

## Lineage delimitation and description of nine new species of bush frogs (Anura: *Raorchestes*, Rhacophoridae) from the Western Ghats Escarpment

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### Abstract

Bush frogs of the genus *Raorchestes* are distributed mainly in the Western Ghats Escarpment of Peninsular India. The inventory of species in this genus is incomplete and there is ambiguity in the systematic status of species recognized by morphological criteria. To address the dual problem of taxon sampling and systematic uncertainty in bush frogs, we used a large-scale spatial sampling design, explicitly incorporating the geographic and ecological heterogeneity of the Western Ghats. We then used a hierarchical multi-criteria approach by combining mitochondrial phylogeny, genetic distance, geographic range, morphology and advertisement call to delimit bush frog lineages. Our analyses revealed the existence of a large number of new lineages with varying levels of genetic divergence. Here, we provide diagnoses and descriptions for nine lineages that exhibit divergence across multiple axes. The discovery of new lineages that exhibit high divergence across wide ranges of elevation and across the major massifs highlights the large gaps in historical sampling. These discoveries underscore the significance of addressing inadequate knowledge of species distribution, namely the “Wallacean shortfall”, in addressing the problem of taxon sampling and unknown diversity in tropical hotspots. A biogeographically informed sampling and analytical approach was critical in detecting and delineating lineages in a consistent manner across the genus. Through increased taxon sampling, we were also able to discern a number of well-supported sub-clades that were either unresolved or absent in earlier phylogenetic reconstructions and identify a number of shallow divergent lineages which require further examination for assessment of their taxonomic status.

**Key words:** Species delimitation, *Raorchestes*, Multi-criteria, Wallacean shortfall, Taxon sampling, Western Ghats

## Introduction

Bush frogs refer to a group of frog species distributed in South and Southeast Asia that were until recently all assigned to the genus *Philautus* (Biju & Bossuyt, 2009; Bossuyt & Dubois, 2001). Recent molecular phylogenetic reconstructions have shown that these frogs are polyphyletic and consist of three major clades, with the distribution of species concentrated in the island of Sri Lanka, Western Ghats Escarpment of Peninsular India and the island of Borneo (Yu *et al.*, 2009; 2010; Bossuyt *et al.*, 2004). The Sri Lankan clade has been assigned the generic name *Pseudophilautus* (Yu *et al.*, 2010), and a majority of the species in the Western Ghats Escarpment of Peninsular India, along with a few species distributed in Southeast Asia, have been assigned to the genus *Raorchestes* (Biju *et al.*, 2010). The third clade, with its distribution in the island of Borneo and other adjoining islands, has been assigned the generic name *Philautus* (Li *et al.*, 2009). *Pseudophilautus* and *Raorchestes* are sister clades, and recent reconstruction shows that they are polyphyletic with respect to *Philautus* (Li *et al.*, 2009).

The bush frog clades from South and Southeast Asia are nested within a larger clade, the family Rhacophoridae, which is largely composed of arboreal frogs (Yu *et al.*, 2009; Li *et al.*, 2009; Frost, 2014). Apart from the three bush frog genera, 14 other genera have been recognized under the family Rhacophoridae (Frost, 2014). Notably, the three bush frog clades comprise nearly half (180 species) of the total number of species (380 species) in this family at this time (Frost, 2014). However, the inventory of lineages of bush frogs from these centres of distribution is incomplete and several new species have been described recently, mainly from the Western Ghats and Sri Lanka (Biju & Bossuyt, 2009; Manamendra-Arachchi & Pethiyagoda, 2005; Meegaskumbura & Manamendra-Arachchi, 2005).

In the Western Ghats, the discovery of bush frogs has been sporadic and patchy over time since Jerdon's recognition of three species: *Ixalus* ? *glandulosa* (uncertain locality), *Phyllomedusa* ? *tinniensi* (from 'Neelgherries'), *Phyllomedusa* ? *wynaadensis* (uncertain locality) (Jerdon, 1854). In subsequent decades, a series of species were described by researchers affiliated to the British Museum (Günther, 1875; Boulenger, 1890; 1891). A majority of these descriptions, with the exception of Jerdon's personal collections and a single species collected by Ferguson (Boulenger, 1891), were based on specimens collected by Lt. Col. Beddome from the southern regions of the Western Ghats. In these early species descriptions, a lack of exact locality data and the use of broad regional names (e.g. Malabar, Travancore) inadvertently created confusion about taxonomy that has remained until recent times (Bossuyt & Dubois, 2001; Biju & Bossuyt, 2009).

Species discovery in this group in the early part of the 20<sup>th</sup> century continued, albeit sporadically, with Annandale's description of a species (Annandale, 1919), followed by Ahl's recognition of three species (under the generic name *Rhacophorus*) (Ahl, 1927). On the pretext of nomenclatural priority over *Ixalus*, Stejneger reallocated the members to a generic name *Philautus* Gistel, 1848, for bush frogs of South and Southeast Asia in 1905 (Bossuyt & Dubois, 2001). This was followed by the first contribution by an Indian researcher, C.R.N. Rao, who described eight species under the generic name *Philautus* based on his own field collections from parts of the central Western Ghats (Rao, 1937). In recognition of Rao's pioneering batrachological studies, Biju *et al.* (2010) coined the generic name *Raorchestes* for the members of the bush frog clade in the Western Ghats. Recent studies have found uncertainty in Rao's generic allocation of selected species under *Philautus*, which have been assigned to *Micrixalus* (Bossuyt & Dubois, 2001). Remarkably, Rao's work also marks the last publication in the 20<sup>th</sup> century on species descriptions of bush frogs in the Western Ghats. There were few or no attempts by taxonomists to revisit the type localities of specimens or attempt a revision, and most researchers who encountered bush frogs as part of other studies assigned available names to a number of potentially new species sampled by them (reviewed in Biju, 2001).

The beginning of the 21<sup>st</sup> century can be regarded as the most important period for bush frog discovery in the Western Ghats. Some of the findings over the previous decade (e.g. Biju & Bossuyt, 2009) overshadow the sporadic discoveries made during the last hundred and fifty years in this region. Reports on major discoveries of hundreds of potentially new species of frogs in the Western Ghats and Sri Lanka (Meegaskumbura *et al.*, 2002; Biju, 2001) likely provided a much needed impetus for renewed interest and exploration in this region. During this time, in a major nomenclatural revision of the bush frogs, Bossuyt and Dubois (2001) revisited all the valid names of *Philautus*, assigned neotypes for lost types, and provided morphological description of types (Bossuyt & Dubois, 2001). A number of isolated species descriptions resulted in a steady increase in the number of new species for the Western Ghats (Bossuyt, 2002; Kuramoto & Joshi, 2003; Gururaja, 2007; Biju & Bossuyt, 2005 a,b; 2006).

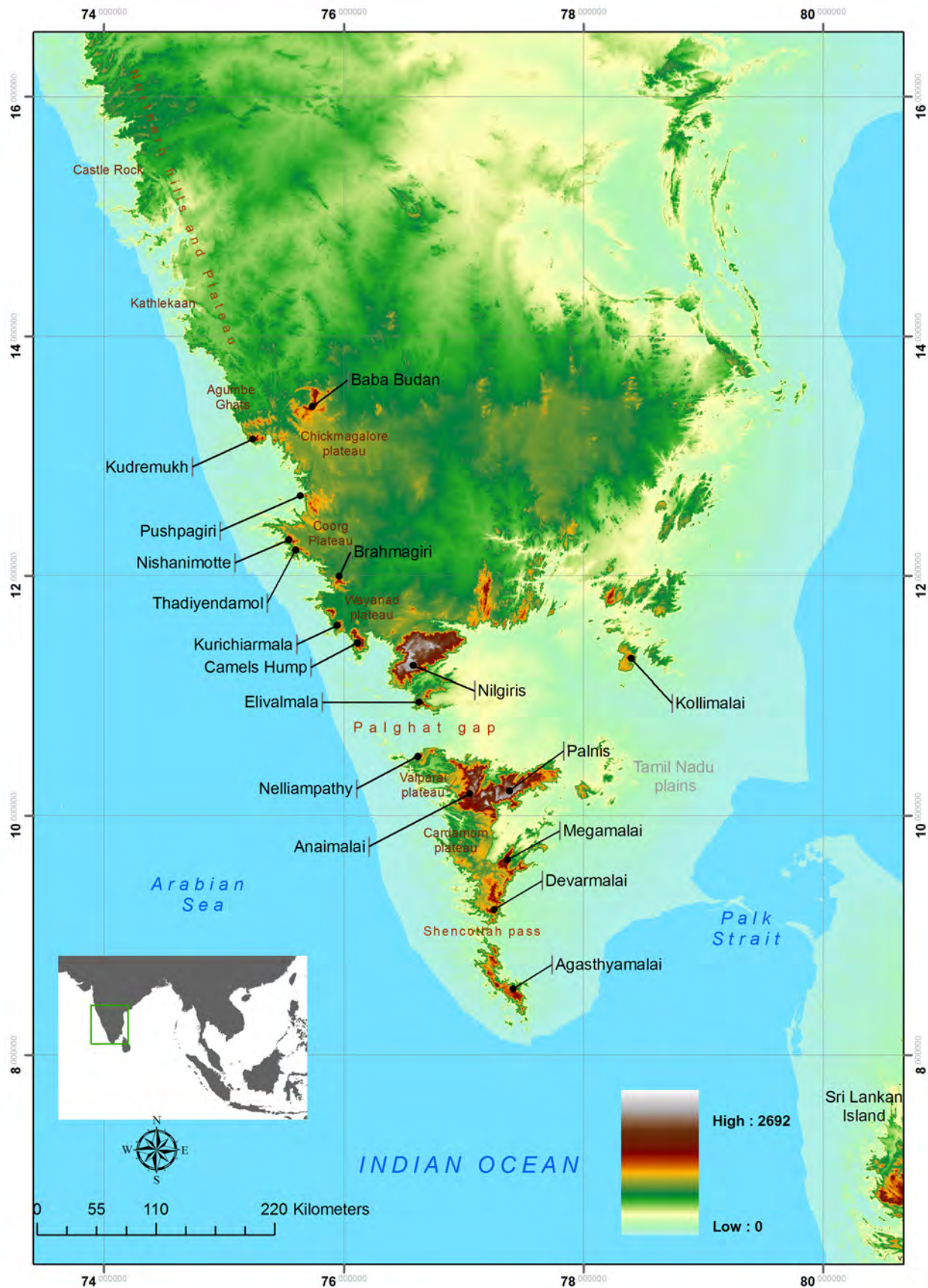
However, all these reports were based on morphological criteria and the systematic status of most of these species remained uncertain. In one of the most significant contributions to bush frog systematics, Biju and Bossuyt (2009) described twelve new species and revised all the species of *Philautus* for the Western Ghats, based on fresh field collections, molecular phylogenetic analysis and morphological criteria. Subsequently, Biju *et al.* (2010) assigned the generic name *Raorchestes* for the Western Ghats clade. Recent studies have also established the presence of a few species in the Western Ghats which share a common ancestor with the *Pseudophilautus* clade (Bossuyt *et al.*, 2004; Biju *et al.*, 2010), confirming the presence of members of both *Raorchestes* and *Pseudophilautus* in the Western Ghats. As part of this trend of ongoing discovery, recent studies by other researchers have added 11 additional species to this group (Zachariah *et al.*, 2011; Seshadri *et al.*, 2012; Padhye *et al.*, 2013). However, since there are no morphological characteristics to differentiate members of *Raorchestes* and *Pseudophilautus*, the recent reports of new species based on morphology (e.g. Zachariah *et al.*, 2011; Seshadri *et al.*, 2012) await independent validation.

Since 2008, overlapping temporally with the above reports of bush frog species discoveries, we carried out field sampling across the major massifs in the Western Ghats. Our sampling was primarily designed to address evolutionary biogeographic questions pertaining to bush frogs of the Western Ghats (Vijayakumar, 2014) and warranted a field sampling design that facilitated comprehensive sampling of lineages. The Western Ghats is a highly heterogeneous escarpment with massifs rising to over 2000 m in the southern region (Fig 1). Topographically, the southern region is also the most heterogeneous part of the Western Ghats with a gradient in vegetation communities, and isolated massifs separated by valleys and plateaus of different depths. The northern regions, characterized by the Deccan plateau, are composed of relatively low-lying plateau with no strong gradient in vegetation. We incorporated this physiographic and ecological heterogeneity in the Western Ghats in our design to sample bush frogs. We therefore accounted for allopatric and parapatric differentiation of lineages, and maximized the discovery of taxa. We then used a combination of phylogeny, genetic distance, geographic range and morphology to delimit bush frog lineages. Our analyses revealed vast underestimation of bush frog species in the Western Ghats (Vijayakumar, 2014). Here, we formally describe nine of these new lineages which could be recognized along multiple axes, namely phylogeny, genetic divergence, geography, acoustic and morphological divergence.

## Methods and material

**Western Ghats Geography. A brief introduction.** The Western Ghats Escarpment occurs on the western most edge of Peninsular India (Fig 1) and runs parallel to the west coast for a distance of around 1500 km. The southern and central parts of the Western Ghats Escarpment are topographically highly heterogeneous and contain the tallest massifs south of the Himalayas (Fig 1). The northern Western Ghats is characterized by the Deccan traps and consists of relatively low lying eroded plateaus and a few high elevation areas. The eastern boundary between the Escarpment and other regions of Peninsular India are more pronounced, with arid lowlands, towards the southern regions. The massifs in the south rise sharply from close to sea level (0 – 100 m) to a montane zone (> 1800m) at a few places. This topographic heterogeneity has also resulted in a number of vegetation categories ranging from lowland wet evergreen forests to a mosaic of stunted evergreen forests (shola) and grasslands in the montane zone. While this is characteristic of the windward side of the southern and central regions, the leeward side is a rain-shadow region and is composed of tropical dry forest mosaic on the lower elevations with wet evergreen forests appearing at intermediate (>900m) elevations. Valleys and plateaus of different depths dissect the adjoining massifs and act as potential biogeographic barriers, the most prominent being the Palghat Gap (Fig 1). The latitudinal extent of the Escarpment also gives rise to a strong rainfall gradient with wetter conditions towards the southern massifs and progressively drier conditions towards the northern plateau.

**Sampling design.** Our sampling design incorporated the above geographical and associated environmental and ecological heterogeneity. We initially identified all the major massifs that had an elevation of > 1400 m in the Western Ghats from relief maps generated using digital elevation data (ASTER by METI (Japan) and NASA (USA)) in a GIS domain. Further, elevational zones (low, medium and high) were identified, coinciding with the broad vegetation categories using available syntheses of vegetation distribution in the Western Ghats (Pascal *et al.*, 2004). Field sampling was initiated in the year 2008 and a total of 13 of the 14 identified massifs were intensively



**FIGURE 1.** A map of the Western Ghats showing the major physiographic features referred to in the text.

sampled for bush frogs till 2011 by SPV followed by collections made as part of general herpetological expeditions during 2012–2013. The research presented here is based predominantly on collections obtained between 2008–2011. Within each massif, sites were identified and sampled within the broad elevation zones. Given the logistic constraints of nocturnal sampling in forests with elephant populations, the expedition sites were largely dictated by availability of accessible trails and camping locations, which were sourced from topographic maps, local inhabitants and forest department staff. In the case of sites that were found to potentially contain greater numbers of species than sampled (based on initial call recordings), multiple visits were made during different years to maximize species detection. We also sampled multiple sites within elevation zones to account for potential turnover within massifs. A total of 249 sites (till 2011) were sampled for bush frogs in the Western Ghats (Fig 2).

**Field sampling.** Within each site, we used available trails to access different vegetation categories, usually within few kilometers of the base camp. Most sampling was carried out during the rainy season (June to September), when bush frogs are highly vocal and active. Individuals were largely located by their advertisement call. Geographic coordinates were obtained for each individual sighting and call detection. A few attempts were also made to access the canopy to obtain potential species that occurred there. Before capture, digital recordings of the calls were made, for select individuals, using a Marantz PMD671 digital recorder with a shotgun microphone, Sennheiser ME67/K6, fitted with a Sennheiser MZW-67 windscreen. During the non-breeding season, active searches were carried out on the forest floor in suitable microhabitats to locate individuals. The total number of individuals captured from a population was restricted to three, but numbers varied on the basis of preliminary observations on encounter rates in the field and observations of morphological variability within populations.

**Specimen processing.** Captured individuals were photographed in controlled conditions to obtain images of prominent features and coloration. Also, for selected species, coloration and body patterns were sketched in the field for species description. Individuals were fixed either in 70% alcohol or 4% formalin solution. Before fixing, especially for formalin medium, tissues (live and thigh muscles) were extracted and stored in 95% alcohol. Specimens are currently housed in the CES repository with the tag number prefixed CESF (Centre for Ecological Sciences—Frogs). Types are deposited at the Zoological Survey of India (ZSI) Western Ghats Regional Centre (WGRC)-Calicut, Kerala, India.

**Lineage delimitation.** We initially used DNA sequences of the mitochondrial 16S rRNA gene (Table 1) (16Sar-L: 5'-CGCCTGTTTATCAAAAACAT-3', 16Sbr-H: 5'-CCGGTCTGAACTCAGATCACGT-3') (Yu et al. 2009) of specimens to identify all the extant species by matching them with available published sequences (Biju & Bossuyt, 2009). This further allowed us to verify all the operational taxonomic units (OTU) i.e. potential species that could be discerned in the field, based on either call or morphological characteristics, from other sympatric species in an elevational zone on a massif. For all the species, both extant and OTUs, we sequenced two additional mitochondrial genes, Cytochrome b, Cytb (CB-J-10933: 5'-TATGTTCTACCA TGAGGACAAATATC-3', BSF4: 5'-CTTCTACTGGTTGTCCTCCGATTCA-3') (Bossuyt and Milinkovitch 2000); and NADH Dehydrogenase subunit 1, ND1 (NDH-L: 5'-AACTATTTAYYAAAGARCC-3', NDH-T: 5'-GGGTATGANGCTCGNACCCA-3', NDH-H-W: 5'-GGGTATGANGCTCGNATTCA-3') (Roelants and Bossuyt 2005). For ND1, we also used sequences from published information for extant species. The final data matrix consisted of 1760bp from 131 individuals of bush frogs from our collection, and individuals from seven species of the family Rhacophoridae. These were used to reconstruct a phylogenetic tree. RaxML was used to run maximum likelihood analyses and generate a tree with the data partitioned by gene under a GTR+G model with 500 rapid bootstraps.

We then followed a hierarchical approach towards delineating lineages (Vijayakumar, 2014):

*Step 1:* The phylogenetic tree was used to identify exclusive haplotypes (Wiens & Penkrot, 2002) based on individual clusters with strong bootstrap values (>70).

*Step 2:* Genetic distance was obtained for all the haplotype clusters with their sister lineage or the closest branch on the tree obtained from step 1. Analyses were conducted using Kimura 2-parameter model. The rate variation among sites for 16S rRNA was modeled with a gamma distribution (shape parameter = 0.2128); the analysis involved 137 nucleotide sequences for 16S rRNA, with a total of 579 positions in the final dataset. All ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011). For lineages for which sister-pairs could not be assigned, as evidenced in the lack of bootstrap support (<70), the minimum pair-wise distance from related lineages within the next well-supported clade was used.

- Step 3:* Since allopatric populations cannot be subject to the test of reproductive isolation under the conventional biological species criterion, one can use the degree of difference between ‘good’ species to make inferences about allopatric taxa within a genus (Mayr & Ashlock, 1991). Hence, for comparison, we selected a sympatric sister—lineage pair (from step 1) that exhibited the lowest genetic divergence and showed strong morphological divergence and could also be distinguished by their advertisement calls in the field.
- Step 4:* The genetic distance of the sympatric sister lineage was used as cut-off to filter haplotype clusters identified in Step 1. Lineages that met the cut-off distances in at least two genes (step 2) were retained. Potential new lineages were selected for further analyses.
- Step 5:* Lineages identified in step 4 were further classified into low (1% to <2%), moderate (2% to <4%), high (4% to <6%) and very high (6% and >6%) levels of divergence based on 16S rRNA distance values. A majority of the lineages that fell under the moderate to very high divergence levels could be easily distinguished in field. To further test the independence of these lineages, we chose the low divergent lineages and tested them for overlap in geographic space (step 6a) and morphological space (step 6b).
- Step 6a:* Based on the geographical range overlap, low divergence lineages were identified as allopatric (with zero overlap), parapatric (with partial overlap on range margins or abutting ranges) and sympatric (overlapping ranges) and uncertain (the data to assign them to a category was inadequate).
- Step 6b:* Shallow divergent sister lineages were also examined for separation in multivariate morphological space using principal component analysis.

**TABLE 1.** GenBank accession numbers of gene sequences for 16S, Cytb and ND1 generated as part of this study. Sequences accession numbers for ND1, downloaded from GenBank along with the source is also listed.

Gene	GenBank Accession Numbers
16S	JX092646–JX092712, JX092714–JX092724, KM596530–KM596580
Cytb	JX092778–JX092850
ND1	JX092851–JX092859, JX092862–JX092863, JX092865, JX092868–JX092870, JX092872–JX092875, JX092877–JX092878, JX092880, JX092882, JX092884, JX092886, JX092888–JX092889, JX092892–JX092893, JX092895, JX092897–JX092902, JX092904–JX092905, JX092907–JX092912, JX092914, JX092918
ND1 (Biju and Bossuyt 2009)	EU450030.1, EU450050.1, EU450039.1, EU450047.1, EU450065.1, EU450033.1, EU450056.1, EU450054.1, EU450058.1, EU450041.1, EU450067.1, EU450063.1, EU450032.1, EU450049.1, EU450059.1, EU450057.1, EU450040.1, EU450036.1, EU450037.1, EU450035.1, EU450055.1, EU450060.1, EU450061.1

The above analyses resulted in a diversity of lineages that could be differentiated along different axes or combinations thereof. For this paper, we choose to limit taxonomic description to a set of lineages that could be easily discerned in phylogenetic, genetic, geographical and morphological space. For a single shallow divergence lineage, we also considered acoustic divergence for species delimitation. We provide here the phylogenetic tree, based on all three genes, with the potential new lineages identified in the analyses.

**Species descriptions.** Each species account has been elaborated under four categories. For lineage diagnosis, we provide characteristics for the recognition of a species as independently evolving lineages in an evolutionary biogeographic framework. For two species, described in the past, *Raorchestes flaviventris* and *R. thodai*, we do not have sequences. *Raorchestes flaviventris* has not been sighted since its description in 1882 (Biju & Bossuyt, 2009). This species was described from the Malabar region and all of the lineages described here that could potentially overlap with the distributional range of *R. flaviventris*, including *Raorchestes aureus* **sp. nov.** (SVL, HL/SVL, coloration and pattern), *Raorchestes leucolatus* **sp. nov.** (SVL, HL/SVL, FOL/SVL, coloration and pattern), *Raorchestes primarrumpfi* **sp. nov.** (SVL, HL/SVL, ShL/SVL, coloration and pattern), *Raorchestes indigo* **sp. nov.** (SVL, coloration and pattern), *Raorchestes echinatus* **sp. nov.** (SVL, HL/SVL, ShL/SVL, coloration and pattern), could be discerned based on morphometric and coloration characteristics from the *R. flaviventris* type description provided in Bossuyt and Dubois (2001). In the case of *R. thodai*, the species can be assigned, based on morphological description, as a relative of *R. signatus* (Zachariah *et al.*, 2011) and a likely member of Signatus clade. In this paper, we do not deal with any new lineages from this clade. Regarding the hidden synonyms for



*Raorchestes* species in the Western Ghats, in this present account, a nomen *Raorchestes hassanensis* (described as *Philautus montanus* by Rao in 1937, followed by a replacement name *Philautus hassanensis* erected by Dutta in 1997) is assigned to a member within the Hassanensis clade; nomen *Raorchestes montanus* (described as *Ixalus montanus* by Gunther in 1876) is assigned to a member within the Tinniens clade. Redescription of these lineages will be dealt with elsewhere (Dinesh and Vijayakumar *in prep*). *Ixalus pulcher* Boulenger, 1882 currently under the synonym of *R. glandulosus* is a member of Glandulosus clade. In this paper, we do not describe any member from this clade.

For field diagnosis, we present the pertinent characteristics that can be used to diagnose the species from its closest relative(s) in the field. A conventional description of the type specimen is presented as holotype description. Finally, we provide a brief summary of natural history and distribution based on primary field data.

**Lineage diagnosis.** Lineage diagnosis is based on the position of lineages on the haplotype phylogenetic tree and its level of genetic divergence, either with its closest sister lineage or, in the case of unresolved nodes, from other related lineages within a sub-clade inferred in this analyses. Where pertinent, additional diagnosis in geographical and morphological space is presented. For a single species (*Raorchestes flaviocularis* **sp. nov.**), a shallow divergent lineage, we used acoustic data to infer the independence of the lineage. Digital call recordings were used to extract temporal and spectral properties of calls. The following variables were measured: call length (in seconds), number of pulses/call, pulse rate/s and dominant frequency of a call (Cocroft and Ryan 1995). Measurements of temporal variables for each call were extracted from a waveform in Raven 1.4 (Bioacoustics Research Program 2011). Dominant frequency was measured using a power spectrum generated using Hanning function (window size 1024) in version 2.0.5 of Audacity ® recording and editing software (Audacity Team 2014).

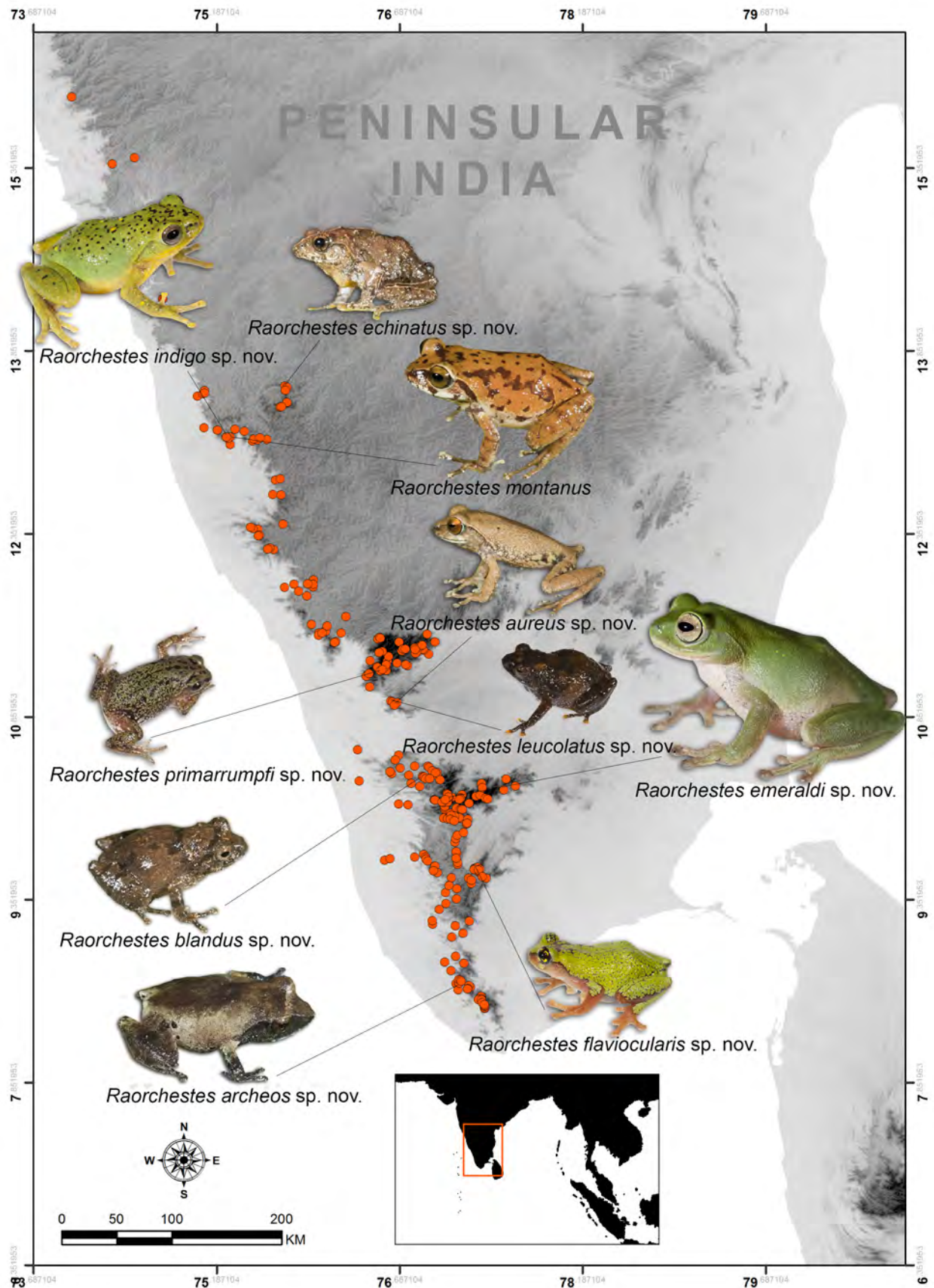
**Field diagnosis.** Field diagnosis is largely written in the context of discerning a species in the field and depending on the availability of data, diagnosis is provided under three independent variables: morphological, geographical and ecological. For a single species, behavioral data consisting of acoustic analysis is included.

**Natural history and distribution.** Depending on the availability of natural history information, we have provided details on micro-habitat (vertical position) and macro-habitat (broad vegetation types). Most data were based on field records obtained from the position of calling individuals. For distribution, we have provided the geographical entity (Massif, Hill, Plateau) as indicated on the map (Fig 1) and the elevational range of lineages derived from a DEM dataset based on the geographic coordinates. For aiding future research, uncertainties associated with range boundaries and presence of potential populations or related lineages in adjoining massifs have been indicated where relevant.

**Abbreviations used:** SVL (Snout to Vent Length); HW (Head Width); HL (Head Length); MN (Mandible to Nostril); MFE (Mandible to Front of Eye); MBE (Mandible to Back of eye); SL (Snout Length); EL (Eye Length); IUE (Shortest distance between the upper eyelids); UEW (Upper Eyelid Width); IFE (Distance in Front of Eyes); IBE (Distance at Back of Eyes); PPL (Papilla); TYD (Tympanum Diameter); FLL (Fore Limb Length); HAL (Hand Length); HeTo (Heels with hind limbs folded at right angles to the body); TFL (Third Finger Length); Fdiii (Finger Disk three Width); Fwiii (Finger three Width); ShL (Shank length/Tibia length); TL (Thigh length/Femur length); FOL (Foot Length); TFOl (Tarsus and Foot Length); HeTo (Heels when folded at right angles To body)

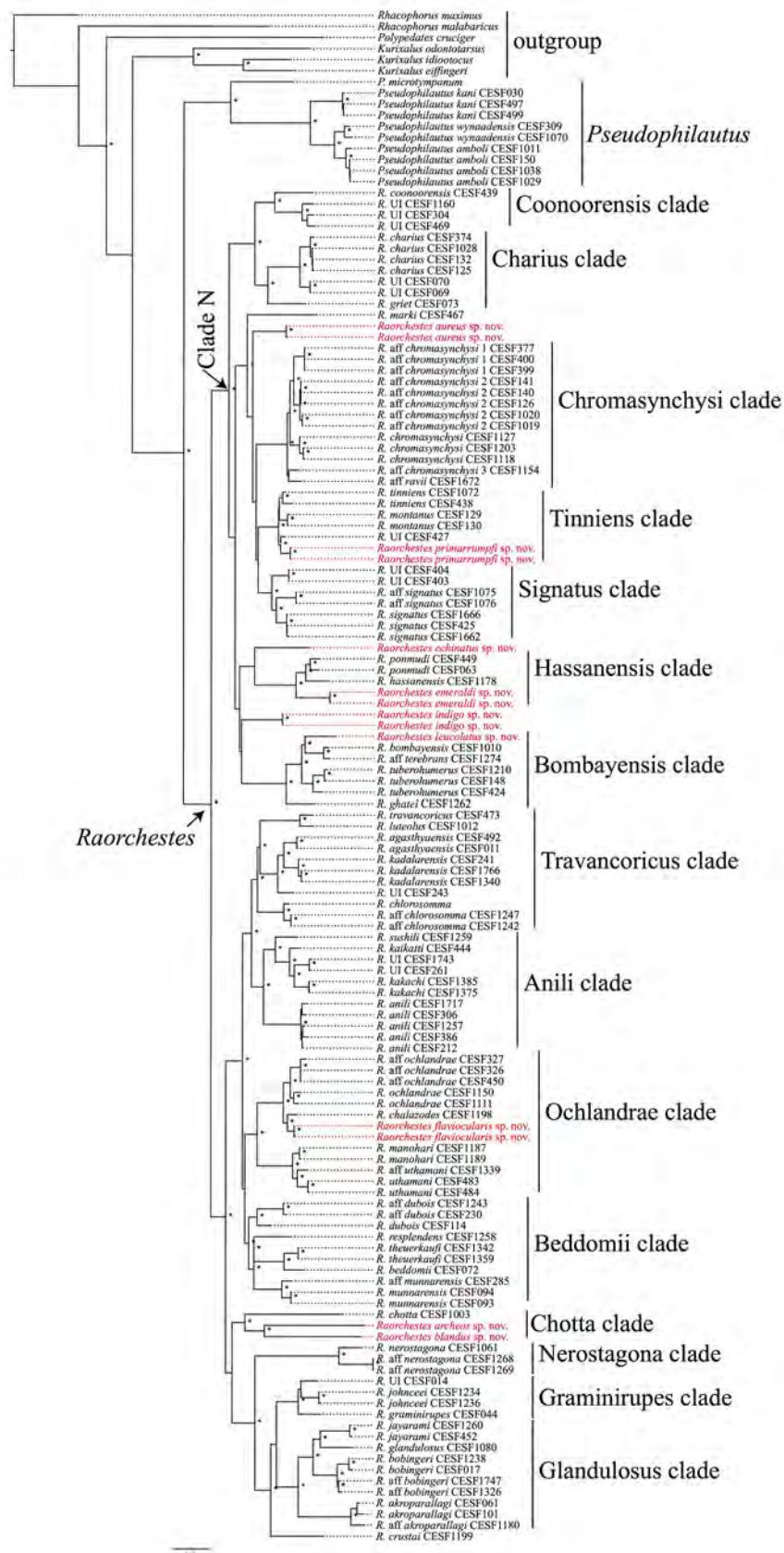
## Results

Phylogenetic analyses confirmed the results of recent studies (Biju *et al.*, 2010) and revealed two well-supported clades in the Western Ghats. This includes a larger clade consisting of all the members of extant species currently placed under the genus *Raorchestes* and three species with affinities towards the Sri Lankan bush frog clade *Pseudophilautus*. Within the clade *Raorchestes*, our species delimitation exercise reveals the existence of a large number of potential new lineages (Fig 3, see below under sub-clade composition) that varied in their levels of genetic and morphological divergence and also in their patterns of geographical distribution (Vijayakumar, 2014). For this work, a total of nine species (marked in red, Fig 3) that showed divergence on multiple axes were chosen for description. The members of these lineages belong to either various sub-clades or were deeply divergent with uncertain clade membership (Fig 3). These new lineages, described here, showed no spatial clustering in their occurrence and were discovered across all major massifs sampled in the Western Ghats Escarpment (Fig 2).



**FIGURE 2.** Geographic distribution of new species of bush frogs (*Raorchestes*) from Western Ghats. Orange filled circles represent the distribution of sampling sites.





**FIGURE 3.** A maximum likelihood tree for bush frogs, and in the Western Ghats, based on three MtDNA genes: 16S, Cytb and ND1. The lineages described are marked in red. Sub-clades are named on the right.

**Sub-clade composition.** We recognized fifteen well supported smaller clades, each composed of a minimum of two lineages (Fig 3). We have assigned names to these sub-clades and list below the component lineages. Five deeply divergent lineages with uncertain clade membership were not assigned to any sub-clade. These include three new lineages described here: *Raorchestes aureus* **sp. nov.**, *Raorchestes indigo* **sp. nov.**, *Raorchestes echinatus* **sp. nov.** and two extant species *R. crustai* and *R. marki* (Fig 3). The well-supported clades include:

1. Coonoorensis clade: comprising two lineages, *Raorchestes coonoorensis* and an unidentified deeply divergent sister lineage complex.
2. Charius clade: comprising two lineages, *Raorchestes charius* and a shallow divergent potential lineage.
3. Chromasynchysi clade: comprising *Raorchestes chromasynchysi* and four shallow divergent potential lineages, including *Raorchestes* aff. *ravii*.
4. Tinniensi clade: comprising four lineages, *Raorchestes tinniensi*, *R. montanus* (a formal redescription of this lineage will be addressed elsewhere) and two sympatric shallow divergent potential lineages. One of these lineages is described here as *Raorchestes primarrumpfi* **sp. nov.**
5. Signatus clade: comprising three lineages including *R. signatus* and two moderately divergent potential new lineages.
6. Hassanensis clade: comprising two shallow divergent potential lineages: *R. ponmudi*, *R. hassanensis* and an unidentified divergent lineage described here as *Raorchestes emeraldi* **sp. nov.**
7. Bombayensis clade: comprising five lineages including *R. bombayensis*, *R. tuberothumerus*, *R. ghatei*, *R. aff. terebrans* from Eastern Ghats Escarpment, and an unidentified divergent lineage described here as *Raorchestes leucolatus* **sp. nov.**
8. Travancoricus clade: comprising six lineages including *R. travancoricus*, *R. luteolus*, *R. agasthyaensis*, *R. kadalarensis*, *R. chlorosomma*, and a deeply divergent unidentified lineage.
9. Anili clade: comprising five lineages including *R. anili*, *R. sushili*, *R. kakachi*, *R. kaikatti* and an unidentified divergent lineage.
10. Ochlandrae clade: comprising five lineages including *R. ochlandrae*, *R. chalazodes*, *R. manohari*, *R. uthamani* and a shallow divergent potential lineage described as *Raorchestes flaviocularis* **sp. nov.**
11. Beddomii clade: comprising seven lineages which includes morphologically highly divergent lineages, *R. beddomii*, *R. munnarensis*, *R. theurkaufi*, *R. dubois*, *R. resplendens* and two shallow divergent potential lineages.
12. Chotta clade: comprising three deeply divergent lineages, *R. chotta* and two unidentified lineages described here as *Raorchestes archeos* **sp. nov.** and *Raorchestes blandus* **sp. nov.**
13. Nerostagona clade: comprising two deeply divergent lineages, *R. nerostagona* and an unidentified sister lineage.
14. Graminirupes clade: comprising three lineages, *R. graminirupes*, *R. johnceei* and an unidentified divergent lineage.
15. Glandulosus clade: comprising four deeply divergent lineages, *R. glandulosus*, *R. akroparallagi*, *R. jayarami*, *R. bobingeri* and a shallow divergent potential lineage.

## Species accounts

### 1. *Raorchestes archeos* **sp. nov.**

(Figures 2, 3 & 4; Tables 2 & 3)

**Holotype:** ZSI/WGRC/V/A/865 (CESF1190), a female (SVL 23.0 mm), collected by S.P. Vijayakumar in August 2010 from a forest site (8.6894 N, 77.1870 E), Agasthyamalai Massif (Fig 1), Western Ghats, Peninsular India.

**Paratype:** ZSI/WGRC/V/A/866 (CESF2060), a male (SVL 20.1), collected by K.P. Dinesh in May 2012 from a forest site (8.6914 N, 77.1823 E), Agasthyamalai Massif (Fig 1), Western Ghats, Peninsular India.

**Lineage diagnosis.** *Raorchestes archeos* **sp. nov.** can be readily discerned on a phylogenetic tree (Fig 3), and is characterized by high genetic divergence (16S—15.3%) from its closest relatives *R. chotta* and *Raorchestes blandus* **sp. nov.** Also very distinct on major aspects of morphology from *R. chotta* and *Raorchestes blandus* **sp. nov.** (see field diagnosis).

TABLE 2. Morphometric measurements for the exemplary specimens of new species of *Raorchestes*.

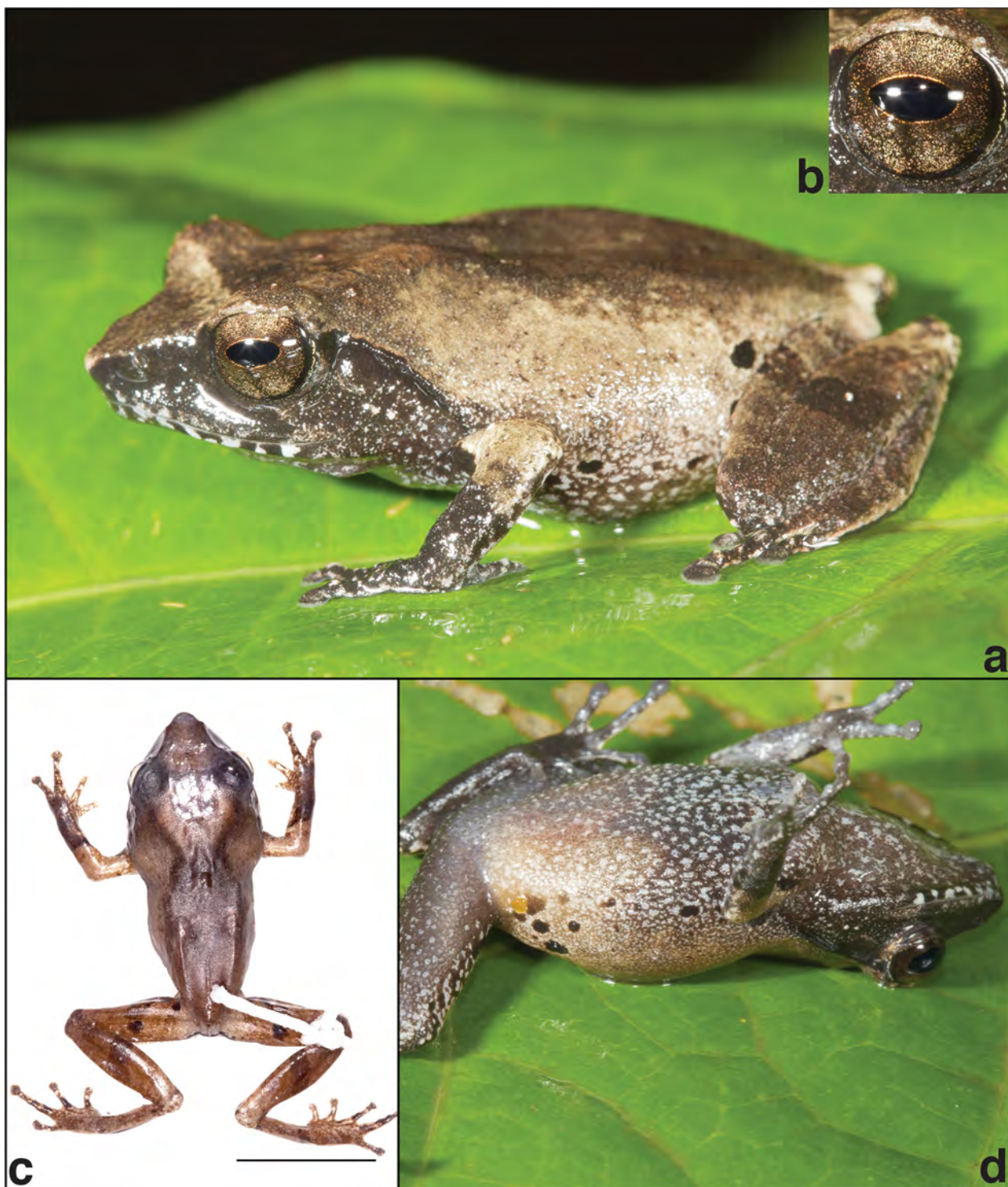
Species	Reg. No.	SEX	PPL	SVL	HW	HL	MN	MFE	MBE	SL	EL	IUE	UEW	IFE	IBE
<i>Raorchestes</i> <i>archeas</i> sp. nov.	ZSI/WGRC/V/A/865 CESF1190 (HT) ZSI/WGRC/V/A/866 CESF2060 (PT)	F M	0 0	23.0 20.1	9.5 8.3	7.7 7.4	6.4 6.0	4.6 4.2	1.5 1.7	3.3 3.5	3.7 3.3	2.7 3.2	2.1 2.0	4.9 5.1	8.0 8.3
<i>Raorchestes</i> <i>aureus</i> sp. nov.	ZSI/WGRC/V/A/867 CESF1165 (HT) ZSI/WGRC/V/A/868 CESF1164 (PT)	M F	1 1	24.8 28.3	10.3 11.1	8.3 9.1	7.1 7.4	5.3 5.3	2.3 2.1	3.4 4.4	3.7 3.6	2.9 3.5	2.6 2.5	5.0 5.4	9.1 9.7
<i>Raorchestes</i> <i>blandus</i> sp. nov.	ZSI/WGRC/V/A/869 CESF104 (HT) ZSI/WGRC/V/A/870 CESF329 (PT)	M M	1 1	18.4 19.1	7.7 6.6	5.1 6.3	4.4 4.1	3.2 3.6	1.5 1.1	2.4 3.1	2.7 2.6	2.0 2.1	2.0 1.8	3.6 4.2	6.4 6.9
<i>Raorchestes</i> <i>echinatus</i> sp. nov.	ZSI/WGRC/V/A/871 CESF1412 (HT) ZSI/WGRC/V/A/872 CESF1414 (PT)	M M	0 0	18.0 19.0	6.9 7.6	4.8 4.6	3.9 3.9	2.8 2.8	1.4 1.6	2.2 2.1	1.9 1.9	2.2 2.1	1.5 1.5	3.7 3.4	5.9 5.8
<i>Raorchestes</i> <i>emeraldi</i> sp. nov.	ZSI/WGRC/V/A/873 CESF1353 (HT) ZSI/WGRC/V/A/874 CESF1365 (PT)	M F	1 1	36.5 50.5	15.2 21.0	12.9 16.2	10.1 14.0	7.6 10.3	2.9 4.1	5.0 6.5	5.1 6.9	4.0 5.0	3.3 4.4	7.0 9.2	13.3 17.8
<i>Raorchestes</i> <i>flaviocularis</i> sp. nov.	ZSI/WGRC/V/A/875 CESF1406 (HT) ZSI/WGRC/V/A/876 CESF1251 (PT)	M F	1 1	26.5 23.9	9.2 9.5	6.7 6.6	6.0 5.9	4.5 4.5	1.8 2.2	2.6 2.4	3.2 2.8	2.7 2.5	1.7 2.1	4.5 4.5	8.2 8.3
<i>Raorchestes</i> <i>indigo</i> sp. nov.	ZSI/WGRC/V/A/877 CESF1437 (HT) ZSI/WGRC/V/A/878 CESF123 (PT)	F F	1 1	25.7 24.4	10.1 9.8	7.0 7.7	5.9 6.5	4.1 4.6	2.0 2.0	3.2 3.5	2.8 3.2	3.2 3.2	2.3 1.7	5.2 5.2	8.9 8.8
<i>Raorchestes</i> <i>leucolatus</i> sp. nov.	ZSI/WGRC/V/A/879 CESF1146 (HT) ZSI/WGRC/V/A/880 CESF1147 (PT)	M M	0 0	16.9 17.1	6.2 6.7	5.2 5.2	4.3 4.0	3.0 2.8	1.2 1.0	2.2 2.4	2.3 2.6	2.1 2.1	1.5 1.6	3.5 3.8	5.8 6.1
<i>Raorchestes</i> <i>primarrumpfi</i> sp. nov.	ZSI/WGRC/V/A/881 CESF1276 (HT) ZSI/WGRC/V/A/882 CESF441 (PT)	M M	1 1	21.0 19.9	7.5 7.5	5.5 5.2	4.7 4.5	3.4 3.2	1.6 1.3	2.5 2.3	2.4 2.1	2.2 2.4	1.8 1.0	3.5 3.5	6.6 6.2

.....continued on the next page

TABLE 2. (Continued)

Species	Reg. No.	SEX	TYD	FLL	HAL	TFL	Fdiii	Fwiii	TL/FL	Sh/TL	FOL	TFOL	HeTO
<i>Raorchestes</i> <i>archeos</i> sp. nov.	ZSI/WGRC/V/A/865 CESF1190 (HT)	F	1.1	4.8	5.0	3.6	1.0	0.6	8.5	9.3	7.5	12.8	3
	ZSI/WGRC/V/A/866 CESF2060 (PT)	M	0.9	4.2	4.7	8.9	0.9	0.5	7.9	7.7	7.6	12.9	3
<i>Raorchestes</i> <i>aureus</i> sp. nov.	ZSI/WGRC/V/A/867 CESF1165 (HT)	M	1.4	5.9	6.3	4.1	1.4	0.7	11.2	12.1	9.7	16.0	3
	ZSI/WGRC/V/A/868 CESF1164 (PT)	F	1.5	5.4	7.0	4.5	1.7	0.8	13.0	13.1	11.1	17.6	3
<i>Raorchestes</i> <i>blandus</i> sp. nov.	ZSI/WGRC/V/A/869 CESF104 (HT)	M	0.9	4.3	4.3	3.2	0.8	0.5	8.8	8.6	6.8	11.4	2
	ZSI/WGRC/V/A/870 CESF329 (PT)	M	0.9	4.6	4.3	2.9	1.2	0.6	9.0	8.8	6.8	11.6	2
<i>Raorchestes</i> <i>echinatus</i> sp. nov.	ZSI/WGRC/V/A/871 CESF1412 (HT)	M	1.0	3.7	5.2	3.4	0.9	0.7	7.4	6.6	7.3	11.4	1
	ZSI/WGRC/V/A/872 CESF1414 (PT)	M	1.3	3.7	4.5	3.8	0.7	0.6	7.4	6.4	7.2	10.7	1
<i>Raorchestes</i> <i>emeraldii</i> sp. nov.	ZSI/WGRC/V/A/873 CESF1353 (HT)	M	2.2	8.6	10.7	7.4	2.7	1.4	17.0	18.2	16.0	26.0	2
	ZSI/WGRC/V/A/874 CESF1365 (PT)	F	3.1	12.1	15.0	9.7	3.4	1.8	22.0	23.9	21.0	34.0	2
<i>Raorchestes</i> <i>flavioocularis</i> sp. nov	ZSI/WGRC/V/A/875 CESF1406 (HT)	M	1.3	4.5	8.3	6.1	2.0	1.1	10.2	10.1	10.9	15.4	2
	ZSI/WGRC/V/A/876 CESF1251 (PT)	F	1.3	5.9	6.1	5.0	1.9	1.2	9.6	10.0	9.7	14.9	2
<i>Raorchestes</i> <i>indigo</i> sp. nov.	ZSI/WGRC/V/A/877 CESF1437 (HT)	F	1.1	5.1	7.0	5.1	1.4	0.9	10.7	11.1	9.7	15.1	2
	ZSI/WGRC/V/A/878 CESF123 (PT)	F	1.1	5.6	6.4	5.3	1.3	0.8	11.8	11.8	11.1	17.3	2
<i>Raorchestes</i> <i>leucolatus</i> sp. nov.	ZSI/WGRC/V/A/879 CESF1146 (HT)	M	0.8	3.3	4.4	3.1	0.8	0.5	7.8	7.5	6.1	10.2	2
	ZSI/WGRC/V/A/880 CESF1147 (PT)	M	0.8	3.4	4.6	3.0	0.9	0.5	7.7	7.9	6.0	9.7	2
<i>Raorchestes</i> <i>primarrumpfi</i> sp. nov.	ZSI/WGRC/V/A/881 CESF1276 (HT)	M	NA	4.5	5.4	2.8	0.6	0.6	7.0	6.5	6.6	10.8	1
	ZSI/WGRC/V/A/882 CESF441 (PT)	M	NA	3.8	4.3	2.4	0.6	0.5	6.5	6.3	6.2	9.6	1

HT (Holotype), PT (Paratype), M (Male), F (Female), PPL (Papilla; present=1, absent=0), HeTo (Hells touch when hind limbs folded against the body; 1=do not touch, 2=barely touch, 3=overlap)



**FIGURE 4.** *Raorchestes archeos* **sp. nov.** (a) profile in life; (b) eye profile in life; (c) dorsal profile of holotype; (d) ventral profile in life.

**Field diagnosis. Morphology.** The new species can be easily distinguished from both *Raorchestes blandus* **sp. nov.**, and *R. chotta* by a combination of the following coloration characters (Fig 4) ; (1) skin on dorsum, lateral and ventral side smooth; (2) canthus rostralis, loreal region, region of supra tympanic fold and dorsal surface of hand black; (3) region of groin in front of thighs and vent with circular black spots; (4) ventrally mottled brown.

Additionally, it exhibits strong morphological divergence from *Raorchestes blandus* **sp. nov.** and *R. chotta* in a number of size related variables. *Raorchestes archeos* **sp. nov.** can be differentiated from *Raorchestes blandus* **sp.**



**nov.** in the large size of the adult males, SVL 20.6 (20.1–21.5, n=3) (vs. 18.5 (18.0–19.5, n=4) in *Raorchestes blandus* **sp. nov.**); longer head length, HL/SVL=0.37 (0.36–0.37, n=3) (vs. 0.30 (0.28–0.32, n=4) in *Raorchestes blandus* **sp. nov.**); wider distance between upper eyelids, IUE/SVL=0.16 (0.15–0.17, n=3) (vs. 0.10 (0.09–0.11, n=4) in *Raorchestes blandus* **sp. nov.**); longer snout length SL/SVL=0.17 (0.15–0.18, n=3) (vs. 0.14 (0.13–0.15, n=4) in *Raorchestes blandus* **sp. nov.**);

*Raorchestes archeos* **sp. nov.** can be differentiated from *R. chotta* in the size of the adult males, SVL 20.6 (20.1–21.5, n=3) (vs. adult male SVL 16.6 (16.0–17.2, n=7) in *R. chotta*); wider distance between upper eyelids, IUE/SVL=0.16 (0.15–0.17, n=3) (vs. ratio of IUE/SVL=0.13 (0.13–0.14, n=7) in *R. chotta*); shorter length of the tibia, ShL/SVL=0.45 (0.38–0.49, n=3) (vs. ratio of ShL/SVL=0.54 (0.52–0.57, n=7) in *R. chotta*); shorter femur length, TL/SVL=0.46 (0.391–0.499, n=3) (vs. ratio of TL/SVL=0.53 (0.505–0.556, n=7) in *R. chotta*).

**Description of holotype (all measurements in mm).** A medium sized bush frog (SVL = 23.0 mm), width of head broader than head length (HW = 9.5 mm; HL = 7.7 mm), flat dorsally; snout acutely pointed in total profile, slightly protruding beyond mouth. Snout length is sub equal to diameter of eye (SL = 3.3 mm, EL = 3.7 mm). Canthus rostralis angular, loreal region concave. Interorbital space (IUE = 2.7 mm) flat and sub equal to upper eyelid (UEW = 2.1 mm). Interorbital space between posterior margins of the eyes 1.6 times that of anterior margins (IFE = 4.9, IBE = 8.0 mm). Nostrils oval, nearer to tip of snout. Weak symphyseal knob. Pupil horizontal. Tympanum distinct, small, rounded, 3.4 times less than the eye diameter (TYD = 1.1 mm). Tongue bifid, granular without papilla. Supratympanic fold from behind eye to shoulder.

Relative length of fingers I<II<IV<III. Finger tips with well developed disks ( $fd_3 = 1.0$  mm;  $fw_3 = 0.6$ ) with distinct circum-marginal grooves, fingers with dermal fringes on both sides. Webbing on palm absent, subarticular tubercles distinct, rounded and pre-pollex tubercle oval, distinct. Supernumerary tubercles absent.

Hind limb long, heels overlap when folded at right angles to the body. Thigh/Femur (TL = 8.5 mm), sub equal to Shank/Tibia (ShL = 9.3 mm); longer than foot (FOL = 7.5 mm) and less than heel to tip of fourth toe (TFOL = 12.8 mm). Relative toe length I<II<III<V<IV, webbing poor, web formula (I 1-1 II 1-2 III 1½-2 IV 2½-1 V). Tibiotarsal articulation reaches posterior corner of eye. Outer metatarsal tubercle, supernumerary tubercles and tarsal tubercle absent.

**Color in life.** Dorsum maroon, light brown laterally with a horizontal light brown band between the orbits; canthus darker, black and lower lip with white bands. Lower half of arms black. Ventrally dark with white scattered speckled patches, distinct towards upper half of abdomen, ventral part of femur, tibia and tarsus dark, distinct black blotches towards the lateral sides of the groin. Distinct small black spot behind fore arm and a black spot at the inguinal region. Vent and posterior femur darker. Iris golden brown with a brown wash, prominent towards the anterior and posterior sides of the black pupil which is edged with varying rufous shades.

**Etymology.** The species is named after its deep divergence from its closest relatives (Greek: *archeo* = 'ancient').

**Natural history and distribution.** The species is an inhabitant of the mid-elevation (840–1071 m, n=7) wet evergreen forests of the Agasthyamalai Massif and observed from a site (9.0392 N, 77.1191 E) in Devarmalai Massif, which currently forms the northern limit of its range. One could look for additional populations in the Periyar plateau (Fig 1).

## 2. *Raorchestes aureus* **sp. nov.**

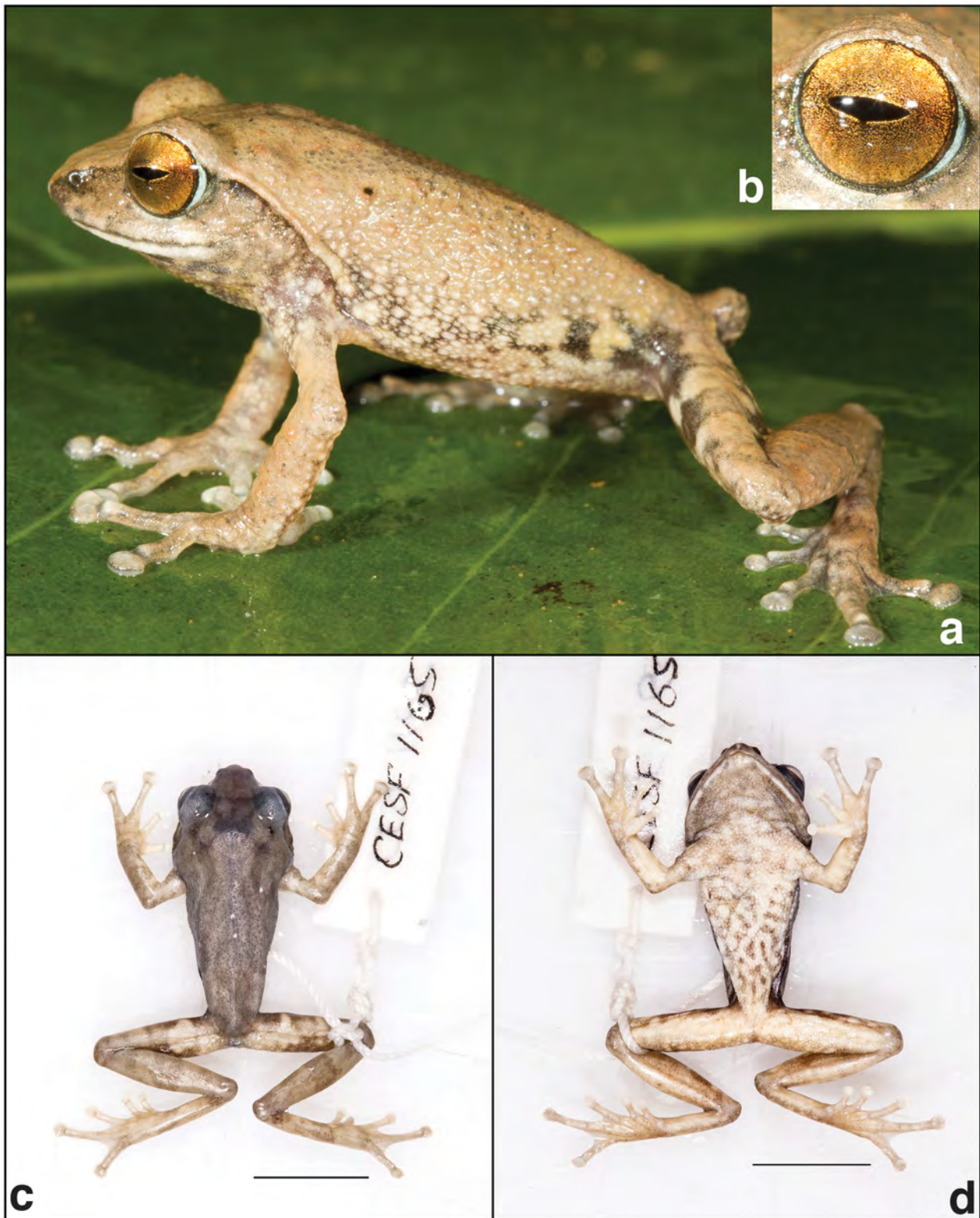
(Figures 2, 3 & 5; Tables 2 & 3)

**Holotype:** ZSI/WGRC/V/A/867 (CESF1165), an adult male (SVL 24.8 mm), collected by S.P. Vijayakumar and Mrugank V. Prabhu in July 2010 from a high elevation site (10.9452 N, 76.6446 E) in Elivalmalai Massif (Fig 1), Western Ghats, Peninsular India.

**Paratype:** ZSI/WGRC/V/A/868 (CESF1164), an adult female (SVL 28.3), collected by S.P. Vijayakumar and Mrugank V. Prabhu in July 2010 from a high elevation site (10.9452 N, 76.6446 E) in Elivalmalai Massif (Fig 1), Western Ghats, Peninsular India.

**Lineage diagnosis.** *Raorchestes aureus* **sp. nov.** can be diagnosed as a deeply divergent (16S—7.3%) lineage nested within a larger clade N (Fig 3). The lineage is isolated on the high elevations of Elivalmalai Massif (Fig 1 & 2). Morphologically, it shows strong signatures of divergence from other similar relatives within clade N (see

below). We use all the above criteria, genetic divergence, geographical range and morphology to diagnose this lineage. The relatives that potentially overlap in morphology and hence could be confused with this lineage within the clade N are discussed below.



**FIGURE 5.** *Raorchestes aureus* sp. nov. (a) profile in life; (b) eye profile in life; (c) dorsal profile of holotype; (d) ventral profile of holotype.

**Field diagnosis. Morphology.** *Raorchestes aureus* **sp. nov.** could be confused with *R. chromasynchysi* which occurs in sympatry (see remarks). However, the new species can be differentiated based on the shorter thigh length, TL/SVL=0.45 (0.44–0.45, n=4) (vs. TL/SVL=0.52 (0.50–0.54, n=3) in *R. chromasynchysi*); shorter tibia length, ShL/SVL=0.46 (0.45–0.47, n=4) (vs. ShL/SVL=0.51 (0.50–0.51, n=3) in *R. chromasynchysi*); in having a distinct golden iris (vs. silvery to light brown in *R. chromasynchysi*); dorsal coloration shades of brown (vs. very variable from shades of brown to green in *R. chromasynchysi*); anterior and posterior region of thigh (femur) characterized by distinct or faint cross bar with alternating darker and lighter shades of brown (vs. plain coloration on the posterior thigh and dark coloration with yellow blotches on the anterior thigh in *R. chromasynchysi*); lateral sides of irregular mottling of brown/yellow and green extending from groin to base of supratympanic fold (vs. distinct separation of dorsal and ventral coloration without any such mottling).

**Geography.** Current data suggests a narrow restricted range to the high elevation of Elivalmalai Massif in the Western Ghats (see natural history and distribution for details).

**Description of holotype (all measurements in mm).** A small sized bush frog (SVL = 24.8 mm), width of head broader than head length (HW = 10.3 mm; HL = 8.3 mm), flat dorsally; snout acutely pointed in total profile, slightly protruding beyond mouth. Snout length is sub equal to diameter of eye (SL = 3.4 mm, EL = 3.7 mm). Canthus rostralis angular, loreal region slightly concave. Interorbital space (IUE = 2.9 mm) flat and sub equal to upper eyelid (UEW = 2.6 mm). Interorbital space between posterior margins of the eyes 1.8 times that of anterior margins (IFE = 5.0, IBE = 9.1 mm). Nostrils oval, nearer to tip of snout. Weak symphyseal knob. Pupil horizontal. Tympanum distinct, rounded, small, barely visible behind the eye. Tongue bifid, granular with a papilla. Supratympanic fold from behind eye to shoulder.

Relative length of fingers I<II<IV<III, finger tips with well developed disks ( $fd_3 = 1.4$  mm;  $fw_3 = 0.7$ ) with distinct circum-marginal grooves, fingers with dermal fringes on both sides. Webbing on palm absent, subarticular tubercles distinct, rounded and pre-pollex tubercle oval, distinct. Supernumerary tubercles absent.

Hind limb long, heels overlap when folded at right angles to the body. Thigh/Femur (TL = 11.2 mm), sub equal to Shank/Tibia (ShL = 12.1 mm); longer than foot (FOL = 9.7 mm) and less than heel to tip of fourth toe (TFOL = 16.0 mm). Relative toe length I<II<III<V<IV, webbing poor, web formula (I 1-1 II 1-2 III 1-2½ IV 2½-1 V). Tibiotarsal articulation reaches anterior corner of eye. Outer metatarsal tubercle, supernumerary tubercles and tarsal tubercle absent.

**Color in life.** Limbs faintly cross-barred, pattern extending towards the anterior and posterior parts of the thigh. Lateral sides characterized by irregular mottling of yellow and light green extending from groin to base of supratympanic fold. Ventral parts of head, body, hand and foot mottled, but more pronounced at the region of belly and throat. Iris distinct golden with brown edged coarse speckles around the pupil, visible even in the preserved specimens.

**Etymology.** The species is named after the consistent golden iris coloration (Latin: *aureus* = golden).

**Natural history and distribution.** All the individuals were collected from forest edges in a grassland site and all males located were found calling at the ground level. It appears to be a range restricted species, recorded from a single high elevation (1524 m) site in Elivalmalai Massif (Fig 1 & 2). The elevational range within Elivalmalai needs additional field sampling.

**Remarks.** *R. chromasynchysi* was known only from the type locality (Biju and Bossuyt 2009) and a recent record from north of its type locality (Dinesh and Radhakrishnan, 2012). We have uncovered multiple potential lineages across various Massifs and hill ranges in the central Western Ghats (see above under sub-clade composition). For the above quantitative comparison, we have used individuals from a shallow divergent lineage that overlap with the range of *Raorchestes aureus* **sp. nov.**

### 3. *Raorchestes blandus* **sp. nov.**

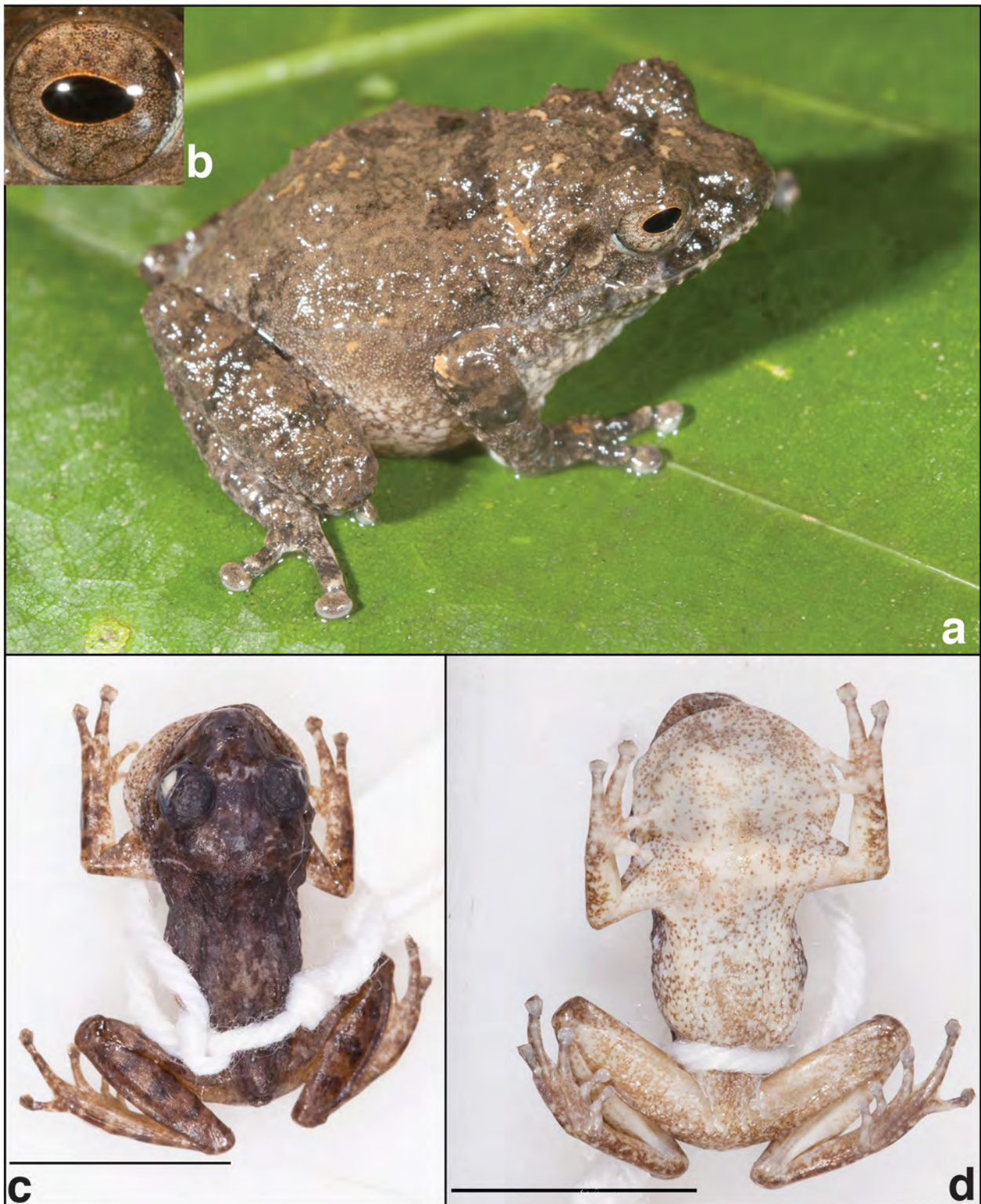
(Figures 2, 3 & 6; Tables 2 & 3)

**Holotype:** ZSI/WGRC/V/A/869 (CESF104), an adult male (SVL 18.4 mm), collected by S.P. Vijayakumar and Mayavan during August 2008, from a fragmented lowland wet evergreen forest site (10.1284 N, 76.7588 E) on the western side of the Anaimalai Massif (Fig 1), Western Ghats, Peninsular India.

**Paratype:** ZSI/WGRC/V/A/870 (CESF329), an adult male (SVL 19.1 mm), collected by S.P. Vijayakumar and Mayavan in June 2009 from an evergreen forest site (8.6667 N, 77.1833 E) in the Parambikulam protected area, western slopes of Anaimalai Massif (Fig 1), Western Ghats, Peninsular India.



**Lineage diagnosis.** *Raorchestes blandus* **sp. nov.** can be readily discerned on a phylogenetic tree (Fig 3), and is characterized by high genetic divergence (16S—14.1%) from its close relative *R. chotta*. Morphologically, it could be confused with *R. chotta*, but could be discerned using their morphological characteristics (See Field diagnosis). It is also very distinct on major aspects of morphology and coloration from *Raorchestes archeos* **sp. nov.** (Fig 4, also see field diagnosis section of *Raorchestes archeos* **sp. nov.**).



**FIGURE 6.** *Raorchestes blandus* **sp. nov.** (a) profile in life; (b) eye profile in life; (c) dorsal profile of holotype; (d) ventral profile of holotype.

**Field diagnosis. Morphology.** *Raorchestes blandus* **sp. nov.** could be superficially confused with *Raorchestes chotta*. However *Raorchestes blandus* **sp. nov.** can be distinguished from *R. chotta* in the larger male adult size of SVL 18.5 (18.0–19.5, n=4) (vs. 16.6 (16.0–17.2, n=7) in *R. chotta*); shorter head length, HL/SVL=0.30 (0.28–0.32, n=4) (vs. HL/SVL=0.38 (0.36–0.41, n=7) in *R. chotta*); Head width (HW=7.5, 7.2–7.7, n=4) longer than head length (HL=5.6, 5.1–6.0, n=4) (vs. head width (HW=6.5, 6.0–6.9, n=7) equal to head length (HL=6.4, 5.8–6.7, n=7) in *R. chotta*); shorter snout length, SL/SVL=0.14 (0.13–0.15, n=4) (vs. SL/SVL=0.17 (0.16–0.18, n=7) in *R. chotta*). Presence of rufous irregular glandular patches on the dorsal surface of skin in the shoulder, posterior side of the body, and in the joints of arms (vs. absence of glandular patches in *R. chotta* (Biju and Bossuyt 2009)).

**Ecology.** Restricted to the under-storey in wet evergreen forests.

**Geography.** This species is restricted in distribution to the low and medium elevations of the western slopes of Anaimalai massif (see natural history and distribution for details).

**Description of holotype (all measurements in mm).** A small sized bush frog (SVL = 18.4 mm), width of head broader than head length (HW = 7.7 mm; HL = 5.1 mm), flat dorsally; snout acutely pointed in total profile, slightly protruding beyond mouth. Snout length is sub equal to diameter of eye (SL = 2.4 mm, EL = 2.7 mm). Canthus rostralis angular, loreal region flat. Interorbital space (IUE = 2.0 mm) flat and equal to upper eyelid (UEW = 2.0 mm). Interorbital space between posterior margins of the eyes 1.8 times that of anterior margins (IFE = 3.6, IBE = 6.4 mm). Nostrils oval, nearer to tip of snout. Weak symphyseal knob. Pupil horizontal. Tympanum minute, rounded, barely visible behind the eye. Tongue bifid, granular with papilla. Supratympanic fold from behind eye to shoulder.

Relative length of fingers I<II<IV<III. Finger tips with well developed disks ( $fd_3 = 0.8$  mm;  $fw_3 = 0.5$ ) with distinct circum-marginal grooves, fingers with dermal fringes on both sides. Webbing on palm absent, subarticular tubercles moderate and pre-pollex tubercle oval, distinct. Supernumerary tubercles absent.

Hind limb long, heels touch when folded at right angles to the body. Thigh/Femur (TL = 8.8 mm), sub equal to Shank/Tibia (ShL = 8.6 mm); longer than foot (FOL = 6.8 mm) and less than heel to tip of fourth toe (TFOL = 11.4 mm). Relative toe length I<II<III<V<IV. Webbing weak, web formula, (I 1-1 II 1-2 III 1½-2 IV 2-1 V). Tibiotarsal articulation reaches anterior corner of eye. Outer metatarsal tubercle, supernumerary tubercles and tarsal tubercle absent.

**Color in life.** Dorsum rufous or brown with irregular dark brown blotches; small orange/rufous glandular patches on the head (Fig 6a), shoulder, elbow, on the dorsum, posterior part of back and on the finger; throat finely speckled with brown; fore and hind arm barred. Skin on dorsum finely granular.

**Etymology.** The species named after its pleasant (Latin: *bland*=pleasant) call notes. Common in the lowland wet forests of Anaimalai.

**Natural history and distribution.** A forest dwelling lineage, it is usually observed calling from understory shrubs. The distribution ranges from low to mid elevations (45–806 m, n=13) of Anaimalai Massif (Fig 1 & 2). Current data suggests that there is no overlap in the geographical range of *Raorchestes blandus* **sp. nov.** with its close relatives *R. chotta* and *Raorchestes archeos* **sp. nov.** An additional population that resembles this lineage was encountered in the medium and low elevations of Periyar Plateau. Considering a potential zone of overlap with the northern range *R. chotta*, we reserve the identity of this population for further verification.

#### 4. *Raorchestes echinatus* **sp. nov.**

(Figures 2, 3 & 7; Tables 2 & 3)

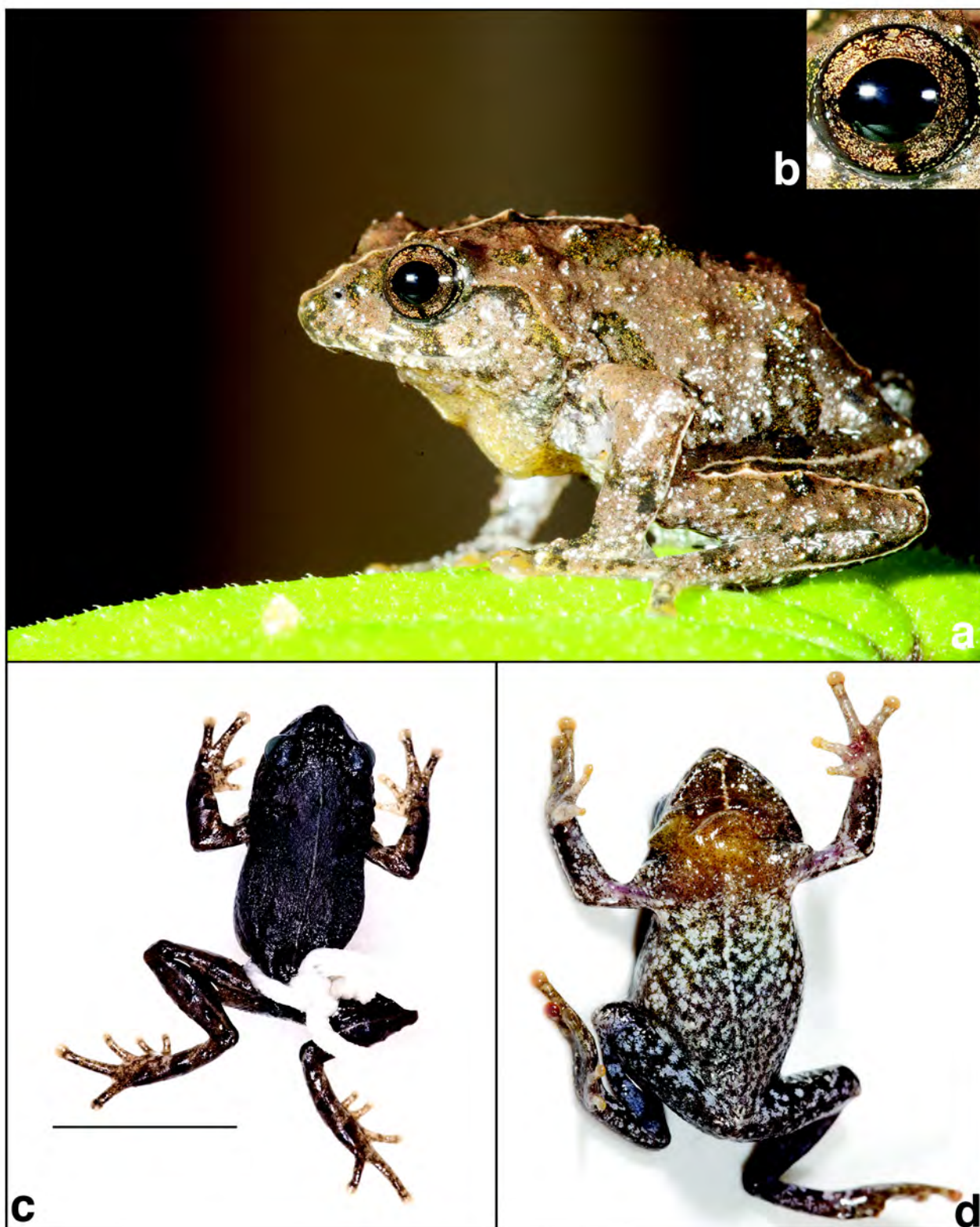
**Holotype:** ZSI/WGRC/V/A/871 (CESF1412), an adult male (SVL 18.0 mm), collected by S.P. Vijayakumar and K.P. Dinesh in September 2011 from a grassland site (13.4228 N, 75.7695 E), Baba Budan Massif (Fig 1), Western Ghats, Peninsular India.

**Paratype:** ZSI/WGRC/V/A/872 (CESF1414), an adult male (SVL 19.0), collected by collected by S.P. Vijayakumar and K.P. Dinesh in September 2011 from a grassland site (13.4228 N, 75.7695 E), Baba Budan Massif (Fig 1), Western Ghats, Peninsular India.

**Lineage diagnosis.** This lineage belongs to the larger clade N (Fig 3), with an unresolved relationship status within this clade, but with very high levels (16S—7.3%) of divergence from all other lineages. It exhibits a number of unique morphological characteristics (Fig 7) (see field diagnosis) and shows strong affinity towards grasslands



and is restricted geographically to the high elevations of Baba Budan Massif in the Western Ghats. We diagnose this lineage based on the deep genetic divergence, morphology, ecology and geography.



**FIGURE 7.** *Raorchestes echinatus* sp. nov. (a) profile in life; (b) eye profile in life; (c) dorsal profile of holotype; (d) ventral profile in life.

**Field diagnosis. Morphology.** *Raorchestes echinatus* **sp. nov.** superficially resembles *R. tuberochumerus* in adult male size SVL 18.4 (18.0–19.0, n=3) (vs. adult male size SVL 18.3 (17.7–19.0, n=6) in *R. tuberochumerus*), however a number of divergent characters could be discerned; larger head width, HW/SVL=0.40 (0.38–0.41, n=3) (vs. HW/SVL=0.35 (0.33–0.36, n=6) in *R. tuberochumerus*); shorter head length, HL/SVL=0.27 (0.24–0.29, n=3) (vs. HL/SVL=0.37 (0.36–0.40, n=6) in *R. tuberochumerus*); shorter eye length, EL/SVL=0.104 (0.099–0.108, n=3) (vs. EL/SVL=0.127 (0.111–0.137, n=6) in *R. tuberochumerus*); shorter tibia, ShL/SVL=0.36 (0.34–0.38, n=3) (vs. ShL/SVL=0.46 (0.41–0.49, n=6) in *R. tuberochumerus*); shorter femur length, TL/SVL=0.409 (0.39–0.43, n=3) (vs. TL/SVL=0.50 (0.46–0.52, n=6) in *R. tuberochumerus*); skin, on the dorsum rough with minute horny ridges, more spinular and on the dorsal surface of limbs smooth; lateral and ventral sides granular (vs. sparsely granular dorsum in *R. tuberochumerus*); yellowish throat finely speckled in brown (vs. flesh coloured, speckled in black in *R. tuberochumerus*); ventral coloration, white mottlings on a dark background, the pattern extending into femur, tibia, tarsus (vs. anterior thigh region characterized by dark colouration with distinct yellow blotches in *R. tuberochumerus*).

Additionally the new species, *Raorchestes echinatus* **sp. nov.**, can be easily distinguished morphologically from known congeners in clade N, that potentially overlap with its geographical range, by its size, horny ridges on the dorsum, ventral and throat coloration.

**Ecology.** A species of the open habitat and all individuals were observed in the grasslands.

**Geography.** Distribution data suggests that it is restricted to the Baba Budan Massif (see natural history and distribution for details).

**Description of holotype (all measurements in mm).** A small sized bush frog (SVL = 18.0 mm), width of head broader than head length (HW = 6.9 mm; HL = 4.8 mm), flat dorsally; snout short and pointed, slightly protruding beyond mouth. Snout length is sub equal to diameter of eye (SL = 2.2 mm, EL = 1.9 mm). Canthus rostralis angular, loreal region flat. Interorbital space (IUE = 2.2 mm) flat and sub equal to upper eyelid (UEW = 1.5 mm). Interorbital space between posterior margins of the eyes 1.6 times that of anterior margins (IFE = 3.7, IBE = 5.9 mm). Nostrils oval, nearer to tip of snout. Weak symphyseal knob. Pupil horizontal. Tympanum indistinct, rounded, barely visible behind the eye. Tongue bifid, granular without a papilla. Supratympanic fold from behind eye to shoulder.

Relative length of fingers I<II<IV<III, finger tips with moderate disks ( $fd_3 = 0.9$  mm;  $fw_3 = 0.7$  mm) with distinct circum-marginal grooves, fingers with dermal fringes on both sides. Webbing on palm absent, subarticular tubercles moderate, rounded and pre-pollex tubercle indistinct. Supernumerary tubercles absent.

Hind limb long, heels do not touch when folded at right angles to the body. Thigh/Femur (TL = 7.4 mm) sub equal to Shank/Tibia (FL = 6.6 mm) and foot (FOL = 7.3 mm) and less than heel to tip of fourth toe (TFOL = 11.4 mm). Relative toe length I<II<III<V<IV, webbing absent. Tibiotarsal articulation reaches posterior corner of eye. Outer metatarsal tubercle, supernumerary tubercles and tarsal tubercle absent.

**Color in life.** Dorsum with olive patches on a brown background with rufous tinge, distinct spinular tubercles behind the head; a mid dorsal ridge coloured off-white from the snout tip to vent (Fig 7a); ventrally a distinct mid ventral white line (Fig 7d), belly predominantly white with black irregular interconnected patches. Ventral pattern extends to femur tibia and tarsus, throat yellow finely speckled with brown. Iris with irregular golden speckles with maroon edges and a distinct brown band on the lower half and brown towards anterior and posterior edges.

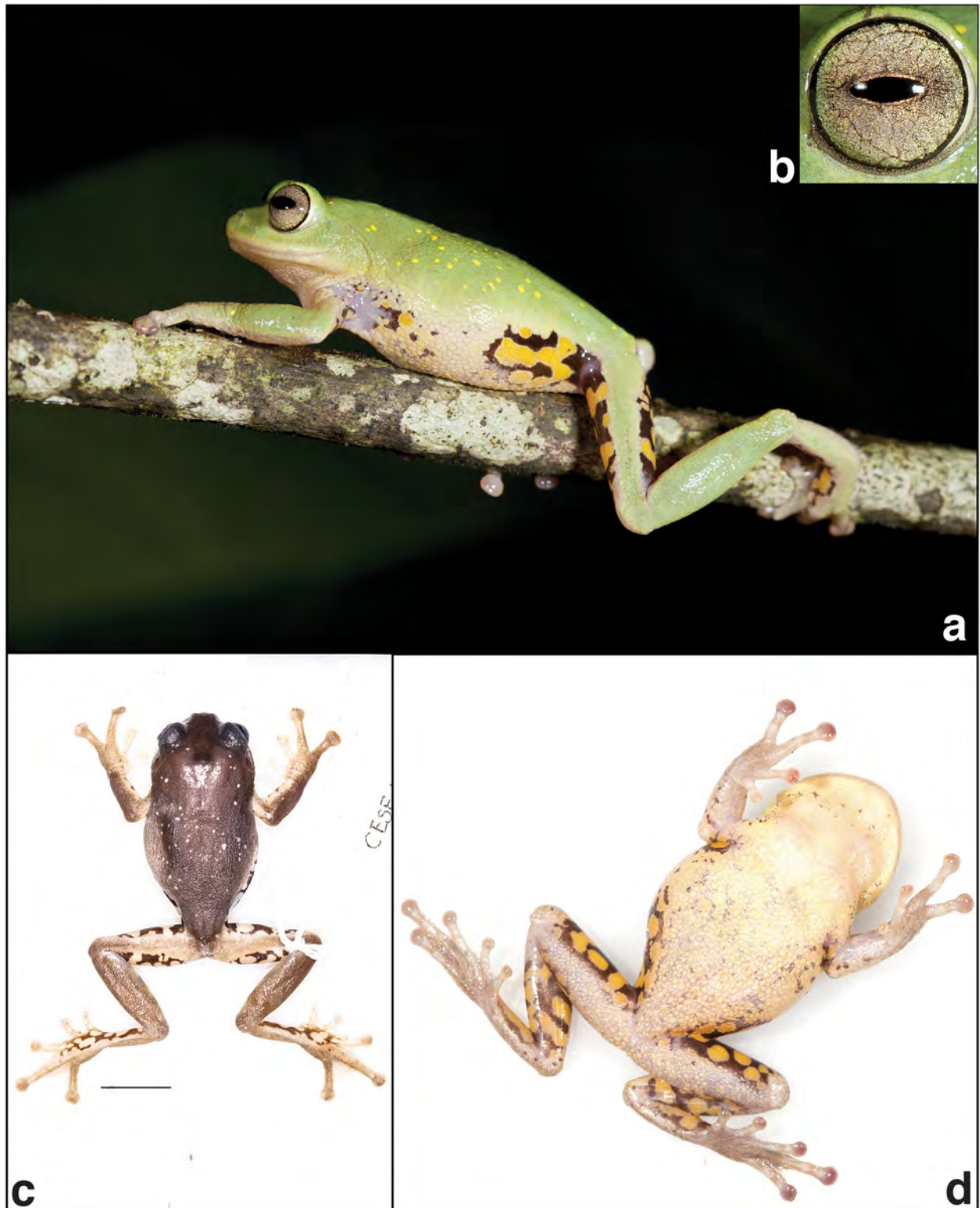
**Etymology.** The species is named after the spinular projections on the dorsum (Latin: *echino*= spiny).

**Natural history and distribution.** All individuals were observed in grasslands on grass blades (30.8 cm, n=5). The species was observed only in the higher elevations (1464–1864 m, n=6) of Baba Budan Massif (Fig 1 & 2). Further surveys are needed to verify the presence of either conspecifics or close relatives of this lineage in the adjoining Massifs of Kudremukh and Pushpagiri.

## 5. *Raorchestes emeraldi* **sp. nov.**

(Figures 2, 3 & 8; Tables 2 & 3)

**Holotype:** ZSI/WGRC/V/A/873 (CESF1353), an adult male (SVL 36.5 mm), collected by S.P. Vijayakumar and Saunak Pal in August 2011 from a site (10.3690 N, 76.9948 E) in a wet evergreen forest fragment, Valparai Plateau, Anaimalai Massif (Fig 1), Peninsular India.



**FIGURE 8.** *Raorchestes emeraldi* sp. nov. (a) profile in life; (b) eye profile in life; (c) dorsal profile of holotype; (d) ventral profile in life.

**Paratype:** ZSI/WGRC/V/A/874 (CESF1365), an adult female (SVL 50.5 mm), collected by S.P. Vijayakumar and Saunak Pal in August 2011 from a site (10.3919 N, 76.9942 E) in a wet evergreen forest fragment, Valparai Plateau, Anaimalai Massif (Fig 1), Peninsular India.

**Lineage diagnosis.** *Raorchestes emeraldi* sp. nov. can be diagnosed by its affinity to the Hassanensis clade

(Fig 3) and in having moderate levels (16S—3.5%) of divergence from its sister lineages *R. ponmudi* and *R. hassanensis*. Morphologically, it shows differences in the dorsum coloration (uniform green), groin patterns and iris coloration (Fig 8). Of the known species of *Raorchestes*, this species was found to be of the largest (50.5 mm: female). Phylogenetic position and morphological distinctness are the two axes on which this lineage is diagnosed.

**Field diagnosis. Morphology.** *Raorchestes emeraldi* **sp. nov.** resembles its sister lineage *R. ponmudi* in overall morphometric characters, however it exhibits strong divergence in coloration from its sister lineages, *R. hassanensis* and *R. ponmudi*. It could be distinguished in having green dorsum (Fig 8a) (vs. dorsum with varying shades of brown in *R. ponmudi* (Biju and Bossuyt, 2009)); region of groin, front and back of thighs, under side of tibia and front of metatarsal with brown and yellow reticulated pattern (vs. posterior surface of thighs light chocolate brown vermiculated with grey patches of variable size in *R. ponmudi* (Biju and Bossuyt, 2009)); Additionally new species can be differentiated from other related congeners by the following combination of characters; (1) large adult size (SVL 36.5–50.5 mm, n=2); (2) head width larger than head length (HW 15.2–21.0 mm & HL 12.9–16.2 mm); (3) snout sub acuminate, sub equal to eye length (SL 5.0–6.5 mm & EL 5.1–6.9 mm); (4) skin on dorsum lateral side smooth and ventral region granular; (5) dorsum green with minute yellow spots.

**Geography.** Restricted to the Anaimalai Massif (see natural history and distribution for details).

Description of holotype (all measurements in mm). A large sized bush frog (SVL = 36.5 mm), width of head broader than head length (HW = 15.2 mm; HL = 12.9 mm), flat dorsally; snout short and sub acuminate, slightly protruding beyond mouth. Snout length is sub equal to diameter of eye (SL = 5.0 mm, EL = 5.1 mm). Canthus rostralis angular rounded, loreal region slightly concave. Interorbital space (IUE = 4.0 mm) flat and equal to upper eyelid (UEW = 3.3 mm). Interorbital space between posterior margins of the eyes 1.9 times that of anterior margins (IFE = 7.0, IBE = 13.3 mm). Nostrils oval and nearer to the tip of the snout. Moderate symphyseal knob. Pupil horizontal. Tympanum moderate, rounded, visible behind the eye, 2.3 times less than the eye diameter (TYD = 2.2 mm). Tongue bifid, granular with a papilla. Supratympanic fold from behind eye to shoulder.

Relative length of fingers I<II<IV<III, finger tips with well developed disks ( $fd_3 = 2.7$  mm;  $fw_3 = 1.4$  mm) with distinct circum-marginal grooves, fingers with dermal fringes on both sides. Webbing on palm absent, subarticular tubercles moderate, rounded and pre-pollex indistinct. Supernumerary tubercles absent.

Hind limb long, heels touch when folded at right angles to the body. Thigh/Femur (TL = 17.0 mm), slightly lesser than Shank/Tibia (ShL = 18.2 mm) length and foot (FOL = 16.0 mm) and much less than heel to tip of fourth toe (TFOL = 26.0 mm). Relative toe length I<II<III<V<IV, webbing medium, web formula (I 1-1 II  $\frac{1}{2}$ -1 III  $\frac{1}{2}$ -1 IV 1-0 V). Tibiotarsal articulation reaches posterior corner of eye. Outer metatarsal tubercle, supernumerary tubercles and tarsal tubercle absent.

**Color in life.** Dorsum uniform green with scattered yellow spots (Fig 8a); green colouration extending to canthus, arm up to  $\frac{1}{4}$ th of outer finger (rest of the fingers flesh coloured, finely speckled with brown), surface of femur, tibia, tarsus and base of outer two toes. Armpits are fleshy, purplish with fine brown specks. Upper lip golden white, lower lip and throat region iridescent off white. Lateral part of mid belly with yellow spots on a dark brown background. Groin, anterior and posterior femur with distinct yellow blotches on a dark brown background. Outer posterior orbital ring bluish green, upper edge of iris dark maroon, interior of iris golden brown with fine markings radiating towards the outer edge. Outer edges of the iris with a green wash (Fig 8b).

**Etymology.** The species is named after its dominant dorsum colour ‘emerald’.

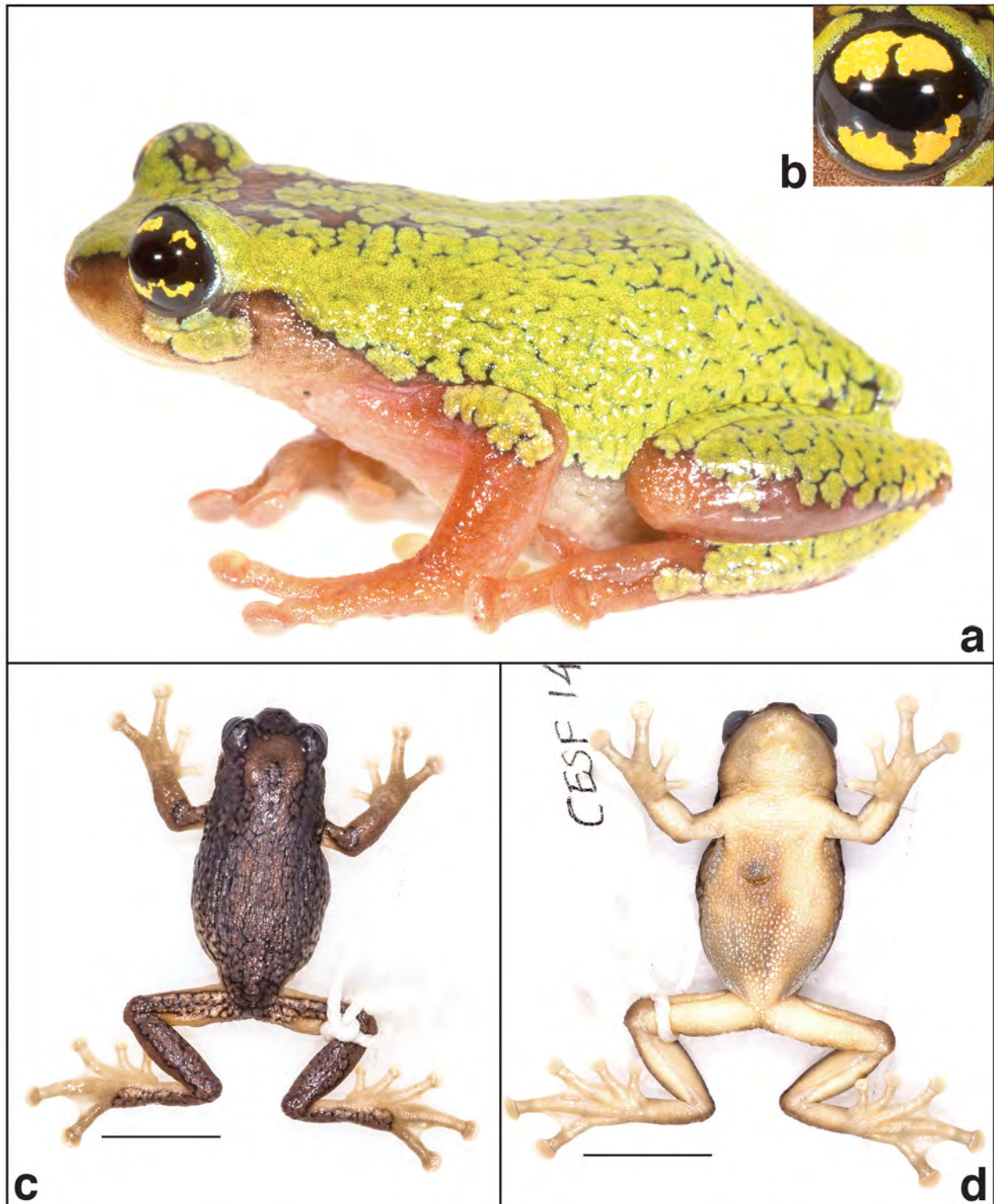
**Natural history and distribution.** We discovered this species from a rainforest fragment at the eastern edge of the Valparai plateau. It appears to be a forest species, occurring in the higher elevation (1249–1488, n = 7) wet evergreen forests of the Anaimalai Massif (Fig 1 & 2). It replaces *R. ponmudi*, a common species of the low and mid-elevations (mean ~ 900 m, n=77) of southern parts of the Western Ghats. We suspect a narrow zone of overlap between these species around 1200–1400 m in the Valparai plateau.

## 6. *Raorchestes flaviocularis* **sp. nov.**

(Figures 2, 3, 9 & 10; Tables 2 & 3)

**Holotype:** ZSI/WGRC/V/A/875 (CESF1406) (SVL 26.5 mm), collected by S.P. Vijayakumar and Varun R Torsekar in September 2011 from a disturbed forest fragment site (9.6064 N, 77.3033 E) located in a tea garden mosaic, Megamalai Massif (Fig 1), Peninsular India.





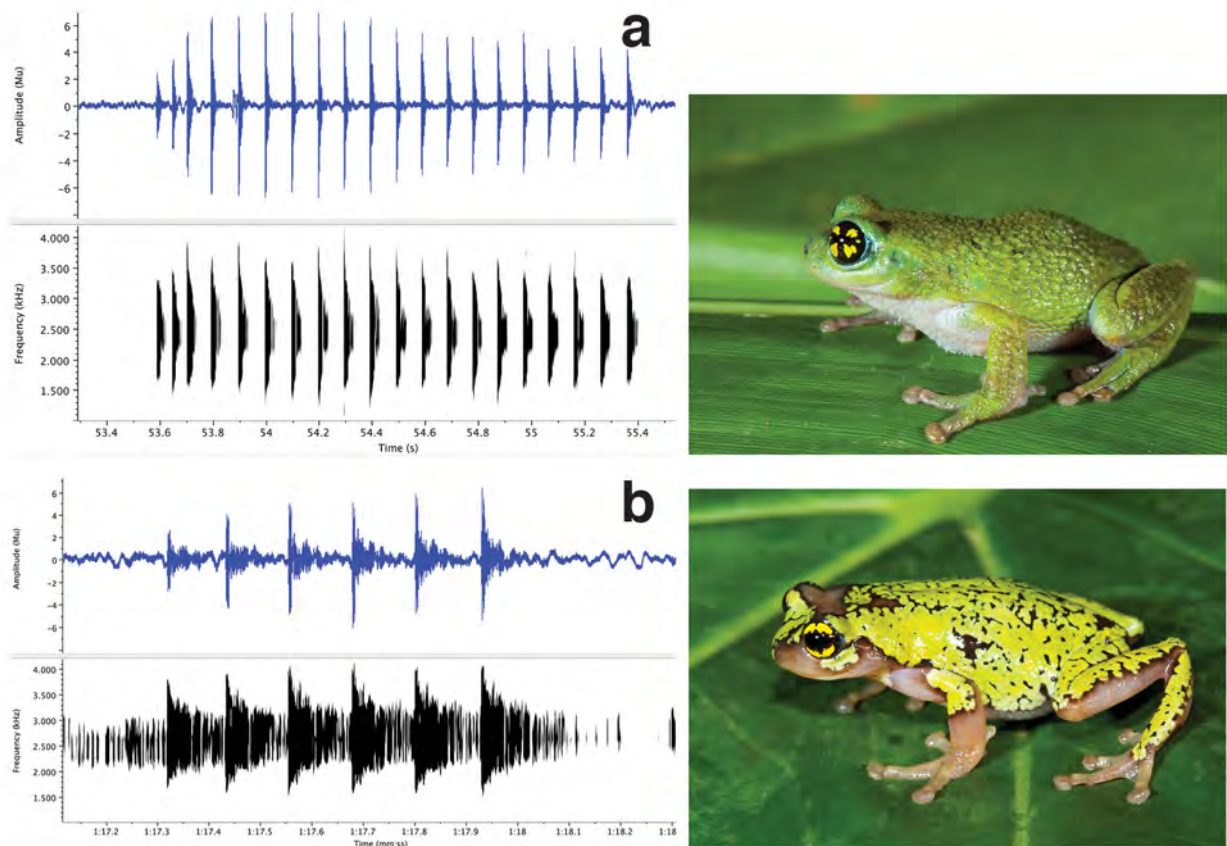
**FIGURE 9.** *Raorchestes flaviocularis* **sp. nov.** (a) profile in life; (b) eye profile in life; (c) dorsal profile of holotype; (d) ventral profile of holotype.

**Paratype:** ZSI/WGRC/V/A/876 (CESF1251) (SVL 23.9), collected by S.P. Vijayakumar, Mrugank V. Prabhu and Mayavan in August 2010 from a disturbed forest fragment site (9.6064 N, 77.3033 E) located in a tea garden mosaic, Megamalai Massif (Fig 1), Peninsular India.

**Lineage diagnosis.** *Raorchestes flaviocularis* **sp. nov.** can be diagnosed phylogenetically as a member of the



Ochlandrae clade (Fig 3), showing sister relationship to *Raorchestes chalazodes* (Fig 10a) (see discussion below). Though it exhibits shallow divergence (16S—1.2 %) with its allopatric sister, we diagnose this lineage and consider it for description based on its phylogenetic position (Ochlandrae clade), distinct morphology (coloration and skin pattern), geographical range and acoustic divergence (Fig 9, 10).



**FIGURE 10.** Waveform and spectrogram of (a. *Raorchestes chalazodes*, top: waveform, bottom: spectrogram) and its sister lineage (b. *Raorchestes flaviocularis* **sp. nov.**, top: waveform, bottom: spectrogram). The two lineages show strong divergence in the temporal structure of the call (see text).

**Field diagnosis. Morphology.** *Raorchestes flaviocularis* **sp. nov.** shows strong similarity with its sister lineage *R. chalazodes* in the morphometric variables considered. However, it exhibits very strong divergence in dorsum skin coloration and patterns (see Fig 9a, 10b). In *Raorchestes flaviocularis* **sp. nov.**, the green dorsum coloration, with a lichen pattern, do not extend on to the hand and foot (vs. dorsal skin colour uniform green extending on to the hand and foot in *R. chalazodes* (Fig 10a). It exhibits signatures of divergence in the limb length (shorter thigh/femur length (TL/SVL=0.40, 0.40–0.40, n=2) in *Raorchestes flaviocularis* **sp. nov.** in comparison to *R. chalazodes* (TL/SVL=0.43, 0.40–0.45, n=3) and shorter tibia/shank length (ShL/SVL=0.42, 0.42–0.43, n=2) in *Raorchestes flaviocularis* **sp. nov.** in comparison to *R. chalazodes* (ShL/SVL=0.45, 0.44–0.45, n=3). Additionally, the new species can be readily distinguished from all other close relatives by its iris pattern (characterized by very distinct small golden yellow patches on a dark background color) and also the dorsum coloration and skin pattern (Fig 9).

**Behaviour.** *Raorchestes flaviocularis* **sp. nov.** shows divergence from its sister lineage in its shorter call length ( $0.59 \pm 0.07$  (N=19) vs.  $2.11 \pm 0.42$  (N=43) in *R. chalazodes*), low number of pulses ( $4.95 \pm 0.52$  (N=19) vs.  $21.08 \pm 3.47$  (N=24) in *R. chalazodes*, lower pulse rate ( $7.36 \pm 0.62$  (N=19) vs.  $9.99 \pm 0.96$  (N=24) in *R. chalazodes* and greater dominant frequency ( $2675.82 \pm 74.19$  (N=38) vs.  $2523.78 \pm 62.93$  (N=23) in *R. chalazodes*) (Fig 10). Considering the short overlap in the range of dominant frequency of the calls of the two lineages, we mainly use strong divergence in the temporal call characteristics as an additional evidence for recognizing and naming this lineage.

**Geography.** Restricted to the Megamalai Massif (see natural history and distribution for details).

**Description of holotype (all measurements in mm).** A small sized bush frog (SVL = 26.5 mm), width of head broader than head length (HW = 9.7 mm; HL = 6.7 mm), arched, flat dorsally; snout short and acuminate in total profile, slightly protruding beyond mouth. Snout length is sub equal to diameter of eye (SL = 2.6 mm, EL = 3.2 mm). Canthus rostralis rounded, loreal region slightly concave. Interorbital space (IUE = 2.7 mm) flat, slightly broader than upper eyelid (UEW = 1.7 mm). Interorbital space between posterior margins of the eyes 1.8 times that of anterior margins (IFE = 4.5, IBE = 8.2 mm). Nostrils oval, nearer to tip of snout. Weak symphyseal knob. Pupil horizontal. Tympanum rather indistinct, rounded, barely visible behind the eye. Tongue bifid, granular with a distinct retractile papilla. Supratympanic fold from behind eye to shoulder.

Relative length of fingers I<II<IV<III, finger tips with well developed disks ( $fd_3 = 2.0$  mm;  $fw_3 = 1.1$  mm) with distinct circum-marginal grooves, fingers with dermal fringes on both sides. Webbing on palm absent, subarticular tubercles indistinct and pre-pollex indistinct. Supernumerary tubercles absent.

Hind limb long, heels barely touch when folded at right angles to the body. Thigh/Femur (TL = 10.2 mm), sub equal to Shank/Tibia (ShL = 10.1 mm) and less than heel to tip of fourth toe (TFOL = 15.4 mm). Relative toe length I<II<III<V<IV, webbing moderate web formula (I 1-1 II 1-1 III 1-2 IV 2-1 V). Tibiotarsal articulation reaches tympanic region. Outer metatarsal tubercle, supernumerary tubercles and tarsal tubercle absent.

**Color in life.** Dorsum with a distinct lichen pattern with uniform green colouration (Fig 9 (a)), the pattern broken irregularly exposing the brown fleshy skin colouration; dorsal pattern extends to mid belly laterally and to dorsal surface of femur, tibia and lower tarsus. Canthus region fleshy brown with occasional green patches in few individuals. Dorsal parts of arms, fingers and disc colour similar to canthus region. Groin, anterior and posterior femur, tibia and tarsus flesh coloured. Iris dark brown with distinct irregular golden yellow patches.

**Etymology.** The species is named after the ‘metallic yellow’ colour of the iris (Latin: *flavin*= yellow; *oculus*= eye).

**Natural history and distribution.** A sub-canopy lineage (325 cm, n=2), it is difficult to locate due to its ventriloquistic call and occurrence in the sub-canopy. Like other members of the Ochlandrae clade, it was also heard calling from *Ochlandra* grass patches. However, the two individuals whose descriptions are given were obtained from a highly disturbed forest fragment, on leaves of short trees (<5 m). Calls were also recorded from high in the canopy. Considering the strong association of Ochlandrae clade lineages with *Ochlandra* reeds, further observations are needed to verify the habitat association of this new lineage. It is a species of high elevations (1459–1569 m, n = 10), and restricted in distribution to the Upper Manalar Plateau, Megamalai Massif (Fig 1 & 2) in the southern Western Ghats. Based on our call recordings in the adjoining Anaimalai Massif (Fig 1), we anticipate a related lineage or an isolated population.

## 7. *Raorchestes indigo* sp. nov.

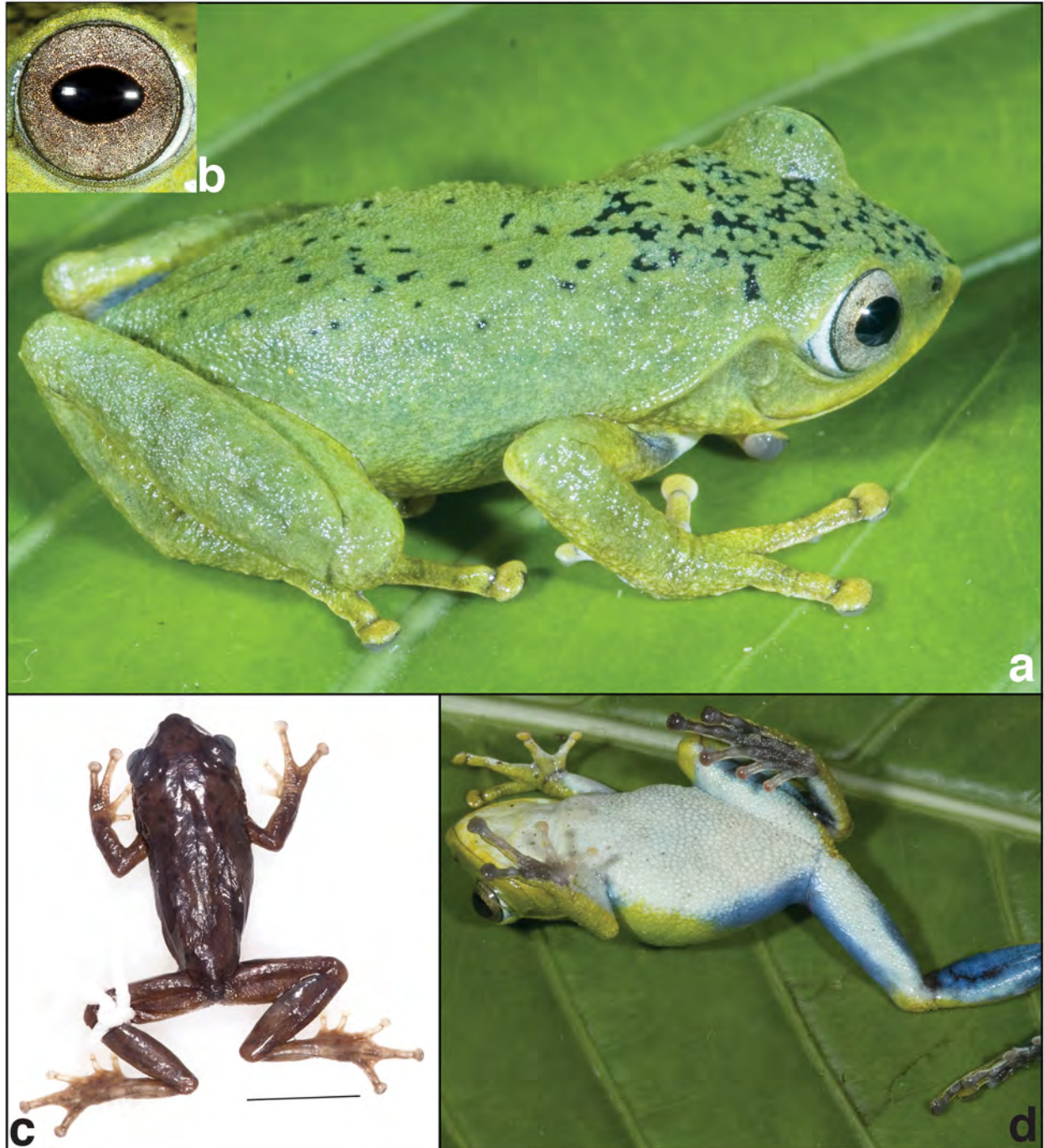
(Figures 2, 3 & 11; Tables 2 & 3)

**Holotype:** ZSI/WGRC/V/A/877 (CESF1437), a female (SVL 25.7 mm), collected by S.P. Vijayakumar and K.P. Dinesh in September 2011 from a stunted forest site (13.1333 N, 75.2704 E), Kudremukh Massif (Fig 1), Western Ghats, Peninsular India.

**Paratype:** ZSI/WGRC/V/A/878 (CESF123), a female (SVL 24.4 mm), collected by S.P. Vijayakumar and M.S. Chaitra in October 2008 from a stunted forest site (13.1347 N, 75.2705 E) Kudremukh Massif (Fig 1), Western Ghats, Peninsular India.

**Lineage diagnosis.** *Raorchestes indigo* sp. nov. could be readily diagnosed by its deep divergence (16S—6.4%) on the phylogenetic tree (Fig 3), with an unresolved relationship status within clade N. It is morphologically distinct (Fig 11) from all other members of the subclade N. It is geographically restricted to the high elevations of the Kudremukh Massif. The lineage is diagnosed based on four axes: phylogenetic position, genetic divergence, morphological distinctness and geographical distribution.

**Field diagnosis. Morphology.** There are no close relatives (within the clade N) that could be confused with this lineage. All can be readily distinguished by a combination of the following characteristics. (1) Size (SVL 24.3–25.7 mm, n=5); (2) Dorsum greenish with irregular black and yellow spots/blotches; (3) ventrally uniform bluish white (Fig 11 (d)) (4) indigo coloration of the groin, posterior arm pits, anterior and posterior femur, tibia and tarsus (unique among the species of *Raorchestes*, Fig 11 (d)); (5) iris silvery brown (Fig 11 (b)).



**FIGURE 11.** *Raorchestes indigo* sp. nov. (a) profile in life; (b) eye profile in life; (c) dorsal profile of holotype; (d) ventral profile in life.

**Geography.** Restricted in distribution to the high elevations of the Kudremukh Massif (see natural history and distribution for details).

**Description of holotype (all measurements in mm).** A small sized bush frog (SVL = 25.7 mm), width of head broader than head length (HW = 10.1 mm; HL = 7.0 mm), flat dorsally; snout acutely pointed, slightly protruding beyond mouth. Snout length is sub equal to diameter of eye (SL = 3.2 mm, EL = 2.8 mm). Canthus rostralis angular, loreal region flat. Interorbital space (IUE = 3.2 mm) flat and broader than upper eyelid (UEW = 2.3 mm). Interorbital space between posterior margins of the eyes 1.7 times that of anterior margins (IFE = 5.2, IBE = 8.9 mm). Nostrils oval, nearer to tip of snout. Weak symphyseal knob. Pupil horizontal. Tympanum indistinct,



barely visible behind the eye. Tongue bifid, granular with a retractile papilla. Supratympanic fold from behind eye to shoulder.

Relative length of fingers  $I < II < IV < III$ , finger tips with well developed disks ( $fd_3 = 1.1$  mm;  $fw_3 = 0.9$  mm) with distinct circum-marginal grooves, fingers with dermal fringes on both sides. Webbing on palm absent, subarticular tubercles moderate and pre-pollex tubercle moderate. Supernumerary tubercles absent.

Hind limb long, heels barely touch when folded at right angles to the body. Thigh/Femur (TL = 10.7 mm) sub equal to Shank/Tibia (ShL = 11.1 mm); sub equal to foot (FOL = 9.7 mm) and less than heel to tip of fourth toe (TFOL = 15.1 mm). Relative toe length  $I < II < III < V < IV$ , webbing moderate, web formula (I 1-1 II 1-2 III 1-2 IV 2-1 V). Tibiotarsal articulation reaches posterior corner of eye. Outer metatarsal tubercle, supernumerary tubercles and tarsal tubercle absent.

**Color in life.** Dorsum, canthal region, lateral parts distinctly green with bluish tinge; posterior arm pits, groin, anterior and posterior femur, tibia and tarsus distinctly indigo varying in lighter to darker (at groin) shades. Dorsum with irregular fine black blotches, denser towards head region. Ventrally uniform bluish white (Fig 11d), throat white with yellow at the lip margins. Iris with a distinct silvery background, coarsely speckled with shades of brown.

**Etymology.** The species is named after the unique ‘indigo’ colour of the groin and other under parts of the body. The specific epithet “indigo” is used as a noun in apposition to generic name.

**Natural history and distribution.** A range restricted species, observed in the high elevation (>1700 m) stunted forests around the highest peak in the Kudremukh Massif (Fig 1 & 2). All the individuals were observed on the forest floor on leaves (avg. 116 cm above ground level,  $n=6$ ). Further surveys are needed to locate populations in other high elevations zones within Kudremukh Massif and also to explore the presence of any allied lineages in the adjacent Pushpagiri Massif.

## 8. *Raorchestes leucolatus* sp. nov.

(Figures 2, 3 & 12; Tables 2 & 3)

**Holotype:** ZSI/WGRC/V/A/879 (CESF1146), an adult male (SVL 16.9 mm), collected by S.P. Vijayakumar, Mrugank V. Prabhu and Mayavan in July 2010 from a wet evergreen forest site (10.9731 N, 76.6289 E), Elivalmalai Massif (Fig 1), Peninsular India.

**Paratype:** ZSI/WGRC/V/A/880 (CESF1147), an adult male (SVL 17.1 mm), collected by S.P. Vijayakumar, Mrugank V. Prabhu and Mayavan in July 2010 from a wet evergreen forest site (10.9731 N, 76.6289 E), Elivalmalai Massif (Fig 1), Western Ghats, Peninsular India.

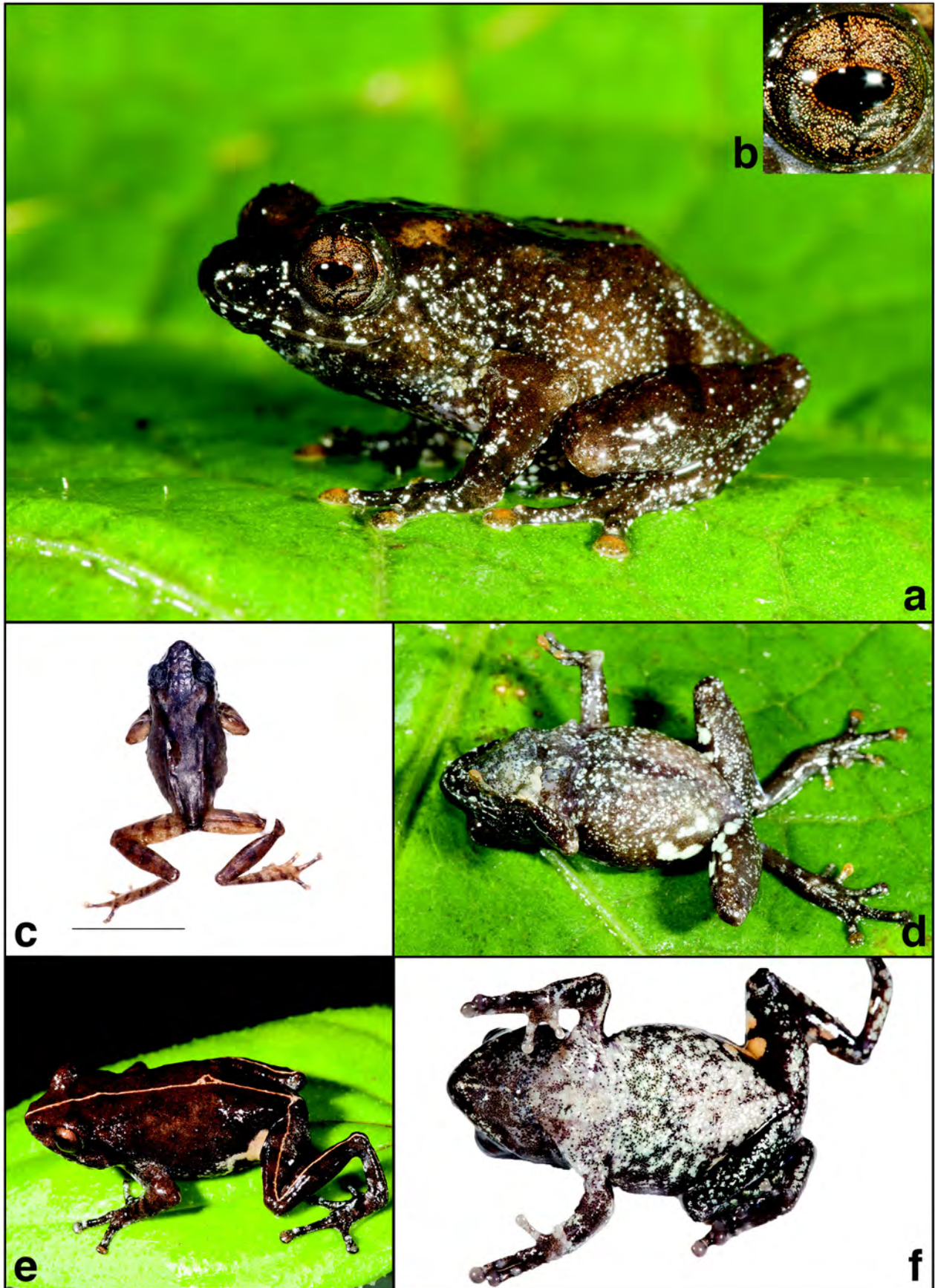
**Lineage diagnosis.** *Raorchestes leucolatus* sp. nov. can be diagnosed by its phylogenetic position within the Bombayensis clade (Fig 3) and exhibits moderate levels (16S—2.9%) of divergence from its closest relative *R. tuberochumerus*. It also shows strong differences in morphology (Fig 12 a,d,e,f). The lineage is diagnosed based on its phylogenetic position, genetic divergence and morphological distinctness.

**Field diagnosis. Morphology.** *Raorchestes leucolatus* sp. nov. could be morphologically confused with its close relative *R. tuberochumerus*. However, it can be distinguished from *R. tuberochumerus* on many aspects of morphology. *Raorchestes leucolatus* sp. nov. can be distinguished by its smaller size (males) 16.9 mm (16.2–17.1,  $n=4$ ) (vs. 18.4 mm (17.7–19.0,  $n=6$ ) in *R. tuberochumerus*); head width, HW/SVL=0.38 (0.37–0.39,  $n=4$ ) greater than head length, HL/SVL=0.29 (0.28–0.31,  $n=4$ ) (vs. HW/SVL=0.35 (0.33–0.36,  $n=6$ ) almost equal to head length (HL/SVL=0.37 (0.36–0.40,  $n=6$ ) in *R. tuberochumerus*); shorter thigh length, TL/SVL=0.45 (0.43–0.46,  $n=4$ ) (vs. TL/SVL=0.50 (0.46–0.52,  $n=6$ ) in *R. tuberochumerus*); shorter foot length, FOL/SVL=0.36 (0.35–0.36,  $n=4$ ) (vs. FOL/SVL=0.40 (0.37–0.43) in *R. tuberochumerus*); groin region with white blotches (vs. groin region with yellow blotches in *R. tuberochumerus*); disc colour orange (vs. disc colour grey to brown in *R. tuberochumerus*).

**Geography.** Found to be restricted to the mid-elevations of Elivalmalai Massif (see natural history and distribution for details).

**Ecology.** Found to be an understory forest species ( $n=4$ ) and was observed in short grasses and shrubs along the forest edges.

**Description of holotype (all measurements in mm).** A small sized bush frog (SVL = 16.9 mm), width of head sub equal to head length (HW = 6.2 mm; HL = 5.2 mm), flat dorsally; snout acutely pointed in total profile,



**FIGURE 12.** *Raorchestes leucolatus* sp. nov. (a) profile in life; (b) eye profile in life; (c) dorsal profile of holotype; (d) ventral profile in life; (e & f) dorsal and ventral profile of *R. tuberothumerus* (note groin and disc coloration).



slightly protruding beyond mouth. Snout length is sub equal to diameter of eye (SL = 2.2 mm, EL = 2.3 mm). Canthus rostralis angular, loreal region flat. Interorbital space (IUE = 2.1 mm) flat and sub equal to upper eyelid (UEW = 1.5 mm). Interorbital space between posterior margins of the eyes 1.7 times that of anterior margins (IFE = 3.5, IBE = 5.8 mm). Nostrils oval, nearer to tip of snout. Weak symphyseal knob. Eyes small, pupil horizontal. Tympanum indistinct, rounded, barely visible behind the eye. Tongue bifid, granular without papilla. Supratympanic fold from behind eye to shoulder.

Relative length of fingers I<II<IV<III. Finger tips with well developed small disks ( $fd_3 = 0.8$  mm;  $fw_3 = 0.5$ ) with distinct circum-marginal grooves, fingers with dermal fringes on both sides. Webbing on palm absent, subarticular tubercles moderate and pre-pollex moderate. Supernumerary tubercles absent.

Hind limb long, heels touch when folded at right angles to the body. Thigh/Femur (TL = 7.8 mm), sub equal to Shank/Tibia (ShL = 7.5 mm); longer than foot (FOL = 6.1 mm) and less than heel to tip of fourth toe (TFOL = 10.2 mm). Relative toe length I<II<III<V<IV, webbing poor; web formula (I 1-1 II 1-2 III 1-2 IV 2-1 V). Tibiotarsal articulation reaches posterior corner of eye. Outer metatarsal tubercle, supernumerary tubercles and tarsal tubercle absent.

**Color in life.** Dorsum maroon with a pair of distinct orange patch on the shoulder. An orange coloured horizontal broken band between the upper eyelids. Groin with distinct white blotches, ventrally varying shades of brown with irregular white spots on the belly. Throat darker towards lips, disks on finger and toes distinctly orange. Iris coarsely speckled with varying shades of golden brown, overlaid on an irregular brown markings. Distinct rufous edged speckles around the pupil (Fig 12 (b)).

**Etymology.** The species is named after one of its distinct characteristics, the ‘white patch’ on the groin (Greek: *leukos* = white).

**Natural history and distribution.** The species was discovered in the mid elevations (894–958 m,  $n=2$ ) and was observed at forested sites in the Elivalmalai Massif (Fig 1 & 2) situated north of Palghat Gap. Currently there are no reports of any allied species from north of its range. The southern most range of *R. tuberochumerus*, its geographically closest relative, appears to be Wayanad plateau (Fig 1). Further surveys are needed to verify the occurrence of this species or any close relatives in the lower elevations of Nilgiri Massif.

### 9. *Raorchestes primarrumpfi* sp. nov.

(Figures 2, 3, 13, 14 & 15; Tables 2 & 3)

**Holotype:** ZSI/WGRC/V/A/881 (CESF1276), a male (SVL 21.0 mm), collected by S.P. Vijayakumar and Mayavan June 2011 from a grassland site (11.2331 N, 76.5443 E), Nilgiri Massif (Fig 1), Western Ghats, Peninsular India.

**Paratype:** ZSI/WGRC/V/A/882 (CESF441), a male (SVL 19.9 mm), collected by S.P. Vijayakumar, August 2009 from a grassland site (11.2331 N, 76.5443 E), Nilgiri Massif (Fig 1), Western Ghats, Peninsular India.

**Lineage diagnosis.** *Raorchestes primarrumpfi* sp. nov. can be phylogenetically diagnosed as belonging to the Tinniens clade (Fig 3), showing a well supported sister relationship with an another unidentified lineage occurring in sympatry. Despite its shallow genetic divergence (1–2 % on 16S gene) from its sympatric sister lineage, it exhibits high divergence in morphological characteristics both in the multivariate morphological space (Fig 14) as well in the dorsal and ventral coloration (Fig 13). Iris coloration and patterns (Fig 13 (b)) were also found to show distinct differences from its sister lineage.

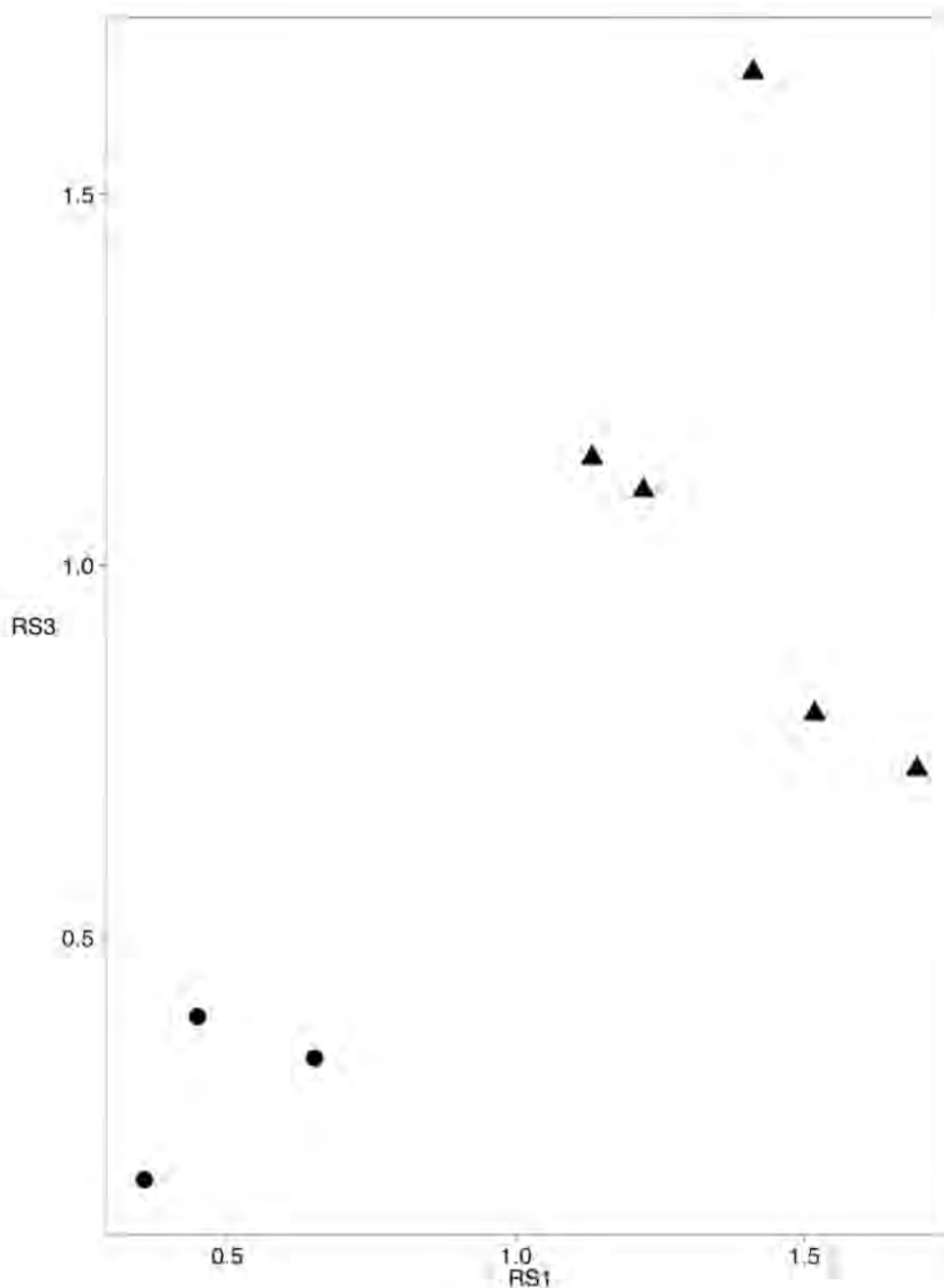
**Field diagnosis.** *Raorchestes primarrumpfi* sp. nov. can be distinguished from the related congeners by the following combination of characters.

**Morphology.** *Raorchestes primarrumpfi* sp. nov. can be distinguished by its (1) shorter tibia length ( $ShL/SVL=0.32$  (0.31–0.35,  $n=5$ ) (vs.  $ShL/SVL=0.45$  (0.43–0.46,  $n=3$ ) in the unidentified lineage and  $ShL/SVL=0.41$  (0.41–0.42,  $n=3$ ) in *R. tinniens*); (2) shorter thigh length ( $TL/SVL = 0.35$ ,  $n=5$ ) (vs.  $TL/SVL=0.45$ ,  $n=3$  in the unidentified lineage and  $TL/SVL=0.44$ ,  $n=3$  in *R. tinniens*); (3) smaller size (males) ( $SVL=20$  (18.4–21.0,  $n=5$ ) (vs. 22 (21–22.8,  $n=3$ ) in the unidentified lineage); (4) shorter head length ( $HL/SVL=0.26$  (0.24–0.28,  $n=5$ ) (vs. 0.37 (0.35–0.38,  $n=3$ ) in *R. tinniens*); (5) shorter snout length ( $SL/SVL=0.11$  (0.10–0.13,  $n=5$ ) (vs. 0.15,  $n=3$ ) in the unidentified lineage); (6) unique iris coloration, lower part dark maroon and upper half speckled with iridescent golden and silvery colour (vs. uniform brown in the unidentified lineage and uniform brown with golden speckles



**FIGURE 13.** *Raorchestes primarrumpfi* sp. nov. (a) profile in life; (b) eye profile in life; (c) dorsal profile of holotype; (d) ventral profile (in life); (e) *R. UI 427* (life); (f) *R. tinniens* (in life); (g) *R. UI 427* (eye profile—in life); (h) *R. UI 427* (ventral profile—in life)

in *R. tinniens* ; (7) dorsum largely granular, dark olive and with a consistent pattern of three distinct maroon longitudinal discontinuous stripes (vs. highly variable dorsum coloration with no distinct pattern in the related lineages); (8) two distinct maroon blotches on the eyelids extending slightly into inter-orbital space (vs. absent in the other lineages) (9) ventral coloration is white with a bluish wash towards sides (vs. shades of yellow, areolate skin, semi-transparent with yellow blotches in some individuals and shades of yellow with black spots in *R. tinniens* (Biju and Bossuyt 2009)).



**FIGURE 14.** Separation of male individuals of *Raorchestes primarrumpfi* **sp. nov.** (triangle) and *Raorchestes* UI 427 (circle) in multivariate space. Axes represent principal component scores.

**Geography.** Restricted in range to the very high elevations of the Nilgiri Massif (see natural history and distribution for details). Overlaps broadly in its geographical range with an unidentified lineage and *R. signatus*.

**Ecology.** Observed to be restricted to grasslands and swamps.

**Description of holotype (all measurements in mm).** A small sized squat bush frog (SVL = 21.0 mm), width of head broader than head length (HW = 7.5 mm; HL = 5.5 mm), arched, flat dorsally; snout rounded in total



profile, slightly protruding beyond mouth. Snout length is equal to diameter of eye (SL = 2.5 mm, EL = 2.4 mm). Canthus rostralis rounded, loreal region slightly concave. Interorbital space (IUE = 2.2 mm) flat and sub equal to upper eyelid (UEW = 1.8 mm). Interorbital space between posterior margins of the eyes 1.9 times that of anterior margins (IFE = 3.5, IBE = 6.6 mm). Nostrils oval, nearer to tip of snout. Weak symphyseal knob. Pupil horizontal. Tympanum indistinct. Tongue bifid, granular with a papilla. Supratympanic fold from behind eye to shoulder.

Relative length of fingers I<II<IV<III. Finger tips with small disks ( $fd_3 = 0.6$  mm,  $fw_3 = 0.6$  mm) with distinct circum-marginal grooves, fingers with dermal fringes on both sides. Webbing on palm absent, subarticular tubercles indistinct, pre-pollex indistinct and supernumerary tubercles absent.

Hind limb short, heels fall apart when folded at right angles to the body. Thigh/Femur (TL = 7.0 mm), sub equal to Shank/Tibia (ShL = 6.5 mm) and foot (FOL = 6.6 mm) but less than heel to tip of fourth toe (TFOL = 10.8 mm). Relative toe length I<II<III<V<IV, webbing rudimentary, web formula (I 1-1 II 1-2 III 1½-3 IV 2½-1½ V). Tibiotarsal articulation reaches shoulder region. Outer metatarsal tubercle, supernumerary tubercles and tarsal tubercle absent.

**Color in life.** Dorsum, background olive with a dark maroon longitudinal disconnected striped pattern, the pattern extending on to outer two fingers on the forelimb and femur, tibia, tarsus and outer toe on the hind limb. Canthus ridge darker extending to the tip of snout and flesh coloured, with olive patches, in a few individuals. Laterally, behind shoulders a distinct flesh coloured patch hidden in resting position; bluish white small blotches along the lateral sides and distinct blotches towards groin and anterior femur on a maroon background, this pattern is variable across individuals. Ventrally white, with a light bluish wash towards lateral edges. Ventral parts of tibia and tarsus with elongated white patches on a fleshy background. Iris, lower 1/3<sup>rd</sup> dark maroon, upper half speckled with iridescent golden and silvery colour and outer posterior orbital ring blue.



**FIGURE 15.** A typical habitat of *Raorchestes primarrumpfii* sp. nov. Detection of the species was greater in the swampy grasslands. Locality: Avalanche zone, Nilgiri Massif.

**Etymology.** Derived and modified from ‘*Primarrumpf*’, a German term used by geomorphologists to refer to remnant primitive surfaces of Gondwanaland. In the Western Ghats Escarpment, these surfaces occur in the Nilgiri and Anaimalai massifs.

**Natural history and distribution.** All the calling males were observed amidst dense grass clumps and herbs in the montane grasslands and the detection of this species was higher in swampy grasslands (Fig 15). It exhibits a



narrow geographical range and is restricted in distribution to the montane zone (2212–2359 m, n=13) towards the western edge of the Nilgiri Massif (Fig 1 & 2). The higher elevations of the Camels Hump Massif, adjacent to the Nilgiri Massif, might hold a relative of this lineage and needs further exploration.

**TABLE 3.** Extant valid species of *Raorchestes* in Western Ghats.

Sl. No	Species
1	<i>Raorchestes agasthyaensis</i> Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot and Kalesh, 2011
2	<i>Raorchestes akroparallagi</i> (Biju and Bossuyt, 2009)
3	<i>Raorchestes anili</i> (Biju and Bossuyt, 2006)
4	<i>Raorchestes archaeos</i> sp. nov.
5	<i>Raorchestes aureus</i> sp. nov.
6	<i>Raorchestes beddomii</i> (Gunther, 1876)
7	<i>Raorchestes blandus</i> sp. nov.
8	<i>Raorchestes bobingeri</i> (Biju and Bossuyt, 2005)
9	<i>Raorchestes bombayensis</i> (Annandale, 1919)
10	<i>Raorchestes chalazodes</i> (Gunther, 1876)
11	<i>Raorchestes charius</i> (Rao, 1937)
12	<i>Raorchestes chlorosomma</i> (Biju and Bossuyt, 2009)
13	<i>Raorchestes chotta</i> (Biju and Bossuyt, 2009)
14	<i>Raorchestes chromasyrchysi</i> (Biju and Bossuyt, 2009)
15	<i>Raorchestes coonoorensis</i> (Biju and Bossuyt, 2009)
16	<i>Raorchestes crustai</i> Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot and Kalesh, 2011
17	<i>Raorchestes dubois</i> (Biju and Bossuyt, 2006)
18	<i>Raorchestes echinatus</i> sp. nov.
19	<i>Raorchestes emeraldi</i> sp. nov.
20	<i>Raorchestes flaviventris</i> (Boulenger, 1882)*
21	<i>Raorchestes flaviocularis</i> sp. nov.
22	<i>Raorchestes ghatei</i> Padhye, Sayyed, Jadhav and Dahanukar, 2013
23	<i>Raorchestes glandulosus</i> (Jerdon, 1853)
24	<i>Raorchestes graminirupes</i> (Biju and Bossuyt, 2005)
25	<i>Raorchestes griet</i> (Bossuyt, 2002)
26	<i>Raorchestes hassanensis</i> (Rao, 1937) <sup>#</sup>
27	<i>Raorchestes indigo</i> sp. nov.
28	<i>Raorchestes jayarami</i> (Biju and Bossuyt, 2009)
29	<i>Raorchestes johnceei</i> Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot and Kalesh, 2011
30	<i>Raorchestes kadalarensis</i> Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot and Kalesh, 2011
31	<i>Raorchestes kaikatti</i> (Biju and Bossuyt, 2009)
32	<i>Raorchestes kakachi</i> Seshadri, Gururaja and Aravind, 2012
33	<i>Raorchestes leucolatus</i> sp. nov.
34	<i>Raorchestes luteolus</i> (Kuramoto and Joshy, 2003)
35	<i>Raorchestes manohari</i> Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot and Kalesh, 2011
36	<i>Raorchestes marki</i> (Biju and Bossuyt, 2009)
37	<i>Raorchestes montanus</i> (Jerdon, 1875) <sup>#</sup>

.....continued on the next page

**TABLE 3.** (Continued)

Sl. No	Species
38	<i>Raorchestes munnarensis</i> (Biju and Bossuyt, 2009)
39	<i>Raorchestes nerostagona</i> (Biju and Bossuyt, 2005)
40	<i>Raorchestes ochlandrae</i> (Gururaja, Dinesh, Palot, Radhakrishnan and Ramachandra, 2007)
41	<i>Raorchestes ponmudi</i> (Biju and Bossuyt, 2005)
42	<i>Raorchestes primarrumpfi</i> sp. nov.
43	<i>Raorchestes ravii</i> Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot and Kalesh, 2011
44	<i>Raorchestes resplendens</i> Biju, Shouche, Dubois, Dutta and Bossuyt, 2010
45	<i>Raorchestes signatus</i> (Boulenger, 1882)
46	<i>Raorchestes sushili</i> (Biju and Bossuyt, 2009)
47	<i>Raorchestes theuerkaufi</i> Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot and Kalesh, 2011
48	<i>Raorchestes thodai</i> Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot and Kalesh, 2011*
49	<i>Raorchestes tinniensi</i> (Jerdon, 1853)
50	<i>Raorchestes travancoricus</i> (Boulenger, 1891)
51	<i>Raorchestes tuberothumerus</i> (Kuramoto and Joshy, 2003)
52	<i>Raorchestes uthamani</i> Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot and Kalesh, 2011

\* species not included in the phylogenetic tree; # details of revalidation will be dealt elsewhere (under preparation)

## Discussion

The Western Ghats, an escarpment on the west coast of Peninsular India, has been recognized as one of the primary areas for amphibians on Earth (Le Saout *et al.*, 2013). This region, along with the geographically adjacent island of Sri Lanka, was brought into the limelight following reports of discovery of hundreds of potentially new species of frogs (Pethiyagoda & Manamendra-Arachchi, 1998; Biju, 2001). A majority of the discovered species in these reports were bush frogs that are currently assigned to the genera *Raorchestes* and *Pseudophilautus*, and belong to the tree frog family Rhacophoridae (Frost, 2014). However, subsequent studies suggested that those numbers of new species, based on morphology, might be over-estimates. Later studies from Sri Lanka suggested that there might be about 100 rather than 200 species (Meegaskumbura *et al.*, 2002). Similarly, in the Western Ghats, despite initial claims of around 100 species, a recent revision documented 33 species of bush frogs (Biju & Bossuyt, 2009). A large number of species recognized on the basis of morphology remain valid (Meegaskumbura & Manamendra-Arachchi, 2005; Biju & Bossuyt, 2009), and the uncertainties cannot be assigned to a single cause. However, the history of description within this lineages highlights the chronic problems associated with species delimitation (Sites & Marshall, 2003), as different kinds of data have been shown to result in different outcomes (Wiens & Penkrot, 2002). Species delimitation based on a single criterion is prone to inconsistency, and agreement across multiple axes can provide a strong case for identifying independent lineages (Alström *et al.*, 2008).

An associated problem with species delimitation, as exemplified by amphibians in the tropics, is the “Linnean Shortfall” or the occurrence of many unknown lineages (Bini *et al.*, 2006; Vieites *et al.*, 2009). Numerous field studies (e.g. Zachariah *et al.*, 2011) have highlighted the fact that the inventory of bush frogs is incomplete. We attempted to address the problem of the “Linnean Shortfall” for bush frogs in the Western Ghats by focusing on the closely related problem of inadequate knowledge of species distribution, namely the “Wallacean Shortfall”. An assessment of previous reports (Biju & Bossuyt, 2009; Zachariah *et al.*, 2011) indicates the occurrence of large gaps in the areas that have been sampled for bush frogs. We managed to sample a majority of the massifs in the Western Ghats and our sampling was designed to account for the underlying topographic variation and associated ecological heterogeneity in this region. The explicit spatial approach allows us to compare lineages with their closest sisters with some degree of certainty, which provides the critical component of consistency in species delimitation.

Our analyses, based on multiple criteria, especially facilitated by explicit incorporation of data on species

ranges, in conjunction with phylogenetic relationships, shows a significant increase in the number of potential new lineages of bush frogs in the *Raorchestes* clade (Fig 3). Here, we describe nine potential lineages that could be diagnosed as independently evolving lineages based on strong support from a minimum of three of the four axes examined: phylogenetic, genetic distance, geographical separation and morphological separation. For a single shallow divergent lineage, we used divergence in call characteristics as an additional variable for delimitation. We attribute the discovery of a large number of unknown lineages and also our ability to sample >95% of the described lineages, in a short span of three years, to our spatial sampling design. The efficacy of the sampling is also evidenced by the discovery of deeply divergent new lineages from almost all the major massifs identified as potential regions during the initial stages of our work (see Fig 2).

We also report the rediscovery of *Raorchestes montanus* (Jerdon 1875) collected by Lt.-Col. Beddome from Kudremukh at an elevation of around 1800m (6000 feet). We located a population from close to the highest peak on the Kudremukh Massif. This isolated lineage was found to be a member of the Tinniens clade and is closely related to *R. tinniens*, distributed in the montane zone of the Nilgiri and Vellarimala Massifs. Bossuyt and Dubois (2001) considered this nomen as a synonym of *R. tinniens*, but with a comment on the morphological differences exhibited by individuals of *R. montanus* in the BMNH (British Natural History Museum). We were also able to rediscover multiple populations that closely matched the description of *R. hassanensis* (Fig 3), described as *Philautus montanus* by Rao (1937). This belongs to the Hassanensis clade (Fig 3), composed of *R. ponmudi*, *Raorchestes emeraldi* **sp. nov.** and *R. hassanensis*. It shares a direct sister species relationship with *R. ponmudi*, described recently from the southern Western Ghats (Biju and Bossuyt 2009). This sister-pair exhibits shallow divergence with an uncertain range boundary, which suggests that further work is required to validate the taxonomic status of *R. ponmudi*. Based on current knowledge, we expect the boundary between *R. hassanensis* and *R. ponmudi* to lie on the southern edge of the Coorg Plateau (Fig 1). We could assign populations that occur in Wayanad Plateau, south of Coorg Plateau, to *R. ponmudi* (see also (Biju & Bossuyt, 2009). A redescription of *R. hassanensis* and *R. montanus* species will be dealt with elsewhere (Dinesh and Vijayakumar *in prep.*).

All the new species described herein belong to the genus *Raorchestes*, and our analysis reaffirms the occurrence of three lineages of *Pseudophilautus* in the Western Ghats (Fig 3). Within the *Raorchestes* clade, we could also discern two well-supported sister clades at the basal end of the tree. These sister clades could be assigned a generic status which we defer for future work. With the addition of nine new lineages and rediscovery of two species, the number of described species of bush frogs in the Western Ghats would increase to a total of 55 species, with 52 species under the genus *Raorchestes* (Table 3) and three under the genus *Pseudophilautus*. The taxonomic status and phylogenetic position of *R. flaviventris* and *R. thodai* awaits further collections. *R. flaviventris* has not been sighted since its initial description by Boulenger in 1882 from Malabar (Biju & Bossuyt, 2009). We also located a species, tentatively assigned to *R. chalazodes*, whose identity needs further validation. Our analyses also reveal a large number of unknown lineages, with varying levels of divergence from their sister lineages. Some of these potential lineages, especially the shallow divergent lineages, require additional data, while others will be dealt with in subsequent work.

The results presented here also reveal the phylogenetic affinities of the species that were morphologically discerned in a recent study (Zachariah *et al.*, 2011) and confirms their initial generic allocation under *Raorchestes*. Our analyses also suggests that 50% of the lineages reported by Zachariah *et al.* (2011) consist of shallow divergent lineages, whose taxonomic status needs further additional exploration along other axes.

Based on our increased taxon sampling, we were also able to discern a number of well supported sub-clades within the larger clade of *Raorchestes* that were absent in the earlier reconstruction (see Biju & Bossuyt, 2009). We have assigned names for all the supported sub-clades recovered here to facilitate future species descriptions.

In the conventional morphology based diagnosis of new species, target species are generally compared with all other species in the group, for example the genus (Biju & Bossuyt, 2009; Zachariah *et al.*, 2011; Biju *et al.*, 2011). Herein, we take a different approach and show that, for species diagnosis, comparisons can be restricted to close relatives within the well-supported sub-clades recognized here. To facilitate this, we have split the diagnosis section into lineage diagnosis and field diagnosis. For lineage diagnosis, which is central to our delineation of taxa, we provide justification using multiple criteria including phylogenetic position, genetic divergence, geographical isolation, morphology and acoustic characteristics, that they are independently evolving lineages (*sensu de* Queiroz, 2007). Under field diagnosis, we compare and highlight the characters that distinguish a lineage from its close relative(s) using multiple criteria that can be measured/observed in the field: morphological, ecological,

geographical and behavioral. In the case of well-supported clades, the comparison is restricted to the sister lineages, while for lineages with poorly supported close relatives, comparisons are made within a larger pool of related lineages nested within the next well-supported clade to which the target lineage can be assigned.

This approach towards lineage diagnosis deviates from earlier studies and explicitly uses multiple variables and incorporates geography as one of the prominent axes in discerning lineages, especially shallow divergent lineages. Though we recovered a large number of potential lineages through our hierarchical method, we have adopted a conservative approach towards describing species, and have, at this time, only described highly divergent lineages and shallow divergent lineages that exhibited deep divergences in morphological and/or acoustic axes. Since we only used mitochondrial markers in delimiting lineages, one important caveat is the problem of mitochondrial introgression and incomplete lineage sorting that could potentially affect recently divergent complexes (Vences and Wake 2007). However, given our conservative approach to species described, we believe that the mitochondrial introgression and incomplete lineage sorting are less likely to affect the lineages recognized here.

The historical pattern of frog species discovery in the Western Ghats is marked by episodes of sudden discovery with intervals of long stasis (Aravind *et al.*, 2004). The bush frog discovery patterns follow this general trend, with a number of species described during latter part of the 18<sup>th</sup> century and early part of the 19<sup>th</sup> century followed by a long gap of several decades before researchers initiated fresh collections (eg. Kuramoto & Joshi, 2003; Biju & Bossuyt, 2005a; 2006; Gururaja, 2007; Seshadri *et al.*, 2012; Padhye *et al.*, 2013). The use of molecular based phylogenetic tools can be considered as one important reason for the recent burst in species discovery (Biju & Bossuyt, 2009). Though these tools facilitated our work, the incorporation of spatial sampling is equally or more important in increasing taxon sampling. The significance of this factor in the Western Ghats (and other similar regions of the world) cannot be overstated given the high topographic heterogeneity and the underlying geological and climatic history that has created ample opportunities for speciation in amphibians. The results here exemplify the significance of spatial sampling, and geographical range of species, that were used effectively in conjunction with recent molecular based phylogenetic tools and classical morphological variables, in uncovering deeply divergent unknown lineages.

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