Two novel genera and one new species of treefrog (Anura: Rhacophoridae) highlight cryptic diversity in the Western Ghats of India

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Abstract

Amphibian diversity in the Western Ghats-Sri Lanka biodiversity hotspot is extremely high, especially for such a geographically restricted area. Frogs in particular dominate these assemblages, and the family Rhacophoridae is chief among these, with hundreds of endemic species. These taxa continue to be described at a rapid pace, and several groups have recently been found to represent unique evolutionary clades at the genus level. Here, we report DNA sequences, larval and breeding data for two species of rhacophorid treefrog (*Polypedates bijui* and a new, hitherto undescribed species). Remarkably, they represent unique, independent clades which form successive sister groups to the *Pseudophilautus* (Sri Lanka) + *Raorchestes* (India, China & Indochina) clades. We place these species into two new genera (*Beddomixalus* **gen. nov.** and *Mercurana* **gen. nov.**). Both of these genera exhibit a distinct reproductive mode among Rhacophoridae of peninsular India and Sri Lanka, with explosive breeding and semiterrestrial, unprotected, non-pigmented eggs oviposited in seasonal swamp pools, which hatch into exotrophic, free-living aquatic tadpoles. Relationships and representation of reproductive modes in sister taxa within the larger clade into which these novel genera are placed, is also discussed. These results suggest that more undescribed taxa may remain to be discovered in South Asia, and the crucial importance of conserving remaining viable habitats.

Key words: Rhacophoridae, anuran reproductive modes, Western Ghats, India, *Beddomixalus, Mercurana*

Introduction

The frog family Rhacophoridae constitutes a radiation of almost 350 treefrogs and allied species (Frost, 2013; Van der Meijden *et al.*, 2001), with members distributed from Asia to Africa (Duellman, 1999). In particular, the Western Ghats-Sri Lanka biodiversity hotspot represents a major area of endemic radiations, with hundreds of described species (Biju & Bossuyt, 2009; Bossuyt *et al.*, 2004; Meegaskumbura *et al.*, 2002). This diversity is characterized by numerous independent clades, with novel and previously unrecognized endemic genera such as *Ghatixalus* and *Taruga* having been described from the region in recent years (Biju, Roelants & Bossuyt, 2008; Meegaskumbura *et al.*, 2010). The rate at which species and genera are being described in the region suggests that numerous taxa may remain yet to be discovered.

Here, we report the discovery of two new genera of rhacophorid frogs, identified by their unique phylogenetic placement and distinctive reproductive mode, along with morphological, anatomical and other physiological data. The generic allocation of many rhacophorid species is complicated by occasionally ambiguous definition of genera, which generally lack distinct morphological synapomorphies or show a high degree of homoplasy (Biju, Roelants & Bossuyt, 2008). However, recent molecular work has shown great progress in resolving the phylogeny of the group (Biju & Bossuyt, 2009; Meegaskumbura *et al.*, 2002; Meegaskumbura & Manamendra-Arachchi,

2005). Reproductive mode, life history, and larval characters can also be useful indicators in determining taxonomic allocation, and as characters for identification in the field (Duellman & Trueb, 1994). One of the genera newly described here comprises a described species, *Polypedates bijui* Zachariah *et al.*, 2011, which exhibits semiterrestrial oviposition with aquatic tadpoles, a reproductive mode that has previously been reported only once in Rhacophoridae, in a related clade. A hitherto undescribed rhacophorid species, also described here as another new genus exhibits a comparable reproductive mode, with slight variations.

Material and methods

Field surveys and specimen collection. We collected field data and specimens during repeated field trips in the Western Ghats of India in 2011-2012. We gathered information on reproductive behaviour, activity patterns and distributions of the observed species during the months of April-August. Our fieldwork yielded specimens and data for two similar rhacophorid species, one of which had been previously placed in the genus *Polypedates* (*P. bijui*) and the other being a hitherto undescribed frog. Adult and tadpole specimens were euthanized and preserved in 10% neutral-buffered formalin (NBF) for 2 days, and then transferred to 70% ethanol. Measurements (rounded to 0.1 mm) and terminology follow Zachariah *et al.* (2011) for adult frogs. Tadpoles of '*P.*' *bijui* were monitored and recorded till metamorphosis in July. Tadpole specimens taken for measurements were directly fixed in 10% NBF and preserved in a 1:1 mixture of 70% ethanol and 10% NBF. Live colouration for individual animals was recorded within an hour after collection. Terminology used to describe external features of tadpoles follows standard references (Altig & McDiarmid, 1999a). Samples for molecular analyses were taken from muscle tissue, preserved in 100% ethanol and stored at –20°C. Measurements (rounded to 0.1 mm) were made with a Mitutoyo Digimatic caliper. Specimens were deposited in the Natural History Museum, Trivandrum, India. Museum abbreviations: ZSI/WGRC = Zoological Survey of India/Western Ghats Regional Centre, Calicut; TNHM = Trivandrum Natural History Museum.

Molecular phylogenetics. We constructed a molecular phylogeny for Rhacophoridae based on the mitochondrial 12S/16S ribosomal RNA fragment and five nuclear loci (BDNF, POMC, RAG1, RHOD, and TYR), for which we gathered single representatives for all available species from GenBank. For the two species of rhacophorid frogs (adults and tadpoles) collected during our fieldwork, we used standard PCR and sequencing protocols to generate sequence data for a target region of ~970bp of 12S, ~570bp 16S, ~320bp of RHOD, and ~1300bp of RAG1 (San Mauro et al., 2004; Frost *et al.*, 2006). The sequences were deposited in GenBank under the accession numbers KC594289 — KC594296. The combined sequence data from GenBank and our sequencing efforts were aligned using the default parameters of the MUSCLE algorithm (Edgar, 2004). We did not partition by stems and loops, as we are not aware of robust models available for rhacophorids, but the protein-coding loci were partitioned by codon.

The final alignment consisted of 5962 bp from 185 of ~350 species of rhacophorids from 16 of 17 recognized genera, with an average of 40% completeness per species (mean length = 2412 bp). We included the ranoids *Rana catesbeiana* and *Mantella madagascarensis* as outgroups. Species names and GenBank accession numbers are available in Appendix 1. We estimated phylogenies using Maximum Likelihood (ML) in RAxMLv7.2.8 (Stamatakis, 2006), using the rapid-bootstrapping (BS) algorithm with 1000 replicates, yielding 200 independent searches from random starting points. In accordance with the established literature, we consider BS > 70 to represent strong support.

Results

Phylogenetic relationships

Overall, our phylogeny (Fig. 1) is highly similar to recent estimates with respect to the higher-level phylogeny of Rhacophoridae, and the monophyly and composition of most genera (Li, Che, Bain, Zhao, & Zhang, 2008; Pyron & Wiens, 2011; Wilkinson, Drewes, & Tatum, 2002; Yu, Rao, Zhang, & Yang, 2009; Yu, Zhang & Yang, 2013), with two novel and surprising results. There is a strongly supported clade (BS = 97) containing *Pseudophilautus,*
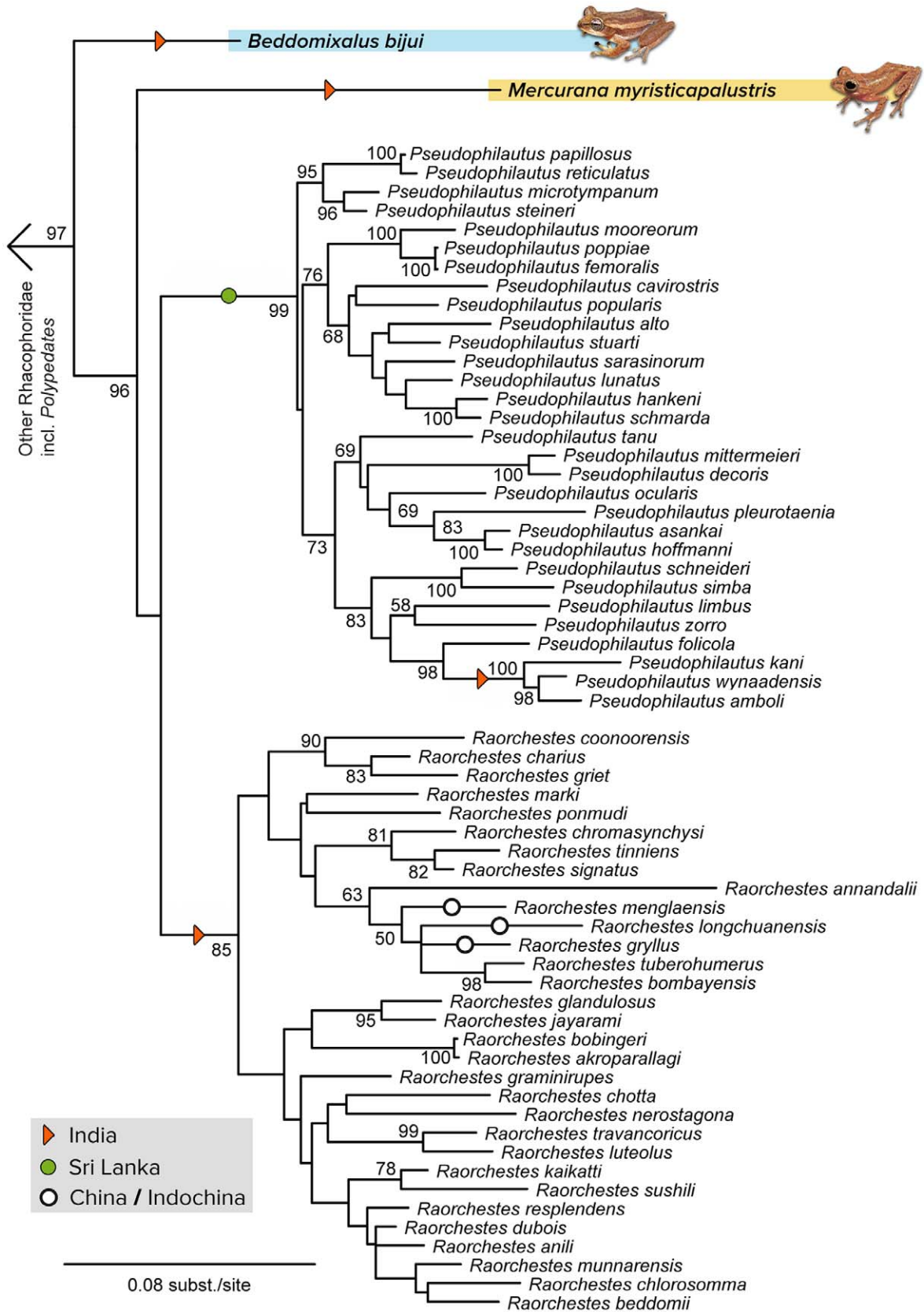
FIGURE 1. Phylogenetic relationships among the South Asian rhacophorid genera that possess simple, tubular Wolffian duct as supported by the maximum-likelihood tree; marked branches highlight the phylogenetic positions of *Beddomixalus* **gen. nov.** *bijui* and *Mercurana myristicapalustris* **gen. et sp. nov.** See Appendix 2 for larger tree.

Raorchestes, Polypedates bijui, and the undescribed species. Both *Pseudophilautus* and *Raorchestes* are strongly supported (BS = 99 and 85, respectively), and the two other taxa are not members of those genera. Instead, *Polypedates bijui* is strongly supported (BS = 96) as the sister group to the undescribed rhacophorid species + (*Pseudophilautus* + *Raorchestes*). The undescribed rhacophorid is the sister lineage to a weakly supported clade of *Pseudophilautus* + *Raorchestes*. Exploratory analyses using only the mitochondrial (12S/16S) and nuclear (RAG1/RHOD) gene partitions separately both recovered the isolated position of the two taxa (results not shown).

Based on this phylogenetic placement, as well as behavioural and biological differences (see below), both taxa thus necessitate generic reassignment and assignment, respectively. As there are no available genus names for either taxon, we assign *P. bijui* and the novel taxa to new genera (see below). Note that it is possible that the undescribed taxon is actually the sister lineage to *Raorchestes* or *Pseudophilautus*. However, this hypothesis is currently rejected (albeit weakly) by our ML results, and a new generic name for the taxon would still be warranted in either case due to its deep molecular differentiation and its distinct natural history.

Our larger tree (Appendix 2) also suggests taxonomic problems and non-monophyly in *Theloderma, Chiromantis,* and *Rhacophorus*, some of which had been identified in previous studies (Li *et al.*, 2008; Pyron & Wiens, 2011; Yu, Rao, Zhang, & Yang, 2009). However, these are not informed by new data generated in this project, and we do not address them here.

Beddomixalus gen. nov.
(Fig. 2C–D)

Type species: *Polypedates bijui* Zachariah *et al.*, 2011

Etymology: The generic epithet is derived from a combination of the cognomen of Colonel Richard Henry Beddome, in honor of his pioneering and extensive fieldwork, which contributed to a fundamental understanding of amphibian diversity of the Western Ghats during the colonial period, and *Ixalus* (Dumeril & Bibron, 1839), often used as a suffix for names of rhacophorid genera. Gender is masculine.

Material examined: ZSI/WGRC/ V/A/842, 843, 844, adult males, Kadalar; ZSI/WGRC/ V/A/849, adult female, Kadalar; TNHM (H) 12.6.18/54, adult male, Kadalar; TNHM (H) 12.6.18/73, tadpole, Kadalar. Measurements of specimens provided in Table 1.

Diagnosis: *Beddomixalus* differs from all other rhacophorid genera by a combination of the following characters: a slender, elongated medium-sized frog (average male SVL 40.1, N=3; female SVL 60.8mm, N=1); dorsum yellowish-buff or reddish-brown in colour with two distinct yellowish-cream coloured parallel longitudinal stripes, extending from the supratympanic fold to the vent along the dorsolateral margin; vomerine teeth and lingual papilla absent; supratympanic fold and tympanum distinct; canthus rostralis rounded, loreal region obtusely concave (the canthus rostralis and the loreal region had been erroneously interpreted in the type description as being concave and angular, respectively); Wolffian duct simple, tubular (Fig. 6). Additionally, early development of non-pigmented eggs occur exposed on moist swamp beds, without any form of protection, neither in the form of foam nests or parental care; has free-living aquatic tadpoles adapted to lentic conditions; inhabits mid- to high-elevation forests. *Beddomixalus* currently contains one species and this diagnosis is valid for the genus and species.

Colour in life: Male: dorsum buff-brown with a dark stripe between the eyes in the inter-orbital region; a dark stripe extending medially from inter-orbital region to vent; two prominent light-coloured, yellowish-cream longitudinal stripes extending along the dorsolateral region, from the outer border of upper eyelid to the groin, on either side of the body, bordered on the lower side by a series of black dots, which appear as a line; a broad dark band extending from below the snout along the tympanum up to the middle of the flank, where it transforms into a pale patch covered in dark-brown to black vermiculations. Female: reddish- to orange-brown dorsum; similar dark and light bands and vermiculations as in the male; ventral surface white.

Geographic distribution: *B. bijui* is found in mid- to high-elevation (1100 – 1600m ASL) evergreen forests along the western slopes of the Eravikulam plateau (Fig. 7). We recorded the species in the Mankulam forest division (10.1344N, 76.9947E; 1431m ASL) of Idukki district and the higher parts of the Malayattoor forest division (10.1869N, 76.9928E; 1584m ASL) of Ernakulam district in Kerala, and also on the Valparai plateau in the Anamalai Hills of Tamil Nadu (10.2756N, 77.0067E; 1246m ASL).

Breeding behaviour and reproductive mode: The species is primarily arboreal and inhabits trees in and around seasonal highland swamps or marshes (Fig. 2B). We carried out observations on breeding and development from April to July 2012. During the early part of the breeding season (late April to early June), hundreds of males aggregated around optimal breeding locations in moist swamp beds that were not yet filled with water. Males begin migrating towards breeding sites (swamp beds) (Fig. 2B) during the onset of pre-monsoon showers in late April from surrounding forest (Fig. 2A), where they congregate. These highland swamps are few in number and form part of the headwaters of regional streams and the substrate is mostly moist clay in April, when the pre-monsoon showers begin. Most males perch on shrubs and bushes (at up to 2m height) along the periphery of the dry swamp and in unison make a pulsating breeding chorus (audio file of call deposited in the Dryad Repository as Supplementary file 1; http://dx.doi.org/10.5061/dryad.pm166). But, individual males that occupy territories on shrubs within the swamp bed make a different call that could not be recorded (Fig. 2C), akin to that of some *Polypedates* spp. from Southern India. The air was dominated by a strong odour, which was reminiscent of the 'burnt rubber' smell characteristic of aggregating males of *Hylarana malabarica* (Daniel, 1976). Close examination of individual males suggested this odour to be produced by glands on their dorsolateral margin. Such an ambient odour and loud pulsating call could be reason for prompting males to form mass aggregations and attracting females, but further observations are needed to validate this. Males descend from their calling perches as the night progresses and occupy positions closer to grass patches in the muddy swamp bed.

In spite of several nights (n = 7) of observation, only two individual females could be located and followed to make observations of reproductive behaviour. Amplexus is pectoral (Fig. 2D) and an amplectant pair was observed moving within the swamp. The pair initiated oviposition on wet grass within the swamp basin. Non-pigmented, semiterrestrial eggs (terminology of Altig and McDiarmid 2007) (average diameter of an egg when freshly laid = 3.2mm) were scattered on moist soil or a grassy substrate, either as scattered loose arrays or in clumps (terminology of Altig and McDiarmid 2007; Fig. 2E). Total clutch size was 175 eggs. Clutches of eggs deposited by other individuals were observed on wet clay. Eggs scattered on exposed rocks were seen to have desiccated the next day. Subsequent rains over the next few days raised the water level of the swamp, after which free-swimming tadpoles could be observed.

Tadpole morphology: Five tadpoles at various stages were collected within or close to grassy agglomerations in a swamp pool on 22nd June, 2012. Sample tissue from a tadpole was DNA sequenced and analyzed to determine its species status. Description is based on a single tadpole [TNHM (H) 12.6.22/73] in Stage 38 (Fig. 3A-C). The small exotrophic, nektonic tadpole is oval and depressed (Fig. 2F) and is consistently dark pigmented from the snout to the tip of the tail, including fins. The ventral and ventrolateral body sides are pale pink and less pigmented. Eyes are reddish and of moderate size, positioned dorsolaterally and directed more laterally than anteriorly; not visible in ventral view. In lateral view, the body is slightly depressed, and snout slightly rounded. The *musculus interhyoideus* and intestine are visible through the ventral parts of the body. Naris equidistant from both snout and eye. Spiracle sinistral, ventrolaterally positioned at midbody. Myotomes of the tail musculature of moderate development. Tail fin moderate, rounded at the end. Upper fin smaller than lower fin. Oral disc anteroventrally positioned, of triangular shape in relaxed state, of oval shape in expanded state, and slightly emarginated laterally. Marginal papillae frame the oral disc ventrally and laterally, but absent on the upper labium. Small submarginal papillae cover the entire inner rim of oral disc. Upper jaw sheath narrow and stretched into a wide U-shaped arch. Lower jaw sheath V-shaped. Labial tooth row formula (LTRF) is 5(2-5)/3 (see Fig. 3B).

Measurements (mm) of the representative tadpole were as follows: 29.1 total length from tip of snout to tip of tail, 9.9 body length as the distance from the tip of the snout to the junction of the body and tail, 20.3 tail length from the bodytail junction to the tip of the tail, 5.6 body width at the plane of the spiracle, 4.1 body height at the plane of the eyes, 2.7 tail muscle height at the base of the tail, 2.4 tail muscle width at the base of the tail, 1.2 maximum upper fin height, 1.3 maximum lower fin height, 4.9 maximum tail height including the fins, 1.1 eye-naris distance between the centres of the nares to the anterior edge of the eyes, 1.8 naris-snout distance between the centres of the narial apertures to tip of snout, 7.0 snout-spiracle distance from the tip of the snout and the terminal end of the spiracle, 1.7 internarial distance between the centres of the narial apertures, 1.5 interorbital distance between the medial borders of the eyes, and 1.5 eye diameter.

Larval metamorphosis: We observed tadpoles with completely developed hindlimbs at Stage 40 (Fig. 3D) in the first week of July. A pale dorsolateral band appears at Stage 42 (Fig. 3E). Eyes are bright red and overall body pigmentation changes to greenish-yellow by Stage 44 (Fig. 3F), when metamorphs begin emerging on land. At Stage 46, froglets can be found among vegetation surrounding the swamp (Fig. 3G).
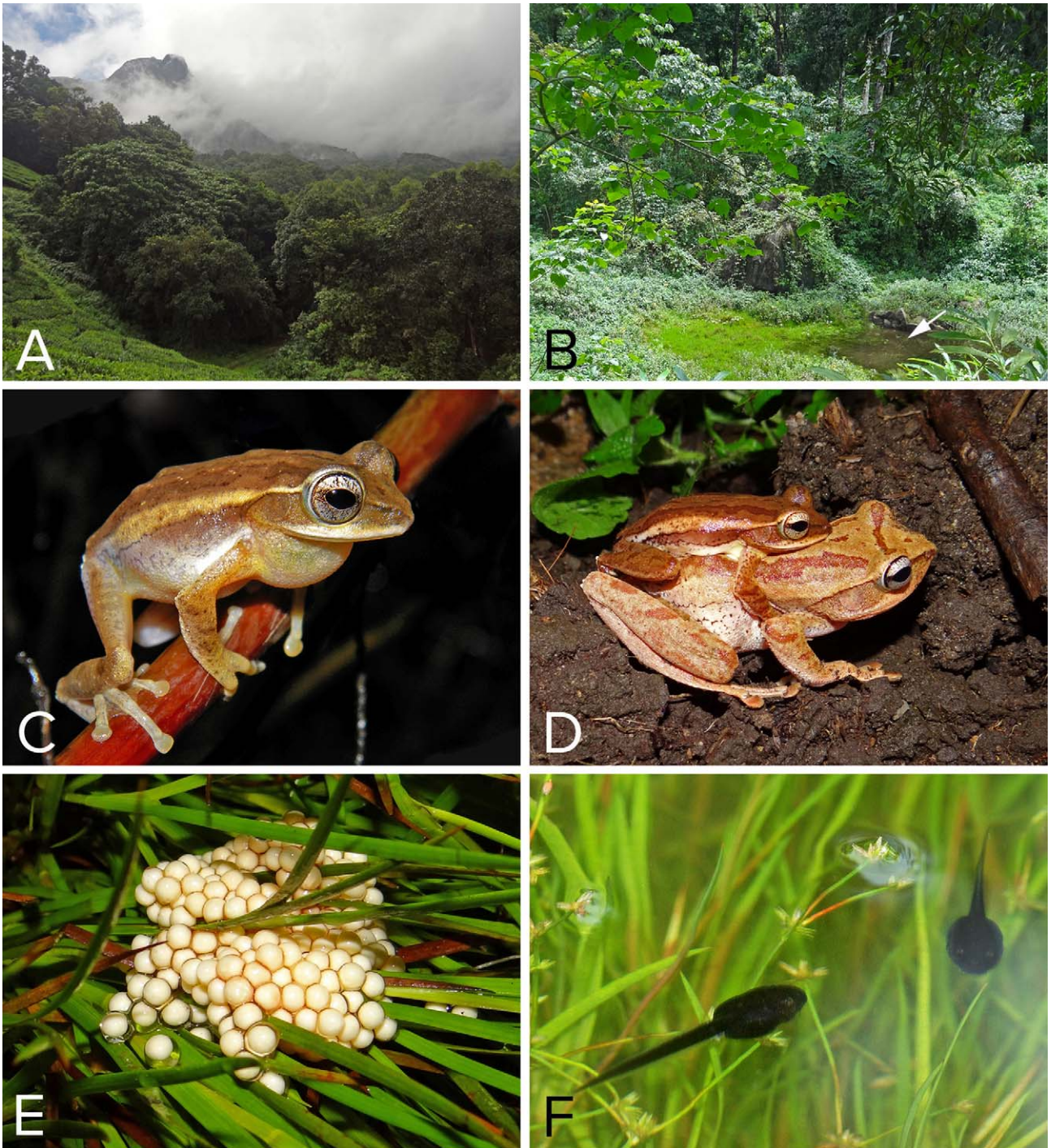
FIGURE 2. *Beddomixalus* **gen. nov.** *bijui* **A.** general habitat comprising of tropical montane wet forests interspersed with tea plantations **B.** highland swamp, arrow = swamp pool after pre-monsoon showers; **C.** vocalizing male; **D.** pair in amplexus; **E.** eggs laid as clumps on grass in the swamp bed; **F.** free-swimming tadpoles in swamp pool.
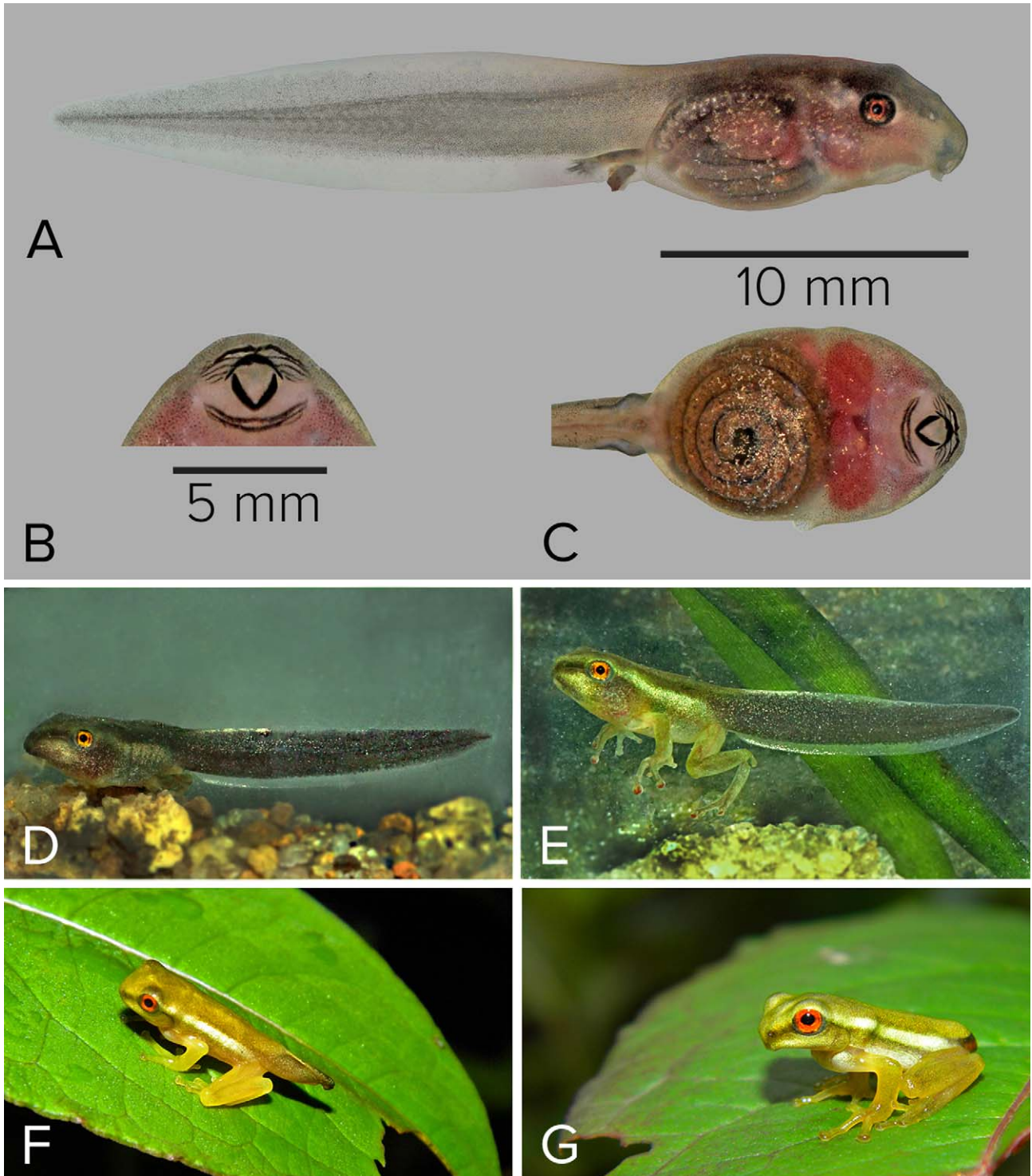
FIGURE 3. Tadpole of *Beddomixalus bijui;* **A.** lateral view, **B.** details of oral apparatus (LTRF: 5(2-5)/3) and **C.** ventral view (Stage 38, 29.1 mm TL); metamorphic development of tadpole, **D.** Stage 40; **E.** Stage 42; **F.** Stage 44; **G.** Stage 46.

Mercurana myristicapalustris **gen. et sp. nov.**
 (Fig. 4C)

Holotype: TNHM (H) 12.6.18/69, an adult male (SVL 35.4 mm) (Fig. 5), collected by Ansil B. R. on 18th May, 2012, from bushes at the periphery of a temple compound, Arippa (8.8392N, 77.0314E, altitude approximately 184m ASL), near Kulathupuzha Reserve Forest, Kollam district, Kerala, India.

Paratypes: TNHM (H) 12.6.18/70 and TNHM (H) 12.6.18/74, two adult males collected by Robin K. Abraham on 12th July, 2012 from the type locality. Measurements of all specimens provided in Table 1.

Etymology: The generic epithet is derived from 'Mercury' as a tribute to Freddie Mercury, late lead singer of the British rock band Queen, whose vibrant music inspires the authors, in combination with *Rana* (Linnaeus, 1758), a suffix commonly used for many frog taxa. Gender is masculine. The specific epithet, a combination of the words 'myristica' for the nutmeg family, and 'palustris', which is latin for swampy, emphasizes the Myristica swamp forest habitat of this frog, which is a fragile and threatened habitat type of the Western Ghats.

Diagnosis: The diagnosis is valid for the genus and species. *Mercurana myristicapalustris* can be distinguished from other rhacophorid genera by the following combination of characters: medium sized frog (average male SVL 36.1, N=3; female SVL 65.1mm, N=1); weakly-developed vomerine teeth present; simple tubular Wolffian duct; eggs non-pigmented; early embryonic development occurs in wet mud after pre-monsoon showers; free living aquatic tadpoles; arboreal adults inhabit low-elevation swamp forests. *Mercurana*, which could potentially be confused with *Beddomixalus,* differs from the latter in the following characters: presence of vomerine teeth and lingual papilla (vs. absence); symphysial knob not sharply pointed (vs. sharply pointed); no stripe on the dorsolateral margin (vs. pale distinct stripe on the dorsolateral margin); canthus rostralis indistinct (vs. rounded); toes 3/4th webbed (vs. half/moderately webbed); finger discs large (vs. moderate); subarticular tubercle on finger IV bifid (vs. rounded); oviposited eggs mixed with mud in shallow pit (vs. eggs openly scattered on ground substrates) and; inhabits low-elevation forests (vs. mid- to high- elevation forest).
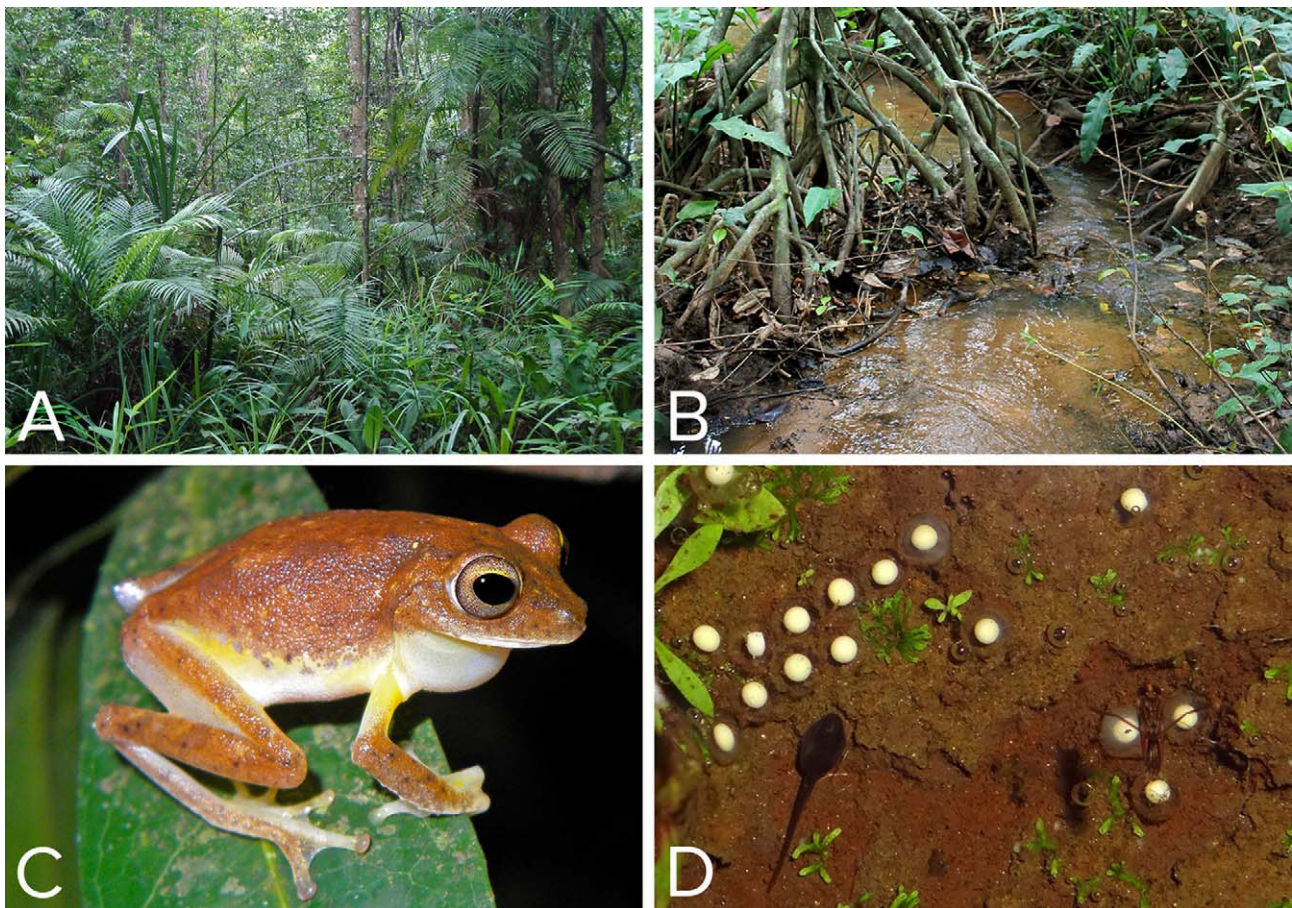

FIGURE 4. *Mercurana myristicapalustris* **gen. et sp. nov. A.** General habitat comprising primarily of lowland *Myristica* swamp forest; **B.** typical breeding habitat on the muddy banks of small rivulets; **C.** vocalizing male; **D.** eggs with enlarged jelly (upon hydration after rains) and tadpole.
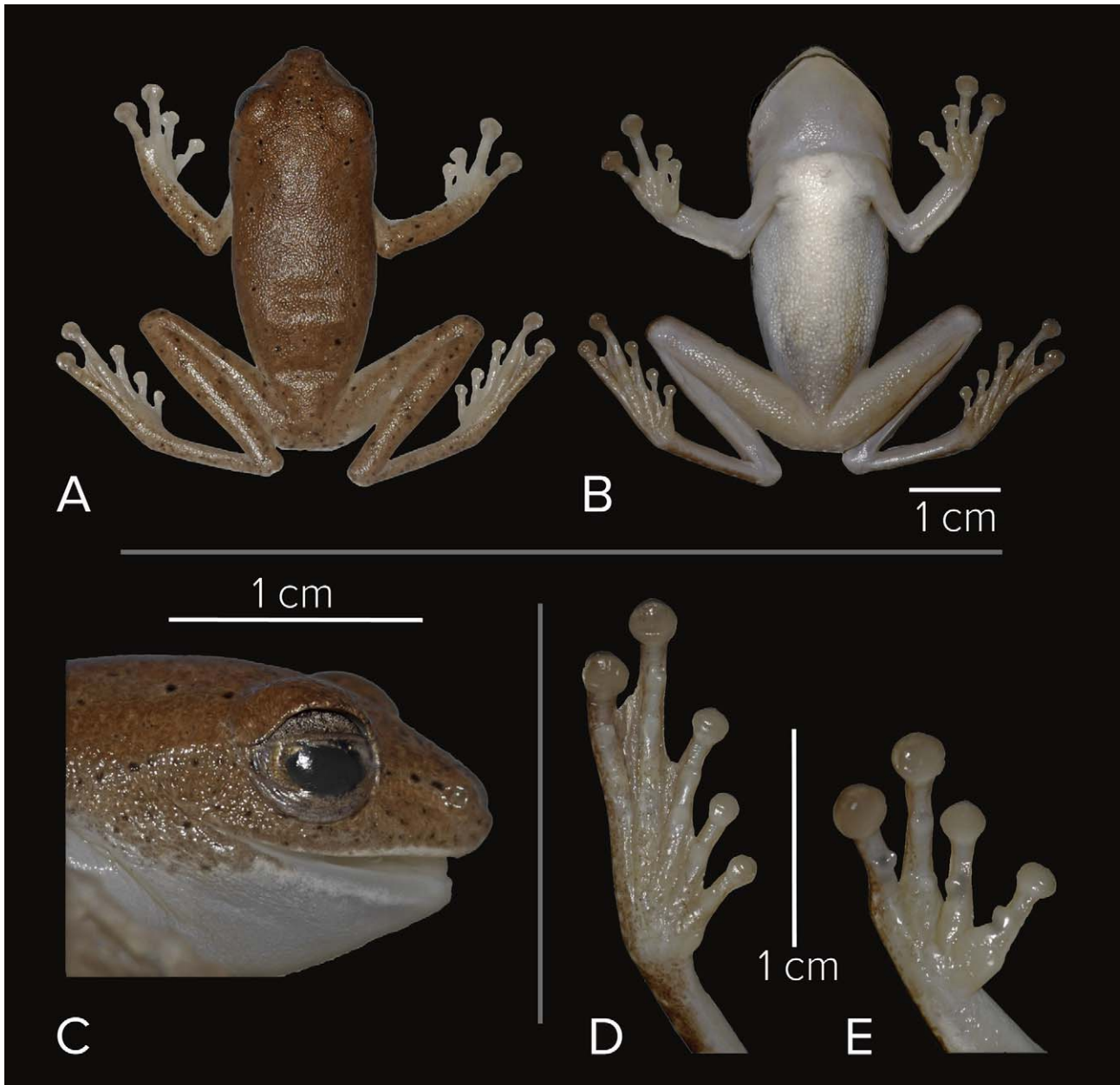
FIGURE 5. Holotype of *Mercurana myristicapalustris* **gen. et sp. nov. A.** dorsal view; **B.** ventral view; **C.** lateral view of head; **D.** ventral view of foot; **E.** ventral view of hand.

Description of Holotype: A slender, medium sized frog (average male SVL 35.4mm); dorsal skin texture shagreened; ventral surface including chest, belly and posterior surface of thighs coarsely glandular; throat slightly glandular; lateral profile of snout rounded and protruding; canthus rostralis indistinct, loreal region obtusely concave; pupil oval and horizontal; tympanum rounded and faintly distinct, faint supratympanic fold from back of eye to shoulder; weakly-developed vomerine teeth oblique and anterior to choanae; tongue lanceolate; lingual papilla absent; small vocal sac; fingers free, toes 3/4ᵗʰ webbed (from inner aspect of disc on Toe V reaching up to the distal subarticular tubercle of Toe IV, resuming from penultimate subarticular tubercle of Toe IV to distal subarticular tubercle of Toe III); subarticular tubercles prominent, rounded, except on finger IV, which is strongly bifid; supernumerary tubercles well developed; discs well developed and distinct; Wolffian duct simple, tubular (not illustrated, but similar to that of *Beddomixalus;* Fig. 6).

Colour in life: Male: Dorsal surface of forearm, hindlimbs and entire body rusty-brown with small black speckles scattered throughout; handful of tiny, sparsely spaced yellow blotches on dorsum; axillary region on the underside of arms white, suffused with yellow splotches; upper-arm from shoulder to elbow and hand yellowish;

flank white with diffused yellow on groin; upper lip whitish; ventral surface uniformly off-white; forelimbs and hindlimbs without any crossband pattern; anterior and posterior border and ventral surface of thigh whitish; vent fold absent; iris silvery-brown with a yellowish tinge. In non-breeding condition, the dorsum of the male is pale yellowish-brown, but with the black speckles. Female: pale greenish-yellow dorsum; ventral surface white.

Geographic distribution and habitat: The species *Mercurana myristicapalustris* was documented only in lowland swampy areas of the western foothills of the Agasthyamalai Hill Range in Kerala (see Fig. 7). These areas are dominated by *Myristica* swamp formations (Fig. 4A), a unique biotope of the Western Ghats. The type locality is outside the protected area network, but at the periphery of the Kulathupuzha Reserve Forest. We have also recorded the species in lowland areas of the Shendurney Wildlife Sanctuary in Kollam district (8.8678N, 77.0939E; 188m ASL) and further south in Peppara Wildlife Sanctuary, Thiruvananthapuram district (8.6697N, 77.1197E; 208m ASL).

Breeding behaviour: The frog *Mercurana myristicapalustris* is an arboreal species whose breeding behaviour is remarkably similar to that of *Beddomixalus bijui*, in terms of large male aggregations at breeding sites during the pre-monsoons and oviposition on the swamp floor. Vocalizing males (Fig. 4C) (audio file of call deposited in the Dryad Repository as Supplementary file 2; http://dx.doi.org/10.5061/dryad.pm166) call from atop perches inside the swamp forest with standing water or intermittent streams, at 2-5m height from the forest floor. As the night progresses, the males make their way slowly towards the forest floor, where competition for space is greater and on one occasion, we witnessed physical combat between males. We were able to make only a brief observation of amplectic behaviour in *M. myristicapalustris*. A male and female in amplexus were seen descending to the swamp floor, from the base of a shrub. On reaching the ground, the pair moved into the leaf litter where both individuals slowly changed colour, becoming almost inconspicuous on the forest floor. The female was seen digging into the slushy soil and ovipositing into the resulting shallow burrow in the mud, following which she mixed the semiterrestrial eggs with muddy soil. Digging and mixing was aided by employing her well-developed hindlimb webbing. Ensuing oviposition, the pair moved away, after which they could not be relocated. Photographic documentation of breeding behaviour was not possible because of failure of camera equipment at the time. However, clutches of developing eggs washed loosely in the swamp floor along a rivulet, and a single early stage tadpole (Fig. 4D), which did not yield an accurate LTRF, were photographed after a few days of pre-monsoon showers. The tadpole was sequenced to determine species identity, and confirmed to be that of *Mercurana*.
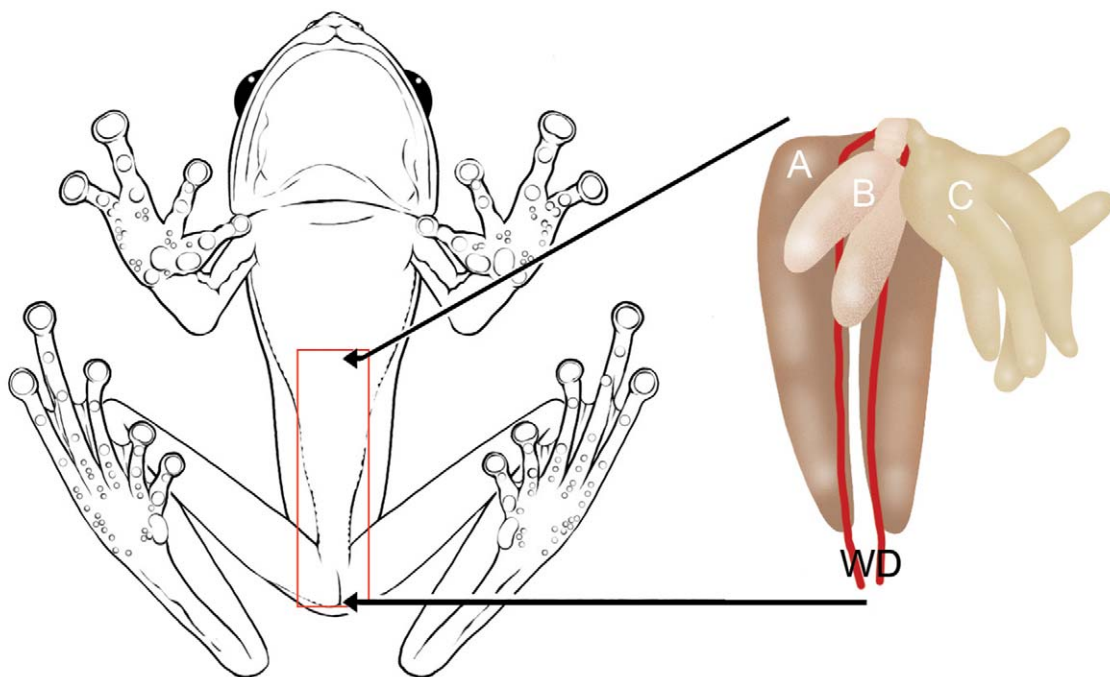

FIGURE 6. Diagram of a pair of simple tubular Wolffian Ducts and associated organs of a male *Beddomixalus bijui*. Notations: **A** = Kidneys, **B** = Testes, **C** = Fat Bodies, **WD** = Wolffian Duct.

TABLE 1. Morphometric measurements (mm) of specimens studied.

Museum Voucher. No.	Sex	SVL	HW	HL	IUE	UEW	SL	EL	FLL	HaL	TL	ShL	TaL	FoL
Beddomixalus bijui														
ZSI/WGRC/V/ A/842	Male	40.1	14.1	12.0	4.7	3.6	5.7	5.0	9.0	11.6	20.0	20.0	11.0	17.1
ZSI/WGRC/V/ A/843	Male	42.0	14.4	12.0	4.7	3.9	5.6	5.0	8.9	12.0	20.0	21.4	11.0	17.3
ZSI/WGRC/V/ A/844	Male	40.3	14.3	11.9	4.4	3.8	5.7	5.0	8.9	12.1	20.0	20.1	11.0	17.2
ZSI/WGRC/V/ A/849	Female	60.8	24.4	21.6	7.3	5.3	8.1	6.5	13.8	18.8	32.1	32.3	17.7	28.5
Mercurana myristicapalustris														
Holotype TNHM (H) 12.6.18/69	Male	35.4	11.9	9.8	4.1	3.0	5.2	5.1	8.4	8.2	17.8	17.7	9.8	13.0
TNHM (H) 12.6.18/70	Male	35.1	12.9	12.0	4.3	2.8	5.0	5.0	8.5	8.0	21.1	18.2	11.7	14.5
TNHM (H) 12.6.18/74	Male	37.8	14.1	15.7	4.8	4.2	5.6	5.5	9.9	10.6	18.7	20.5	12.0	13.4
Mean		36.1	13.0	12.5	4.4	3.4	5.2	5.2	8.9	8.9	19.2	18.8	11.2	13.6

Beddomixalus bijui Tadpole														
Gosner Stage 38		TL	BL	TAL	BW	BH	NSD	END	TMH	TMW	SSD	IND	IOD	ED
TNHM (H) 12.6.22/73		29.1	9.9	20.3	5.6	4.1	1.8	1.1	2.7	2.4	7.0	1.7	1.5	1.5

Abbreviations for adult measurements: SVL = snout-vent length; HW = head width, at angle of jaw; HL = head length, from rear of mandible to tip of snout; IUE = inter upper eyelid width, the shortest distance between the upper eyelids; UEW = maximum upper eyelid width; SL = snout length, from tip of snout to anterior orbital border of eye; EL = eye length; FLL = forelimb length, from elbow to base of outer palmar tubercle; HaL = hand length, from base of outer palmar tubercle to tip of third finger; TL = thigh length; ShL = shank length; Tal = tarsal length; FoL = foot length, from base of inner metatarsal tubercle to tip of fourth toe. Abbreviations for tadpole measurements: TL = total length; BL = body length; TAL = tail length; BW = body width; BH = body height; NSD = naris-snout distance; END = eye-naris distance; TMH = tail muscle height at the base of the tail; TMW = tail muscle width; SSD = snout-spiracle distance; IND = internarial distance; IOD = interorbital distance; ED = eye diameter.

Discussion

Here, we describe two new genera, adding to the known diversity of South Asian Rhacophoridae. Astonishingly, we find that *Beddomixalus* gen. nov. proposed here for *Polypedates bijui* Zachariah et al., 2011 and *Mercurana myristicapalustris* gen. et sp. nov actually represent two ancient, independent clades, which are the sister groups, respectively, to the Sri Lankan and Indian-Chinese-Indochinese radiations of bushfrogs (*Pseudophilautus + Raorchestes*) (Fig. 1). This supports previous findings that the region is a centre of megadiversity and endemism (Bossuyt *et al.*, 2004; Meegaskumbura *et al.*, 2002), and suggests that yet further obscure taxa potentially remain to be discovered. More importantly, the region apparently harbors numerous relict clades above the species level, as the new genera described here demonstrate. Although we cannot reject a sister-clade relationship to *Raorchestes* or *Pseudophilautus* for *Mercurana*, our analyses indicate that it forms a distinct clade that is not nested among species currently allocated to these genera. Recognition of a new genus for species that possess a distinctive breeding mode, restricted distribution and other phylogenetically distinct characters is thus consistent with the generic status of other branches of this radiation.

Consistent with phylogenetic results in previous studies, our larger tree (Appendix 2) corroborates the polyphyletic nature of the Asian bushfrog clade (*Philautus*, *Raorchestes* and *Pseudophilautus*) with these genera nested amongst *Kurixalus* and the two newly described genera. This large clade is united by the presence in males,

of a simple, tubular Wolffian duct (Fig. 6) (Grosjean *et al.*, 2008; Liem, 1970), as compared to the convoluted form in foam-nesting rhacophorids. Liem (1970) and Grosjean *et al.* (2008) have justified using the shape of the Wolffian duct as a valid taxonomic character in Rhacophoridae, helping to distinguish between foam-nesting and non-foam-nesting taxa. Species of the genera *Polypedates, Rhacophorus, Ghatixalus* and *Taruga*, which occur in Southern India and Sri Lanka, are foam nesters (Biju, Roelants, & Bossuyt, 2008; Grosjean *et al.*, 2008; Liem, 1970; Meegaskumbura *et al.*, 2010), while *Pseudophilautus* and *Raorchestes* are direct developers (Bahir *et al.* 2005; Biju *et al.* 2010). In overall morphology, both *Beddomixalus bijui* and *Mercurana myristicapalustris* resemble foam-nesting rhacophorids, including in size dimorphism between males and females, the reason for the earlier allotment of the former to *Polypedates* based solely on morphological characters (Zachariah *et al.*, 2011). Such purely morphology-based classifications of amphibians have often been misled by plesiomorphic traits (Köhler & Günther, 2008).
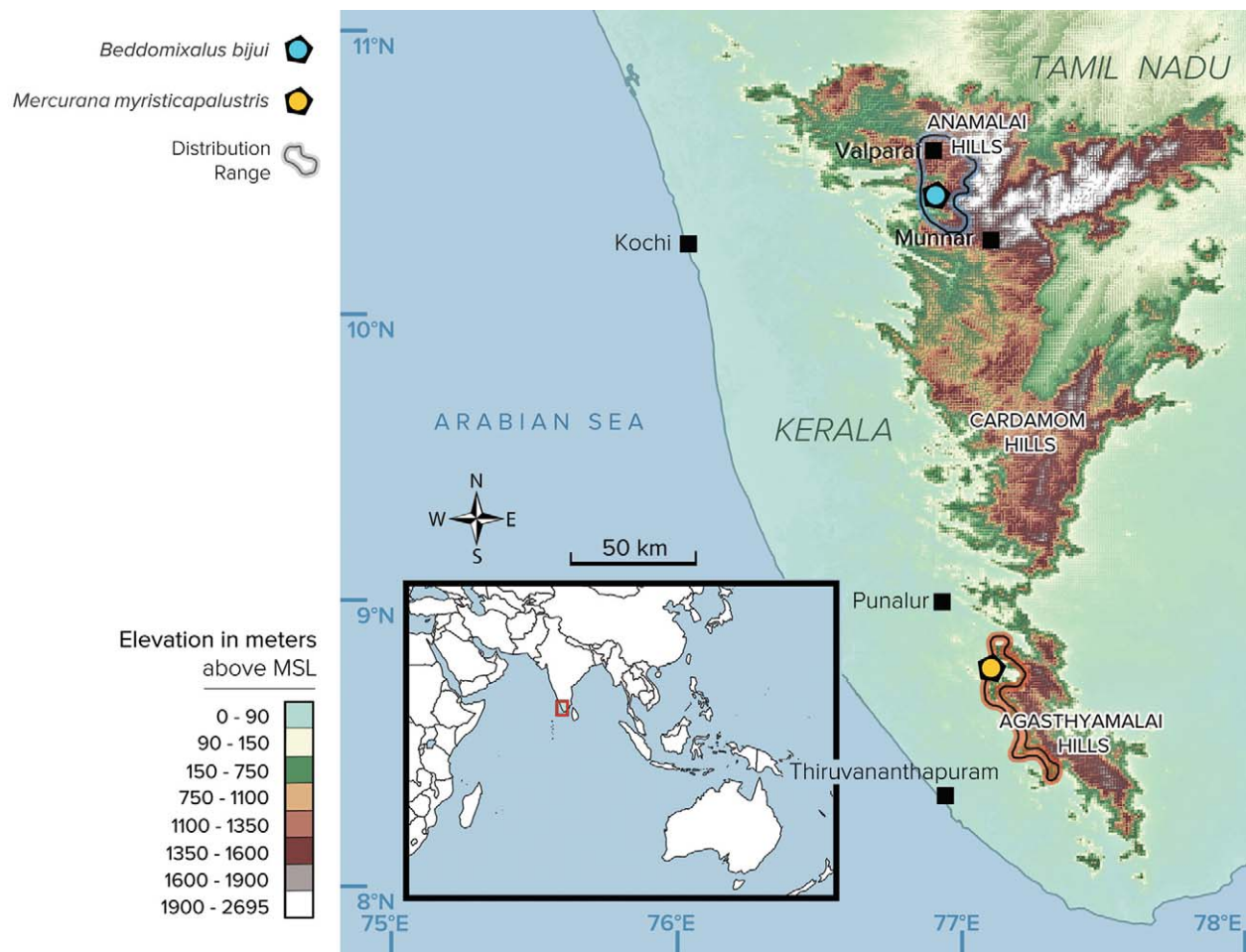

FIGURE 7. Map showing distribution of *Mercurana myristicapalustris* **gen. et sp. nov.** and *Beddomixalus* **gen. nov.** *bijui* in the southern Western Ghats, India; coloured markers indicate type localities.

But, despite the *Polypedates*-like external bauplan, and similarity of the tadpole to that of *Rhacophorus* (Altig & McDiarmid, 1999b), *Beddomixalus bijui* and *Mercurana myristicapalustris* are significantly different from species of other South Asian rhacophorid genera. And though both genera are superficially similar to each other in being sexually dimorphic (especially in colour and body size) and also in overall habitat and breeding preferences, they are also very different to each other in many aspects (see diagnosis of *M. myristicapalustis* gen. et sp. nov). While *Beddomixalus* abandons its terrestrial non-pigmented eggs exposed on the substrate surface of swamps, *Mercurana* mixes its non-pigmented eggs with mud in self-made shallow burrows, making them relatively inconspicuous. Similar oviposition behaviour in swampy terrain, akin to these novel rhacophorid genera, has also been documented in the genera *Mantella* and *Mantidactylus* (*Brygoomantis*) of the family Mantellidae of Madagascar (Miguel Vences, pers. comm; Glaw & Vences, 1994). But, within Rhacophoridae, *Kurixalus* (*Chirixalis) idiootocus* from Taiwan has been recorded to lay pigmented eggs in shallow depressions or holes at the

edges of temporary ponds (Kuramoto and Wang, 1987). Embryonic development in this species is apparently stimulated by heavy rain, with the tadpoles completing their development in a pond, just like in the new genera described here. Also, *Kurixalus appendiculatus* (Yu, Zhang & Yang, 2013) is known to occupy swamp forests and produce breeding choruses (Diesmos et al, 2004) just as observed in the new genera. The description of the habitat preferences and breeding behaviour of the recently described *Polypedates ranwellai* (Wickramasinghe *et al.*, 2012) from Sri Lanka resembles that of *Mercurana*, but including this species in future phylogenetic analysis is necessary to discern its actual taxonomic position. So, within the larger paraphyletic clade comprising *Philautus*, *Raorchestes*, *Pseudophilautus, Kurixalus, Beddomixalus* and *Mercurana,* unprotected terrestrial oviposition with aquatic tadpoles is the reproductive mode assumed by the latter three genera, while direct development seems to have evolved independently, twice among *Philautus* and *Raorchestes + Pseudophilautus* (see Appendix 2). It is imperative to have a clear understanding of the reproductive behaviours in the other *Kurixalus* species, in order to help elicit clear relationships between phylogenetic history and evolution of reproductive modes in this larger rhacophorid clade. Gomez-Mestre, Pyron, & Wiens (2012) found evidence suggesting that many seemingly intermediate reproductive modes (e.g., terrestrial eggs, endotrophic larvae, protected eggs and larvae) are sometimes skipped in the sequence leading to direct development and vice versa. Such rapid transitions may have resulted in the contrasting reproductive strategies we see within different paraphyletic sub-clades of Rhacophoridae.

The reproductive strategy of *Beddomixalus* and *Mercurana* can be noted among the South Asian rhacophorids in terms of its strong association with the climatic conditions of the region. With tadpole development being dependent on ephemeral swamp pools, where oviposition is triggered at the onset of the pre-monsoons (immediately before the pools have water in them), the breeding cycles of both new genera is finely attuned to the arrival of the southwest monsoon, akin to *Nasikabatrachus sahyadrensis* (Zachariah *et al.*, 2012). Early breeding in these frogs may be a mechanism to establish motile larvae ahead of establishment of predators and a competitive aquatic community (in contrast to foam-nesters and direct developers who guarantee protection of early stage larvae in foam nests and by egg attendance, respectively). But, such a distinct reproductive mode, in combination with a narrow breeding season and specialized breeding habitats may have also limited diversification in these clades, unlike direct developing taxa, which can potentially disperse widely and colonize multiple wide-ranging, humid tropical habitats. Hence, despite being sister clades to the speciose bushfrog radiation of South Asia, both newly identified clades are so far monotypic, with only a singular representative species for each genus known.

With the addition of more taxa which had previously not been included in a comprehensive molecular analysis of the family, our results point to the need for further morphological, behavioural and molecular work, which is essential to fully delineate diversity in the group at the genus level. Distressingly though, these groups (and many other Sri Lanka-Western Ghats taxa) are similar to other diverse global amphibian assemblages in facing severe pressure from habitat loss and other anthropogenic disturbances (Stuart *et al.*, 2004), and many have already been lost (Meegaskumbura, Manamendra-Arachchi, Schneider, & Pethiyagoda, 2007). The habitats of both new genera are greatly threatened, consequently endangering them as well. The lowland forests of the western slopes of the Agasthyamalai Hills, which is the only known habitat of *Mercurana myristicapalustris*, have been reduced to a handful of fragmented forest patches, including inside protected areas, as a result of widespread habitat conversion. Similarly, the habitat of *Beddomixalus bijui* is also under threat. The type specimen was recorded in disturbed forest in a cardamom plantation in the High Ranges of Idukki, Kerala. Habitat in the Valparai plateau is also highly fragmented by tea plantations. It is imperative that these threatened ecosystems are protected and conservation programs be initiated to restore critical habitats of such highly endemic and evolutionarily distinct taxa.

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Species	12S	16S	BDNF	POMC	RAG1	RHOD	TYR
Buergeria buergeri	AB127977	AB127977	AB728215	AB728249	AB72871	AB728288	AB612033
Buergeria japonica	DQ283055	DQ283055	GQ285691	GQ285722	GQ285754	AY880624	GQ285801
Buergeria oxycephala	EU215524	EU215524	GQ285695	GQ285726	GQ285758	EU924536	EU924564
Buergeria robusta	AF458125	AF458125	--	--	--	--	--
Chiromantis doriae	DQ283135	DQ283135	GQ285716	GQ285747	GQ285779	DQ347379	EU215584
Chiromantis nongkhorensis	GQ204774	GQ204723	GQ204475	--	GQ204604	GQ204659	--
Chiromantis petersii	GQ204784	GQ204733	--	--	--	--	--
Chiromantis rufescens	DQ347004	AF458126	GQ204476	--	GQ204605	AY341807	DQ347139
Chiromantis vittatus	DQ283134	GQ285684	GQ285711	GQ285742	GQ204603	GQ285793	GQ285811
Chiromantis xerampelina	AF458132	AF458132	--	--	--	DQ284012	--
Feihyla palpebralis	GQ285681	GQ285681	GQ285709	GQ285740	GQ285772	GQ285792	GQ285810
Ghatixalus asterops	EU178098	EU178091	--	--	--	--	--
Ghatixalus variabilis	EU178099	EU178092	--	--	--	--	--
Gracixalus ananjevae	--	JN862546	--	--	--	--	--
Gracixalus carinensis	GQ285670	GQ285670	GQ285699	GQ285730	GQ285762	GQ285788	GQ285806
Gracixalus gracilipes	DQ283051	DQ283051	GQ285701	GQ285732	GQ285764	DQ283780	EU924565
Gracixalus guyeti	--	EU871429	--	--	--	--	--
Gracixalus jinxiuensis	EU215525	EU215525	GQ285700	GQ285731	GQ285763	EU215557	EU924571
Gracixalus quangi	--	JN862541	--	--	--	--	--
Kurixalus banaensis	GQ285667	GQ285667	GQ285689	GQ285720	GQ285752	GQ285781	GQ285799
Kurixalus bisacculus	GU227280	GU227334	--	--	--	--	--
Kurixalus eiffingeri	DQ283122	DQ283122	--	--	--	DQ283830	DQ282931
Kurixalus idiootocus	DQ283054	DQ283054	GQ285688	GQ285719	GQ204569	DQ283783	EU215607
Kurixalus odontotarsus	EU215549	EU215549	GQ285687	GQ285718	GQ285750	AY880638	EU924566
Kurixalus verrucosus	EU178093	AF285226	--	--	--	--	--
Liuixalus ocellatus	--	GQ285672	--	--	--	--	--
Liuixalus romeri	EU215528	EU215528	GQ285693	GQ285724	GQ285756	EU215559	EU924570
Mantella madagascariensis	NC_007888	NC_007888	AB612046	HM998971	DQ019500	AY263284	AB612045
Nyctixalus margaritifer	EU178094	EU178087	--	--	--	--	--
Nyctixalus pictus	DQ283133	AF458135	GQ285698	GQ285729	GQ285761	AY880634	GQ285805

APPENDIX 1. (Continued)

Species	12S	16S	BDNF	POMC	RAG1	RHOD	TYR
Nyctixalus spinosus	DQ283114	DQ283114	--	--	--	DQ283827	--
Philautus abditus	GQ285673	GQ285673	GQ285712	GQ285743	GQ285775	GQ285794	GQ285812
Philautus acutirostris	AY326059	AF458137	--	--	--	--	--
Philautus acutus	JN705337	JN705366	--	--	--	--	--
Philautus aurantium	JN705338	JN705367	GQ204460	--	GQ204587	GQ204642	--
Philautus aurifasciatus	AY141804	GQ204702	GQ204458	--	GQ204584	GQ204640	--
Philautus bunitus	JN705339	JN705368	--	--	--	--	--
Philautus davidlabangi	JN705357	JN705387	--	--	--	--	--
Philautus disgregus	GQ204755	GQ204704	GQ204459	--	GQ204586	GQ204641	--
Philautus everetti	--	JN705377	--	--	--	--	--
Philautus hosii	JN705353	JN705384	--	--	--	--	--
Philautus ingeri	JN705354	JN705385	GQ204461	--	GQ204588	AY880629	--
Philautus juliandringi	JN705347	JN705378	--	--	--	--	--
Philautus macroscelis	JN705345	JN705375	--	--	--	--	--
Philautus mjobergi	JN705349	JN705380	GQ204463	--	GQ204590	GQ204644	--
Philautus petersi	AF026349	JN705381	--	--	--	--	--
Philautus refugii	JN705351	JN705383	--	--	--	--	--
Philautus surdus	AF458138	AF458138	--	--	--	--	--
Philautus tectus	JN705341	JN705369	--	--	--	--	--
Philautus umbra	JN705348	JN705379	--	--	--	--	--
Philautus worcesteri	GQ204758	GQ204707	GQ204462	--	GQ204589	GQ204643	--
Polypedates braueri	AB728015	AB728015	AB728194	AB728234	AB728252	AB728274	AB728308
Polypedates colletti	AB728189	AB728189	AB728212	AB728246	GQ204579	AB728285	AB728319
Polypedates cruciger	DQ346973	AF215357	JQ073140	HM998970	HQ260712	AF249124	AF249187
Polypedates leucomystax	AB728115	AB728115	AB728202	AB728241	GQ204583	AB728279	AB728313
Polypedates macrotis	AB728187	AB728187	AB728210	AB728244	GQ204577	AB728283	AB728317
Polypedates maculatus	AB728188	AB728188	AB728211	AB728245	GQ204575	AB728284	AB728318
Polypedates megacephalus	AB728091	AB728091	AB728201	AB728239	AB728259	GQ285791	AB728311
Polypedates mutus	EU215551	HQ588946	GQ285707	GQ285738	GQ285770	EU215581	EU215611
Polypedates otilophus	AB728190	AB728190	AB728213	AB728247	GQ204578	AB728286	AB728320

Species	12S	16S	BDNF	POMC	RAG1	RHOD	TYR
Pseudophilautus amboli	--	EU450025	--	--	--	--	--
Pseudophilautus asankai	FJ788141	FJ788160	--	--	--	--	--
Pseudophilautus cavirostris	GQ204737	FJ788156	GQ204439	--	GQ204561	GQ204622	--
Pseudophilautus decoris	FJ788144	FJ788163	--	--	--	--	--
Pseudophilautus femoralis	AY141787	AY141833	--	--	--	--	--
Pseudophilautus folicola	--	GQ204680	--	--	GQ204564	GQ204625	--
Pseudophilautus hankeni	GU593346	GU593348	--	--	--	--	--
Pseudophilautus hoffmanni	GQ204736	GQ204673	GQ204436	--	GQ204558	GQ204619	--
Pseudophilautus kani	--	EU449994	--	--	GQ204553	--	--
Pseudophilautus limbus	--	GQ204668	--	--	GQ204560	GQ204621	--
Pseudophilautus lunatus	FJ788150	GQ204675	GQ204438	--	GQ204563	DQ019566	AF249189
Pseudophilautus microtympanum	DQ346974	AF249046	GQ204441	--	GQ204565	GQ204626	--
Pseudophilautus mittermeieri	GQ204741	GQ204681	GQ204443	--	--	--	--
Pseudophilautus mooreorum	FJ788134	FJ788153	--	--	--	--	--
Pseudophilautus ocularis	FJ788145	FJ788164	--	--	--	--	--
Pseudophilautus papillosus	FJ788151	FJ788170	--	--	--	--	--
Pseudophilautus pleurotaenia	FJ788146	FJ788165	--	--	GQ204555	GQ204616	--
Pseudophilautus poppiae	FJ788136	GQ204670	GQ204437	--	--	--	--
Pseudophilautus popularis	FJ788149	FJ788168	--	--	GQ204559	GQ204620	--
Pseudophilautus reticulatus	--	GQ204674	GQ204435	--	GQ204552	GQ204614	--
Pseudophilautus sarasinorum	--	GQ204667	GQ204442	--	GQ204554	GQ204615	--
Pseudophilautus schmarda	AY880617	GQ204669	--	--	--	--	--
Pseudophilautus schneideri	GU593345	GU593347	--	--	--	GQ204624	--
Pseudophilautus simba	GQ204740	GQ204679	--	--	GQ204557	GQ204618	--
Pseudophilautus steineri	FJ788138	FJ788157	--	--	--	--	--
Pseudophilautus stuarti	GQ204735	GQ204672	--	--	GQ204568	AY364384	AF249190
Pseudophilautus tanu	FJ788152	FJ788171	--	--	GQ204556	GQ204617	--
Pseudophilautus wynaadensis	DQ346966	GQ204685	AB612040	AY819103	AB612037	DQ283926	DQ360044
Pseudophilautus zorro	FJ788147	GQ204671					
Rana catesbeiana	M57527	M57527					

Species	12S	16S	BDNF	POMC	RAG1	RHOD	TYR
Raorchestes amandalii	AY880588	--	--	--	--	--	--
Raorchestes beddomii	--	EU450013	--	--	--	--	--
Raorchestes bobingeri	--	AB530558	--	--	--	--	--
Raorchestes bombayensis	--	EU450019	--	--	--	--	--
Raorchestes charius	DQ346967	AY141840	--	--	--	AF249128	AF249191
Raorchestes chlorosomma	--	EU450017	--	--	--	--	--
Raorchestes chotta	--	EU450022	--	--	--	--	--
Raorchestes chromasynchysi	--	EU450018	--	--	--	--	--
Raorchestes coonoorensis	--	EU449999	--	--	--	--	--
Raorchestes dubois	--	EU449996	--	--	--	--	--
Raorchestes glandulosus	--	EU450020	--	--	--	--	--
Raorchestes graminirupes	--	EU450015	--	--	--	--	--
Raorchestes griet	AY706108	EU449997	--	--	--	--	--
Raorchestes gryllus	GQ285674	GQ285674	GQ285714	GQ285745	GQ285777	GQ285796	GQ285814
Raorchestes jayarami	--	EU450023	--	--	--	--	--
Raorchestes kaikatti	--	EU450021	--	--	--	--	--
Raorchestes longchuanensis	GQ285675	GQ285675	GQ285713	GQ285744	GQ285776	GQ285795	GQ285813
Raorchestes luteolus	AB167904	AB530554	--	--	--	--	--
Raorchestes marki	--	EU450028	--	--	--	--	--
Raorchestes menglaensis	GQ285676	GQ285676	GQ285715	GQ285746	GQ285778	EU924544	GQ285815
Raorchestes munnarensis	--	EU450016	--	--	--	--	--
Raorchestes nerostagona	--	EU450012	--	--	--	--	--
Raorchestes ponmudi	--	EU450011	--	--	--	--	--
Raorchestes resplendens	--	GU808563	--	--	--	--	--
Raorchestes signatus	AY141795	GQ204684	GQ204444	--	GQ204567	GQ204629	--
Raorchestes sushili	--	EU450027	--	--	--	--	--
Raorchestes tinniens	AY706112	EU450001	--	--	--	--	--
Raorchestes travancoricus	--	EU450029	--	--	--	--	--
Raorchestes tuberohumerus	--	EU450004	--	--	--	--	--
Rhacophorus angulirostris	JN705322	JN377348	--	--	--	--	--

Species	12S	16S	BDNF	POMC	RAG1	RHOD	TYR
Rhacophorus arboreus	AF458142	AF458142	--	--	--	AY880653	--
Rhacophorus belalongensis	JN705324	JN377352	--	--	--	--	--
Rhacophorus bipunctatus	AY843750	JX219444	GQ204469	--	--	AY844737	EU924574
Rhacophorus burmanus	JX219422	JX219422	--	--	--	EU215567	EU924589
Rhacophorus calcaneus	DQ283380	DQ283380	--	--	GQ204600	DQ283999	DQ282991
Rhacophorus chenfui	JX219431	JX219431	GQ204467	--	GQ204594	EU215564	EU924575
Rhacophorus chuyangsinensis	JX219451	JX219451	--	--	--	--	--
Rhacophorus cyanopunctatus	--	JN377367	--	--	--	--	--
Rhacophorus dennysi	JX219433	JX219433	HM998941	HM998972	DQ019512	EU924548	EU924576
Rhacophorus dorsoviridis	JX219427	JX219427	--	--	--	--	--
Rhacophorus duboisi	JX219413	JX219413	--	--	--	--	--
Rhacophorus dugritei	JN688875	JN688875	GQ285705	GQ285736	GQ285768	EU215571	EU924577
Rhacophorus dulitensis	JX219434	JX219434	--	--	GQ204597	GQ204651	--
Rhacophorus exechopygus	--	GQ469979	--	--	--	--	--
Rhacophorus fasciatus	JN705331	JN377356	--	--	--	--	--
Rhacophorus feae	EU215544	EU215544	--	--	--	EU215574	EU924578
Rhacophorus gauni	JX219456	JX219456	--	--	GQ204596	GQ204650	--
Rhacophorus harrissoni	JN705332	JN377358	--	--	--	--	--
Rhacophorus hongchibaensis	JN688883	JN688883	--	--	--	JN688897	JN688906
Rhacophorus hui	JN688877	JN688877	--	--	--	JN688901	JN688911
Rhacophorus hungfuensis	JN688879	JN688879	--	--	--	EU215568	EU215598
Rhacophorus kajau	--	JN377362	--	--	--	--	--
Rhacophorus kio	EU215532	EU215532	GQ285703	GQ285734	GQ285766	EU215562	EU215592
Rhacophorus lateralis	AY880612	AB530548	--	--	--	AY880655	--
Rhacophorus malabaricus	DQ346957	AB530549	--	--	AY948912	AY880656	AF249188
Rhacophorus maximus	JX219411	JX219411	--	--	--	EU924552	EU924580
Rhacophorus minimus	EU215539	EU215539	--	--	--	EU924553	EU924581
Rhacophorus moltrechti	AF458145	EU215543	--	--	--	EU215573	EU215603
Rhacophorus monticola	AY326060	AY326060	--	--	--	--	--

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Species	12S	16S	BDNF	POMC	RAG1	RHOD	TYR
Rhacophorus nigropalmatus	JX219437	JX219437	GQ204465	--	GQ204592	GQ204646	--
Rhacophorus omeimontis	JX219421	JX219421	--	--	--	EU924556	EU924584
Rhacophorus orlovi	DQ283049	DQ283049	--	--	--	DQ283778	--
Rhacophorus pardalis	JX219454	JX219454	GQ204466	--	GQ204593	GQ204647	--
Rhacophorus penanorum	JN705323	JN377350	--	--	--	--	--
Rhacophorus puerensis	JN688893	JN688893	--	--	--	EU215572	JN688908
Rhacophorus reinwardtii	JX219443	JX219443	GQ204472	--	GQ204601	EU924559	EU924587
Rhacophorus rhodopus	JX219442	JX219442	--	--	--	EU215561	EU215591
Rhacophorus rufipes	JX219455	JX219455	--	--	--	--	--
Rhacophorus schlegelii	NC_007178	NC_007178	--	--	--	AY880658	--
Rhacophorus translineatus	JX219449	JX219449	--	--	--	--	--
Rhacophorus vampyrus	--	HQ656818	--	--	--	--	--
Rhacophorus verrucopus	JX219436	JX219436	--	--	--	--	--
Rhacophorus wui	JN688881	JN688881	--	--	--	JN688896	JN688910
Taruga eques	AY880469	GQ204689	GQ204447	--	GQ204571	GQ204633	--
Taruga fastigo	AY880562	GQ204690	GQ204448	--	GQ204572	GQ204634	--
Taruga longinasus	GQ204745	GQ204691	GQ204449	--	GQ204573	GQ204635	--
Theloderma asperum	AF458148	AF458148	GQ285697	GQ285728	GQ285760	GQ285786	GQ285804
Theloderma bicolor	AY880616	AY880529	--	--	--	AY880659	--
Theloderma corticale	DQ283050	DQ283050	--	--	--	DQ283779	DQ282904
Theloderma gordoni	--	JN688167	--	--	--	--	--
Theloderma moloch	GQ285679	GQ285679	GQ285690	GQ285721	GQ285753	GQ285782	GQ285800
Theloderma nebulosum	--	JN688168	--	--	--	--	--
Theloderma palliatum	--	JN688172	--	--	--	--	--
Theloderma rhododiscus	EU215530	DQ283393	GQ285696	GQ285727	GQ285759	DQ284007	DQ282998
Theloderma stellatum	--	JN688170	--	--	--	--	--
Theloderma truongsonense	--	JN688174	--	--	--	--	--
Beddomixalus bijui	KC594292	KC594291	--	--	KC594289	KC594290	--
Mercurana myristicapalustris	KC594296	KC594295	--	--	KC594293	KC594294	--

APPENDIX 2. Full ML phylogeny for Rhacophoridae (outgroups not shown), showing BS proportions >50%, and well-supported (BS >70%) branches indicated with bold lines. Red arrow indicates clade containing novel genera.

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