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Phylogenetic position of the montane treefrog *Polypedates variabilis* Jerdon, 1853 (Anura: Rhacophoridae), and description of a related species

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Abstract

Since its original description, the Indian treefrog species *Polypedates variabilis* Jerdon, 1853 has been assigned variously to one of the widespread genera *Polypedates* Tschudi, 1832, *Rhacophorus* Kuhl & van Hasselt, 1822, and *Philautus* (*Kirtixalus*) Dubois, 1987. Here we present phylogenetic analyses based on 1.4 kb of mitochondrial DNA showing that *P. variabilis* and a previously undescribed relative are not nested within any of those genera, but stem from a lineage that originated relatively early in the rhacophorid radiation. We propose the name *Ghatixalus* gen. n. for this lineage, whose known members are restricted to high altitudes in the Western Ghats of India. The sister species of *G. variabilis* (Jerdon), comb. n. is described as *Ghatixalus asterops* sp. n. The morphological and ecological features of both species are discussed.

Keywords: Biodiversity; India; Molecular phylogenetics; New genus; New species; Old world treefrogs

Introduction

The Rhacophoridae constitute a radiation of over 270 treefrog species (Frost 2006), whose divergence from the Madagascan Mantellidae has been associated with the breakup of Gondwana (Bossuyt and Milinkovitch 2001; Van Bocxlaer et al. 2006). Primary centres of rhacophorid diversity are now located in Southeast Asia and the Indian subcontinent (Inger 1999), the latter centre being characterised by remarkable species abundance and endemism (Dutta 1997; Biju 2001; Kuramoto and Joshy 2003; Biju and Bossuyt 2003, 2005a–c; Das and Kunte 2005; Meegaskumbura and Manamendra-Arachchi 2005; Das and Dutta 2006).

The generic allocation of many rhacophorid species is complicated by the poor definition of the recognized genera, which generally lack distinct morphological synapomorphies or show a high degree of homoplasy. A particularly problematic species is *Polypedates variabilis* Jerdon, 1853, described from the Nilgiri Hills in the Indian Western Ghats. Since its original description, *P. variabilis* (or its junior synonym, *P. pleurostictus* Günther, 1864) has been assigned variously to *Rhacophorus* (e.g. Inger and Dutta 1986; Daniel and Sekar 1989; Dutta 1997; Bossuyt and Dubois 2001), to *Philautus* (*Kirtixalus*) (Dubois 1987) or to *Polypedates* (Ravichandran 1997; Biju 2001). This instability was due to the fact that none of those assignments is supported by convincing morphological or ecological characters. To clarify the evolutionary position of *Polypedates variabilis* and an undescribed relative, we performed

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molecular phylogenetic analyses of approximately 1.4 kilobases (kb) of mitochondrial DNA from both species, as well as from representatives of the major rhacophorid lineages.

Material and methods

Field survey and specimen collection

Ecological surveys and collection of specimens were performed during field trips in the Western Ghats between 1997 and 2000. Specimens were preserved in 5% formaldehyde for 2 days, then transferred to 70% ethanol. Samples for molecular analyses were taken from muscle tissue, preserved in 100% ethanol and stored at –20 °C.

Phylogenetics

For phylogenetic inference, we assembled a mitochondrial DNA matrix of approximately 2000 base pairs (bp), covering part of the *12S RNA* gene, the complete $tRNA^{Val}$ gene, and part of the *16S RNA* gene. The relevant sequences were compiled for *Polypedates variabilis*, and 32 other rhacophorid ingroup species (Appendix A). This taxon set comprises all genera recognized in Dubois (2005), and includes 11 type species. Because the phylogenetic position of *Polypedates variabilis* is particularly relevant with respect to *Polypedates* and *Rhacophorus*, these genera are represented by an increased number of taxa (five and nine species, respectively). Five species from families closely related to Rhacophoridae served as outgroup taxa. For most species, DNA sequences were retrieved from previous studies (Richards and Moore 1998; Meegaskumbura et al. 2002; Wilkinson et al. 2002); others were newly obtained by whole-genome extraction (Sambrook et al. 1989), PCR amplification, and cycle sequencing along both strands. The new sequences are deposited in GenBank under accession numbers EU178086–EU178099. Alignments were created using the program ClustalX 1.81 (Thompson et al. 1997); minor corrections were made with MacClade 4.06 (Maddison and Maddison 2000).

Phylogenetic relationships were estimated using heuristic maximum-parsimony (MP) and maximum-likelihood (ML) searches, both executed with the program PAUP* 4.0b10 (Swofford 2002). The MP analysis involved equal character weighting, 1000 replicates of random taxon addition and tree-bisection-reconnection branch swapping. The ML search included 10 replicates of random taxon addition and was performed using a general time-reversible (GTR) model of DNA evolution, with gamma correction for among-site rate heterogeneity and an estimated proportion of invariable sites. Clade confidence was assessed by non-parametric bootstrap analyses, under MP using PAUP* and under ML using PHYML 2.4.4 (Guindon and Gascuel 2003), with 1000 sampling replicates in both cases.

Morphology

Measurements (in mm) and terminology follow Bossuyt and Dubois (2001).

The following measurements were taken to the nearest 0.1 mm, using a digital slide-calliper or a binocular microscope with a micrometre ocular: EL = eye length, horizontal distance between bony orbital borders of eye; EN = eye to nostril distance, i.e. from nostril to anterior orbital border of eye; $FD_{I–IV}$ = disk width on fingers I–IV; FFTF = distance from maximum incurvation of web between fourth and fifth toe to tip of fourth toe; FLL = forelimb length, from elbow to base of outer palmar tubercle; FOL = foot length, from base of inner metatarsal tubercle to tip of fourth toe; FTL = length of fourth toe, from base of first subarticular tubercle to tip of fourth toe; $FW_{I–IV}$ = width of fingers I–IV, at base of disk; HAL = hand length, from base of outer palmar tubercle to tip of third finger; HL = head length, from rear of mandible to tip of snout; HW = head width, at angle of jaw; IBE = internal back of eyes, shortest distance between posterior orbital borders of eyes; IFE = internal front of eyes, shortest distance between anterior orbital borders of eyes; IMT = inner metatarsal tubercle length; IN = internarial distance, i.e. between internal borders of nostrils; ITL = inner toe length; IUE = inter upper eyelid width, the shortest distance between the upper eyelids; MBE = distance from rear of mandible to posterior orbital border of eye; MFE = distance from rear of mandible to anterior orbital border of eye; MN = distance from rear of mandible to nostril; MTFF = distance from distal edge of metatarsal tubercle to maximum incurvation of web between fourth and fifth toe; MTTF = distance from distal edge of metatarsal tubercle to maximum incurvation of web between third and fourth toe; NS = distance from nostril to tip of snout; ShL = shank length; ShW = maximum shank width; SL = snout length, from tip of snout to anterior orbital border of eye; SVL = snout-vent length; $TD_{I–V}$ = disk width on toes I–V; TFL = third finger length, from base of first subarticular tubercle; TFOL = distance from heel to tip of fourth toe; TFTF = distance from maximum incurvation of web between third and fourth toe to tip of fourth toe; TL = thigh length; $TW_{I–V}$ = width of toes I–V, at base of disk; TYD = largest tympanum diameter; TYE = tympanum to eye distance, i.e. from posterior orbital border of eye to tympanum; UEW = maximum upper eyelid width.

Live colouration was recorded for individual animals within 1 h after collection. Drawings of the holotype were made using a stereomicroscope with camera lucida. Museum abbreviations: BNHS = Bombay Natural History Society, Bombay, Maharashtra, India; IRSNB = Institut Royal des Sciences Naturelles de Belgique, Brussels, Belgium; TNHC = Texas Natural History Collections, University of Texas, Austin, USA; VUB = Vrije Universiteit Brussel, Brussels, Belgium.

Results

Phylogenetic position of *Polypedates variabilis* Jerdon

Alignment resulted in a data matrix of 2081 nucleotide sites, 1392 of which could be aligned unambiguously. Of these, 605 bp are variable and 454 bp are parsimony-informative. Parsimony and likelihood analyses produced optimal trees that are broadly congruent with previous molecular phylogenetic hypotheses for Rhacophoridae (Richards and Moore 1998; Wilkinson et al. 2002). In both analyses, *Polypedates variabilis* is recovered as the closest relative of an undescribed rhacophorid from the southern part of the Western Ghats (100% bootstrap support under MP and ML). Together, these two species constitute a distinct lineage among a poorly resolved arrangement of several rhacophorid genera. The MP analyses (6 MP trees; length = 2743) suggest two equally parsimonious alternative origins for this newly identified lineage: as the sister-branch of *Polypedates* (5 trees) or as the sister-branch of a *Feihyla* + *Rhacophorus* assemblage (1 tree). Both arrangements, however, receive poor MP bootstrap support (< 50%). The single maximum-likelihood tree (–*lnL* = 13479.41) pairs the relevant lineage with a well-defined *Polypedates* clade, and this relationship is moderately supported by a Bayesian posterior probability of 90%. The short internal branches and overall low intergeneric resolution indicated by our analyses are consistent with the results of Wilkinson et al. (2002). These observations suggest that the primary diversification of rhacophorid treefrogs may have happened within a relatively short time span in the Eocene (Bossuyt et al. 2006), but additional nuclear genes, together with a statistical test, will be necessary to test this hypothesis. Rather than being nested within *Polypedates* or *Rhacophorus*, *Polypedates variabilis* and its sister species have diverged from other rhacophorids at or shortly after the time of this radiation. Moreover, they occur only at high altitudes on isolated mountains of the Western Ghats. Therefore, to facilitate communication in rhacophorid taxonomy, we propose to allocate both species to a new genus. It has been proposed that the category of genus, though artificial, should reflect evolutionary history and be "a monophyletic group composed of one or more species separated from other generic taxa by a decided gap" (Mayr and Ashlock 1991). Although we cannot reject a sister-clade relationship to *Polypedates* or *Rhacophorus*, our analyses indicate that *Polypedates variabilis* and its relative form a distinct clade that is not nested among species currently allocated to these genera. Recognition of a new genus for species that are morphologically and phylogenetically distinct, though subjective, is consistent with the generic status of other branches of this radiation.

Ghatixalus gen. n.

(Figs. 1–4; Table 1)

Etymology

The generic epithet is derived from the word 'Ghats' and the name of the genus *Ixalus* Duméril & Bibron, 1841. The former is the Sanskrit word for 'steps' and refers to the Western Ghats mountain range; the latter is often used as a suffix in rhacophorid genus names. Gender of genus name for the purposes of nomenclature: male.

Type species

Polypedates variabilis Jerdon, 1853, p. 532.

Definition

Ghatixalus is the most inclusive clade that contains *Ghatixalus variabilis* but not *Aquixalus gracilipes*, *Buergeria buergeri*, *Chirixalus doriae*, *Chiromantis xerampelina*, *Kurixalus eiffingeri*, *Nyctixalus margaritifer*, *Philautus aurifasciatus*, *Polypedates leucomystax*, *Rhacophorus reinwardtii*, *Feihyla palpebralis* and *Theloderma leporosa*. This is a stem-based clade definition, excluding the type species of the currently recognized rhacophorid genera (Frost 2006).

Diagnosis

In practice, *Ghatixalus* can be distinguished from other rhacophorid genera by the combination of the following characters: medium-sized adults (male SVL 38.8–51.3 mm, female 58.1–66.7 mm) having a dorsal colour pattern with dark brown prominent blotches; egg development in foam nests, followed by a free-swimming tadpole stage; an ecology that is strongly associated with mountain streams throughout the life cycle (for details see "Ecology and reproduction" below). Additionally, the geographic restriction to high altitudes (approximately 1700–2650 m asl) distinguishes representatives of *Ghatixalus* from those of *Polypedates* and *Rhacophorus* inhabiting the Western Ghats. *Ghatixalus* currently contains two species, one of which is described as new below.

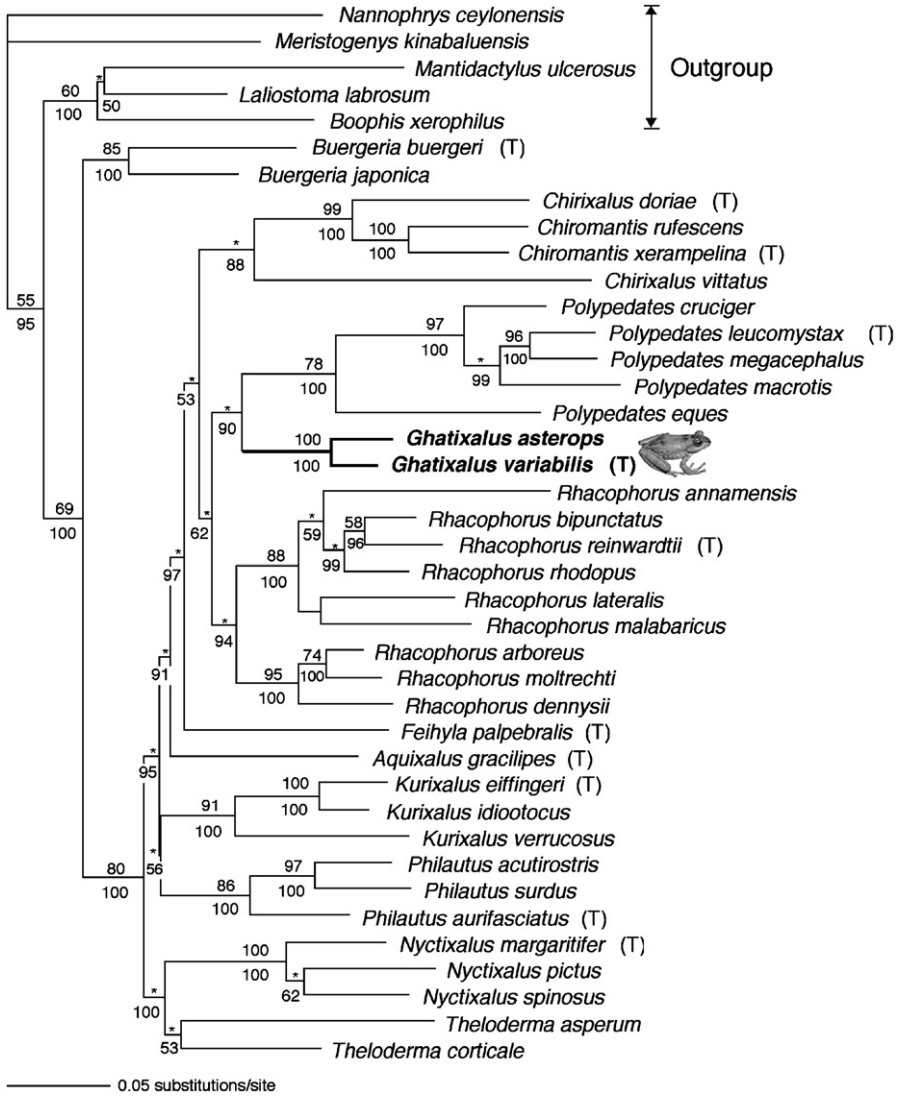

Fig. 1. Phylogenetic relationships among rhacophorid genera, as supported by the maximum-likelihood tree. Type species names labelled "(T)". Branches in bold highlight phylogenetic position of *Ghatixalus* gen. n., proposed here for *Polypedates variabilis* Jerdon and a newly discovered species. Numbers above and below branches represent non-parametric bootstrap values under MP, and Bayesian posterior probabilities, respectively.

Ghatixalus variabilis (Jerdon, 1853)

Polypedates variabilis Jerdon – Jerdon (1853, p. 532)

Polypedates pleurostictus Günther (in part) – Günther (1864, p. 430), Ravichandran (1997, p. 415), Biju (2001, p. 19)

Rhacophorus pleurostictus (Günther) – Boulenger (1882, p. 79), Inger and Dutta (1986, p. 140), Daniel and Sekar (1989, p. 200), Dutta (1997, p. 107)

Rhacophorus parkeri Ahl – Ahl (1927, p. 38)

Philautus (Kirtixalus) variabilis (Jerdon) (in part) – Dubois (1987, p. 73)

Rhacophorus variabilis (Jerdon) – Bossuyt and Dubois (2001, p. 13)

Material examined

Tamil Nadu: IRSNB 1918, neotype adult male, "Nilgiri Hills, Botanical Garden Udhagamandalam"; BNHS 4261–4262, adult males, Udhagamandalam (Ooty); BNHS 4263, adult male, Avalanche; BNHS 4270, adult male, Pandiyar; BNHS 4268, adult male, Naduvattam; BNHS 4269, adult male, Doddabetta; BNHS 4271, adult female, Avalanche.

Diagnosis and comparison

A detailed description of the neotype was published by Bossuyt and Dubois (2001, p. 63). Measurements from eight specimens are provided in Table 1. For

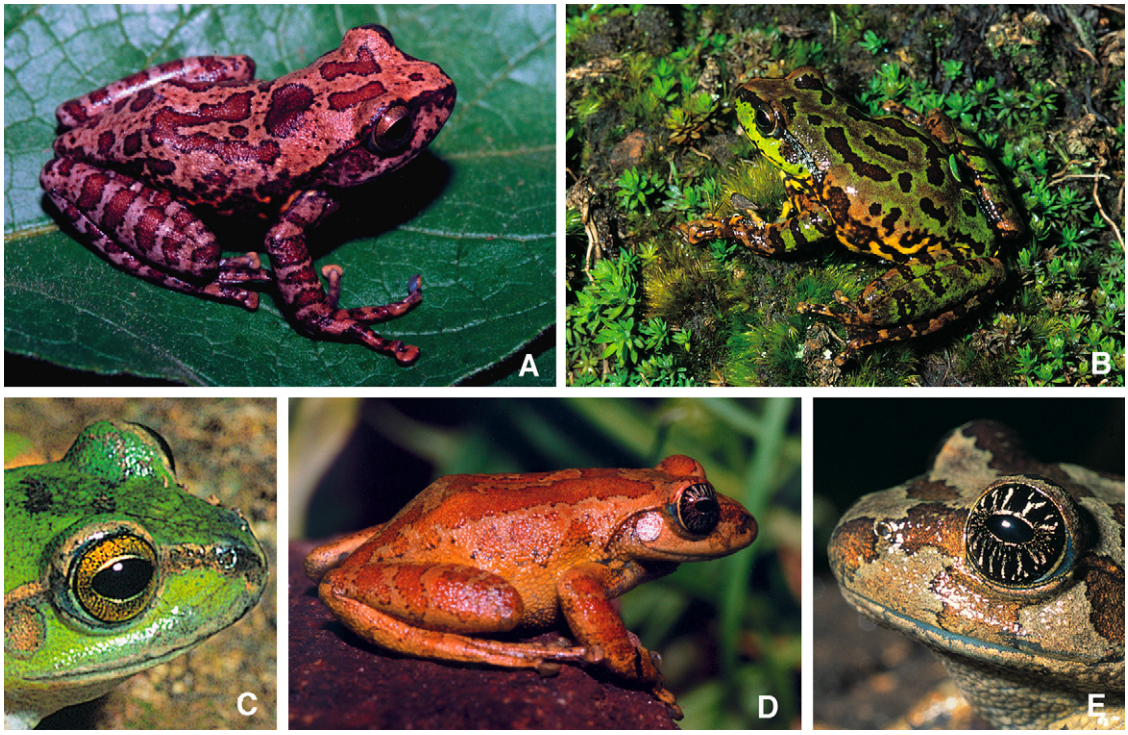

Fig. 2. *Ghatixalus* gen. n. (A–C) *G. variabilis*: (A) brown colour variation, (B) green colour variation, (C) close-up of eye showing unicoloured golden iris. (D and E) *G. asterops* sp. n.: (D) brown colouration of adult frogs, (E) close-up of eye showing golden star-like pattern in iris.

detailed comparison with *Ghatixalus asterops* sp. n., see below.

Colour in life. Dorsally brownish grey with irregular blotches (BNHS 4261–4262; Fig. 2A), unicolorous brownish green (BNHS 4268) or dark green with brownish irregular blotches (BNHS 4263: Fig. 2B); loreal and tympanic region dark grey with brown spots, lateral side light yellowish brown with dark reticulation; iris brownish, encircled by a golden ring (Fig. 2C); posterior margin of thighs light bluish-grey (BNHS 4261–4262) or dark brown with prominent yellow reticulation (BNHS 4263, 4268); webbing dark grey with brown blotches.

Secondary sexual characters. Male: nuptial spines present (Fig. 3C) on finger I, weakly spinular, yellowish white.

Intraspecific variation. Measurements representing the morphological variation among eight specimens from different localities are provided in Table 1. There is a high degree of colour variation among individuals of this species (Figs. 2A–C), even within a single locality. The background colour of the dorsum, head, and limbs varies from light brown to bright green. Individuals found under leaf litter, rocks or logs during the winter season (December–February) are dark or blackish brown. The blotches are always dark or reddish brown, but seem to fade and become less contrasted in captivity. The yellow or yellowish green colouration of the lateral sides is sometimes extended as a bright streak across the loreal region.

Geographic distribution. We recorded this species at several highland localities in the Nilgiri hills (Tamil Nadu District; Fig. 4): Udhagamadalam (“Ooty”; 11°24′N, 76°42′E; 2000 m asl), the neotype locality; Naduvattam (11°28′N, 76°32′E; 1900 m asl); Avalanche (11°17′N, 76°35′E; 2100 m asl); Doddabetta (11°24′N, 76°44′E; 2630 m asl); Pandiyar (11°24′N, 76°31′E; 2300 m asl).

Ghatixalus asterops sp. n.

(Figs. 1, 2D–E, 3E–H and 4; Table 1)

Etymology

The specific epithet combines the ancient Greek words ‘aster’ and ‘ops’, meaning ‘star’ and ‘eye’, respectively. It refers to one of the most conspicuous characters of this species.

Materials examined

Holotype. BNHS 4247, adult male, collected by SDB on 20 August 1999, in Bear Shola, Kodaikanal, 10°13′N, 77°29′E, 2000 m asl, Dindigal district, Tamil Nadu, India.

Paratypes. BNHS 4248, adult male; BNHS 4249, adult female; both collected together with the holotype.

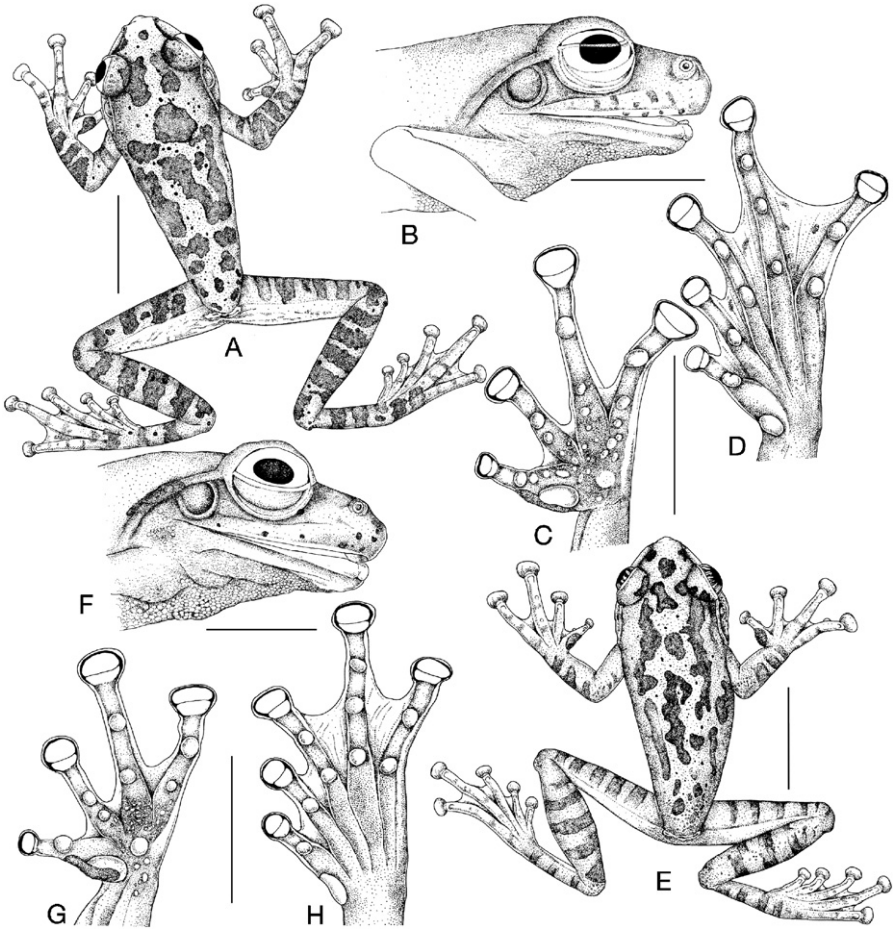

Fig. 3. *Ghatixalus* gen. n. (A–D) *G. variabilis*: (A) dorsal view, (B) lateral view of head, (C) ventral view of left hand. (D) ventral view of left foot. (E–H) *G. asterops* sp. n.: (E) dorsal view, (F) lateral view of head, (G) ventral view of left hand, (H) ventral view of left foot. Scale bars = 10 mm.

Additional material. BNHS 4250–4251, adult males, Mattupetti, Idukki district, Kerala, India.

Diagnosis

Ghatixalus asterops is distinguished from *G. variabilis* by a golden star-like pattern on a black-coloured iris (Fig. 2E) (vs. an overall golden brown iris; Fig. 2C); rather pointed snout in dorsal view (Fig. 3E) (vs. snout oval in outline; Fig. 3A); acute loreal region (vs. obtuse loreal region); supratympanic fold from posterior corner of upper eyelid to upper level of forearm (Fig. 3F) (vs. supratympanic fold from posterior corner of upper eyelid to just below level of forearm, Fig. 3B); shank equal to thigh length, ShL 20.6 ± 1.0 mm vs. TL 20.6 ± 1.3 mm, $N = 4$ (vs. shank shorter than thigh length, ShL 22.7 ± 1.2 mm vs. TL 24.0 ± 1.0 mm, $N = 7$).

This diagnosis also confirms that neither *Rhacophorus parkeri* Ahl, 1927 nor *Polypedates pleurostictus* Günther, 1864 – both currently in synonymy with *Rhacophorus variabilis* (= Ghatixalus *variabilis*) (Bossuyt and Dubois 2001) – is a senior synonym of *Ghatixalus asterops*.

Genetic divergence

Pairwise comparison of the sequenced mtDNA fragment in *Ghatixalus asterops* and *G. variabilis* reveals an uncorrected genetic distance of 7.4% and a GTR-corrected distance of 8.1%. In addition, *G. asterops* differs from *G. variabilis* and all other rhacophorids by a unique 10-bp insertion in the *16S RNA* gene.

Description of holotype

Medium-sized treefrog (SVL 42.9); body rather robust (Fig. 3E); head longer than wide (HW 14.8; HL 15.9; MN 13.3; MFE 11.1; MBE 6.4), slightly convex above; outline of snout rather pointed in dorsal view, slightly protruding; snout longer than horizontal diameter of eye (SL 6.9; EL 5.0); canthus rostralis rounded, loreal region acutely concave; interorbital space convex, larger than upper eyelid and internarial distance (IUE 4.9; UEW 3.4; IN 3.8); distance between anterior margins of eyes 1.7 times in distance between posterior margins of eye (IFE 7.9; IBE 13.7); nostril oval, without flap of skin, closer to tip of snout than to front of eye (NS 2.3; EN 3.3); pupil oval, horizontal;

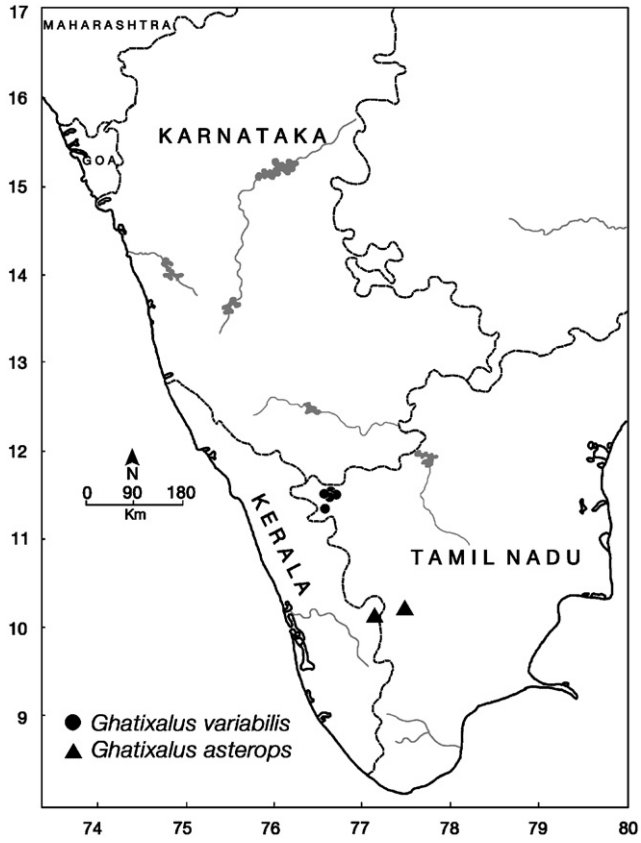

Fig. 4. Distribution map for *Ghatixalus* gen. n., showing disjunct distribution of its two known species, *G. variabilis* (circles) and *G. asterops* sp. n. (triangles).

tympanum (TYD 1.7) distinct, rounded, horizontal diameter 2.9 times less than eye diameter (Fig. 3F), almost 1.3 times larger than distance from tympanum to eye (TYE 1.3); pineal ocellus present between eyes; vomerine teeth present, bearing five large teeth, with an angle of 60° relative to body axis, closer to choanae than to each other, slightly longer than distance between them; tongue large (13.1 × 8.0), rounded to cordate, emarginate, lingual papilla absent; supratympanic fold distinct, from posterior corner of upper eyelid to upper level of forearm; no co-ossified skin on skull. Forelimbs (FLL 8.9), 1.6 times shorter than hand (HAL 13.9; TFL 7.9); relative length of fingers: I < II < IV < III; tips of fingers enlarged with disks ($FD_I = 1.5$, $FW_I = 0.8$; $FD_{II} = 2.3$, $FW_{II} = 1.1$; $FD_{III} = 2.8$, $FW_{III} = 1.3$; $FD_{IV} = 2.8$, $FW_{IV} = 1.3$), with distinct circummarginal grooves; fingers with lateral dermal fringe on both edges, webbing present, moderate; subarticular tubercle prominent, rounded, single, all present; prepollex distinct, oval; single palmar tubercle, rounded, rather distinct; supernumerary tubercles present, prominent on fingers III and IV (Fig. 3G). Hind limbs moderately long, heels touch when limbs are folded at right angles to body, shank four times longer than wide (ShL 20.7; ShW 5.2), shorter than thigh (TL 21.2), length of toe IV (FTL 10.1) 1.8 times in distance from base of tarsus to tip of toe IV (FOL 18.2; TFOL 29.2); relative length of toes: I < II < III < V < IV; tips of toes with disk ($TD_I = 1.7$, $TW_I = 0.9$; $TD_{II} = 2.0$, $TW_{II} = 0.9$; $TD_{III} = 1.9$, $TW_{III} = 0.9$; $TD_{IV} = 2.4$, $TW_{IV} = 1.0$; $TD_V = 2.1$, $TW_V = 0.9$), with a distinct circummarginal groove, webbing extensive (Fig. 3H, reaching above distal subarticular tubercle on either side of toe IV, (MTTF = 9.3, MTFF = 11.3, TFTF = 7.2, FFTF = 5.8); dermal fringes or ridge along toe V absent; subarticular tubercles prominent, rounded, simple; inner metatarsal tubercle rather distinct (IMT 2.2), oval, 2.3 times shorter than Toe I (ITL 5.1); supernumerary tubercle absent. Skin of snout and between eyes shagreened, upper eyelids shagreened to granular, side of head shagreened, anterior and posterior part of back shagreened to slightly granular; upper and lower part of flank slightly granular; dorsal parts of forelimb, thigh, leg and tarsus shagreened; ventral parts of throat and chest shagreened to granular, belly and thigh granular.

Colouration in life. Dorsally dark grey with brown irregular blotches, loreal and tympanic region light grey with brown spots, lateral side yellow with numerous brown patches; iris brownish, with golden yellow vertical stripes, surrounded by a thin golden ring; limbs dark grey with dark brown cross-bands, fingers and toes with cross-bands, tips light grey, posterior side of thigh bluish-brown without reticulation; ventrally light greyish with a tinge of blue; foot and hand light bluish-white, webbing bluish-brown with brown blotches.

Colouration in preservation. Dorsum light brownish grey with dark irregular blotches, tympanic region light brown, upper eyelid black; lateral area light grey with dark spots; ventrally unicolorous light grey.

Male secondary sexual characters. Nuptial spines present on finger I (Fig. 3G), weakly spinular, white; vocal sac present, a pair of openings present at base of lower jaw.

Variation

Measurements representing the morphological variation among five specimens from different localities are provided in Table 1. The dorsal colouration of BNHS 4248 is yellowish brown with reddish brown blotches (Fig. 2D), whereas BNHS 4250 is light brownish grey with dark brown blotches. The metamorphosed juveniles are variable in colour from light green without dorsal markings (except for a light brown streak from snout to forearm through upper eyelid on either side) to light brown (with pale markings). The green colour variant is evident only in juvenile and sub-adult frogs, and was not found in adults ($N = 57$ males from two different localities: Kodaikanal and Mattupetti). Compared to the male, the female (BNHS 4249) has prominently more granular skin on the dorsum and on

Table 1. Morphometric data (in mm) of the specimens studied

Species/locality	Museum no.	Sex	SVL	HW	HL	IFE	IBE	IUE	UEW	SL	EL	FLL	HAL	ShL	TL	FOL
Ghatixalus variabilis																
Udagamandalam (NT)	IRSNB 1918	M	48.6	17.2	17.2	9.3	14.5	5.5	3.9	6.1	5.1	11.1	15.3	23.1	23.9	23.4
Udagamandalam	BNHS 4261	M	51.1	17.8	17.3	9.1	15.5	5.4	4.4	7.9	6.4	9.5	16.0	23.6	24.5	23.0
Udagamandalam	BNHS 4262	M	45.9	17.1	16.7	8.1	13.8	5.8	3.9	6.6	6.2	8.6	12.5	22.7	24.3	19.0
Avalanche	BNHS 4263	M	42.9	16.9	16.4	8.7	13.6	5.2	3.4	6.1	4.9	8.0	15.4	22.7	23.5	21.1
Naduvattam	BNHS 4268	M	41.4	16.8	16.1	7.6	12.7	5.0	3.6	6.5	5.0	8.4	13.6	21.0	22.5	20.9
Doddabetta	BNHS 4269	M	50.3	17.7	17.0	9.9	15.0	6.0	3.5	7.4	6.0	10.4	16.5	24.5	25.8	22.9
Pandiyar	BNHS 4270	M	46.5	17.2	16.8	7.7	13.3	5.1	3.9	6.8	5.4	9.1	15.0	21.6	23.5	21.1
Avalanche	BNHS 4271	F	66.7	22.4	26.6	12.7	18.8	7.3	5.5	10.3	6.6	14.0	22.6	34.2	34.0	32.3
Ghatixalus asterops																
Kodaikanal (HT)	BNHS 4247	M	42.9	14.8	15.9	7.9	13.7	4.9	3.4	6.9	5.0	8.9	13.9	20.7	21.2	18.2
Kodaikanal (PT)	BNHS 4248	M	41.2	14.7	14.3	7.6	12.6	4.7	3.2	6.7	5.2	7.4	13.7	20.9	20.4	17.1
Mattupetti	BNHS 4250	M	38.8	13.8	13.9	7.4	12.3	4.6	3.8	6.4	4.4	7.5	11.0	19.2	18.9	16.6
Mattupetti	BNHS 4251	M	44.8	17.1	16.7	9.0	13.9	6.7	3.2	7.0	5.9	8.2	15.4	21.8	22.1	20.4
Kodaikanal (PT)	BNHS 4249	F	58.1	20.5	19.8	11.7	17.8	7.5	4.5	9.4	5.7	12.1	19.7	28.7	28.7	27.9

Type status indicated behind respective locality name: HT = holotype, NT = neotype, PT = paratype. Sex: F = female, M = male. For all other abbreviations, see text (Material and methods).

the ventral side of the thighs and forearms, as well as larger subarticular and supernumerary tubercles.

Geographic distribution

This species has been observed in two high-altitude localities in the Palani Hills and surrounding areas, south of the Palghat Gap (Fig. 4): Kodaikanal (10°13′N, 77°29′E; 2000 m asl), Tamil Nadu, and Mattupetti (10°05′N, 77°10′E; 1700 m asl), Kerala.

Ecology and reproduction

The two species of *Ghatixalus* share similar ecological preferences and seem to be restricted to disturbed evergreen sholas (isolated montane forest patches). Juveniles and adult frogs (*G. variabilis*, $N=32$; *G. asterops*, $N=17$) were always found in the direct proximity of mountain streams, in habitats close to the ground, such as leaf litter, rock patches, tall grass clumps and the undergrowth of shrub vegetation. When disturbed, these frogs tend to jump into the water and hide at the bottom for several minutes. To our knowledge, most other rhacophorid treefrogs tend to aggregate in the proximity of water bodies only during the mating season and hardly ever enter the water.

In both species, males were heard calling after 18:00 h in August–September. The mating calls differ considerably between the two species. In *G. variabilis* populations studied near Ooty, the call was a sharp 'terr, chick-chick-chick' sound, repeated in intervals of 2–4 min. Males of *G. asterops* produce a distinct series of five to seven bird-like whistle notes (Phu-phu-phu-phu-phu), which is repeated every 2–3 min during the breeding season. The amplexus is axillary in both species. In *G. variabilis,* foam nests are spherical in shape (98 ± 63 mm length × 70 ± 21 mm width; $N=4$) and can be found suspended up to 3 m above the water against near-vertical surfaces of moss-covered banks. Eggs examined in one nest were non-pigmented white and measured 2.8 ± 0.6 mm ($N=208$) in diameter. *Ghatixalus asterops* foam nests are also spherical in shape (77 ± 23 mm length × 53 ± 14 mm width; $N=11$). Of the 11 nests, seven were found on rocks, three on plain earth up to 2 m above the water level, and one at the base of a tree near the stream. Eggs are non-pigmented white and measured 2.3 ± 0.4 mm ($N=185$) in diameter. In both species, tiny tadpoles hatched from the eggs inside the foam and dropped into the water 4 days after spawning. Hatching success of *G. variabilis* and *G. asterops* was 93.5% ($N=208$) and 78.7% ($N=185$), respectively.

Discussion

In this study, we use a molecular phylogenetic approach to identify and describe a new clade of treefrogs endemic to the Western Ghats. Molecular phylogenetic analyses have played an important role in the recognition of distinct evolutionary lineages among Western Ghats frogs and the corresponding definition of supra-specific taxa (Biju and Bossuyt 2003; Roelants et al. 2004). In the absence of morphological evidence, such approaches probably represent our best chances to reach a phylogenetically stable taxonomy for rhacophorid treefrogs. Additional taxon sampling within the Western Ghats or the adjacent Oriental region will most likely identify other, previously unrecognized rhacophorid lineages.

Our field observations suggest that *Ghatixalus* gen. n. only inhabits a few montane localities in isolated hill ranges of the Western Ghats. This further stresses the

importance of isolated forest patches as reservoirs of unique evolutionary history within the Western Ghats biodiversity hotspot (Roelants et al. 2004).

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Appendix A

See Table A1.

Table A1. Taxa included in the phylogenetic analyses, with corresponding sequence source (specimen voucher or reference) and GenBank accession number(s)

Genus	Species	Sequence source	Accession number
Aquixalus	*gracilipes* (T)	Frost et al. (2006)	DQ283051
Buergeria	*buergeri* (T)	Wilkinson et al. (2002)	AF458122
	japonica	Wilkinson et al. (2002)	AF458123
Chirixalus	*doriae* (T)	Wilkinson et al. (2002)	AF458127
	vittatus	Wilkinson et al. (2002)	AF458131
Chiromantis	*rufescens*	Wilkinson et al. (2002)	AF458126
	xerampelina (T)	Wilkinson et al. (2002)	AF458132
Feihyla	*palpebralis* (T)	Wilkinson et al. (2002)	AF458130
Kurixalus	*eiffingeri* (T)	Wilkinson et al. (2002)	AF458128
	idiootocus	Wilkinson et al. (2002)	AF458129
	verrucosus	VUB 0695	EU178086, EU178093
Nyctixalus	*margaritifer* (T)	TNHC JAM 3030	EU178087, EU178094
	pictus	Wilkinson et al. (2002)	AF458135
	spinosus	Wilkinson et al. (2002)	AF458136
Philautus	*acutirostris*	Wilkinson et al. (2002)	AF458137
	aurifasciatus (T)	Meegaskumbura et al. (2002)	AY141804, AY141850
	mjobergi	Richards and Moore (1998)	AF026348, AF026365
Polypedates	*cruciger*	VUB 0125	AF249028, AF249045
	eques	VUB 0153	EU178088, EU178095
	leucomystax (T)	Wilkinson et al. (2002)	AF458140
	macrotis	VUB 0613	EU178089, EU178096
	megacephalus	Wilkinson et al. (2002)	AF458141
Rhacophorus	*annamensis*	Wilkinson et al. (2002)	AF458143
	arboreus	Wilkinson et al. (2002)	AF458142
	bipunctatus	Wilkinson et al. (2002)	AF458144
	dennysi	Wilkinson et al. (2002)	AF458139
	lateralis	BNHS 4260	EU178090, EU178097
	malabaricus	VUB 0001	DQ346957, AF249050
	moltrechti	Wilkinson et al. (2002)	AF458145
	reinwardtii (T)	Wilkinson et al. (2002)	AF458146
	rhodopus	Wilkinson et al. (2002)	AF458147
Theloderma	*asperum*	Wilkinson et al. (2002)	AF458148
	corticale	Richards and Moore (1998)	AF268254, AF268256
Ghatixalus	*asterops*	VUB 0025	EU178091, EU178098
	variabilis (T)	VUB 0061	EU178092, EU178099
OUTGROUP			
Boophis	*xerophilus*	VUB 0935 (M. Vences)	DQ346999, AF249038
Laliostoma	*labrosum*	VUB 0934 (M. Vences)	DQ346998, AF249037
Mantidacylus	*ulcerosus*	VUB 0932 (M. Vences)	DQ346996, AF249035
Nannophrys	*ceylonensis*	VUB 0172	DQ346975, AF249047
Meristogenys	*kinabaluensis*	VUB 0627	DQ346983, AY322292

(T) = type species. For collection abbreviations, see text (Material and methods).

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