10.8-11.0, n=13	Calder & Peters (1975); Marques et al. (1997)
Chiropsalmus n. sp. A (N. QLD)	Kinsey: Identical to *Chironex*, though uniformly smaller Carrette: Same types as *Chironex*, except lacking large class mastigophores; mastigophores 35-55 long; Fewer mastigophores and more isorhizas than *Chironex* LG: 1) Banana-form microbasic mastigophore, 38.90-45.98 x 9.39-10.26, n=6 2) Large football-shaped isorhizas, 20.77-24.07 x 11.76-13.77, n=7 3) Small football-shaped isorhizas, 8.61-9.70 x 6.77-7.89, n=14 4) Small football-shaped euryteles, 9.20-10.09 x 7.93-8.68, n=3 5) Small rod-shaped isorhizas, 10.97-13.08 x 3.64-4.64, n=16	N/A	Not examined	Not examined	Kinsey, 1986; Carrette et al., 2002; Hartwick (ID poster); Herein
Chiropsalmus n. sp. B (Gove)	Currie et al.: Baseball bat-shaped microbasic mastigophores similar to *Chironex*; trirhopaloids LG: 1) Banana-form microbasic mastigophores, 39.28-44.84 x 8.79-10.92, n=12 2) Large football-shaped mastigophores, 21.27-26.29 x 12.73-14.37, n=10 3) Small football-shaped isorhizas, 9.04-9.94 x 6.88-7.71, n=5 4) Small rod-shaped isorhizas, 13.21-14.20 x 6.54-7.27, n=3	N/A	Not examined	Not examined	Currie et al., 2002; Herein

	Tentacles	**Exumbrellar warts**	**Gastric cirri**	**Manubrium**	**Citations**
Chiropsoides buitendijki, Sri Lankan specimen	1) Banana-form microbasic mastigophores, 60.79-78.92 x 9.51-11.78, n=11 2) Small football-shaped isorhizas with a beehive-form tubule, 9.26-11.26 x 7.39-8.30, n=6 3) Small rod-shaped isorhizas, 14.34-15.25 x 3.76-4.22, n=3 4) Small spherical isorhizas, 6.97-9.71, n=11 5) Very small spherical isorhizas, 3.53-4.21, n=8	N/A	Not examined	Not examined	Herein
Chironex fleckeri	Cleland & Southcott: Elongate microbasic mastigophores, ca 50 long. Rifkin & Endean: Microbasic p-mastigophores 22-90 long, large and small heterotrichous microbasic euryteles, holotrichous and atrichous isorhizas. Hartwick: Cigar-shaped microbasic p-mastigophores 50-95 long; straight-sided isorhizas 12 long; sub-spherical euryteles 11; football-shaped euryteles 20-35 long. Williamson et al.: At least 6 types, including 2 size classes of mastigophores Carrette: Atrichous and holotrichous isorhizas, large and small trirhopaloids, and two sizes of microbasic mastigophores. LG (6 cm BH): 1) Type 3 banana-form microbasic mastigophores, 64.90-77.96 x 9.58-12.08, n=25 2) Football-shaped trirhopaloids, 31.50-38.77 x 14.53-19.03, n=11 3) Sub-spherical trirhopaloids, 11.14-17.31 x 8.64-13.26, n=19 4) Straight-sided isorhizas, 12.00-19.55 x 4.47-6.55, n=55 5) Ovoid isorhizas with spiraled tubule, 13.19-16.94 x 8.08-9.94, n=4	N/A	Not examined	Not examined	Cleland & Southcott, 1965 (Pl. 2, 3); Rifkin & Endean 1983 (excellent description of types); Hartwick (ID poster); Williamson et al., 1996 (p. 156); Carrette et al., 2002; Herein

Table 2.14a. Comparison of main diagnostic characters in the Carybdeida. These characters, if assessed correctly, will give accurate diagnosis at the familial and generic levels in the revised classification, but will only give partial accuracy at the species level; it would not necessarily be possible to discern new species based on these few characters. See text for explanation of characters and character states.

Species	**Phacellae**	**Rhopalial niche ostium**	**Pedalial canal bend**	**Velarial canals (per octant)**	**Principal tentacular nematocysts**	**Other distinguishing features**
Alatina mordens gen. et sp. nov.	Crescentic, long cirri on several roots	T-shaped	Rounded or angular	3, simple	Lemon-shaped euryteles	Tall, narrow body of thin gelatinous texture; black eyes; gonads attached full BH
Alatina rainensis gen. et sp. nov.	Crescentic, long cirri singly rooted or paired	T-shaped	90° angle	3, simple	Lemon-shaped euryteles, large and small sub-spherical isorhizas, tiny spherical amastigophores	Small body with butterfly-wing-shaped gonads attached in the middle only
Carybdea alata species complex	Crescentic, with long cirri	T-shaped	Simple	Ca. 3, typically simple or only slightly branched distally	Euryteles, isorhizas	Tall, narrow, flimsy body, very wide pedalial wings; lacking perradial mesenteries
Manokia stiasnyi	Crescentic	T-shaped	Small nub	4, more or less simple; undulating	Sub-spherical euryteles	Tentacle bands with lateral extensions; with scalpel-shaped pedalia
Carybdea rastonii	Multiple stalked oblique row	Heart-shaped	Knee	2, dendritic	Football-shaped euryteles and small ovoid isorhizas	Single row of nematocyst freckles on outer pedalial wing; pedalia scalpel-shaped
Carybdea marsupialis	Multiple-stalked corner bunches	Heart-shaped	Knee	3-4, dendritic	Microbasic euryteles, and two types of isorhizas	Single row of nematocyst freckles on outer pedalial wing; pedalia scalpel-shaped
Carybdea xaymacana	Single-stalked corner bunch	Heart-shaped	Knee	2, of two types	Large club-shaped euryteles, small ovoid isorhizas	Single row of nematocyst freckles on outer pedalial wing; pedalia scalpel-shaped
Tripedalia binata	Single-stalked corner bunch	Shallow frown-shaped	Simple	3-4, simple	Euryteles, stenoteles and spherical & non-spherical isorhizas	Two pedalia per corner; with weak mesenteries; pedalia narrow
Tripedalia cystophora	Single-stalked corner bunch	Shallow frown-shaped	With small nub	3, simple	Euryteles, stenoteles and spherical & non-spherical isorhizas	Three pedalia per corner; with weak mesenteries; pedalia narrow

Species	**Phacellae**	**Rhopalial niche ostium**	**Pedalial canal bend**	**Velarial canals (per octant)**	**Principal tentacular nematocysts**	**Other distinguishing features**
Carybdea sivickisi	Crescentic, cirri singly rooted	Vertical keyhole	Simple	2, paw-like	Football-shaped & round euryteles, large and small ovoid isorhizas	Adhesive pads on exumbrella apex; single row of nematocyst bars on outer pedalial keel; narrow pedalia
Carukia barnesi	Lacking	Frown-shaped	Simple	2, simple; adperradial with one wart	Lemon-shaped tumiteles	Neckerchief-like tentacle bands; with rhopalial "horns"; narrow pedalia
Carukia sp. A (Russell's)	Lacking	Open smile	Knee-like	1, triforked	Lemon-shaped tumiteles	Neckerchief-like tentacle bands
Carukia sp. B (Broome)	Lacking	Frown-shaped	Knee-like	1, twice biforked, with one large wart	Lemon-shaped tumiteles	Neckerchief-like tentacle bands; long, narrow capitate horns
"Broome Irukandji"	Lacking	Frown-shaped	Oblique, blunt projection	Paw-like with 4 simple to end-biforked fingers	Club-shaped Type 4 microbasic p-mastigophores	With short, broad rhopalial "horns", with one-third mesenteries; large robust body, fine tentacles
"Dampier Irukandji"	Lacking	Frown-shaped	(obscured)	2, of different forms, diverticulated	Rice-shaped microbasic p-mastigophores	Halo-like tentacle bands; short wide horns; broad round lappets
"Halo Irukandji"	Lacking	Frown-shaped	Simple	Paw-like into 4 dendritic fingers	Club-shaped Type 4 microbasic p-mastigophores	Halo-like tentacle bands, with rhopalial "horns"
"Pseudo-Irukandji"	Lacking	Frown-shaped	With small nub	Paw-like into 3-4 fingers	Club-shaped Type 4 microbasic p-mastigophores	With rhopalial "horns", with strong mesenteries
"Darwin carybdeid"	Lacking	Frown-shaped	Prominent thorn	About 7, with laminar branching, lacking lateral diverticula	Club-shaped Type 4 microbasic p-mastigophores	With crescentic rhopalial "horns"; pedalia broadly rounded; robust mesenteries; stout body, heavy tentacles
"Morbakka" (Port Douglas)	Lacking	Frown-shaped	Prominent thorn	Feather-like, too many to count, with lateral diverticula	Club-shaped Type 4 microbasic p-mastigophores; football-shaped isorhizas	2 eyes per rhopalium; with perradial lappets and rhopalial "horns"; mesenteries well developed; pedalia narrow
Tamoya haplonema	Vertical	Frown-shaped	Prominent thorn	Numerous, broad, dendritically branched, diverticulated	Two types, club-shaped and sub-spherical (further ID could not be made)	6 eyes per rhopalium; lacking perradial lappets and rhopalial "horns"; with stout mesenteries

Table 2.14b. Comparison of main diagnostic characters in the Chirodropida. These characters, if assessed correctly, will give accurate diagnosis to the species level, but will not necessarily discern new species. Nematocysts are not included because they are largely homogeneous among chirodropid species. See text for explanation of characters and character states.

Species	**Saccule shape**	**Saccule consistency**	**Pedalial canal bend**	**Pedalial branching**	**Tentacles**	**Other features**
Chirodropus gorilla	Feather-like, with filaments	Solid?, pendulous	Thorn-like diverticulum	Doubly uniserial, alternate	Wide, flat tentacles	
Chirodropus sp. A	Absent	N/A	Thorn-like diverticulum	Central "palm" reduced	Flat, heavy	Spotted exumbrella; subumbrella w/ conspicuous muscle bands; gonads filamentous; phacellae in vertical patch of horizontal rows
Chironex fleckeri	Cock's combs to more complex in grape-like clusters	Solid, pendulous	Thorn-like diverticulum	Doubly uniserial, alternate	Wide, flat tentacles	Lethal to humans
Chiropsalmus quadrumanus	Short and finger-like	Hollow or solid, pendulous	Volcano-like diverticulum	Biserially opposite	Fine, round tentacles	Lethal to humans
Chiropsalmus quadrigatus	Unknown	Unknown	Thorn-like diverticulum	Unilaterally branched on the axis	Wide, flat tentacles	Holotype is a juvenile; Unrecognizable
Chiropsalmus sp. A (N. QLD)	Hemispherical	Solid, knob-like, sessile	Knee-like, lacking diverticulum	Doubly uniserial, opposite	Fine, round tentacles	Not lethal to humans
Chiropsalmus sp. B (Gove)	Kidney-bean	Solid, knob-like, sessile	Volcano-like diverticulum	Doubly uniserial, opposite, terminal	Fine, round tentacles	Common during dry season
Chiropsoides buitendijki	Long and finger-like	Hollow, pendulous	Thorn-like diverticulum	Unilaterally branched abaxially	Wide, flat tentacles	

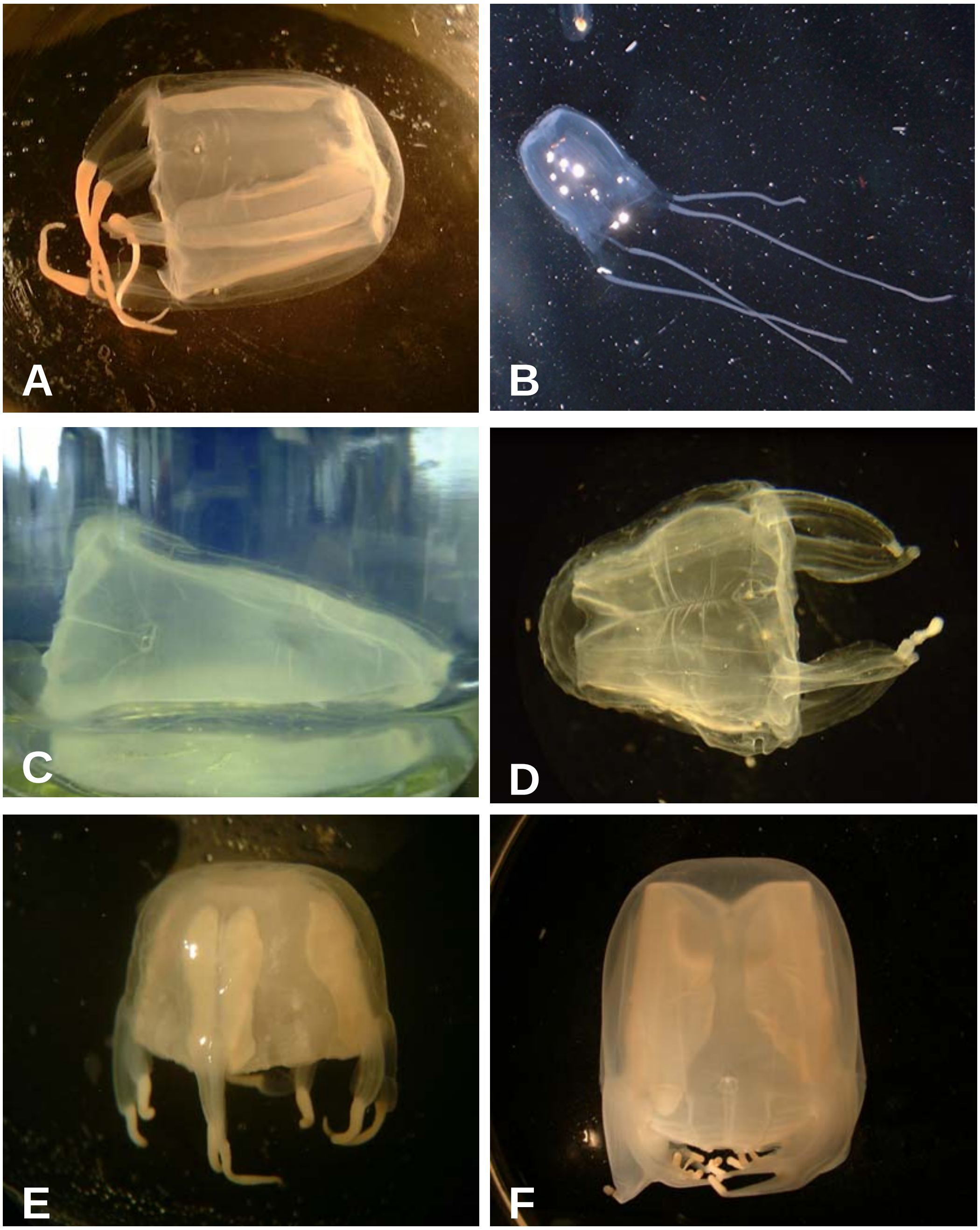

Plate 2.1. Bell shape and apical morphology (Sections 2.3.1, 2.3.2; Table 2.1). A. Cylindrical, with dome-shaped apex (*Carybdea xaymacana*, Puerto Rico, Neotype, USNM coll'n.). B. Cylindrical, with flat apex (Broome Irukandji, copyright M. Alexander). C. Tall and pyramidal (*Alatina mordens* gen. et sp. nov., see Chapter 5). D. Short and pyramidal, with gelatinous apex (*Carukia barnesi*, Cairns). E. Short and wide, with apical depression (*Tripedalia binata*, Paratype, NHM coll'n.). F. Nearly perfectly cuboid (*Chiropsalmus* n. sp. B, Holotype, Gove, N.T.).

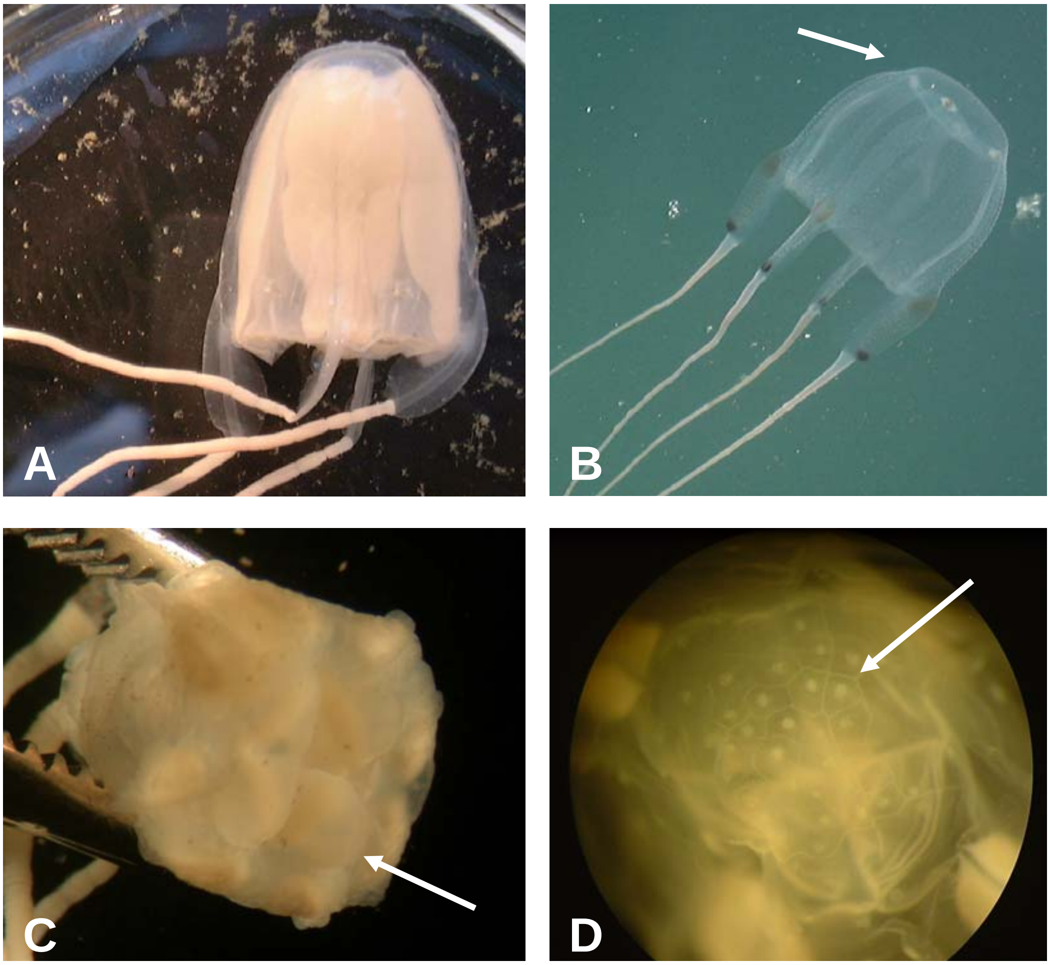

Plate 2.2. Apical decorations (Section 2.3.2). A. Lacking decorations (Pseudo-Irukandji, Port Douglas, Paratype, SAM coll'n.). B. Sub-apical coronal furrow (*Carybdea* n. sp., Cape Town). C. Exumbrellar adhesive pads (*Carybdea sivickisi*, Townsville, MTQ colln.). D. Apical reticulations (*Carukia barnesi,* Barnes coll'n.).

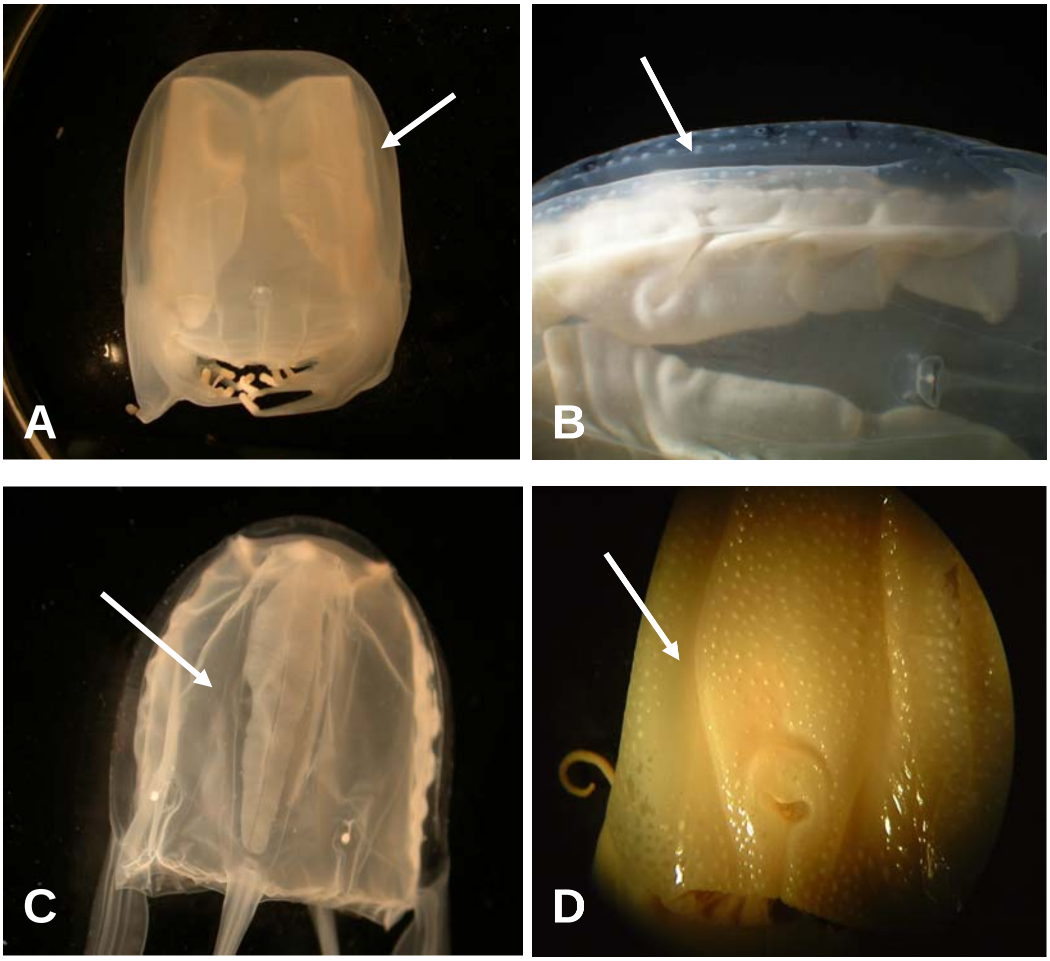

Plate 2.3. Exumbrellar furrows (Section 2.3.2). A. Interradial furrow shallow (*Chiropsalmus* n. sp. B, Holotype, Gove). B. Interradial furrow deep (Darwin carybdeid, Paratype, NTM coll'n, copyright P. Alderslade). C. Adradial furrows shallow (*Carybdea xaymacana*, Busselton, WA). D. Adradial furrows deep, defining pillars and "smile lines" (*Carybdea* n. sp., Cape Town, SAM coll'n).

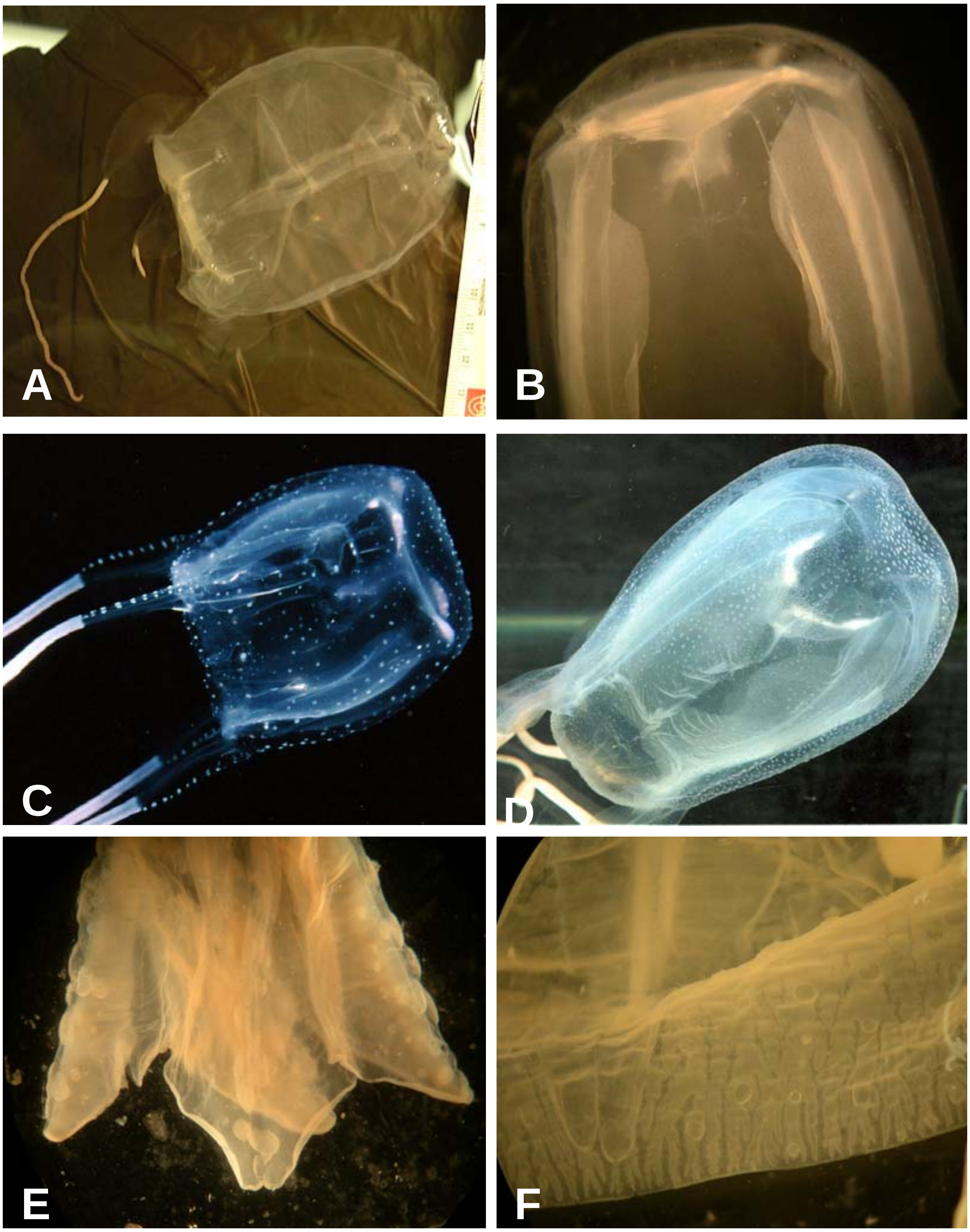

Plate 2.4. Nematocyst warts and freckles (Section 2.3.3; Table 2.2). A. Bell lacking warts or freckles (*Alatina mordens* gen. et sp. nov., see Chapter 5). B. Bell with flush nematocyst freckles (*Carybdea xaymacana*, Puerto Rico, USNM coll'n). C. Bell with scattered warts (*Carybdea rastonii*, copyright K. Gowlett-Holmes). D. Bell very warty (*Tamoya haplonema*, Neotype, copyright A. Migotto).E. Mouth warty (*Tamoya* n. sp., South Carolina, QM coll'n). F. Velarial canals with warts (Morbakka, Holotype, Port Douglas).

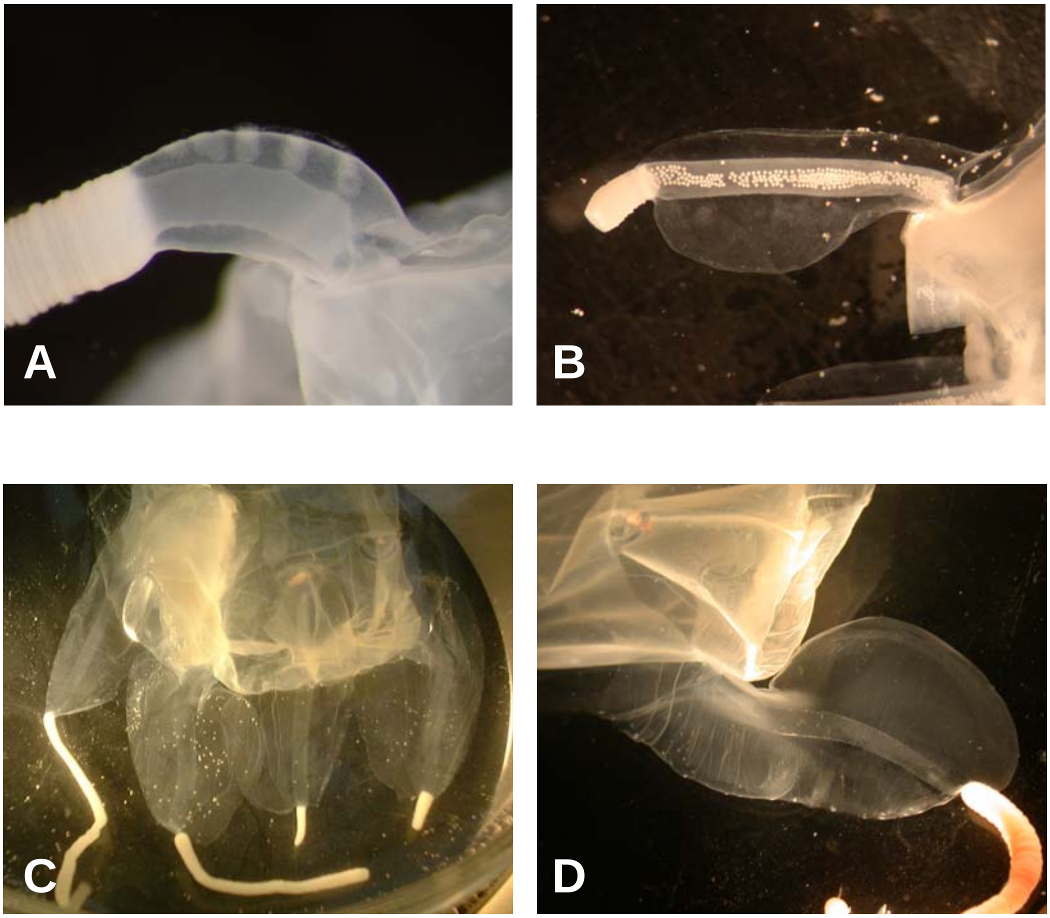

Plate 2.5. Carybdeid pedalia inner wing shape (Section 2.3.4). A. Narrow (*Carybdea sivickisi*, Townsville). B. Scalpel-shaped (*Pseudo-Irukandji*, Townsville). C. Hemispherically rounded (*Alatina* sp. indet., NHM coll'n.). D. Inner wing overhanging tentacle insertion (*Alatina mordens* gen. et sp. nov., see Chapter 5).

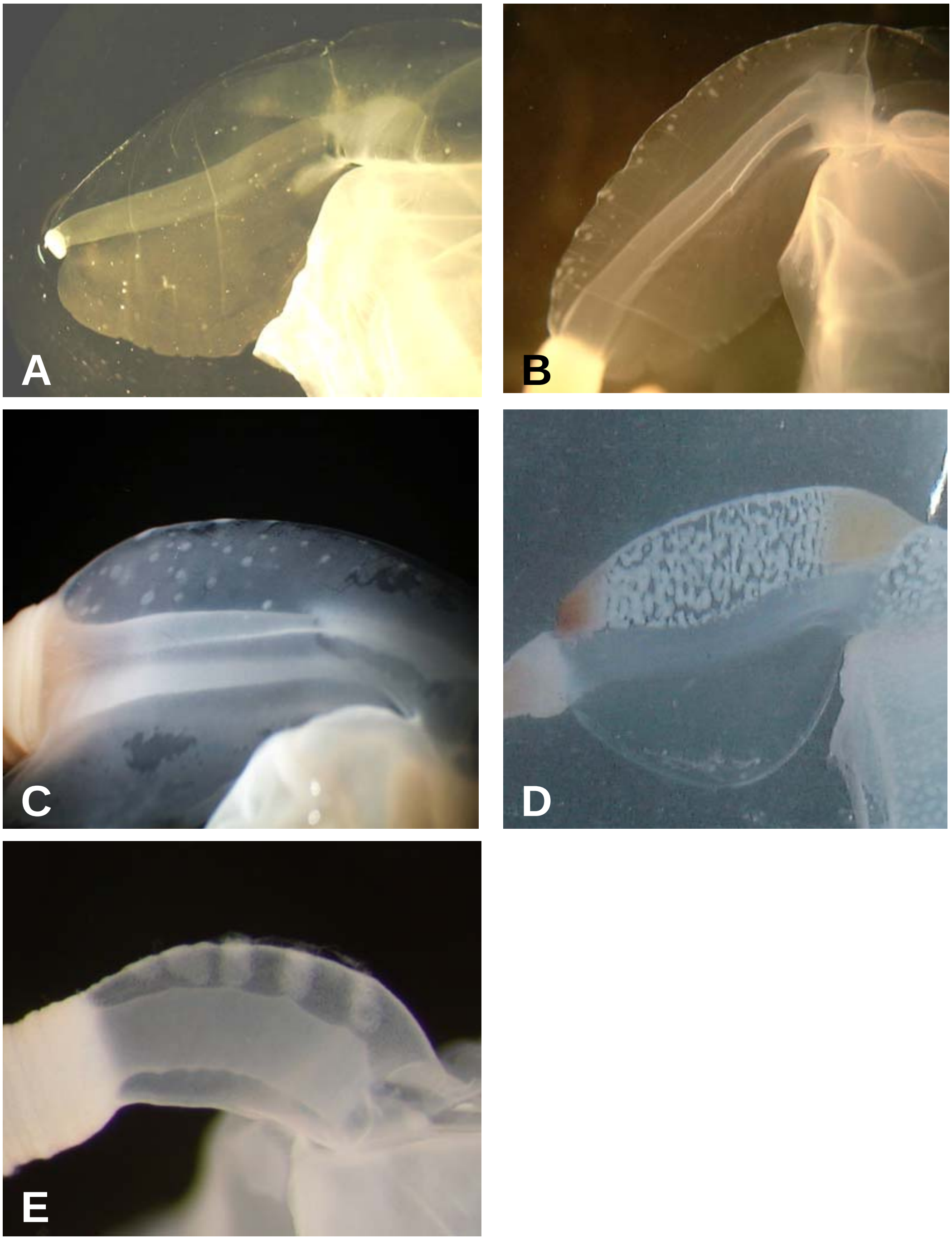

Plate 2.6. Pedalial nematocyst patterns (Section 2.3.4). A. Lacking nematocysts (*Alatina mordens* gen. et sp. nov., see Chapter 5). B. 1 row of nematocyst warts or freckles on outer keel (*Carybdea xaymacana*, Puerto Rico, USNM coll'n). C. Scattered nematocysts on outer keel (Darwin carybdeid, Paratype, NTM coll'n, copyright P. Alderslade). D. Scattered nematocysts on outer keel (*Carybdea* n. sp., Cape Town). E. Row of nematocyst bars on outer keel (*Carybdea sivickisi*, Townsville, MTQ coll'n).

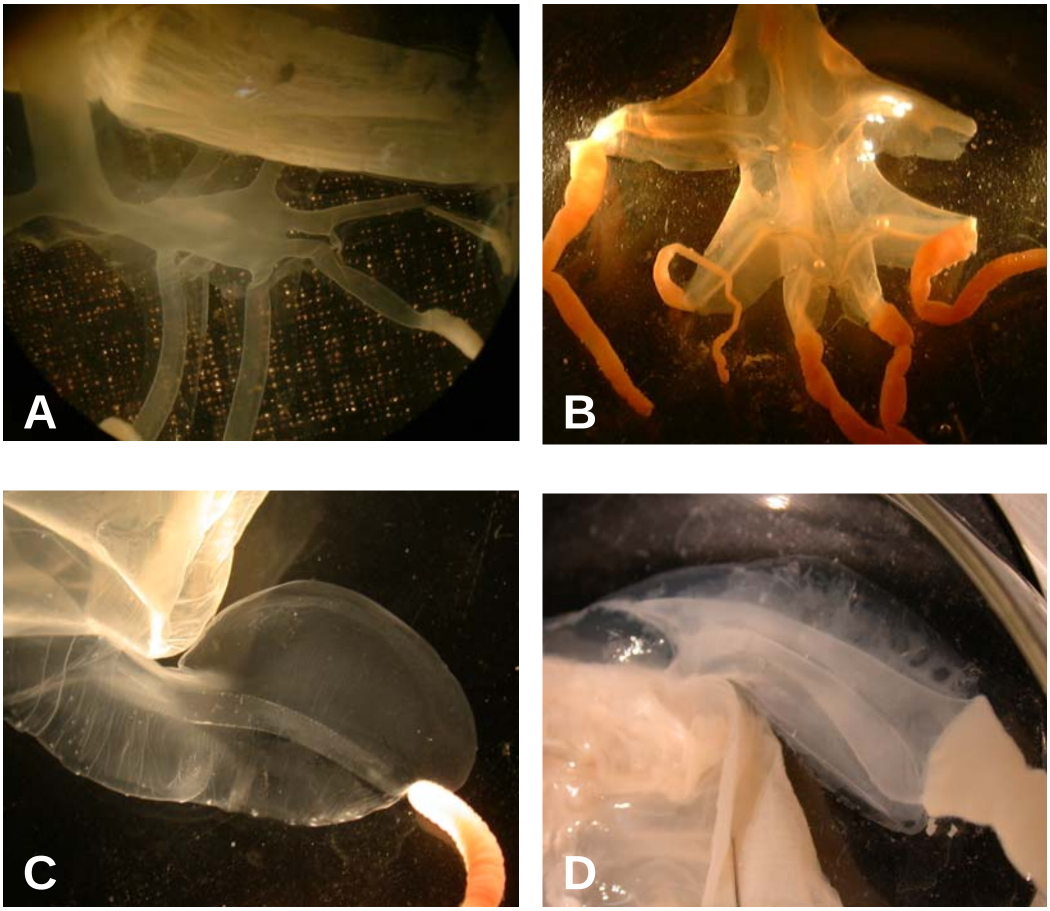

Plate 2.7. Pedalial canal characters (Section 2.3.5; Table 2.3). A. Bilateral branching canal undivided (*Chiropsalmus quadrumanus,* Univ. Sao Paulo coll'n). B. Bilateral branching canal divided (*Chironex* n. sp., Philippines, ZMUC coll'n.). C. Flat in cross section, straight at tentacle insertion (*Alatina mordens* gen. et sp. nov., see Chapter 5). D. Quadratic in cross section, flared at tentacle insertion (Morbakka, Holotype, Port Douglas).

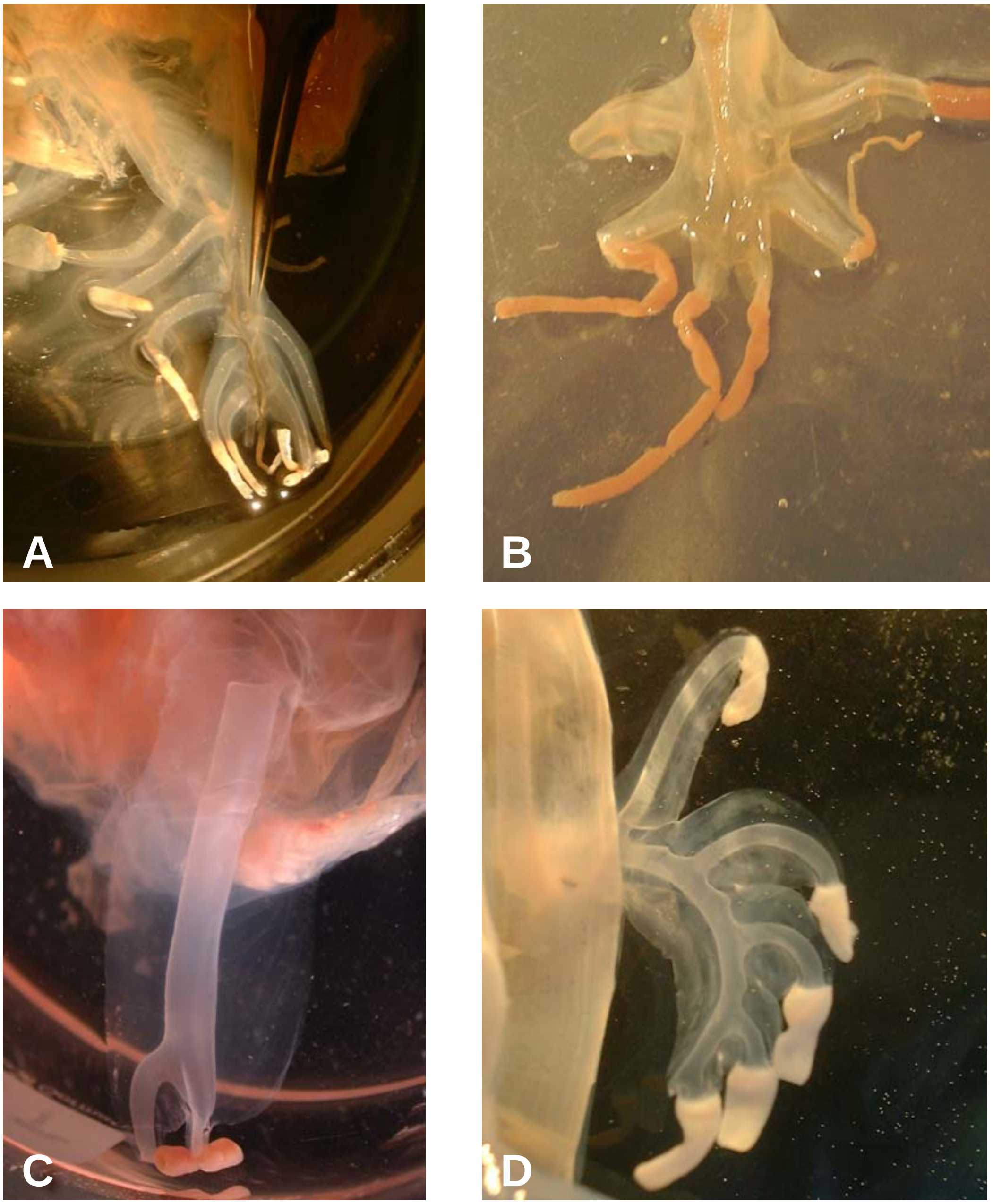

Plate 2.8. Chirodropid pedalia branching patterns (Section 2.3.5; Table 2.3).
A. Branching alternate (*Chiropsalmus quadrumanus*, Brazil, ZMUC coll'n.).
B. Branching opposite (*Chironex* n. sp., Philippines, ZMUC coll'n.).
C. Branching at end (*Chiropsalmus* n. sp., Mexico, Scripps coll'n.).
D. Unilateral branching (*Chiropsoides buitendijki*, Holotype, Leiden coll'n.).

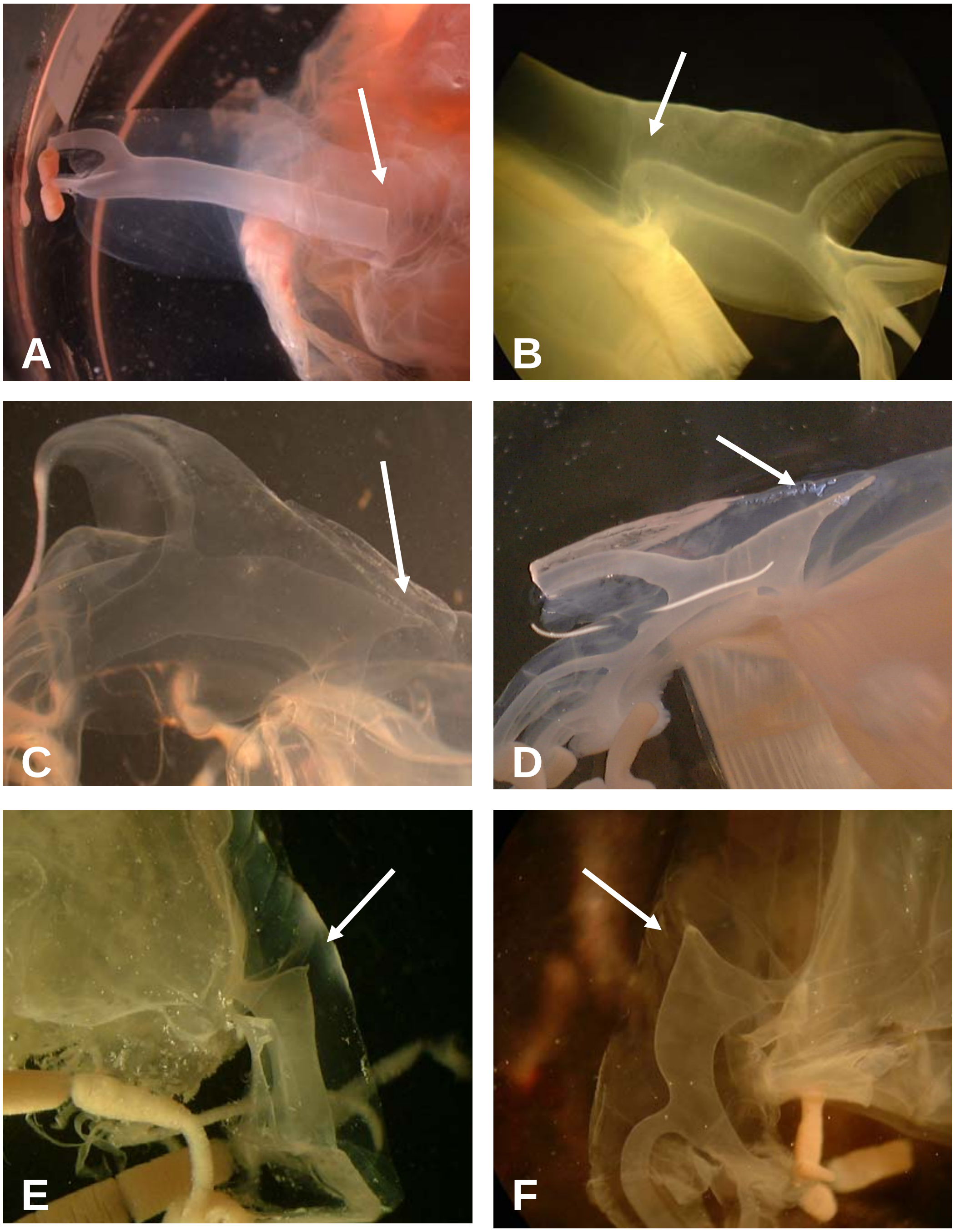

Plate 2.9. Pedalial canal bend forms (Section 2.3.5; Table 2.3). A. 90º bend (*Chiropsalmus* n. sp., Mexico, Scripps coll'n.). B. Smoothly rounded knee-like bend (*Chiropsalmus* n. sp. A, N. QLD). C. Sharp upward spike (*Chironex fleckeri*, Cairns). D. Very narrow long spike (*Chiropsoides buitendijki,* Holotype, Leiden coll'n.). E. Short lateral spike (*Carybdea* n. sp., Cape Town, SAM coll'n). F. Prominent volcano (*Chironex* n. sp., Philippines, USNM coll'n).

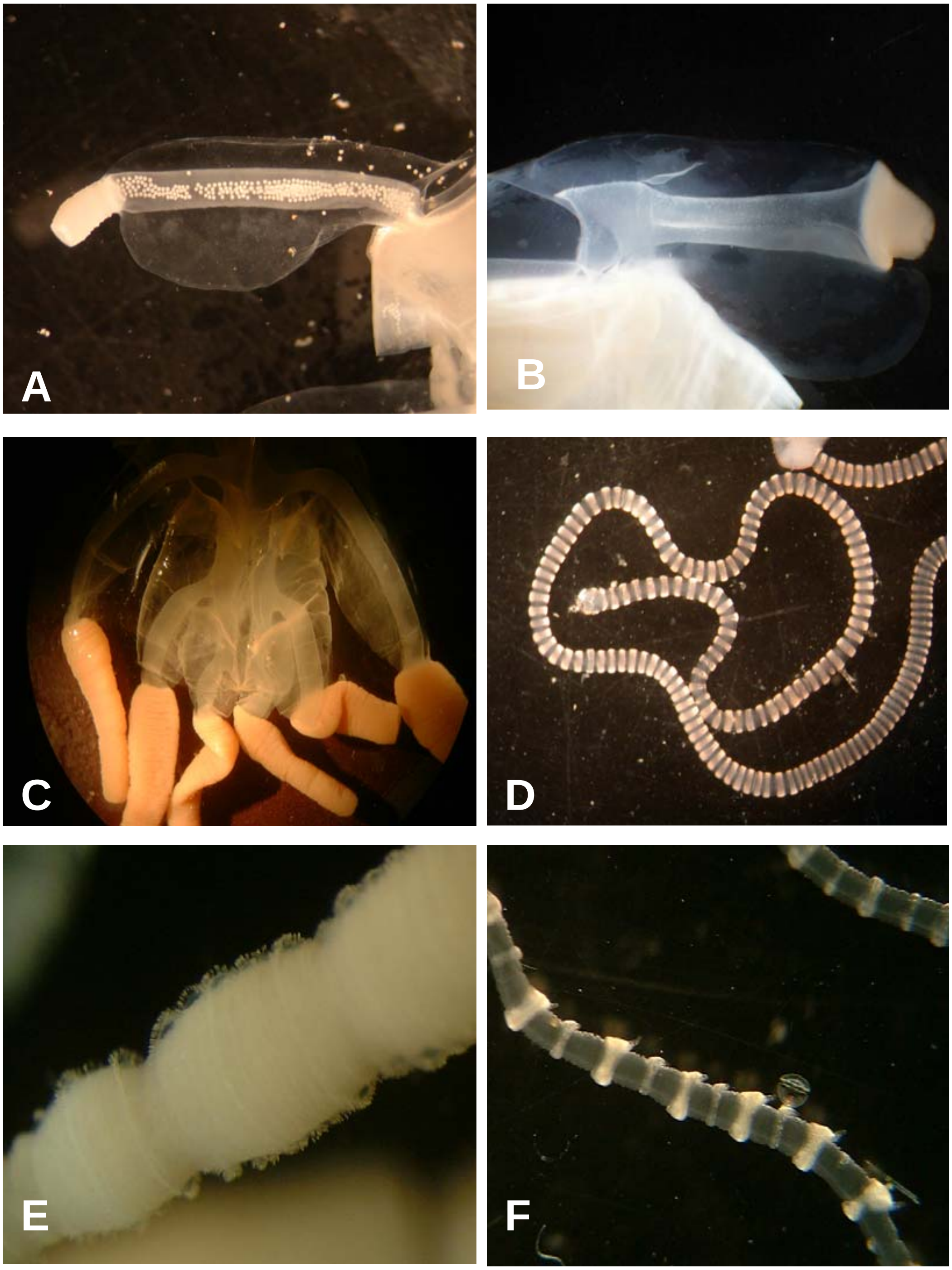

Plate 2.10. Tentacle characters (Section 2.3.6; Table 2.4). A. Tentacle base straight (Pseudo-Irukandji, Townsville). B. Tentacle base flared greatly (Darwin carybdeid, Paratype, NTM coll’n, copyright P. Alderslade). C. Tentacle base tapered (*Chironex* n. sp., Philippines, USNM coll’n.). D. “Normal” banding (Pseudo-Irukandji, Port Douglas). E. “Halo” banding (Halo-Irukandji, Holotype, Port Douglas, QM coll’n.). F. “Handkerchief” tailed banding (*Carukia barnesi*, Cairns).

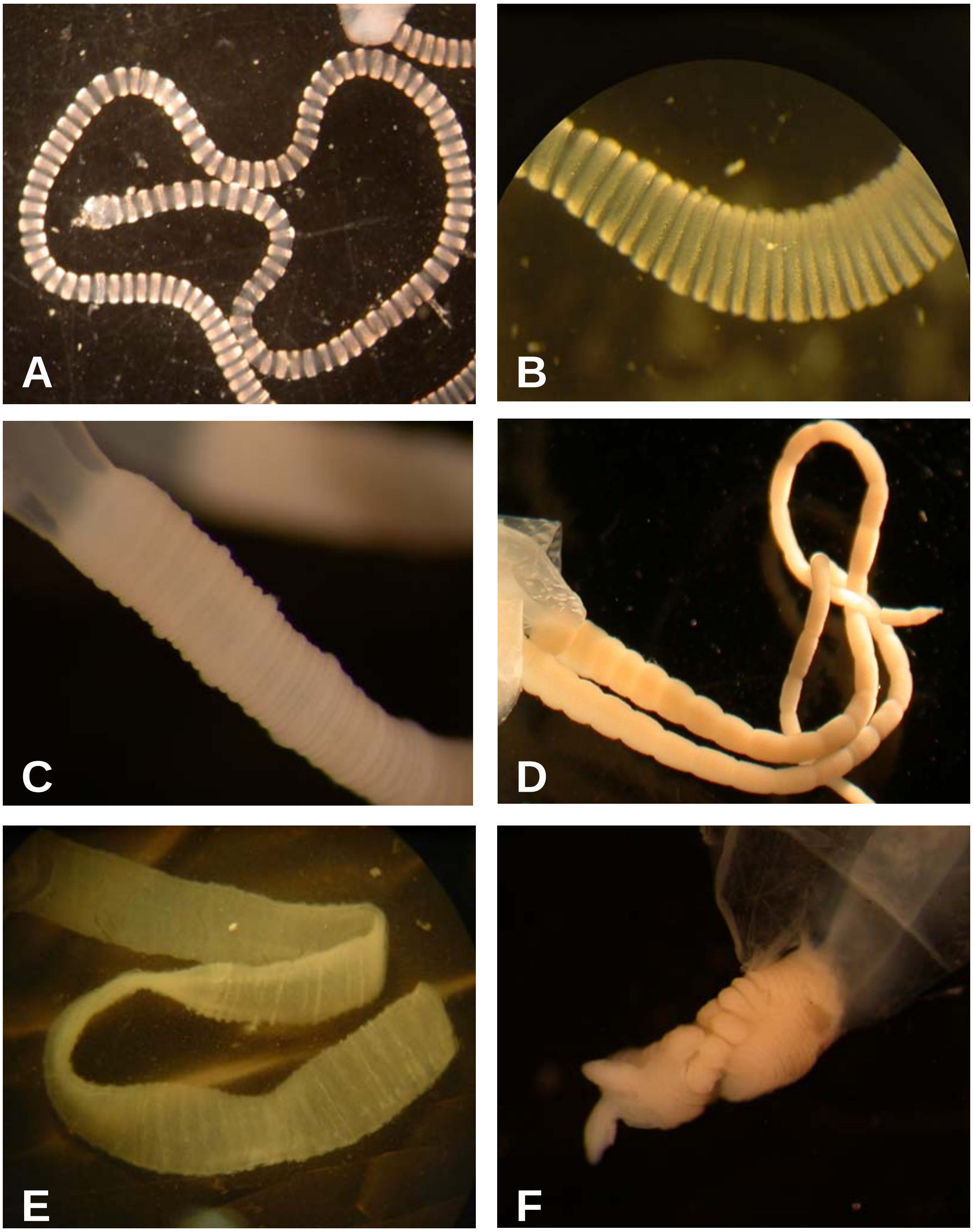

Plate 2.11. Tentacle banding characters (Section 2.3.6; Table 2.4). A. 1-1-1-1 (Pseudo-Irukandji, Port Douglas). B. 1-2-1-2 (Darwin carybdeid, N.T.). C. Complex banding pattern, cross section round (*Carybdea sivickisi*, Townsville). D. Segmented appearance (*Carybdea* sp., Japan). E. Flat and ribbon-like (Morbakka, Mackay). F. Tentacle "branched" (*Manokia stiasnyi*, Holotype, IRSNB coll'n.).

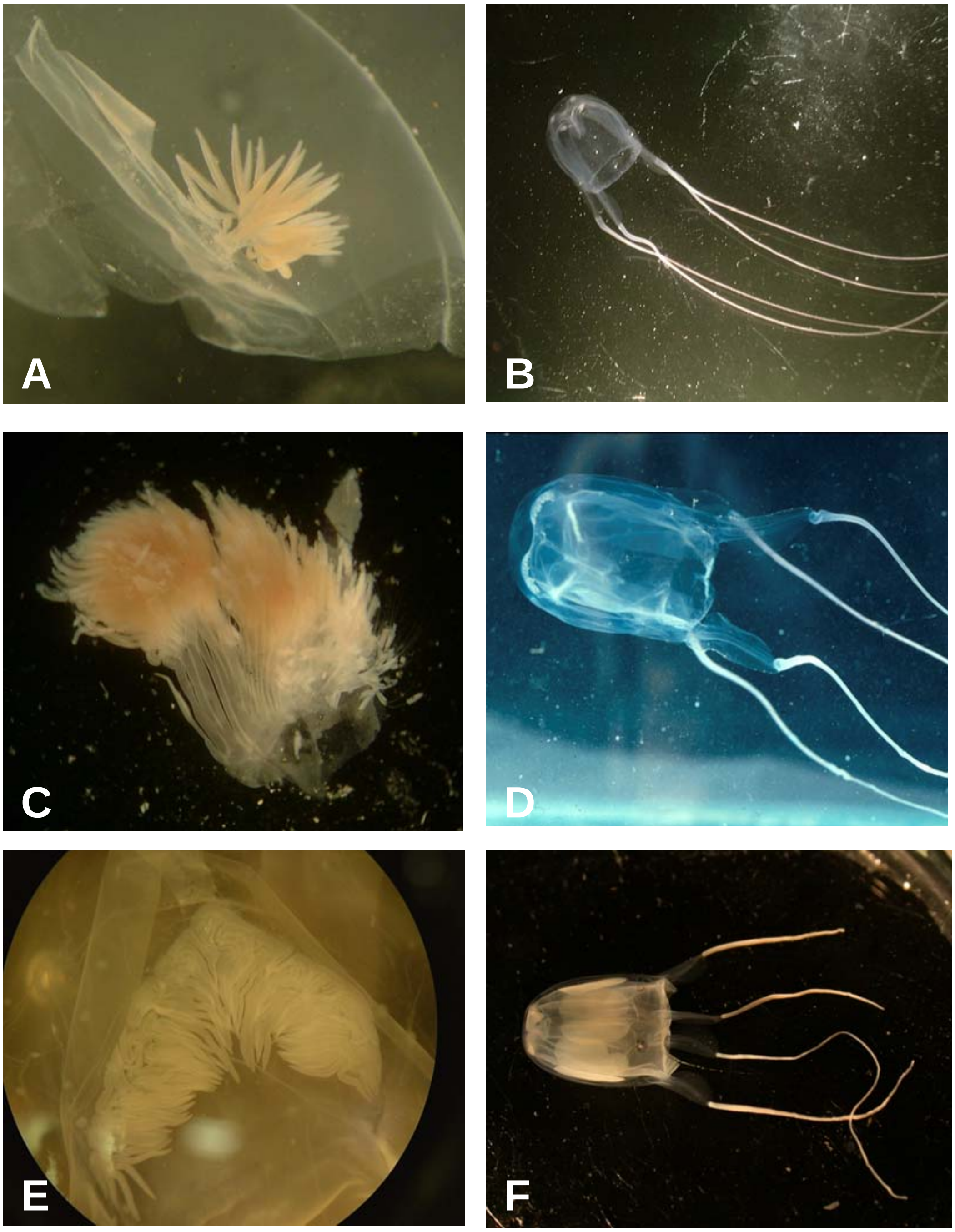

Plate 2.12a. Carybdeid phacellae (Section 2.3.7; Table 2.5). A. Single-stalked epaulette of short cirri (*Carybdea xaymacana*, ZMUC coll'n.). B. Single-stalked epaulette appearance in situ (*Carybdea xaymacana*, WA). C. Multiple-stalked epaulette (*Carybdea* n. sp., Cape Town, Leiden coll'n.). D. Horizontal rows oblique across corners in situ (*Carybdea rastonii*, Sydney, copyright I. Bennett). E. Crescentic phacellus of long cirri (*Alatina mordens* gen. et sp. nov., Barnes coll'n.). F. Crescentic phacellae in situ (*Alatina rainensis* gen. et sp. nov., MTQ coll'n.).

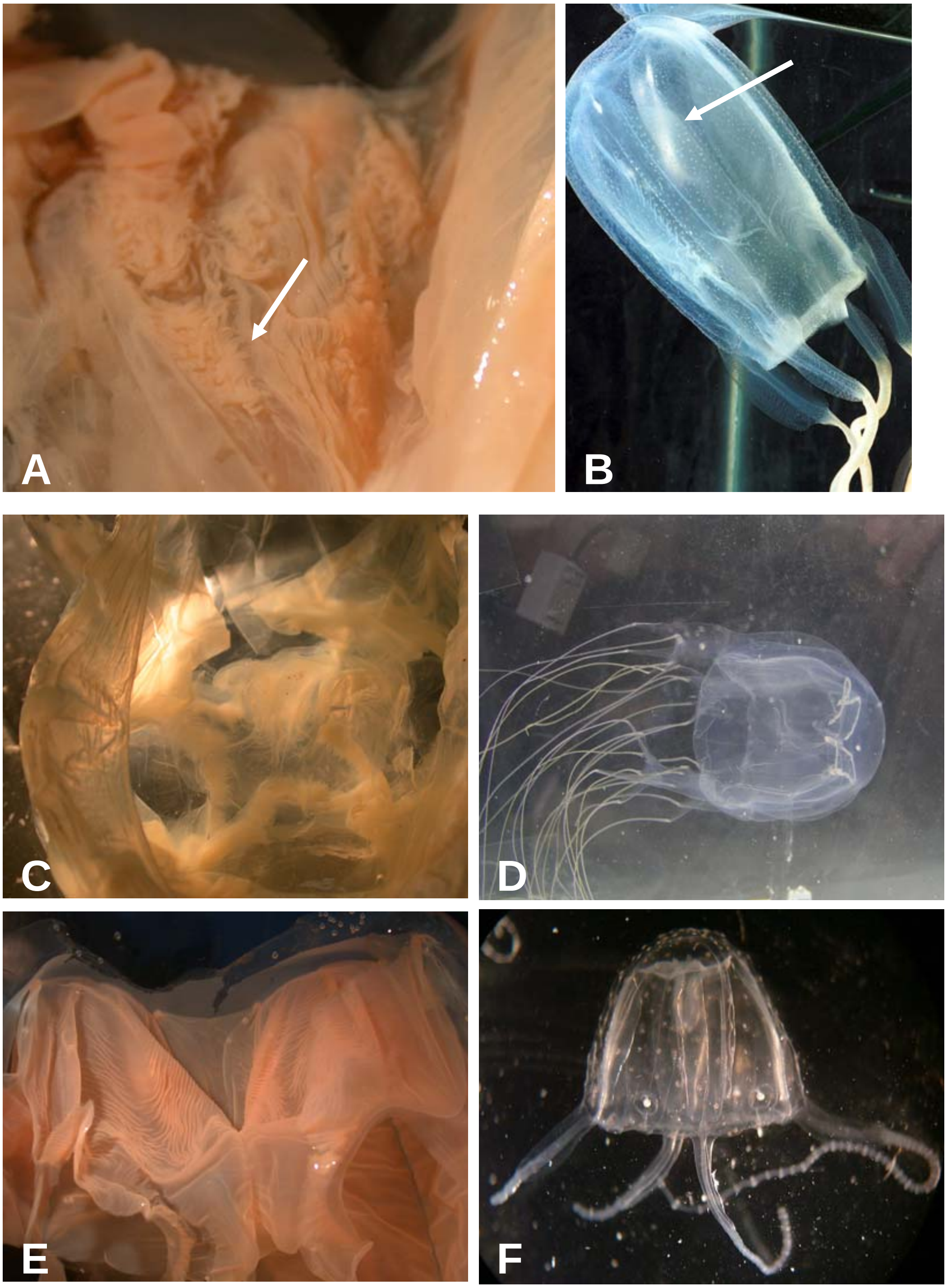

Plate 2.12b. Carybdeid phacellae (Section 2.3.7; Table 2.5). A. Vertical phacellae (*Tamoya* n. sp., QM coll'n.). B. Vertical phacellae appearance in situ (*Tamoya haplonema*, copyright A. Migotto). C. Chirodropid V-shaped phacellae (*Chiropsalmus* n. sp. B, Gove, Hartwick coll'n.). D. V-shaped phacellae in situ (*Chiropsalmus* n. sp. A, Townsville). E. Phacellae lacking (Morbakka, Moreton Bay, SAM coll'n.). F. Phacellae lacking in situ (*Carukia barnesi*, Cairns).

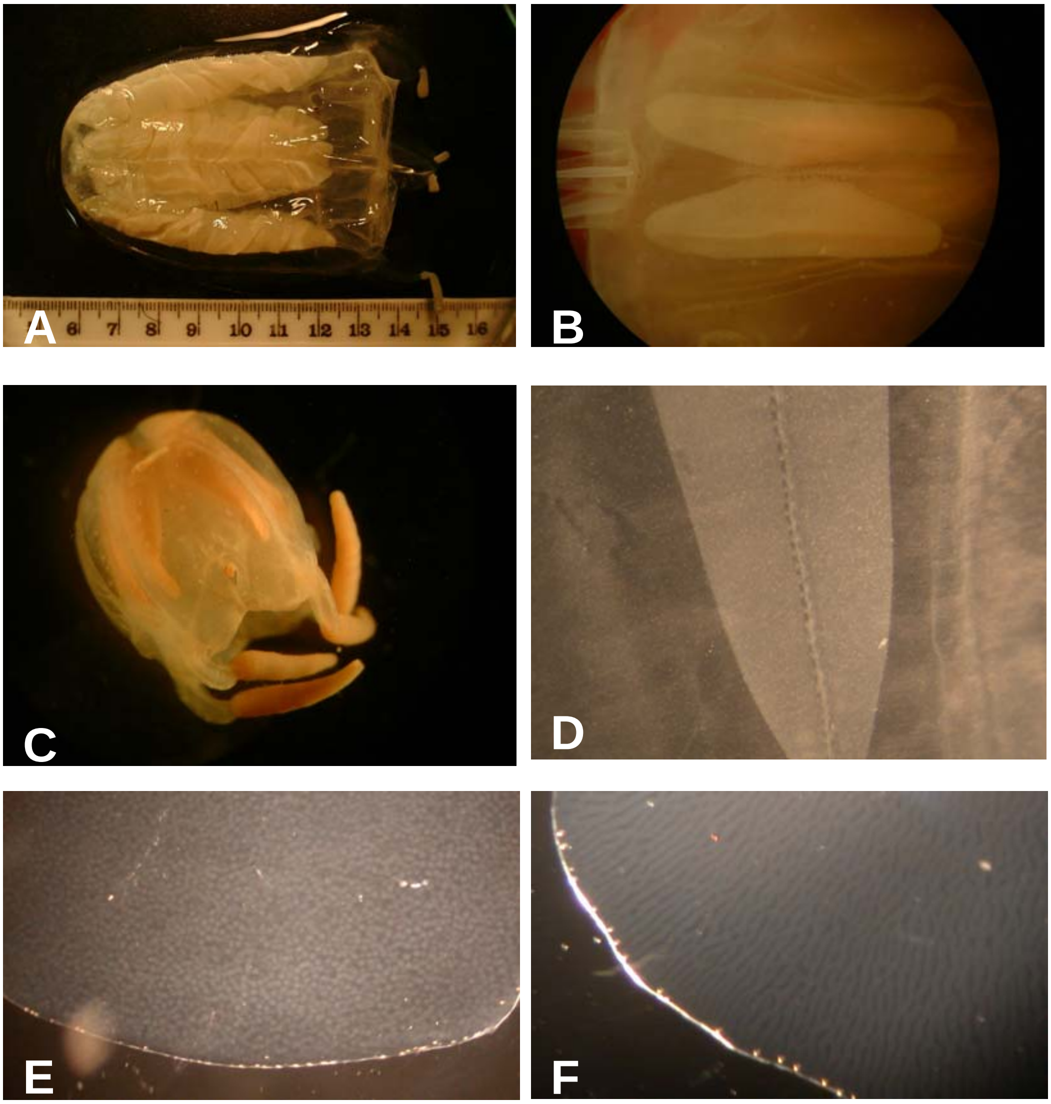

Plate 2.13. Gonad characters (Section 2.3.8). A. Leaf-form, attached full length (*Alatina mordens* gen. et sp. nov., see Chapter 5). B. Butterfly-form, attached in the center only (*Tripedalia cystophora*, Leiden coll'n.). C. Pendant, attached only at the top (*Carybdea sivickisi*, ZMUC coll'n.). D. Interradial septa with perforations (*Alatina mordens* gen. et sp. nov., see Chapter 5). E. Female gonad (Broome Irukandji). F. Male gonad (Broome Irukandji).

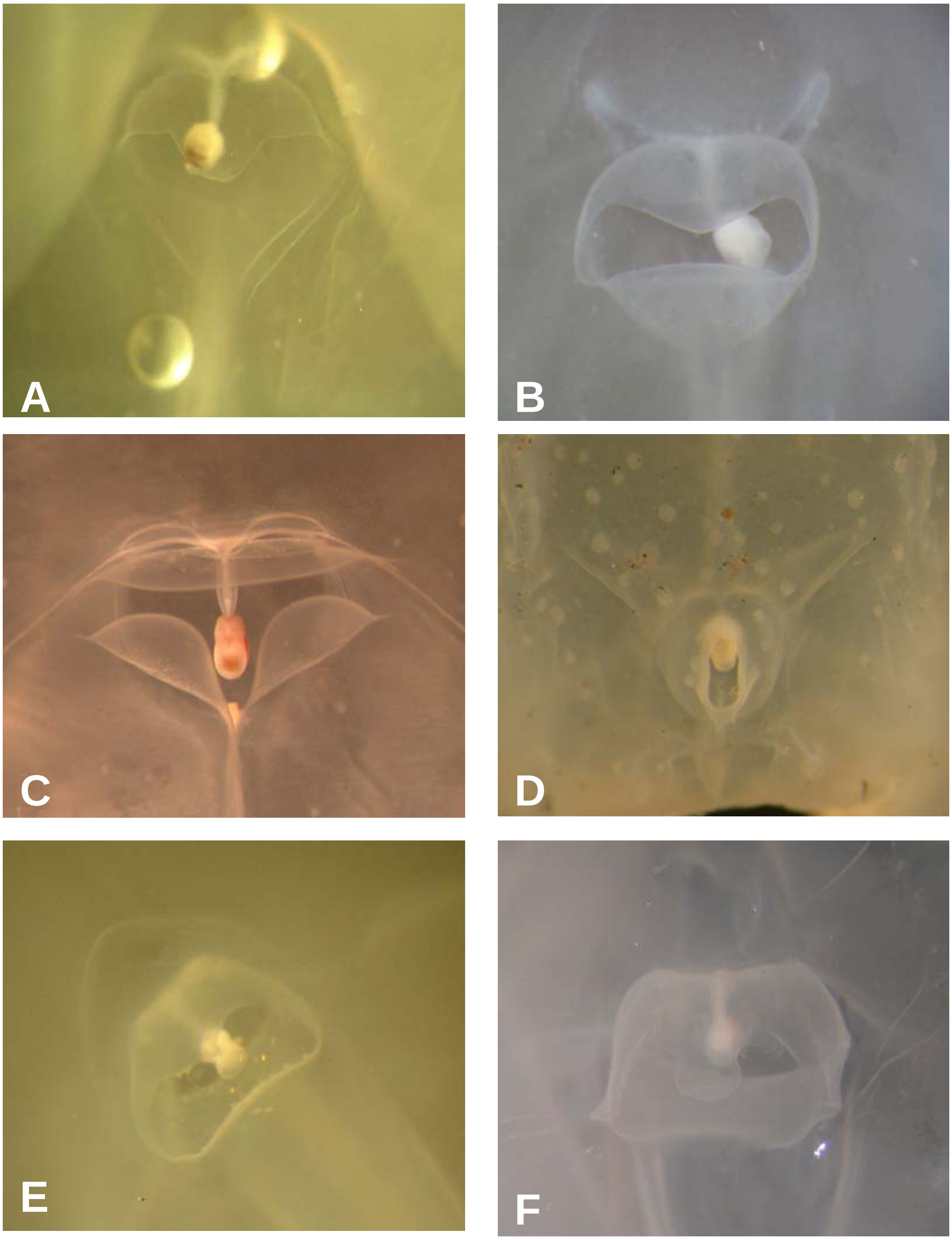

Plate 2.14. Rhopaliar niche ostium shape (Section 2.3.9; Table 2.6). A. Heart-shaped (*Carybdea xaymacana*, Cairns). B. Frown-shaped, upper scale convex (Darwin carybdeid, Paratype, NTM coll'n, copyright P. Alderslade). C. T-shaped, upper scale straight (*Alatina mordens* gen. et sp. nov., see Chapter 5). D. Vertical key-hole-shaped (*Carybdea sivickisi*, MTQ coll'n.). E. Dome-shaped chirodropid form (*Chiropsalmus* n. sp., NTM coll'n.). F. Upper scale with central tab (*Chiropsalmus* n. sp. A, N. QLD).

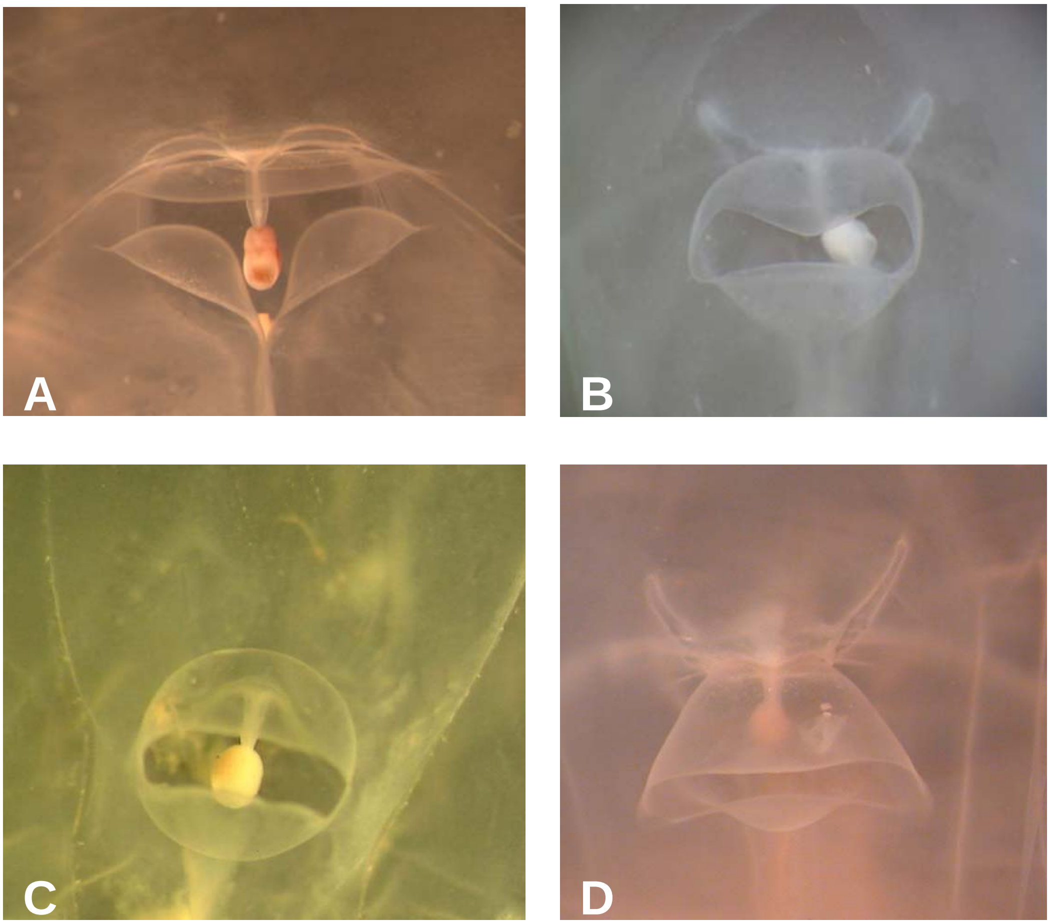

Plate 2.15. Rhopalial horns (Section 2.3.10). A. Absent (*Alatina mordens* gen. et sp. nov., see Chapter 5). B. Short, thick, and curved like Viking horns (Darwin carybdeid, Paratype, NTM coll'n., copyright P. Alderslade). C. Long and very narrow (*Carukia barnesi*, Holotype, SAM coll'n). D. Long and pointy (Morbakka, Moreton Bay, SAM coll'n.).

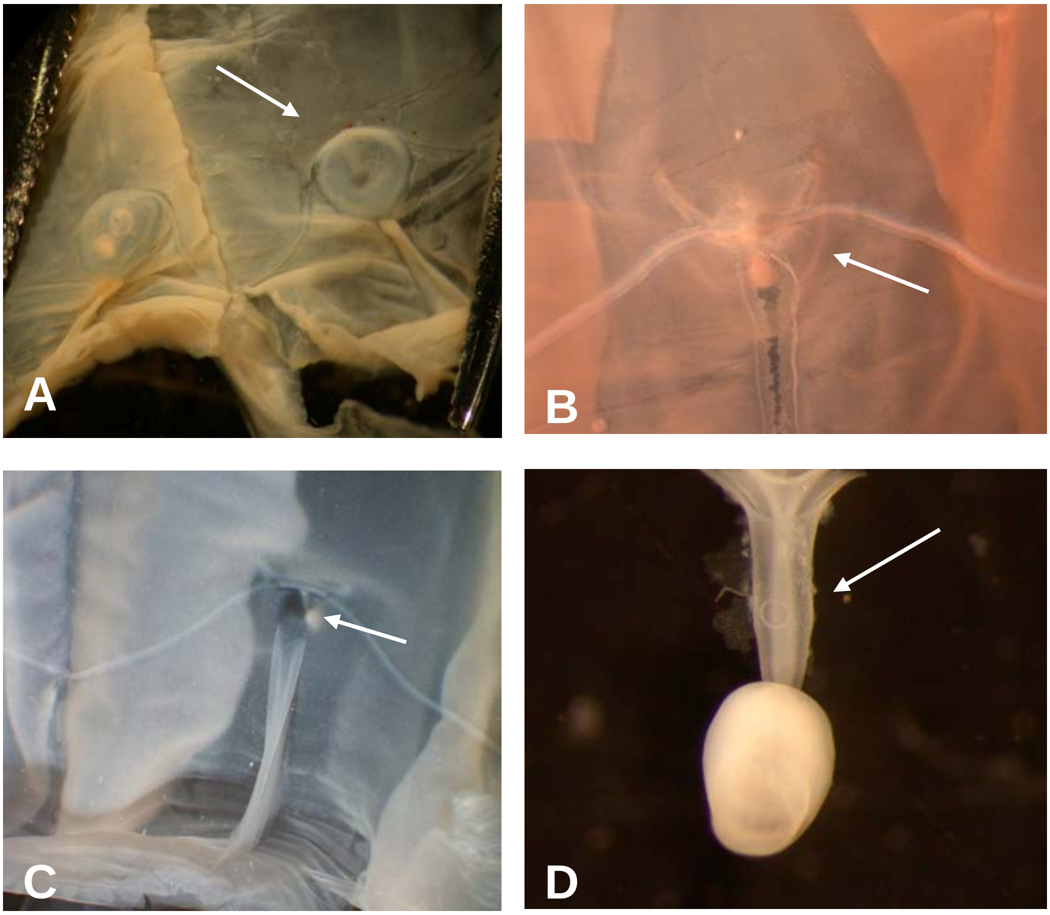

Plate 2.16. Rhopalial windows and warts (Section 2.3.11, 2.3.12). A. Windows broadly convex (*Manokia stiasnyi*, Holotype, IRSNB coll'n.). B. Windows flat (Morbakka, Moreton Bay, SAM coll'n.). C. Windows indented at top (Darwin carybdeid, Paratype, NTM coll'n., copyright P. Alderslade). D. Rhopalial wart on "back" of stem (Morbakka, Holotype, Port Douglas).

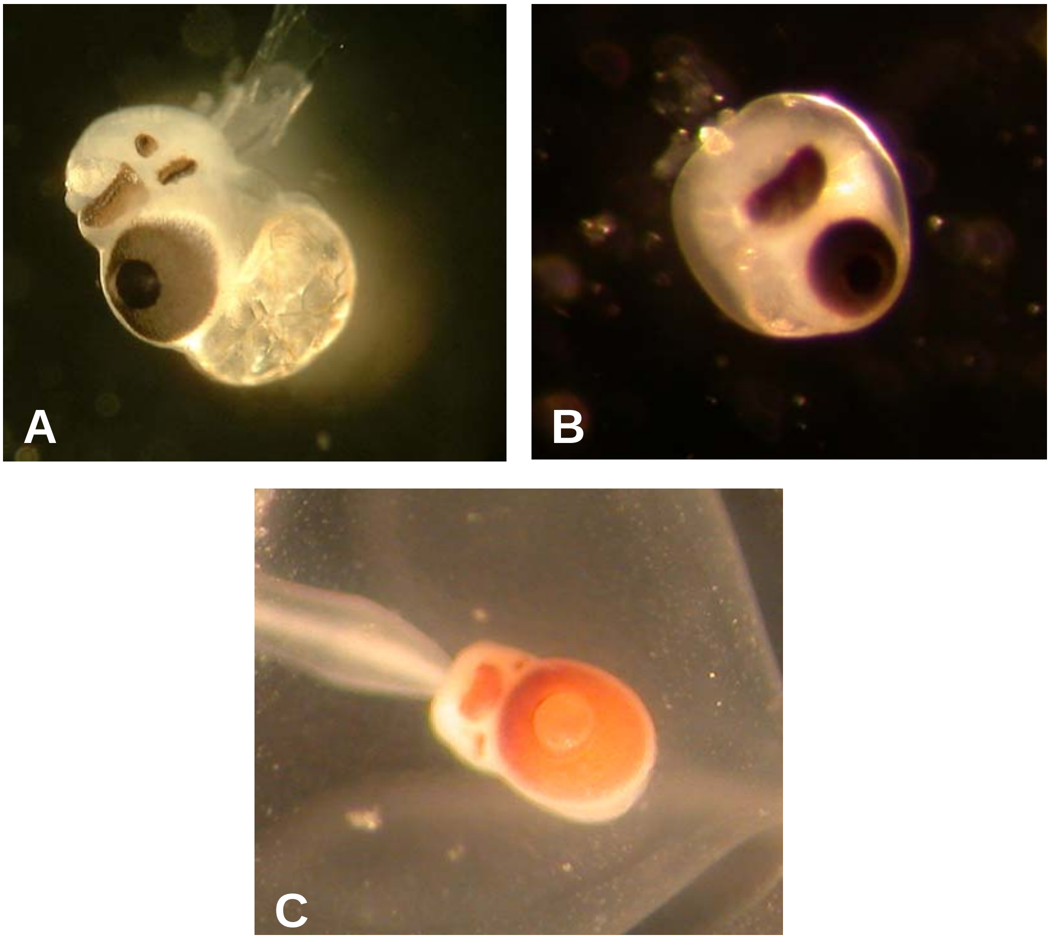

Plate 2.17. Eyes (Section 2.3.12; Table 2.7). A. 6 eyes, i.e., 2 median lensed eyes plus 2 pairs of lateral eyes (2 hidden in photo) (*Chiropsalmus quadrumanus*, Sao Paulo). B. 2 eyes, i.e., 2 median lensed eyes only, lacking lateral eye spots (Pseudo-Irukandji, Townsville). C. 4 eyes, i.e., 2 median lensed eyes plus 1 pair of lateral eye spots (*Alatina mordens* gen. et sp. nov., see Chapter 5).

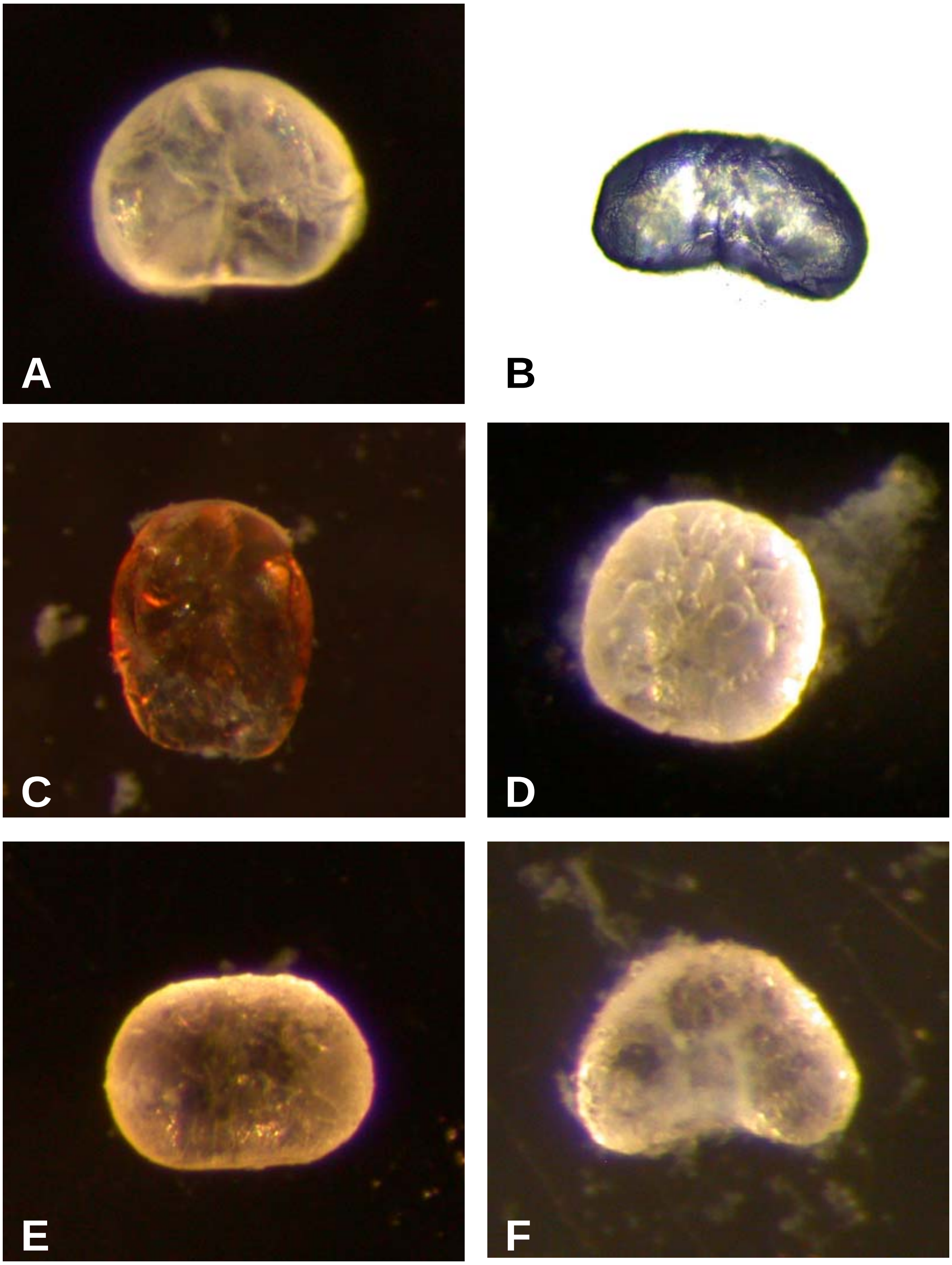

Plate 2.18. Statoliths (Section 2.3.13; Table 2.8). A. Bean-shaped (*Carybdea xaymacana*, Busselton, WA). B. Long and narrow (*Carybdea sivickisi*, Townsville). C. Tall and cylindrical (*Alatina mordens* gen. et sp.nov., see Chapter 5). D. Sub-spherical (*Carukia barnesi*, Cairns). E. Short and cylindrical (*Chironex fleckeri*, Townsville). F. V-shaped (*Tripedalia binata*, NTM coll'n.).

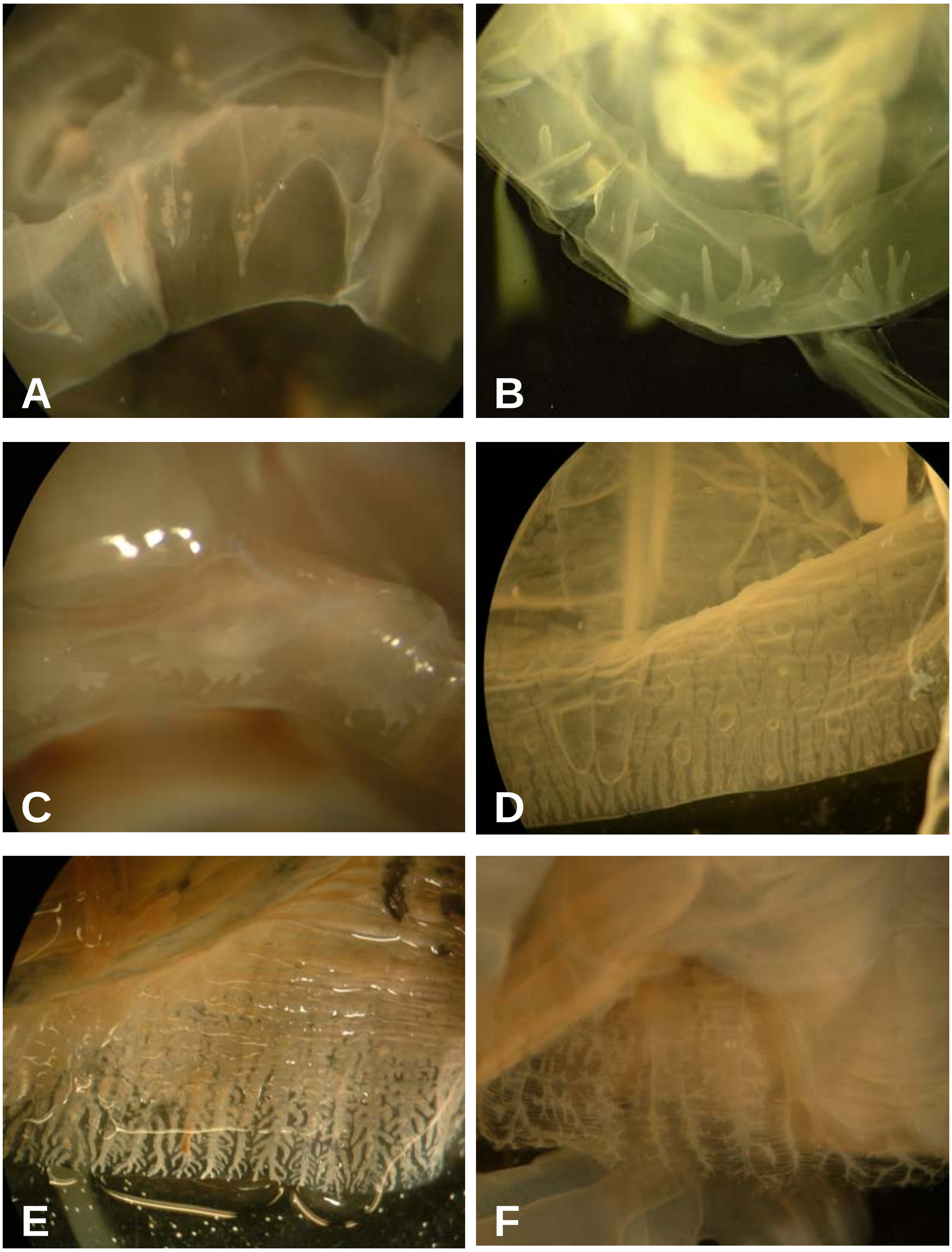

Plate 2.19. Velarial canals (Section 2.3.13; Table 2.9). A. Simple (*Tripedalia cystophora*, Leiden coll'n). B. Biforked (*Carybdea xaymacana*, Cairns). C. Paw-like (*Carybdea sivickisi*, Syntype, Leiden coll'n). D. Dendritic (Morbakka, Holotype, Port Douglas). E. With lateral diverticula (un-ID, ZMUC coll'n). F. With complex diverticula (*Chiropsoides buitendijki*, Sri Lanka, QM coll'n).

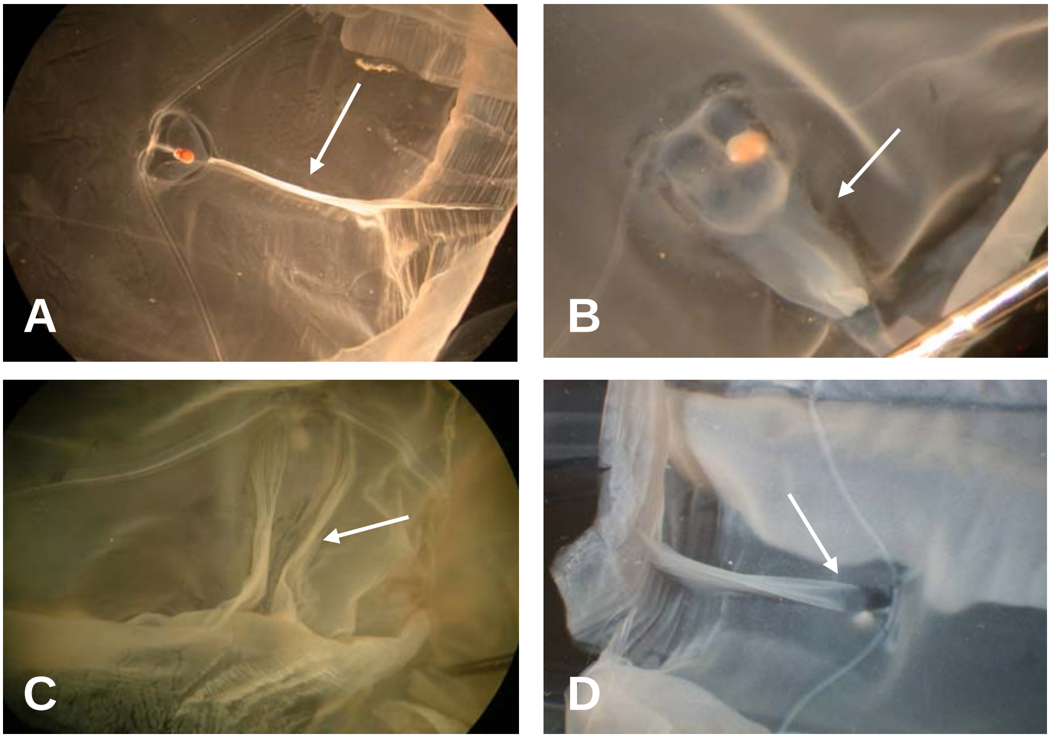

Plate 2.20. Frenulae (Section 2.3.16). A. Single simple sheet (*Alatina mordens* gen. et sp. nov., see Chapter 5). B. Single solid gelatinous substance (*Carybdea rastonii*, Neotype, SAM coll'n). C. Two sheets (*Tamoya haplonema*, Leiden coll'n). D. Hollow (Darwin carybdeid, Paratype, NTM coll'n, copyright P. Alderslade).

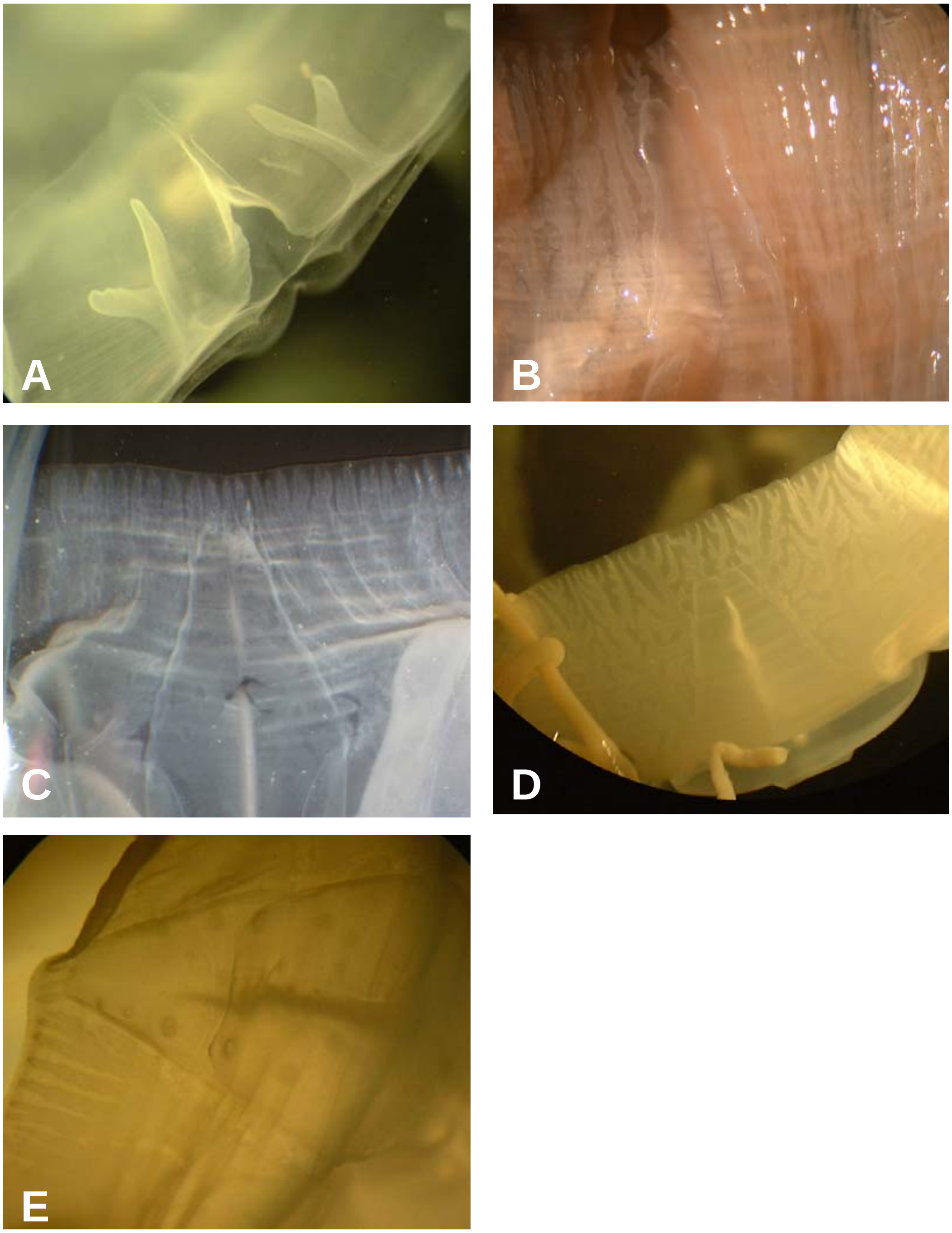

Plate 2.21. Perradial lappets (Sections 2.3.17, 2.3.18; Table 2.10). A. Absent (*Carybdea xaymacana*, Cairns). B. Narrow, with canal extensions (Morbakka, QM coll'n). C. Moderate, without side branches but with canal extensions (Darwin carybdeid, Paratype, NTM coll'n, copyright P. Alderslade). D. Broad, with side branches (*Chiropsalmus* n. sp., Darwin). E. With 2 rows of nematocyst warts (Darwin carybdeid, Paratype, NTM coll'n).

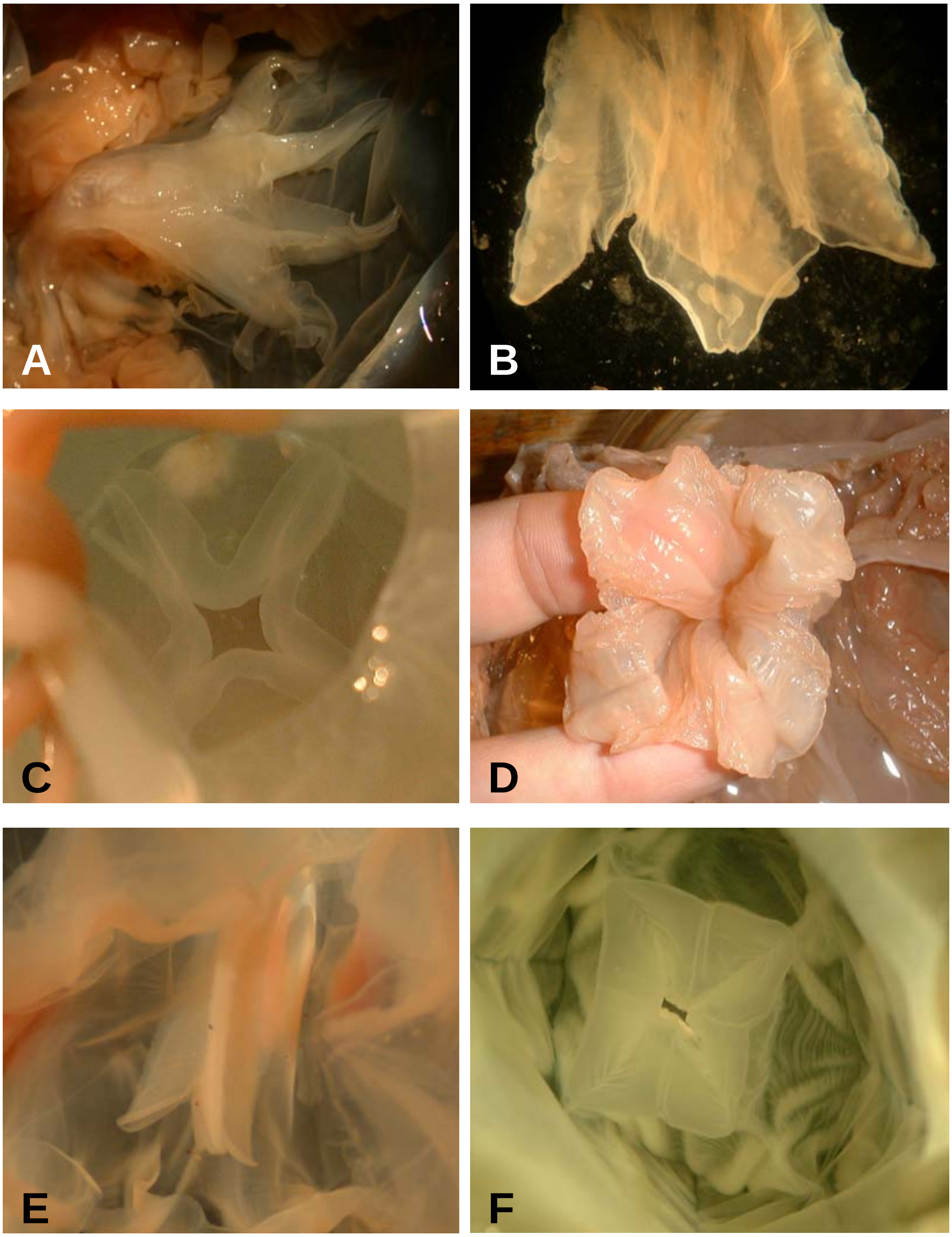

Plate 2.22. Lips shape (Section 2.3.20). A. Narrow pointy (*Chironex* n. sp., Okinawa, QM coll'n). B. Broadly pointy (*Tamoya* n. sp., South Carolina, QM coll'n). C. Narrow rounded (*Carybdea* n. sp., Cape Town, SAMA coll'n). D. Broadly rounded (*Chirodropus* sp., ZMUC coll'n). E. Short and pointy (*Tripedalia cystophora*, QM coll'n). F. Quadrate (Darwin carybdeid, Paratype, NTM coll'n).

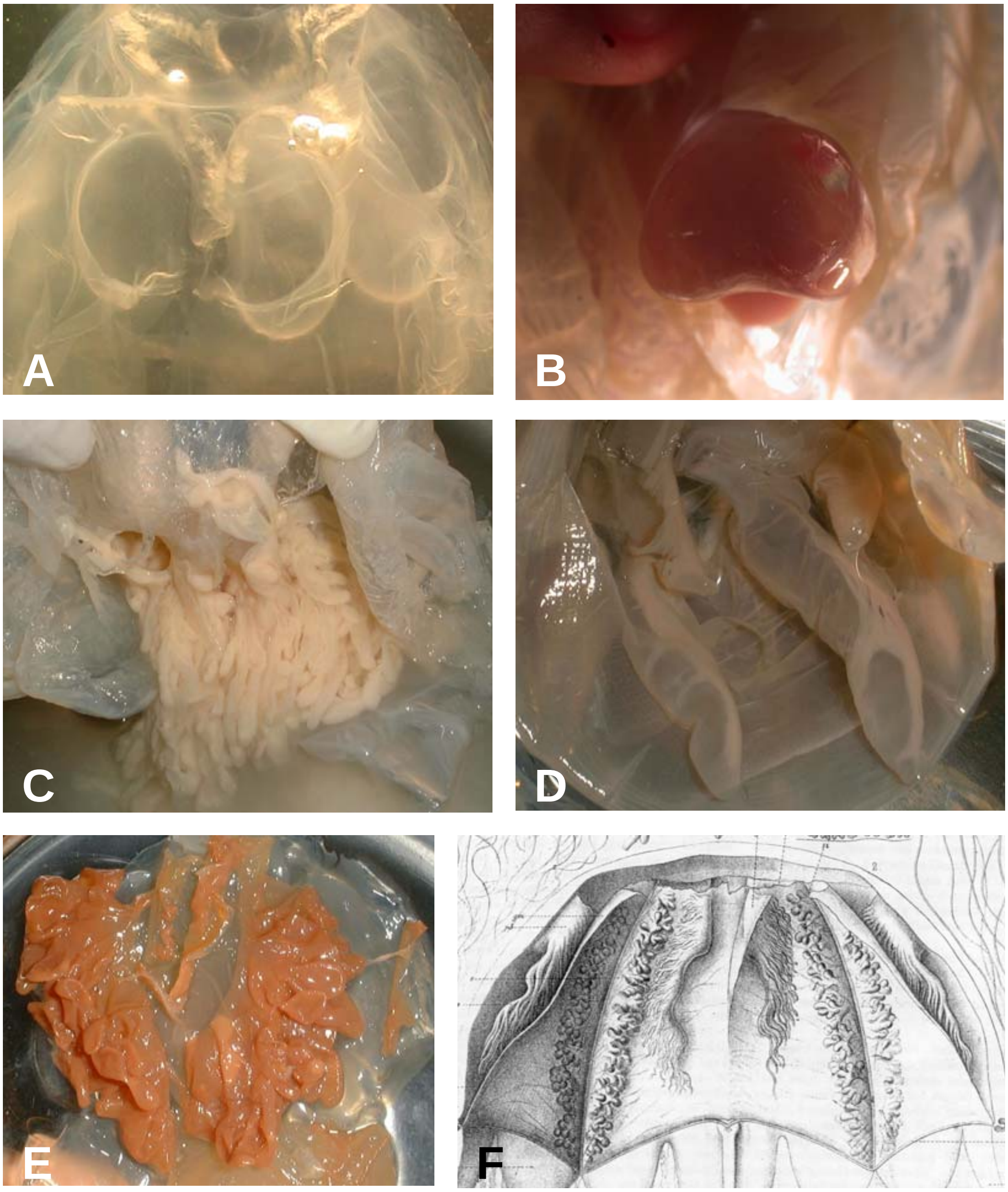

Plate 2.23. Gastric saccules (Section 2.3.21; Table 2.11). A. Round and hemispherical (*Chiropsalmus* n. sp. A, Cairns). B. Coalesced heart shaped (*Chiropsalmus* n. sp. B, Paratype, Gove, N.T.). C. Highly branched, like clumps of grapes (Chirodropida sp. indet., SAMA coll'n). D. Finger-shaped (*Chiropsoides buitendijki*, Holotype, Leiden coll'n). E. Laterally flattened and cock's-comb-shaped (*Chironex* n. sp., ZMUC coll'n). F. Feather-like, with numerous filaments (*Chirodropus gorilla*, original illustration, Haeckel, 1880).

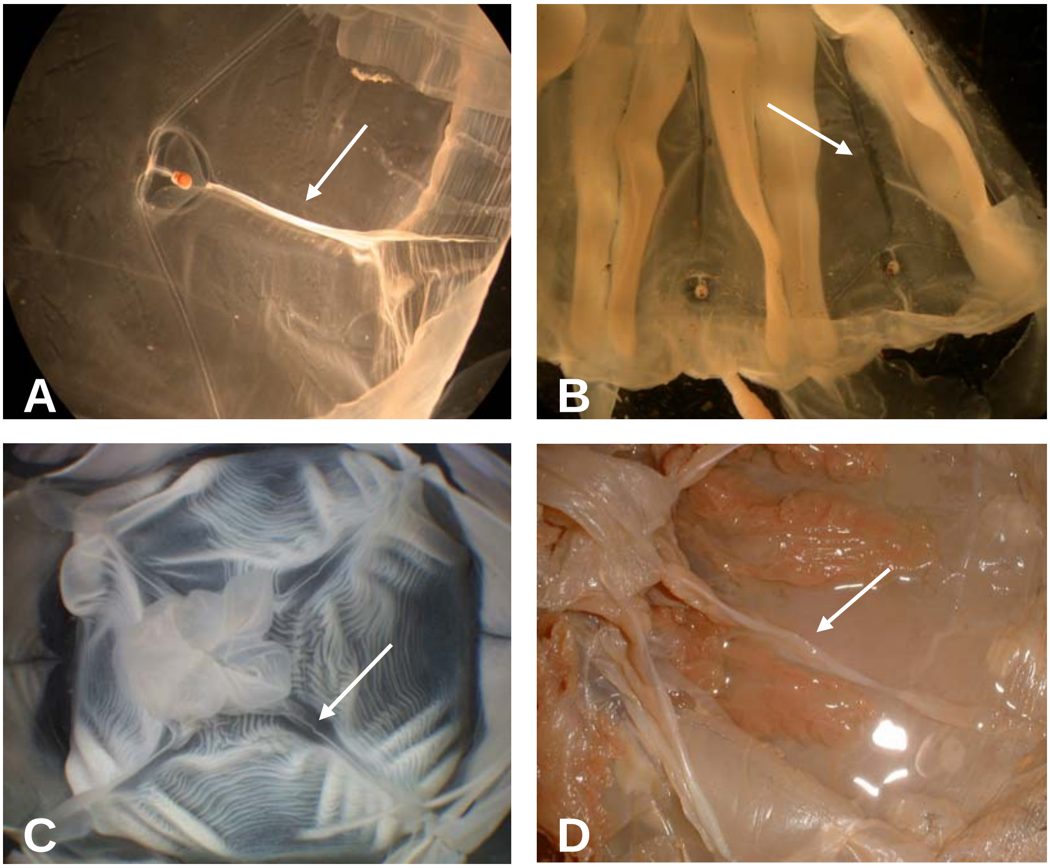

Plate 2.24. Mesenteries development (Section 2.3.22; Table 2.12); note that particular species may have different development of mesenteries at different locations along the subumbrellar perradii, e.g., flap-like proximally and cord-like distally. A. Absent (*Alatina mordens* gen. et sp. nov., see Chapter 5). B. Poorly developed with clear strip of tissue (Pseudo-Irukandji, Port Douglas). C. Moderately developed and cord-like (Darwin carybdeid, Paratype, NTM coll'n, copyright P. Alderslade). D. Well developed and flap-like (*Chirodropus* sp., ZMUC coll'n).

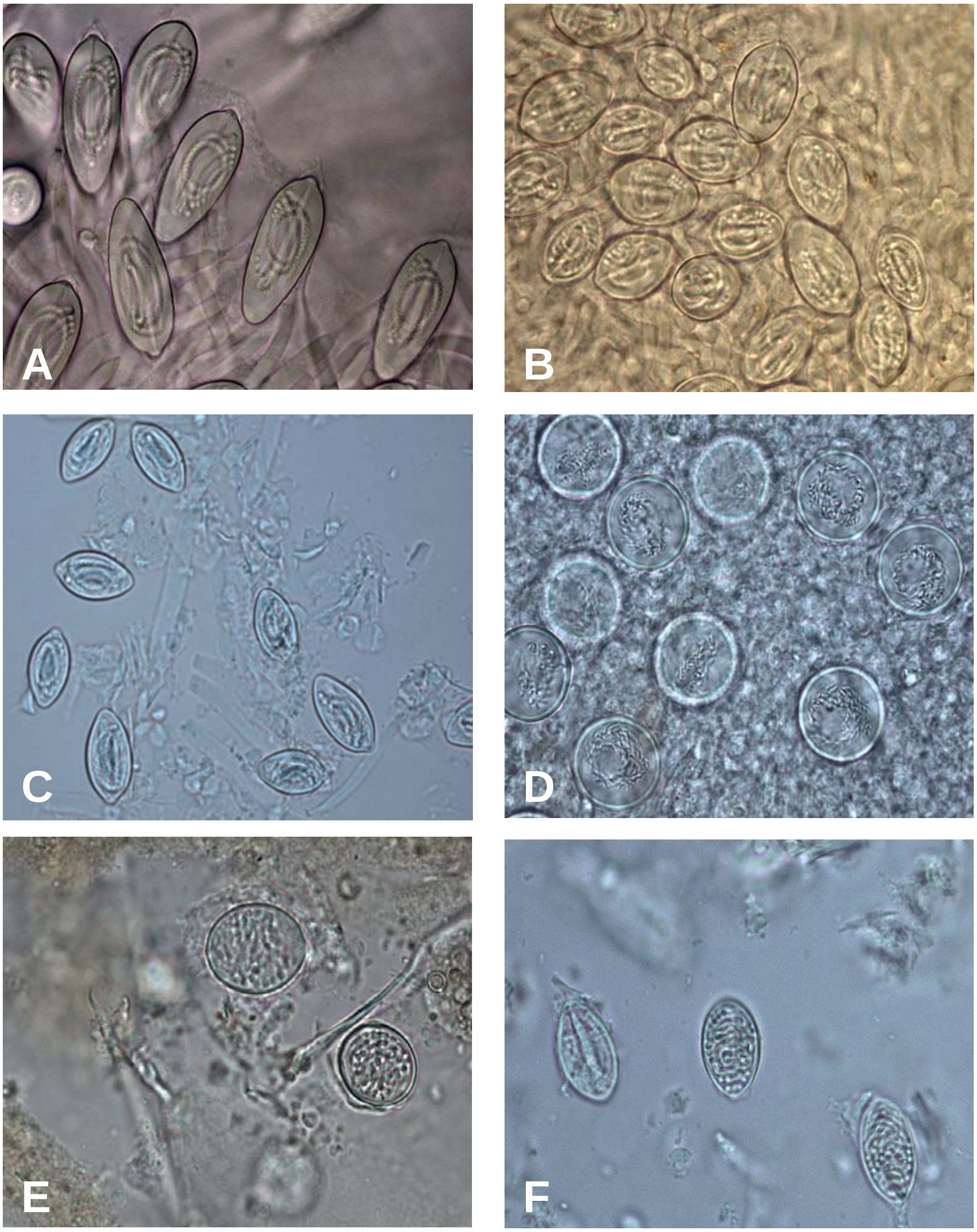

Plate 2.25. Carybdeid nematocysts, undischarged, 400x (Section 2.3.24, Table 2.13). A. Club-shaped microbasic p-mastigophores (Broome Irukandji). B. Lemon-shaped tumiteles (*Carukia* n. sp. Broome). C. Football-shaped microbasic euryteles (*Alatina mordens* gen. et sp. nov., see Chap. 5). D. Spherical isorhizas from the bell (*Alatina mordens* gen. et sp. nov., see Chap. 5). E. Sub-spherical isorhizas from the tentacles (*Alatina mordens* gen. et sp. nov., see Chap. 5).

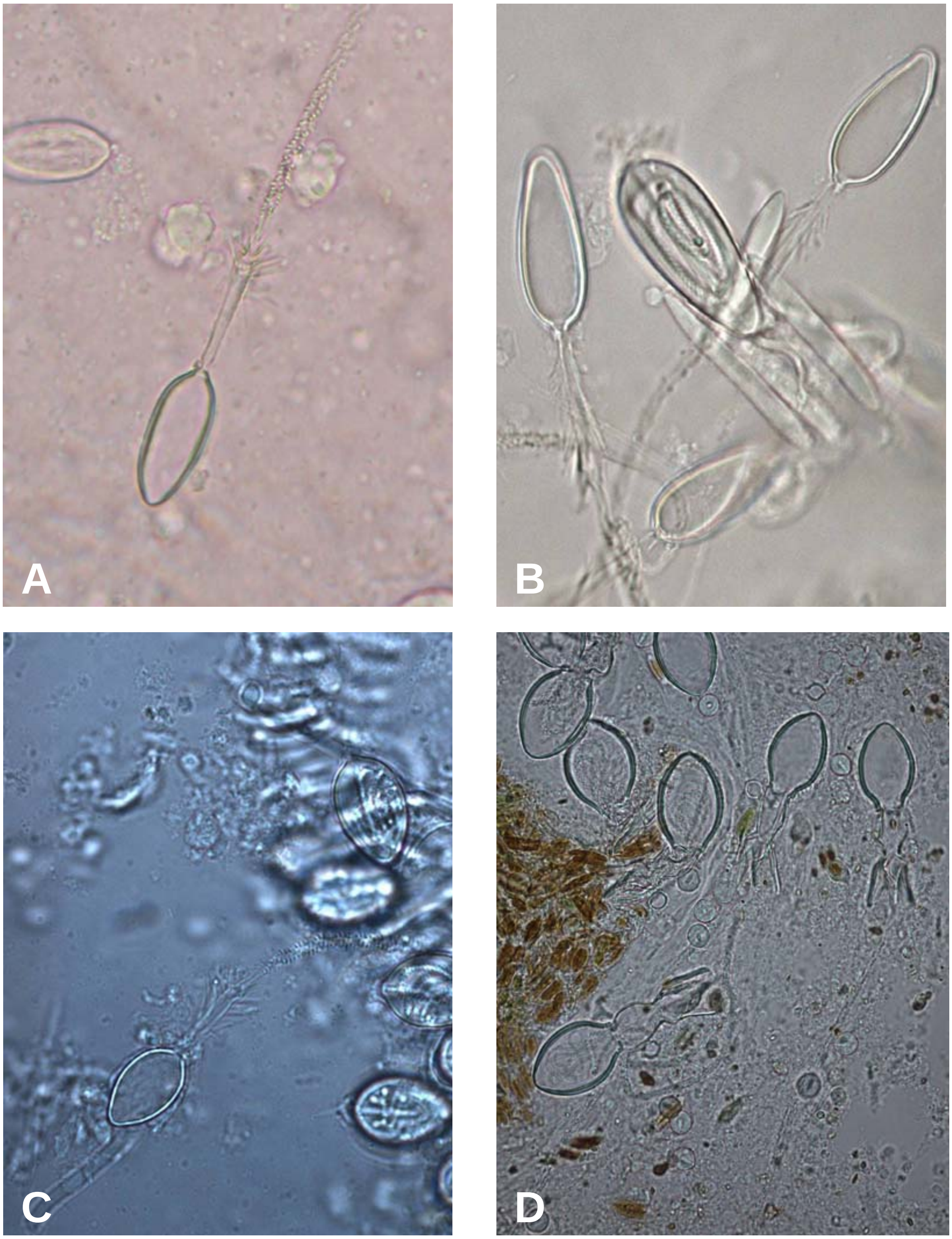

Plate 2.26. Carybdeid nematocysts, discharged, 400x (Section 2.3.24, Table 2.13). A. Type 4 microbasic p-mastigophores, with spines only at end of butt (Pseudo-Irukandji, N. QLD). B. Type 4 microbasic p-mastigophores, with spines along whole butt (Broome Irukandji). C. Tumiteles (*Carukia* n. sp. B, Broome). D. Stenoteles (*Carukia barnesi* polyp).

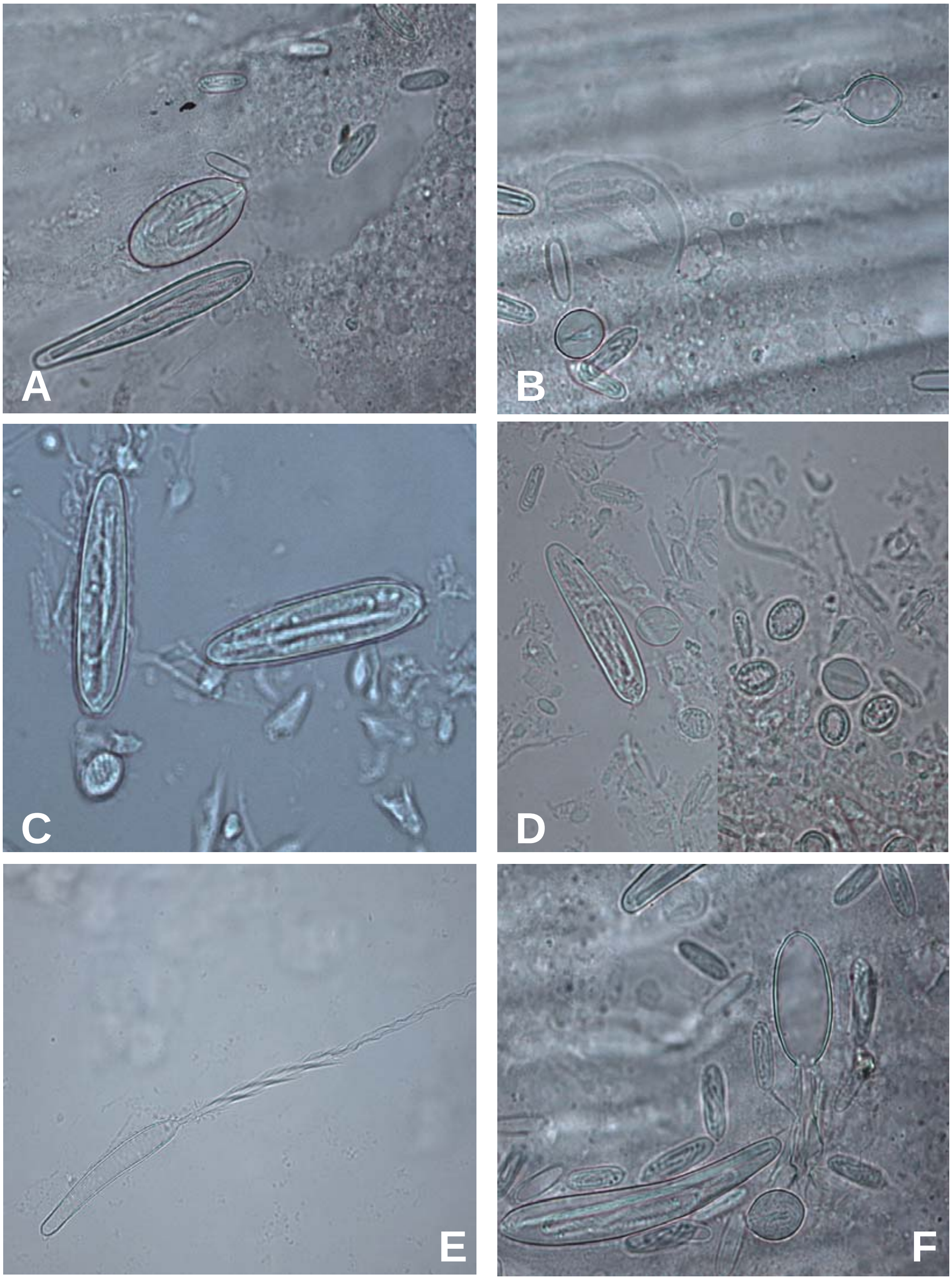

Plate 2.27. Chirodropid nematocysts, undischarged A-D, discharged E-F, 400x (Section 2.3.24, Table 2.13). A. Ovoid and banana-form microbasic p-mastigophores, rod-shaped isorhizas (*Chironex fleckeri*, Townsville). B. Undischarged and discharged spherical trirhopaloids (*Chironex fleckeri*, Townsville). C. Club-shaped microbasic p-mastigophores and small isorhizas (*Chiropsalmus* n. sp. B, Gove). D. Banana-form microbasic p-mastigophores, spherical trirhopaloids, and small ovoid isorhizas (*Chiropsalmus* n. sp. A, N QLD). E. Type 3 banana-form microbasic p-mastigophores (*Chironex fleckeri*, Townsville). F. Type 3 ovoid p-mastigophore (*Chironex fleckeri*, Townsville).

CHAPTER 3: MOLECULAR AND MORPHOLOGICAL PHYLOGENY OF AUSTRALIAN CUBOZOA

3.1 INTRODUCTION

The box jellies and Irukandjis are the world's deadliest animals (Cropp and Cropp, 1984; Endean, 1988), and yet, most of the species in Australia and their relationships to each other are unknown. A well supported phylogeny, coupled with a sound taxonomy, is essential to understanding and managing this biologically intriguing and medically important group. All other types of comparative cubozoan studies will build on this foundation.

The current taxonomy has been widely used since its erection by Haeckel (1880). A few additional species have been added, and a few species have been declared unrecognizable or identical to other taxa, but for the most part, the framework of Haeckel has stood the test of time. However, I argue that this test of time has been one of passive acceptance rather than of active support, and that when subjected to scrutiny, this taxonomic framework is proving far too general to fully express the diversity of species that are observable in nature. As a starting point of comparison, I have translated the current taxonomy (from Kramp, 1961, and later descriptions) into a branching diagram (Figure 3.1).

Many recent studies have shown the utility of comparative data sets for phylogenetic inference, juxtaposing nucleotides with morphology, for example, in angiosperms (Soltis et al., 2002), corals (Wolstenholme et al., 2003), and dinoflagellates (Taylor, 2004), or multiple molecular data sets from different genes, for example, in angiosperms (Soltis et al., 1999), scyphozoan jellyfishes (Dawson and Jacobs, 2001; Schroth et al., 2002), and fishes (Martin, 2001; Mattern, 2004). Sometimes, these comparative data sets support the patterns hypothesized by the traditional taxonomy, for example, in salamanders (Moritz et al., 1992), tardigrades (Garey et al., 1999), marsupials (Krajewski et al., 2000), echinoderms (Janies, 2001), carnivorous plants (Cameron et al., 2002), and the Medusozoa (Marques and Collins, 2004), whereas other times, whole new patterns are elucidated, for example, in corals (van Oppen et al., 2001), dinoflagellates (Costas et al., 1995), moulting invertebrates (Aguinaldo et al., 1997), cnidarian parasites (Siddall et al., 1995), and bats (Ammerman and Hillis, 1992). Herein, I capitalize on the comparative approach with both a comprehensive set of morphological characters and DNA sequences.

Haeckel (1866) regarded the Cubomedusae as the sister group to the Stauromedusae, together forming the sister group to the hydrozoan Trachymedusae and Narcomedusae. Thiel (1936) speculated that *Carybdea* gave rise to *Chiropsalmus* and *Tripedalia*. While there is

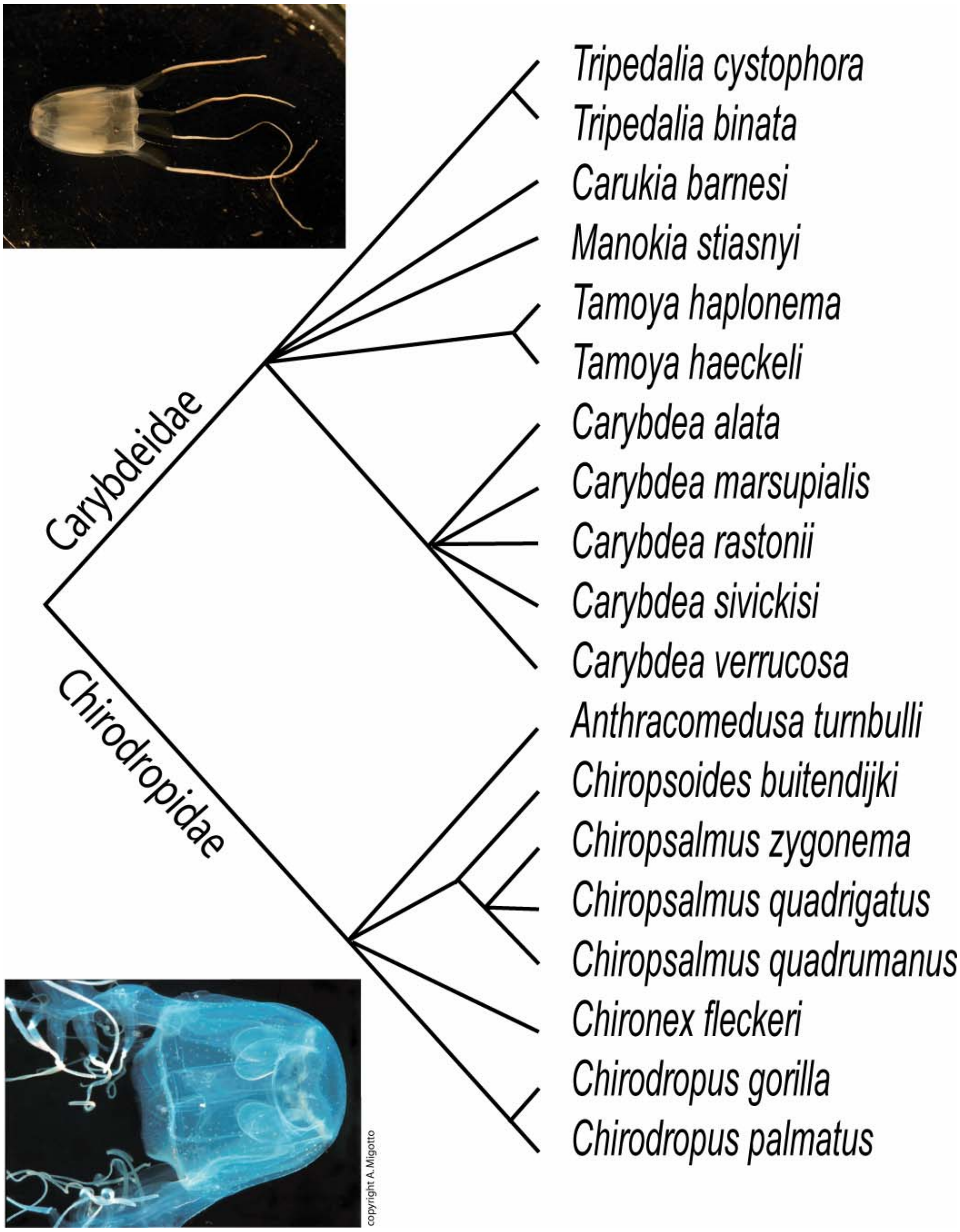

Figure 3.1. Branching diagram of the taxonomic relationships of the Cubozoa, according to the classification of Kramp (1961) and later authors. Note two main groups, the families Carybdeidae and Chirodropidae (= orders Carybdeida and Chirodropida, respectively).

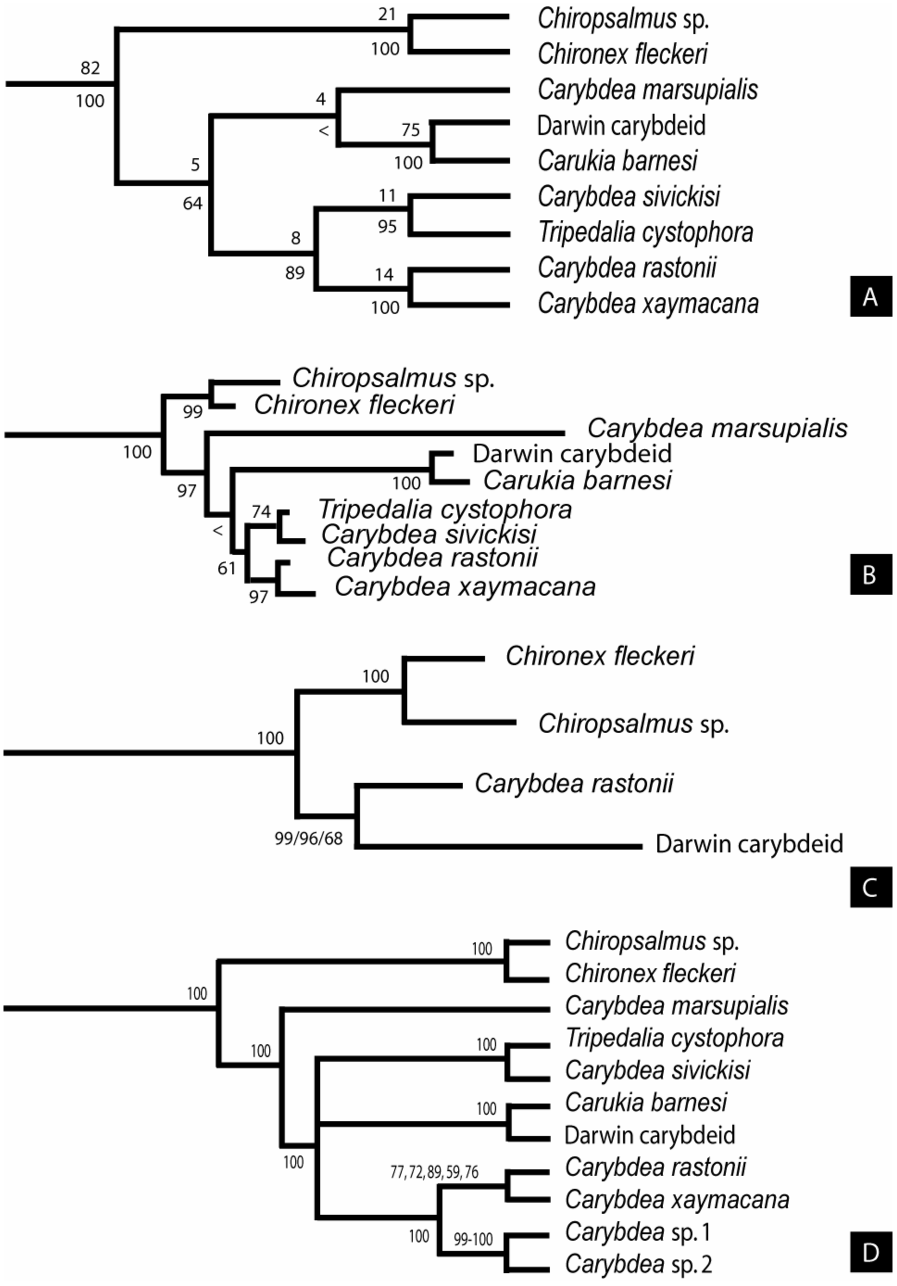

Figure 3.2. Phylogenetic hypotheses based on 18S rDNA (A and B redrawn from Collins, 2002; D redrawn from Collins et al., in review) and 28S rDNA (C from Collins et al., in review). A. Maximum parsimony analysis; Bremer support and bootstrap values indicated above/below the lines, respectively. B. Maximum likelihood analysis; ME bootstrap values shown at nodes. C. Maximum likelihood analysis; bootstrap values under ML, ME, and MP criteria are shown at the nodes. Nodes with bootstrap indices of 100 under all three criteria are indicated with a bold 100. D. Preferred topology based on the Bayesian analysis with three GTR models and a gamma parameter fit to the SSU data.

some support to indicate that the Cubozoa and Staurozoa might be each other's closest relatives (Collins, 2002; Marques and Collins, 2004), there is no modern evidence to support either Haeckel's hypothesis that the Cubozoa and Staurozoa descended from the Trachymedusae, or Thiel's hypotheses that *Chiropsalmus* and *Tripedalia* share a close relationship, or that together they descended from *Carybdea*. The most comprehensive estimate of cubozoan phylogeny to date was made by Collins (2002; Figure 3.2A, B, herein), based on complete 18S rDNA gene sequences from each of nine species of cubozoans, including six from Australia, as part of a larger study on the Medusozoa. His focus was not on the Cubozoa, and he devoted only minor discussion to their relationships, but several interesting patterns were evident from his results: A) the Cubozoa as well as the Chirodropida and Carybdeida are monophyletic groups, B) the genus *Carybdea* is not monophyletic, and C) two other groups clustered consistently that had not been previously recognized as sharing relationships, i.e., the Irukandjis (represented in Collins's analysis by *Carukia barnesi* and the "Darwin carybdeid") and *Carybdea sivickisi* + *Tripedalia cystophora*. Collins recognized the monophyletic relationships enumerated in "A" above, but did not comment on these other patterns. Recently, Collins and his colleagues (In review) tested the earlier 18S work with new 28S rDNA sequences for four of the taxa (Figure 3.2C, herein), and reworked the earlier 18S data set (Figure 3.2D, herein); their findings were robustly supported and consistent with the earlier topologies. An affinity had been noted between *C. sivickisi* and *Tripedalia* by Hartwick (1991) based on developmental observations, but no nomenclatural changes were suggested.

Werner (1973b) proposed that the Cubozoa be regarded as a sister Class to the Scyphozoa, rather than nested within, based upon unique developmental characteristics. Calder and Peters (1975) agreed, based on cnidome characteristics, though proposed alternatively retaining the Cubozoa as a subclass of the Scyphozoa. In contrast, the 18S rDNA sequence analysis of Collins (2002) yielded ambiguous results, with the Cubozoa either as the sister group to the Staurozoa, or as the sister group to the Hydrozoa. While the external relationships of the Cubozoa are still unclear, the internal relationships have been even less resolved. This is the first study to examine the Cubozoa comprehensively in a phylogenetic context. The objective of this chapter is to test the hypothesis of the current cubozoan taxonomy (i.e., Figure 3.1), and the specific aims are 1) to develop a morphological phylogeny based on as many characters as possible, 2) to develop a robust molecular phylogeny based on the 18S rDNA gene, and 3) to qualitatively compare them for an evolutionary framework on which to build an updated classification.

3.2 MATERIALS AND METHODS

3.2.1 Morphological data collection

Characters were scored on specimens preserved in dilute formalin (i.e., 2-5% saturated formaldehyde solution), except in rare cases where live observations were preferred (e.g., to score colour patterns and discharged nematocysts) or ethanol preservation was necessary (e.g., for statoliths). Specimens were examined under a dissecting microscope, with a strong side light for best illumination. Foreign language original descriptions were translated electronically with Globalink Power Translator for Windows.

3.2.2 Scoring of morphological data

In order to avoid tautologous species definitions, data were scored from holotypes or neotypes when possible, or from specimens destined to become them, or otherwise from a combination of original descriptions and paratypes or specimens from nearby the type locality. Characters were chosen with the intention of evaluating all of the structural information that is inherent in cubozoan species. Character states were chosen based on logical structural variability, with breaks between states reflecting the observed differences among specimens. No *a priori* assumption was made as to character usefulness for taxonomic discrimination. Comparison of specimens yielded 85 characters (enumerated and defined as characters 1-85 in the Character Results and Discussion (Section 2.3). A matrix (Appendix 2) of characters and character states was constructed in Nexus Data Editor for Windows v. 0.5.0 (Page, 2001) following the criteria in Chapter 2. Non-applicable data were scored as dashes (-); missing data were scored as question marks (?); combined polymorphisms (i.e., x and y) were scored inside brackets {xy}, whereas either/or polymorphisms (i.e., x or y) were scored inside parentheses (xy).

3.2.3 Morphological data analysis

PAUP*4.0b10 for Macintosh (Swofford, 2002) was used for parsimony analysis with a heuristic search using random stepwise addition of 100 replicates. All characters were treated as unweighted and unordered. Support values for internal nodes of the trees were estimated with 1000 bootstrap replicates (Felsenstein, 1985), using Maximum Parsimony (MP) criteria.

Morphological phylogenetic trees were rooted using the Chirodropida as a monophyletic outgroup following the results of molecular analyses (this thesis; Collins, 2002). A cnidarian outgroup could not be scored as part of the morphological data matrix (see Section 3.2.2), because most of the hypothesized synapomorphic character states used to analyze cubozoan taxa have no counterpart in the other classes, but those that do have non-equivalent states. For example, the velarium, and therefore all velarial structures, is absent in the other classes; as explained in Section 2.3.14, the hydrozoan velum is non-homologous with the cubozoan velarium, i.e., the hydrozoan velum is non-vascularized and is comprised of exumbrellar and subumbrellar epithelia, divided by a mesolamella, whereas the cubozoan velarium is vascularized and is entirely of subumbrellar origin, with the mesolamella located above the pedalia and rhopalia. In another example, rhopalia are present in only the Cubozoa and Scyphozoa, but the latter lack comparable eyes and statoliths; specifically, when present, scyphozoan ocelli are singular and lacking lenses, and statoliths are comprised of multiple tiny parts rather than a single large stone, thus shape is inapplicable. Furthermore, all structures associated with the rhopalia such as rhopalial niche ostia, rhopalial horns, and rhopalial windows, have no counterpart in the other classes. Finally, even nematocysts might not be a legitimate comparison, because the major groupings (i.e., isorhizas, euryteles, and mastigophores) are not well resolved in the Cubozoa. Specifically, carybdeid euryteles are more properly classified as microbasic p-mastigophores, but are still retained as euryteles to distinguish them from the wholly different banana-form microbasic p-mastigophores, but are dissimilar structurally to the euryteles of other classes (Hartwick, unpublished; Gershwin, unpublished). Thus, rather than score homoplasies inadvertently as homologies, it seemed more conservative to exclude cnidarian outgroups and root the tree following molecular analyses.

3.2.4 DNA extraction, PCR and sequencing

Total genomic DNA was extracted from match-head-sized samples of ethanol-preserved tissues according to the methods of Wilson et al. (2002), with the following modifications: I down-scaled the reaction to correspond to the reduced tissue sample size, and I omitted the RNAse treatment. Tissues from the velarium were empirically found to be superior, with tentacular samples often being problematical and stomach or manubrium samples having potential prey contamination; rhopalia also yielded very good product, but provided less tissue to start with (L. Peplow, pers. comm., 2004).

The 18S ribosomal DNA gene was targeted, based on the earlier results of Collins (2002) and preliminary studies by AIMS (L. Peplow and M. van Oppen, pers. comm., 2004) indicating that typical “species level” markers such as Cytochrome Oxidase I (COI) and Internal Transcribed Spacer (ITS) are evolving too fast to align across all Cubozoa. PCR primers were adopted from Collins (2002: 18SAf 5' CCG AAT TCG TCG ACA ACC TGG TTG ATC CTG CCA GT3' and int6 5' GAA TTA CCG CGG CTG CTG 3'). PCR products were generated with 30 μL reactions containing 1.0 μL of 1:10 dilution of DNA, 1.7 μL 10mM forward primer, 1.7 μL 10mM reverse primer, 25.8 μL master mix (containing 3.0 μL 10x PCR buffer, 1.2 μL

25mM $MgCl_2$, 3.0 µL 2mM dNTP's, and 17.88 µL sterile MilliQ water), and 0.12 µL 5 units/µL of *Taq* DNA Polymerase (Fisher Biotech) or the Qiagen Taq PCR Core Kit (Qiagen cat. no. 201223). The first 585 bp fragment at the 5' end of the 18S gene was amplified using a block thermal-cycler (Perkin Elmer Applied Biosystems GeneAmp PCR system 9700), with the following profile: an initial heating (3 min, 94ºC), then 30 cycles of denaturation (30 sec, 94ºC), annealing (30 sec, 62ºC), and extension (30 sec, 72ºC), followed by a final extension (5 min, 72ºC), then held at 4°C.

Once the PCR reactions were completed, a 3 µl sample was run on a 1% TAE-agarose gel to check the quality of the product, and the remainder of each PCR reactions was purified using DNAce (Astral Scientific), following manufacturer's directions. Quantity and quality of the DNA were measured spectrophotometrically (260/280 ratio).

Sequencing reactions were performed with ½ quantity of Dynamic ET (containing 4.0 µL ET Terminator mix, 0.5 µL 10mM primer, 42 ng PCR product, and sterile MilliQ water to equal a total volume of 10.0 µL). The sequencing reactions were cleaned up with Sephadex G-50 resin columns. Samples were sequenced on the Amersham MegaBace at the James Cook University Advanced Analytical Centre.

Chromatograms were truncated as necessary in MEGA3 (Kumar et al., 2004), then blasted (NCBI: http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/ information3.html) to ensure that they were of cubozoan origin. Sequences were proofread and aligned (Appendix 2) in MEGA3 using built-in CLUSTALW implementation (http://www.ebi.ac.uk/clustalw/), then refined by eye on a base-by-base basis. The chromatograms were then compared base-by-base with the sequences to ensure optimum accuracy.

Three specimens of each species were targeted for sequencing; however, numerous species of interest were not sampled due to lack of material, or to preservation which precluded DNA retrieval (i.e. formalin, methanol, too long in ethanol or dried); some of the more important missing taxa include *Tamoya haplonema,* the true Adriatic *Carybdea marsupialis, Tripedalia binata, Chiropsalmus quadrumanus, C. quadrigatus, Chiropsoides buitendijki,* and *Chirodropus gorilla*. A total of 33 specimens were sequenced, representing 13-16 hypothesized species. Most sequences correspond with voucher specimens that have been or will be deposited in the state museums closest to where they were collected. Sequences will be deposited in the GenBank database prior to publication of these results.

3.2.5 Molecular phylogenetic analysis

Algorithmic methods are hotly debated in the scientific literature. There are three common methods currently employed for phylogenetic inference of sequence data: Bayesian inference, Maximum Parsimony, and Maximum Likelihood. Maximum Parsimony gives the arrangement that best fits the data given the lowest number of steps, whereas Maximum Likelihood and Bayesian both allow for a model of character evolution to be specified. Bayesian analysis was selected because it allows for posterior probabilities to be estimated conditional on the observed data, based on the designated model. Furthermore, it uses Markov Chain Monte Carlo sampling, which is thought to be a less biased predictor of phylogenetic accuracy than the more traditional bootstrapping method (Alfaro et al., 2003). Bayesian analysis has been used by many recent authors in exploring questions of phylogenetic inference (Eick et al., 2005; Luan et al., 2005). For this analysis, sequences were analyzed using MrModeltest 2.0 (Nylander, 2004) to find the most appropriate model of sequence evolution for the data. Posterior probabilities for phylogenetic clades were then calculated in MrBayes 3.0 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). No prior probabilities were defined. The analysis was run with four independent chains and for 5 million generations, of which 1.25 million were discarded (burn-in). The following parameters were used: K = 1; Base frequencies = equal; substitution model = all rates equal; proportion of invariable sites = 0; variable sites (G); gamma distribution shape parameter = 0.0135. An unrooted consensus tree was derived, then run through PAUP* (version 4.0b10; Swofford, 2002) to obtain a printable tree.

3.3 RESULTS

Patterns evident from the molecular analysis were largely congruent with the traditional interpretation (compare Figure 3.1 with Figure 3.3). However, two novel groupings were revealed with strong support, namely, "*Carybdea alata*" spp. (= *Alatina* spp.), and the Irukandji species clade; furthermore, *Carybdea sivickisi* grouped outside the *Carybdea* proper with *Tripedalia* spp., a grouping with some further support based on several shared sequence motifs unique from the remaining taxa. Within the Irukandji clade, two conspicuous groupings were further revealed, namely the *Carukia* spp. and the "Pseudo-Irukandjis". In addition to these groupings, two separate cases of incomplete sorting (in the general sense of the phrase) of morpho-species were brought to light, i.e., in the "Pseudo-Irukandji" and *Chiropsalmus* groups. Finally, the molecular results strongly indicate that there may be cryptic species within "*Carybdea sivickisi*" as circumscribed. These patterns are discussed more thoroughly below (Section 3.4). The molecular data set included a total of 33 new sequences, plus the nine from Collins (2002). The aligned data set consisted of 568 positions (Appendix 3). The number of variable sites was 85. Bayesian maximum likelihood analysis of the molecular data set under the best-fitting model (JC+G) generated a consensus tree (Figure 3.3; -lnL = 1503.3009).

The morphological topology was largely congruent with the molecular topology, and was supported by moderate or better bootstrap values (e.g., Chirodropida, Irukandjis, *Carybdea*, Alatinidae fam. nov. (see Chapter 5), and *Carybdea sivickisi* + *Tripedalia*), but there were several important differences. Of the 85 morphological characters, two were invariant, 7 were parsimony-uninformative, and 76 were parsimony-informative. Parsimony analysis of this data set generated 45 equally most parsimonious trees (Length = 474; Consistency index = 0.5169; Retention index = 0.5933; Figure 3.4, strict consensus).

The major differences (Figure 3.5) between the morphological consensus tree and the molecular tree included, in the former: A) the three Irukandji groups were still recovered intact but were paraphyletic with respect to the carybdeids; B) the “Morbakka” + “Darwin carybdeid” grouped together and shifted to an ancestral position among the Irukandjis, rather than as nested sister taxa to the *Carukia* spp.; C) *Carybdea xaymacana* was represented as the sister group to the other *Carybdea* spp., rather than as a nested member; and D) a rearrangement of the carybdeids such that *Tripedalia* + *sivickisi* became a clade. The broadly used genera *Chiropsalmus* and *Carybdea* were not congruent with traditional interpretations: in the morphological analysis, *Chiropsalmus* auct. was divided in the poorly resolved Chirodropida (whereas it was cohesive in the molecular analysis); and *Carybdea* auct. was consistently disrupted in both analyses, with both *C. sivickisi* and “*C. alata*” outside the traditionally circumscribed genus.

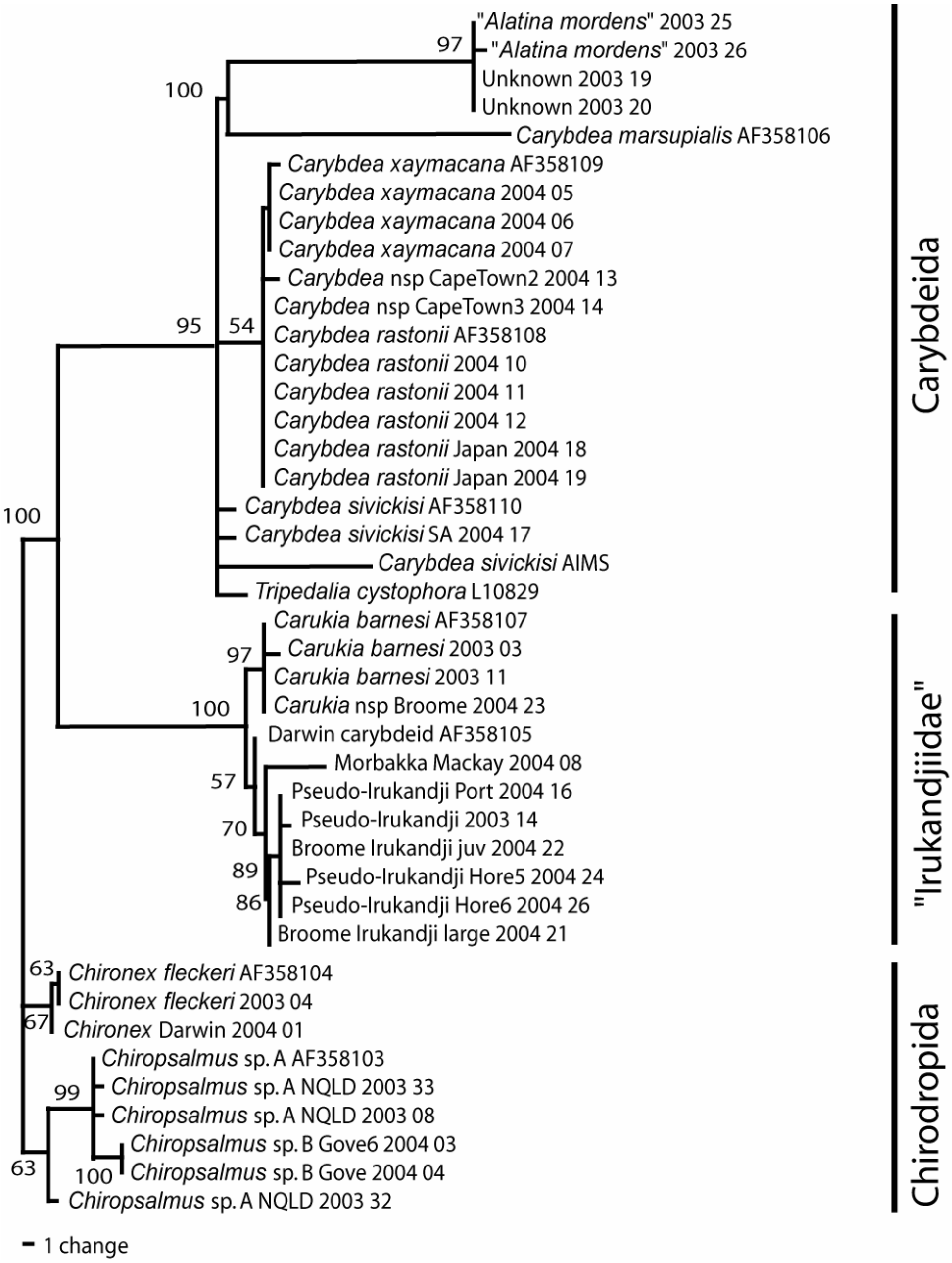

Figure 3.3. Proposed preliminary molecular phylogeny of the Cubozoa, based on partial 18S rDNA, Bayesian Maximum Likelihood analysis (-lnL = 1503.3009). Posterior probabilities are shown at the nodes. Species groups are as indicated, with the traditional taxa of the "Carybdeida" noted as such.

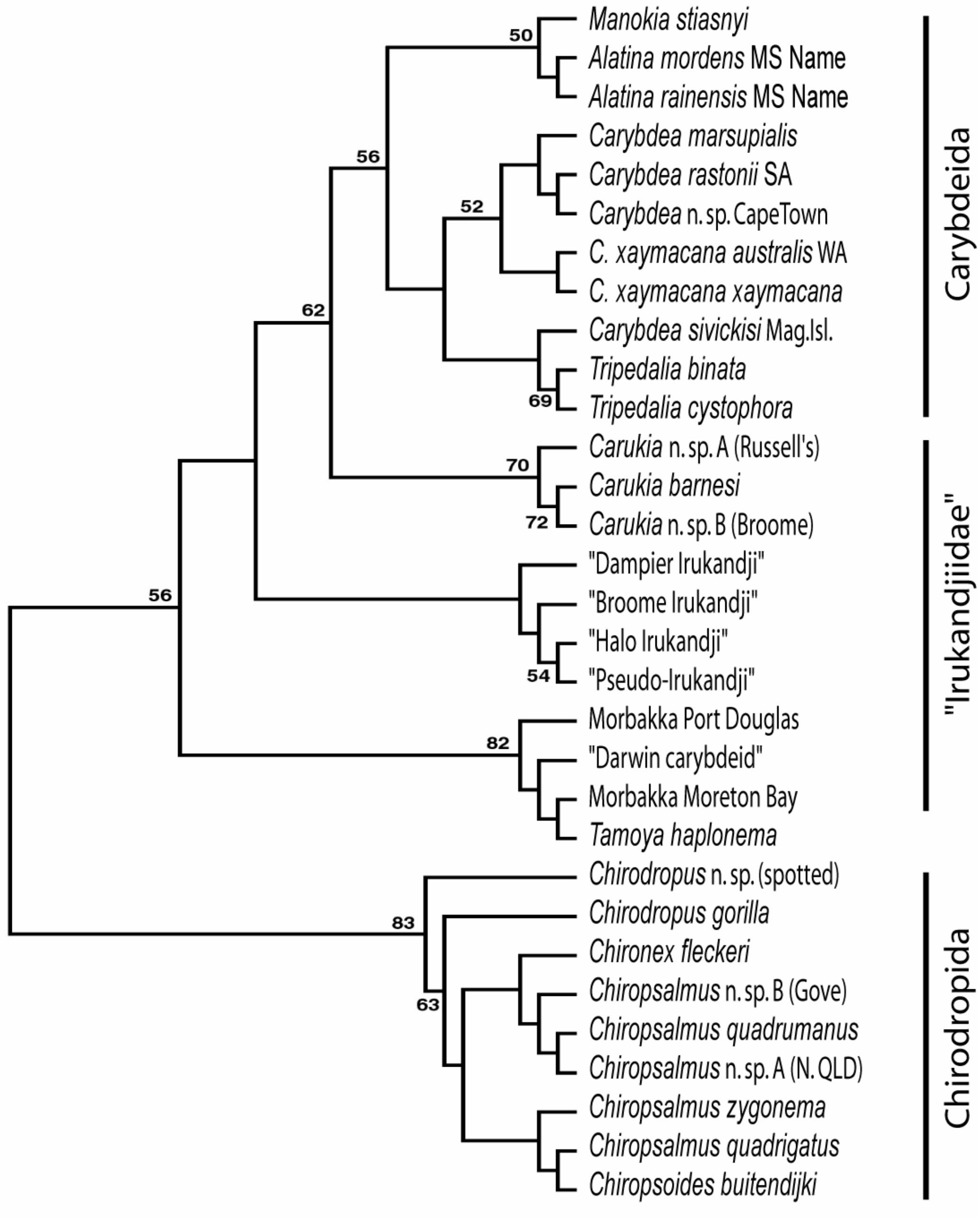

Figure 3.4. Proposed morphological phylogeny of the Cubozoa. Strict consensus of 45 most parsimonious trees, from Maximum Parsimony analysis of 85 morphological characters. Length = 474; CI = 0.5169; RI = 0.5933. Bootstrap values greater than 50% are shown at each node. Species groups are as indicated, with the traditional taxa of the "Carybdeida" excepting *Tamoya haplonema* noted as such.

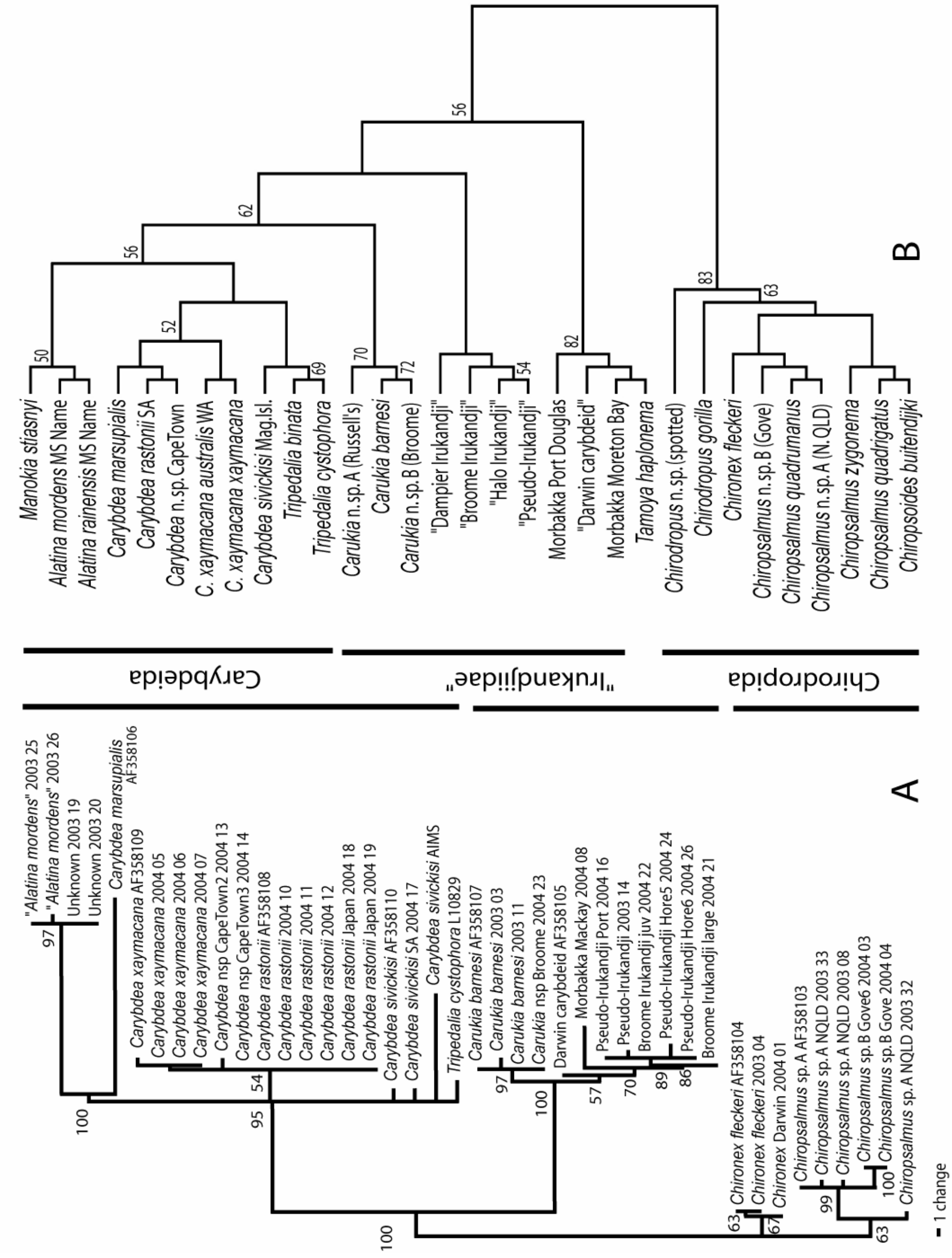

Figure 3.5. Comparison of molecular and morphological phylogenetic results from Figures 3.3.and 3.4. A. Proposed preliminary molecular phylogeny of the Cubozoa, based on partial 18S rDNA, Bayesian ML analysis (-lnL = 1503.3009). B. Strict consensus of 45 most parsimonious trees, from morphological analysis of 85 characters. Length = 474; CI = 0.5169. Posterior probabilities and bootstrap values greater than 50% are shown at each node. Species groups are as indicated, with the traditional taxa of the “Carybdeida” noted as such.

3.4 DISCUSSION

The phylogenetic patterns revealed in these analyses were, for the most part, consistent between morphology and molecular data. The relatively congruent conclusions obtained from two independent data sets increases our confidence in the reliability of these analyses. This study corroborates with previous work based on the 18S rDNA gene (Collins, 2002), as well as previous morphological work (Gershwin, In press; Gershwin, unpublished). A summary of phylogenetic relationships inferred from these analyses follows.

3.4.1. Monophyly of Cubozoa, Carybdeida and Chirodropida

Both types of analyses herein support the hypothesis that the Chirodropida is a cohesive group, in agreement with traditional taxonomy and the molecular phylogenies of Collins (2002; In review). However, because the root was artificially forced between the Chirodropida and the remaining taxa, monophyly of the Chirodropida was not tested.

There exists discordance between the two present data sets and the traditional view as to the interpretation of the Carybdeida. The Carybdeida is traditionally interpreted as comprising all non-chirodropid taxa, i.e., *Carybdea* spp., *Tripedalia* spp., *Tamoya*, *Carukia*, all of equal rank. In the unrooted molecular analysis, a three-pronged node divides the chirodropids, the Irukandjis, and the rest of the carybdeids. This deep divergence of the Irukandjiidae from the remaining Carybdeida may or may not represent a third order – this question cannot be answered by these analyses. But to assume that the root is between the Irukandjiidae and the Chirodropida is subjective (I think it very well might be, but I do not have evidence that it is). In the classification I have kept the Irukandjiidae in the Carybdeida because the nomenclatural implications of not doing so are enormous, but I think the genetics should not be assumed in the absence of evidence. Given their morphology, there is every reason to consider the possibility that they are unique from the remaining Carybdeida.

Before rooting the morphological analysis, the chirodropids resolved as nested within the Irukandji clade, whereas when I forced the chirodropids as the root, the carybdeids resolved as nested within the Irukandji clade. Either way, the traditional interpretation of the Carybdeida is not supported, i.e., the Irukandji group is a whole new entity that has not been previously recognized, and the molecular analysis herein does not conclusively support or reject its position inclusive or exclusive to the traditional Carybdeida. Monophyly of the Cubozoa was not tested in the present analyses, but was upheld by Collins (2002; In review).

3.4.2 Monophyly of "*Carybdea alata*", i.e., *Alatina* gen. nov.

Based on overwhelming morphological data, the "*Carybdea alata*" group should be classified as a separate family from the rest of the Carybdeidae (Chapter 5), a conclusion corroborated by both of the present analyses. I only have one species of known identity in the present molecular analysis, i.e., *Alatina mordens* gen. et sp. nov. (see Chapter 5), plus two unknown specimens and Collins's (2002) curious "*Carybdea marsupialis*" (see below for more on the latter). While the two unknowns are too dehydrated for positive identification, their sequences and localities make it highly probable that they were *A. mordens* as well.

The phylogenetic position of this clade is less certain than the cohesiveness of the clade itself. In my molecular Bayesian ML and morphological MP analyses, the clade appears to bear a sister-group relationship to the *Carybdea* + *Tripedalia* + *sivickisi* clade (morphological MP), or is part of an unresolved comb with these other taxa (Bayesian ML; see comparative Figure 3.5). Either way, it appears more closely related to the carybdeids *sensu stricto* than to the Irukandjis. However, assuming that Collins's problematical "*Carybdea marsupialis*" (see Section 3.3.4 below) is actually a member of this clade, then his analyses (see Figure 3.2 herein) place it as either ancestral to the carybdeids + Irukandjis (Collins, 2002, fig. 3, ML; Collins et al., In review, fig. 6, Bayesian ML) or as the sister group to the Irukandji clade (Collins, 2002, fig. 2, MP).

3.4.3 Identification of Collins's problematical "*Carybdea marsupialis*"

The sequence that Collins (2002) identified as *Carybdea marsupialis* consistently grouped outside the *Carybdea* clade in his analyses and in mine. While this sequence should have been very similar to that of his "*C. xaymacana*", the opposite was true, leading to a host of bizarre possible hypotheses. In fact, it shares numerous identical motifs with *Alatina mordens* gen. et sp. nov. (see Chapter 5; Appendix 3), and is thus most likely one of the "*Carybdea alata*" species. However, it is interesting to note that in Collins's MP analysis (Figure 3.2A, herein), it clustered with the Irukandji group (*Carukia barnesi* + "Darwin carybdeid", but with low support values), whereas in his ML analysis (Figure 3.2B, herein), it resolved basal to the remainder of the Carybdeida (with high support). In contrast, it resolved with high support herein as the sister taxon to *Alatina mordens* gen. et sp. nov. (see Chapter 5), in a comb-like relationship to the *Carybdea* spp., *sivickisi*, and *Tripedalia* (Figure 3.3). Tree topology can be affected by the addition or removal of samples, possibly explaining the reason for the discordance between my results and those of Collins.

A minor other nomenclatural issue relates to Collins's "*C. marsupialis*". The sequence in question was from polyps meant to be the Caribbean form (A. Collins, pers. comm., 2002), i.e., originally described as *Carybdea xaymacana* Conant, 1897, and variously synonymized with *C. marsupialis* (Linnaeus, 1758) from the Mediterranean (see synopsis in Kramp, 1961). This is potentially confusing since Collins used the name *C. xaymacana* for the southwestern Australian form of *Carybdea* based on my identification. I have examined all three medusa forms (Caribbean, Mediterranean, and SW Australian), and am unable to tell the Caribbean and Australian forms apart structurally, but they differ substantially from the Mediterranean form. We may never know the true identity of the "*C. marsupialis*" sequence with certainty, as no voucher material exists that would make it identifiable, and there currently exists no taxonomic scheme for cubopolyps.

3.4.4 *Carybdea sivickisi* groups with *Tripedalia* spp.

These results indicate that *C. sivickisi* groups naturally with *Tripedalia* instead of with the "true carybdeas", i.e., *C. marsupialis*, *C. rastonii* and *C. xaymacana*, and that there may further be significant structure within what we currently recognize as the species *C. sivickisi*. Compared with Collins's (2002) Guamanian *C. sivickisi* sequence, the South Australian *C. sivickisi* differed by 4 bp, and the Magnetic Island (QLD) *C. sivickisi* differed in 16 bp plus 6 separate insertion clusters (6, 4, 2, 1, 6, and 1 bp). Hartwick (1991) noted the similarity of *C. sivickisi* and *T. cystophora* based on sexual dimorphism and spermatophore formation and transfer, but no taxonomic changes were made. In qualitative comparison, they do not share an overall similarity, so it is particularly interesting that this grouping has been suggested by these analyses.

3.4.5 Monophyly of Irukandjis

Perhaps the most intriguing outcome that has come from the present analyses is in the relationship of the known and suspected Irukandji syndrome-causing species to each other. In the molecular analysis, most of the Irukandji-causing jellyfishes form a monophyletic group sister to the other Carybdeida, indicating a tripartite arrangement comprising the carybdeids, the Irukandjis, and the chirodropids. In the morphological analysis, the Irukandjis are *between* the other two groups, i.e., prior to enforcing the Chirodropida as the ancestor, the Irukandjis were paraphyletic with respect to the Chirodropida, and when I enforced the Chirodropida as the ancestor, the Irukandjis appeared as paraphyletic with respect to the remaining Carybdeida. By traditional morphological examination, the group is cohesive based on numerous shared characters. For ease of communication I have dubbed this evolutionary group of genera and species which cause or potentially cause Irukandji syndrome "the Irukandjiidae"; however, the name itself has no nomenclatural status.

Spread throughout this Irukandji clade, *Carukia barnesi*, “Morbakka”, “Darwin carybdeid”, “Pseudo-Irukandji”, “Broome Irukandji”, and an offshore undescribed *Carukia* have all been connected with Irukandji syndrome or Irukandji-like syndrome of varying intensities (Southcott, 1967; Fenner et al., 1985; Kinsey, 1988; Currie, 2000a; Little and Seymour, 2003; Pearl divers, 2004). No species in this natural group are known to not give systemic symptoms (although the precise syndrome parameters are not well understood for most of these species). One could, therefore, reasonably hypothesize that other species in this clade might give systemic symptoms, thus using phylogeny to predict the potential risks of species of unknown toxicity.

Although all species in this clade should be regarded as potentially capable of causing Irukandji syndrome, that is not to say that all Irukandji-causing jellyfishes are in this clade. *Alatina mordens* gen. et sp. nov. (Carybdeida: Alatinidae fam. nov., described in Chapter 5 below) has also been connected with Irukandji syndrome, and according to Williamson et al. (1996), so have diverse taxa such as some blue bottles (*Physalia* spp.; Hydrozoa: Siphonophora), *Gonionemus* spp. (Hydrozoa: Limnomedusae), and *Nemopilema nomurai* (Scyphozoa: Rhizostomeae).

3.4.6 Resolution within the “Irukandjiidae” clade

Another interesting result of this study is that the Irukandji clade is divided into three different morphological groups, roughly corresponding to genetic groupings (Figure 3.5). Specifically, the *Carukia* and “Pseudo-Irukandji” groups are resolved in both analyses, while the “Darwin carybdeid” + “Morbakka” group evident in the morphological tree is unresolved in the molecular tree, possibly due to the small number of samples.

In the molecular data set, the *Carukia* species cluster separately from the apparently more severe “Pseudo-Irukandji” species (i.e., “Pseudo-Irukandji”, “Broome Irukandji”, “Dampier Irukandji”, and “Halo-Irukandji”), separated by the milder “Morbakkas” and “Darwin carybdeid” (see Appendix 4 for syndrome comparison by species). In the morphological data set, the three groups resolve more completely, although in a hierarchical relationship, with the “Pseudo-Irukandjis” placed in the middle of the other two groups. The paraphyletic arrangement of the morphological Irukandji groups with respect to the remaining Carybdeida has low to moderate support, whereas the molecular sister-group arrangement of the *Carukia* spp. to the remaining Irukandjis has very high support. In scoring the characters for the morphological analysis without *a priori* assumptions about their utility, the drawback is that some characters are probably not reliable for phylogenetic inference (see also Section 6.1.5). I believe that this occurred in this case, and is largely responsible for the muddled pattern in Figure 3.4., especially when compared with the results in Chapter 2.

Besides molecular and morphological concordance, the *Carukia* and "Pseudo-Irukandji" groups can be distinguished on numerous other features. Their cnidomes and statolith morphology (see Chapter 2) were used in a coarse treatment in the morphological data set, but finer comparison reveals even more differences. For example, nematocysts were scored as "lemon-shaped tumiteles" for *Carukia* spp., but "Type 4 microbasic p-mastigophores" for the "Pseudo-Irukandjis"; side-by-side comparison of undischarged and discharged cells reveals the following differences: 1) *Carukia* cnidae are half the size of "Pseudos"; 2) *Carukia* cnidae are lemon-shaped rather than club-shaped, as in "Pseudos"; 3) the tubule winding patterns of *Carukia* have a single main loop, whereas those of "Pseudos" have four main loops; 4) *Carukia* cnidae have a single swelling midway on the shaft, whereas "Pseudos" are cylindrical with no such swelling; and 5) the spines of *Carukia* cnidae emanate from the swelling, whereas those of "Pseudos" are confined to the distal end of the shaft ("Pseudo-Irukandji" and Halo-Irukandji") or along its entire length ("Broome Irukandji").

For the statoliths, only general statolith shape was scored in the phylogenetic data set, but closer comparison reveals additional differences here as well. While the current data set is preliminary (*Carukia* spp., n = 10, "Pseudos", n = 14), some generalizations can be made: "Pseudo" statoliths are somewhat triangular-globular shaped, with an indentation along the "bottom" edge and a projection from the "top" edge; *Carukia* statoliths are somewhat more evenly rounded, lacking both the indentation and the apical projection.

Other differences between the two groups include their distribution, i.e., *Carukia* spp. are generally found on-shore, whereas "Pseudos" are more common off-shore (Gershwin and Hartwick, unpublished collection data). The geographical distribution of syndrome severities, as well as those linked to different species, follows this same pattern (Appendices 4, 5). Furthermore, the groups can be separated on numerous behavioural patterns, such as swarming, apparent preferred water depth, and bloom cycles (Kinsey, 1988; Barnes, unpublished notes; Hartwick and Gershwin, unpublished collection data).

3.4.7 Incomplete sorting of morpho-species in the "Pseudo-Irukandji" group

The two sequenced specimens of "Broome Irukandjis" did not cluster together within the "Pseudo-Irukandji" group. The "juvenile" was captured onshore in Broome in early April following a cyclone, whereas the "adult" was captured about 25 miles offshore from 80 Mile Beach south of Broome in late May. At the times of capture, the smaller specimen, which had only rudimentary gonads, was identified by me as a juvenile of the larger form, which had full gonads. Whether this sorting pattern is due to the genetic phenomenon sometimes referred to as "incomplete sorting" (i.e., differential sorting of ancestral polymorphisms) is unknown. Alternative hypotheses, in light of the results herein, are that A) they represent two different species, and possibly the smaller gonads were a result of recent spawning rather than a juvenile stage, or B) the whole "Pseudo-Irukandji" clade represents a single genetic species, and the morphological differences among "species" are merely population variation. This latter hypothesis seems unlikely given how different they are by comparative morphology (Plate 4.7; Appendix 2); however, more samples of both forms would be necessary to test these hypotheses.

The issue of identification of juveniles is problematical in all groups, but has been particularly troublesome in the "Pseudo-Irukandji" clade. The aforementioned "Broome Irukandjis" may well prove to be different species rather than juvenile and adult of the same species, and similar confusion exists as to whether the "Pseudo-Irukandji" and "Halo Irukandji" are juvenile and adult, respectively, of the same species, or actually different species. At the heart of this "Pseudo"-"Halo" problem is whether the tentacles change through ontogeny – "Halo Irukandji" has, as the name implies, halo-form bands on the tentacles, while the tentacles of "Pseudo-Irukandji" have no such structures (Plate 2.10E and D, respectively). However, I am otherwise unable to tell them apart. To confound the problem, most of the "Pseudo-Irukandji" specimens I have studied lack full gonads, whereas the "Halo Irukandji" specimens I have studied are typically spawning, leading me to wonder whether these are ontogenetic rather than phylogenetic forms.

3.4.8 Incomplete sorting of morpho-species in Australian *Chiropsalmus*

At least two species of *Chiropsalmus* occur in northern Australian waters, one common to North Queensland and the other common to the Gove Peninsula in eastern Arnhem Land, Northern Territory. These species are separable morphologically and genetically, and are compared synoptically in Chapter 4. It is note-worthy, however, that one of the sequences of the North Queensland *Chiropsalmus* did not cluster with the group for reasons that are not currently clear. As above, whether this is due to the genetic concept of "incomplete sorting" is unknown, but not assumed.

It is also interesting to note that these two species of *Chiropsalmus* are geographically separated by a form of *Chironex* (commonly regarded as *C. fleckeri*) which is not deadly (Weipa Hospital staff, pers. comm. 2004), whereas *Chironex* to the West and South of these *Chiropsalmus* species are known to be deadly but are not morphologically identical. Further to the West, the Broome form of "*Chironex*" is not deadly, nor does it morphologically group with the genera *Chironex* or *Chiropsalmus* as defined herein (Chapter 4). And another poorly known form from the northern Kimberley region of Western Australia is different again, based on anecdotal observations from fishermen. Thus, it would seem likely that there are several different species comprising what are currently recognized as *Chironex fleckeri* and *Chiropsalmus* sp. in northern Australia. Clearly, our understanding of the identities and relationships of the chirodropids of northern Australia would benefit greatly from closer examination genetically and morphologically.

3.4.9 Data and analysis considerations

Most of the groupings as mentioned above are congruent, which lends support to those hypotheses. However, two main issues with the analysis became evident that could confound the conclusions. First, maximum likelihood analysis was used for the molecular data set because it allows for application of a model of evolution that best fits that set of data. However, these results do not agree with various analyses by Collins (2002) with respect to the placement of the "*Carybdea alata*" (*Alatina*) clade, leading to the question of reliability of any of these topologies. Clearly more work needs to be done on the *Alatina* group and its phylogenetic position in the Cubozoa; more samples and additional species internal and external to the *Alatina* clade will likely give better resolution. The Bayesian ML tree presented herein (Figure 3.3) should be regarded as one of many possible hypotheses of cubozoan phylogeny, and should be further corroborated with an independent data set, e.g. mitochondrial DNA sequences.

The other main confounding issue is with the scoring of morphological characters, and the assumptions of homology inherent in this process. For identification it is desirable to use as many characters as possible, but this is not equally so for phylogenetic inference. For example, characters such as bell height (Char. 1), bell rigidity (Char. 5), or tentacle base width (Char. 29), to name just a few, are helpful in identification but potentially problematical for phylogenetic study. Specifically, while the body itself is obviously homologous, the reasons controlling its actual height limit might not be; *Carukia* spp. are all about the same size, but members of the "*Carybdea alata*" species complex may be anywhere from 2 cm to 50 cm tall at maturity. Bell rigidity can be a function of thickness of the mesoglea, as in the chirodropids, or stiffness of the mesoglea, as in the *Carybdea* spp. or the very young of all species. And tentacle base width can be a function of small overall size, as in the *Carukia* spp. and *Tripedalia* spp., or it can also be independent of body size, as in *Alatina mordens* gen. et sp. nov. (see Chapter 5) or *Chiropsalmus* n. spp. A and B. All of the characters chosen for analysis are believed to have merit as homologous characters at some scale in the Cubozoa, but many of them clearly are not universally applicable for phylogenetic inference. Future studies should take this issue into careful consideration when selecting characters for comparison.

3.4.10 Applicability of phylogenetic results

While some uncertainties are evident from the analyses, many of the groupings were well supported across analyses and are consistent with qualitative morphological conclusions (e.g., the Alatinidae group, the Irukandji group and *Carukia* + "Pseudo" sub-groups, and the *Tripedalia* + *sivickisi* group). These conclusions are used in turn to better understand the taxonomy of the Cubozoa at all levels, feeding into the revised classification proposed at the end of this chapter (Section 3.4.11) and the monographic revision that will be published separately.

Although the molecular phylogeny herein is still missing some important taxa (e.g., *Tamoya haplonema*, the true *Carybdea marsupialis*, *Tripedalia binata*, *Chiropsalmus quadrumanus*, *Chirodropus gorilla*, *Chiropsoides buitendijki*, the spotted chirodropid, and many new species), it is sufficiently robust and concordant with morphological data to be used predictively for many types of scientific enquiry.

3.4.11 Proposed revised classification of the Cubozoa

Phylum Cnidaria Verrill, 1865 (Hatschek, 1888)
Subphylum Medusozoa Petersen, 1979
Class Cubozoa Werner, 1973
Order Carybdeida Gegenbaur, 1857 (Werner, 1984)
Superfamily Carybdeoidea Gegenbaur, 1857, superfamily nov.
Family Alatinidae fam. nov.
- **Genus *Alatina* gen. nov.**
  - *Alatina mordens* n. sp. – Queensland
  - *Alatina rainensis* n. sp. – Queensland
  - *Alatina alata* (Reynaud, 1830) comb. nov.
  - *Alatina grandis* (Agassiz and Mayer, 1902) comb. nov. = revalidate
  - *Alatina madraspatana* (Menon, 1930) comb. nov. = revalidate
  - *Alatina moseri* (Mayer, 1906) comb. nov. = revalidate
  - *Alatina obeliscus* (Haeckel, 1880) comb. nov. = unrecognizable
  - *Alatina philippina* (Haeckel, 1880) comb. nov. = unrecognizable
  - *Alatina pyramis* (Haeckel, 1880) comb. nov. = revalidate
  - *Alatina tetraptera* (Haeckel, 1880) comb. nov. = revalidate
  - *Alatina turricula* (Haeckel, 1880) comb. nov. = unrecognizable
- **Genus *Manokia* Southcott, 1967**
  - *Manokia stiasnyi* (Bigelow, 1938)

Family Carybdeidae Gegenbaur, 1857, sens. emend.
- **Genus *Carybdea* Peron and Lesueur, 1810, sens. emend.**
  - *Carybdea marsupialis* (Linnaeus, 1758)
  - *Carybdea murrayana* Haeckel, 1880 [Haeckel, 1877 MS]

Carybdea rastonii Haacke, 1886
Carybdea n. sp. – South Africa
Carybdea xaymacana Conant, 1897 var. *xaymacana* n. subsp.
Carybdea xaymacana Conant, 1897 var. *australis* n. subsp.

Family Tripedaliidae Conant, 1897
Genus *Tripedalia* Conant, 1897
Tripedalia binata Moore, 1988
Tripedalia cystophora Conant, 1897
Tripedalia n. sp. – Cuba
Genus *Collostemma*, new genus
Collostemma sivickisi (Stiasny, 1926), comb. nov.

Superfamily Tamoyoidea Haeckel, 1880, superfamily nov.
Family Tamoyidae Haeckel, 1880 (1877 unpublished, as subfamily), new definition
Subfamily Tamoyinae Haeckel, 1880, new subfamily
Genus *Tamoya* F. Müller, 1859
Tamoya haeckeli Southcott, 1967 = should be disregarded
Tamoya haplonema F. Müller, 1859

Subfamily Carukinae, new subfamily – i.e., "the Irukandjiidae"
Genus *Carukia* Southcott, 1967
Carukia barnesi Southcott, 1967
Carukia n. sp. A (Russell's) – Queensland
Carukia n. sp. B (Broome) – Western Australia
New genus A – the mild Irukandjis
"Darwin Carybdeid" sensu Williamson et al., 1996
"Morbakka" spp., sensu Williamson et al., 1996
New genus B – the "pseudo-Irukandjis"
"Broome Irukandji" – Western Australia
"Halo Irukandji" – Queensland
"Pseudo-Irukandji" sensu Barnes in Kinsey, 1988 – Queensland
"Dampier Irukandji" – Western Australia

Order Chirodropida Haeckel, 1880 (Werner, 1984)
Family Chirodropidae Haeckel, 1880 [1877, unpublished] sens. emend.: with branched saccules
Genus *Chirodropus* Haeckel, 1880: with feathered or absent saccules
Chirodropus gorilla Haeckel, 1880
Chirodropus palmatus Haeckel, 1880
Chirodropus n. sp. (spotted) – Great Barrier Reef
Genus *Chironex* Southcott, 1956: with cock's-comb saccules
Chironex fleckeri Southcott, 1956
Chironex n. sp. – Philippines

Family Chiropsalmidae Thiel, 1936, new definition: with simple saccules
Genus *Chiropsalmus* L. Agassiz, 1862: with pendant saccules and bilateral pedalia
Chiropsalmus quadrumanus (Müller, 1859)
Chiropsalmus zygonema Haeckel, 1880
Chiropsalmus n. sp. – Philippines
Chiropsalmus n. sp. – western Africa
Chiropsalmus n. sp. – Mexican Pacific

New genus A: with sessile saccules and bilateral pedalia

"*Chiropsalmus*" sp. A (N QLD) – Queensland
"*Chiropsalmus*" sp. B (Gove) – Northern Territory

Genus *Chiropsoides* Southcott, 1956: with pendant saccules and unilateral pedalia

Chiropsoides buitendijki (Horst, 1907)
Chiropsoides quadrigatus (Haeckel, 1880) comb. nov.

Chirodropida incerta sedis

Genus *Anthracomedusa* Johnson and Richardson, 1968

†*Anthracomedusa turnbulli* Johnson and Richardson, 1968

CHAPTER 4: SYNOPSIS OF THE AUSTRALIAN CUBOZOA

4.1 INTRODUCTION

The objective of this chapter is to provide synoptic tools to facilitate identification of cubozoan species. A brief diagnosis of each species, including information on their distribution and comparison with taxa that they might be confused with, plus a dichotomous key to the Australian species, are given. It is tempting for the non-expert to rely on these tools as a definitive method of identification. However, reliable species identification of cubozoans cannot be made with these guides alone; they provide only a starting point. Accurate identification to the species level must be made comparatively against comprehensive descriptions of known species, based on study of all possible characters. With many unknown forms still to be found, these synoptic tools merely tell what is known to-date; it is up to the investigator to discern the similarities and differences and to decide whether he or she has found something new.

I must caution future workers against adding to the confusion with careless or ill-informed identifications; it has taken me over six years of intensive study to un-knot some of the problematical conclusions of my predecessors, and most will never be resolved. Where there is some doubt as to the identification of a species, it is appropriate to leave the conclusion uncertain, e.g., *Carybdea* sp., or *Carybdea* cf. *rastonii*.

4.2 MATERIALS AND METHODS

This chapter follows the materials and methods already given in Chapters 2 and 3. The characters used herein to diagnose and key the species are distilled from the criteria defined and analysed in Chapters 2 and 3. The arrangement of taxa follows the classification derived from the phylogenetic analyses in Chapter 3. Abbreviations used in the key follow those outlined in Section 2.2.

At various places below the terms “species”, “taxa”, and “forms” are used nearly interchangeably to group individuals; the terms “species” and “taxa” denote described forms, whereas “forms” generally denotes undescribed species and taxa. The term “taxa” may refer to species or to higher taxonomic units, e.g., genera, families.

I have chosen to use common names for undescribed species where they have been previously published or widely used, e.g., “Darwin carybdeid”, in the interest of continuity. Focus herein is given to the Australian taxa, comprising five described species plus 13 conspicuous undescribed forms; the other 14 currently valid species from elsewhere in the world, i.e., those recognized as valid by Kramp (1961) and later authors (Southcott, 1967; Moore, 1988), are treated here to give the new taxa a proper context. Numerous other species previously described from around the world are not included herein for various reasons, including vague descriptions that have rendered the species questionable by previous authors, or in some cases, adequate material is available for revalidation, but the species is outside Australian waters, and thus beyond the scope of this thesis. Numerous additional undescribed forms, which I believe to be worthy of specific recognition, are not included due to their geographical occurrence outside Australian waters.

A tabular comparison of the main diagnostic characters for recognizable cubozoan species and forms is given in Tables 2.14a (Carybdeida) and 2.14b (Chirodropida). Nematocysts are included in the synopses below, and are compared more thoroughly in Table 2.13; in some cases they will be helpful in distinguishing between two species, but using them to define species would require study of more comparative material. The key presented below is limited to the Australian Cubozoa, and is artificial, i.e., the groupings are based on similarity rather than on phylogeny.

4.3 RESULTS

4.3.1 Artificial Key to Australian Cubozoa

1a. Stomach area with gelatinous protrusions into bell cavity, smoothly rounded or cock's-comb-shaped; tentacles numerous, clustered on 4 branched pedalia 15

1b. Stomach area lacking gastric saccules or knob-like protrusions; with 4 or 8 tentacles, borne singly on 1 or 2 separate unbranched pedalia per corner……..…………………...…… 2

2a. With single tentacle on each of 4 interradial corners ……………..……………..……..…… 3

2b. With 2 tentacles per interradial corner; Darwin region ……………..……... *Tripedalia binata*

3a. Gastric cirri lacking; rhopaliar niche ostium frown-shaped (with 1 upper and 1 lower covering scales) ………………………………………………………..…………...…. 4

3b. Gastric cirri present in brush-like bundles or in horizontal or crescentic rows in the corners of the stomach; rhopaliar niche ostium heart-shaped or T-shaped …………………... 11

4a. Body small (ca 1-1.5 cm) and warty; tentacles decorated with widely-spaced handkerchief-like nematocyst bands; pedalial canals simple ………………..……………………….. 5

4b. Body medium-sized (2-6 cm); tentacles otherwise; nematocyst bands with many fine regularly-spaced halo-like bands which contain nematocysts inserted end-wise, or with unadorned bands; pedalial canals with or without upward-pointing spike……………...6

5a. Body small (1 cm), with conspicuous gelatinous mammillations; tentacles with or without minor nematocyst bands, but lack unarmed bands; velarial canals 2 per octant, simple; rhopaliar horns long and narrow; N. QLD …....................................... *Carukia barnesi*

5b. Body about 1.5 cm, smooth but with low rounded nematocyst warts; tentacles with about 14 fine unarmed bands between successive handkerchief bands; velarial canals 2 per octant, branched, each with 1 large nematocyst wart; rhopaliar horns long and narrow, capitate; Broome region ……….……..… *Carukia* n. sp. B

6b. Tentacles with halo-like nematocyst bands ……………….……………………………..…. 7

6a. Tentacles with "normal" cubozoan nematocyst bands, lacking halos …..………….……… 8

7a. Body 3-4 cm, lacking gelatinous protuberances; with complexly branched velarial canals; with narrow triangularly-shaped perradial lappets with 2 rows of 3-4 nematocyst warts; with relatively short, broad, inwardly-curved rhopalial horns; northern QLD ………...…………………………….…………………………..….. "Halo-Irukandji"

7b. Body about 2 cm tall, with halo-like tentacle bands; with 2 velarial canal in each octant, single main canal broadly bifurcated, and a narrow ad-interradial; with large, rounded beehive-shaped perradial lappets, with a single large nematocyst wart on one side of the lappets; with broad, nearly vertical rhopaliar horns; Dampier region …………………...…………………..……………………………..…. "Dampier Irukandji"

8a. Pedalial canal with prominent upward pointing spike at the bend …………….……...…. 9

8b. Pedalial canal without prominent spike at the bend ……………………...…………..…… 10

9a. Bell extremely large (10-15 cm or more); tentacles wide and flat, flaring at top; velarial canals extremely numerous and pinnate; coastal QLD & NSW .... "Morbakka"

9b. Bell smaller (3-5 cm); pedalial canals square in cross section along most of length; velarial canals dendritic and diverticulated, with 2 rows of conspicuous round nematocyst patches on perradial lappets; Darwin area ..…................................ "Darwin carybdeid"

10a. Body up to about 5 cm, with a noticeably flat top; with variable pedalial canal bend form, but never spiked; 1 velarial canal root branching into about 4 simple, crooked canals per octant; rhopalial horns short and broad, often curved inward like Viking horns; offshore Broome area ………………...…… "Broome Irukandji"

10b. Pedalial spike reduced to a nub; bell typically 3-4 cm, taller than wide, with a rounded top; 1 palmate velarial canal per octant, with numerous simple branches; northern QLD ………………………….………………………………………… "Pseudo-Irukandji"

11a. Stomach flat, completely lacking mesenteries; phacellae in large crescentic bundles in corners of stomach, with long cirri; velarial canals 3 per octant, of variable form typically simple; rhopaliar niche ostium T-shaped (with 1 upper and 2 well developed lower covering scales) ..……………………………...…. 12

11b. Phacellae in brush-like bundles or in oblique rows across stomach corners; stomach flat, with poorly developed mesenteries; velarial canals 2 per octant, biforked or complexly branched; rhopaliar niche ostium heart-shaped (with 1 upper and 2 vestigial lower covering scales), or open (lacking covering scales) ……….. 13

12a. Bell to 10 cm, exceedingly taller than wide; cirri in crescentic rows of long parallel filaments; nematocyst freckles very tiny, evenly scattered; velarial canals simple, 3 per octant; rhopalial niches T-shaped; GBR ……………………..... *Alatina mordens* n. sp.

12b. Bell to about 2 cm, with very minute sparsely scattered nematocyst freckles; with butterfly-form gonads, attached only in the center region of the interradial septa; with very long pedalial stalks; GBR …………..……………………………… *Alatina rainensis* n. sp.

13a. Rhopaliar niche ostium heart-shaped ………………...…...…………...………………..… 14

13b. Rhopaliar niche ostium like a vertical key-hole, lacking covering scales; bell minute, typically less than 1 cm, with adhesion pads near apex which may or may not be visible; tentacles banded brown and orange; N. QLD …………………......… *Carybdea sivickisi*

14a. Phacellae in single-rooted brush-like dendritic bundles; bell about 1-2 cm, with scattered nematocyst freckles; velarial canals 2 per octant, of 2 forms: those nearest the rhopaliar radii simply dichotomous, those nearest the pedalial radii complexly dichotomous; rhopalial niches heart-shaped; Southern WA and Far North QLD ……………………………………...…………………….…*Carybdea xaymacana*

14b. Phacellae in elongated rows, set obliquely across stomach corners; bell 3-4 cm; cirri in horizontal rows of small bundles; nematocyst freckles scattered over bell and along pedalial outer keel; velarial canals 2 per octant, biforked; rhopalial niches heart-shaped; SA and Southern WA ……………………………………….....…… *Carybdea rastonii*

15a. Body well pigmented with brownish spots; subumbrella with horizontal muscle bands; gastric saccules absent; gonads filamentous; GBR……….....……. *Chirodropus* n. sp. A

15b. Body typically transparent and colourless ……………………...……………………... 16

16a. Pedalial canal with upward-pointing thorn at bend; with cockscomb-like saccules that are functioning gonads; up to 4 x 15 tentacles, flat in cross section and thick; body size to 38 cm, lethal above 8-10 cm; tropical Australia …............................... *Chironex fleckeri*

16b. Pedalial canal lacking thorn, typically knee-shaped; tentacles fewer than 10 per corner, round and fine; gastric saccules sessile, knob-shaped or coalesced ....… 17

17a. Pedalial canal bend rounded or with sharp 90° angle, lacking thorn; gastric saccules solid, smoothly rounded, separate knob-like swellings; up to 4 x 9 fine, round tentacles; body size 8-10 cm; northern QLD ……………….....……… *Chiropsalmus* n. sp. A (N. QLD)

17b. Pedalial canal bend rounded, lacking thorn; gastric saccule pairs coalesced into a single, solid, kidney bean-shaped swelling; up to 4 x 6 fine, round tentacles; body size to about 5 cm; Arnhem Land, N.T. ……..….................................*Chiropsalmus* n. sp. B (Gove)

4.3.2 Synopsis of Cubozoa plus new Australian taxa

Class Cubozoa Haeckel, 1880 (Werner, 1973b)

Order Carybdeida Gegenbaur, 1857 (Werner, 1984)

Superfamily Carybdeoidea Gegenbaur, 1857, superfamily nov.

Family Alatinidae fam. nov.

Alatina mordens gen. et sp. nov. (Plate 4.1A)

IDENTIFICATION. Commonly called the "Outer Reef Irukandji", this species belongs to the "*Carybdea alata*" species complex, with crescentic phacellae, T-shaped rhopaliar niche ostia, and 3 velarial canals per octant. This species is characterized by having a very tall, narrow body (typically reaching about 12 cm), of a thin gelatinous consistency; large, conspicuous phacellae visible through the body wall; large, black eyes; and broadly-rounded pedalia. The gonads are attached along the entire bell height and grow outward into the bell cavity, becoming heavy and pleated when near spawning. The upper covering scale of the rhopalial niche is broadly rounded. The statoliths are the only type known so far that is taller than wide, of a peculiar truncate teardrop-shape, and deep garnet in colour. The tentacular nematocysts are of one type only, i.e., lemon shaped euryteles, 19-27 x 11-15 µm.

DISTRIBUTION. Common all months of the year on the outer Great Barrier Reef, especially around the 9th night after the full moon (R. Hore, pers. comm., 2004).

OTHER TAXA TO BE CONFUSED. This species might be confused with its congeners outside QLD, but is quite distinct within the Australian Cubozoa. It has a tall body like some of the "Morbakkas", but the "Morbakkas" are very robust and the exumbrellar surface is very warty, whereas *A. mordens* has a narrow, flimsy body and the bell is smooth.

Alatina rainensis gen. et sp. nov. (Plate 4.1B)

IDENTIFICATION. This is a peculiar and remarkable little medusa, belonging to the "*Carybdea alata*" species complex with crescentic phacellae, T-shaped rhopaliar niches, and 3 velarial canals per octant. The body is about 2.5 cm tall, sparsely scattered with minute nematocyst freckles; with large, conspicuous phacellae visible through the bell wall; with butterfly-wing-shaped gonads attached only at the center; and with very long pedalial stalks. The upper covering scale of the rhopalial niche is W-shaped. Three types of tentacular nematocysts are present: 1) microbasic euryteles, 15-20 x 10-13 µm; 2) sub-spherical isorhizas of two size classes, 16-24 x 13-18 µm and 6-8 x 6-7 µm; and 3) tiny nearly spherical microbasic amastigophores, 6-7 µm. DISTRIBUTION. Only recorded from Raine Island on the outer Great Barrier Reef.

OTHER TAXA TO BE CONFUSED. Because of its small size (about 2 cm bell height), this species might be mistaken at first glance for the *Carybdea* spp. or some of the undescribed Irukandjis such as "Pseudo-Irukandji" or "Halo-Irukandji". The Irukandji species do not have phacellae and the rhopaliar niche ostia are frown-shaped; the *Carybdea* spp. have bush-like or linear phacellae and the rhopaliar niche ostia are heart-shaped; *A. rainensis* has crescentic phacellae and the rhopaliar niche ostia are T-shaped.

Alatina spp. (= *"Carybdea alata"* auct. species complex; Plate 4.1C)

IDENTIFICATION. All forms have a tall, narrow bell, with a thin consistency, T-shaped rhopalial niche ostia, and crescentic gastric phacellae. They differ in the number of eyes on the mature rhopalium, the number and complexity of the velarial canals, the rhopalial window size and shape, and whether they have bell nematocysts. Preliminary study indicates that the number of trunks to the gastric phacellae is variable; further study over more species may prove that this is an important character as well. The statoliths and nematocysts of the different species have not yet been studied.

DISTRIBUTION. Members of the *Alatina* group are relatively common throughout the tropics of the world (see Chapter 5 and references therein).

OTHER TAXA TO BE CONFUSED. Members of this group would not easily be confused with other taxa, based on the unique crescentic phacellae and T-shaped rhopaliar niches, though the precise characters to distinguish taxa within the group have not been entirely resolved. I have identified at least 6 different morphological forms among museum collections, each of which is segregated geographically. It is, however, a difficult group to resolve morphologically, given the large number of earlier named species based on poor descriptions. The species are treated in more depth in Chapter 5.

Manokia stiasnyi (Bigelow, 1938) (Plate 4.1D)

IDENTIFICATION. Small (ca. 2 cm bell height), with crescentic phacellae, and T-shaped rhopalial niches. The upper rhopaliar covering scale possesses a median flap, rather than being smooth as in other carybdeids. The velarial canals are 4 per octant, simple, and undulating in outline, and the bell is moderately sculptured with thickened interradii. The fine, round tentacles are said to bear lateral branches, but in fact, it is not the tentacles themselves that are branched, but rather the nematocyst bands, each being drawn out adaxially into a short, blunt projection. Furthermore, this species has the full complement of 2 median lensed eyes and 4 lateral eye spots. The tentacular nematocysts are of a single type, i.e., sub-spherical euryteles with a thick capsule wall, 13-17 x 12-14 µm.

DISTRIBUTION. Type locality: Manokwari, Papua New Guinea. Not reported since.

OTHER TAXA TO BE CONFUSED. The branched appearance of the tentacles would immediately preclude this species from being easily mistaken for any other.

Family Carybdeidae Gegenbaur, 1857, sens. emend.

Carybdea marsupialis (Linnaeus, 1758) (Plate 4.2E)

IDENTIFICATION. Medium sized (ca. 4-5 cm bell height), with heart-shaped rhopaliar niche ostia, epaulette-shaped phacellae, scalpel-shaped pedalia, and 3-4 dendritic velarial canals per octant. The statoliths of *C. marsupialis* have not yet been studied. The tentacular nematocysts are of three types: 1) heterotrichous microbasic euryteles, 17-42 x 12-24 µm; 2) atrichous isorhiza haplonemes, 9-18 x 4-10 µm; and 3) holotrichous isorhizic haplonemes, 15-23 x 14-23 µm (Avian et al., 1997).

DISTRIBUTION. Type locality: Rimini, Italy; common throughout the Adriatic and Mediterranean (Plancus, 1739; Linnaeus, 1758; Kramp, 1961; Boero and Minelli, 1986; Mizzan, 1993); also reported from the Aegean (Geldiay and Balik, 1977). Kramp (1961) reported *C. marsupialis* from Malaya; this was actually a misprint from his 1955 revision of Haeckel's material from Malaga, Spain. Often reported from the Caribbean and California, but these erroneous reports actually apply to *C. xaymacana* and an undescribed species, respectively.

OTHER TAXA TO BE CONFUSED. Overall this species is most similar to *Carybdea xaymacana*, though it is typically considerably larger, about the size and shape of *Carybdea rastonii*. However, the phacellae are in a tight corner bundle of numerous roots (8-10), whereas in *C. xaymacana* the corner bundle has only 1 root, and in *C. rastonii* the phacellus is stretched across the corner rather than bunched into it. Furthermore, *C. rastonii* and *C. xaymacana* both have only 2 velarial canals per octant, whereas *C. marsupialis* typically has 3-4.

Carybdea rastonii Haacke, 1886 (Plate 4.2A)

IDENTIFICATION. Medium sized (ca. 3-4 cm bell height), with heart-shaped rhopalial niche ostia, scalpel-shaped pedalia, and linear phacellae orientated obliquely across the corners of the stomach. The velarium has 2 canals per octant, and they tend to be quite complexly branched, even at a relatively immature stage. The bell is fairly robust, and protected with numerous nematocyst patches, which may be on slight gelatinous warts. The statoliths are broad and dome-shaped (or thick, kidney-bean-shaped) with rounded edges. The tentacular nematocysts are of two types: 1) football-shaped microbasic euryteles or tumiteles, 20-30 x 13-18 µm; and 2) egg-shaped isorhizas, 9-13 x 7-8 µm.

DISTRIBUTION. Type locality: Port Victoria, South Australia. Common along the southern coasts of Australia as far west as Albany, WA, and up into the Sydney region on the east coast. Outside Australia it has been reported from Japan (Maas, 1909; Yatsu, 1917; Yatsu, 1918; Okada, 1927; Uchida, 1927; Uchida, 1928; Uchida, 1929; Stiasny, 1931; Ishida, 1936; Uchida, 1938a; Uchida, 1938b; Uchida, 1938c; Uchida, 1947b; Uchida, 1954; Uchida, 1955; Yamazi, 1958; Uchida, 1970; Ueno et al., 1995; Ueno et al., 1997; Kubota, 1998; Oishi et al., 1999; Nagai et al., 2000; Ueno et al., 2000); Honolulu, Hawaii (Mayer, 1906; Devaney and Eldredge, 1977); the Philippine Islands (Mayer, 1915; Stiasny, 1922); Malayan Archipelago (Stiasny, 1935; Stiasny, 1937b); China (Chiu, 1954); Marquesas Islands (Kramp, 1956a); Southern California, USA (Stiasny, 1922; Satterlie, 1979; Satterlie and Spencer, 1979; Matsumoto, 1995); and Vietnam (Kramp, 1962). California material has proven to belong to other species (Larson and Arneson, 1990), as have Japanese specimens (Gershwin, unpublished); remaining non-Australian reports should be considered with caution.

OTHER TAXA TO BE CONFUSED. This species is immediately distinguishable from all other known cubozoans by its possession of obliquely orientated, linear phacellae, with many separate tufts of cirri. When young, it might be mistaken for *C. xaymacana*, but is immediately distinguishable by the form of the phacellae. It might also be mistaken for young chirodropids, or any of the undescribed Irukandjis, but would be immediately distinguishable by the shape of the rhopaliar niche ostia.

Carybdea xaymacana Conant, 1897 (Plate 4.2B, C)

IDENTIFICATION. Body small to medium (about 2-3 cm tall), with heart-shaped rhopaliar niche ostia, scalpel-shaped pedalia, and epaulette-like phacellae. The velarial canals are 2 per octant, with the adperradial typically being much less branched than the adinterradial. The South Western Australian form of *C. xaymacana* has statoliths of a similar shape to *C. rastonii*, i.e., rounded pentagonal shape, broad and dome-shaped, or thick, kidney-bean-shaped, depending on one's perspective. The tentacular nematocysts of the southwestern Australian form are of two types: 1) large club-shaped microbasic euryteles, range 26-37 x 12-15 µm; and 2) small oval isorhizas, 9-12 x 5-7 µm. The tentacular euryteles of the Caribbean form are about the same size (29-33 x 12-15 µm), but have a much finer tubule; isorhizas could not be found.

DISTRIBUTION. Type locality: Jamaica; common throughout the Caribbean. Curiously, there appears to be two separate populations of *C. xaymacana* in Australian waters, for which I am at a loss of explanation. They are exceedingly common along the southern Western Australian Indian Ocean coastline, and they were captured at least once by R. Hartwick and twice by J. Seymour in Far North Queensland (unpublished collection records). I am unable to tell the Australian and Caribbean forms apart structurally, though the Caribbean form reaches a slightly greater bell height and, as stated above, has slightly different nematocysts.

OTHER TAXA TO BE CONFUSED. Quite similar to, and often mistaken for, the Mediterranean *Carybdea marsupialis*, but the two species are easily identifiable. At maturity, *C. marsupialis* can get much larger, to about 40-50 mm, whereas *C. xaymacana* rarely reaches over 30 mm, typically more in the 20-25 mm range. The phacellae of *C. xaymacana* have only a single root into the floor of the stomach, whereas in *C. marsupialis* each has numerous roots. Finally, *C. marsupialis* typically has 3-4 quite branched velarial canals per octant, whereas in *C. xaymacana* they are 2 per octant and much less complex. The species is also often mistaken for *C. rastonii*, but in *C. rastonii* the phacellae are linear across the stomach corners, rather than epaulette-like in the corners.

Carybdea aurifera Mayer, 1900 (Plate 4.2D)

REMARKS. Kramp (1961) regarded this species as possibly the young of *C. sivickisi*, whereas Arneson and Cutress (1976) considered it to be a juvenile of "*C. alata*". However, *C. sivickisi* and "*C. alata*" are in very different family groups (see Chapters 2, 4, 6), underscoring the problem of describing juvenile forms. In fact, *C. aurifera* as described can be attributed to just about any cubozoan species for which the early juvenile form is known. To help stabilize cubozoan nomenclature, this and other names belonging to unrecognizable juveniles should be permanently abandoned.

Other *Carybdea* spp. (Plate 4.3A, B)

REMARKS: Several other forms of *Carybdea* are known but not yet described; two of these are used comparatively in the molecular and morphological treatments of Chapters 2 and 3. One (Plate 4.3A), from Cape Town, South Africa, is related to *Carybdea marsupialis*, but has been erroneously identified as "*Carybdea alata*" (Uchida, 1970; Branch et al., 1994) and *Tamoya* *haplonema* (Pagès et al., 1992) as well. This form differs from *C. marsupialis* in having a much larger, more robust body; phacellae comprised of many roots; and an unmistakable colouration pattern.

The other (Plate 4.3B), from Japan, is related to and often erroneously identified as *Carybdea rastonii* (Ueno et al., 1995; Ueno et al., 1997; Ueno et al., 2000). It differs from *C. rastonii* in having a larger, more robust body with a much wartier exumbrellar surface; furthermore, the nematocysts are quite remarkably different. Whether this is identical to a suppressed species or is new to science is still being ascertained.

Family Tripedaliidae Conant, 1897

Collostemma gen. nov.

Collostemma sivickisi (Stiasny, 1926) comb. nov. (Plate 4.4A)

IDENTIFICATION. Small (less than 1 cm bell height), with a highly sculptured bell and vertical keyhole-shaped rhopalial niche ostia, lacking the upper and/or lower covering scales typical of other species. Other conspicuous characters include the pedalial keels being very narrow and the outer keel is decorated with a series of horizontal nematocyst bars; the phacellae comprise a crescent-shaped bundle of cirri which are individually rooted in the floor of the stomach; and the velarial canals are 2 per octant and paw-like in shape. Furthermore, the exumbrella has four adhesive pads, one over each interradial quadrant of the stomach, though these are not always conspicuous. The gonads grow from the stomach downward, having a somewhat pendant appearance inside the coelenteron. The North Queensland form of *C. sivickisi* has horizontally elongate, sausage-shaped statoliths. The tentacular nematocysts are of four different types: 1) football-shaped euryteles, 13-19 x 10-12 μm; 2) football-shaped isorhizas with full tubules, 16-19 x 10-12 μm; 3) round euryteles, 11-12 x 10-11 μm; and 4) long oval isorhizas, 9-11 x 4-6 μm.

DISTRIBUTION. Type locality: Philippines and Thailand (Stiasny, 1922; Stiasny, 1926a; Kramp, 1968b). It has since been reported from Vietnam (Kramp, 1962), Japan (Uchida, 1929; Uchida, 1970; Kubota, 1998), New Zealand (Hoverd, 1985), northeastern Australia (Hartwick, 1991), Hawaii (Matsumoto et al., 2002), and Guam (Gershwin, 2003). In Australia, the species has been found at Magnetic Island off Townsville; at the Whitsundays; at Palm Cove north of Cairns; at Ulverstone on the Tasmanian side of the Bass Strait; and at Port Victoria, Yorke Peninsula, South Australia.

OTHER TAXA TO BE CONFUSED. This species is immediately distinguishable from all other cubozoans by its vertical oval or key-hole-shaped rhopalial niche ostium; it would be impossible to mistake this for any other species.

Tripedalia binata Moore, 1988 (Plate 4.4B)

IDENTIFICATION. Body small (ca. 1 cm tall), with two simple, narrow pedalia per corner, each bearing a single tentacle. The rhopalial niche ostia are of a very shallow frown shape, and the rhopalial stems are adherent to the roof of the rhopalial niche. The velarial canals are 3-4 per octant, and perradial lappets are lacking. The statoliths are of a thickened V-shape or heart-shape form. The tentacular nematocysts are of two types: 1) stenoteles, 18-20 x 10-15 µm; and 2) spherical atrichous isorhizas, 12 µm, with a 10:1 ratio of stenoteles to isorhizas (Moore, 1988).

DISTRIBUTION. Type locality: Darwin region, Northern Territory. Also reported from Weipa on Cape York and from the Cairns region (J. Seymour, pers. comm.).

OTHER TAXA TO BE CONFUSED. This species is similar to *Tripedalia cystophora* except that it has only two pedalia per corner, and thus only a total of 8 tentacles instead of 12, and the velarial canals may be 3 or 4 per octant, instead of 2.

The meristic difference in the pedalia appears to be a mutation from the tripedalial form, which has become fixed in the population. In this Northern Territory species, it is always the left-side pedalium which is lost, leaving the centermost and the right-side intact. This same condition can be obtained in laboratory raised specimens of *T. cystophora* (unpublished obs., Monterey Bay Aquarium 2002), and has been seen once in a population from India (Jambu River, Orissa State, N. Annandale, unpublished coll. 1901, NHM).

Tripedalia cystophora Conant, 1897 (Plate 4.4C, D)

IDENTIFICATION. The body is wider than tall (about 1 cm bell height), with numerous minute nematocyst patches. The most obvious character separating this species from all others is that it has three separate pedalia on each corner of the bell, and in typical carybdeid form, each pedalium has but a single tentacle. Other distinctive characters include the unusual butterfly appearance of the gonads, and the 3 simple velarial canals per octant. The rhopalial niche ostium is atypically-frown-shaped, i.e., with a single covering scale above and an indented, V-shaped margin on a shallow scale below. The phacellae are similar to those of *Carybdea xaymacana*, i.e., on a single brush-like corner bundle. The tentacular nematocysts are of two types: 1) heterotrichous microbasic euryteles, 16-23 x 12-16 µm; and 2) holotrichous haplonemes of two size classes, 9-14 x 6-7 µm and 12-15 x 11-14 µm (Werner, 1975).

DISTRIBUTION. Type locality: Jamaica; it has since been reported from the Philippines (Stiasny, 1926a), Japan (Uchida, 1970), Puerto Rico (Werner et al., 1971), and Brazil (Morandini and

Marques, 1996). I have also examined museum specimens from the Seychelles, Indonesia (near Borneo), and Grand Bahama (Gershwin, unpublished).

OTHER TAXA TO BE CONFUSED. It would be unlikely to mistake this species for any other, given the peculiar replication of pedalia on each corner of the bell.

Superfamily Tamoyidea Haeckel, 1880, superfamily nov.

Family Tamoyidae Haeckel, 1880, sens. emend.

Subfamily Carukiinae subfamily nov. – i.e. "The Irukandjiidae" (informal)

Carukia barnesi Southcott, 1967 (Plate 4.5A, B)

IDENTIFICATION. Originally distinguished primarily based on the absence of phacellae, *Carukia barnesi* is quite distinctive in a number of features. The body is small (ca. 1 cm bell height) and quite mammillated, with an interesting reticulated pattern partitioning off each wart on the apex. The tentacles have unmistakable "handkerchief-like" or "tailed" rings, with fairly long regions of unadorned tentacle shaft in between. The rhopalial niche ostia are of the frown form, with the "rhopalial horns" very long at a strong upward angle, and the pedalial canals are simple, lacking any sort of diverticula at the bend. The velarium is quite distinctive, with 2 canals per octant, all alike in the form of simple triangles, with a single nematocyst wart on the one nearest the perradius, and the perradial lappets are present but lacking nematocyst warts. The statoliths are sub-circular in outline, without a basal concavity and lacking an apical "tooth" projection. The tentacular nematocysts are of a single type only, i.e., egg-shaped euryteles or tumiteles, 25-26 x 15-18 µm (Southcott, 1967).

DISTRIBUTION. Type locality: Cairns region, far north QLD. The species has been frequently reported across northern Australia, based on the occurrence of Irukandji syndrome rather than on the identification of specimens. So far, it has only been confirmed from Port Douglas to the Whitsundays in North Queensland.

OTHER TAXA TO BE CONFUSED. The small, pyramidal, warty bell and the tailed tentacle bands make this species difficult to confuse with any species outside the genus. Several new species are being added to the genus; see below for morphological differences.

Carukia n. sp. A (Russell's) (Plate 4.5C)

IDENTIFICATION. Small (ca. 1 cm bell height), with a thimble-shaped bell and narrow pedalia. Velarial canals 1 per octant, more or less palmate with several branches, lacking nematocyst warts. Tentacles with tailed bands as described for *C. barnesi*. Rhopaliar niche ostia large open smile-shaped, i.e., frown-shaped but with the lower covering scale concave instead of convex. The statoliths are sub-circular without a basal concavity, similar in general shape to those of

C. barnesi. The tentacular nematocysts are of a single type, i.e., lemon-shaped euryteles or tumiteles, with distal-facing spines at the shaft's distal end, 18-25 x 13-15 µm.

DISTRIBUTION. Rare; only reported from the outer Great Barrier Reef region.

OTHER TAXA TO BE CONFUSED. This species is about the size of *Carukia barnesi*, and has similar tentacles, but differs in having only a single velarial canal in each octant, and it is branched instead of simple. Furthermore, this form lacks the perradial mesenteries that are found in *C. barnesi* and *Carukia* sp. B.

Carukia n. sp. B (Broome) (Plate 4.5D)

IDENTIFICATION. Small (ca. 1.5 cm bell height), with a thimble-shaped bell and narrow pedalia. Velarial canals branched, with a single large nematocyst wart on each velarial canal and perradial lappet pair. Rhopaliar horns long, narrow, capitate. Tentacles with similar tailed bands as described for *C. barnesi*, but with 14 naked ringlets between adjacent bands. The tentacular nematocysts are of a single type, i.e., lemon-shaped euryteles or tumiteles, 19-28 x 13-18 µm.

DISTRIBUTION. Only recorded once off Broome, Western Australia.

OTHER TAXA TO BE CONFUSED. The tailed tentacle bands might confuse this species with *C. barnesi*, but this species is half again as tall and wide and much less warty than *C. barnesi*, and the velarial canals are dendritically branched, whereas they are simple in *C. barnesi*. Furthermore, this species has nematocyst warts on all canals and the lappets, whereas in *C. barnesi* they are confined to one canal and lacking on the lappets. This species might also be superficially confused with *Carybdea xaymacana* because of its small size, but the tentacles and rhopaliar niche ostia would immediately distinguish the two forms.

Unnamed new Genus A: The Mild Irukandjis

"Darwin Carybdeid" (Plate 4.6A)

IDENTIFICATION. This species is the most robust of the medium-sized carybdeids. The body is about the size of a matchbox (ca. 4-6 cm tall), quite boxy in 3-dimensional shape, the rhopalial niche ostia are frown-shaped, horn-shaped blind-ending canals project upward from the upper back edge of the rhopalial niche, the pedalial canals have a rose-thorn-like diverticulum, the mesenteries are well developed, the phacellae are lacking, and the velarial canals are 7 per octant and branch so heavily in older specimens that they cover the velarium. Furthermore, scattered nematocyst freckles are found on the outer keel of the pedalia. The statoliths and nematocysts have not yet been studied.

DISTRIBUTION. Reported primarily from the Darwin Harbour region of the Northern Territory, with a couple of reports from North Queensland.

OTHER TAXA TO BE CONFUSED. The robust nature and medium size of this species would make it difficult to mistake for another species. It could possibly be confused with the taller, narrower "Broome Irukandji", but the pedalial canals of the "Darwin carybdeid" possess a thorn and the velarial canals are quite complexly branched, whereas the pedalial thorn structure is lacking in the "Broome Irukandji", and the velarial canals of the latter are far less complex. Because of the pedalial canal thorn, it might be possible to mistake this species for the "Morbakkas" or even *Chironex fleckeri*, but the large, warty bell of the "Morbakkas" and the branched pedalia of *Chironex* would make either unlikely.

"Morbakka" (Plate 4.6B, D)

IDENTIFICATION. There appears to be at least 3, possibly 4, forms of this undescribed group. Collectively, they are recognizable as having a large, robust warty body; flattened, ribbon-like tentacles; a prominent "rose-thorn" extension of the pedalial canal bend; and completely lacking gastric phacellae. The regional forms differ in body size, and other characters are currently being studied. The tentacular nematocysts of the North Queensland form were studied on a specimen from Port Douglas, and were found to be of three types: 1) club-shaped microbasic p-mastigophores, 61-70 x 14-19 μm; and 2 & 3) oval isorhizas of two types, one with a loose tubule, the other with a tight tubule, both 49-57 x 28-34 μm.

DISTRIBUTION. Rare; different forms are found in northern Queensland from Port Douglas to Mackay, the Moreton Bay region, and the Sydney region.

OTHER TAXA TO BE CONFUSED. "Morbakkas" are similar in overall appearance to, and often wrongly identified as, the western Atlantic *Tamoya haplonema*, but differ in their lack of gastric phacellae, their possession of perradial lappets on the velarium and rhopalial "horns", and in lacking lateral eye spots on the rhopalia. Among Australian taxa, "Morbakkas" could possibly be confused with *Alatina mordens* gen. et sp. nov. based on their tall bodies, but the two groups differ in almost every other scorable character. "Morbakkas" might also be confused with the "Darwin carybdeid" because of the pedalial canal thorn, but "Morbakkas" are relatively taller and wartier.

Unnamed new Genus B: The "Pseudo-Irukandjis"

"Broome Irukandji" (Plate 4.7A)

IDENTIFICATION. This species is characterized by a tall (to ca. 5 cm), narrow, robust body, though not as robust as the "Darwin carybdeid"; tentacles are remarkably fine and round. Rhopalial niche ostia frown-shaped, with short, broad rhopaliar horns. Pedalial canals squared or diamond-shaped in cross section, with a short, flat-topped, oblique extension at the bend.

Velarial canals about 4 per octant, simple to end-biforked, somewhat undulating in outline, all emanating from a single root; perradial lappets with 1-4 (typically 2) round warts per side. Rhopalia with 2 median eyes only, lacking lateral pigment spots; statoliths are rounded pyramidal in shape, with a deep basal concavity. The tentacular nematocysts are of a single type only, i.e., club-shaped sub-ovate microbasic p-mastigophores (Type 4); spines full length of the shaft, 35-49 x 15-20 µm.

DISTRIBUTION. Common offshore from Broome, Western Australia (Gershwin, unpublished).

OTHER TAXA TO BE CONFUSED. This species might be confused with the "Darwin carybdeid" from the Northern Territory or with "Pseudo-Irukandji" or "Halo-Irukandji" from N. QLD. The "Darwin carybdeid" has a prominent thorn at the bend of the pedalial canal, whereas the thorn is lacking in the present species. This species is much larger and more robust than either of the two comparable Irukandjis, and the pedalia of the "Broome Irukandji" are much broader, though the tentacles of are much finer.

"Dampier Irukandji" (Plate 4.7B)

IDENTIFICATION. Small body (ca. 1.5 cm bell height), with halo-like tentacles, i.e., the nematocysts are inserted end-on in tightly spaced shelf-like bands of tissue. Velarial canals two per octant, a main broadly bifurcated and diverticulated velarial canal centrally located in each octant, plus a narrower canal on each side of the interradii. Perradial lappets broadly rounded, beehive-shaped, with a single large nematocyst wart on one side only of the perradial lappets. Rhopalial horns broad and nearly vertical. The tentacular nematocysts are rice-shaped sub-ovate microbasic p-mastigophores, 32-40 x 14-17 µm.

DISTRIBUTION. From the Dampier region of Western Australia.

OTHER TAXA TO BE CONFUSED. The tentacles are similar to those of the "Halo-Irukandji", but this species is only about half as tall as the latter. It might also be superficially confused with any of the other smaller carybdeids, e.g., *Carybdea xaymacana*, *Alatina rainensis*, or the young of just about any species, but the peculiar tentacle banding would immediately distinguish this species.

"Halo-Irukandji" (Plate 4.7C)

IDENTIFICATION. Body medium-sized (ca. 2-3 cm tall) and fairly robust, with frown-shaped rhopaliar niche ostia, scalpel-shaped pedalia, and phacellae lacking. The tentacle bands of this species are closely spaced and halo-like, each possessing a narrow shelf of tissue projecting outward, with a row of large mastigophore nematocysts inserted end-on and arrayed out from the periphery of the tissue. Furthermore, the pedalial canal may have a hump at the bend, and the velarial canals are typically 4 per octant and complexly branched. The tentacular nematocysts are of a single type only, i.e., club-shaped sub-ovate microbasic p-mastigophores, 33-38 x 12-16 µm.

DISTRIBUTION. Reported only in North Queensland from the Mackay-Whitsundays region and from Port Douglas.

OTHER TAXA TO BE CONFUSED. This species has sometimes been mistaken for *Carukia barnesi*, though the former is considerably larger and more robust, and the tentacles are entirely different. It would be most likely to be confused with "Pseudo-Irukandji", with the two types differing in the form of the tentacles. In fact, it is not yet clear whether these two are merely growth stages of the same species or actually separate but closely related species.

"Pseudo-Irukandji" (Plate 4.7D)

IDENTIFICATION. Very similar to the "Halo-Irukandji", with a medium-sized (ca. 2-3 cm tall) and fairly robust body, with frown-shaped rhopaliar niche ostia, scalpel-shaped pedalia, and phacellae lacking. The pedalial canals are rounded or squared proximally in cross section, flattened with axis of pedalia distally; with small raised nub at the bend. The velarial canals are only 1 per octant, but with numerous distal digitations, and the perradial lappets have 2 rows of 2-3 nematocyst warts. The rhopalia lack the 4 lateral eye spots, having only the two median lensed eyes. Statoliths are of a sub-circular three-dimensional shape, with a basal concavity and an "apical tooth". The tentacular nematocysts are of a single type only, i.e., club-shaped sub-ovate microbasic p-mastigophores (Type 4), with spines confined to the distal end of the shaft, 30-37 x 13-16 µm.

DISTRIBUTION. Relatively rare; recorded in the North Queensland region from Port Douglas to Mackay. Preliminary data suggest that this species might be more common late in the summer and through the winter.

OTHER TAXA TO BE CONFUSED. This species has been confused with *Carukia barnesi*, though they are really quite different. The bell of the former is less pyramidal in shape and much less warty, and this form is about twice the size of *C. barnesi*, with tentacles of the unadorned "normal" banded type, rather than the tailed *Carukia barnesi* type. This species may or may not be an immature form of the Halo-Irukandji, with tentacles that lack the halo-like shelves of tissue.

Subfamily Tamoyinae Haeckel, 1880, subfamily nov.

Tamoya haeckeli Southcott, 1967 (No figure available)

REMARKS. This name was given to the *Tamoya gargantua* of Haeckel (1880), to distinguish it from the *T. gargantua* of Lesson (1829). Bigelow (1938) and Kramp (1956b) both assigned all Indo-Pacific *Tamoya* to *T. gargantua* sensu Haeckel, in order to maintain stability. However, the medusae to which they refer are not *Tamoya*, and not all Indo-Pacific "*Tamoya gargantua*" are identical. Furthermore, because this species exists in name only, i.e., there are no type specimens and no formal description, a more stable solution would be to disregard the problematical names *T. gargantua* and *T. haeckeli*, and build a classification based on type specimens and comparative analysis.

Tamoya haplonema Müller, 1859 (Plate 4.6C)

IDENTIFICATION. This is the largest and most robust of the known carybdeids, reaching over 130mm BH. The exumbrella is studded with numerous conspicuous gelatinous warts, typically armed with nematocysts. The most obvious characters of this species include the vertically arranged phacellae along the side wall of the stomach; the frown-shaped rhopaliar niche ostium; the well developed perradial mesenteries; and the thick tentacles, which are round in cross section but may be somewhat flattened. Furthermore, the pedalial canals are strongly quadrate in cross section throughout their length, with a prominent thorn-shaped diverticulum at the bend, and considerably flared at the tentacle insertion. The statoliths and nematocysts of the Brazilian form have not yet been studied; however, the tentacular nematocysts of a similar form from South Carolina are as follows: all discharged such that full identification could not be made, 1) club-shaped, 56-59 x 16-17 µm; and 2) sub-spherical, 30-36 x 20-28 µm.

DISTRIBUTION. Type locality: Desterro, Isla Santha Catharina, Brazil. Subsequently reported from the West Indies, Beaufort NC, and Long Island Sound NY (Mayer, 1910); Bahamas, Cuba, and Bimini (Boone, 1933); equatorial western Africa (Stiasny, 1934; Kramp, 1959); northwestern Africa (Ranson, 1949; Kramp, 1955a); Argentina (Pastorino, 2001); and Montevideo, Uruguay (Goy, 1979). Numerous reports are erroneous, including Cape Town, South Africa (Pagès et al., 1992); Bay of Bengal and Singapore (Kramp, 1968a); Japan (Uchida, 1970; Kubota, 1998); and probably the Arabian Sea and Gulf of Oman (Stiasny, 1937a). Often reported from Australia, but all reports, where verifiable, have proven to be "Morbakka".

OTHER TAXA TO BE CONFUSED. In comparison with the "Morbakkas" which are superficially similar, this species lacks the rhopalial "horns" as well as the perradial lappets, and the rhopalia have lateral eye spots as well as complex lensed eyes. The vertical phacellae make *Tamoya* unique among the Cubozoa.

Order Chirodropida Haeckel, 1880 (Werner, 1984)

Family Chirodropidae Haeckel, 1880, sens. emend.

Chirodropus gorilla Haeckel, 1880 (Plate 4.8A)

IDENTIFICATION. Haeckel (1880) distinguished this species from others based on the grape-like swellings of the free margins of the gonads, and the gastric saccules being long and tapered, with long filaments. The original specimen was 15 cm tall, with 9 tentacles per pedalium; later specimens have ranged up to about the same height, with up to 11 tentacles per pedalium. The nematocysts were reported by Kingston and Southcott (1960), but I have examined this specimen and do not agree with the identification; thus, the nematocysts of a verified *C. gorilla* have not yet been studied.

DISTRIBUTION. Type locality: Loango in Gabon, equatorial western Africa; subsequently reported from various localities along the western coast of Africa, though some of these erroneous reports actually belong to other species.

OTHER TAXA TO BE CONFUSED. Haeckel's original specimens no longer exist, and no later collections exist of specimens that unequivocally match his description and figures. This species is badly in need of revision based on new material in order to determine the true species parameters.

Chirodropus palmatus Haeckel, 1880 (No figure available)

IDENTIFICATION. This species was defined on having a body about 10 cm tall, 21 fingers and tentacles on each pedalium, and the gastric saccules fused in the upper 2/3, bearing numerous filaments. The statoliths and nematocysts have not yet been studied.

DISTRIBUTION. Type locality: South Atlantic near St. Helena.

REMARKS. It is unlikely that this species would be the young of *C. gorilla*, because at a smaller size, it has a much more advanced state of tentacle development. Unfortunately, only a brief description exists, accompanied by no illustrations and no extant type material; furthermore, the species has never been recorded again. Due to its distinct morphology, it should be regarded as provisionally valid until a redescription can be made on new material.

Chirodropus n. sp. A (Plate 4.8B)

IDENTIFICATION. This undescribed species is immediately identifiable in having brownish pigment spots of varying sizes all over the body; the gastric saccules are lacking, replaced by vertical fields of horizontal muscle bands on the subumbrellar bell wall and horizontal rows of gastric cirri inside the stomach; and the gonads are comprised of numerous fine filaments rather than the typical leaf-shaped form. It is further unique in having the palm-like part of the pedalia

greatly reduced, so that the clusters of heavy, purple tentacles appear to arise together near the bases of the pedalia. A complete species description is being prepared by P.F.S. Cornelius, R. Hore, and P.J. Fenner. The tentacular nematocysts are apparently of only one type, namely, banana-form microbasic mastigophores, 43-61 x 6-12 µm; other types were not observed.
DISTRIBUTION. Only reported once from the outer Great Barrier Reef, following a cyclone.
OTHER TAXA TO BE CONFUSED. The brownish mottling pattern, subumbrellar muscle fields, lack of gastric saccules, and filamentous gonads, make this species impossible to mistake for any other.

Chironex fleckeri Southcott, 1956 (Plate 4.8C)

IDENTIFICATION. The body is massive, often larger than the size of an adult human head (reported up to 380 cm!). There may be up to about 15 wide, flat tentacles on each pedalium, and the pedalial canals possess a conspicuous upward-pointing thorn at the bend. In younger specimens, the saccules appear as solid cock's-combs, whereas in larger, more mature individuals, the saccules are complexly diverticulated in many planes, like bunches of small, flat grapes. The gonads develop from the top of the septum, and taper downward, but overgrow the saccules as "superior gonads". Furthermore, the pedalial canals are divided, such that the branching to the tentacles is doubly-unilateral and the branches tend to alternate in position side to side. The statoliths of *Chironex* are of a short, sausage-form shape, like a short cylinder with perfectly rounded ends. The tentacular nematocysts of *C. fleckeri* have been differentially reported by numerous authors (Cleland and Southcott, 1965; Rifkin and Endean, 1983; Williamson et al., 1996; Carrette et al., 2002; Hartwick, Unpublished); it appears that there are at least five types: 1) banana-form microbasic mastigophores (type 3) in two size classes, the largest up to 95 µm long; 2) football-shaped trirhopaloids, 32-39 x 15-19 µm; 3) sub-spherical trirhopaloids, 11-17 x 9-13 µm; 4) straight-sided isorhizas, 12-20 x 4-7 µm; and 5) ovoid isorhizas with spiraled tubule, 13-17 x 8-10 µm.
DISTRIBUTION. Type locality: Cardwell, N. QLD. Commonly reported from across northern Australia, Yeppoon, QLD, to Broome, WA; however, recent distributional, morphological and molecular studies indicate that regional species occur in all three states across the North (Gershwin et al., unpublished).
OTHER TAXA TO BE CONFUSED. This species is often confused with the local Australian *Chiropsalmus* (sp. A, herein; often mistakenly identified as *C. quadrigatus*), but the two forms are quite different in numerous characters. Whereas *Chironex* has a large, heavy body; flat, robust, ribbon-like tentacles; branched gastric saccules; and a prominent thorn-like diverticulum at the pedalial canal bend, *Chiropsalmus* sp. A has a smaller, more delicate body; fine, round

tentacles; spherical, knob-like gastric saccules; and no thorn at the pedalial canal bend. Currently, this is the only well-defined species of the forms with branched gastric saccules, but others are known from elsewhere in Australia, Japan, the Philippines, and Indonesia; the complete diagnostic morphology is not yet clear for many of these forms.

Family Chiropsalmidae Thiel, 1936, sens. emend.

Chiropsalmus quadrumanus (Müller, 1859) (Plate 4.9A)

IDENTIFICATION. This is the only species of chirodropid with exumbrellar nematocysts, although at least one population in northern Brazil does not possess bell nematocysts; whether this represents a new species has not yet been fully ascertained. The bell nematocysts occur on conspicuous gelatinous warts, and could not be easily overlooked. One other useful character is the gastric saccules, which are short to medium length, solid, unbranched bulges into the subumbrellar cavity. Furthermore, there is but a single undivided main pedalial canal, such that all the tentacular branches arise from the same axis, opposite one another. The diverticulum of the pedalial canal at the bend is more of a "volcano" than a "thorn", and the tentacles are fine and round in cross section. The tentacular nematocysts are similar between two disparate populations, southeastern U.S. by Calder and Peters (1975) and southern Brazil by Marques et al. (1997); in summary, four types were found (based on Brazilian material): 1) microbasic mastigophores, 55-187 x 11-42 µm; 2) ellipsoid isorhizas 11-14 x 4-5 µm; 3) ovoid isorhizas, 6-8 x 3-4 µm; and 4) microbasic euryteles of two size classes: 16 x 12 µm and 7 x 6 µm.

DISTRIBUTION. Type locality: Santa Catharina, Brazil; reported from throughout the tropical and subtropical western Atlantic, from South Carolina to southern Brazil.

OTHER TAXA TO BE CONFUSED. It would be difficult to mistake this species for any other known chirodropid, based on the exumbrellar nematocysts and warts. However, there exists a new species from the western coast of Mexico that also possesses exumbrellar warts, but the pedalia are curiously carybdeid-like with axial keels, and the pedalial branches are confined to the extreme distal end.

Chiropsalmus zygonema Haeckel, 1880 (No figure available)

IDENTIFICATION. The species was originally described based on having a body about 60 mm tall, two asymmetrical tentacles on each pedalium, and rudimentary oval gastric saccules. The statoliths and nematocysts have not yet been studied.

DISTRIBUTION. Type locality: the Argentinian coast. Not reported since.

OTHER TAXA TO BE CONFUSED. Interestingly, Haeckel (1880) regarded it as closer to *Tamoya* than to *Chiropsalmus*, whereas Thiel (1928) believed it to be the young of *C. quadrigatus*.

Based on distribution, it would be more likely to be related to *C. quadrumanus* than to *C. quadrigatus*; however, based on morphology, it seems distinct from both. However, with only a brief description and no figure or extant material, it is presently impossible to adequately compare this species with its congeners.

Chiropsalmus n. sp. A (N. Qld) (Plate 4.9B)

IDENTIFICATION. This form is the one often referred to as *Chiropsalmus quadrigatus* of northern Queensland. Body small to medium for a chirodropid (ca. 6-8 cm tall). It differs from other species of cubozoans in the gastric saccules, which are solid, hemispherical knobs, rather than the simple or branched extensions characteristic of other chirodropids. Other characters include up to 9 tentacles per pedalium, fine and round in cross section; the bend of the pedalial canal lacks a diverticulum but is instead knee-shaped; and the pedalial canal branching is doubly unilateral with opposite branches. The tentacular nematocysts are similar to those of *Chironex fleckeri*, but lacking the large class of banana-form mastigophores: 1) banana-form microbasic mastigophore, 39-46 x 9-10 µm; 2) football-shaped isorhizas in two size classes, 21-24 x 12-14 µm and 9-10 x 7-8 µm; 3) small football-shaped euryteles, 9-10 x 8-9 µm; and 4) small rod-shaped isorhizas, 11-13 x 4-5 µm.

DISTRIBUTION. Reported from Cooktown to Townsville.

OTHER TAXA TO BE CONFUSED. See remarks for *Chironex*, above, and for the "Gove chirodropid" (= *Chiropsalmus* n. sp. B), below.

Chiropsalmus n. sp. B (Gove) (Plate 4.9C)

IDENTIFICATION. This form, often referred to as the "Gove chirodropid", is characterized by its small body (typically only 4-5 cm tall); its long pedalia with the fingers and tentacles clustered near the end; and its peculiar gastric saccules, which are short, solid, and coalesced into a single kidney-bean-shaped knob-like structure. The species has full gonads by the time the body is 4 cm in height, whereas most chirodropids don't *start* developing gonads until about 6-8 cm. The tentacular nematocysts are similar to other chirodropids, with four types evident: 1) banana-form microbasic mastigophores, 39-45 x 9-11 µm; 2) large football-shaped mastigophores, 21-26 x 13-14 µm; 3) small football-shaped isorhizas, 9-10 x 7-8 µm; and 4) small rod-shaped isorhizas, 13-14 x 6-7 µm.

DISTRIBUTION. Only reported from the Gove Peninsula, Arnhem Land, Gulf of Carpentaria.

OTHER TAXA TO BE CONFUSED. This species might be confused superficially with the N. QLD *Chiropsalmus*, based on the smallish body; fine, round tentacles; and simple, solid, sessile gastric saccules. However, the saccules of the mature Northern Territory form are coalesced into a single kidney-bean-shaped structure, whereas those of the mature QLD form are separate, spherical structures. Furthermore, the pedalia are remarkably different, with the QLD form having typical hand-like or claw-like pedalia, but those of the NT form having a longer, leaner appearance, with the branches clustered near the distal end.

Chiropsoides buitendijki (Horst, 1907) (Plate 4.10A, B)

IDENTIFICATION. This species is immediately distinguishable from all other chirodropids by the unilateral branching of the pedalia, i.e., they are branched in a single series along the main axis of the pedalia, rather than biserially along both sides of the main axis. Other characters include a well developed thorn-shaped pedalial canal diverticulum, very long, unbranched, hollow gastric saccules, and wide, flat, ribbon-like tentacles. The tentacular nematocysts are of four types: 1) banana-form microbasic mastigophores, 61-79 x 10-12 µm; 2) small football-shaped isorhizas with a beehive-form tubule, 9-11 x 7-8 µm; 3) small rod-shaped isorhizas, 14-15 x ca. 4 µm; and 4) spherical isorhizas in two size classes, small, 7-10 µm, and very small, 3-4 µm.
DISTRIBUTION. Type locality: Java; subsequently reported from the Malay Archipelago (Stiasny, 1919), India (Menon, 1936; Nair, 1951; Chakrapani, 1984), and Indochina (Dawydoff, 1936; Ranson, 1945). Reports from Australia are erroneous (Stiasny, 1926b; Pope, 1953).
OTHER TAXA TO BE CONFUSED. The peculiar serial branching of the pedalia makes this species unique. However, see comments below for *C. quadrigatus*.

Chiropsoides quadrigatus (Haeckel, 1880) comb. nov. (Plate 4.10C, D)

IDENTIFICATION. The badly damaged juvenile holotype possesses serially branching pedalia such as those characteristic of *C. buitendijki*. Further similarities with *C. buitendijki* include an upward-pointing thorn on the bend of the pedalial canal, and flat, ribbon-like tentacles. Other discernable characters in the holotype include 3-4 branches of the pedalia, and the rhopalial niche ostium of the typical chirodropid form with a w-shaped upper covering scale. The gonads and gastric saccules were not observable, and were presumed to be undeveloped in this juvenile specimen. The statoliths and nematocysts have not yet been studied.
DISTRIBUTION. Type locality: near Rangoon, Indian Ocean.
REMARKS. This species has been the subject of great confusion over the last 100 years. The adult morphology that characterizes the species is currently unknown; therefore, the nominal species should be regarded as unrecognizable until it can be properly diagnosed. The redescriptions of "*Chiropsalmus quadrigatus*" given by Mayer (1910; 1915; 1917) from Philippine material were apparently based on two different species, neither of which possesses the most obvious scorable character in the badly damaged juvenile holotype of *C. quadrigatus*,

namely the unilateral branching of the pedalia, which most resembles that of *Chiropsoides buitendijki*.

Whether *Chiropsoides buitendijki* from Java is the adult form of *C. quadrigatus* from Rangoon cannot at this time be ascertained and should not be assumed. However, regardless of its ultimate fate, it is clear from the morphology that it should be classified in the genus *Chiropsoides* rather than *Chiropsalmus*. I am currently aware of at least 4 different forms throughout the Indo-Pacific which are all erroneously said to be *C. quadrigatus*.

Anthracomedusa turnbulli Johnson and Richardson, 1968 (Plate 4.9D)

IDENTIFICATION. This species, known only by fossil impressions, is characterized by its small size (up to 100 mm), with many tentacles issuing from a short pedalium at each corner of the bell. The tentacle length is described as not exceeding the width of the bell, although tentacles were likely to be as contractile 300 mya as they are today. The bell has a simple margin, and appears somewhat wider than tall and relatively rounded.

DISTRIBUTION. Type locality: Middle Pennsylvanian, near Essex, Illinois. The Essex Fauna of the Mazon Creek Formation are found in the Francis Creek shale, a member of the Livepool cyclothem of the Carbondale group (Johnson and Richardson, 1966; Johnson and Richardson, 1968).

REMARKS. *Anthracomedusa turnbulli* is, in my opinion, the only undisputable fossil cubozoan. Although the preservation is inadequate to place it to family, the rigid cuboid body shape and clumps of tentacles on corner pedalia firmly place this in the Chirodropida. Because of the short pedalia, it appears to be most similar to the currently undescribed spotted form (*Chirodropus* n. sp. A, herein) found only once at the Great Barrier Reef.

4.4 DISCUSSION

The species and forms diagnosed herein should help guide the end-user toward better understanding the species boundaries in the Cubozoa. While the criteria herein should give reasonable accuracy in identification at the familial and generic levels for the taxa treated as well as most others, the same level of confidence cannot yet be claimed at the species level. These tools will work for the species that they are meant to work for, but because of the large number of undescribed forms, compounded by differential preservation, allometric growth, and ontogenetic character changes, accurate species identification can only be accomplished with character-by-character comparison with known species based on as many characters as possible, e.g. those outlined in Chapter 2. Thus, these tools are provided as a starting point for comparison, but the level of accuracy is ultimately determined by the amount of effort spent in identification.

A comprehensive monographic revision of the Cubozoa of the world is forthcoming, which will fully describe all these forms and more. This thesis was originally approved to include this revision, but departmental page limit requirements now prevent such a lengthy chapter from being included. A representative section from the monograph is presented in Chapter 5, comprising a revision of the "*Carybdea alata*" group and proposal to elevate the group to family status. Furthermore, this work spawned a web-based interactive key to the Cubozoa, which shall be published shortly.

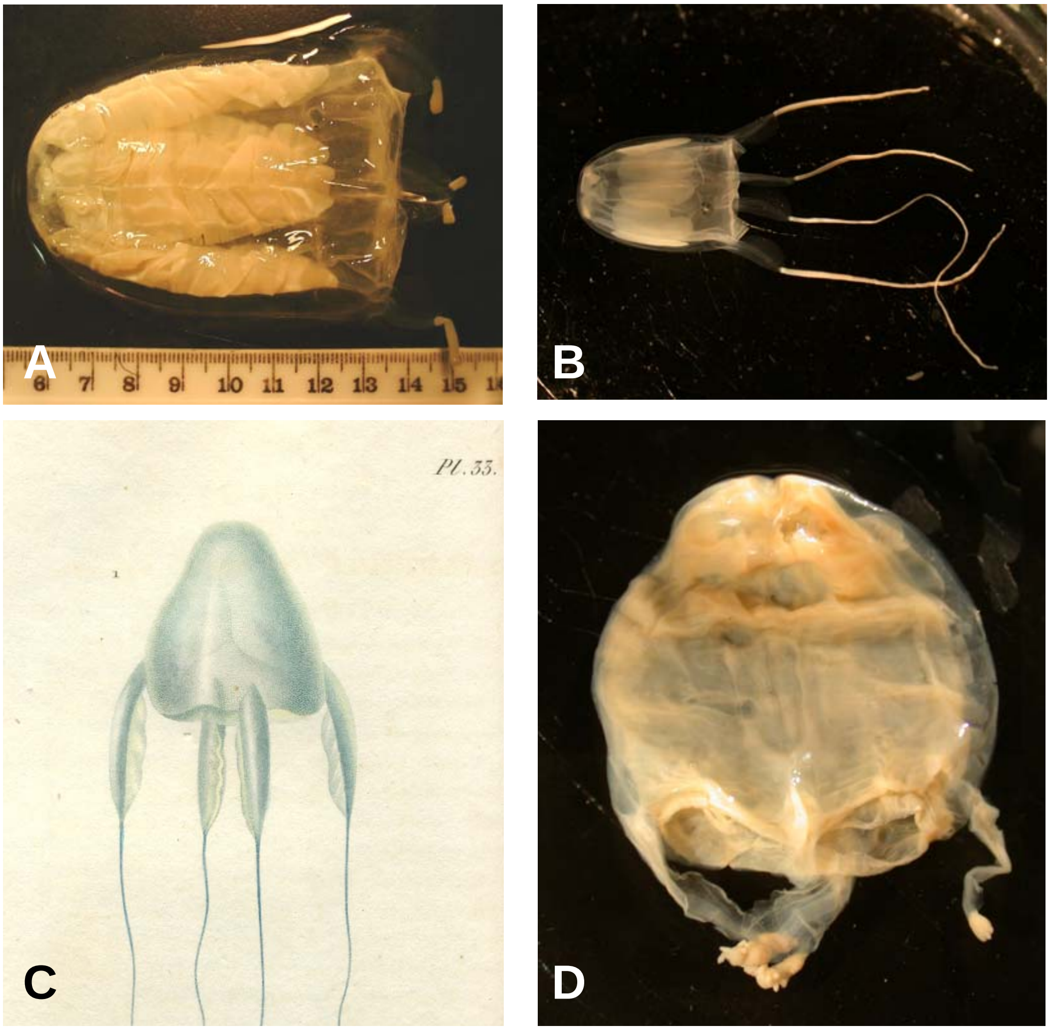

Plate 4.1. Comparison of *Alatina* spp. A. *Alatina mordens* n. sp., Outer Great Barrier Reef (Paratype, SAM coll'n). B. *Alatina rainensis* n. sp., Raine Island, Queensland (Holotype, MTQ coll'n). C. *Alatina alata* comb. nov. (watercolour by Reynaud, in Lesson, 1830). D. *Manokia stiasnyi* (Holotype, IRSNB coll'n).

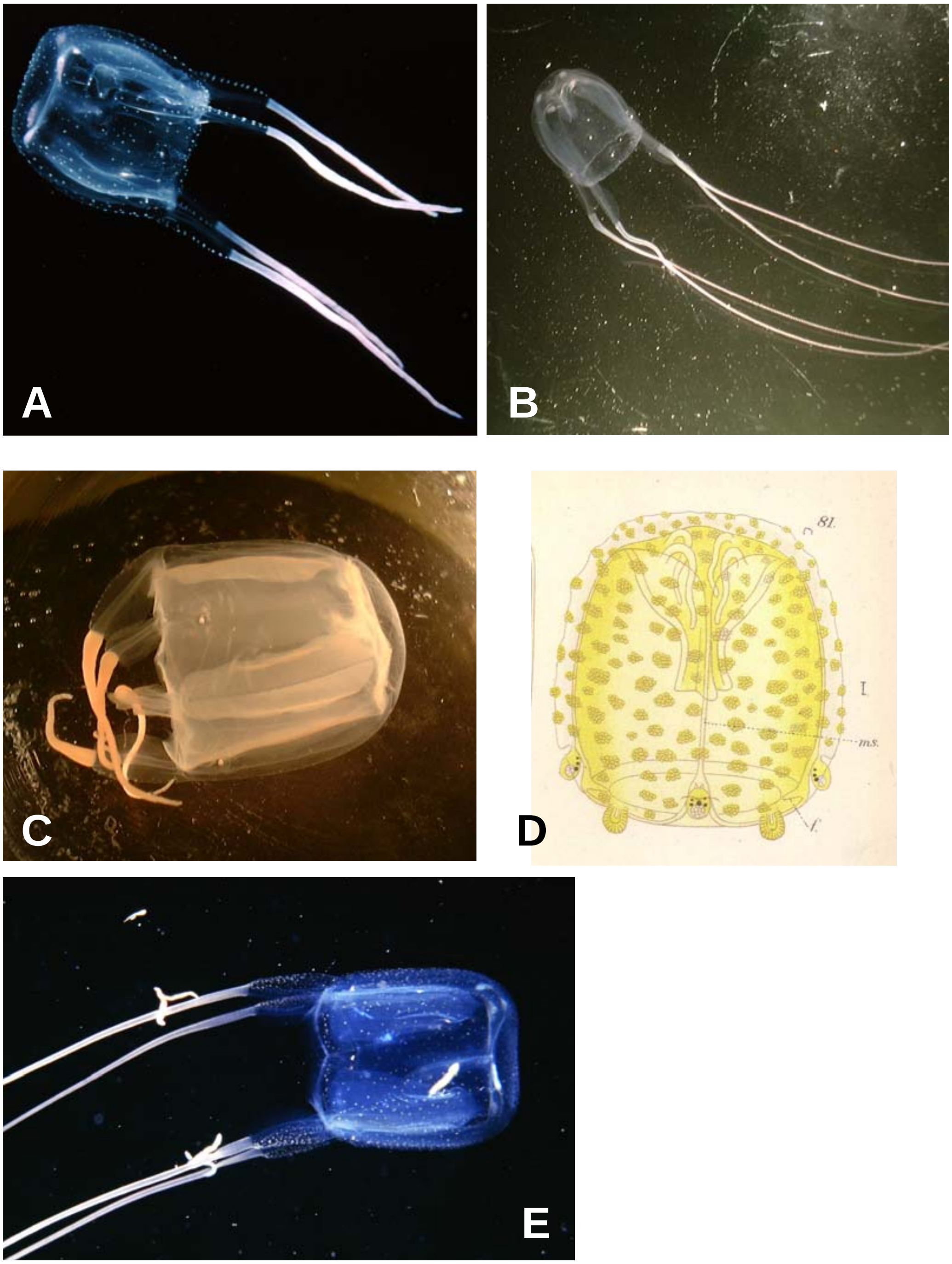

Plate 4.2. Comparison of *Carybdea* spp. A. *Carybdea rastonii*, Edithburg South Australia (copyright K. Gowlett-Holmes). B. *Carybdea* cf. *xaymacana*, Busselton, Western Australia. C. *Carybdea xaymacana*, La Parguera, Puerto Rico (Neotype, USNM coll'n). D. *Carybdea aurifera* (original illustration, Mayer, 1900). E. *Carybdea marsupialis*, Tyrrhenian Sea, Italy (copyright P.Gay).

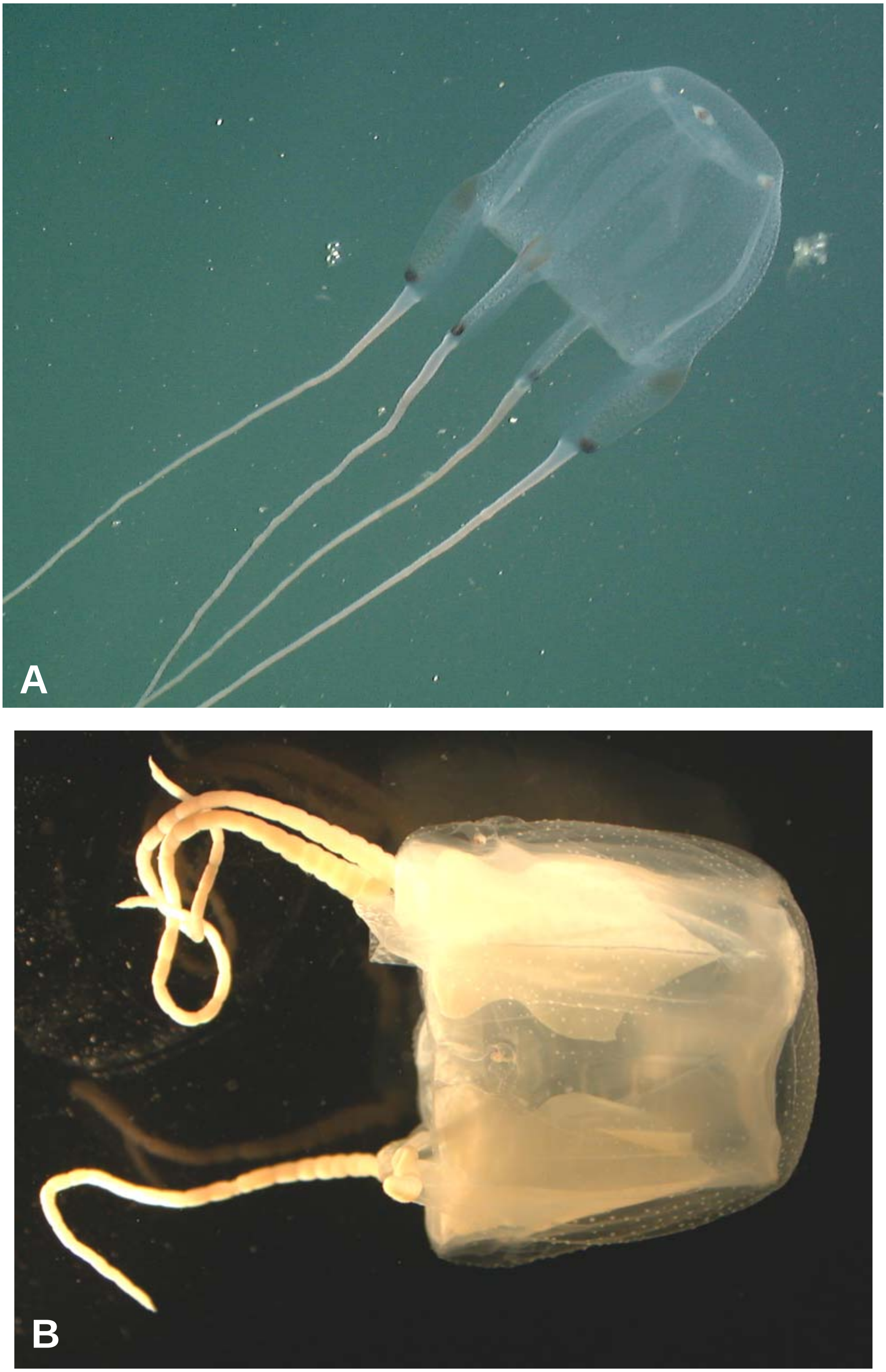

Plate 4.3. Comparison of “other” *Carybdea* spp. A. Undescribed carybdeid, Cape Town, South Africa. B. Undescribed carybdeid, Japan.

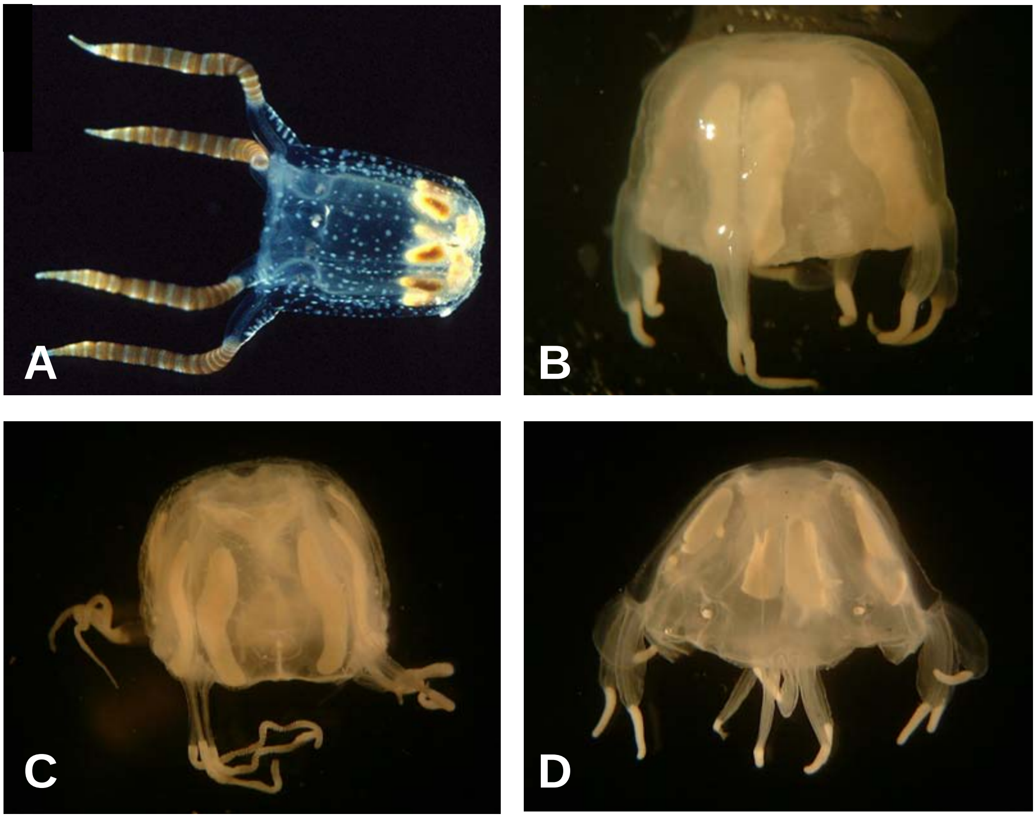

Plate 4.4. Comparison of *Tripedalia* spp. and “*Carybdea sivickisi*”.
A. *Collostemma sivickisi* (gen. nov., comb. nov., copyright B. Hamner).
B. *Tripedalia cystophora*, La Parguera, Puerto Rico (Neotype, USNM coll’n.).
C. *T. cystophora*, La Parguera, different view (USNM coll’n.). D. *Tripedalia binata*, Darwin, Northern Territory (Paratype, NHM coll’n.).

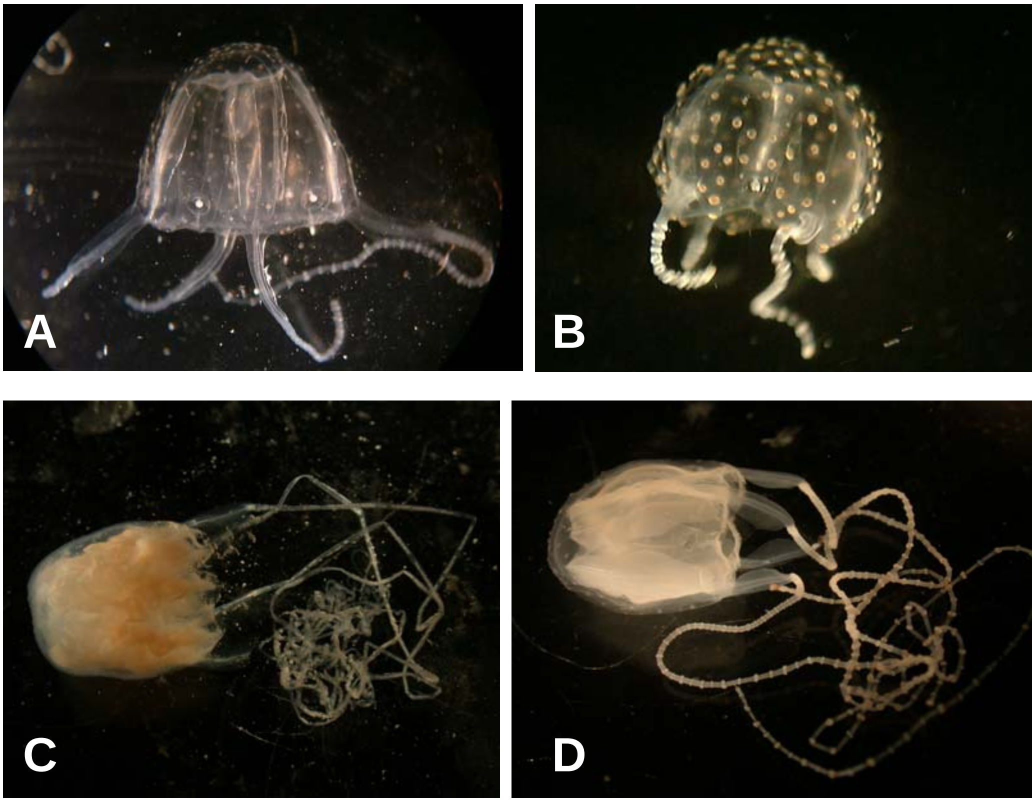

Plate 4.5. Comparison of *Carukia* spp. A. *Carukia barnesi*, Palm Cove, Queensland. B. *Carukia barnesi*, juvenile, ca. 3 mm bell height, Palm Cove. C. *Carukia* n. sp. A, Outer Great Barrier Reef. D. *Carukia* n. sp. B, Broome, Western Australia.

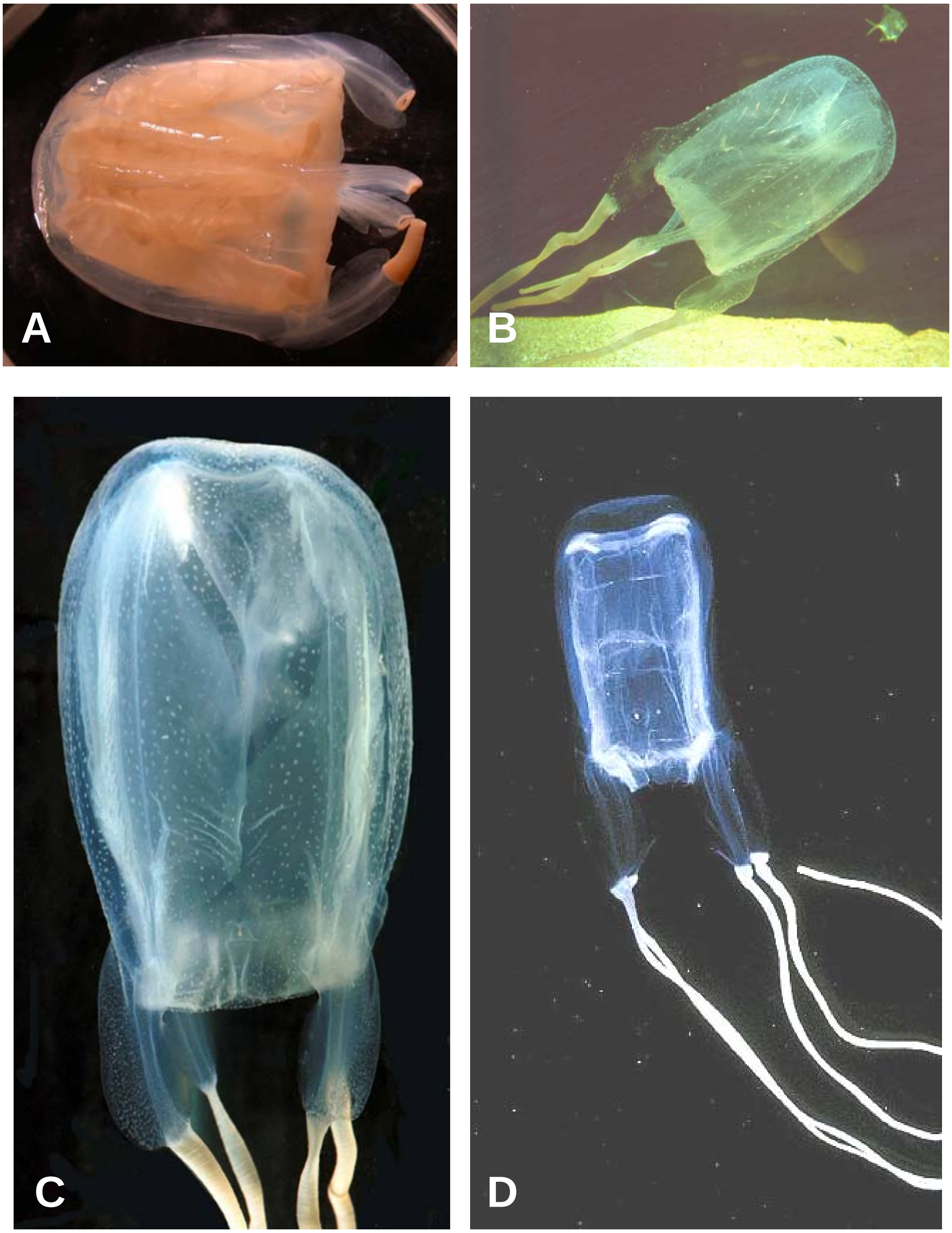

Plate 4.6. Comparison of species in the Tamoyidae. A. Darwin carybdeid (Holotype, NTM coll'n.). B. Morbakka, Port Douglas, Queensland (copyright B. Cropp). C. *Tamoya haplonema*, San Sebastion Channel, Sao Paulo, Brazil (Neotype, copyright A. Migotto). D. Morbakka, Coffs Harbour, New South Wales (copyright C. Buchanan).

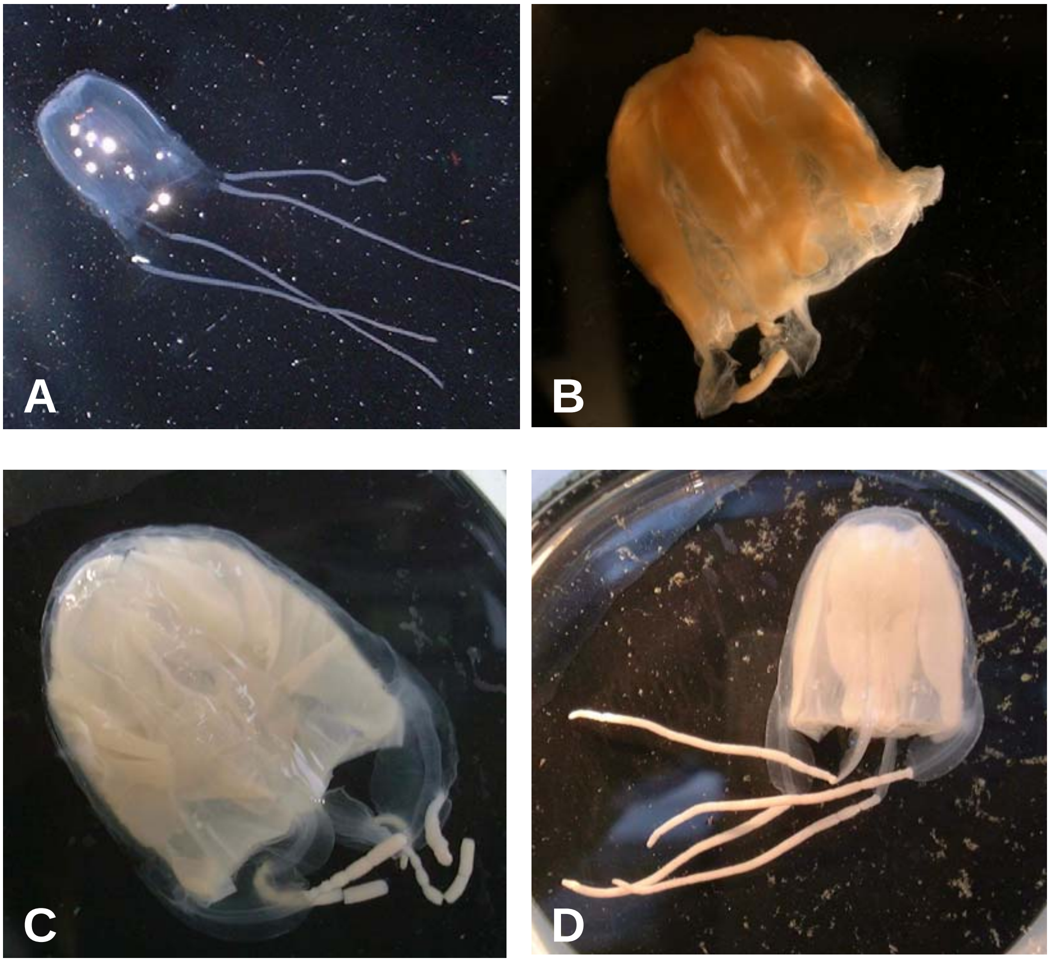

Plate 4.7. Comparison of “other Irukandji” spp. A. “Broome Irukandji,” Broome, Western Australia (copyright M. Alexander). B. “Dampier Irukandji,” Dampier, Western Australia (Holotype, NTM coll’n.). C. “Halo-Irukandji,” Port Douglas, Queensland (Holotype, QM coll’n.). D. “Pseudo-Irukandji,” Port Douglas, Queensland (Paratype, SAM coll’n.).

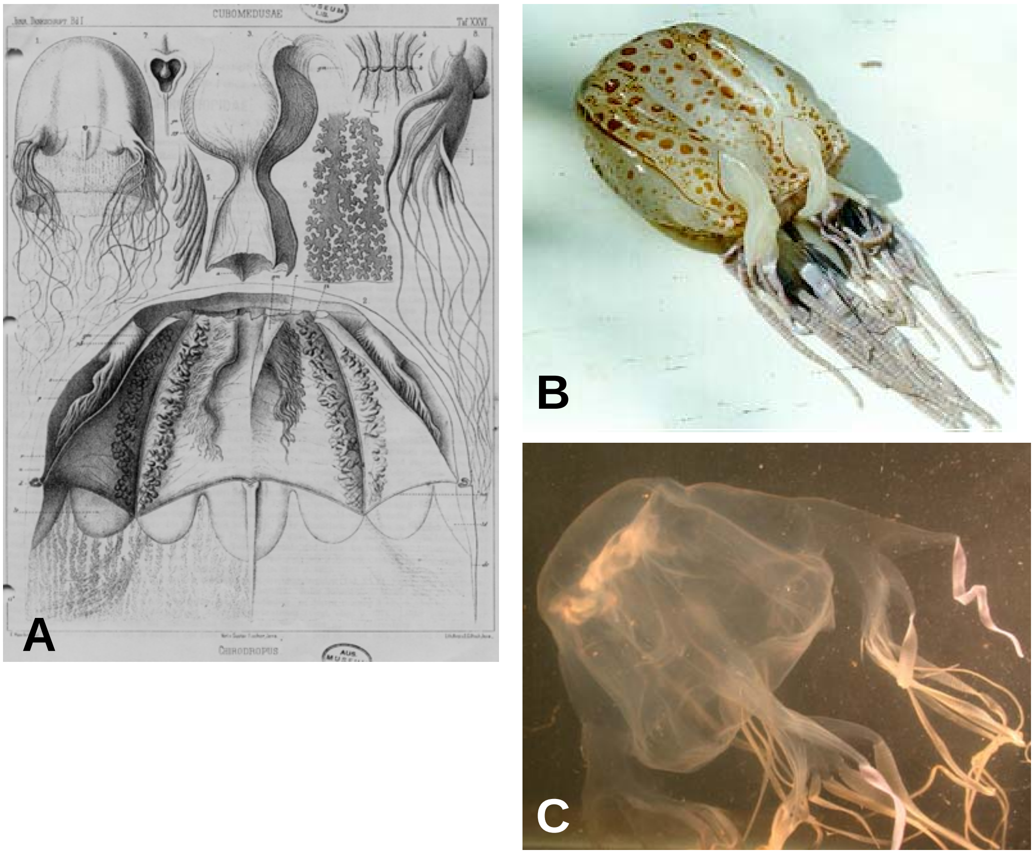

Plate 4.8. Comparison of *Chironex* and *Chirodropus* spp. A. *Chirodropus gorilla* (Illustration by Haeckel, 1880). B. *Chirodropus* n. sp. A, Great Barrier Reef (Holotype, QM coll'n.; copyright R. Hore). C. *Chironex fleckeri*, Cairns, Queensland.

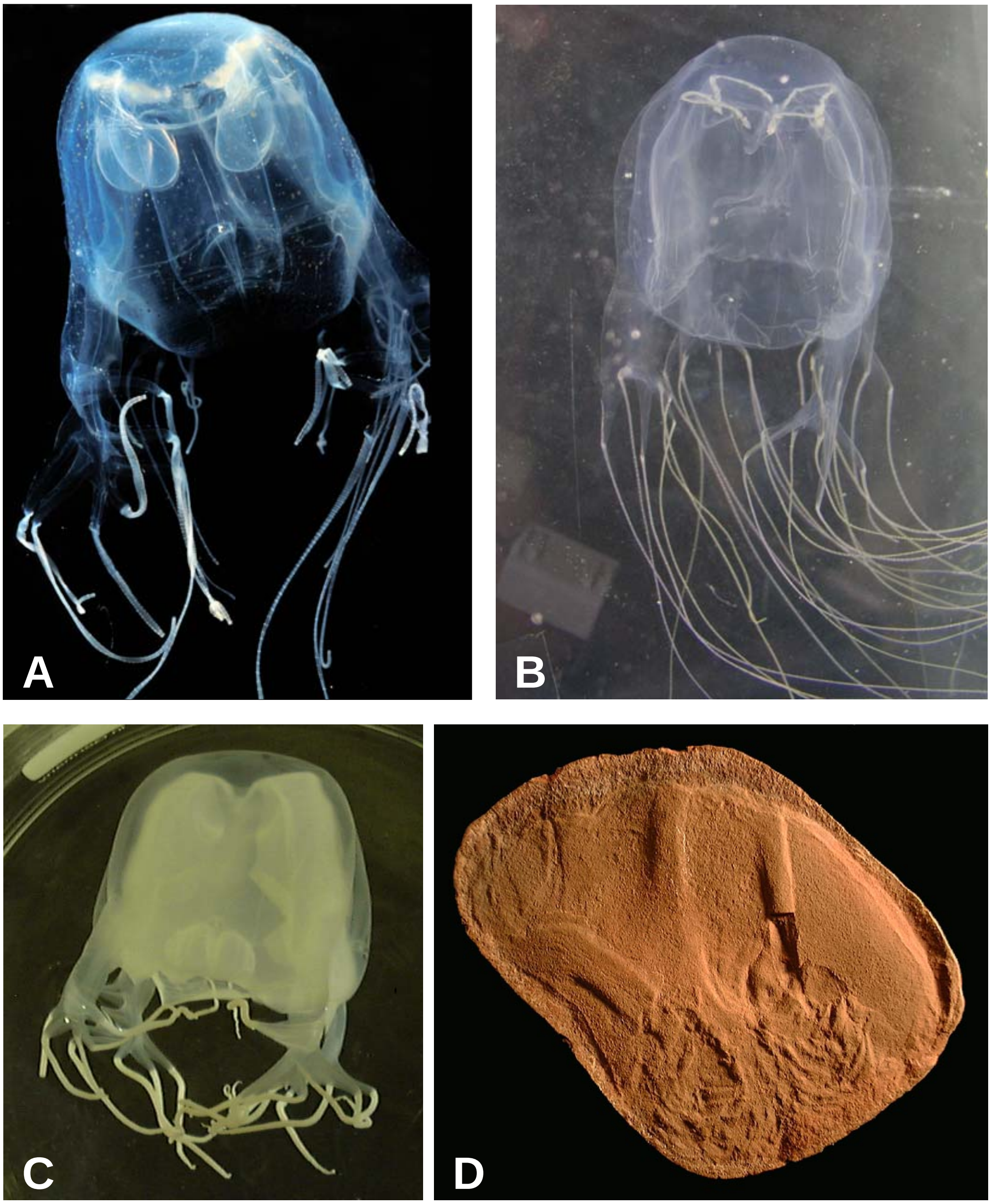

Plate 4.9. Comparison of *Chiropsalmus* spp. and the fossil species *Anthracomedusa turnbulli*. A. *Chiropsalmus quadrumanus*, Ubatuba, SP Brazil (Neotype, MZUSP coll'n.; copyright A. Migotto). B. *Chiropsalmus* n. sp. A, North QLD (Horseshoe Bay, Magnetic Island). C. *Chiropsalmus* n. sp. B, Gove Peninsula, Northern Territory (NTM coll'n.). D. *Anthracomedusa turnbulli*, Middle Pennsylvanian, Mazon Creek, Illinois (UCMP coll'n, copyright A. Collins).

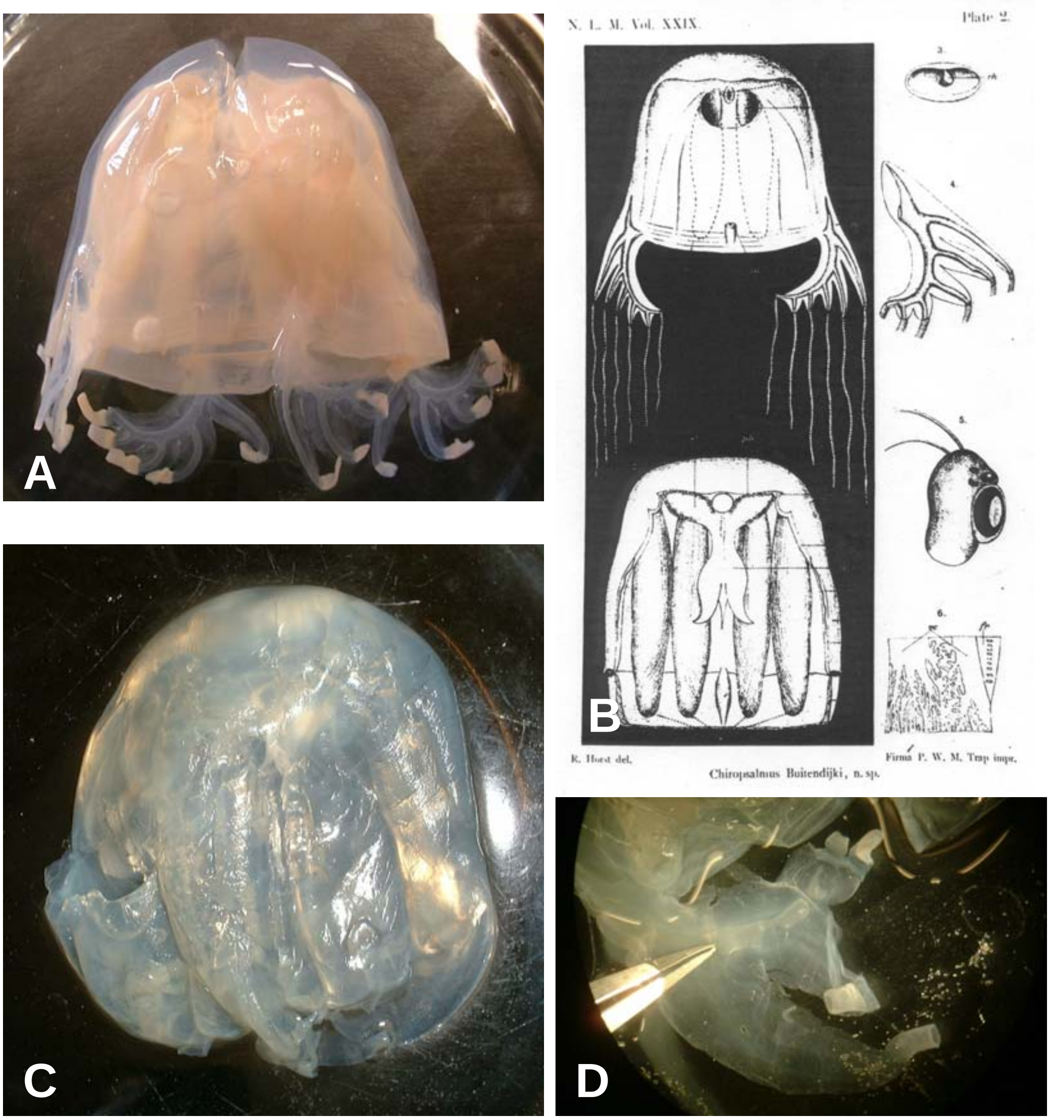

Plate 4.10. Comparison of *Chiropsoides* spp. A. *Chiropsoides buitendijki*, Java (Holotype, Leiden coll'n.). B. *Chiropsoides buitendijki* (original illustrations, Horst, 1907). C. *Chiropsoides quadrigatus* comb. nov., Rangoon, Burma (Holotype, ZMUC coll'n.). D. *Chiropsoides quadrigatus*, pedalium, holotype.

CHAPTER 5: *CARYBDEA ALATA* AUCT. AND *MANOKIA STIASNYI*, RECLASSIFICATION TO A NEW FAMILY WITH DESCRIPTION OF A NEW GENUS AND TWO NEW SPECIES

5.1 INTRODUCTION

The nominal species *Carybdea alata* Reynaud, 1830, has been the most problematical of all cubozoan species, from both nomenclatural and practical perspectives. The species was originally founded from a medusa collected somewhere in the South Atlantic, on the basis of a watercolour by Reynaud in Lesson's Centurie Zoologique (Lesson, 1830). It was widely disregarded as unrecognizable, until the name was revived by Vanhöffen (1908) in the interest of stability. However, Vanhöffen failed to assign a particular specimen or suite of characters to the name, and the species originally assigned that name is unrecognizable. By the time of Kramp's Synopsis (1961), the nominal species *C. alata* was recognized by its crescentic phacellae and three well developed scales enclosing the rhopalial niches. Other authors additionally recognized three velarial canals per octant as diagnostic (e.g., Mayer, 1910; Bigelow, 1938). The problem is that many different forms all share these characters, even though they barely resemble one another overall. On the one hand, this makes identification very easy for the lay person, but on the other hand, it does not reflect biological reality and makes for confusion that has yet to be resolved.

At least nine different nominal species have been described with crescentic phacellae and/or three canals per octant and/or three well developed covering scales. Haeckel (1880) recognized six nominal species with these characters, including five new ones (*Procharybdis tetraptera*, *Procharybdis turricula*, *Carybdea pyramis*, *Carybdea philippina*, and *Carybdea obeliscus*). Agassiz and Mayer (1902) added *Carybdea grandis*, based on its very large size. Mayer (1906) added *Carybdea moseri*, based on short basal stalks of the pedalia and differently shaped pedalial wings, though Bigelow (1909) regarded it as a young stage of *Carybdea grandis* and Mayer (1910) concurred. Menon (1930) added *Carybdea madraspatana*, based on its apical concavity and greater number of velarial canals. Kramp (1961) lumped them all under the oldest available name, *C. alata*. The historical confusion and instability concerning *alata*-group relationships have apparently been based on a failure to recognize the crescentic phacellae as common to more than one species, combined with a reluctance to regard geographically variable characters as informative.

Another species, *Manokia stiasnyi* (Bigelow, 1938), also possesses crescentic phacellae and three well developed covering scales, though the former character was not previously appreciated. The species was defined on the basis of its branched tentacles, and its similarity to *Carybdea alata* was not noted. Different authors have held quite different opinions about the validity of this species without examining the type specimen (Southcott, 1967; Kramp, 1968a). I have examined the holotype, and believe the species is worthy of recognition.

It is difficult to say at this time how many different species under the name *Carybdea alata* can be distinguished worldwide, because typically every specimen with crescentic phacellae has been lumped into the one nominal species, with typically very little discussion about other characters. However, it is clear that at least three quite different species exist in the southwestern Pacific of Australia and Papua New Guinea, and that these differ from several earlier described forms. In order to stabilize the nomenclature of the various species associated with *Carybdea alata* auct., I propose reclassification of the collective species with crescentic phacellae and T-shaped rhopaliar niche ostia, defined on the basis of a well characterized type species for which type specimens and DNA sequences are available (see Chapter 3). Here I propose the new genus *Alatina*, and describe two new species, *Alatina mordens* sp. nov. and *Alatina rainensis* sp. nov. The other nine species previously associated with the name "*Carybdea alata*" are reevaluated and determinations are made as to their validity. Furthermore, the problematical species *Manokia stiasnyi* (Bigelow, 1938) is resurrected and redescribed based on examination of the type specimen. Together, species of *Alatina* and *Manokia* appear to form a natural group separate from those of *Carybdea* and other carybdeids, and for this group I propose the new family Alatinidae; the separation of the groups is supported by 18S rDNA analysis (Chapter 3). Revised definitions of the family Carybdeidae and the genus *Carybdea* are given below.

5.2 MATERIALS AND METHODS

All taxonomic observations and measurements were made on preserved material, unless otherwise noted, based on the criteria set forth in Chapters 2 and 3. Measurements were made with Max-Cal digital calipers to the nearest 0.01 mm, then rounded to one decimal place; in some cases, an available absolute measurement was clearly not the correct full measurement (e.g., in the case of a brittle, folded specimen), denoted with a "+" following the measurement. Bell height (BH) was measured from the apex of the bell to the velarial turnover. Diagonal bell width (DBW) was measured across diagonally opposite pedalia on a flattened specimen, at the height of the pedalial lamella. Interrhopalial width (IRW) was measured between adjacent rhopalia, with the specimen flattened. Tentacle base width (TBW) was measured across the widest diameter at the uppermost part of the tentacle, immediately below the pedalium. Pedalial dimensions are as follows: pedalial length (PL) from the subumbrellar lamella to the tentacle insertion, pedalial width (PW) and pedalial canal width (CW) at the vertical midpoint. Sex was determined, when possible, by biopsy. Female gonads have obvious ova; male gonads have a conspicuous "finger-print" appearance of many fine more-or-less parallel lines. Phacellae were examined by making a small incision in the upper corners of the bell, and then pulling back a small amount of mesoglea to expose the phacellae in situ, or by opening up the full length of the body wall to expose the stomach. Nematocysts were examined and measured with a Leica DMLB compound microscope and Leica IM-50 Image Manager v. 1.20 for Windows; all observations and photographs were made through a 40x objective, unless otherwise specified. Nematocysts were identified following the keys of Calder (1974), Mariscal (1971), and Williamson et al. (1996). Translations of the original German descriptions of *Manokia stiasnyi* and Haeckel's species were made with Globalink Power Translator 6.0 for Windows.

Throughout the text, usage of the form of the name "*alata*" has very restricted meaning. "*Alatina*" refers to the new genus with its present definition. "*Carybdea alata*" refers to the species named by Reynaud, or to specific contextual meanings of other authors, but "*Carybdea alata* auct." refers collectively to the species grouped under this name by various other authors. The term "*alata*-group" refers to the loosely defined collection of described and undescribed forms that have been identified as *Carybdea alata* by traditional criteria (including museum collections and published identifications), but are not yet clearly internal or external to the genus *Alatina*.

Abbreviations follow those defined in Chapter 2 (Section 2.2). For the synonymy section, I have used the special notation introduced by Matthews (1973); "v" indicates that I have seen the material referenced and "v*" indicates that I have seen the type.

5.3 SYSTEMATICS

Phylum Cnidaria Verrill, 1865

Subphylum Medusozoa Petersen, 1979

Class Cubozoa Werner, 1973

Order Carybdeida Maas, 1909 sensu Werner, 1984

5.3.1 Alatinidae, family nov.

TYPE GENUS. *Alatina* gen. nov.

DIAGNOSIS. Carybdeida with crescentic gastric phacellae, comprised of long cirri arranged more or less parallel in a single plane; with T-shaped rhopalial niche ostia, comprised of a single upper covering scale and 2 lower, well developed covering scales; with 3 or 4 more or less simple velarial canals per octant; with a shallow stomach, completely lacking perradial mesenteries.

5.3.2 *Alatina* gen. nov.

TYPE SPECIES. *Alatina mordens* n. sp., designated herein.

ETYMOLOGY. The genus name *Alatina* (feminine, Latin) is taken from the nominal species *Carybdea alata* Reynaud, 1830, which has been confused as the senior synonym for many other species. The genus name *Alata* is preoccupied for Mollusca (Linck, 1783) and Lepidoptera (Walker, 1863), so the suffix *-ina* (diminutive, Latin) has been added.

DIAGNOSIS. Alatinidae with 3 velarial canals per octant, with simple tentacles.

REMARKS. *Alatina* spp. are easily distinguished from other carybdeids by several conspicuous features, especially the crescentic shape of the phacellae and their long cirri, the hemispherical shape of the adaxial pedalial wings, the T-shaped rhopaliar niche ostia, the three simple or end-branched velarial canals per octant, and the flimsy gelatinous consistency of the body. In contrast, *Carybdea* spp. have epaulette-shaped or linear phacellae of short cirri, scalpel-shaped pedalia, heart-shaped rhopalial niche ostia, two to four branched velarial canals per octant, and a relatively springy bell consistency that holds its shape in water. *Tripedalia* spp. have multiple simple pedalia. *Tamoya* is characterized by having vertical phacellae in the interradial corners of the stomach wall. *Carukia* and several other undescribed forms are characterized by lacking phacellae altogether. *Tripedalia*, *Tamoya* and *Carukia* all have frown-shaped rhopaliar niche ostia. The main characters that separate the functional groups of Carybdeida are outlined in Table 1, and are typically observable with the naked eye or by dissecting scope. The species of *Carybdea* are detailed because there is historically great confusion on how to tell *Carybdea* species apart. A revision of the genus *Carybdea* with additional species shall soon be published elsewhere, and is beyond the scope of this thesis.

It is impossible to say with certainty which of the Atlantic forms is the true *Carybdea alata* of Reynaud, but it is clear that not all forms of *Carybdea alata* auct. should be considered the same species. One must start sorting out the confusion somewhere, and because I have multiple local specimens at my disposal, along with their DNA, it seems most prudent to begin with these. It is my hope that by establishing a workable system in which to incorporate other species, it will eventually be possible to differentiate the large number of regionally distinct forms.

Alatina moseri (Mayer, 1906) from Hawaii is redescribed below based on examination of type and non-type material. The remaining eight species of *Alatina* are treated briefly below, and a comparison of their characters is given in Table 2. Although some remain unidentifiable, most forms previously described and associated with *Carybdea alata* auct. should be considered valid until proper comparison of material from the different type localities can be made.

5.3.3 *Alatina mordens* sp. nov.

Plates 5.1, 5.2

Unidentified species – v?Mulcahy, 1999: 88 [Irukandji syndrome case requiring life support]. – v?Little et al., 2001: 178-180 [case history of sting, life support required for 8 days]. – ?Taylor et al., 2002: 175-180 [Irukandji syndrome with persistent symptoms over 7 months].

MATERIAL EXAMINED. *Holotype*: MTQ G55282, Moore Reef, GBR, QLD (approx. 16°52.1S 146°12.3E), coll. 13 November 1998; immature male, BH 80.8 mm, DBW 64.4 mm, IRW 30.1 mm, TBW 2.5 mm; forwarded by J. Seymour; captured within minutes of severe Irukandji sting reported by Mulcahy, 1999 (Plate 5.1A).
Paratypes: SAM H1013, same data as holotype; BH 74.1 mm, DBW 54.2 mm, IRW 25.3 mm, TBW 1.9 mm (Plate 5.1B). SAM H1053, Osprey Reef, Coral Sea, QLD (approx. 13°54.1S 146°38.9E), 29 January 2000; BH uninterpretable due to damage, DBW 47.8 mm, IRW 24.1 mm, TBW 1.9 mm; forwarded by P. Colwell; captured within minutes of Irukandji stings; examined live and preserved. QM G317058, Agincourt Reef, GBR (approx. 16°01.9S 145°51.2E), coll. 24 April 1998, forwarded by R. Hore; BH 80.4 mm, DBW 64.1 mm, IRW 30.9 mm, TBW 2.1 mm. QM G317059, same data as QM G317058; BH 59.2 mm, DBW 41.2 mm, IRW 22.4 mm, TBW 1.1 mm. AIMS 2003-10, Agincourt Reef, GBR, 28 April 2003; BH ca. 75 mm, DBW ca. 55 mm; forwarded by R. Hore, captured following superficial sting of 12 year old boy. MTQ G55288, Agincourt Reef, GBR, 25 August 2003; immature male, 85 mm BH, 65 mm DBW, 32 mm IRW; forwarded by R. Hore.

OTHER MATERIAL EXAMINED. [JCU 1] Unregistered specimen at JCU Cairns, QLD, same data as holotype; BH 61.3 mm, DBW 53.5 mm, IRW 22.8 mm, TBW 1.8 mm (Plate 5.1C). [JCU 2] Unregistered specimen at JCU Cairns, QLD, coll. Agincourt Reef, GBR, 6 July 1991; BH 96.1 mm, DBW 69.7 mm, IRW 31.8 mm, TBW 1.8 mm. Approximately 20 specimens frozen or in ethanol, caught at various times at Agincourt Reef, GBR, 2000-2004, examined casually before being forwarded for venom analysis.

TYPE LOCALITY. Moore Reef, outer Great Barrier Reef.

ETYMOLOGY. From the Latin *mordax*, meaning “biting”, in reference to the painful sting.

DIAGNOSIS. *Alatina* with a tall, tapered, apically truncate bell, with exumbrellar nematocyst freckles; with crescentic phacellae, comprising many tufts of long cirri which branch only near the root; with 3 straight, simple to triforked velarial canals in each octant, bearing a row of 1-5, typically 1-3, small, round nematocyst freckles on root area; with broadly rounded adaxial pedalia keels; with simple rounded pedalial canal.

DESCRIPTION OF HOLOTYPE. Bell much taller than wide, with overall shape of a truncate tall pyramid, of thin and flimsy gelatinous consistency (Plate 5.1A-C). Apex much narrower than velarial aperture; flat to slightly rounded. Exumbrella with sparsely scattered minute, unraised, round nematocyst freckles; with interradial furrows shallow and wide, extending along entire bell height. Adradial furrows lacking. Phacellae (Plate 5.2A) 4, interradial, crescentic, broad, with numerous long gastric filaments arranged in a more or less parallel fashion in a single plane.

Rhopalial niches (Plate 5.2B) 4, perradial, flush with exumbrellar bell wall, shallowly convex on subumbrellar wall; with T-shaped ostia, i.e., 1 covering scale above and 2 well-developed scales below. Upper covering scale thickened, protruding slightly from bell wall, broadly convex in contour; lower scales well developed, with vertical opposing edges, separated by a furrow continuing to velarium; upper and lower scales separated horizontally by a discontinuity on each side of ostium. Rhopalial horns lacking. Rhopalial stem without warts. Rhopalial lens and eyespot morphology indeterminable in holotype without dissection; due to very large size of lower eye, rhopalium appearing as a single black spot to unaided eye.

Pedalia (Plate 5.2C) 4, interradial, with short stalk; with large round adaxial keel approximately 3-4x pedalial canal width, overhanging at point of tentacle insertion; with narrow abaxial keel approximately 2x canal width; with few scattered minute nematocyst freckles.

Pedalial canals bowed slightly toward abaxial keel, narrow, laterally flattened; with rounded to slightly angular bend near point of origination from bell, straight or slightly tapered into tentacle.

Velarium wide, with nematocysts confined to single row of small, round warts on adperradial velarial canals only. Velarial canals (Plate 5.2D) 3 per octant, simple throughout most of length, may be biforked or triforked at distal end; 4-6 extensions reaching margin or nearly so in each octant; canal roots (velarial extensions of gastric pouches) 3 per octant, narrow, extending onto velarium approximately halfway to margin. Perradial lappets lacking. Frenulum a single, narrow, stiff sheet, extending only about halfway onto velarium, with a narrow strip of thickened tissue extending almost to velarial margin.

Gonads attached along entire length of interradial septa; leaf-like, extending laterally into radial stomach pouches; narrow in this immature male specimen. Tentacles (Plate 5.2E) 4, 1 per pedalium, hollow, round in cross section, with nematocysts in alternating bands (larger, smaller, larger, etc.); preserved, tentacle constricted approximately every 10 bands, giving segmented appearance, though unclear whether this is an artifact of contraction and preservation; length in life unknown. Stomach small, flat, extending into 4 large coelenteric pouches, divided by interradial septa. Interradial septa without minute perforations. Manubrium short.

Color: preserved in formalin, the gonads, tentacles, rhopalia, and phacellae opaque whitish; body slightly cloudy.

The exact nature of several characters could not be ascertained in the holotype without dissection, the privilege of which was denied by the MTQ, e.g., the phacellae (number of roots, branching pattern of cirri bundles), the eyes (number and arrangement), the manubrium (cross section shape, length and shape of lips), and mesenteries (length and state of development). With strong side light, I was able to determine with reasonable confidence that the mesenteries do not extend down along the middle regions of the bell wall; however, it is not unusual for taxa to have weakly developed mesenteries in the perradii of the manubrium which only extend a short distance. Whether this is the case in *A. mordens* cannot be determined from the holotype. It is unfortunate that the number of roots to the phacellae could not be examined; this will likely be an important character in determining the species boundaries within the genus. In paratype MTQ G55288, these missing characters were scored as follows: phacellae with about 20 roots per group; cirri long, bundled near the base in clusters of several; eyes 4 per rhopalium, two lensed median plus one on slit-eye each side; manubrium open and amorphous; lips wide triangular; mesenteries completely absent in flap or cord form. Some specimens of this species have only two median eyes and no lateral eye spots, while one specimen has the normal cubozoan 6 eyes (2 median lensed eyes, 4 lateral eye spots); it would appear that the lateral eye spots are somewhat variable, but the lower main eye is always unusually large in comparison to other Carybdeida. The gonads of sexually ripe specimens are overlapping and pleated (Plate 5.1B). In life, the body is completely transparent and colorless, and the tentacles are bright pink.

STATOLITH (Plate 5.2F, paratype #AIMS 2003.10). Tear-drop-shaped, with a medially incised, truncate basal border, deep garnet reddish in colour; enclosed in lower portion of rhopalium, situated immediately behind the main lens rather than below it. The statolith is orientated truncate-side down for standardized comparison with statoliths of other species; however, *in situ* the truncate side is orientated up toward the rhopalial stalk and the flat side facing the camera in the photograph is the unexposed side, facing the back of the main eye *in situ*.

CNIDOME (Plate 5.7A, B; from paratype MTQ G55288). The tentacular nematocysts are exclusively lemon-shaped euryteles, with an arithmetic mean of 23.0 µm long by 12.8 µm wide (range 19.3-27.3 µm long by 11.1-14.9 µm wide, N= 57). The bell nematocysts are exclusively large spherical isorhizas with a short, loosely coiled tubule, averaging about 12 per nematocyst freckle; the arithmetic mean size is 30.3 µm (range 28.3-31.7 µm, N= 24). Nematocysts of the lips and gastric cirri were not observed, despite efforts to find them.

NUCLEOTIDE SEQUENCES. Most of the 18S rDNA gene has been sequenced for four specimens of *A. mordens* (L. Peplow, unpublished data); these sequences will be published elsewhere as part of a comprehensive phylogeny of the Australian Cubozoa. The first 568 bp are given in Appendix 2 and analyzed phylogenetically in Chapter 3.

DISTRIBUTION. Anecdotal reports from throughout the northern outer Great Barrier Reef (GBR) region; currently confirmed only from Moore Reef and Agincourt Reef, on the outer GBR, and from Osprey Reef in the Coral Sea.

REMARKS. *Alatina mordens*, as the type species of the genus, is distinctive from other carybdeid genus types in nearly every scorable character (Table 2.14). Only rudimentary comparison can be made with other species that have previously been associated with *Carybdea alata* auct., due to insufficient descriptions and lack of comparative material; a brief synopsis of characters of *Alatina* spp. is given in Table 5.1.

The relationships of the *Alatina* spp. will not be fully understood until fresh collections are made from type localities and rigorous morphological and molecular comparative studies are made on many forms. Particular attention should be given to the number of roots to the gastric phacellae, the branching pattern or lack thereof to the velarial canals, the number of lensed eyes and eye spots at maturity, and patterns of exumbrellar, pedalial, and velarial nematocyst clusters.

LIFE CYCLE. The life cycle of *Alatina mordens* is presently unknown. However, Arneson and Cutress (1976) described the life cycle of "*Carybdea alata*" from Puerto Rico. Similarities may emerge when the life history of *A. mordens* is resolved, presumably being closely related.

ECOLOGY. The general ecology of *Alatina mordens* is presently unknown. However, the ecology of another species of uncertain identity in the *Alatina* clade has been studied in Hawaii, and has been documented to occur in large numbers the 9th-10th day after the full moon (Thomas et al., 2001). Preliminary study indicates that *A. mordens* has a similar predictability (R. Hore, pers. comm., 2004); the ability to predict this species is of great importance if this is, as suspected, a species dangerous to humans.

SEASONALITY. As illustrated from collection records indicated in the material examined section, *Alatina mordens* can be encountered any time of the year on the outer Great Barrier Reef, contrary to local folklore which holds that one is only at risk of stings during the summer months, and then only onshore. Furthermore, collection records do not appear to correlate season with size or maturity, indicating that perhaps *A. mordens* breeds and grows all year. Additional attention to the ecology of this species should be given priority, in order to test these observations in a statistically meaningful way.

MEDICAL NOTES. *Alatina mordens* may pose a serious human health risk; however, correlations are ambiguous and experimental evidence supporting or refuting this hypothesis is lacking. There are currently several known cases of severe Irukandji symptoms following envenomation on the outer reef, with similar case histories and *A. mordens* being captured or sighted at the time. Typically, this species is encountered at night, when it swarms near lights used for scuba diving; some stings occur when scuba divers return to the boat and swim up into a swarm of jellyfishes. As elaborated by Williamson (1985) and Hadok (1997), severe envenomations in cases involving diving may be mistaken for decompression sickness. The outer reef symptoms typically onset quickly (ca. 5-10 minutes), and resemble a more severe version of the Irukandji Syndrome than is typically associated with coastal envenomation, often involving severe hypertension (Gershwin et al., unpublished).

In at least one outer reef case, the victim saw herself get stung by a large jellyfish in the water (Anonymous, pers. comm., Dec. 1998); three specimens retrieved at the time of the incident comprise the holotype, a paratype (SAM H986), and the first of the two JCU non-type specimens listed above. The patient was critically ill for more than a week following envenomation (Mulcahy, 1999; Little et al., 2001). A single nematocyst recovered from the victim's skin was later reported by Little and Seymour (2003) and attributed to an unnamed species in the Queensland Museum collection previously identified by me. The nematocyst from the sting event figured by Little and Seymour cannot be differentiated from those characteristic of *Alatina mordens* (Plate 5.7); thus, the possibility that this species was responsible for the sting must be considered.

In another case from which the same species was recovered, five divers were stung at Osprey Reef the night of 29 January 2000 while surfacing into the swarm at the end of the dive; the onset of symptoms was rapid (ca. five minutes) and all required medical treatment (P. Colwell, pers. comm., 2000). A single specimen was captured from those that were swarming at the time, and was forwarded to me for study (paratype #SAM H1053). A third case of similar circumstances at Hastings Reef on New Years Eve 1999, involving two victims, was reported to me (P. Colwell, pers. comm., 2000); no specimens were retained. Other anecdotal cases exist in which similar jellyfish were sighted but not captured, and which involved severe Irukandji syndrome (Gershwin, unpublished). While the evidence from multiple sting events lends support to this species being the stinging agent, it is important to note that this species is only possibly the cause of these cases of Irukandji syndrome; testing this hypothesis experimentally should be considered a high priority for stinger management.

There is also some indication that perhaps *A. mordens* is not especially dangerous. Paratype #AIMS 2003.10 was captured following a superficial sting to a 12 year old boy, in which no systemic symptoms were reported (R. Hore, pers. comm., 2003). In another incident, a 38 year old woman was stung on the arm, with no systemic symptoms; her husband saw the jellyfish and a biologist familiar with *A. mordens* saw what he believed to be this species in the water shortly after (R. Hore, pers. comm., 2003).

Several explanations exist which may account for the differential symptoms. First, it is possible that a different species than *A. mordens* is responsible for the stings. This seems unlikely because the same species was recovered from multiple sting events with similar sting characteristics. Second, it is possible that different intensities of stings or different susceptibilities in the sting victims have led to very different outcomes. While differential susceptibilities and severities should not be ignored, it seems unlikely that these alone would fully explain the wide range of symptoms from mild to potentially lethal, all in previously healthy individuals. Third, it is possible that the medusae go through ontogenetic, seasonal, or reproductively-related changes in toxicity. No data currently exist as to whether this is or is not the case, but it should be a priority for collaborative study by ecologists, toxinologists and taxonomists.

STING MANAGEMENT. A thorough treatment of stings and sting management is given by Williamson et al. (1996). When swimming in areas where cubozoans may occur, common-sense sting precautions should be employed, such as wearing protective clothing over exposed skin areas, e.g., a Lycra body suit. There is currently no medical or scientific evidence that commercial sting repellants or swimming like a turtle are effective methods of sting prevention. If stung, the best-known treatment for minimizing further envenomation is to pour vinegar liberally over the sting area for 30 seconds; this has been shown to be effective for a wide range of cubozoan stings (Williamson et al., 1996, and references therein). If tentacles are present on the skin, they can then be safely removed. For severe stings, or those thought to be potential Irukandji envenomations, the patient should be made comfortable and medical treatment should be sought as quickly as possible.

There are many sources dispensing advice on jellyfish sting treatments, many of which are simply inaccurate. Contrary to popular belief, rubbing with sand or washing with fresh water often do more harm than good, causing additional nematocysts to discharge into the victim's skin. Metholated spirit is still often said to work well, but has been scientifically shown with *Chironex fleckeri* to cause immediate, massive discharge of nematocysts rather than to inhibit discharge (Hartwick et al., 1980); thus, it should not be used. There is also a common misbelief that vinegar stops the effect of the sting (i.e., relieves the pain, stops the illness) – this is untrue. It only disables undischarged nematocysts from discharging, eliminating further envenomation; vinegar should be used in all cases where stings from dangerous cubozoans are suspected.

There is no cause for alarm with the recognition of *Alatina mordens*. Most stings attributable to *A. mordens* occur at night, when the medusae are attracted to artificial lights (R. Hore, pers. comm., 2000; P. Colwell, pers. comm., 2000). Perhaps the most urgent action that should come from formally recognizing this species and its potential for harm, is the quest for an antivenom for severe cases of Irukandji syndrome. Presently the only jellyfish antivenom that has been developed is for *Chironex fleckeri*, and this was shown by Fenner and his colleagues (1986) to be ambiguous in managing Irukandji envenomation. Recent efforts to develop an antivenom to the Irukandji syndrome have been hampered by the sporadic occurrence of *Carukia barnesi*, combined with its small size (and thus, low venom yield per animal). *Alatina mordens* may provide a more stable subject, as it occurs all year and has a much larger, more robust body and tentacles, and thus more venom yield. However, *C. barnesi* and *A. mordens* do not appear to be closely related to one another, so the relationship between their venoms is unclear.

5.3.4 *Alatina rainensis* sp. nov.

Plates 5.3, 5.4

MATERIAL EXAMINED. *Holotype*: MTQ G55286, Raine Island, Great Barrier Reef, 11º35'34"S 144º02'12"E, Dec. 2002, collected by J. Seymour; gravid female, BH 17.8 mm, DBW 15.9 mm, IRW 8.0 mm, TBW 0.6 mm, PL 8.8 mm, PW 4.3 mm, CW 0.7 mm.
Paratype: MTQ G55287, same locality as holotype; gravid female, BH 17.2 mm, DBW not taken prior to sectioning, IRW 7.9 mm, TBW 0.6 mm, PL 8.7 mm, PW 3.7 mm, CW 0.7 mm.

TYPE LOCALITY. Raine Island, outer Great Barrier Reef.

ETYMOLOGY. The specific name, *rainensis*, is derived from the name of the island where this species was found.

DIAGNOSIS. *Alatina* with small body height at maturity, with butterfly-form gonads, attached in the central portion of the interradii only; phacellae with cirri rooted singly or in pairs.

DESCRIPTION OF HOLOTYPE. Bell somewhat taller than wide, with domed apex; with wide, shallow interradial furrows, lacking circum-aboral groove (Plate 5.3A). Adradial furrows absent. Exumbrella sparsely sprinkled with minute unraised nematocyst freckles, absent on pedalia and velarium.

Pedalia 4, interradial, with long stalk; inner keel quite rounded, outer keel more or less straight (Plate 5.3B). Pedalial canals somewhat quadrate in cross section through stalk portion, flat through remainder; running along lower edge of pedalial lamella to about halfway, then leaving it perpendicularly, producing a 90° bend. Canal of fairly even width throughout length, bowing somewhat adaxially in a large shallow curve; straight at tentacle insertion. Tentacles 4, 1 per pedalium, round in cross section; straight-sided at the base. Tentacular banding pattern of two types: proximally, every 10th or 11th band smaller than others, thus giving tentacle a segmented appearance; distally, bands more or less alternate smaller with larger.

Gonads butterfly-shaped, approximately one half BH in length, but restricted to centre portions of interradii, approaching neither stomach nor pedalia, overlapping slightly at perradii; not pleated (Plate 5.3C). Interradial septa with extremely minute perforations.

Velarium narrow; with three canals per octant, simple throughout length (Plate 5.4A). Perradial lappets lacking. Frenulae composed of a single narrow sheet of tissue, extending on to velarium nearly to margin.

Rhopalial niche ostium T-shaped, with a shallow M-shaped covering scale above, and two shallow scales below, open at both sides between upper and lower scales; lower scales separated by a deep furrow extending to velarium (Plate 5.4B). Rhopaliar niche flush with exumbrellar wall; subumbrellar window bulging considerably. Rhopalia with 4 eyes, 2 median lensed eyes plus 2 elongate lateral eye spots. Lower of two lensed eyes large and round; upper laterally flattened into a strip distal to and cupping the lens. In preserved specimen, faint shadows occur where the 2nd pair of eye spots should be if present; thus, unknown whether eye spots faded or simply lacking. Statolith situated behind main eye, not below it; statolith shape indeterminable in preserved specimen. Rhopalial horns and warts lacking.

Stomach small, shallow, completely lacking mesenteries (Plate 5.4C). Manubrium very short, quadrate in cross section; mouth cruciform with 4 rounded lips. Gastric phacellae crescentic in interradii, opening toward midline; cirri approximately 50 per phacellus, long, simple, rooted singly or in pairs (Plate 5.4D).

Color in life not reported; preserved, the gonads, phacellae, and tentacles whitish, rhopalia dark brown, all other parts transparent and colourless.

CNIDOME (Plate 5.7D-H; from holotype and paratype). The tentacular nematocysts are of two primary types and two very small secondary types. The largest are isorhizas, with a sub-spherical capsule and tightly packed tubule occupying the whole inside; the tubule morphology could not be determined due to lack of discharged capsules. The arithmetic mean of these undischarged capsules is 20.1 x 15.7 µm (range 16.4-23.8 µm long by 13.5-18.3 µm wide, N=42; Plate 5.7G). The other primary nematocysts are medium-sized microbasic euryteles, with a relatively narrow lemon-shaped capsule, with a distinct nipple at the distal end, and a distinctly visible shaft but poorly defined tubule; the arithmetic mean of these undischarged capsules is 17.4 x 11.5 µm (range 15.0-19.7 µm long by 10.4-13.1 µm wide, N= 37; Plate 5.7E, undischarged, Plate 5.7F, discharged). The secondary nematocysts include a smaller size class of ovoid isorhizas (arithmetic mean 7.4 x 6.2 µm, range 6.2-8.2 µm long by 5.6-6.8 µm wide, N=13; not figured) and a nearly spherical type with a short, straight shaft and no visible tubule, presumed to be microbasic amastigophores (arithmetic mean 6.8 x 6.4 µm, range 6.2-7.2 µm by 6.0-6.8 µm, N=6; Plate 5.7E).

The exumbrellar nematocyst freckles have about 20-30 small spherical isorhizas per cluster, with an arithmetic mean of 9.7 (range 9.2-10.5 µm, N= 22; Plate 5.7H). Other loose nematocysts were found on a bell fragment from the apical portion of the paratype's dissected exumbrella; these included large sub-spherical isorhizas similar to those found on the tentacles (mean of 21.9 x 17.9 µm), microbasic euryteles (16.6 x 13.0 µm), and large sub-ovate microbasic p-mastigophores (mean of 28.3 x 13.8 µm). It is presumed that the isorhizas and euryteles were transferred from the tentacles during instrument handling, but whether the mastigophores were even from this species is not known; this type of nematocyst is characteristic of several undescribed species of Irukandjis. The nematocysts of the gastric cirri are extremely small euryteles, averaging 7.7 x 5.5 µm (range 6.5-9.0 µm long by 4.6-6.6 µm wide, N= 24; Plate 5.7D). Nematocysts were not observed on the lips, despite exhaustive searching.

VARIATION. The paratype is extremely similar to the holotype in most respects, but differs in the tentacle banding pattern, which is more strongly alternate, lacking the 10-band groupings.

DISTRIBUTION. Presently known only from the type locality.

REMARKS. *Alatina rainensis* differs from all other carybdeids in its unique combination of crescentic phacellae and butterfly-form gonads. The crescentic phacellae are typically associated with the *Alatina* group, but the butterfly-form gonads are typically associated with the distantly related *Tripedalia* group. However, this species does not appear to be an evolutionary intermediate between the two groups, because the rhopalial niche ostia and windows, as well as the velarial canals, are also of the *Alatina* form. Thus, the species seems clearly of *Alatina* affinity, and the odd gonad shape appears to be convergent.

5.3.5 *Alatina moseri* (Mayer, 1906) comb. nov.

Plate 5.5A

Charybdea moseri v*Mayer, 1906: 1135-1136, pl. 1, figs. 2-2c; n. sp., description and illustrations. – Bigelow, 1909: 19-20; young stage of *C. grandis*. – Bigelow, 1938: 144, junior synonym of *Carybdea alata*. – Chu and Cutress, 1954: 9, cause of dermatitis, Hawaii. – Kramp, 1961: 304; in synonymy of *Carybdea alata*.

Carybdea moseri Mayer, 1915: 171, probably young of *C. alata* var. *grandis*. – Mayer, 1917: 189 [in part], fig. 3; only half-grown stage of *C. alata*.

Carybdea alata var. *moseri* Mayer, 1910: 512; probably a variety or young stage of *C. grandis*; probably identical with *C. philippina*. – Light 1914: 196; = *C. philippina*, Philippines. – Stiasny, 1919: 34, 37-38, fig. 5, Sumatra. – Bigelow, 1938: 144, in synonymy of *C. alata*.

MATERIAL EXAMINED. *Syntype*: USNM #21800, Str. Albatross, sta. 3829, Avalu Pt., Lanai Island, Hawaii; 23 specimens; poor condition, uninterpretable.

Other material examined: USNM #22311, Albatross Station 3931, from Honolulu to Laysauld, Hawaiian Islands, 2535 fathoms; 1 specimen in very fine condition, BH 39.4 mm, DBW 29.9 mm, IRW 13.9 mm, TBW 1.2 mm. USNM #29632, Albatross Station 3829, South coast of Molokai Island, Hawaii, 1 April 1902, at surface; 2 specimens in very fine condition, A) BH 85.4 mm, DBW 51.5 mm, IRW 26.8 mm, AR 74.7 mm, TBW 1.5 mm, B) BH 73.4 mm, DBW 43.3 mm, IRW 22.9 mm, AR 62.5 mm, TBW 1.9 mm (Plate 5.5A).

TYPE LOCALITY. Avalu Pt., Lanai Island, Hawaii.

DIAGNOSIS. *Alatina* with or without nematocysts on exumbrella and pedalia, but lacking on velarium, with two median and two lateral eyes, with phacellae comprised of numerous cirri pairs, velarial canals 3 per octant and either simple or of two forms.

REVISED DESCRIPTION. Bell to about 85 mm in height, 27 mm wide, tall, narrow; with bluntly rounded apex, without circum-aboral groove; with thin but rigid body (Plate 5.5A). Exumbrella lacking nematocysts and warts in most specimens. Interradial and adradial furrows lacking.

Pedalia 4, approximately one-fourth BH, nearly as wide as long, with widely rounded adaxial keel and narrow abaxial keel. Pedalial canals simple at bend; not flaring at tentacle insertion, flat throughout length. Tentacles 4, simple, round in cross-section, with equal-sized nematocyst rings; straight-sided at the base.

Rhopalial niche flush with exumbrella; T-shaped, with a single broadly rounded covering scale above and two well developed scales below. Rhopalia with 2 round median eyes with lenses and 2 lateral, elongate eye spots slanting upward away from between the two median eyes. Statolith disintegrated, uninterpretable in preserved specimens. Rhopalial horns lacking.

Velarium wide; nematocyst warts lacking. Velarial canals 3 per octant, simple and unbranched. Perradial lappets lacking. Frenulum very broadly webbed when viewed laterally, narrow and pointed along velarium; nearly reaching velarial edge.

Phacellae in crescentic rows at interradii; cirri long, arranged in parallel manner, rooted together in pairs. Mouth with 4 simple lips. Gonads attached along entire length, reaching from stomach to level of rhopalia, not extending to pedalial canal. Interradial septa perforations lacking. Stomach wide and shallow. Mesenteries lacking.

Color in life unknown; preserved body hyaline, tentacles pink, eye spots dark brown, gonads milky yellow.

VARIATION. One specimen (USNM #29632-A) has nematocyst freckles in a single row of the outer pedalial keel, and also a few scattered upon the exumbrellar surface. The relationship of this specimen to the others is not presently well understood. The velarial canals nearest the pedalia are biforked in one specimen (USNM #22311).

DISTRIBUTION. Not reported outside Hawaiian Islands.

REMARKS. This species clearly falls within the genus *Alatina*, with crescentic gastric phacellae, 3 velarial canals per octant, and broadly rounded abaxial pedalia wings. However, it may be distinguished from the other species by several characters. First, the gastric cirri are rooted in pairs, whereas in other forms they are typically rooted in bunches. Second, the velarial canals are straight and unbranched, similar to most species in the *alata*-group but differing from others such as *A. grandis* comb. nov. and at least two undescribed forms. Third, the rhopalia of *A. moseri* have only 4 eyes, 2 median eyes with lenses and 2 lateral ocelli. This feature was also described for immature specimens of *Alatina grandis* (Agassiz and Mayer, 1902). And indeed, several workers have thought that *A. moseri* is merely the young of *A. grandis* (Bigelow, 1909: 19-20; Bigelow, 1938: 138, 144-145; Mayer, 1910: 507, 512; Mayer, 1915: 171; Mayer, 1917: 189); however, *A. moseri* specimens have fully mature gonads, so they are unlikely to be the young of another species. Bigelow (1938: 138, 145) thought that *A. moseri* might be a dwarf race of *A. grandis*; there is limited value in recognizing races among diagnosably different forms.

Mayer (1906) thought this species might be the same as “*Carybdea* sp.” of Semper (1860: fig. 9) from the Philippines, subsequently named by Haeckel (1880) as *Procharybdis turricula*. Oddly enough, Mayer (1910: 512) and Stiasny (1919: 37) both confused Semper’s and Haeckel’s forms, attributing Haeckel’s name “*C. philippina*” to Semper’s fig. 9, whereas

Haeckel gave the name to Semper's fig. 8. To whichever one Mayer intended to refer, he (Mayer, 1910: 512) stated that "*C. moseri*" and Semper's form were "probably identical". Light (1914: 196) regarded the two species as identical. I have translated Haeckel's descriptions, and studied them and Semper's figures extensively; I am unable to find any characters that definitively characterize either of Semper's species, whereas *A. moseri* is diagnosable based on the peculiar paired phacellae bunches, having only four eyes, and the lack of exumbrellar nematocysts.

There are some discrepancies between the reported station data and the data on the specimen labels; it is difficult at this point to say with certainty what is accurate. First, Station 3931 was not included in the published list, but was indicated on the label in Mayer's writing as being "*Charybdea moseri* n. sp." Second, specimens from Station 3829 are indicated on two samples, USNM #29632 and #21800, unfortunately with different localities. Furthermore, the specimen numbers do not match those published. Specifically, Mayer lists 10 specimens from Station 3829, collected 1-2 April at Avalu Point, Lanai Island. However, neither of the two lots of specimens is a match. USNM #29632 matches the date, 1 April, but gives the locality as Molokai Island, and only contains two specimens. USNM #21800 matches the locality, Avalu Pt., Lanai Island, but there are 23 specimens. The remaining samples could not be found. Mayer (1906: 1136) indicated that USNM #21800 was to be the type, which is unfortunate, since the specimens are now completely fragmented and uninterpretable; he indicated in handwriting on the specimen labels in USNM #29632 that that lot was to be the type, but he did not indicate this in publication. The two lots #29632 and #22311 are in excellent condition.

5.3.6 Other nominal species of *Alatina*

Alatina alata (Reynaud, 1830) comb. nov., is completely unrecognizable based on the original description and illustration, but because of the prevalence of the name in the literature, should be stabilized by declaration of a neotype; a full redescription of a South Atlantic specimen will serve as the basis for identification of the taxon in the future. Accordingly, an application to the I.C.Z.N. to conserve the name *Alatina alata* will be submitted as soon as a suitable neotype is located.

Alatina obeliscus (Haeckel, 1880) comb. nov., seems to fall within the *Alatina* group, based on the large phacellae as described; however, its exact identity with respect to its congeners cannot be determined. Even with a specimen to study, it is unlikely that the species could be properly diagnosed, based on its uneven development of the velarial canals, and the size of Haeckel's specimen (BH 35 mm, BW 20 mm), both of which indicate that it was very likely to be a juvenile in the absence of information to the contrary. Assuming it is one of the branched-canal species, it would be relatively easy to diagnose, but only with mature specimens. Haeckel described a “button-form” thickening at the end of the tentacles; this is found occasionally in specimens that have spent too long in captivity in sub-optimal conditions (e.g., too warm, too confined, poor circulation; Gershwin, unpublished), and does not appear to be a diagnostic species character. Because this species is unlikely to be recognizable under any circumstances, the name is best abandoned.

Alatina philippina (Haeckel, 1880) comb. nov., was described based on a line drawing by Semper (1860, pl. 39, fig. 8). At a mature bell height of 30 mm, one might expect that the species would be diagnosable, but Semper’s line drawing and Haeckel’s description are too vague to allow for differentiation of this species from any other. It seems closest to Haeckel’s earlier named species *Alatina tetraptera* (Haeckel, 1880) comb. nov., based on the long pedalia and the wing-like phacellae; however, whether the two are identical or different cannot be concluded based on the available information. Thus, in the interest of stability, it seems most conservative to permanently abandon the name.

Alatina pyramis (Haeckel, 1880) comb. nov., appears distinctive based on several good features that can be discerned from Haeckel’s (1880) description and illustrations. First, being only 30 mm tall and having full gonads, the only other *Alatina* spp. in this adult size range would be *A. rainensis*, which has butterfly-shaped gonads, and *A. tetraptera*, which has peculiarly long pedalia and wing-shaped phacellae. Second, *A. pyramis* is the only species with frizzy lips. Third, the pedalia are considerably narrower than is typically found in other species in the genus *Alatina*. I have no doubt that when this species is encountered again, it will be immediately recognizable based on these combined characteristics.

Alatina tetraptera (Haeckel, 1880) comb. nov., seems to fall within the *Alatina* group, based on the large pedalia with “mighty” wings and the phacellae with long cirri. Furthermore, the rhopalial niche ostia were described as “heart-shape”, which could be easily mistaken for T-shaped if the specimen were not well preserved. Finally, only a single large eye was observed on the rhopalia, which is characteristic of some *Alatina* spp. Haeckel classified this species into his genus *Procharybdis*, based on the absence of the velarial canals and frenulum. I am unsure what to interpret from the missing velarial structures, but it would be wrong to regard the species as anything other than distinct based on the split, wing-like phacellae (Haeckel, 1880: pl. 25, fig. 4) and the extremely long, uniquely shaped pedalia (pl. 25, fig. 3). These two characters are not known in any other cubozoan, except possibly the later-described *A. pyramis*. This species has not been recognized in the scientific literature for almost 100 years, but it seems appropriate to revalidate it awaiting fresh material that can be studied for a proper redescription. At a mature bell height of 30 mm, with the structures described, I think this species would be recognizable if found again.

Alatina turricula (Haeckel, 1880) comb. nov., described from a line drawing by Semper (1860, pl. 39, fig. 9), is unrecognizable. It is clear from the widely rounded pedalia in the original illustration that this medusa is a member of the *Alatina* group. Furthermore, due to its extremely tall body (170 mm), it is possible that it is referable to *Alatina grandis*. However, this is merely speculation, as it is impossible to diagnose with certainty from the drawing and vague description. There are no structural characters described that would serve to differentiate this species from any of the others; thus, it seems most conservative to permanently abandon the name.

Alatina grandis (Agassiz and Mayer, 1902) comb. nov.: The type material of this species from the Paumotus, has been lost; neither MCZ nor USNM know of its whereabouts. Specimens from the Society Islands (MCZ 1043 and MCZ 342), identified by H. Bigelow as "*C. grandis*", match the original description but are too poorly preserved to be usefully interpretable. One (MCZ 1043, BH 184.6 mm, DBW 59.6 mm, IRW 33.2 mm), bears the following collection data: Pacific Ocean, Society Islands, coll. A. Garrett, 29.ix.1861; originally preserved in alcohol, now preserved in formalin, and was delivered to the MCZ by A. Garrett in 1864 (Plate 5.5B). The other (MCZ 342, BH 170.9 mm, DBW 57.8 mm, IRW 33.6+ mm), was apparently collected at the same time (Plate 5.5C). The species seems distinctive based on its extremely large size, and in having only one median eye and short, branched velarial canals.

Alatina madraspatana (Menon, 1930) comb. nov., is described as having up to 5 branched velarial canals per octant, an apical concavity, and 6 eyes on each rhopalium, one of the median bearing a lens. This combination of characters is unique, and thus, the species is regarded herein as valid. However, I remain curious about the velarial interpretation, as it seems rather odd for an *Alatina* to have 5 velarial canals per octant.

5.3.7 *Manokia* Southcott, 1967

Manokia stiasnyi (Bigelow, 1938)

Plate 5.6

Charybdea spec. v*Stiasny, 1930: 3-5, figs. 1-7; occurrence in New Guinea, and description of species. – Stiasny, 1937a: 216; brief comparison of branched tentacles.

Carybdea stiasnyi v*Bigelow, 1938: 136; n. sp., in reference to Stiasny's (1930) description. – Kramp, 1961: 306. – Southcott, 1963: 51; tentacle comparison. – Kramp, 1968a: 69 [doubtful species].

Manokia stiasnyi Southcott, 1967: 667; new genus comb. nov., and discussion of characters.

Charybdea stiasnyi Payne, 1960: 6, 28, 32-33.

MATERIAL EXAMINED. *Holotype*: IRSNB IG 9223, Manokwari, New Guinea, 10 March 1929; male, BH 23.6 mm, DBW 20.1 mm at the top of the pedalium, DBW 21.7 mm at the widest point, IRW 10.2 mm, TBW 1.3 mm, 12.5 mm Pedalial length, 2.2 mm pedalial width at widest part.

TYPE LOCALITY. Manokwari, New Guinea.

REVISED DESCRIPTION. Body barrel-shaped, widest in middle region, with conspicuous apical depression (Plate 5.6A). Interradial furrows deep, nearly meeting pedalia. Adradial furrows deep, demarcating rhopaliar region and interradial thickenings. Bell with scattered gelatinous nematocyst warts, extending onto velarium but warts not specific to any canal or pattern.

Pedalia 4, interradial, scalpel-shaped, with relatively narrow inner keel, lacking nematocyst warts or freckles. Pedalial canals flat throughout length, with slight upward-pointing nub projecting into sub-lamellar space; straight at tentacle insertion. Tentacles 4, interradial, round in cross section, with evenly-sized nematocyst bands. Nematocyst bands drawn out adaxially into short, blunt extensions, approximately 8 per tentacle (Plate 5.6B); one tentacle having extensions in 2 alternating rows, other tentacles having them in more or less a single row.

Rhopaliar niche flush with surrounding bell wall; with T-shaped ostia, comprised of a single thickened covering scale above and two well developed covering scales below. Upper scale with a median flap hanging down into ostium in front of rhopalium. Rhopalial horns absent. Subumbrellar wall of rhopalial niche made of a thick window of un-muscled mesoglea, hemispherically convex on subumbrellar side, concave on rhopaliar niche side (Plate 5.6C). Rhopalia with two median lensed eyes, distal-most larger than proximal, and two pairs of unevenly sized lateral eye spots, distal pair larger than proximal pair.

Phacellae crescentic, with numerous long cirri, arranged more or less parallel; number of trunks in each phacellus indeterminable without damaging specimen, but appears to be between 5 and 10. Stomach shallow, with short manubrium; specimen with large amphipod high inside the subumbrellar cavity. Mouth shape indeterminable without damaging specimen. Mesenteries lacking. Gonads leaf-like, attached along nearly whole length of interradial septa, projecting laterally into coelenteric cavity.

Velarium 2.7 mm wide, with 4 undulating, unbranched canals per octant. Frenulae well developed, but short, reaching only approximately halfway to velarial margin. Perradial lappets absent.

Colour in life unknown.

CNIDOME (Plate 5.7F). The tentacles of *Manokia stiasnyi* have a monocnidome of sub-spherical euryteles with a thick capsule wall, with an arithmetic mean of 15.2 x 12.4 µm (range 13.4-16.5 µm long by 11.5-13.6 µm wide, N=12). Nematocysts from other parts of the body were not examined due to brittleness of the specimen.

REMARKS. Stiasny (1930) commented that the exumbrella of this species was smooth, lacking nettle-warts. However, this is inaccurate. The holotype specimen has a few scattered warts, and while most of these have become flattened through the passage of time, several are still raised. I could not observe any particular pattern to their arrangement, but they are present on the velarium as well as the body.

The tentacles are worthy of discussion, as they have always been the chief character used to separate this species from others. The tentacles are typically said to be branched (Stiasny, 1930; Bigelow, 1938; Kramp, 1961; Southcott, 1967). However, the tentacles are not branched in the conventional sense, and referring to them as such is somewhat misleading. In branched tentacles, one would expect that the central lumen would be branched, in order to maintain the flow of nutrients and various fluids. However, in the tentacles of the present specimen, it is the actual nematocyst bands that are branched rather than the tentacle itself. Therefore, the "branches" are not true branches, but rather, they are merely elongations of one side of the tentacular nematocyst bands. Each band is drawn out a short distance adaxially like a little tail, with these extensions primarily arranged in two vertical rows. This character is somewhat reminiscent of the neckerchief-shaped tentacle bands of *Carukia barnesi*, but the resemblance is apparently only superficial. In *M. stiasnyi*, the bands are apparently normal around most of the tentacle, and only extended in a bluntly rounded, almost herniated manner along the adaxes. In *C. barnesi*, the bands are widely spaced, and the adaxial extensions are quite remarkably triangular in form, extending distally.

The peculiar branching of the tentacles led Stiasny (1937a: footnote p216) to think that the medusa might be the young of an unusual chirodropid he identified as *Chiropsalmus quadrigatus*. His specimen from the Maldives had numerous filaments on the tips of the tentacles. He misunderstood Mayer's (1910) redescription of *C. quadrigatus*, thinking that each pedalial finger should bear numerous tentacles, rather than a single one. In fact, *M. stiasnyi* bears no resemblance whatsoever to *C. quadrigatus*, nor does Stiasny's Maldivian specimen; the latter will be formally described in a forthcoming chirodropid revision.

Two particular characters suggest a strong affinity to the *Alatina* species group, namely, the T-shaped rhopaliar niche ostia and the crescentic-shaped phacellae of long cirri. Although similar, the ostia are also quite different, in that the upper covering scale has a central flap that hangs down, rather than the typical straight scale of *Alatina*. The two lower covering scales are quite robust, and the indentation between them extends down to the velarium.

Southcott (1967) erected the genus *Manokia* based on the branched tentacles, four undulating velarial canals per octant, and horizontal phacellae. He commented that the branched tentacles alone would be insufficient basis to establish a new genus, because they could simply be aberrant; however, the velarial canals were quite distinct. He went on to compare the canals with those of *Carybdea rastonii* and *C. marsupialis*, citing that those of *M. stiasnyi* are more numerous but simpler in nature. However, any species in the *Alatina* group would have been a closer comparison (though *M. stiasnyi* still would have proven unique). The crescentic phacellae and T-shaped rhopalial niche ostia are more reminiscent of *Alatina*, as are the more or less simple velarial canals and lack of mesenteries.

Kramp (1968a) remarked that Bigelow's species was doubtful, but did not elaborate why. Less than a decade earlier, he had considered it valid (1961). The point is moot anyway, for reexamination of the specimen has revealed a combination of characters unlike those of any other known species.

Table 5.1. Comparison of *Alatina* species characters. Data based on original descriptions and figures. Question marks indicate unavailable data.

	Max. BH	Bell freckles	Velarial canal # per octant	Velarial canal form	Pedalial canal bend	Pedalial freckles	# of eyes	Other characters
A. alata comb. nov.	?	?	?	?	?	?	?	
A. grandis comb. nov.	230mm	Absent?	3	Short, branched tree-like	90°	Absent?	1 in adults; 3 in juvs	
A. madraspatana comb. nov.	110mm	Small	5	Branched	Simple	Small when present	6 (1 lensed)	Apical concavity
A. mordens n. sp.	96mm	Small	3	Simple, biforked, triforked at tip	Round	Minute	2 median	Pedalial wings wide
A. moseri comb. nov.	80mm	Present	3	Simple/ biforked	Simple	Absent or 1 row oblong	4	Paired cirri in phacellae
A. obeliscus comb. nov.	35mm	?	3	Simple beside frenula, forked beside pedalia	?	?	?	Tentacles thickened at end
A. philippina comb. nov.	30mm mature	Absent?	3	Simple, short	Simple to angular	Absent?	?	Split phacellae; long pedalia
A. pyramis comb. nov.	30mm mature	Absent?	3	Simple, wide	Simple?	Absent?	6?	Pedalial wings narrow; frizzy lips
A. rainensis n. sp.	18mm mature	Minute	3	Simple	90°	Absent	4	Butterfly-form gonads
A. tetraptera comb. nov.	30mm mature	Absent?	Lacking	Lacking	Simple	Absent?	1	Split wing-like phacellae; very long, large pedalia
A. turricula comb. nov.	170mm	Absent?	Absent	--	Simple to angular	Absent?	?	Overhang-ing pedalial wings

5.4. DISCUSSION

The molecular and morphological phylogenetic analyses in Chapter 3 clearly separate the *Alatina* spp. from the *Carybdea* spp. proper, plus the *Tripedalia* spp., and *Carybdea sivickisi*. Together with *Manokia stiasnyi*, the *Alatina* spp. appear to form a natural group diagnosable by numerous major structural characters. The family Alatinidae is erected herein to accommodate this group.

The species of the *Alatina* group all share the conspicuous characters of crescentic phacellae and T-shaped rhopaliar niches; however, the internal and external relationships of this group have been debated in the past. Traditionally, *Carybdea alata* auct. was diagnosed by focusing on the crescentic phacellae, with varieties sometimes named based on size differences (see Mayer, 1910; Kramp, 1961). Other characters occasionally used for diagnosis have been inconsistent and often misinterpreted, for example, rhopaliar niche shape (Mianzan and Cornelius, 1999). However, Gershwin (2001) and Gershwin and Collins (2002) showed that analysis of numerous characters in jellyfishes can highlight relationships that were previously overlooked with narrower analyses; consideration of multiple characters is not a new approach, but its application is new to the Cubozoa. Numerous forms of "*Carybdea alata*" from disparate locations, all with crescentic phacellae, differ with regard to umbrellar and velar nematocysts, number and degree of branching of the velarial canals, number of eyes, number of phacellae roots, shape of the rhopaliar niche ostium scales, and tentacle banding patterns. No doubt additional differences will be found with closer morphological study, cnidome comparison, and molecular analysis of these and other forms.

Within the *Alatina* clade, the most attention historically has fallen on the interpretation of *Alatina grandis*. According to Bigelow (1938), "*Carybdea grandis*" was the first in the group to be positively identifiable, because "*C. alata*" as described by Reynaud (1830) is unrecognizable and the name was revived by Vanhöffen (1908) for stability. Unfortunately, Vanhöffen failed to assign a particular specimen or suite of characters to the name; thus, we are left with the name of an unrecognizable species, and stability was not served. Bigelow went on to conclude that all the forms within the "*C. alata*" group are but one species, with "*C. grandis*" being the adult form. However, three decades earlier, Bigelow (1909) thought that "*C. grandis*" was valid and that the Pacific complex of "*C. philippina*", "*C. grandis*", and "*C. moseri*" could be easily separated as follows: "*C. philippina*" matures at only 30mm BH, whereas "*C. moseri*" does not begin to develop gonads until 60mm BH; "*C. moseri*", in turn is closely allied with "*C. grandis*", being separable, if at all, on the velarial canals being simple in the former, branched in the latter. More often than not, "*C. grandis*" has been interpreted as a gigantic variety of "*C. alata*" (Mayer, 1910, 1915, 1917; Light, 1921; Thiel, 1928), although Kramp

(1961), without comment, regarded "*C. grandis*" and all the nominal species in the group as junior conspecifics of "*C. alata*".

While most authors have argued over which crescentic-phacellaed forms should be considered species and which should be considered varieties, the specific name *alata* also became the subject of a large and confusing misunderstanding that spanned fourteen decades and never was completely resolved. Agassiz (1862) assigned Reynaud's species *Carybdea alata* to the genus *Tamoya*, rather than to his new genus *Marsupialis*, for reasons that are wholly unclear. *Tamoya haplonema*, the type species of the genus, could not possibly be confused with "*C. alata*" under any reasonable circumstances. Haeckel (1880) moved it back to *Carybdea*, a combination which has been widely adopted since. However, Uchida (1929) identified his local large carybdeid as *Tamoya alata*. It is clear from his illustrations how he arrived at the *Tamoya* part of his identification, but there is no indication of how he came to think that they were *alata*. His medusae were not *alata*-like in the sense of the crescentic phacellae and T-shaped rhopalial niche ostia. He further misidentified the small species *Carybdea sivickisi* to be the young of his *Tamoya alata*; in fact, *Carybdea sivickisi* shares only the crudest resemblance to any *Alatina*, in that both have 4 tentacles. All other characters, from the rhopalial niche ostia to the phacellae, from the velarial canals to the pedalia, are quite different. Over forty years later Uchida remained confused on "*Carybdea alata*", for he erroneously assigned to this species a single specimen from Cape Town with *Carybdea marsupialis*-type gastric phacellae and *Tamoya haplonema*-like rhopaliar niche ostia (Uchida, 1970). Apparently Uchida's error was what led Branch and his colleagues (1994) to erroneously identify the common Cape Town carybdeid as "*Carybdea alata*", and Pagès and his colleagues (1992) to identify it as *Tamoya haplonema*, when it should have been recognized as a new species (Gibbons and Gershwin, unpublished data).

In the process of sorting out which of the *Alatina* species should be retained and which are unrecognizable, many other new species are likely to be found. For example, a single Indonesian specimen (SAM H967) from the Te Vega Expedition, and specimens from Sri Lanka (QM G317054, G317055) and Madagascar (QM G317053), all match the standard descriptions for *C. alata* auct. but differ in numerous other structural characters. Sorting out the true biodiversity of the *Alatina* group will require fine feature study of a large number of specimens from regions throughout the world's tropics.

Removal of several species from the genus *Carybdea* and the family Carybdeidae, in particular the well published *C. alata* auct. and the obscure *Manokia stiasnyi*, in effect redefines both the genus *Carybdea* and the family Carybdeidae. I propose, therefore, the following revised descriptions, pending a comprehensive revision. I am further excluding *Carybdea* *sivickisi* from the *Carybdea* definition, and the Irukandjis and *Tamoya* from the Carybdeidae definition, based on overwhelming morphological and molecular evidence (Chapter 3); the full explanation and reclassification for these exclusions is beyond the scope of this paper, but is forthcoming (Gershwin, unpublished).

> Carybdeidae. Carybdeida with gastric phacellae; with poorly defined rhopaliar niche covering scales; with nematocyst clusters on the pedalia; with unbranched tentacles.
>
> *Carybdea*. Carybdeidae with epaulette-shaped or linear phacellae, comprised of short gastric cirri; with heart-shaped rhopaliar niche ostia; with usually two, sometimes 3-4, dendritically branched velarial canals per octant; with scalpel-shaped pedalia, typically with nematocyst clusters on the outer keel.

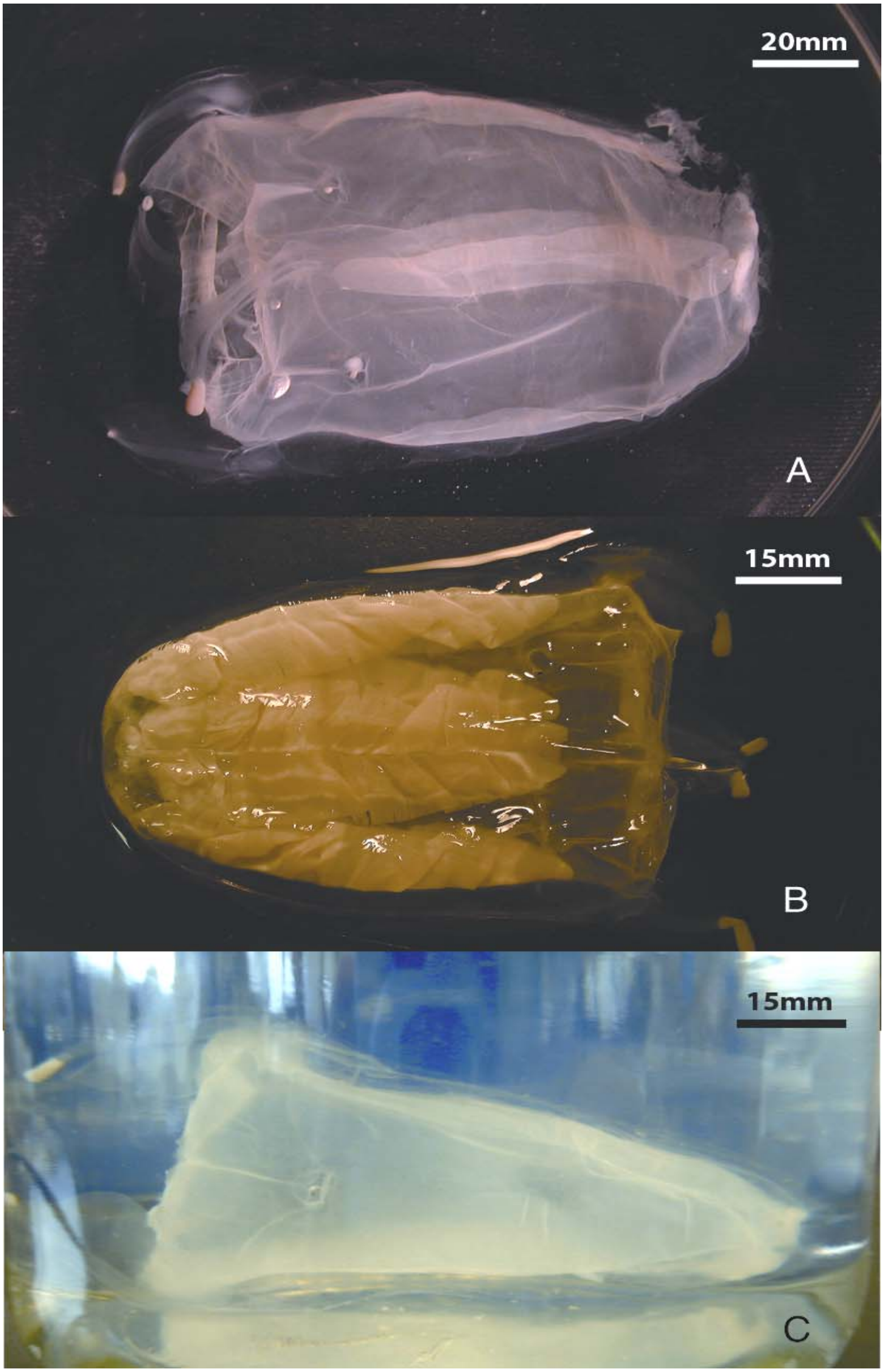

Plate 5.1. *Alatina mordens* gen. et sp. nov., different forms of general appearance. A. Holotype (MTQ coll'n), laying flat, with immature gonads. B. Paratype (SAM H1013), laying flat, with ripe, pleated gonads. C. Unregistered JCU specimen from Moore Reef, GBR, normal pyramidal appearance, as in life.

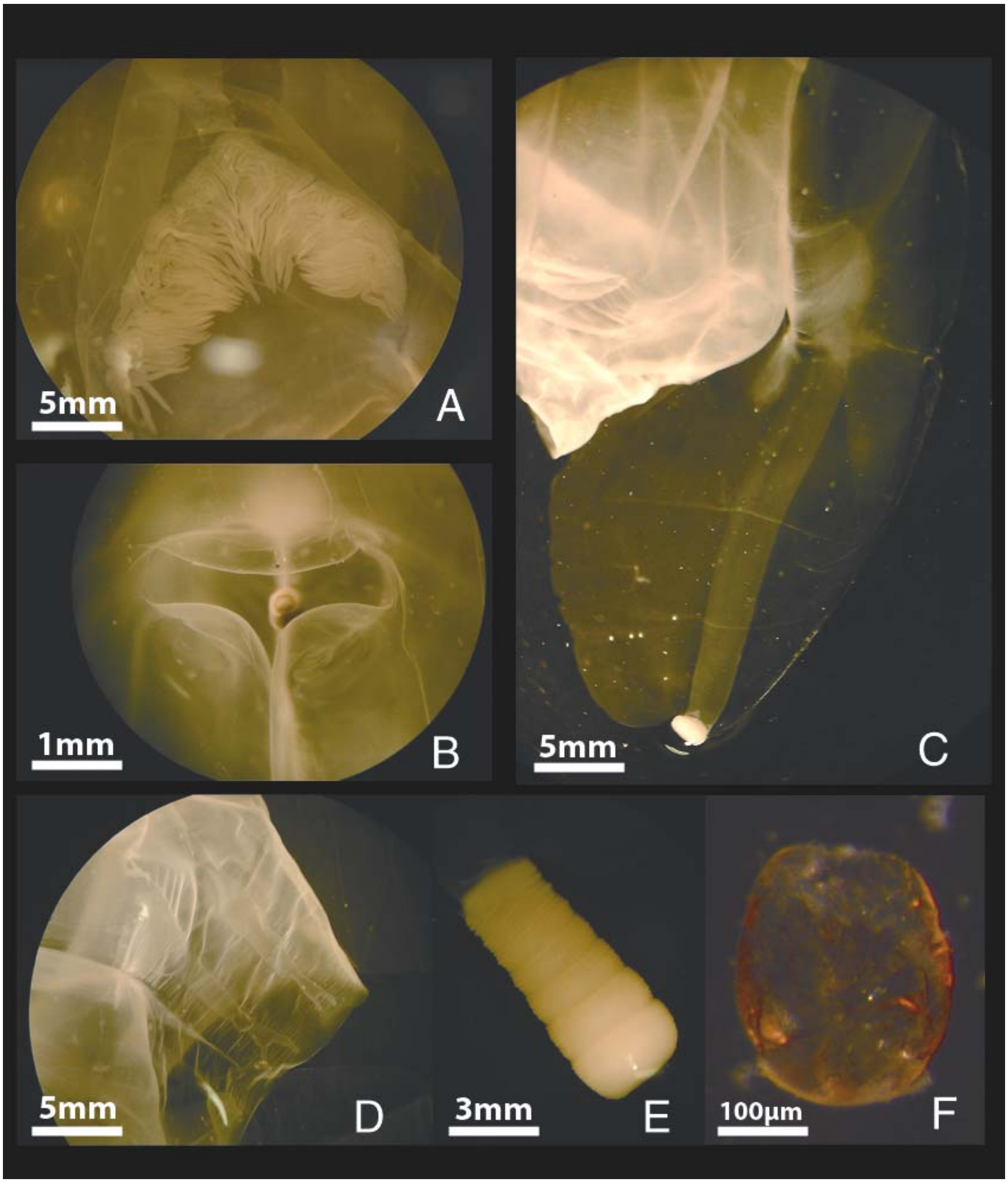

Plate 5.2. *Alatina mordens* gen. et sp. nov. A. Crescentic phacellus with long cirri. B. Expanded lower covering scales, producing a T-shaped rhopalial niche. C. Pedalium, with greatly rounded adaxial keel. D. Velarial canals. E. Contracted tentacle, with regular constrictions. F. Statolith, dissected out of rhopalium; note that the statolith in this figure is orientated as it is in life, i.e., with the truncate border facing upward, whereas for inter-species comparison the truncate border is standardized to be the base.

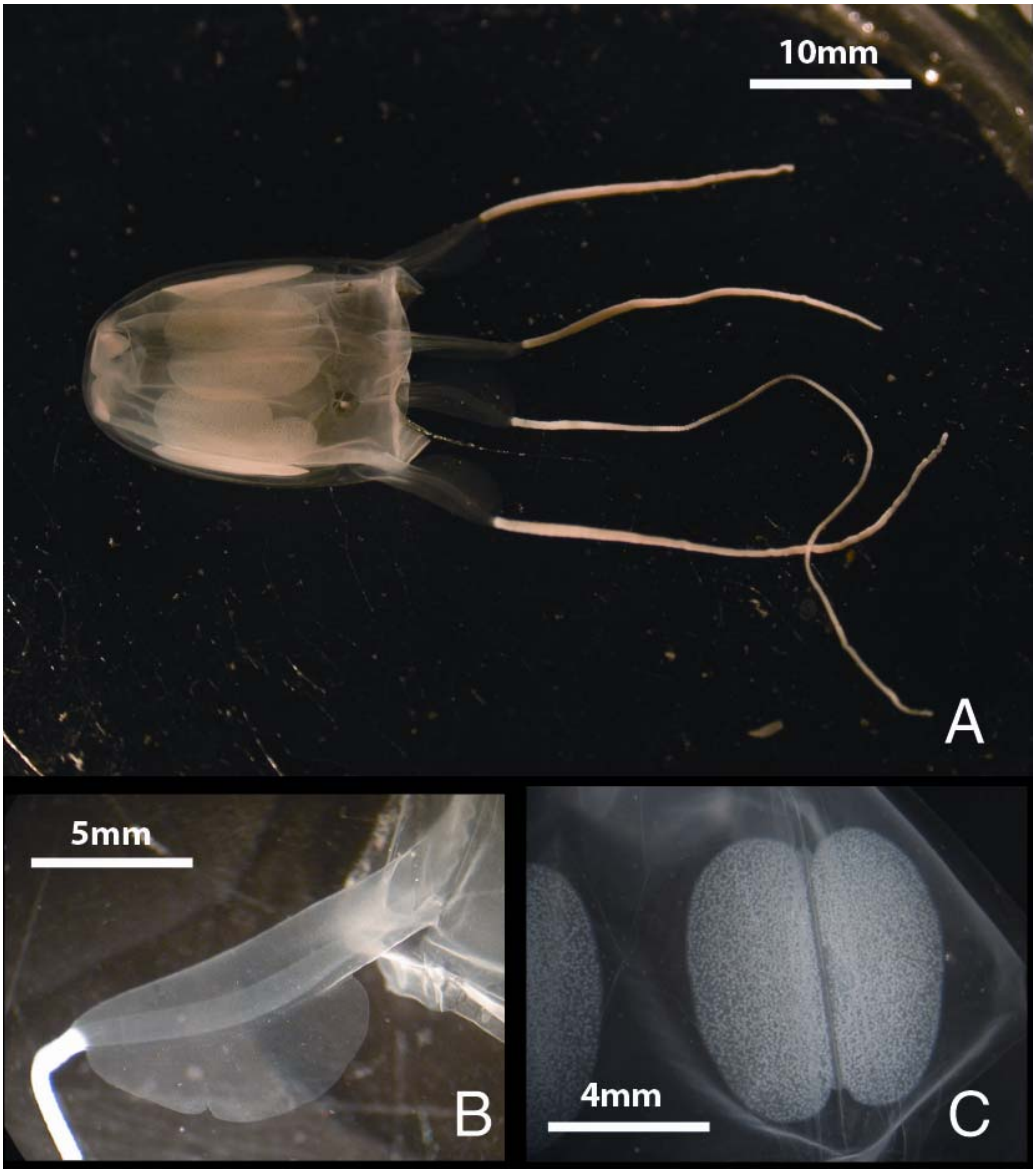

Plate 5.3. *Alatina rainensis* sp. nov. A. Holotype specimen, laying flat. B. Pedalium, with long stalk. C. Gonads, showing “butterfly” appearance.

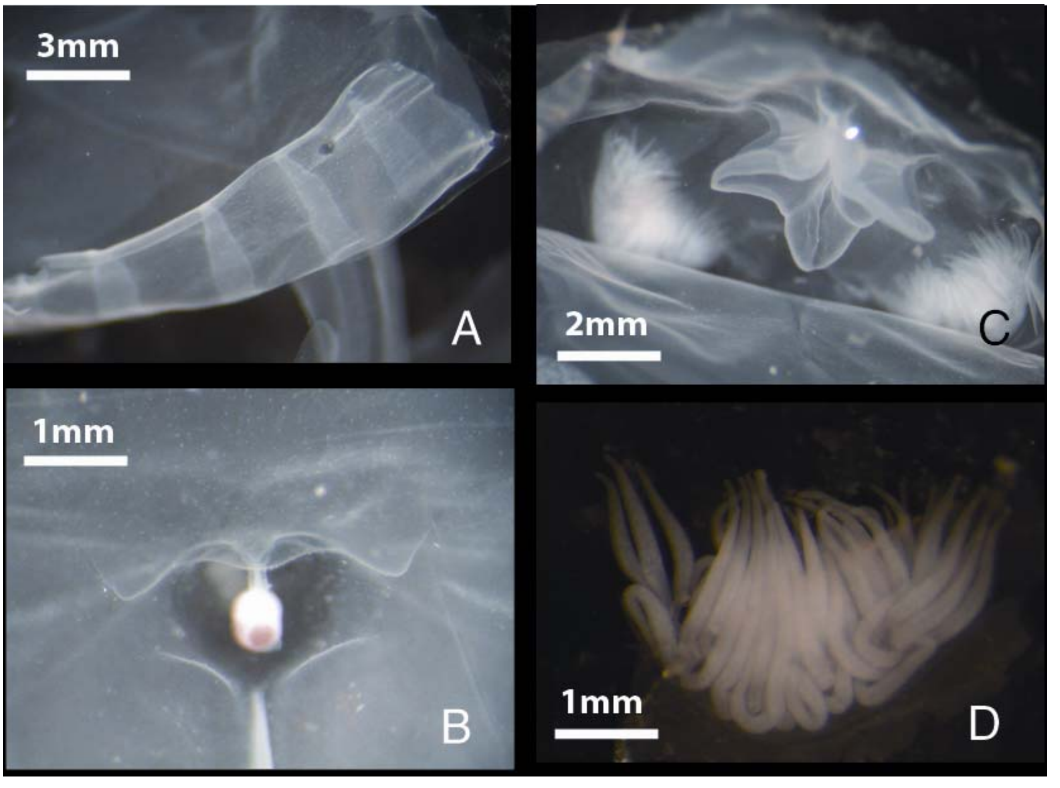

Plate 5.4. *Alatina rainensis* sp. nov. A. Portion of velarium showing 3 simple canals per octant. B. Rhopaliar niche with W-shaped upper scale and poorly developed lower scales. C. Bell wall dissected away to show short, flat stomach. D. Phacellus, dissected out of stomach.

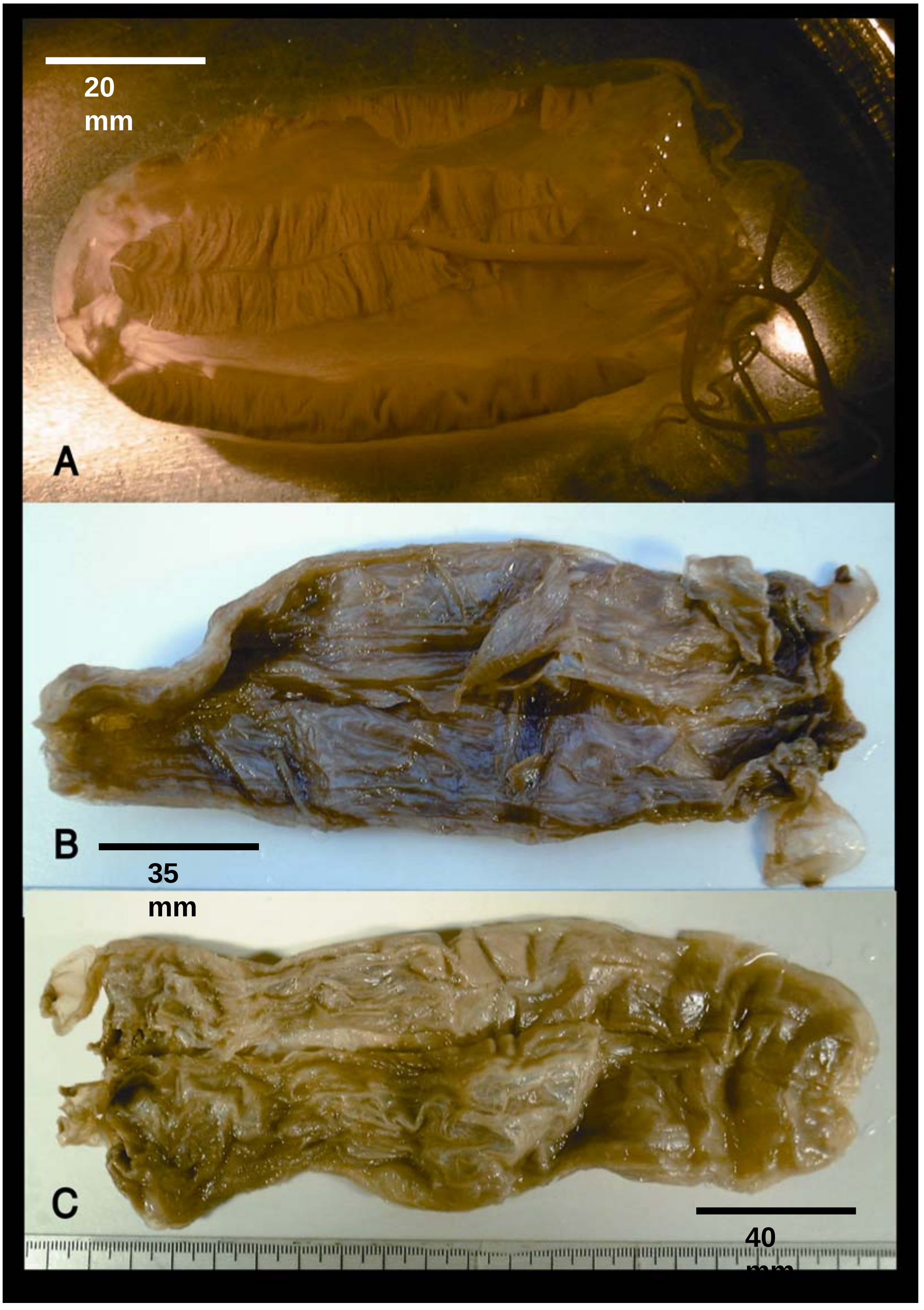

Plate 5.5. *Alatina moseri* (Mayer, 1906) comb. nov., and *Alatina grandis* (Agassiz and Mayer, 1902) comb. nov. A. *Alatina moseri*, non-type specimen from Molokai Island (USNM #29632). B (MCZ #1043) & C (MCZ #342). *Alatina grandis*, non-type specimens from the Hawaiian Islands, caught in 1861 and identified by Bigelow (1909, p20).

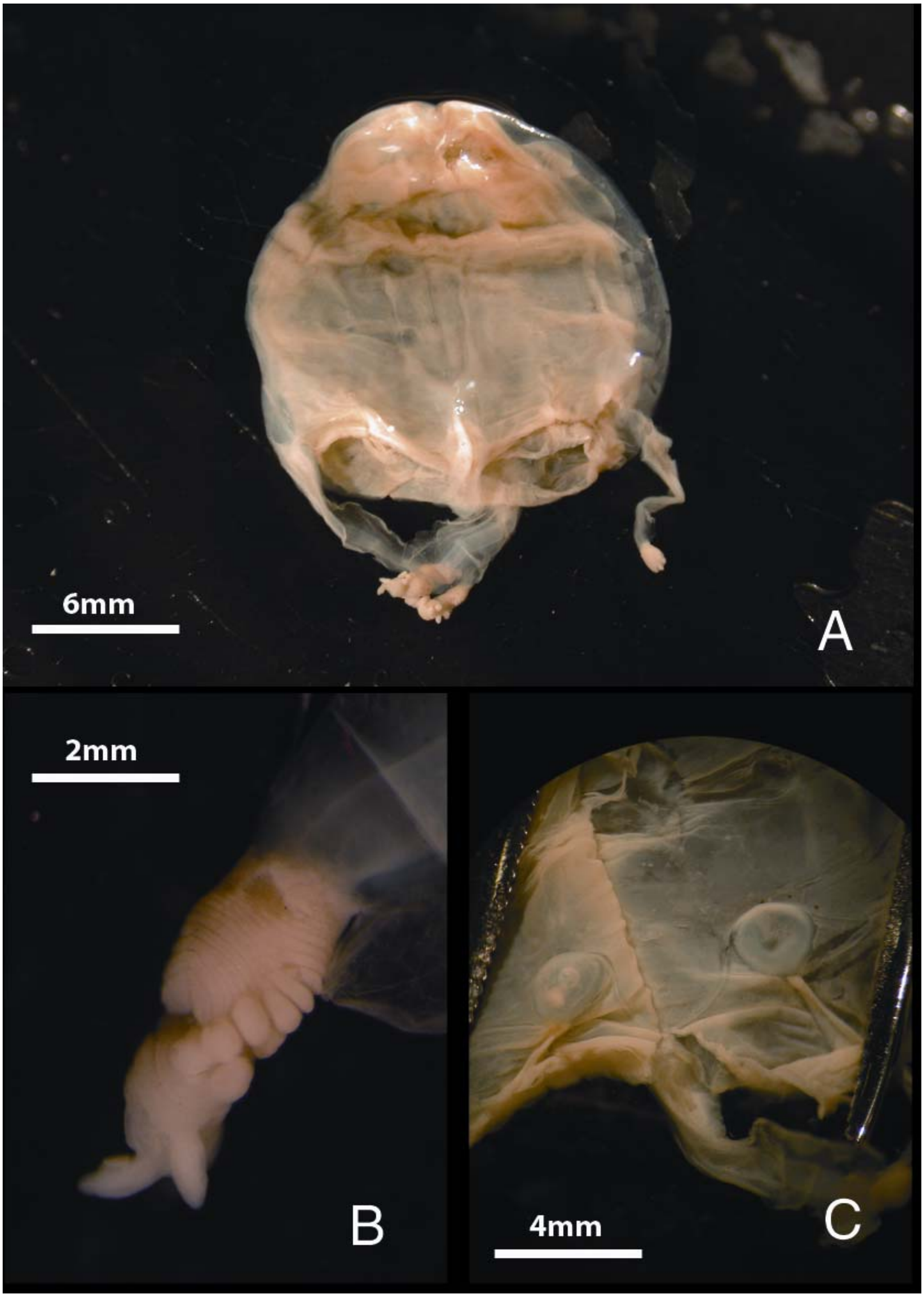

Plate 5.6. *Manokia stiasnyi* (Bigelow, 1938). A. Holotype specimen. B. One tentacle, showing peculiar branching pattern. C. Subumbrellar view of rhopalium, with convex windows.

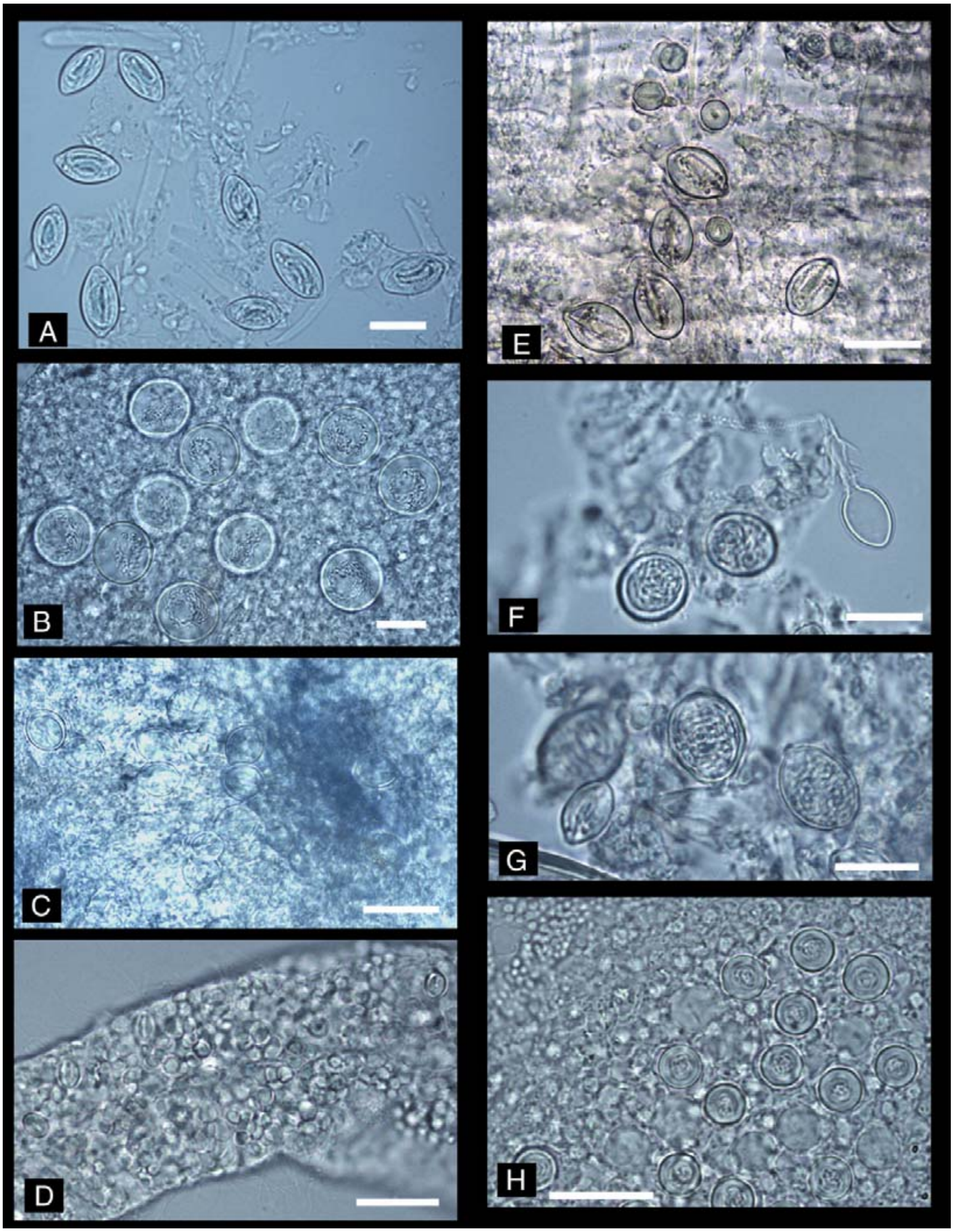

Plate 5.7. Nematocysts of Alatinidae. A. *Alatina mordens*, tentacle. B. *A. mordens*, bell wart. C. *Manokia stiasnyi*, tentacle. D. *A. rainensis*, gastric cirrus. E-G. *A. rainensis*, tentacle. H. *A. rainensis*, bell wart. See text for complete descriptions and measurements. Scale bar = 25µm.

CHAPTER 6: GENERAL DISCUSSION

At the start of this study, I had only fragmented bits of information that collectively hinted toward inaccuracy in the existing taxonomy, curious patterns in the phylogeny, and unrealized species diversity. However, I was still unaware of the magnitude of the issue and just how complex and stimulating the answer would become. In studying the Cubozoa, I have identified many intriguing patterns of morphological, geographical, and phylogenetic distribution, some of which support existing hypotheses, and others which introduce entirely new ideas. It is on the basis of needing a robust framework for further research that I have undertaken the task of sorting out the relationships and nomenclature of this group. Although a study such as this is obviously ongoing by its very nature, several compelling answers and insights have come to light in terms of my original thesis objective and specific questions. The objective of this thesis was to develop a solid foundation for the taxonomy (i.e., identification and classification) and phylogeny (i.e., evolutionary relationships) of the Cubozoa.

6.1 REVIEW OF SPECIFIC QUESTIONS

6.1.1 Question 1: *Does the current classification scheme accurately represent evolutionary patterns and genetic biodiversity of the group?*

The current classification (cf. Kramp, 1961, transcribed as Figure 3.1, herein) has some regions that are strongly supported by this study, as well as others at all levels within the class necessitating revision (Section 3.4.11). The patterns most strongly supported are the division between the multi-tentacled chirodropids and single-tentacled carybdeids, and the separation of most of the genera as circumscribed. This pattern of accuracy in Kramp's classification should not be discounted; however, these results suggest that it is accurate at the wrong scale. Specifically, many of the genera that he recognized are herein raised to families, and many of the species he recognized are now indicative of species complexes or higher groupings (Table 6.1).

Of the changes indicated, first, the families Carybdeidae and Chirodropidae are restricted to include the genera *Carybdea* and *Chirodropus* + *Chironex*, respectively, and the larger groupings typically recognized under these names are now recognized at the ordinal level as the Carybdeida and Chirodropida, respectively. Werner (1984) recognized this arrangement, but his conclusions have not been widely adopted.

Second, numerous families are recognized, some being resurrected from older classifications and some being newly erected. For example, the Chiropsalmidae, Tamoyidae, and Tripedaliidae are resurrected, to accommodate *Chiropsalmus* + a new genus, *Tamoya* + the Irukandji clade, and *Tripedalia* + *Carybdea sivickisi*, respectively. Furthermore, the various species belonging to the "*Carybdea alata*" clade are reclassified to a new genus and family (see Chapter 5).

Third, many new genera are proposed. For example, it is clear from molecular and morphological analyses in Chapter 3 that *Carybdea sivickisi* needs to be reclassified to a different genus, as it consistently groups outside the *Carybdea* proper and shares several characters with *Tripedalia* spp. As noted in Section 3.4.4, Hartwick (1991) noted the similarity of these taxa based on sexual dimorphism and spermatophore formation and transfer, but made no taxonomic changes. Reclassifying *C. sivickisi* presents a bit of a challenge, since it may have been described earlier by Haeckel (1880) as *Procharagma aurea*, and subsequently disregarded. However, this species was not the type species of the genus *Procharagma*; *P. prototypus* Haeckel, 1880, is unrecognizable, leaving the genus name unavailable. I propose the new genus name *Collostemma* (collo = sticky, stemma = head), in reference to the exumbrellar adhesive patches that make this species (group?) unusual. I further propose that the Tripedaliidae be broadened to accommodate *Collostemma*; it may seem counterintuitive to group a single-pedalia form into a family based on multiple-pedalia forms, but the grouping had some support in both molecular and morphological analyses herein, as well as Collins's analyses using different algorithms, and is further supported by the reproductive criteria outlined above. Even though the molecular analyses strongly suggest multiple distinct lineages within *C. sivickisi*, I am as yet unable to tell them apart morphologically. Sorting out the species boundaries in *C. sivickisi* will likely require studies of their population genetics and detailed morphological examination of a large number of specimens from each locality where the species occurs.

One of the biggest changes to the classification is in the addition of the Irukandji clade, which represents a previously unrecognized major group with an apparent deep divergence from the other recognized families, comprising nine new species and three new genera (see Section 6.1.6). Only one of the Irukandji species is currently named and classified (*Carukia barnesi*), but it is evident from both analyses that this group is well supported and faunistically diverse. The two main groups within the Irukandji clade (*Carukia* spp. and the wholly undescribed "Pseudo-Irukandjis") may correspond to different sets of Irukandji symptoms, as elaborated below (Section 6.3; Appendix 5).

Fourth, I have contributed multiple species additions; here I discuss some of the major clusters. The aforementioned Irukandji group comprises at least nine new species; the morphological differences between these species are numerous and robust, and it is possible that additional species will be found with further examination. Another notable large cluster is the

Alatinidae; many species are revalidated in Chapter 5, pending full examination of new material, based on uniqueness as indicated in the original descriptions. Furthermore, I have identified at least six different forms of alatinids from museum collections, none of which satisfactorily match up with any of the known species (Gershwin, unpublished). Finally, as indicated above, chirodropid diversity is likely to increase dramatically. Preliminary comparison of *Chironex* and *Chiropsalmus* populations across northern Australia reveals numerous morpho-species; southeast Asian chirodropids are largely unstudied but examination of sporadic museum specimens suggests unrealized diversity; and none of the six known "*Chirodropus gorilla*" specimens from western Africa resemble each other in gross morphology.

In summary, the existing classification appears to be mostly accurate in terms of identifying the major groups, but inaccurate in terms of species resolution and phylogenetic relationships.

6.1.2 Question 2: *What are the molecular relationships of species within the Cubozoa?*

The molecular relationships presented in Chapter 3 indicate a much richer fauna than originally appreciated, with well supported relationships often different from those previously recognized. Perhaps one of the most exciting results is that the Irukandjis form a monophyletic clade with a deep divergence from the other carybdeids, and that the structure within this clade corresponds to morphological groupings and possibly toxins (Section 6.1.6). It is currently unclear whether this clade is part of a trichotomy (chirodropids + Irukandjis + remaining carybdeids; e.g., Figure 3.5A), or is the sister group to the remaining carybdeids with the Chirodropida at the root. There is some reason to doubt the nesting pattern of the "Irukandjiidae" in Figure 3.5B: some morphological characters were used despite appearing "floppy", but character reliability analyses were not able to be employed (e.g., trait mapping; see Section 6.1.3). Future analyses should focus on determining which characters are of phylogenetic utility.

Other interesting groupings revealed by this study are also well supported and concordant between data sets. For example, in all cases, the alatinids (formerly "*Carybdea alata*") grouped separately from the remaining species of *Carybdea* (Section 3.3.2 and Chapter 5). Thus, the prevailing definition of the genus *Carybdea* is inconsistent with the phylogenetic patterns herein.

In another example, the chirodropids consistently grouped separately from the other cubozoans, and *Chironex* spp. grouped separately from *Chiropsalmus* spp., both patterns which are consistent with the current taxonomy. However, the *Chiropsalmus* and *Chironex* samples from different regions seem to indicate genetic differences; population-level genetic studies on these forms may reveal even greater differences, i.e., additional species that have gone undetected.

Finally, the samples of *Carybdea sivickisi* used herein were from quite different locations and habitats, and the species is more or less benthic throughout its life cycle (Hartwick, 1991); thus, the possibility must be considered that *C. sivickisi* comprises cryptic species (or possibly, with re-examination, not so cryptic!). The division of *C. sivickisi* from the other species of *Carybdea*, and its grouping with *Tripedalia*, are also supported by reproductive-developmental observations (Hartwick, 1991), as explained above. I am awaiting sequences of another species of *Tripedalia* (*T. binata*) that may give additional resolution to the *Tripedalia* + *C. sivickisi* group.

The molecular relationships suggested in this study are mostly concordant with those of Collins (2002) and Collins et al. (In review, 2005). The only topological difference is in the placement of the Alatinidae, which is thoroughly discussed above (Section 3.4.3). The primary difference between my results and those of Collins is in the number and taxonomic variety of samples treated, and therefore, the resolution of certain groups.

6.1.3 Question 3: *Is there congruence between the morphological and molecular datasets, and if so, can we combine them for a stronger dataset?*

Many of the major and minor groupings are largely concordant between the two data sets, but some notable exceptions are evident. First, the separation of the Chirodropida from the remaining cubozoans is congruent, although the cohesion of the genus *Chiropsalmus* is not, forming a sister clade to *Chironex* in the molecular data set, but ambiguously paraphyletic or polyphyletic in the morphological data set.

Second, as previously mentioned, the Irukandji species group together separately from the other carybdeids in both data sets, but the structure within the clade is not concordant. In the molecular data set, the *Carukia* spp. are the sister group to the remaining Irukandjis, with the “Pseudo-Irukandjis” nested monophyletically distal to the “Darwin carybdeid” and “Morbakka”. In contrast, in the morphological data set, the same three groupings are evident, but the “Darwin carybdeid” + “Morbakka” clade is basal to the “Pseudo-Irukandji” clade, which is basal to the *Carukia* clade in a nested arrangement.

Finally, although the groups themselves are concordantly supported, the arrangement within the *Carybdea* + *Tripedalia* + *Alatina* clade lacks concordance. In the molecular data set, a 6-pronged comb-like arrangement is evident, comprising the *Alatina* clade, the *Carybdea* clade, *Tripedalia*, and three samples of *Carybdea sivickisi*. In contrast, in the morphological data set, the Alatinidae clade is basal to the others, and the *Tripedalia* + *Carybdea sivickisi* clade is the sister group to the species of *Carybdea*.

As far as whether the data sets can be combined, some incongruence lies in the tree topology, but a much more serious issue lies in the data sets themselves. Specifically, the molecular data set comprises multiple samples of 14 nominal species, whereas the morphological data set comprises a single sample of each of 31 species. If I were to prune the molecular data so that each species was represented only once, and similarly prune the morphological data so that only the species represented in the molecular set were included, two different sources of error would be introduced. First, homoplasies evident in the larger data set would be masked by the small number of included taxa, giving misleading results for certain characters. For example, Character 39 (cylindrical isorhizas), are present in some taxa but not in others closely related (*Tripedalia binata*: present; *Tripedalia cystophora*: absent; all other carybdeids absent; *Chironex fleckeri*: present; *Chiropsalmus quadrumanus*, *C.* n. sp. A, and *C.* n. sp. B: present; *Chiropsoides buitendijki* and *Chirodropus* n. sp. A: absent); however, due to the limited number of taxa with this feature included in the pruned analysis (i.e., *Chironex fleckeri*, *Chiropsalmus* n. spp. A and B), this homoplasious character would appear as diagnostic for the Chirodropida and its absence would appear as diagnostic for the Carybdeida. A similar problem would occur with Character 11 (bell nematocysts), with their presence defining the Carybdeida and their absence defining the Chirodropida; however, in reality, two species of chirodropids possess them, while numerous species of carybdeids do not, but none of these “aberrant” taxa would be represented in the combined analysis.

The opposite problem would also manifest, namely, character states that clearly diagnose certain clades would be unrepresented or poorly represented, appearing as autapomorphies in cases of a single included species, or simply lost in the noise of other character states. For example, Character 27 (tentacle complexity: branched) diagnoses *Manokia stiasnyi*, but would be lost because *M. stiasnyi* would not be represented in the combined analysis. In another example, Character 48 (gonad attachment) is an extremely reliable character to diagnose different groups. Most taxa have leaf-like gonads, but *Tripedalia* spp. have butterfly-like gonads, the *Collostemma sivickisi* species complex has pendant gonads, and the spotted *Chirodropus* n. sp. (and quite possibly the other *Chirodropus* spp.) has filamentous gonads; however, this character would resolve as autapomorphic for each *C. sivickisi* and *T. binata*, and unrepresented for *Chirodropus*.

These same issues preclude any statistically supported, robust approach to trait mapping as well. In order to have a comparable data set for either use, the characters would need to be scored from the actual specimens sequenced, which is often simply not possible. Most species are known only from museum specimens, precluding DNA retrieval, and many of the specimens with retrievable DNA have been ethanol preserved, frozen, or dried, making it impossible to score most of the 85 characters. Obtaining specimens that are useful to both morphological and molecular analyses should be a high priority for future studies, but we are still a long way off from having a dual data set of all species for total evidence analyses or robust trait mapping.

6.1.4 Question 4: *What are the morphological characters useful for operational taxonomy?*

Using the criteria and synoptic tools in Chapters 2, 3, and 4, I believe it is simpler than ever to accurately identify existing and newly discovered cubozoan species. However, there is no simple combination of morphological features that can be reliably compared across all species. For example, presence of gastric saccules and forking of the pedalia have been long used to separate the chirodropids from the carybdeids. However, the undescribed spotted chirodropid (*Chirodropus* sp. A) does not possess saccules, and immature specimens of *Chiropsalmus zygonema* or the undescribed Mexican chirodropid (see Plate 2.8C) would most certainly not have forked pedalia, having only 2-3 fingers and tentacles at maturity. Furthermore, occasional aberrant specimens of carybdeids with forked pedalia are found (e.g., “Halo-Irukandji”, QM coll’n). So-called reliable carybdeid characters such as oar-like pedalia and four tentacles are similarly not completely reliable; three species of chirodropids have oar-like pedalia and *Tripedalia* spp. have more than four tentacles. At the lower taxonomic levels these problems are compounded. Rhopalial niche ostium shape seems to be an excellent character in separating carybdeid families, except for the tripedaliids and tamoyids; rhopalial horns are exclusive to the Irukandjis, except that they also occur in the very distantly related *Collostemma sivickisi*; and perradial lappets are diagnostic of the Irukandjis as well as the chirodropids. The list goes on. Thus, for the species treated herein, different sets of characters are meaningful for different groups, and at different levels; for other species yet to be discovered, the diagnostic sets of characters are likely to be somewhat dissimilar.

Besides the lack of universally comparable characters, identification can be hampered by type of preservation and state of maturity. Many characters can be studied on formalin-preserved specimens, but statoliths disintegrate in formalin, nematocysts cannot be discharged for proper identification, and morphometric characters are differentially distorted by different percents of formalin and the degree to which the specimen contorted during preservation. Ethanol preserves statoliths quite well, and discharges nematocysts, but eliminates the possibility of studying almost all structural and certainly all morphometric characters; furthermore, if all the nematocysts are discharged, then measurements on undischarged capsules cannot be made. The answer might seem clear to only study live specimens, but many characters are invisible until preserved, e.g., velarial canals, rhopalial horns, phacellae. Thus, different parts of each specimen need to be preserved differently; however, this is often unrealistic. Similarly, many characters develop as the specimen grows, e.g., bell size, gonad shape, number of tentacles in chirodropids, and velarial canal branching. Many examples exist of overlap between immature characters for one species and mature characters for another; thus, it is imperative to sample mature specimens for morphological identification.

The question “what species is this?” can have two quite different meanings with quite different approaches. Some workers may simply want to know which known species a given specimen is similar to, whereas others may need to assess whether it is likely to be a new species. To assess similarity, one need only examine enough characters to satisfy their curiosity that their species is more similar to A than to B; the synoptic tools in Chapters 2, 3, and 4 will be sufficient to answer this question. On the other hand, to assess whether a specimen is *actually the same* as one of the species herein, one must compare a much broader data set including less obvious traits.

The tradeoff between taxonomically useful data and phylogenetically useful data also plays heavily on this question. The character matrix used herein was developed in order to have a parallel data set among species from which to track, sort, and interpret morphological characters. I believed initially that it would be subjectively inappropriate to “select the characters I believed were most useful” for phylogenetic analysis; I believed it would be more objectively appropriate to let the analysis tell me which characters were most useful. However, it has recently become clear that not all characters are equally appropriate for both needs, i.e., some homoplasious characters are nonetheless taxonomically useful. Further studies should seek to resolve this dilemma.

So, one might ask, which characters are the most important, and how many are necessary for accurate identification (“Certainly not all 85?”). While, theoretically, some of the characters are not necessary for examination, or are even possibly misleading, I have as yet identified only one that I can comfortably place in this category, i.e., Char. 49, the interradial suture. All other characters appear to have at least one state that defines a group, and in many cases, different states define different groups at different levels. Thus, the decision of how much effort to put into identification (i.e., whether to use the limited number of features presented in the keys and synopses, or whether to use the more complete data set), is ultimately at the discretion of each researcher to diagnose the species at the accuracy level for their needs. I recommend using the complete data set in Chapter 2 for cases in which absolute accuracy in identification is necessary.

6.1.5 Question 5: *What is the species diversity of known or suspected Irukandji causing jellyfish, and do they form a monophyletic group within the Cubozoa?*

At least ten species currently comprise the Irukandji group, which I refer to as the "Irukandjiidae" (Chapter 3) – there is no family called Irukandjiidae, nor will there ever be, because there is no genus called "*Irukandji*", and even if there were, Haeckel's family name Tamoyidae would have nomenclatural priority. The species grouped in Chapter 3 as the "Irukandjiidae" are all readily identifiable on gross morphology. Each species has multiple structural autapomorphies that serve to make it immediately recognizable from other species in this group. Currently, ten species are known, but only one is classified (*Carukia barnesi*); the remainder shall be published shortly. An additional half dozen new species are suspected based on atypical geographical occurrences of unique sting symptoms.

Whether the "Irukandjiidae" is monophyletic is ambiguous in the present analyses; the molecular analysis supports this hypothesis, whereas the morphological phylogenetic analysis suggests a paraphyletic arrangement with respect to the remaining carybdeids. As stated above (Sections 3.4.6, 6.1.4), there was some doubt in my mind while I was scoring the characters that they were all of equal utility – the morphological character matrix was originally developed as a taxonomic tool for my morphological work, but some characters did not "seem" appropriate for phylogenetic inference. However, in the interest of not making *a priori* assumptions about character utility, I fought the urge to filter out what I believed were inappropriate characters; I have since come to believe that perhaps science is not well served by a completely objective approach, i.e., some expert knowledge may be helpful. In overall natural appearance, the Irukandji species do appear to form a monophyletic group based on the following autapomorphic major structural characters: lack of gastric phacellae (Character 41: state 0); frown-shaped rhopalial niche ostia (51:1); and possession of rhopalial horns (53:1). Either way, whether to recognize the group because it is monophyletic, i.e., that the Irukandji taxa form a natural group without descendants, or whether to not recognize the group because it is paraphyletic, i.e., that they form a cohesive group from which other groups descend, is a semantic cladistic argument rather than a biological argument (Greene, 1998; Pearse, 1998). They are morphologically similar because of their shared evolutionary history; whether or not other taxa have descended and modified from them does not negate their cohesion as a natural group.

While monophyly of the group itself is still somewhat ambiguous, the distinction of the sub-groups is also unclear. By qualitative morphological analysis, three groups are evident; the *Carukia* group (3 species currently known, all with "tailed" tentacle bands and red exumbrellar warts), and the "Pseudo-Irukandji" group (4 species currently known, with "normal" or "halo" tentacle bands and purple exumbrellar warts) are clearly separate from each other and from the "Morbakka" spp. and the "Darwin carybdeid" group (3 species currently known, all with robust bodies, flared tentacle bases, and thorn-shaped pedalial canal extensions). The morphological phylogenetic analysis supports this hypothesis of three separate groups, as does the morphological qualitative analysis in Chapter 2. In contrast, the molecular phylogenetic analysis does not resolve the "Morbakka" + "Darwin carybdeid" group, where it is paraphyletic with respect to the "Pseudo-Irukandji" group; however, this may be the result of too few sequences in these taxa. Based on numerous structural differences that separate the "Morbakkas" and "Darwin carybdeid" from the other Irukandjis, I hypothesize that additional sequences from these taxa will bring better resolution as a monophyletic group.

6.2 PROGRESS IN IRUKANDJI RESEARCH

No experimental evidence currently exists differentiating the factors responsible for variability in syndrome expression; hypothesized factors include venom load, differential victim sensitivities, differential reporting, seasonal or ontogenetic fluctuations in venom potency, and species-specific differences in toxicity. It is interesting to note that while the Irukandjis discussed herein clearly group together, other Irukandji syndrome-causing species are known from diverse groups including the cubozoan Alatinidae (see Chapter 5), the scyphozoan Stomolophidae, and within the Hydrozoa, both the siphonophoran Physaliidae, and the limnomedusan Olindiidae (Williamson et al., 1996).

The identification of the Irukandji clade and separation of the medically important Irukandjis, i.e., the *Carukia* spp. and "Pseudo-Irukandji" spp., into two quite different subgroups (Section 3.3.5) may prove to be an important breakthrough. The division of the two species groups has well supported concordance from morphological and molecular analyses (Chapter 3), as well as cnidome characteristics (Table 2.13; Plate 2.25A, B; Plate 2.26A-C) and statolith shape (Section 2.3.13; Table 2.8; Plate 2.18), and is consistent with behavioural and distributional patterns (Gershwin, unpublished). Recent preliminary studies have suggested two different groups of Irukandji syndrome as well (Appendix 5): one with a mild non-welting initial sting, delayed onset of about 20-30 minutes, profuse nausea and vomiting, pain that responds well to analgesics, and mild hypertension (in the 100's); the other with a moderate to severe welting initial sting, rapid onset of typically about 5-10 minutes, rarely with vomiting, but with uncontrollable pain and severe hypertension (in the high 200's). Interestingly, in the first instance, the blood pressure seems to come down when the pain is treated, whereas in the second instance, the pain comes down when the blood pressure is treated, although there is apparently no link between pain and hypertension in the body (M. Corkeron, pers. comm.

2004). Of further intrigue, the milder syndrome appears to be more common on the coast, whereas the more severe syndrome is more typically reported on the reefs and islands, corresponding to the general distribution pattern of *Carukia* spp. and “Pseudo-Irukandji” spp., respectively. What remains to be shown is whether the division in syndromes is clinically real, and if so, whether the two evolutionary groups correspond with the different syndrome types, but if this is someday verified, the implications of this would be immense. For example, both known fatalities were due to brain hemorrhages that occurred a couple of hours after the stings; if the early signs and symptoms (e.g., painful sting, lack of vomiting) could be used to predict later complications (e.g., life threatening hypertension), management decisions could be made for remote stings during the critical early minutes, such as whether or not to request an air ambulance. Recognizing the two groups may have implications to other fields of study as well, such as:

- Medical: refine treatment based on prediction of different syndromes and possible complications
- Pharmacology: broaden efforts toward two different anti-venoms, with comparative understanding gained from the two groups
- Genetics: demonstrates the need for population genetics to better understand the species boundaries, and opens up exciting questions about evolution of venoms
- Ecology: much insight to be gained from comparative study of two different systems rather than as a single homogenous group
- Taxonomy: ability for experts and non-experts to predict group relationship and potential toxicity of unknowns based on morphological appearance
- Education and media presentation: focus on altering public perception about likelihood and management of risks

If the evolutionary pattern of species suggested by the molecular analysis is correct, then a surprising pattern of venom evolution would also be suggested. Specifically, one might reasonably expect that venom would be ancestrally mild, and evolve to be more potent as predator or prey items evolve more tolerance, or that alternatively, venom might be ancestrally potent, becoming less so in descendants. However, our current state of knowledge on Irukandji species and their syndromes (Appendix 4) indicates that the “Morbakka” + “Darwin carybdeid” group is the milder of the three, with the “Pseudo-Irukandji” group apparently the most potent, and the *Carukia* group serious but probably not deadly. Thus, the phylogenetic pattern hypothesized by the molecular analysis suggests that the ancestral state of Irukandji toxin may be more in the mid-range, so to speak, and evolved to be more severe and milder in different descendant lineages. This also brings up the often-asked question of why Irukandji toxin is so potent to humans. The most-cited answer is that the toxin evolved against vertebrate prey (i.e., fishes), and that we are sensitive to it because we are vertebrates (J. Seymour, pers. comm., 2003). We know from the fossil record that chirodropids were present in the Pennsylvanian (Johnson and Richardson, 1968), and molecular phylogenies suggest that the Irukandji group diverged relatively early in the Cubozoa (Figure 3.4; Collins, 2002; Collins et al., In review). However, we do not know how far back the Chirodropid-"Irukandjiidae" divergence might have been, or more to the point, when the divergence of Irukandji sub-groups might have been. It is conceivable, therefore, that the split between Irukandji groups, and thus the probable presence of their powerful toxin, predates the fishes, leaving one to wonder about the question of potency.

Confusion exists regarding the meaning and scope of the word "Irukandji". The term historically refers to an Aboriginal tribe in the coastal region north of Cairns, and was applied to the constellation of systemic symptoms that occurred in the summertime oceans of this region (Southcott and Powys, 1944; Flecker, 1952). When a species of jellyfish was later proven to cause the syndrome (Barnes, 1964), the name was co-opted to refer to that species, *Carukia barnesi* (Southcott, 1967). *Carukia barnesi* is often said to be widespread based on the distribution of the syndrome rather than on specimens identified as that species (Edmonds, 1975; Sutherland, 1981; Sutherland, 1983; Marsh and Slack-Smith, 1986; Sutherland and Sutherland, 1999; Sutherland, 2001), thus confounding the accuracy of the literature. Today the term "Irukandji" is used nearly interchangeably to refer to different degrees of symptom severity, a species which causes these symptoms or a group of such species, or a philosophical assumption of these species or syndromes, but is rarely associated in the general context with the aboriginal tribe from which the name was derived. Herein I use the term to refer to a morphologically and genetically cohesive group of species which cause or potentially cause systemic illness.

Our understanding of the syndrome, too, has been affected by the confusion over species terminology, with the range in symptom variability often attributed to *C. barnesi* instead of being viewed in terms of potentially different syndromes attributable to different species. Of these ten species, four are known to cause systemic symptoms associated with Irukandji syndrome and none are known not to. Some authors have preferred the term "Irukandji-like syndrome" for mild symptoms (Williamson et al., 1996), or for relatively severe symptoms thought not to be attributable to *Carukia barnesi* (Cheng et al., 1999), thus drawing the division line between perceived severity rather than between systemic versus non-systemic. However, this seems unconstructive because it is not hard to imagine that a species capable of causing systemic symptoms might, under certain conditions, cause more severe sickness (e.g., venom load, ontogenetic toxicity fluctuations of the species, individual reactions, differential reporting). Thus, it seems more practical to draw the line at “causes sickness” vs. “does not cause sickness” rather than between the harder to delineate “mild sickness” vs. “severe sickness”. Furthermore, all but one of the species currently linked with Irukandji syndrome fall within the phylogenetic Irukandji grouping, and there is some indication that “mild” and “severe” might sort along phylogenetic boundaries as well (see above).

6.3 AREAS OF FUTURE FOCUS

I have studied a significant portion of the Australian Cubozoa, as well as type specimens and new collections from many parts of the world. However, the species remain poorly circumscribed in Australia’s remote regions and through most of the world; we are still in the initial descriptive taxonomic phase, trying to sort out the basic questions of how many species we have and how to tell them apart. Moving forward into the refinement phase will entail detection of ecotypes where genotypic and phenotypic clusters correspond; population genetics to define the parameters of gene flow among species; ecological studies to better understand the temporal and spatial factors that govern isolating mechanisms; and reproductive studies to determine the level of clonality and promiscuity in cubozoans and what effect, if any, these have on species recognition.

One of the most urgently needed studies to follow from the primary descriptive work is to better understand evolutionary species boundaries, especially in the Irukandji group, the Carybdeidae sens. emend., and the Chirodropida, through sequence analysis of faster evolving genes and through population genetics. Several of the species that I have treated herein have already shown at least some indication of genetic and/or morphological polymorphism diagnosable by geographical boundaries, e.g., *Chironex fleckeri*, *Carybdea rastonii*, *Carybdea xaymacana*, *Chiropsoides buitendijki*, *Chirodropus gorilla*, and *Carybdea sivickisi*. Most of these taxa are probably intimately associated with a certain substrate type, and thus would not be expected to freely cross oceans or have wide distributions. For example, *Chironex* is most often found confined to certain bays nearby river systems, and often alternates its populations with different species of *Chiropsalmus* across northern Australia (Gershwin, unpublished). In another example, *Carybdea sivickisi* is more or less benthic throughout its life cycle (Hartwick, 1991), and apparently associated with algae; I have recently found *C. sivickisi* at disparate habitats in Tasmania and South Australia, leading one to wonder how a tropical form might adapt to cold temperate waters, and what effect this adaptation might have on speciation.

Finally, *Chirodropus gorilla* is a highly problematical form, being reported only a handful of times in the literature, from nearly one tip of Africa to the other, with none of the forms matching in morphology! It would be interesting, and no doubt highly informative, to properly survey the coastline of western Africa and resolve the taxonomy of this "species".

Another field of study urgently needing attention is linking the different forms of Irukandji syndrome with different species, and understanding the factors responsible for different severities of sickness. In theory, it doesn't change the treatment all that much, i.e., a hospital will still treat a patient's symptoms, but knowing which species give which complications, and under what conditions, will allow for predictive management that will be useful to Surf Life Saving, city councils, shire councils, and fishing and research organizations. For example, all growth stages and species of small northern Australian carybdeids are currently treated with similar potential for harm. However, as detailed above, some species appear to be more dangerous than others, and there is some indication that toxicity might increase as the animal grows. Thus, treating them all as equally dangerous might conceivably result in unnecessary beach closures, lost industrial time, and media sensationalism. At such a time as growth stages and species can be accurately ranked according to their risk potential, Irukandjis will become a manageable hazard similar to any other danger. A summary of the current state of knowledge of species-syndrome relationships is presented in Appendix 4.

The question of using statoliths shows much promise for identification of previously unidentifiable specimens, e.g., ethanol-preserved, cryo-preserved, fragmented, or even fossil specimens. The preliminary statolith study herein (Section 2.3.13; Table 2.8; Plate 2.18) reveals that generic distinction is likely, and possibly even in some cases to species, based on gross shape comparison. Further studies employing Fourier analysis may reveal finer scale reliable differences than are obvious qualitatively, as has been shown successful in other soft-bodied taxa, e.g., beaks and pens of cephalopods, statoliths of worms and squids, and fish otoliths (see Section 1.4.2).

The Cubozoa are an ancient group, with the Chirodropida fully recognizable at least as far back as the Pennsylvanian (385-286 mya; Johnson and Richardson, 1968). Historically, the group has not been regarded as speciose, due to the small number of characters that have been used diagnostically. However, even by examining many more characters than ever before, and thus opening up opportunities to discern additional species, the morphological diversity of cubozoans is still relatively low compared to other groups, e.g., hydrozoans, insects, and fishes. Curiously, the Cubozoa seems reasonably well resolved at the species level with the 18S rDNA gene, which is typically regarded as a higher-level discriminator. Whether this pattern of morphological-genetic homogeneity is due to recent divergence of extant lineages, or due to a slow rate of evolution in cubozoan taxa, or due to a persistent underestimation of morphological diversity, cannot be answered from the current analyses. Thus, the argument that I made at the beginning of this thesis, i.e., that my predecessors have underestimated the cubozoan biodiversity, may still exist at even finer resolution. Without doubt, greater species diversity will be resolved by studying the group at a global scale.

Finally, quantitative exploration of morphological-genetic congruence and analysis of character evolution as discussed above (Section 6.1.3) will be extremely valuable pieces of the cubozoan puzzle; however, like with so many other questions, our ability to compile useful data sets relies on availability of difficult-to-obtain specimens. Morphological characters and DNA sequences need to be obtained from the same specimens, and these data need to be sampled from a greater number and taxonomic diversity of taxa. But acquiring these specimens and tissue samples is not simply a matter of collaborating with the right person or collecting in the right location. Many of these species have not been seen in well over a hundred years, and many others are likely to have problematical identification issues such as ontogenetically non-comparable character scoring or unrealized species richness. Thus, this thesis provides a qualitative means of identifying different species, and phylogenetic hypotheses to test in future studies, but our breadth of understanding based on quantitative analyses of the Cubozoa still relies on acquiring the numerous poorly known forms.

6.4 IN CONCLUSION

The major outputs of this study include a substantial review of morphological features and robust phylogenetic hypotheses based on molecular and morphological data sets. This study provides a reliable means of identifying existing and new cubozoan taxa, using standard morphological and molecular discriminators as well as innovative approaches to previously unappreciated features. These phylogenetic results are applicable both as a powerful predictive tool for almost all types of scientific enquiry and as a testable basis for future systematic work. Furthermore, several larger works were spawned from this study, and will be soon published: a full monographic revision of the Class Cubozoa; detailed descriptions of 26 new species, including nine new Irukandji species and four new box jellies; and a web-based interactive key to the Cubozoa.

As stated earlier, a detailed taxonomy and robust phylogeny are the basic building blocks for all other types of biological enquiry, forming the basis of communication and comparison about species. Many diverse fields of study relating to stinger management and treatment will benefit from being able to more accurately identify and phylogenetically predict the species of interest. For example, ecologists trying to predict distributions, bloom cycles, and behaviours of poorly known species may find robust predictive power in the phylogeny. Toxinologists and biochemists trying to understand the different syndrome effects or working toward antivenom development, or bioprospectors looking for therapeutic compounds, will benefit by reliably identifying the correct target species. And life guards, life savers, and charter operators will be better equipped to make on-the-spot judgments as to the relative risk associated with captured specimens, using the identification criteria herein.

The field of cubozoology is wide open to the curious student, from basic knowledge of life cycles and histology, to medical and pharmacological implications and bioprospecting applications; from the questions of where the cubopolyps spend the winter and what causes them to bloom, to learning more about the marvelous array of spawning and mating mechanisms among the different species; from understanding species boundaries through population genetics to macro-evolutionary questions about the age of the group and who its ancestors were; and the riveting paradox of having image-forming eyes and the ability to see, and yet having no brain for interpretation.

“There is grandeur in this view of life...” Darwin wrote these words nearly 150 years ago (Darwin, 1859, p. 490), the same year that the total number of recognizable cubozoans was expanded from one to three. The number has since increased ten-fold, and yet we still lack even basic understanding of the relationships among many of the species, how they reproduce, what factors govern their appearance and disappearance, and what effect their toxins have on humans and why. It’s a pretty safe bet that Darwin was unaware of the interesting biologies and fascinating paradoxes of the Cubozoa, but as we stand now at the dawn of cubozoan understanding, there is grandeur indeed.

Table 6.1. Summary of taxonomic changes in this thesis over the current system (i.e., Kramp, 1961). New taxa are denoted by an asterisk (*). Taxa are arranged in descending taxonomic order of the revised name, and alphabetically within rank. The many new species being added are not included, unless they correspond to new higher taxa or other nomenclatural changes.

Old classification	**Revised classification**
Order Cubomedusae	Not recognized
Family Carybdeidae	Order Carybdeida
Family Chirodropidae	Order Chirodropida
N/A	*Family Alatinidae
Genus *Carybdea* in part	Family Carybdeidae sens. emend.
Genera *Chirodropus* + *Chironex*	Family Chirodropidae sens. emend.
Not recognized	Family Chiropsalmidae
Not recognized	Family Tamoyidae
Not recognized	Family Tripedaliidae
N/A	*Subfamily Tamoyinae
N/A	*Subfamily Carukiinae
N/A	*Unnamed subfamily (“Pseudo-Irukandjis”)
Carybdea alata species complex	*Genus *Alatina*
N/A	*Genus *Collostemma*
N/A	*Unnamed genus 1 (“Darwin Carybdeid”)
N/A	*Unnamed genus 2 (“Morbakka” spp.)
N/A	*Unnamed genus 3 (“Pseudo-Irukandji” spp.)
N/A	*Unnamed genus 4 (“*Chiropsalmus*” spp QLD & NT)
Carybdea alata	*Alatina alata* comb. nov.
Carybdea grandis	*Alatina grandis* comb. nov.
Carybdea madraspatana	*Alatina madraspatana* comb. nov.
Carybdea moseri	*Alatina moseri* comb. nov.
Carybdea obeliscus	*Alatina obeliscus* comb. nov.
Carybdea philippina	*Alatina philippina* comb. nov.
Carybdea pyramis	*Alatina pyramis* comb. nov.
Carybdea turricula	*Alatina turricula* comb. nov.
Chiropsalmus quadrigatus	*Chiropsoides quadrigatus* comb. nov.
Carybdea sivickisi	*Collostemma sivickisi* comb. nov.
Carybdea xaymacana	**Carybdea xaymacana xaymacana* n. ssp.
N/A	**Carybdea xaymacana australis* n. ssp.

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APPENDIX 1. GLOSSARY

I have arranged this glossary by functional categories, i.e., taxonomic abbreviations and terms (relates largely to Chapters 2 and 5), general systematics terms (relates primarily to Chapter 3), nematocyst terminology (relates almost entirely to Chapter 2), and a general glossary of cubozoan terms. The reason for structuring this appendix this way is to facilitate browsing of related terminology. Within each section, all terms are arranged alphabetically.

A1.1. TAXONOMIC TERMS AND LATIN ABBREVIATIONS

Auct. (auctorum): Literally, "of authors", used nomenclaturally to refer to a name used by different authors in different ways.

Gen. (genus, pl. genera): A formal grouping of species.

Holotype: The individual specimen formally designated by the original author to represent a species. Compare with paratype, syntype, and neotype.

Neotype: An individual specimen formally designated after the original description to represent a species; can only be designated in the absence of a holotype.

Nov. (nova): Literally, "new", used as a formality in taxonomic descriptions to identify a new name.

Paratype: One or more specimens formally designated by the original author as supporting material to represent a species.

Sp. (species, pl. spp.): A formal designation of an identifiable unit observable in the natural world.

Syntype: Multiple specimens formally designated by the original author to represent a species; a single holotype is not selected.

A1.2. TERMINOLOGY OF SYSTEMATICS AND CLASSIFICATION

Apomorphy: Derived character (apo = derived, morph = character).

Autapomorphy: Unique derived character; not informative for cladistic analysis.

Cladistics: The branch of systematics that analyses branching order of taxa by grouping by synapomorphy.

Monophyletic: A natural group comprising an ancestor and all of its descendants, e.g., mammals.

Paraphyletic: A group comprising an ancestor and some, but not all, of its descendants, e.g., reptiles (excluding birds and mammals). Some workers do not regard paraphyletic groups as natural, whereas others do.

Phylogenetics: The study of evolutionary relationships among living things.

Plesiomorphy: Ancestral character (plesio = ancestral).

Polyphyletic: An unnatural group comprising taxa that are similar by analogy, e.g., “winged animals”: birds, bats, and insects. Polyphyletic taxa do not share a most recent common ancestor to the exclusion of other taxa.

Symplesiomorphy: Shared ancestral character; not informative for cladistic analysis.

Synapomorphy: Shared derived character; the basis for groupings in cladistic analysis.

Taxonomy: The branch of systematics that names and classifies taxa.

A1.3. NEMATOCYST TERMINOLOGY

The nematocyst nomenclature can be bewildering, but most of the names are quite descriptive and helpful, once their meaning is known. Following is just a brief synopsis of some of the main terms that apply to the Cubozoa; more detail is given in Chapter 2 and Plates 2.25-2.27; for a more comprehensive understanding, refer to Weill (1934), Mariscal (1971), Calder (1974), and Rifkin (in Williamson et al., 1996).

Heterotrichous/Holotrichous/Homotrichous/Atrichous: Refers to the spines on the tubule of the nematocyst. Nematocyst shafts may be atrichous (= without spines), homotrichous (also called Holotrichous) (= spines the same throughout), or heterotrichous (= spines different throughout). The shaft uses a different terminology: for terminology on the length of the shaft, see “Macrobasic/microbasic”; for terminology on the shape of the shaft, see “Mastigophores” and “Rhopaloids”; for terminology on the spination of the shaft, see the discussion of “Types” under “Mastigophores”.

Macrobasic/Microbasic: Refers to the length of the shaft on nematocysts. Traditionally, "microbasic" was defined as 3 times or less the length of the capsule, whereas "macrobasic" was defined as 4 times or more than the length of the capsule. However, I prefer to follow the convention established by Bouillon and Boero (lecture notes, Hydrozoan Workshop, 2000), in which a single capsule length is the critical point; thus, microbasic is herein used to mean those nematocysts in which the shaft does not exceed the length of the capsule, and thus does not wrap or bend inside, and macrobasic is herein used to mean those nematocysts in which the shaft is too long for the capsule, and thus is forced to wrap or bend inside, regardless of the number of times.

Mastigophores: A type of nematocyst (= stinging cell). These are thought to be the primary carrier of the lethal factor in the chirodropid taxa. B-mastigophores, in which the discharged shaft tapers gradually into the distal tubule, are not known in the Cubozoa, whereas p-mastigophores, in which the discharged shaft tapers abruptly into the distal tubule, are quite common. P-mastigophores have been previously documented in three types: Type I [all spines orientated at right angles to the capsule], Type II [all spines orientated towards the capsule], and Type III [shorter spines orientated toward the capsule, longer spines orientated away]; a fourth form, herein designated Type IV [all spines long, orientated away from the capsule], is common in some Irukandji species.

Rhopaloids: A category of nematocysts. Rhopaloids come in three types: **Euryteles**, in which the shaft is dilated at only one point along the length; **Birhopaloids**, in which the shaft has two dilations with regular spines; and **Trirhopaloids**, with three dilations, the middle (largest) swelling bearing spines.

A1.4. GLOSSARY TO THE CUBOZOA

The following list of terms has been compiled from countless sources. This glossary refers to terms used throughout this thesis, especially so in Chapter 2. Most of the terms are still widely used for cubozoan systematics and other studies; those no longer in use have been identified as such.

Abaxial: Away from the axis, i.e., towards the outer body wall rather than towards the inside or middle. Compare with Adaxial (= toward the axis).

Aboral: Generally used as a reference direction, with an aboral orientation being away from the mouth and an oral orientation being toward the mouth. Compare with Oral.

Adaxial: Toward the axis, i.e., towards the inside or middle of the body, rather than towards the outer body wall. Compare with Abaxial (= away from the axis).

Adradial: Tertiary radii, defined as the radii midway between the perradii (containing the mouth-lips and eyes) and the interradii (containing the gonads and tentacles). There are 8 adradii, or two in each quadrant of the body. Important adradial structures include the furrows that characteristically separate the flat sides of the body from the corners, and sometimes extend to sculpt out the areas containing the rhopaliar niches. [See also Perradial and Interradial.]

Alternate: With respect to the chirodropid pedalia, some taxa have the branching pattern "alternate", in which the fingers and tentacles are arranged in a staggered fashion right-left-right-left... Compare with "opposite", in which the fingers and tentacles arise directly opposite one another at the same horizontal level.

Apical: Pertaining to the apex, or the highest point of the body. The apex is often sculpted in species-specific forms, and thus helpful in identification.

Atrichous isorhizas: A type of nematocyst (= stinging cell) [see Isorhizas].

Cnidae: [see Nematocysts].

Cnidome: The full complement of nematocysts characteristic of a species.

Coelenteron: The open space inside the jellyfish between the exumbrellar body wall and the subumbrellar body wall. By analogy, the jellyfish's body is like a sandwich with no filling -- the two pieces of bread in this case are layers of jelly with dermal tissue on both the inside face and the outside face. All surfaces of the jellyfish in contact with the seawater (whether on the outside of the bell or the inside of the dome-like bell) are covered in a layer of ectoderm (= outer skin); the surfaces of the jellyfish that are away from the seawater, i.e., the "inner guts" are covered in a layer of endoderm (= inner skin). Between the ectoderm and endoderm is a layer of mesoglea, the "jelly" of the jellyfish.

Complex eyes: Image-forming eyes with lenses, retinas, and corneas. Compare with ocelli, or merely light-sensitive, non-image-forming eyes. All known cubozoans have complex eyes, and typically some number of ocelli as well. All the eyes are located on the rhopalia, or sensory knobs, along with the statolith, or balance organ. Typically, cubozoans have two median complex eyes and two pairs of lateral ocelli on each rhopalium, and they have 4 rhopalia, for a total of 24 eyes! One of the great mysteries of the invertebrate world is that cubozoans are sort of "pre-brain" evolutionarily; we know experimentally that they can see, but we are not clear how they process the information.

Euryteles: A type of nematocyst (= stinging cell) common in all four medusozoan classes, but lacking in the Anthozoa; characterized by a single dilation on the shaft. [See Rhopaloids.]

Exumbrella: Literally, "outer umbrella", or the outer/upper body wall of the jelly body.

Eyes: [See complex eyes].

Freckles: Unraised nematocyst clusters on the exumbrellar surface, velarium, perradial lappets, or pedalia. Often historically referred to as “nematocyst warts”, but herein redefined as follows: warts are nematocyst clusters which are underlain by gelatinous protuberances, whereas freckles are nematocyst clusters which are not underlain by gelatinous protuberances. [See warts.]

Frenulum: Bracket-like strips of tissue that hold the velarium in a horizontal position, located on the 4 perradii. The thickness, length, and form of the frenulum is often helpful in identification.

Gastric cirri: Individual filaments that comprise the phacellae. [See phacellae.]

Gastric pouches (= coelenteron): The space between the exumbrellar wall and the subumbrellar wall, in which lie the gonads. It is a bit of a misnomer, since the gastric region is primarily confined to the stomach.

Gastric saccules: Solid or hollow subumbrellar structures characteristic of the chirodropids, important in defining the genera. The saccules grow out of the subumbrellar wall and project into the subumbrellar cavity. They may be simple and finger-like, knob-like, cock's-comb-shaped, or highly branched resembling bunches of grapes. These structures are interchangeably termed "Taschen-Armen" by Haeckel (1880); "hernia-like pouches" by Mayer (1910); "diverticula (Umbralsäcke)" by Uchida (1929); "gastric pouches" by Bigelow (1938); "superior gonad" by Southcott (1956); "diverticula", "projections", "gastric saccules", or "hernia-like pouches" by Kramp (1961); "perradial nucleus" by Barnes (1965); "divertikel der gastraltasche" by Werner (1984); and "diverticula" by Mianzan and Cornelius (1999).

Gonads: The tissues that make the sperms and eggs. All cubozoans are thought to be dioecious (= having separate sexes). Most cubozoans have "lateral gonads" attached to the interradial corners of the body, inside the coelenteron. Each of the 4 lateral gonads is comprised of 2 "hemi-gonads", or half-gonads, being one half growing to the left and the other half growing to the right. Some species of chirodropids have the gonads growing up around the saccules rather than down laterally through the body pouches.

Handkerchiefs [see Nematocyst bands].

Heterotrichous/Holotrichous/Homotrichous/Atrichous: Refers to the spines on the shaft of the nematocyst. See above (Section A1.3) for more explanation.

Interradial: By definition, the secondary radii of the body ("inter" = between); in the Cubozoa, the interradii correspond to the 4 corners of the body, and their associated structures (e.g., phacellae, gonads, pedalia, tentacles). [See also Perradial and Adradial.]

Isorhizas: A type of nematocyst (= stinging cell) characterized by the discharged tubule being the same diameter throughout its length, or narrowing slightly toward the distal end. Nematocysts with the tubule slightly dilated proximally are termed "anisorhizas".

Lappets: [see Perradial lappets].

Macrobasic/Microbasic: Refers to the length of the shaft on nematocysts. See above (Section A1.3) for more explanation.

Manubrium: Literally, the throat; the tube that connects the mouth of the jellyfish to the stomach. The manubrium may be long or short, depending on species specific properties, and also on the state of contraction at the time of preservation.

Mastigophores: A type of nematocyst (= stinging cell). These are thought to be the primary carrier of the lethal factor in the chirodropid taxa. See above (Section A1.3) for more explanation.

Mesenteries: Web-like sections of tissue that connect the stomach to the subumbrellar body wall along the perradial axes. In a few species, the mesenteries are extremely well developed, whereas in most species they are more modestly developed and have typically been ignored in the diagnostic literature. It is more properly a case of degree than of presence/absence as has been typically assumed before. The distance toward the rhopalium that the mesenterial tissue reaches is of importance to identification. Also sometimes called perradial suspensoria.

Mesoglea: The jelly that comprises most of the bulk of a jellyfish, sandwiched between two cellular layers. It is made up of collagenous compounds and has relatively few cells in comparison to the dermal tissues.

Microbasic/Macrobasic: Refers to nematocyst shaft lengths [see Macrobasic/Microbasic]. See above (Section A1.3) for more explanation.

Neckerchiefs [see Nematocyst bands].

Nematocysts: The stinging cells, also collectively called "cnidae". Nematocysts occur in a variety of shapes and sizes, and the ratios of different types can be diagnostic for some species. The main cnidae that occur in the Cubozoa are: Mastigophores, Isorhizas, Euryteles, and Stenoteles. Nematocyst identification is properly done on the shafts of discharged capsules. [See also Macrobasic/Microbasic and Heterotrichous/Holotrichous/ Homotrichous/Atrichous for explanation of the shaft terminology. For explanation of shaft morphology, see also Mastigophores and Rhopaloids.]

Nematocyst bands: Fleshy raised bands on the tentacles of all cubozoan species, on which the nematocysts are concentrated. Nematocyst bands repeat throughout the length of the tentacle, and the pattern of banding is highly diagnostic in some species. In some cases the nematocyst bands are of a peculiar form, as in the genus *Carukia*, where the bands may be of a "handkerchief" or "neckerchief" form, so called by Southcott (1967) who likened them to the handkerchief worn around the neck by John Wayne in his movies, with the triangular "tail" that hangs down on one side. In a currently undescribed species, the nematocyst bands have a peculiar "halo" form, with the nematocysts inserted end-on around the periphery of the bands.

Ocelli: Light-sensitive eye spots, without image-forming capabilities. [See Complex eyes for a more thorough explanation.]

Opposite: With respect to the chirodropid pedalia, some taxa have the branching pattern "opposite", in which the fingers and tentacles arise directly opposite one another at the same horizontal level. Compare with "alternate", in which the fingers and tentacles are arranged in a staggered fashion right-left-right-left...

Oral: Pertaining to the mouth, generally used as a reference direction, with an oral orientation being towards the mouth and an aboral orientation being away from the mouth. Compare with Aboral.

Perradial: By definition, the primary radii of the body arrangement; the 4 radii on which the mouth-lips occur. In the case of cubozoans, the rhopalia also occur on the perradii. In some species, mesenteries and/or perradial lappets occur on this radial designation. In the chirodropids, the gastric saccules are perradial. [See also Interradial and Adradial.]

Perradial lappets: Thickened regions on the perradial abaxial surface of the velarium of some species, appearing as a flap extending down from the outer body wall. The frenulum extends along the central axis of each of the four lappets on the subumbrellar side. The presence or absence of the lappets is important in species recognition, and in some cases the lappet shape can be useful as well. In a few taxa, the lappets give rise to lateral and distal canals. They have variously been called "tentacular lobes" (Agassiz, 1862) and "schirmlappen" (Claus, 1878), and were once believed to be homologous with scyphozoan lappets.

Perradial suspensoria [see Mesenteries].

Pedalia: The blade-like or claw-like gelatinous extensions on the four corners of the body, forming the base for the tentacles. Carybdeid pedalia are simple (= unbranched), and each pedalium gives rise to only a single tentacle. Chirodropid pedalia are complex (= branched), with each pedalium branching into numerous finger-like extensions, and each finger giving rise to a single tentacle, but the entire pedalial structure having multiple tentacles. The branching pattern (whether alternate or opposite) of the chirodropids and the shape of the pedalial "keels" in the carybdeids are of taxonomic importance.

Pedalial canals: A single hollow canal runs through each of the pedalia, from the coelenteron of the body to the hollow tentacle. Many features of the pedalial canals are important in species identification, including the shape of any diverticula that may occur near the base, the cross section shape, and whether or not the end is flared where it meets the tentacle.

Pedalial keels: In the Carybdeidae, the abaxial (= inner) wing of the pedalia is typically flared in taxonomically diagnostic ways. This is also the case for some chirodropids in the genus *Chiropsalmus*, but this is only recently beginning to be appreciated.

Phacellae: The bundles of gastric cirri in the stomach corners. The shape of the phacellae and the number of roots is highly diagnostic in the Carybdeidae. The shape can be observed through the body wall without dissection, whereas counting the roots typically requires that a small incision be made in one of the upper corners of the body to gain better access for accuracy.

Planula larva: The larval stage of all members of the sub-phylum Medusozoa. The cubozoan planula is typically about 150 µm long, rounded teardrop-shaped, with a conspicuous row of dark spots encircling the larger end.

Polyp: The sedentary, asexual portion of the cubozoan life cycle. The cubopolyp is formed from metamorphosis of the planula larva, and after asexually budding other cubopolyps, undergoes a total transformation into a juvenile medusa.

Rhopalia: The sensory bodies that contain the eyes and balance organs (statoliths). An individual has 4 rhopalia, one inside each rhopaliar niche on the lower portion of the flat sides of the body wall. Each rhopalium typically has 6 eyes (2 median complex eyes and 2 pairs of lateral eye spots) plus a single statolith, however, some species are lacking the full complement of 6 eyes. The rhopalia are borne on the end of a stalk, such that they dangle inside the rhopaliar niche and can be rotated around by the animal at will. The stalk is connected to a nerve cord that encircles the body in a zig-zag fashion, alternating up to the rhopalia and down to the base of the pedalia.

Rhopalial niche: The cavity in the lower portion of the bell wall that protects the rhopalia. This has also been called the "sensory niche" or "sensory nitch".

Rhopaliar niche ostium: The opening to the rhopaliar niche. Often referred to in the literature simply as rhopaliar niche, but referring to the opening to the cavity rather than the cavity itself. The shape of the ostium, which is a function of how many covering scales it has, is highly diagnostic in the Carybdeida.

Rhopalial horns: Newly discovered structures issuing from the upper part of the rhopalial niche, as blind-ending canals that traject upward and sometimes outward from the rhopalial niche. The length, width and angle appear to be useful for identification. These structures are not mentioned in the older literature, being first described by Gershwin and Alderslade (in review) as "supra-rhopalial canals". They have an appearance reminiscent of devil horns or Viking horns.

Rhopaliar windows: A non-muscularized portion of the subumbrellar bell wall that allows for the rhopalia to see into the bell cavity; the relative concavity of the structure can be taxonomically useful in the Carybdeida. This structure has not been previously identified.

Rhopaloids: A category of nematocysts. See above (Section A1.3) for more explanation.

Saccules: [see Gastric saccules].

Sexual dimorphism: Males and females having different characters or character states, such that they can be identified structurally. Di = two, morph = form.

Species diversity: Species richness.

Stenoteles: A type of nematocyst (= stinging cell) present in many hydrozoans and a few cubozoans, characterized by a constriction on the shaft, with large spines emitting from the constriction.

Statocyst: The balance organ, located on the rhopalium, and containing a statolith. In some species the statocyst is located "behind" the main eye, whereas in other species it is located "below" the main eye.

Statolith: The "rock" or concretion that, along with the statocyst (the cavity it sits in), comprises the balance organ. The statolith is made of calcium sulfate, and grows with the medusa by adding daily growth rings. The shape of the statolith has not been previously used as a taxonomic character, but is proving quite helpful.

Subumbrella: Literally, the "under umbrella", or the part of the jelly body that one has to "lift up the skirt" to see.

Suspensoria: [see Mesenteries].

Tentacles: The whip-like filaments on which the primary stinging cells occur, i.e., the business end of the jellyfish. In cubozoans, the tentacles are attached to the body by means of pedalia; the tentacles are properly defined as the flexible, opaque part, whereas the pedalia are the transparent, gelatinous, stiff part at the base.

Tentacle bands: [see Nematocyst bands].

Tumiteles: A type of nematocyst (= stinging cell) introduced by Southcott (1967) for some carybdeids, characterized by the middle portion of the shaft being wider than it is at either end. This type of nematocyst is not typically recognized by later workers, though it is not completely clear why not.

Velarium: The shelf-like, ring-shaped flap of tissue that narrows the aperture to the main subumbrellar cavity. The velarium is useful to the animal in locomotion, providing the jet propulsion that is important to them in powerful swimming. The velarium has many structures that are important in species identification, e.g., the velarial canals, the perradial lappets, the frenulum, and patterns of nematocyst warts. In *Carybdea sivickisi* the velarium contains sexually dimorphic structures.

Velarial canals: Blind-ending extensions of the gastric pouches down into the velarium. The number and form of the velarial canals can be highly diagnostic, primarily in the Carybdeidae.

Warts: Typically nematocyst clusters which are underlain by gelatinous protuberances on the exumbrellar surface. [See Freckles.]

APPENDIX 2. DATA MATRIX OF CUBOZOAN MORPHOLOGICAL CHARACTERS

'Alatina mordens MS Name'	56{23}0111111 1004242{45}10 --01001040 2110902000 1422110110 340--{02}{02}042 22{34}{04}001110 --{27}00300-- -0032
'Alatina rainensis MS Name'	3410{01}11001 1004{12}41{34}00 --{02}2001010 2{01}13(69)02(12)00 142{01}111000 390--210?2 2220001130 --000300-- -00??
'Manokia stiasnyi'	3{56}00032224 ?005161300 --03002033 2000903000 142?11{02}1?0 3{46}0--200?3 ?330000110 --70???0-- -0???
'Carukia barnesi'	{12}5130(03)22{24}6 1004{12}51400 --01001{01}{01}1 2100702000 0----10114 1{02}?10000?1 001{01}001121 0050{01}000-- -7006
'Carukia n. sp. B (Broome)'	251{03}03222{23} 100{56}1{45}1400 --01001011 1100702000 0----10113 1{49}500000?1 002{12}001141 {03}{12}{35}00300-- -7006
'Carukia n. sp. A (Russell"s)'	33{12}00(23)204{34} ?005241{45}00 --01{02}010{01}1 2100340000 0----10113 1{24}30{01}(14)0060 00{12}{12}001121 00001100-- -{27}00(36)
'Carybdea marsupialis'	43111(03)2??{12} ?005141301 --12001030 200290?(12)00 1102010110 2?0--?00?{23} ?{23}?{15}?11?30 --001??0-- -?032
'Carybdea rastonii SA'	43{12}10(03)212{23} 1002141{34}01 --21001030 2001(69)02200 1212010104 280--(14)0021 1145001230 --000200-- -4{04}32
'Carybdea n. sp. S. Africa'	41110(03)2224 1005222302 --3{49}101060 {12}001(69)0??00 1103010103 2{47}0--(14)00?1 1145101220 --000200-- -7{24}32
'Carybdea sivickisi Mag Isl'	11220(13)2021 ?004040003 --0{02}001000 2022(69)02100 100?0122?3 515002??01 0110001140 --000200-- -2035
'C. xaymacana australis - WA'	25110(03)22{24}{12} 1005141301 --31001000 2{01}01(69)01200 1002010112 2{69}0--(14)0021 113{15}010{12}40 --000000-- -4030
'C. xaymacana xaymacana'	4{34}{12}10(03)1221 1004142401 --22{01}01140 2003(69)?2200 10020101{01}2 2{47}0--100?1 112(01)010140 --000200-- -10??
'"Darwin Carybdeid"'	4{45}210(03)222{14} 1004241212 --37101{12}50 1{01}01{23}?0000 0----101{01}3 1{47}211420?4 0556000311 1141{01}{13}00-- -60?2
'"Broome Irukandji"'	461002122{34} 1004{01}41310 --03{02}01030 2100340000 0----10111 11210(04)2033 0343100221 01410300-- -7{04}2{26}
'"Dampier Irukandji"'	26?001???? ?00{45}{12}???00 --0?201032 2??0340000 0----101?? 12220(14)???{12} 0{12}41110121 42300300-- -7???
'"Halo Irukandji"'	35100211(24)(14) ?00{45}141400 --{23}3(02)01032 2010340000 0----10104 1(28)210(04)?0?3 0035000131 01{24}00100-- -70??
'"Pseudo-Irukandji"'	331002202{34} 100415{01}{23}00 --232010{34}4 2010340000 0----101?4 122004203{23} 0043000121 0(12)401300-- -7022
'Morbakka Port Douglas'	55{12}00220(25)4 20041{45}{01}{12}02 --{23}7{12}01260 0001(36)40100 0----10103 141(01)(02)(04)21?5 0655100301 {01}2(47)11100-- -70?2
'Morbakka Moreton Bay'	45200221(24)(23) (12)00{45}{01}41101 --{23}7101?6? ???04????? 0----10103 14411(04)???5 ??5{56}1004{01}1 0?411100-- -7{04}??
'Tamoya haplonema'	5{56}200{12}22(24)(25) ?004{12}11202 --37101260 {12}00??????? 1332010 1?2 100--30??5 ?655101300 --711100-- -7{04}?2
'Tripedalia binata'	1{01}{02}00(13)2{12}21 1106051300 --00001000 2001(58)00011 10020110{01}2 620--4005{23} 1{23}{23}0000{12}40 --001000-- -60??
'Tripedalia cystophora'	1{01}{01}0012{12}(24)1 2204050100 --01001000 2{01}01(59)02(12)00 1002011003 620--(14)0??2 1220000140 --{07}02000-- -70??
'Chirodropus gorilla'	5120020110 001------0 00?7?510?0 0?0??????? 16400101?3 44???0???5 1656100221 ??01?10153 ?6?-?
'Chironex fleckeri'	{56}1100(03)02{25}0 001------0 0107061060 0405(25)31110 16400121?3 4(34)0--04?15 0?56100221 1200200122 125-(13)
'Chiropsalmus quadrigatus'	401002???0 002------0 1-07?31?40 0?0??????? 1????????3 48???????? ?????????? ???????1?? ?????
'Chiropsalmus maculatus MS Name'	51{12}0022250 -01------0 1107051060 0000{23}?0000 13301103?3 42???0???5 ??56000541 23011000-- -83-4
'Chiropsalmus quadrumanus'	5{01}{12}00(02)1144 (24)01------0 1005241030 2205(25)3(14)210 16200101?3 450--?00?5 0656100111 2270110132 {01}000{36}
'Chiropsalmus n. sp. A (N. QLD)'	{45}{12}1100{12}2{12}0 001------0 0001041030 2504(25)34(12)10 16400101?2 460--00??5 0?56100121 2200000100 130-6
'Chiropsalmus n. sp. B (Gove)'	4{12}100(03)2140 001------0 0006031030 2003(26)30210 16400101?3 460--00??5 0656100221 2200100110 1(7A)0-?
'Chiropsalmus zygonema'	43???????? ?01------? ????11??? ?????????? 164??????? ?????????? ?????????? ???00??131 ?????
'Chiropsoides buitendijki'	5{23}100(03)0{01}10 -02------0 1-0{78}0{34}1150 0101(26)?0000 1640{01}12103 450--00??1 0156{01}01{14}11 1(12)00020133 00{05}-2

APPENDIX 3. PARTIAL 18S rDNA ALIGNMENT

Aligned sequences of 568 bp from the 5’ end of the 18S rDNA sequence in 42 cubozoan specimens representing 13-16 species. Question marks (?) represent missing data; dashes (--) represent gaps; dots (.) indicate identical bases. AF and L numbers are from GenBank; 2003-series sequences were kindly shared by M. van Oppen and L. Peplow; 2004-series sequences were generated by me for this study.

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Alatina mordens 2003 25       ??????GCATGTC-AAGTGTAAGCAC-TGGTACTGTGAAACTGCGAAT-GGCTCATTAAATCAGTTATCGTTTACTTG
Alatina mordens 2003 26       ????GT.......-............-.....................-.............................
Unknown 2003 19               ?GC-AT.......-............-.....................-.............................
Unknown 2003 20               ???????????????????????...-.....................-.............................
Carybdea marsupialis AF358106 AGCCAT.......G............T.-...................-.............................
Carybdea xaymacana AF358109   AGCCAT.......TT...A.......T.T...................-.............................
Carybdea xaymacana 2004 5     ???CAT.......TT...A.......T.T...................-.............................
Carybdea xaymacana 2004 06    ???CAT.......TT...A.......T.T...................-.............................
Carybdea xaymacana 2004 07    AGCCAT.......TT...A.......T.T...................-.............................
Carybdea nsp CapeTown22004 13 ??????????????????????????????????????????????????????????????????????????????
Carybdea nsp CapeTown32004 14 AGCCAT.......TT...A.......T.T...................-.............................
Carybdea rastonii AF358108    AGCCAT.......TT...A.......T.T...................-.............................
Carybdea rastonii 2004 10     AGC-AT.......TT...A.......T.T...................-.............................
Carybdea rastonii 2004 11     AGCCAT.......TT...A.......T.T...................-.............................
Carybdea rastonii 2004 12     AGCCAT.......TT...A.......T.T...................-.............................
Carybdea rastoniiJapan 2004 18AGCCAT.......TT...A.......T.T...................-.............................
Carybdea rastoniiJapan 2004 19AGCCAT.......TT...A.......T.T...................-.............................
Carybdea sivickisi AF358110   AGCCAT.......TT...A.......T.-...................-.............................
Carybdea sivickisi SA2004 17  AGCCAT.......TT...A.......T.-...................-.............................
Carybdea sivickisi AIMS       ????AT.......TT...A.......T.-...................-.............................
Tripedalia cystophora L10829  AGCCAT.......T....A.......T.-...................-.............................
Carukia barnesi AF358107      AGCCAT.......TT...A......-T.T...................-.............................
Carukia barnesi 2003 03       AGC-AT.......TT...A......-T.T...................-.............................
Carukia barnesi 2003 11       AGC-AT.......TT...A......-T.T...................-.............................
Carukia nsp Broome 2004 23    AGCCAT.......TT...A......-T.T...................-.............................
Darwin carybdeid AF358105     AGCCAT.......TT...A......-T.T...................-.............................
Morbakka Mackay 2004 08       AGCC-T.AC....T....A......-T.T...................A.........?...................
Pseudo Irukandji Port2004 16  AGCCAT.......TT...A......-T.T...................-.............................
Pseudo Irukandji 2003 14      ??????????????????????...-T.T...................-.............................
Broome Irukandji large2004 21 AGCCAT.......TT...A......-T.T...................-.............................
Broome Irukandji juv2004 22   AGCCAT.......TT...A......-T.T...................-.............................
Pseudo Irukandji hore52004 24 AGCACT.......TT...A......-T.T...................-.............................
Pseudo Irukandji hore62004 26 AGCCAT.......TT...A......-T.T...................-.............................
Chironex fleckeri AF358104    AGCCAT.......C....A......-T.T...................-.............................
Chironex fleckeri 2003 04     ???????????????...A......-T.T...................T.............................
Chironex Darwin 2004 01       AGCCAT.......T....A......-T.T...................-.............................
Chiropsalmus sp.AF358103      AGCCAT.......T....A.......T.-...................-.............................
Chiropsalmus sp.NQLD 2003 33  ??????????????....A.......T.-...................-.............................
Chiropsalmus sp.NQLD 2003 08  ?????????????????????????????????????????????????????.........................
Chiropsalmus sp.NQLD 2003 32  ?????????????????????????????????????...........-.............................
Chiropsalmus sp.Gove62004 03  AGCCAT.......T....A.......T.-...................-.............................
Chiropsalmus sp.Gove2004 04   AG-CAT.......T....A.......T.-...................-..........?..................
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Alatina mordens 2003 25       ATGT----TGTCCT--TACATGGATAACTGTGGTAATTCTAGAGCTAATACATGCAAGAA-GTCCCGACC-----TCT
Alatina mordens 2003 26       ....----......--............................................-.........-----...
Unknown 2003 19               ....----......--............................................-.........-----...
Unknown 2003 20               ....----......--............................................-.........-----...
Carybdea marsupialis AF358106 ....----......CT.......................................TT...-.........-----...
Carybdea xaymacana AF358109   ..CA----.A....--.......................................G.A..-.........-----...
Carybdea xaymacana 2004 5     ..CA----.A....--.......................................G.A..-.........-----...
Carybdea xaymacana 2004 06    ..CA----.A....--.......................................G.A..-.........-----...
Carybdea xaymacana 2004 07    ..CA----.A....--.......................................G.A..-.........-----...
Carybdea nsp CapeTown22004 13 ????----??....--.......................................G.A..-.........-----...
Carybdea nsp CapeTown32004 14 ..CA----.A....--.......................................G.A..-.........-----...
Carybdea rastonii AF358108    ..CA----.A....--.......................................G.A..-.........-----...
Carybdea rastonii 2004 10     ..CA----.A....--.......................................G.A..-.........-----...
Carybdea rastonii 2004 11     ..CA----.A....--.......................................G.A..-.........-----...
Carybdea rastonii 2004 12     ..CA----.A....--.......................................G.A..-.........-----...
Carybdea rastoniiJapan 2004 18..CA----.A....--.......................................G.A..-.........-----...
Carybdea rastoniiJapan 2004 19..CA----.A....--.......................................G.A..-.........-----...
Carybdea sivickisi AF358110   ..CG----.A....--.......................................G.A..-.........-----...
Carybdea sivickisi SA2004 17  ..CG----.A....--.......................................G.A..-.........-----...
Carybdea sivickisi AIMS       ..CGTACT.A....--.......................................G.A..-.........CCTTTG..
Tripedalia cystophora L10829  ..CG----.A....--.......................................G.A..-.........-----...
Carukia barnesi AF358107      ..CG----.A....--.......................................G.A..-.........-----...
Carukia barnesi 2003 03       ..CG----.A....--.......................................G.A..-.........-----...
Carukia barnesi 2003 11       ..CG----.A....--.......................................G.A..-.........-----...
Carukia nsp Broome 2004 23    ..CG----.A....--.......................................G.A..-.........-----...
Darwin carybdeid AF358105     ..CG----.A....--.......................................G.A..-.........-----...
Morbakka Mackay 2004 08       ..CG----.A....--................?............?.........G.A..-.....A...-----...
Pseudo Irukandji Port2004 16  ..CG----.A....--.......................................G.A..-.........-----...
Pseudo Irukandji 2003 14      ..CG----.A....--.......................................G.A..-.........-----...
Broome Irukandji large2004 21 ..CG----.A....--.......................................G.A..-.........-----...
Broome Irukandji juv2004 22   ..CG----.A....--.......................................G.A..-.........-----...
Pseudo Irukandji hore52004 24 ..CG----.A....--.......................................G.A..-.........-----...
Pseudo Irukandji hore62004 26 ..CG----.A....--.......................................G.A..-.........-----...
Chironex fleckeri AF358104    ..CG----.A...A-T............C..........................G.C..-.........-----...
Chironex fleckeri 2003 04     ..CG----.A...A-T............C..........................G.C..-.........-----...
Chironex Darwin 2004 01       ..CG----.A...A-T............C..........................G.C..-.........-----...
Chiropsalmus sp.AF358103      ..CG----.A....-A............C............................A..-.........-----...
Chiropsalmus sp.NQLD 2003 33  ..CG----.A....-A............C............................A..-.........-----...
Chiropsalmus sp.NQLD 2003 08  ..CG----.A....-A............C............................A..-.........-----...
Chiropsalmus sp.NQLD 2003 32  ..CG----.A...A-T............C..........................G.C..-.........-----...
Chiropsalmus sp.Gove62004 03  ..CG----.A....-C............C............................A..-.....A...-----...
Chiropsalmus sp.Gove2004 04   ..CG----.A....-C............C............................A..-.....A...-----...


Alatina mordens 2003 25       ----GGAAGGGATGTATTTATTAGACTAAAAACCAATAC-CGGCTCTTGCAGCT-------GGTTCAC-TTGGTGATT
Alatina mordens 2003 26       ----...................................-..............-------.......-.........
Unknown 2003 19               ----...................................-..............-------.......-.........
Unknown 2003 20               ----...................................-..............-------.......-.........
Carybdea marsupialis AF358106 ----...................................G...TG...CAC.G.G---CCGC....TTG.........
Carybdea xaymacana AF358109   ----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea xaymacana 2004 5     ----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea xaymacana 2004 06    ----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea xaymacana 2004 07    ----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea nsp CapeTown22004 13 ----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea nsp CapeTown32004 14 ----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea rastonii AF358108    ----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea rastonii 2004 10     ----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea rastonii 2004 11     ----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea rastonii 2004 12     ----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea rastoniiJapan 2004 18----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea rastoniiJapan 2004 19----...........................G.......-...T--..CAC.------ACC.....TA-.........
Carybdea sivickisi AF358110   ----...........................G.......-...T--..C.CA------ACC.....TA-.........
Carybdea sivickisi SA2004 17  ----...........................G.......-...T--..TAC.------ACC.....TA-.........
Carybdea sivickisi AIMS       GC-G...........................G....CG.-...T...CCTC..GGGGCACC..C..GTT.........
Tripedalia cystophora L10829  ----...........................G.......-...T--..CGC.------ACC.....TA-.........
Carukia barnesi AF358107      ----..........C................G.......-T..T--GGCTC.---TCTGCCA...G.A-.........
Carukia barnesi 2003 03       ----..........C................G.......-T..T--GGCTC.---TCTGCCA...G.A-.........
Carukia barnesi 2003 11       ----..........C................G.......-T..T--GGCTC.---TCTGCCA...G.A-.........
Carukia nsp Broome 2004 23    ----..........C................G.......-T..T--GGCTC.---TCTGCCA...G.A-.........
Darwin carybdeid AF358105     ----..........C................G.......-T..T--GGCTC.---TCTGCCA...G.A-.........
Morbakka Mackay 2004 08       ----..........C................G.......-T..T--GGCTT.---TCTGCCA...G.A-.........
Pseudo Irukandji Port2004 16  ----..........C................G.......-T..T--GGCTC.---TCTGCCA...G.A-.........
Pseudo Irukandji 2003 14      ----..........C................G.......-T..T--GGCTC.---TCTGCCA...G.A-.........
Broome Irukandji large2004 21 ----..........C................G.......-T..T--GGCTC.---TCTGCCA...G.A-.........
Broome Irukandji juv2004 22   ----..........C................G.......-T..T--GGCTC.---TCTGCCA...G.A-.........
Pseudo Irukandji hore52004 24 ----..........C................G.......-T..T--GGCTC.---TCTGCCA...G.A-.........
Pseudo Irukandji hore62004 26 ----..........C................G.......-T..T--GGCTC.---TCTGCCA...G.A-.........
Chironex fleckeri AF358104    ----...........................G.......-T..T--..CAC.------ACCA.....A-.........
Chironex fleckeri 2003 04     ----...........................G.......-T..T--..CAC.------ACCA.....A-.........
Chironex Darwin 2004 01       ----...........................G.......-T..T--..CAC.------ACCA.....A-.........
Chiropsalmus sp.AF358103      ----...................................-T..T--..CAC.------ACCA....CT-.........
Chiropsalmus sp.NQLD 2003 33  ----...................................-T..T--..CAC.------ACCA....CT-.........
Chiropsalmus sp.NQLD 2003 08  ----...................................-T..T--..CAC.------ACCA....CT-.C.......
Chiropsalmus sp.NQLD 2003 32  ----...........................G.......-T..T--..CAC.------ACCA....CA-.........
Chiropsalmus sp.Gove62004 03  ----...................................-T..T--..CAC.------ACCA....CT-.........
Chiropsalmus sp.Gove2004 04   ----...................................-T..T--..CAC.------ACCA....CT-.........
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Alatina mordens 2003 25       CATGATAACTGTACGAATCGCACGGGCTTTGTACCGGCGATGTTTCATTCAAATATCTGCCCTATCAACTGTCGATGG
Alatina mordens 2003 26       ..............................................................................
Unknown 2003 19               ..............................................................................
Unknown 2003 20               ..............................................................................
Carybdea marsupialis AF358106 ..........TG..........G....A.C..C.............................................
Carybdea xaymacana AF358109   ..........TCT..............A........T.........................................
Carybdea xaymacana 2004 5     ..........TCT..............A..................................................
Carybdea xaymacana 2004 06    ..........TCT..............A..................................................
Carybdea xaymacana 2004 07    ..........TCT..............A..................................................
Carybdea nsp CapeTown22004 13 ..........?CT..............A..................................................
Carybdea nsp CapeTown32004 14 ..........TCT..............A..................................................
Carybdea rastonii AF358108    ..........TCT..............A..................................................
Carybdea rastonii 2004 10     ..........TCT..............A..................................................
Carybdea rastonii 2004 11     ..........TCT..............A..................................................
Carybdea rastonii 2004 12     ..........TCT..............A..................................................
Carybdea rastoniiJapan 2004 18..........TCT..............A..................................................
Carybdea rastoniiJapan 2004 19..........TCT..............A..................................................
Carybdea sivickisi AF358110   ..........TCT..............-..................................................
Carybdea sivickisi SA2004 17  ..........TCT..............-..................................................
Carybdea sivickisi AIMS       ..........TGT..............-...C..............................................
Tripedalia cystophora L10829  ..........TCT..............-..................................................
Carukia barnesi AF358107      ..........TGTT........A....-..................................................
Carukia barnesi 2003 03       ..........TGTT........A....-..................................................
Carukia barnesi 2003 11       ..........TGTT........A....-..................................................
Carukia nsp Broome 2004 23    ..........TGTT........A....-..................................................
Darwin carybdeid AF358105     ..........TG.T................................................................
Morbakka Mackay 2004 08       ..........TG.T........T....A.A.............?.................................?
Pseudo Irukandji Port2004 16  ..........TG.T...............A.C..............................................
Pseudo Irukandji 2003 14      ..........TG.T...............A.C..............................................
Broome Irukandji large2004 21 ..........TG.T...............A.C..............................................
Broome Irukandji juv2004 22   ..........TG.T...............A.C..............................................
Pseudo Irukandji hore52004 24 ..........TG.T...............A.C..............................................
Pseudo Irukandji hore62004 26 ..........TG.T...............A.C..............................................
Chironex fleckeri AF358104    ..........TCT................A................................................
Chironex fleckeri 2003 04     ..........TCT................A................................................
Chironex Darwin 2004 01       ..........TCT................A................................................
Chiropsalmus sp.AF358103      ...........CT................A................................................
Chiropsalmus sp.NQLD 2003 33  ...........CT................A................................................
Chiropsalmus sp.NQLD 2003 08  ...........CT................A................................................
Chiropsalmus sp.NQLD 2003 32  ...........CT................A................................................
Chiropsalmus sp.Gove62004 03  ...........CT................A................................................
Chiropsalmus sp.Gove2004 04   ...........CT................A................................................


Alatina mordens 2003 25       TAAGATAGAGGCTTACCATGGTTGCAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATGG
Alatina mordens 2003 26       ..............................................................................
Unknown 2003 19               ..............................................................................
Unknown 2003 20               ..............................................................................
Carybdea marsupialis AF358106 ......C.......................................................................
Carybdea xaymacana AF358109   ....G...T.....................................................................
Carybdea xaymacana 2004 5     ....G...T.....................................................................
Carybdea xaymacana 2004 06    ....G...T.....................................................................
Carybdea xaymacana 2004 07    ....G...T.....................................................................
Carybdea nsp CapeTown22004 13 ....G...T.......................A.............................................
Carybdea nsp CapeTown32004 14 ....G...T.......................A.............................................
Carybdea rastonii AF358108    ....G...T.......................A.............................................
Carybdea rastonii 2004 10     ....G...T.......................A.............................................
Carybdea rastonii 2004 11     ....G...T.......................A.............................................
Carybdea rastonii 2004 12     ....G...T.......................A.............................................
Carybdea rastoniiJapan 2004 18....G...T.......................A.............................................
Carybdea rastoniiJapan 2004 19....G...T.......................A.............................................
Carybdea sivickisi AF358110   ....G...T.....................................................................
Carybdea sivickisi SA2004 17  ....G...T.......................A.............................................
Carybdea sivickisi AIMS       ....G...C..................................................................C..
Tripedalia cystophora L10829  ....G...T.......................A.............................................
Carukia barnesi AF358107      ....G...T...............T...........................................C.........
Carukia barnesi 2003 03       ....G...T...............T...........................................C.........
Carukia barnesi 2003 11       ....G...T...............T...........................................C.........
Carukia nsp Broome 2004 23    ....G...T...............T...........................................C.........
Darwin carybdeid AF358105     ....G...T...............T.....................................................
Morbakka Mackay 2004 08       ....G...T...............T.....................................................
Pseudo Irukandji Port2004 16  ....G...T...............T.....................................................
Pseudo Irukandji 2003 14      ....G...T...........T...T.....................................................
Broome Irukandji large2004 21 ....G...T...............T.....................................................
Broome Irukandji juv2004 22   ....G...T...............T.....................................................
Pseudo Irukandji hore52004 24 ....G...T...............T.....................................................
Pseudo Irukandji hore62004 26 ....G...T...............T.....................................................
Chironex fleckeri AF358104    ....G...T...............T.....................................................
Chironex fleckeri 2003 04     ....G...T...............T.....................................................
Chironex Darwin 2004 01       ....G...T...............T.........????????????????????????????????????????????
Chiropsalmus sp.AF358103      ....G...T...............T.......A.............................................
Chiropsalmus sp.NQLD 2003 33  ....G...T...............T.......A.............................................
Chiropsalmus sp.NQLD 2003 08  ....G...T...............T.......A.............................................
Chiropsalmus sp.NQLD 2003 32  ....G...T...............T.......A.............................................
Chiropsalmus sp.Gove62004 03  ....G...T...............T.......A.............................................
Chiropsalmus sp.Gove2004 04   ....G...T...............T.......A.............................................
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Alatina mordens 2003 25       CTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTCAAAGAGGG-AGGTAGTGACAAGAACTAAC-A
Alatina mordens 2003 26       .......................................................-....................-.
Unknown 2003 19               .......................................................-....................-.
Unknown 2003 20               .......................................................-....................-.
Carybdea marsupialis AF358106 .......................................................-....................-.
Carybdea xaymacana AF358109   ..............................................G.C.C....-...............A..G.-.
Carybdea xaymacana 2004 5     ..............................................G.C.C....-...............A..G.-.
Carybdea xaymacana 2004 06    ..............................................G.C.C....-...............A..G.-.
Carybdea xaymacana 2004 07    ..............................................G.C.C....-...............A..G.-.
Carybdea nsp CapeTown22004 13 ..............................................G.C.C....-...............A..G.-.
Carybdea nsp CapeTown32004 14 ..............................................G.C.C....-...............A..G.-.
Carybdea rastonii AF358108    ..............................................G.C.C....-...............A..G.-.
Carybdea rastonii 2004 10     ..............................................G.C.C....-...............A..G.-.
Carybdea rastonii 2004 11     ..............................................G.C.C....-...............A..G.-.
Carybdea rastonii 2004 12     ..............................................G.C.C....-...............A..G.-.
Carybdea rastoniiJapan 2004 18..............................................G.C.C....-...............A..G.-.
Carybdea rastoniiJapan 2004 19..............................................G.C.C....-...............A..G.-.
Carybdea sivickisi AF358110   ..............................................G.C.C....-....................-.
Carybdea sivickisi SA2004 17  ..............................................G.C.C....-....................-.
Carybdea sivickisi AIMS       ..............................................G.C.C....-....................-.
Tripedalia cystophora L10829  ..............................................G.C.C....-....................-.
Carukia barnesi AF358107      ..............................................G.C.C....-...............A....-.
Carukia barnesi 2003 03       ...A..A.......................................G.C.C....G...............A....C.
Carukia barnesi 2003 11       ..............................................G.C.C....G...............A....-.
Carukia nsp Broome 2004 23    ..............................................G.C.C....-...............A....-.
Darwin carybdeid AF358105     ..............................................G.C.C....-...............A....-.
Morbakka Mackay 2004 08       .....................................?........G.C.C....-..........?....A....-.
Pseudo Irukandji Port2004 16  ..............................................G.C.C....-...............A....-.
Pseudo Irukandji 2003 14      ..............................................G.C.C....-...............A....-.
Broome Irukandji large2004 21 ..............................................G.C.C....-...............A....-.
Broome Irukandji juv2004 22   ..............................................G.C.C....-...............A....-.
Pseudo Irukandji hore52004 24 ..............................................G.C.C....-...............A....-.
Pseudo Irukandji hore62004 26 ..............................................G.C.C....-...............A....-.
Chironex fleckeri AF358104    ..G....................................................-...............A....-.
Chironex fleckeri 2003 04     ..G....................................................-...............A....-.
Chironex Darwin 2004 01       ??????????????????????????????????????????????????????????????????????????????
Chiropsalmus sp.AF358103      ..G.............................................C......-...............A....-.
Chiropsalmus sp.NQLD 2003 33  ..G.............................................C......-..T............A....-.
Chiropsalmus sp.NQLD 2003 08  ..G.............................................C......-...............A....-.
Chiropsalmus sp.NQLD 2003 32  ..G.............................................C......-...............A....-.
Chiropsalmus sp.Gove62004 03  ..G.............................................C......-...............A....-.
Chiropsalmus sp.Gove2004 04   ..G..................................?..........C......-...............A....-.
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Alatina mordens 2003 25       ATATGGGGCC-TTTCTAGGT-CCCATAATTGGAATGAGTACAATTTAAATCCTTTAACGAGGATCTATTGGAGGGCAA
Alatina mordens 2003 26       ..........-.........-.........................................................
Unknown 2003 19               ..........-.........-.........................................................
Unknown 2003 20               ..........-.........-.........................................................
Carybdea marsupialis AF358106 ..........-.........-.........................................................
Carybdea xaymacana AF358109   ..........-....-T...-.T.......................................................
Carybdea xaymacana 2004 5     ..........-....-T...-.T.......................................................
Carybdea xaymacana 2004 06    ..........-....-T...-.T.......................................................
Carybdea xaymacana 2004 07    ..........-....-T...-.T.......................................................
Carybdea nsp CapeTown22004 13 ..........-..CTCT...-.T.......................................................
Carybdea nsp CapeTown32004 14 ..........-....-T...-.T.......................................................
Carybdea rastonii AF358108    ..........-....-T...-.T.......................................................
Carybdea rastonii 2004 10     ..........-....-T...-.T.......................................................
Carybdea rastonii 2004 11     ..........-....-T...-.T.......................................................
Carybdea rastonii 2004 12     ..........-....-T...-.T.......................................................
Carybdea rastoniiJapan 2004 18..........-....-T...-.T.......................................................
Carybdea rastoniiJapan 2004 19..........-....-T...-.T.......................................................
Carybdea sivickisi AF358110   ..........-....-T...-.T.......................................................
Carybdea sivickisi SA2004 17  ..........-....-T...-.T.......................................................
Carybdea sivickisi AIMS       ..........-..CGC-...-.T.......................................................
Tripedalia cystophora L10829  ..........-....-T...-.T.......................................................
Carukia barnesi AF358107      .....T....C...-GT...-.G.......................................................
Carukia barnesi 2003 03       .....T....C...TGT...A.G...........................................????????????
Carukia barnesi 2003 11       .....T....C...-GT...-.G.......................................................

Carukia nsp Broome 2004 23    .....T....C...-GT...-.G.......................................................
Darwin carybdeid AF358105     .....T....C...-GT...-.G.......................................................
Morbakka Mackay 2004 08       .....T....C...-GT...-.G...........................................????????????
Pseudo Irukandji Port2004 16  .....T....C..A-GT...-.G.......................................................
Pseudo Irukandji 2003 14      .....T....C..A-GT...-.G.......................................................
Broome Irukandji large2004 21 .....T....C...-GT...-.G.......................................................
Broome Irukandji juv2004 22   .....T....C..A-GT...-.G.......................................................
Pseudo Irukandji hore52004 24 .....T....C..A-GT...-.G.......................................................
Pseudo Irukandji hore62004 26 .....T....C..A-GT...-.G.......................................................
Chironex fleckeri AF358104    ...C......A.C.--T...-.T.G.....................................................
Chironex fleckeri 2003 04     ...C......A.C.--T...-.T.G.....................................................
Chironex Darwin 2004 01       ??????????????????????????????????????????????????????????????????????????????
Chiropsalmus sp.AF358103      ...C......A.C.--T...-.T.G.....................................................
Chiropsalmus sp.NQLD 2003 33  ...C......A.C.--T...-.T.G.....................................................
Chiropsalmus sp.NQLD 2003 08  ...C......A.C.--T...-.T.G.....................................................
Chiropsalmus sp.NQLD 2003 32  ...C......A.C.--T...-.T.G.....................................................
Chiropsalmus sp.Gove62004 03  ...C......A.C.--T...-.T.G.....................................................
Chiropsalmus sp.Gove2004 04   ...C......A.C.--T...-.T.G.............................................?.......
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Alatina mordens 2003 25       GTCTGGTGCCAGCAGCCGCGGT
Alatina mordens 2003 26       ......................
Unknown 2003 19               ......................
Unknown 2003 20               ......................
Carybdea marsupialis AF358106 ......................
Carybdea xaymacana AF358109   ......................
Carybdea xaymacana 2004 5     ..............????????
Carybdea xaymacana 2004 06    ..????????????????????
Carybdea xaymacana 2004 07    ......................
Carybdea nsp CapeTown22004 13 ...............???????
Carybdea nsp CapeTown32004 14 ........??????????????
Carybdea rastonii AF358108    ......................
Carybdea rastonii 2004 10     ..........????????????
Carybdea rastonii 2004 11     ..........????????????
Carybdea rastonii 2004 12     ....................??
Carybdea rastoniiJapan 2004 18...............???????
Carybdea rastoniiJapan 2004 19......................
Carybdea sivickisi AF358110   ......................
Carybdea sivickisi SA2004 17  ......................
Carybdea sivickisi AIMS       ......................
Tripedalia cystophora L10829  ......................
Carukia barnesi AF358107      ......................
Carukia barnesi 2003 03       ??????????????????????
Carukia barnesi 2003 11       ......................
Carukia nsp Broome 2004 23    ................??????
Darwin carybdeid AF358105     ......................
Morbakka Mackay 2004 08       ??????????????????????
Pseudo Irukandji Port2004 16  .......???????????????
Pseudo Irukandji 2003 14      ......................
Broome Irukandji large2004 21 ......................
Broome Irukandji juv2004 22   ...............???????
Pseudo Irukandji hore52004 24 ....??????????????????
Pseudo Irukandji hore62004 26 ............??????????
Chironex fleckeri AF358104    ......................
Chironex fleckeri 2003 04     ....??????????????????
Chironex Darwin 2004 01       ??????????????????????
Chiropsalmus sp.AF358103      ......................
Chiropsalmus sp.NQLD 2003 33  ......................
Chiropsalmus sp.NQLD 2003 08  ......................
Chiropsalmus sp.NQLD 2003 32  ......................
Chiropsalmus sp.Gove62004 03  ...................C..
Chiropsalmus sp.Gove2004 04   ...???????????????????
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APPENDIX 4. SUMMARY OF KNOWLEDGE OF IRUKANDJI SPECIES-SYNDROME RELATIONSHIPS

Species-syndrome relationships are not well understood; however, some reasonable deductions can be made by synthesizing experimental results, published reports, phylogenetic relationships, and known species distributions. Abbreviations follow those defined in Chapter 2.

	Relative severity	**Distribution**	**Source**
WITHIN THE IRUKANDJI CLADE			
Carukia barnesi	Moderate, onset typically in 20-30 minutes, with nausea, vomiting, elevated BP (typically in the 100's)	Coastal north QLD	Barnes, 1964; Williamson et al., 1996 (in part)
Carukia n. sp. A (Russell's)	Linked to severe syndrome by nematocysts, but possibly erroneous (see Section 2.3.24)	Onshore N. QLD, twice offshore	Little & Seymour, 2003; this thesis; Gershwin, unpubl.
Carukia n. sp. B (Broome)	Unknown	Coastal N. WA	
"Darwin carybdeid"	Mild	Coastal NT	B. Currie, pers. comm., 2004
"Morbakka"	Mild	Coastal eastern Australia	Fenner et al., 1985
"Broome Irukandji"	Anecdotally thought to be quite severe; severe initial sting, leaves welts, variable onset of syndrome (5-10-20 minutes)	Offshore N. WA; possibly onshore following cyclones	Pearl divers, pers. comm., 2004; Gershwin, unpubl.
"Dampier Irukandji"	Unknown	Offshore N. WA	
"Halo Irukandji"	Linked with fatality by nematocysts	Offshore and islands N. QLD; (onshore N. QLD)	Huynh et al., 2003; Gershwin & Hartwick, unpubl.
"Pseudo-Irukandji"	Mild (Kinsey, 1988; Gershwin unpublished); possibly linked with fatality (Huynh et al., 2003)	Offshore and islands N. QLD; (onshore N. QLD)	Gershwin & Hartwick, unpubl.
OUTSIDE THE IRUKANDJI CLADE			
Alatina mordens	Linked to severe syndrome by specimens (Chapter 5) and nematocysts (Section 2.3.24). Severe initial sting, leaves welts, rapid syndrome onset (5-10 minutes), high BP	Offshore, GBR	Little & Seymour, 2003; this thesis; Gershwin, unpubl.
Alatina sp. (= *Carybdea alata*)	No details given about severity (but see Yoshimoto & Yanagihara, 2002)	Hawaii	Carrette & Seymour, Internet web page
Physalia spp. (Hydrozoa: Siphonophora)	Mild, lasts about one hour	N. QLD and Pakistan	Fenner et al., 1993
Gonionemus vertens (Hydrozoa)	Moderate	Russia	Williamson et al., 1996, p. 201, and references therein
Nemopilema nomurai (Scyphozoa)	Severe; deadly	China	Williamson et al., 1996, p. 214, and references therein

APPENDIX 5. SUMMARY OF TWO MAIN GROUPS OF IRUKANDJI SYNDROME

Summary of symptom groupings. Compiled from approximately 100 retrospective interviews with patients diagnosed with Irukandji Syndrome by physicians in Cairns, Townsville, Mackay, and Broome. The extent to which these two apparent groups divide or overlap must be confirmed with clinical data. These two groups are hypothesized to correspond to the *Carukia* and "Pseudo-Irukandji" groups (Type 1 and Type 2, respectively), based on known species distributions and scant information from stings from which the species were identified.

	Type 1	**Type 2**
Initial sting	Mild; often unnoticed	Moderate to severe
Welting	None; may be ephemeral "goose pimples"	Often leaves welts
Onset of symptoms	Slow onset: 20-30 minutes	Fast onset: 5-10 minutes, sometimes 20
Nausea and vomiting	Profusely	Rarely
Pain	Intense; responds well to strong analgesics	Severe; does not respond to analgesics; comes down with control of BP
Blood pressure	Minimal to moderate hypertension (in the 100's); comes down with pain relief	Severe hypertension (in the 200's)
Distribution	Most common coastally	Most common offshore
Links to species	Experimental stings of Barnes (1964) with *Carukia barnesi*; many cases coastally during periods when *Carukia barnesi* are collected	Nematocysts from fatal event (Huynh et al., 2003) linked to "Pseudo-Irukandji" or "Halo Irukandji" (Chapter 6)