

Molecular phylogenetic relationships and generic placement of *Dryaderces inframaculata* Boulenger, 1882 (Anura: Hylidae)

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Abstract

Dryaderces inframaculata Boulenger, 1882, is a rare species known only from a few specimens and localities in the southeastern Amazonia rainforest. It was originally described in the genus *Hyla*, after ~130 years transferred to *Osteocephalus*, and more recently to *Dryaderces*. These taxonomic changes were based solely on the similarity of morphological characters. Herein, we investigate the phylogenetic relationships and generic placement of *D. inframaculata* using molecular data from a collected specimen from the middle Tapajós River region, state of Pará, Brazil. Two mitochondrial DNA fragments (16S and COI) were assessed among representative species in the subfamily Lophiohylinae (Anura: Hylidae) to reconstruct phylogenetic trees under Bayesian and Maximum Likelihood criteria. Our results corroborate the monophyly of *Dryaderces* and the generic placement of *D. inframaculata* with high support. *Dryaderces inframaculata* is sister to an undescribed taxon, *Dryaderces pearsoni* Cal Jungfer *et al.*, 2013, and both are sister to *Dryaderces pearsoni* Gaige, 1929. These findings are relevant for further research on the systematics and biogeography of the genus.

Key words

Amazonia, integrative taxonomy, Lophiohylinae, mtDNA, phylogeny, rare species, Santarém tree frog, Tapajós River.

Introduction

Tree frogs of the Amazonian genera *Dryaderces* Jungfer *et al.*, 2013, *Osteocephalus* Steindachner, 1862, and *Tepuihyla* Ayarzagüena, Señaris & Gorzula, 1993 (Lophiohylinae) are among the most speciose Neotropical hylids. These genera are often taxonomically problematic as they contain high levels of cryptic diversity, cases of synonymy and resurrection of names, old descriptions of species, and type series with low representation of the species' variation and distribution (TRUEB & DUELLMAN, 1971; DUELLMAN & MENDELSON, 1995; JUNGFER, 2010; JUNGFER *et al.*, 2013; FERRÃO *et al.*, 2019). Using integrative approaches, recent studies have re-evaluated the spe-

cies boundaries, described new taxa, and transferred taxa among genera and among species groups within *Osteocephalus* (MORAVEC *et al.*, 2009; JUNGFER, 2010; RON *et al.*, 2010, 2012, 2016; JUNGFER *et al.*, 2013, 2016; FERRÃO *et al.*, 2019). Such increasing knowledge has helped to clarify the diversity and evolutionary history of these genera; however, some taxa remain absent from molecular phylogenies.

In a vast systematic revision using molecular data, JUNGFER *et al.* (2013) noted some distinct and early divergent lineages in the genus *Osteocephalus*, and allocated them in a new genus, *Dryaderces*. Currently, *Dryaderces*

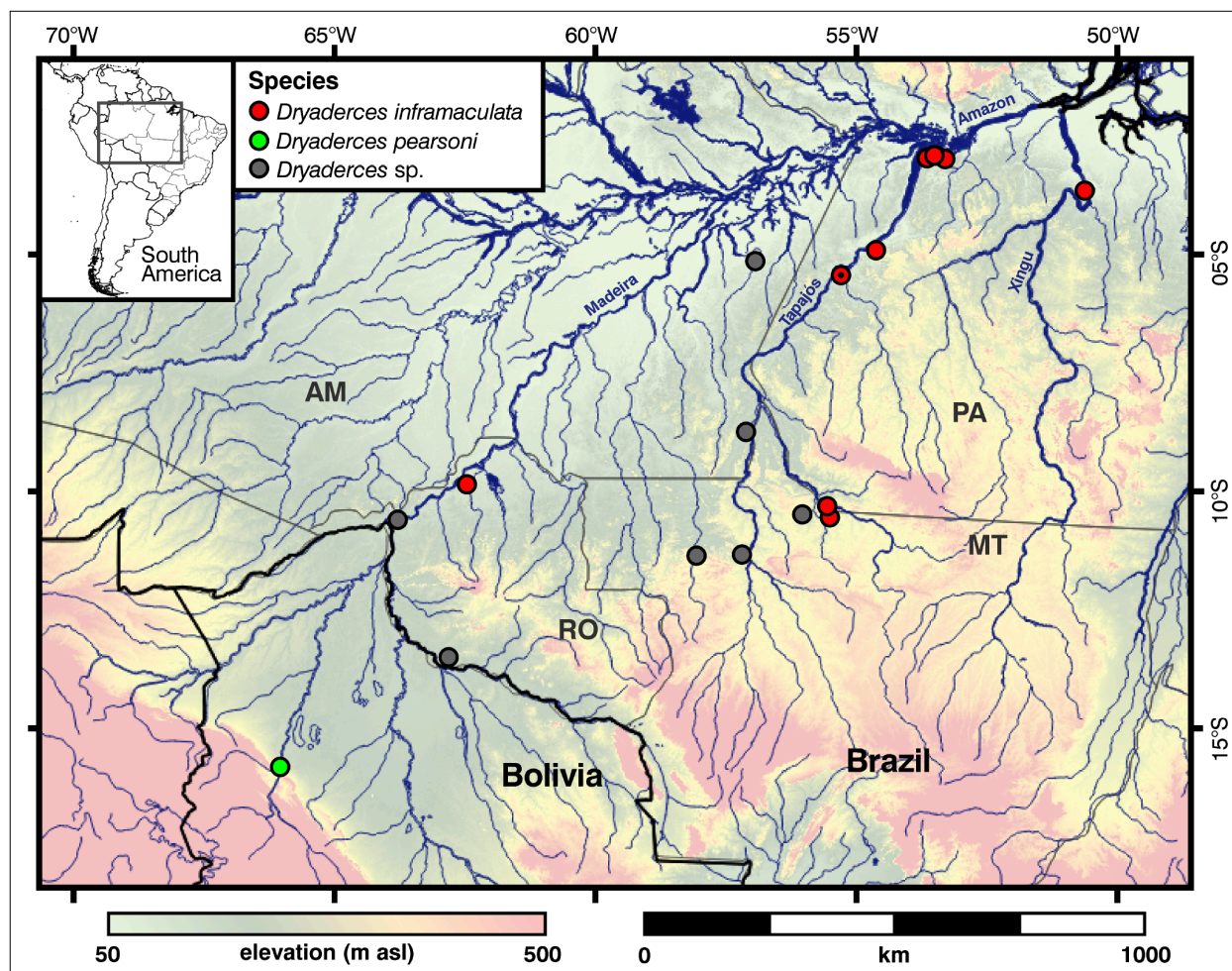


Fig. 1. Map of south-central Amazonia with a hydrographic and elevational background showing the known localities of *Dryaderces*. Data compiled from literature records (JUNGFER *et al.*, 2013; MAFFEI *et al.*, 2018; HOOGMOED, 2019) and material examined at INPA-H collection (Appendix). The black-dotted red symbol represents the locality of the newly collected *D. inframaculata* specimen (INPA-H 41311 with molecular data). Brazilian state abbreviations are: AM (Amazonas), MT (Mato Grosso), PA (Pará), and RO (Rondônia). The type locality of *D. inframaculata* is Santarém, PA (northern-most symbol).

is considered the sister taxon of *Osteocephalus* (DUELLMAN *et al.*, 2016; BLOTTO *et al.*, 2020); and contains two described species (*D. pearsoni* Gaige, 1929, and *D. inframaculata* Boulenger, 1882), and an undescribed species labelled as *D. pearsoni* Ca1 (JUNGFER *et al.*, 2013).

The Santarém tree frog (*Dryaderces inframaculata*) is known from primary flooded and non-flooded forests of southeastern Amazonia, with confirmed localities in the Brazilian states of Pará, Mato Grosso, and Rondônia, south of the Amazon and east of the Madeira rivers (Fig. 1; HOOGMOED, 2013; PINTO *et al.*, 2017a; MAFFEI *et al.*, 2018). Despite its apparent wide geographic distribution, the species has been rarely collected and for more than a century it was only known from the holotype (JUNGFER, 2010). After HOOGMOED (2013), new information was reported on its morphological variation and taxonomy, distribution range, natural history, and reproduction (PINTO *et al.*, 2017a, b; MAFFEI *et al.*, 2018; HOOGMOED, 2019).

Since its formal description in 1882, the absence of new specimens and data led FAIVOVICH *et al.* (2005) to consider the species *incertae sedis* within Hylidae

(as *Hyla inframaculata*). JUNGFER (2010) reviewed the holotype, reported new morphological information, and transferred it to *Osteocephalus* (as *Osteocephalus inframaculatus*), within the *Osteocephalus buckleyi* species group. JUNGFER *et al.* (2013), in the absence of DNA sequences of *O. inframaculatus*, kept the generic placement suggested by JUNGFER (2010). HOOGMOED (2013) studied the morphology of the holotype and new specimens, re-diagnosed the taxon, and transferred it to *Dryaderces* based on the morphological similarity with *D. pearsoni*, the only other described species in this genus. Therefore, the generic placement of *D. inframaculata* has been so far exclusively based on morphological data, and its phylogenetic relationships remain speculative (BLOTTO *et al.*, 2020).

Based on a newly collected *D. inframaculata* specimen from the middle Tapajós River region, we present here the first molecular-based study addressing its generic placement and phylogenetic relationships, through comparison of mitochondrial DNA sequences among related taxa in Lophiohylinae, Hylidae.

Materials and Methods

Study area and specimen examination

We collected an adult female *D. inframaculata* (SVL = 67.9 mm), found perched on a palm leaf in a non-flooded (*terra firme*) primary forest, on the east bank of the middle Tapajós River, municipality of Itaituba, state of Pará, Brazil (4°45'S, 56°36'W, 132 m above sea level) [Fig. 1; see Moraes *et al.* (2016) for further details on locality]. This locality is close (ca. 97 km southwest) to a recently included locality from the known distribution range of the species (Itaituba *sensu* MAFFEI *et al.*, 2018). The new specimen (Figs. 2, 3) was identified based on external morphology according to the known species' variation (JUNGFER 2010; HOOGMOED, 2013; MAFFEI *et al.*, 2018), which we compared to other available specimens of *Dryaderces* deposited at the Amphibians and Reptiles Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA-H), including additional specimens of *D. inframaculata* collected close to the type locality (Fig. 1; Appendix). The specimen with molecular data was deposited in this same collection under the voucher number: INPA-H 41311; field series: DT3584.

Main diagnostic characters used for the species identification included: medium body size; snout truncate in dorsal view, and rounded, slightly protruding in lateral view; granular skin over most of body, except on hidden surfaces of hind limbs (smooth); outer edge of forearms and tarsus with a row of tubercles; brown dorsum with a V-shaped mark extending from upper eyelid to shoulders; lower part of flanks cream with large black spots; ventral surfaces of body bluish-cream, lighter on throat and chest, cream with few black marks on belly; black anastomosing curved lines and spots over a bluish background on hidden surfaces of hind limbs; a cream spot on the upper lip between the eye and tympanum; yellowish iris with wide, triangular and irregular dark marks at each side and below the pupil (these marks are barely discernible and restricted to the upper extreme border of the iris), with radiating black lines more abundant below it (Figs. 2, 3).

DNA extraction, amplification and sequencing

Laboratory work was conducted at the Thematic Laboratory of Molecular Biology (LTBM-INPA), Manaus, Brazil. Genomic DNA was extracted following protocols of the Wizard® extraction kit (Promega). Two mitochondrial DNA fragments (16S rRNA, 523 bp; and COI, 653 bp) were amplified via Polymerase Chain Reaction (PCR). Amplification reactions (total volume: 15 µL) used a mix of 1.2 µL of 10 mM dNTPs, 3 µL of 5X buffer, 1.2 µL of 25 mM MgCl₂, 1.0 µL of template DNA (concentration: 50 ng/µL), 0.5 µL of each primer at 10 mM (16S: 16Sar/16Sbr, PALUMBI *et al.*, 1991; and COI: CHmR4/

CHmL4, CHE *et al.*, 2012), 0.15 µL of Taq polymerase (1U), and 7.45 µL of ddH₂O. Reaction conditions followed standard cycling protocols (PALUMBI *et al.*, 1991), with an annealing temperature of 48 °C and 50 °C for 16S and COI, respectively. PCR products were purified with ExoSAP-IT (USB Corporation) and sequenced using Big Dye Terminator kit (Applied Biosystems) in an automated sequencer ABI 3130 XL (Applied Biosystems). Consensus sequences were assembled in GENEIOUS 8 (KEARSE *et al.*, 2012) and deposited in GenBank (Table 1).

Phylogenetic analyses

We combined our newly generated sequences of *D. inframaculata* with available GenBank material of closely related taxa (Table 1), according to recent molecular systematic reviews of Lophiohylineae (RON *et al.*, 2012, 2016; JUNGFER *et al.*, 2013; DUELLMAN *et al.*, 2016). We included all available homologous sequences of *Dryaderces*, all described species with sequences of *Osteocephalus* and *Tepuihyla* (closest related genera), and one species per genus from other genera within Lophiohylineae (FROST, 2020; Table 1). More recently published, molecular studies propose the transference of species from *Argenteohyla*, *Aparasphenodon* (most of), and *Corythomantis* (part) to *Nyctimantis* (BLOTTO *et al.*, 2020), as well as the inclusion of new species in *Osteocephalus* (FERRÃO *et al.*, 2019; CHASILUISA *et al.*, 2020). We could not account for these modifications in our analyses; however, they were not relevant for *Dryaderces*. The root of Lophiohylineae was defined using as outgroups: *Ceratophrys cornuta* Linnaeus, 1758 (Ceratophryidae), *Allobates femoralis* Boulenger, 1884 (Aromobatidae), and *Adenomera andreae* Müller, 1923 (Leptodactylidae). Sequences were aligned in MAFFT 7 (KATO & STANDLEY, 2013) and manually checked in ALIVIEW 1.26 (LARSSON, 2014). Partition schemes and substitution models were defined using the Bayesian Information Criterion (BIC) as implemented in PARTITIONFINDER 2.1 (LANFAR *et al.*, 2016). Four partitions were found: one for 16S (GTR+I+G), and one for each codon position of COI [1st (SYM+I+G); 2nd (F81); and 3rd (GTR+G)].

Phylogenetic relationships were estimated using Bayesian Inference (BI) and Maximum Likelihood (ML) approaches for each fragment (16S and COI) and for a concatenated dataset (2175 bp). Furthermore, we analyzed two datasets for 16S: one containing sequences of up to 1522 bp as they were available for most GenBank sequences; and a shorter subset from the former one, only containing 536 bp (same length as our *D. inframaculata* sequence) to account for possible missing data impacts. BI analyses were conducted in MRBAYES 3.2 (RONQUIST *et al.*, 2012) using substitution model parametrization as described above, two independent runs with four chains each, and 10 millions of MCMC generations. Sampling was set each 1000 generations and initial 20% was discarded as burn-in. Effective sample sizes (ESS > 200) and stability and convergence between independent runs were



Fig. 2. Dorsolateral view (A) and detail of head and eye (B) of an adult female *Dryaderces inframaculata* (INPA-H 41311; SVL = 67.9 mm) from the east bank of the middle Tapajós River region, state of Pará, Brazil.

evaluated in TRACER 1.7 (RAMBAUT *et al.*, 2018). ML analyses were conducted in RAxML 8 (STAMATAKIS, 2014), using the GTR+I+G model for all partitions and 1000 interactions. Pairwise uncorrected p-distances among *Dryaderces* species were calculated in MEGA 7 (KUMAR *et al.*, 2016) for 16S (522 bp) and COI (651 bp).

Results

Our phylogeny showed high clade support for the monophyly of Lophiohylinae and each of the three targeted genera (i.e., *Dryaderces*, *Osteocephalus*, and *Tepuihyla*;

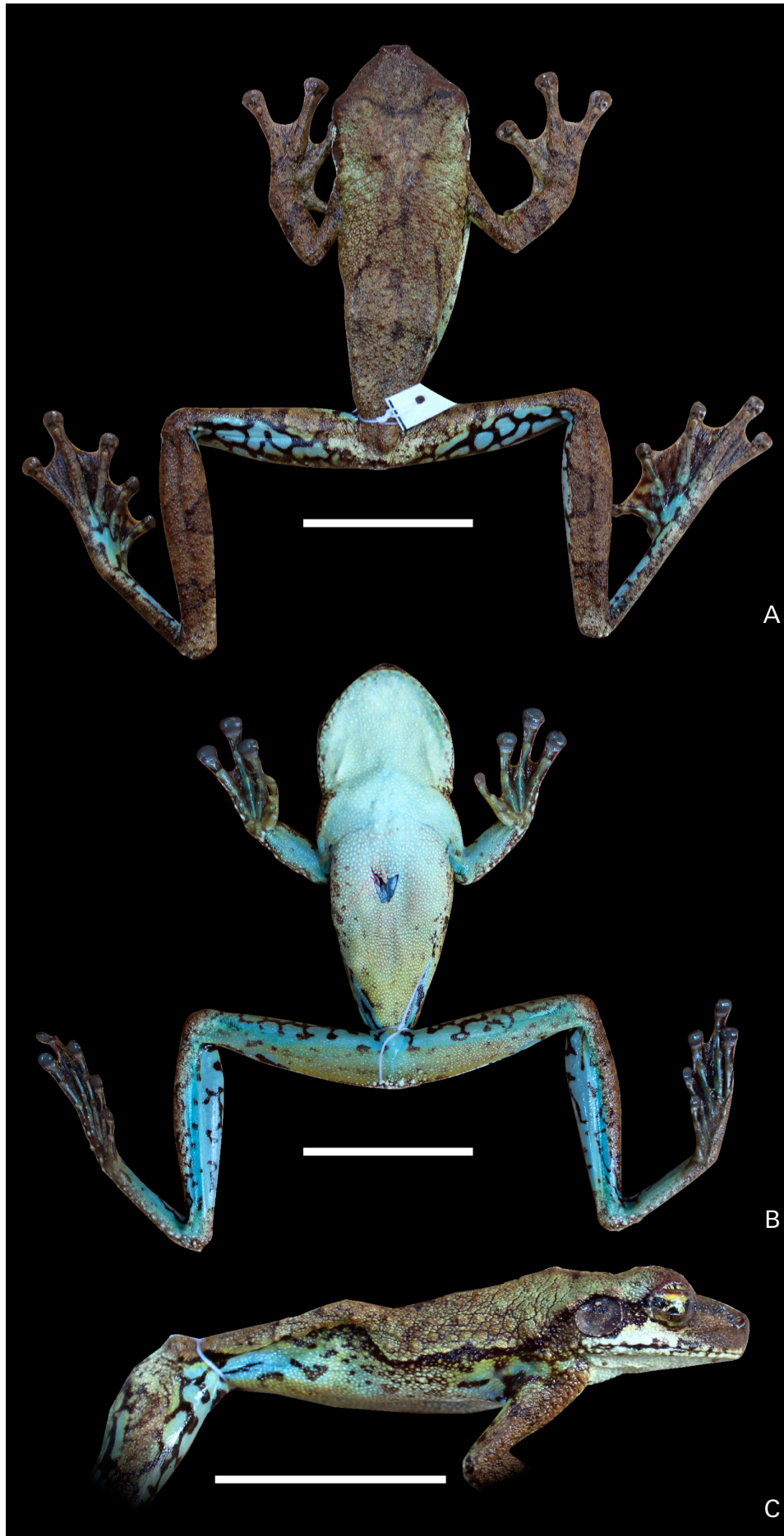


Fig. 3. Dorsal (A), ventral (B), and lateral (C) views of an adult female *Dryaderces in-fraculata* (INPA-H 41311; SVL = 67.9 mm) from the east bank of the middle Tapajós River region, state of Pará, Brazil, showing dark vermiculation over a bluish background at hidden surfaces. Scale bar = 3.0 cm.

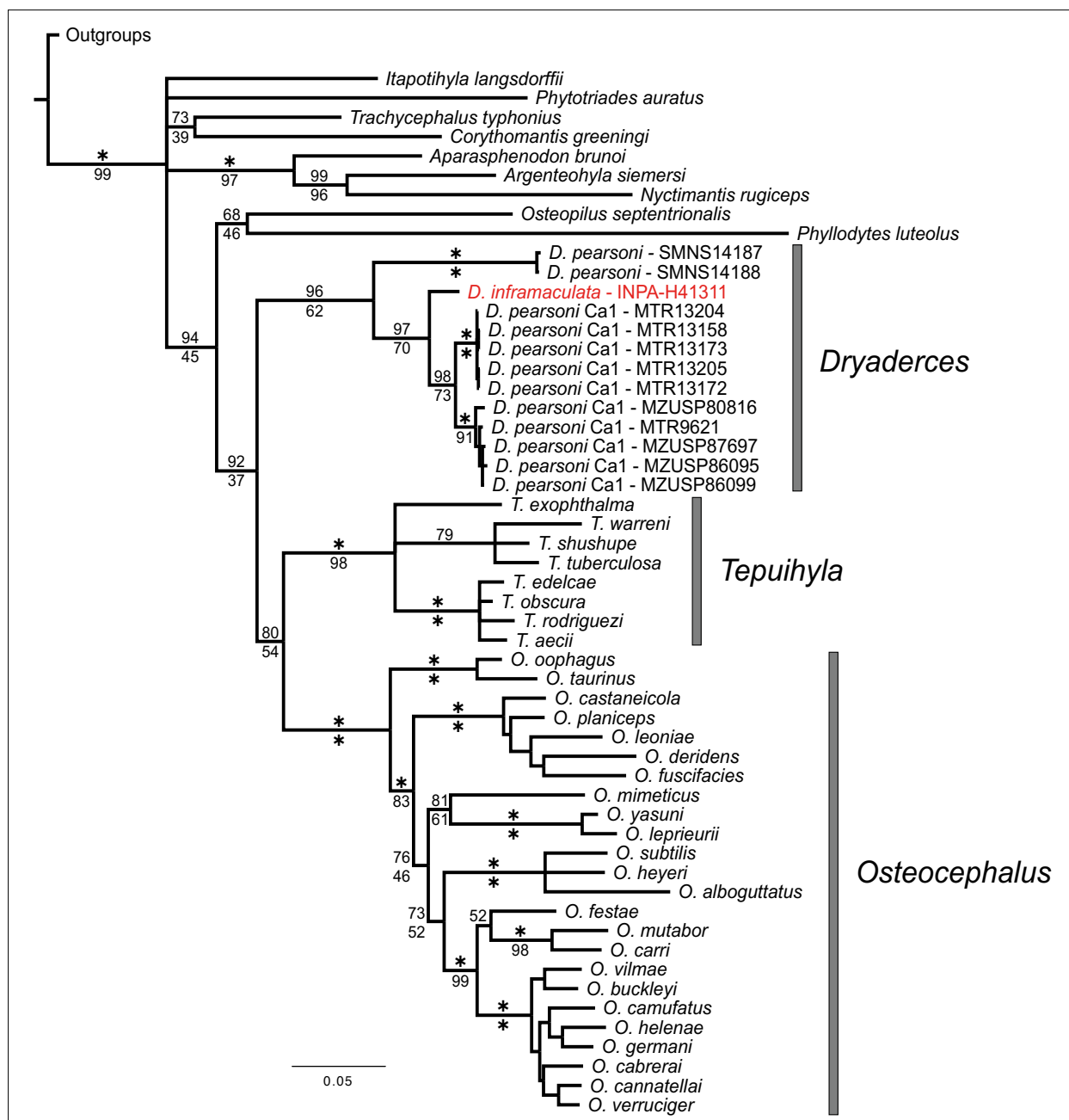


Fig. 4. Phylogenetic reconstruction of a concatenated dataset (2175 bp) of the mitochondrial fragments 16S+COI for representative species in the subfamily Lophiohyllinae (Hyllidae), highlighting the position of *Dryaderces inframaculata*. Topology depicts the BI tree and supporting values are presented as: BI posterior probability \times 100 (above branches) and ML bootstrap support (below branches); values of 100 are represented by asterisks. The position of *Tepuihyla warreni* and *Osteocephalus festae* presented herein was only recovered under BI (similar but not the same under ML).

Fig. 4). Some nodes across the phylogeny (especially internal ones) had higher support under BI than ML. Within *Osteocephalus*, the five traditional species groups (i.e., *O. alboguttatus*, *O. buckleyi*, *O. lepieurii*, *O. planiceps*, and *O. taurinus* groups) were monophyletic and highly-supported, with the exception of *O. mimeticus* that clustered (with low support) with the *O. lepieurii* group instead of with the *O. buckleyi* group.

Dryaderces inframaculata clustered within *Dryaderces* in all analyses. In general, the nodal support (BI posterior probability / ML bootstrap support) for *Drya-*

derces was high across datasets: concatenated 16S+COI (0.96/62; Fig. 4); 16S-1522 bp (0.9/63); 16S-523 bp (0.67/21); and COI (1.0/91).

Dryaderces inframaculata was recovered with high support as the sister species to the undescribed taxon *D. pearsoni* Ca1 in the concatenated 16S+COI dataset (0.97/70), and this clade was sister to *D. pearsoni* (Fig. 4). For individual gene datasets, the relationships for the three species were more uncertain. For example, 16S-1522 bp dataset recovered a polytomy for the three species under BI (0.9); while under ML the relationships

Table 1. GenBank accession numbers of *Dryaderces inframaculata* and related taxa in the subfamily Lophiohylineae (Hylidae) used in this study, including outgroups.

Species	Voucher	16S	COI	Reference
<i>Adenomera andreae</i>	317AF	KC520683	KC520689	FOUQUET <i>et al.</i> (2013)
<i>Allobates femoralis</i>	QCAZ16484 / OMNH36070	AY364543	DQ502811	SANTOS <i>et al.</i> (2003); GRANT <i>et al.</i> (2006)
<i>Aparasphenodon brunoi</i>	CFBH2715	AY843567	—	FAIVOVICH <i>et al.</i> (2005)
<i>Argenteohyla siemersi</i>	MACN38644	AY843570	—	FAIVOVICH <i>et al.</i> (2005)
<i>Ceratophrys cornuta</i>	CFBH20082	KP295608	KP295688	FAIVOVICH <i>et al.</i> (2014)
<i>Corythomantis greeningi</i>	CFBH2968	AY843578	—	FAIVOVICH <i>et al.</i> (2005)
<i>Dryaderces inframaculata</i>	INPA-H41311	MT633109	MT629774	This study
<i>Dryaderces pearsoni</i>	SMNS14187	KF002006	—	JUNGFER <i>et al.</i> (2013)
<i>Dryaderces pearsoni</i>	SMNS14188	KF002007	—	JUNGFER <i>et al.</i> (2013)
<i>Dryaderces pearsoni</i> Ca1	MTR13158	KF002008	KF001880	JUNGFER <i>et al.</i> (2013)
<i>Dryaderces pearsoni</i> Ca1	MTR13172	KF002009	KF001881	JUNGFER <i>et al.</i> (2013)
<i>Dryaderces pearsoni</i> Ca1	MTR13173	KF002010	KF001882	JUNGFER <i>et al.</i> (2013)
<i>Dryaderces pearsoni</i> Ca1	MTR13204	KF002011	KF001883	JUNGFER <i>et al.</i> (2013)
<i>Dryaderces pearsoni</i> Ca1	MTR13205	KF002012	KF001884	JUNGFER <i>et al.</i> (2013)
<i>Dryaderces pearsoni</i> Ca1	MZUSP87697	KF002013	—	JUNGFER <i>et al.</i> (2013)
<i>Dryaderces pearsoni</i> Ca1	MZUSP80816	KF002014	—	JUNGFER <i>et al.</i> (2013)
<i>Dryaderces pearsoni</i> Ca1	MZUSP86099	KF002015	—	JUNGFER <i>et al.</i> (2013)
<i>Dryaderces pearsoni</i> Ca1	MTR9621	KF002016	—	JUNGFER <i>et al.</i> (2013)
<i>Dryaderces pearsoni</i> Ca1	MZUSP86095	KF002017	—	JUNGFER <i>et al.</i> (2013)
<i>Itapotihyla langsdorffii</i>	MACN38643	AY843706	KF001942	FAIVOVICH <i>et al.</i> (2005); JUNGFER <i>et al.</i> (2013)
<i>Nyctimantis rugiceps</i>	—	AY843781	—	FAIVOVICH <i>et al.</i> (2005)
<i>Osteocephalus alboguttatus</i>	SMNS14189	KF002018	—	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus buckleyi</i>	SMNS13714 / QCAZ48827	KF002020	JX875865	JUNGFER <i>et al.</i> (2013); RON <i>et al.</i> (2012)
<i>Osteocephalus cabrerai</i>	KHJ-F082	KF002030	KF001888	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus camufatus</i>	MTR12779	KF002027	KF001887	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus cannatellai</i>	TG1853 / QCAZ39633	KF002032	JX875852	JUNGFER <i>et al.</i> (2013); RON <i>et al.</i> (2012)
<i>Osteocephalus carri</i>	MAR1379	KF002033	—	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus castaneicola</i>	MUSM23918	KF002034	KF001889	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus deridens</i>	KHJF006	KF002037	KF001890	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus festae</i>	QCAZ41039	---	JX875855	RON <i>et al.</i> (2012)
<i>Osteocephalus fuscifacies</i>	SMNS14194 / QCAZ20785	KF002038	JX875818	JUNGFER <i>et al.</i> (2013); RON <i>et al.</i> (2012)
<i>Osteocephalus germani</i>	MHNC7004	KF002049	—	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus helenae</i>	IRSNB14669	KF002048	—	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus heyeri</i>	AJC25813165	KF002054	—	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus leoniae</i>	KHJ-FTUN002	KF002056	KF001893	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus leprieurii</i>	IRSNB14656	KF002064	KF001895	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus mimeticus</i>	MUSM23180	KF002076	KF001901	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus mutabor</i>	EPN-H6659 / QCAZ30926	KF002080	JX875838	JUNGFER <i>et al.</i> (2013); RON <i>et al.</i> (2012)
<i>Osteocephalus oophagus</i>	IRSNB14657 / IRSNB14659	KF002083	KF001902	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus planiceps</i>	GGU752	KF002091	KF001904	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus subtilis</i>	TG2977 / GGU901	KF002092	KF001906	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus taurinus</i>	MTR13081	KF002104	KF001908	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus verruciger</i>	SMNS14197 / QCAZ45344	KF002170	JX875859	JUNGFER <i>et al.</i> (2013); RON <i>et al.</i> (2012)
<i>Osteocephalus vilmae</i>	SMNS14192	KF002019	KF001885	JUNGFER <i>et al.</i> (2013)
<i>Osteocephalus yasuni</i>	KHJ-F113	KF002175	KF001937	JUNGFER <i>et al.</i> (2013)
<i>Osteopilus septentrionalis</i>	USNM317830	AY843712	KF001943	FAIVOVICH <i>et al.</i> (2005); JUNGFER <i>et al.</i> (2013)
<i>Phyllodytes luteolus</i>	—	AY843721	—	FAIVOVICH <i>et al.</i> (2005)
<i>Phytotriades auratus</i>	T521	DQ403730	—	JOWERS <i>et al.</i> (2008)
<i>Tepuihyla aecii</i>	MHNLS12013	JQ868533	—	SALERNO <i>et al.</i> (2012)

Table 1 continued.

<i>Tepuihyla edelcae</i>	PS002	JQ868537	—	SALERNO <i>et al.</i> (2012)
<i>Tepuihyla exophthalma</i>	IRSNB14644	KF002181	KF001939	JUNGER <i>et al.</i> (2013)
<i>Tepuihyla obscura</i>	IRSNB15879	JQ742232	—	KOK <i>et al.</i> (2012)
<i>Tepuihyla rodriguezi</i>	CPI10223	KF002183	KF001941	JUNGER <i>et al.</i> (2013)
<i>Tepuihyla shushupe</i>	CORBIDI12513	—	KY013396	RON <i>et al.</i> (2016)
<i>Tepuihyla tuberculosa</i>	QCAZ53542	—	KY013415	RON <i>et al.</i> (2016)
<i>Tepuihyla warreni</i>	ROM39491	KF002185	—	JUNGER <i>et al.</i> (2013)
<i>Trachycephalus typhonius</i>	AMNHA1411427	AY549362	KF001946	FAIVOVICH <i>et al.</i> (2004); JUNGER <i>et al.</i> (2013)

were similar to the concatenated dataset (*D. pearsoni* (*D. inframaculata* + *D. pearsoni* Ca1)) but with lower support (55) for the sister relationship between these two latter species. In contrast, in 16S-523 bp dataset the relationships were: *D. inframaculata* (*D. pearsoni* + *D. pearsoni* Ca1), but with very low support for the sister relationship between these two latter species (0.5/13). Finally, for COI there were no available sequences of *D. pearsoni* (Table 1), thus only resulting in the sister relationship: *D. inframaculata* + *D. pearsoni* Ca1.

Mean uncorrected pairwise p-distances for 16S were: *D. inframaculata* vs. *D. pearsoni* Ca1: 2.3% (range: 2.3–2.5); *D. inframaculata* vs. *D. pearsoni*: 3.6% (3.6–3.6); and *D. pearsoni* vs. *D. pearsoni* Ca1: 3.9% (3.6–4.2). For COI, mean uncorrected p-distance between *D. inframaculata* and *D. pearsoni* Ca1 was: 5.3% (5.2–5.4).

Discussion

The molecular phylogenetic placement of *D. inframaculata* within *Dryaderces* in both mitochondrial fragments (16S and COI) corroborates its current generic allocation, as determined by HOOGMOED (2013). This taxonomic decision based solely on morphological characters is now confirmed by molecular information. Our analyses did not recover a sister relationship between *Dryaderces* and *Osteocephalus* as observed in other phylogenies with higher taxonomic and character sampling (JUNGER *et al.*, 2013; DUELLMAN *et al.*, 2016). However, the precise determination of the among genera relationships, as well as those among species within *Osteocephalus* and *Tepuihyla* was beyond the scope of our study and would require more data (see RON *et al.*, 2016; BLOTTO *et al.*, 2020).

Within *Dryaderces*, the recovered relationships for the concatenated dataset (16S+COI) were: *D. pearsoni* (*D. inframaculata* + *D. pearsoni* Ca1). This was surprising because *D. pearsoni* and *D. pearsoni* Ca1 are phenotypically more similar, at least concerning to external morphology. Initially, we thought that these relationships could be driven by: 1) the absence of COI sequences for *D. pearsoni*; and 2) a poor resolution/low genetic distances of the 16S analyzed fragment, as observed between some *Osteocephalus* species (JUNGER *et al.*, 2013). When analyzed independently, 16S fragment re-

covered a range of different relationships for *Dryaderces* species including: the concatenated hypothesis (Fig. 4) and a polytomy (in 1522 bp dataset); and the relationships: *D. inframaculata* (*D. pearsoni* + *D. pearsoni* Ca1) (in 533 bp dataset). However, this last configuration received very low support. In addition, the genetic distances in 16S indicated that *D. pearsoni* Ca1 is overall more similar to *D. inframaculata* (mean of 2.3%), than to *D. pearsoni* (mean of 3.9%).

Biogeographically, a closer relationship between *D. inframaculata* and the undescribed taxon *D. pearsoni* Ca1 also seems more likely. These two species are mainly distributed (based on literature records and our analysis of specimens at INPA-H collection; Fig. 1) in the lowlands of the Madeira-Tapajós interfluvium (*D. pearsoni* Ca1; JUNGER *et al.*, 2013), and the Tapajós-Xingu interfluvium (*D. inframaculata*; MAFFEI *et al.*, 2018). A closer phylogenetic relationship and similar species composition has been observed for several terrestrial vertebrates and butterflies distributed in these interfluvial areas from southeastern Amazonia than with western Amazonia and Andean lineages (e.g., RON, 2000; HALL & HARVEY, 2002; RIBAS *et al.*, 2012, 2018; LYNCH ALFARO *et al.*, 2015; OLIVEIRA *et al.*, 2017; ROJAS *et al.*, 2018). This pattern is consistent with the distribution of the three known *Dryaderces* species, as *D. pearsoni* is only known to occur in northern Amazonian Bolivia, close to the Andean slopes (JUNGER *et al.*, 2013; FROST, 2020).

Future systematic studies of *Dryaderces* with a denser geographic and genetic sampling are needed to better understand the species distributions, their diversification and biogeography. In conclusion, our results confirm the generic allocation of *D. inframaculata*, and provide the phylogenetic relationships of this enigmatic Amazonian taxon.

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Appendix

Specimens examined in the INPA-H collection. All specimens from Brazil.

***Dryaderces inframaculata* (n=3).** PARÁ: Itaituba, east bank of middle Tapajós River (INPA-H 41311); Alter do Chão, Andiroba (INPA-H 10736–10737).

***Dryaderces* sp. (n=10).** AMAZONAS: Apuí, Parque Nacional do Sucunduri (INPA-H 17461). RONDÔNIA: Guajará Mirim, linha D (INPA-H 6082–6084, 6094, 6182); Costa Marques, ramal do km 10 da BR-429 (INPA-H 6567); Madeira River (INPA-H 15540–15542).