Received: 8 March 2023 | Revised: 13 August 2023 | Accepted: 16 August 2023

DOI: 10.1111/jfb.15530

REGULAR ARTICLE

JOURNAL OF FISH BIOLOGY fsbi

Utility of body and otolith morphometry to discriminate cryptic juveniles of two sympatric red snappers (Perciformes: Lutjanidae)

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Abstract

The sympatric red snappers, *Lutjanus erythropterus* and *Lutjanus malabaricus*, are highly valued by commercial and recreational fishers along the tropical northern coasts of Australia and throughout their distribution. Studies on the life history and ecology of these congeners are confounded by difficulties in distinguishing the cryptic juveniles of each species (i.e., < 200 mm total length). This study aimed to validate a robust and cost-effective method to discriminate these juveniles using body and/or otolith morphometric data in a multivariate analysis. Juvenile samples were collected from the northwest ($n = 71$) and northeast ($n = 19$) coasts of Australia, and species identification was confirmed using DNA barcoding. The most parsimonious multivariate models achieved accurate species prediction rates of 98.8%, which consisted of just three body variables (dorsal fin length, the distance from the snout to the anterior edge of the eye, and either jaw length or distance from the snout to the preoperculum). The high level of discrimination for these cryptic juveniles highlights the robustness of this morphometric approach. The slightly lower rate of discrimination using otolith morphology (84.9%) was associated with greater regional variation in *L. malabaricus* between the northwest and northeast coasts. Slight variations in otolith shape are typically used to determine stock structure, which highlights the potential need to collect samples over a broader area of a species geographic range when using an otolith morphometric discrimination model. The method outlined in this study could be applied to distinguish other cryptic congeneric fish species, including from archived otolith collections. Moreover, this method has the potential to be utilized in assessing species compositions using body measurements from in situ stereo-video.

KEYWORDS

cryptic species identification, juveniles, Lutjanidae, morphometrics, otoliths, resource assessments

1 | INTRODUCTION

The red snappers, *Lutjanus erythropterus* (Bloch, 1790) and *Lutjanus malabaricus* (Schenider, 1801), are highly valued by commercial, recreational, and artisanal fishers throughout their sympatric distribution in the tropical and subtropical waters of the Indo-Pacific region (Allen, 1985; Blaber et al., 2005). In 2019, approximately 3500 t of these species were landed by commercial fishers across Australia using fish trawls, traps, and lines, with the catch dominated by *L. malabaricus* (~2450 t; Saunders, Roelofs, et al., 2021; Saunders, Trinnie, et al., 2021). The commercial catch of these species in 2019 had an estimated value in excess of $25 million. There is also a substantial recreational angling catch of these species across northern Australia, with the estimated recreational catch in Queensland exceeding the commercial catch for *L. malabaricus* (see Campbell et al., 2021; Ryan et al., 2019; West et al., 2022). The high economic and social value of these species has resulted in several studies being undertaken to understand their life history and ecology (Fry & Milton, 2009; McPherson et al., 1992; Newman, 2002; Newman et al., 2000; O'Neill et al., 2011), with ongoing monitoring requirements in multiple jurisdictions to support stock assessments, and thus contribute to the sustainable management of these species (Saunders, Roelofs, et al., 2021, Saunders, Trinnie, et al., 2021).

In Australian waters, *L. erythropterus* and *L. malabaricus* are sustainably fished (Saunders, Roelofs, et al., 2021, Saunders, Trinnie, et al., 2021); however, accurate species-level identification is fundamental for monitoring and assessment programs, with identification to species level usually being based on morphological characteristics (Hey et al., 2003). Adult *L. erythropterus* and *L. malabaricus* are easily distinguished by their external morphology, particularly evident around the nape, mouth, and caudal peduncle pigmentation. In contrast, juveniles are morphologically indistinguishable (i.e., cryptic), requiring DNA barcoding to discriminate between species (Elliott, 1996; Fry & Milton, 2009; Takahashi et al., 2020). Most higher-level single-species stock assessment models rely on a sound knowledge of life-history attributes (i.e., age, growth, and maturity), with associated input parameters requiring data from juvenile samples to accurately describe their life-history schedules (e.g., Wakefield et al., 2020), to best inform sustainable management. Information associated with recruitment in teleosts is inherently difficult to obtain for the majority of species, but very important for applications such as understanding impacts on growth overfishing (e.g., by-catch of juveniles) or ecosystem-based fisheries management (e.g., assessing impacts on juvenile habitats). Takahashi et al. (2020) identified significant diet partitioning patterns between these lutjanid species during their cryptic juvenile stage. Given that habitat association and diet partitioning are typically exhibited between sympatric species (Cocheret de la Morinière et al., 2003; Szedlmayer & Lee, 2004; Takahashi et al., 2020), this infers that despite the cryptic appearance or phenotype of the juveniles, they occupy different microhabitats that are yet to be defined. To facilitate these assessments at a species level, there is a need to develop a robust, cost-effective identification tool to discriminate between the cryptic juveniles of *L. erythropterus* and *L. malabaricus*.

Previous studies have utilized multivariate morphometric approaches to discriminate cryptic species in a range of taxa, such as Angiosperms (Fisher, 1936, 1938), Reptiles (Sanders et al., 2006), Diptera (Cazorla & Acosta, 2003), and Bivalves (Baker et al., 2003). In fish biology, otolith morphometry has used multivariate analyses to identify species (Bani et al., 2013; Stransky & MacLellan, 2005; Wakefield et al., 2014; Zhuang et al., 2015), and stock structure (population subdivision) within a species (Jemaa et al., 2015; Longmore et al., 2010; Tracey et al., 2006). Otoliths are paired calcareous structures in the inner ear of teleosts, which provide valuable information for biological and ecological studies (Begg et al., 2005; Newman et al., 2015; Williams et al., 2015). Although several studies have concluded that there is a high degree of interspecific variation in otolith morphometric data for cryptic adult teleosts (Stransky & MacLellan, 2005; Wakefield et al., 2014; Zhuang et al., 2015; Zischke et al., 2016), there has been limited application for distinguishing juveniles. Otolith morphometric analyses have been undertaken for *L. erythropterus* and *L. malabaricus* (Sadighzadeh et al., 2012), yet juvenile fish were not included in that study (smallest fish were 316 and 235 mm total length for *L. erythropterus* and *L. malabaricus*, respectively).

The aim of this study was to identify a robust, simple, and cost-effective method to discriminate between the juveniles of *L. erythropterus* and *L. malabaricus* using body and/or otolith morphometric measurements as an alternative to DNA barcoding. Specifically, our objectives were to (1) assess the differences in the body and/or otolith morphometric data between the species, (2) assess the allocation success rates of each prospective model, and (3) identify the most parsimonious model for practical and efficient species differentiation. The findings of this study will facilitate simpler species identification among early life stages and thus contribute toward advancing the current paucity of knowledge on the biology and ecology of these two valuable and important species. Importantly, separation of the juveniles in a cost-effective manner will facilitate further studies that will be able to assess spatial distribution patterns and microhabitat use of the juveniles of each species.

2 | MATERIALS AND METHODS

2.1 | Sample collection and genetic species identification

A total of 90 juvenile fish were sampled from research surveys using demersal trawling from the Pilbara and Kimberley regions of Western Australia (WA), and from the coast of central Queensland (QLD), eastern Australia, between 2014 and 2019 (Figure 1). All the juvenile fish were sampled between 7 and 24 m depth, except for five fish that were sampled between 40.5 and 46.4 m depth in Collier Bay in the Kimberley (WA). Fin clips of each fish were collected and stored in 100% ethanol to genetically identify each species. DNA from each fin clip was extracted and diluted to 1/10 with ultra-pure water. Polymerase chain reaction (PCR) was carried out using the FishBCH forward primer (5′-ACTTCYGGGTGRCCRAARAATCA −3′) and the FishBCL

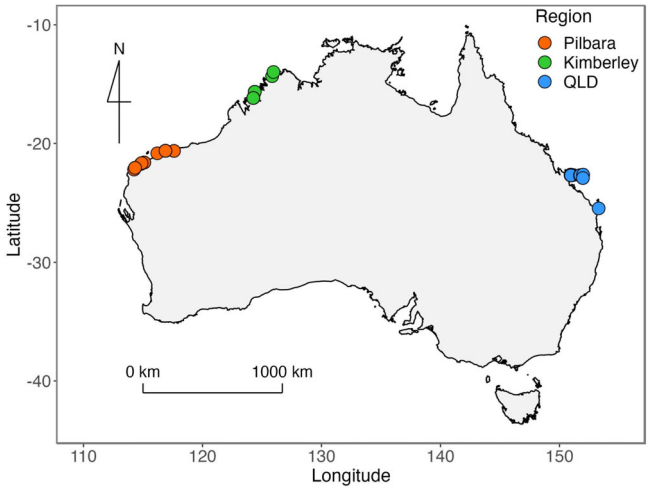

FIGURE 1 Location where samples of juvenile *Lutjanus erythropterus* (LE) and *Lutjanus malabaricus* (LM) were collected in the Pilbara, Kimberley, and central Queensland regions of Australia. Sample sizes are shown for each species and region in Table 1.

reverse primer (5′-TCAACYAATCAYAAAGATATYGGCAC-3′) to target 600 to 800 bp of the cytochrome c oxidase subunit I (COI) region, following the "HotSHOT" technique (Meeker et al., 2007). The choice of the target region and primers was based on the database availability and interspecific diversity within the targeted amplicon for accurate species identification. The following PCR cycling programme was used: (1) 94°C for 4 min; (2) 35 amplification cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 60 s; and (3) a final extension step at 72°C for 10 min. Successful amplification was tested by loading 4 μl of each amplicon onto a 2% agarose gel and analysing the gel image under UV light with a Bio-Rad transilluminator and GelRed nucleic acid staining dye (Molecular Probes). DNA was further diluted to 1/25 if no signature appeared on the gel image at the target size, re-amplified, and visualized on a gel. 10 μl of the amplicons and 2 μl of exonuclease I and FastAP thermosensitive alkaline phosphatase (ExoFAP; USB, Cleveland, OH, USA) were mixed together and purified using the following cycling programme: (1) 37°C for 15 min, (2) 80°C for 15 min, and (3) 4°C for 10 min. The purified amplicons were shipped to Macrogen for Sanger Sequencing (Macrogen Facility, Seoul, Korea) in the forward direction only. A Basic Local Alignment Search Tool (BLASTn) query was carried out to assign each sequence to a species using the customized database of the National Center for Biotechnology Information (NCBI) GenBank nucleotide reference sequences (Benson et al., 2017) and the Western Australian fish database (Nester et al., 2020). All sequences were assigned to either *L. erythropterus* or *L. malabaricus* with over 99.5% fidelity.

After genetic identification it was confirmed that a total of 26 *L. erythropterus* and 17 *L. malabaricus* were collected from the Pilbara region, 22 *L. erythropterus* and 6 *L. malabaricus* from the Kimberley region, and 19 *L. malabaricus* from central QLD (Table 1 and Figure 1). No *L. erythropterus* were collected from QLD.

TABLE 1 Descriptive statistics for total length measurements of *Lutjanus erythropterus* and *Lutjanus malabaricus* collected from Pilbara, Kimberley, and Queensland.

		Total length (mm)	
Species / Region	***n***	**Range**	**Mean ± SE**
L. erythropterus			
Pilbara	26	78.5–166.5	129.0 ± 4.53
Kimberley	22	59.5–185.2	98.4 ± 6.21
Queensland	0	NA	NA
L. malabaricus			
Pilbara	17	103.7–198.7	129.8 ± 6.08
Kimberley	6	136.7–200.0	167.4 ± 10.61
Queensland	19	94.0–179.0	126.5 ± 6.24

Abbreviation: SE, standard error.

2.2 | Morphometric measurements and analyses

Total length (TL), jaw length, length from the snout to the preoperculum, length from the snout to the operculum, length from the snout to the anterior edge of eye, length from the posterior edge of the eye to the base of first dorsal fin ray, dorsal fin length, and body height were measured to the nearest 0.01 mm using digital calipers (Figure 2). Sagittal otoliths were dissected, cleaned in water, and stored dry. Using digital calipers, the otolith length (i.e., the length from the rostrum to post-rostrum), otolith width (i.e., the width at the widest point approximately perpendicular to the length axis), and otolith thickness (i.e., the overall thickness across the width of the otolith taken at the primordium and perpendicular to the sulcus acusticus) were measured to the nearest 0.01 mm (Figure 2). The otolith weight was measured to the nearest 0.001 g using an analytical balance with a glass shield. These morphometric variables were selected because (1) they have successfully distinguished cryptic species in previous studies (i.e., Kerschbaumer & Sturmbauer, 2011; To & Ci, 2015; Wakefield et al., 2014), and (2) the required body features can be easily identified for accurate measurements, with some of the body morphometric variables conspicuously diagnostic in differentiating these two species as adults. Previous studies revealed no significant differences between the shapes of left and right otoliths from the same fish (Stransky & MacLellan, 2005; Zhuang et al., 2015). In this study, the measurements were taken on the left otolith (or right otolith if the left was chipped or broken) for each fish.

Multivariate analyses were carried out on the body and otolith morphometric datasets separately. An additional model combining otolith morphometrics and total length was also explored based on higher allocation success rates achieved in a similar study (Wakefield et al., 2014). A Euclidean distance similarity matrix was constructed for each dataset, and a Canonical Analysis of Principal Coordinates (CAP) was carried out with species as a priori groups, and region and species as factors (Anderson & Willis, 2003). The data did not require transformation as the PCO axes in the CAP use orthonormal axes

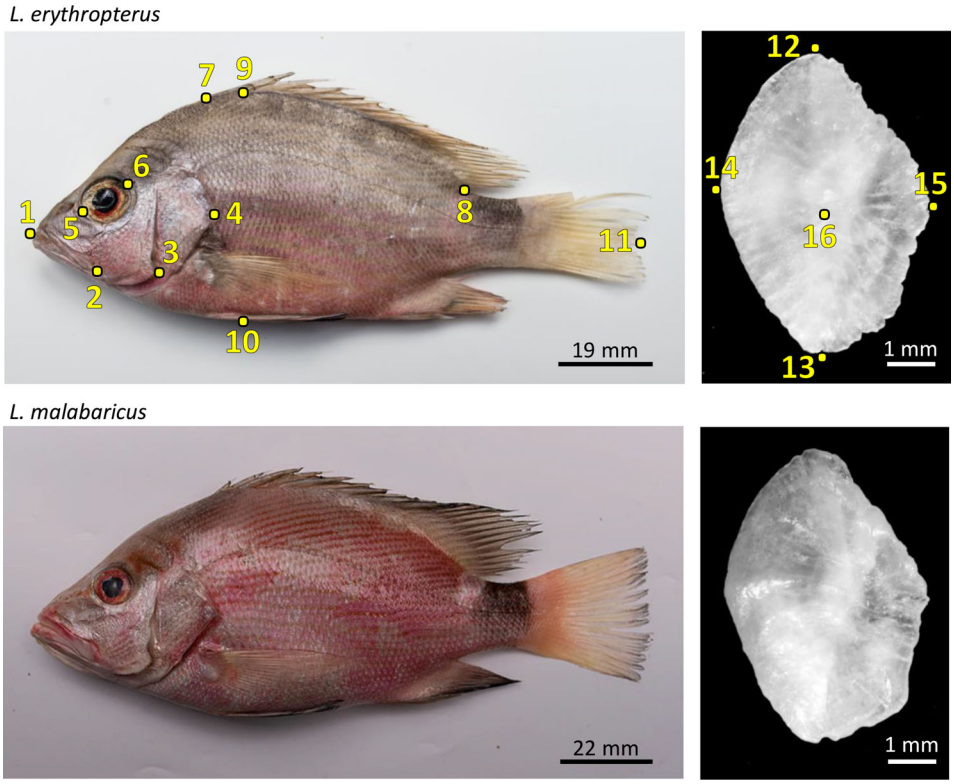

FIGURE 2 Images of the juveniles and the distal aspect of left sagittal otoliths of *Lutjanus erythropterus* and *Lutjanus malabaricus*. The numbers in the images indicate the points where morphometric measurements were taken (i.e., jaw length, 1–2; snout to the preoperculum, 1–3; snout to the operculum, 1–4; snout to the anterior edge of eye, 1–5; posterior edge of eye to base of first dorsal fin ray, 6–7; dorsal fin length, 7–8; body height, 9–10; total length, 1–11; otolith length, 12–13; otolith width, 14–15; otolith thickness, 16).

and are automatically sphericized and not scaled by their respective eigenvalues (Anderson et al., 2008). The number of PCO axes included in the CAP analyses (m) was defined as the number of variables in each test (Anderson & Willis, 2003). The leave-one-out allocation success rates were used to determine the accuracy of each model in predicting the correct species and regions (Anderson & Willis, 2003).

To determine the most parsimonious model, the analyses described above were repeated for all possible combinations of the variables, with the number of variables within each model ranging from two to seven for body morphometrics, and from two to four for otolith morphometrics. The most parsimonious model was considered to have the highest species prediction accuracy with lowest number of variables. Where possible, partial correlation vectors representing each morphometric variable were overlaid on ordinations to ascertain the strength and direction of their influence in separating the data clouds for each species. Trace and delta canonical test statistics were also obtained using 9999 permutations to assess the significant differences in body or otolith morphometric data between species and region. Finally, the CAP analyses were carried out with both body and otolith variables of the most parsimonious models combined and assessed based on the leave-one-out allocation success using the CAPdiscrim function of the biodiversityR package in R (v. 2.12–1) (Kindt, 2020). The software PRIMER 7 (v. 7.0.13, https://www.primer-e.com) (Clarke et al., 2014) was used to plot the selected models and assess the multiple partial correlations of the morphometric variables and test statistics.

TABLE 2 Leave-one-out allocation results using the most parsimonious canonical analysis of principal coordinates (CAP) models with body (a), otolith (b), and body and otolith (c) morphometric variables.

	Predicted		
Observed	**LE**	**LM**	**Correct %**
(a) Body—three variables			
LE	42	0	100
LM	1	41	97.6
(b) Otolith—three variables			
LE	41	7	85.4
LM	11	27	71.1
(c) Body and otolith—six variables			
LE	42	0	100
LM	2	36	94.7

Note: Species was used as the factors for the CAP analyses.
Abbreviations: LE, *Lutjanus erythropterus*; LM, *L. malabaricus*; Ob, observed.

2.3 | Ethics statement

This study did not involve any endangered or protected species. In Western Australia, the Animal Welfare Act 2002 does not require the Department of Primary Industries and Regional Development (DPIRD) to obtain a permit to use animals for scientific purposes unless the

species are outside the provisions of the Fish Resources Management Act 1994 and Fish Resources Management Regulations 1995. Nonetheless, all sampling was undertaken in strict adherence to the DPIRD Policy for the ethical handling, use, and care of marine fauna for research purposes.

3 | RESULTS

Juvenile samples of *L. erythropterus* ($n = 48$) and *L. malabaricus* ($n = 42$) ranged from 60 to 200 mm TL across all samples. The smallest individual (60 mm) and smallest mean TL (98 mm ± 6.21 standard error) were observed in *L. erythropterus* from the Kimberley region, whereas the mean TL of other regions and/or species ranged from 126 to 167 mm (Table 1). The sample sizes varied between the CAP models with different variables (body, otolith, and both combined) due to some samples having missing values (i.e., missing otolith length and weight when both otoliths were chipped) (Tables 2 and 3).

The species prediction accuracy from the CAP analyses using all eight body measurement variables was both very accurate using either species as a factor (i.e., 96.4%, Table S1a) or species and region as factors (i.e., 95.2%, Table S2a; Figure 3). Species allocation success was high for all models with three to seven variables (range 98.8%, Figure 3), with only one *L. malabaricus* sample (110 mm TL)

TABLE 3 Leave-one-out allocation results using the most parsimonious canonical analysis of principal coordinates (CAP) models with body (a), otolith (b), and body and otolith (c) morphometric variables. The total numbers of samples and correct allocation rates (%) are indicated in bold.

	Predicted							
	L. erythropterus			*L. malabaricus*				
Observed	**Pil**	**Kim**	**Total**	**Pil**	**Kim**	**QLD**	**Total**	**Correct %**
(a) Body—three variables								
LE								
Pil	12	8		0	0	0		60
Kim	4	18		0	0	0		81.8
Total			**42**				**0**	**100**
LM								
Pil	0	1		10	1	5		58.8
Kim	0	0		0	3	3		50
QLD	0	0		6	3	10		52.6
Total			**1**				**41**	**97.6**
(b) Otolith—five variables								
LE								
Pil	21	4		1	0	0		80.8
Kim	5	14		3	0	0		63.6
Total			**44**				**4**	**91.7**
LM								
Pil	6	1		7	2	1		41.2
Kim	0	0		2	3	0		60
QLD	1	1		0	0	14		87.5
Total			**9**				**29**	**76.3**
(c) Body and otolith—eight variables								
LE								
Pil	13	6		1	0	0		65
Kim	6	16		0	0	0		72.7
Total			**41**				**1**	**97.6**
LM								
Pil	0	1		12	2	2		70.6
Kim	0	0		4	1	0		20
QLD	0	0		1	1	14		87.5
Total			**1**				**37**	**97.4**

Note: Species and regions were used as the factors for the CAP analyses.
Abbreviations: Kim, Kimberley; LE, *Lutjanus erythropterus*; LM, *L. malabaricus*; Ob, observed; Pil, Pilbara; QLD, Queensland.

misclassified as *L. erythropterus* in each model (Tables 2a and 3a). The species allocation success decreased slightly from 98.8% to 96.4%, when the number of variables was reduced to two (Figure 3). Overall, the three-variable models (including dorsal fin length, snout to the anterior margin of eye, and either jaw length or snout to the preoperculum) were the most parsimonious with the highest species prediction accuracy using body morphometrics (Tables S1a and S2a).

Significant differences in the body morphometrics between species were also identified by the separation of clusters of data points for each species within the ordination, with the first canonical axis describing a large majority of the discrimination ($\delta 2 = 0.90$), and significant differences in the test statistics ($p < 0.001$ for both trace and delta statistics, Figure 4). The overlapping clusters of data points for regions within a species suggested there were little, if any, regional

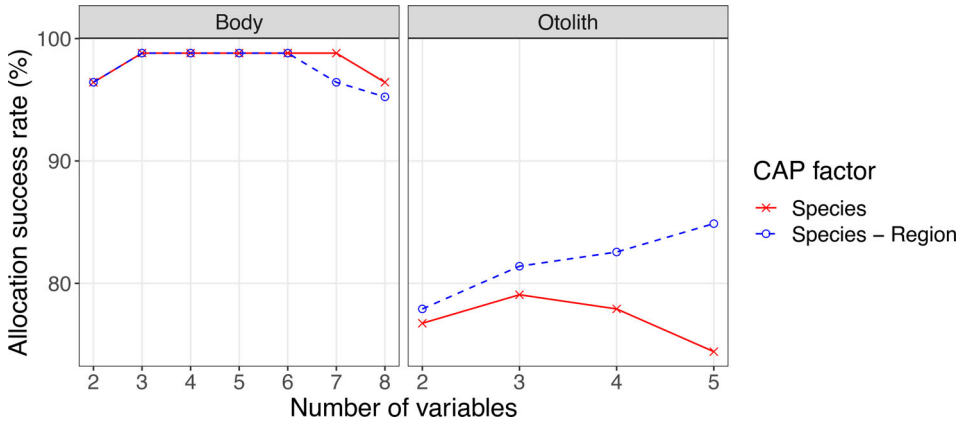

FIGURE 3 Leave-one-out allocation success rates for species predictions (%) from canonical analysis of principal coordinates (CAP) models using different combinations of body (left) and otolith (right) morphometric variables. Species (red) and species and region (blue) were used as a priori factor(s) within the CAP analyses.

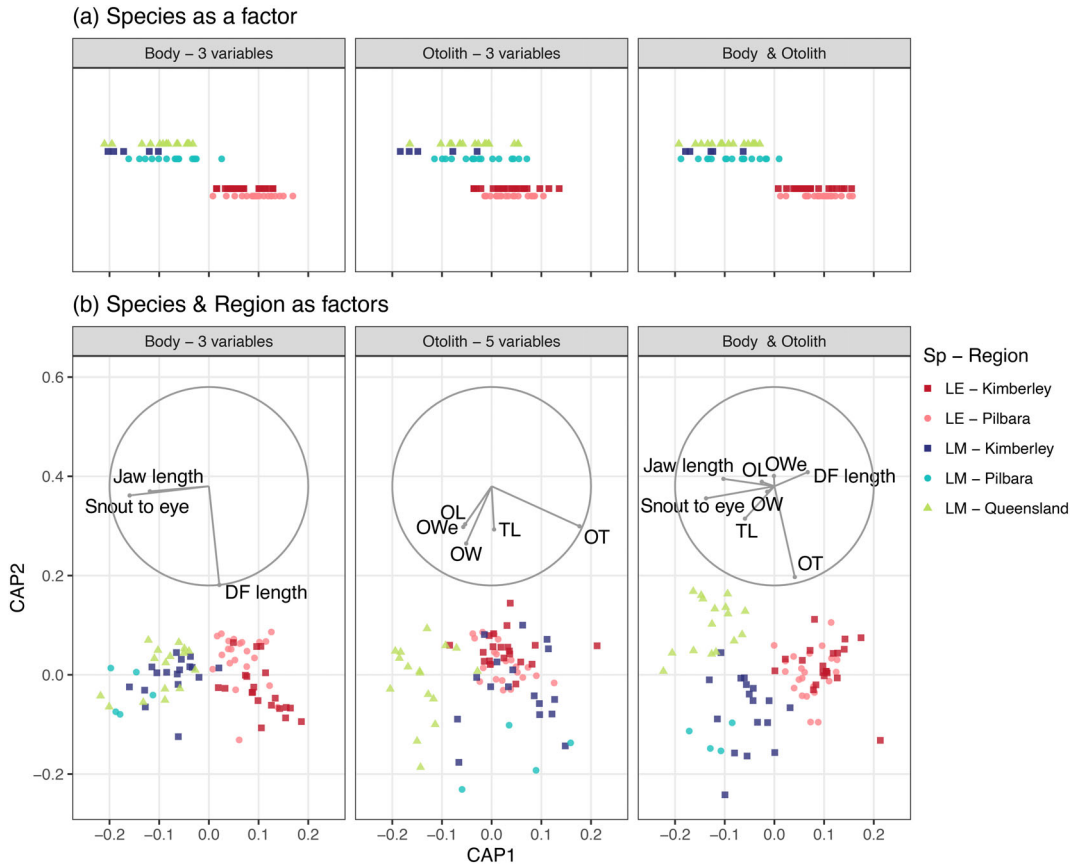

FIGURE 4 Canonical analysis of principal coordinates (CAP) ordinations for the most parsimonious model predicting species (a) and species*region (b) using body (left), otolith (middle), and body and otolith (right) morphometrics combined for juvenile *Lutjanus erythropterus* (LE) and *Lutjanus malabaricus* (LM). The strength and direction of the variables within each model for predicting species*region are shown as vectors in each ordination (circle denotes a correlation coefficient of 1; DF, dorsal fin; TL, total length; OL, otolith length; OW, otolith width; OT, otolith thickness; Owe, otolith weight).

variations in body morphometrics within a species (Figure 4b). The very low regional variations were also indicated by the low allocation success rates (50%–81.8%), with most of the misclassifications occurring at the region and not species level (Table 3a). *L. malabaricus* had a relatively longer jaw length and a longer distance from the snout to the eye compared to *L. erythropterus*, based on the direction and length of their vectors within the ordination (Figure 4b). Dorsal fin length was highly correlated along the second canonical axis and was, therefore, more likely to be related to the size of the fish rather than differences between species (Figure 4b).

The highest species prediction accuracy for otolith morphometric models (i.e., 84.9%) was achieved using all five otolith variables with species and regions as factors ($p < 0.001$ for trace and delta statistics, Table S2b and Figure 3). The leave-one-out allocation success rates were lower for all combinations of otolith morphometric variables compared to those of the body morphometric models (Figure 3). This was also reflected in the overlapping distribution of data points between species in the ordinations along the first canonical axis (Figure 4). The leave-one-out allocation success rates using otolith variables were lower for all models when the single a priori factor of species was used without region (Figure 3 and Table S1b). Total length and otolith width were significant species predictor variables (Table S1b).

The most parsimonious model with body and otolith morphometrics combined achieved a leave-one-out allocation success rate of 97.5% with six variables including species as a factor (Table 2c) and eight variables with species and region as factors (Table 3c). Within the multivariate ordination there was a clear separation in the data clouds for each species along the first canonical axis ($\delta 2 = 0.69$), with the most significant variables influencing this separation being a relatively larger jaw length and distance from the snout to the anterior edge of the eye for *L. malabaricus*, and a relatively larger dorsal fin length for *L. erythropterus* (Figure 4b). Spatial variation in otolith morphometrics between the WA and QLD samples of *L. malabaricus* was evident along the second canonical axis ($\delta 2 = 0.21$), mainly driven by otolith thickness (Figure 4b). Only two samples were misclassified to species in both models, that is, two *L. malabaricus* (110 and 199 mm TL) with species as a factor (Table 2c), and a *L. erythropterus* (130 mm TL) and a *L. malabaricus* (110 mm TL) with species and region as factors (Table 3c).

4 | DISCUSSION

Cryptic species or species that exhibit cryptic phenotypes during parts of their life history (e.g., juvenile stage) pose challenges for biological and ecological studies as they often require expensive and time-consuming molecular analyses for accurate species identification. The multivariate models of body and otolith morphometric features investigated in this study provide a robust and cost-effective alternative to molecular methods for discriminating the cryptic juveniles of *L. erythropterus* and *L. malabaricus*. Among the eight body morphometric variables investigated, the most parsimonious model contained just three variables (dorsal fin length, distance from the snout to the anterior edge of the eye, and either jaw length or distance from the snout to the preoperculum) with a species classification accuracy of 98.8%. This study has revealed that, despite the challenges associated with visual identification (e.g., Elliott [1996] determined that 68% of juvenile fish that were visually classified as *L. malabaricus* were genetically identified as *L. erythropterus*), high levels of discrimination can be achieved for *L. erythropterus* and *L. malabaricus* juveniles with just a few body measurements. This highlights the robustness of the multivariate morphometric approach described herein to discriminate these cryptic juveniles as a robust and accurate alternative to misleading visual identification or the high cost and time-consuming requirements of molecular techniques.

Higher allocation success rates were achieved by the body morphometric models compared to the otolith morphometric models (98.8% cf. 84.9%). The prediction rates of the otolith models were also lower than those reported in previous studies that examined otolith morphometrics to distinguish adult teleosts (i.e., above 95%; Wakefield et al., 2014; Zischke et al., 2016). The lower prediction rates in this study indicate that distinct variations in otolith shape occur later in life, and that the otolith morphometric approach is not as effective for juveniles as it is for adults. The otoliths of commercially important species are often routinely collected for fishery monitoring and assessment purposes, and biological studies. Although adult otolith morphometric models can be applied to discriminate among individual species from archived collections, molecular approach using DNA from the surface of otoliths (if available) would potentially provide more accurate discrimination among juveniles if body morphometrics were not recorded prior to dissection.

Accurate species identification of the juveniles of *L. erythropterus* and *L. malabaricus* is particularly important where the juveniles of these species comprise part of the by-catch of significant fisheries. The juveniles of both species have been found in the by-catch of commercial prawn trawling (Fry et al., 2009; McPherson et al., 1992). Diet partitioning of *L. erythropterus* and *L. malabaricus* juveniles was identified by Takahashi et al. (2020), which may infer potential microhabitat partitioning as diet and habitat are closely linked (Takahashi et al., 2020). Knowledge of the habitat use and residency of the juveniles of these species is an important consideration to determine the level of impact from fishing or other anthropogenic sources. Separation of the juveniles of these species in the cost-effective manner derived from this study will facilitate innovative studies to assess their spatial distribution patterns and microhabitat use.

This study successfully validated robust species identification models for the cryptic juveniles of *L. erythropterus* and *L. malabaricus* on the east and west coasts of Australia. The near-total discrimination of these species highlighted the reliability and accuracy of the body morphometric approach, a viable alternative to time-consuming and expensive molecular analyses. Morphometric analyses also have a wide range of applications. For instance, given the very high level of species prediction accuracy of the models with as few as three body morphometric variables, species discrimination of these cryptic juveniles has the potential to be applied to in-situ stereo-video-based studies (e.g., Harvey et al., 2021; Langlois et al., 2021). Although only preliminary, the spatial variation in the otolith morphometric data of *L.*

malabaricus between the east and west coasts of Australia signifies potential population separation (i.e., limited mixing) that requires further investigation. No spatial variation was found in the body morphometric models, suggesting the application of this model is appropriate across the widespread Indo-Pacific distribution of these species.

AUTHOR CONTRIBUTIONS

Corey B. Wakefield and Stephen J. Newman designed the study. Miwa Takahashi, Corey B. Wakefield, Stephen J. Newman, and Kyle B. Hillcoat collected the samples. Miwa Takahashi, Euan S. Harvey, Corey B. Wakefield and Benjamin J. Saunders conducted data analyses. Miwa Takahashi wrote the manuscript. All authors contributed to reviewing and editing the manuscript.

ACKNOWLEDGMENTS

Logistical support for the project was provided by Curtin University and the Department of Primary Industries and Regional Development, Government of Western Australia (DPIRD). We would like to thank staff at DPIRD and the Department of Agriculture and Fisheries in Queensland for the collection of juvenile fish samples. We acknowledge the Traditional Owners of the land and sea country where this research was conducted and pay respects to Elders past, present, and emerging. Open access publishing facilitated by Curtin University, as part of the Wiley - Curtin University agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST STATEMENT

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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SUPPORTING INFORMATION

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How to cite this article: Takahashi, M., Wakefield, C. B., Newman, S. J., Hillcoat, K. B., Saunders, B. J., & Harvey, E. S. (2023). Utility of body and otolith morphometry to discriminate cryptic juveniles of two sympatric red snappers (Perciformes: Lutjanidae). *Journal of Fish Biology*, *103*(6), 1312–1320. https://doi.org/10.1111/jfb.15530