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A New Frog Species from the Central Western Ghats of India, and Its Phylogenetic Position

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Tropical evergreen forests of Indian subcontinent, especially of the Western Ghats, are known hot spots of amphibian diversity, where many new anuran species await to be identified. Here we describe from the Sharavathi River basin of central Western Ghats a new shrub-frog taxon related to the anuran family Rhacophoridae. The new frog possesses the characteristic features of rhacophorids (dilated digit tips with differentiated pads circumscribed by a complete groove, intercalary cartilages on digits, T-shaped terminal phalanges and granular belly, the adaptive characters for arboreal life forms), but also a suite of unique features that distinguish it from all known congeners in the region. Morphogenetic analysis based on morphological characteristics and diversity in the mitochondrial 12S and 16S rRNA genes revealed it to be a new *Philautus* species that we named *Philautus neelanethrus* sp. nov. The phylogenetic analysis suggests the new frog to represent a relatively early *Philautus* species lineage recorded from the region. The distribution pattern of the species suggests its importance as a bioindicator of habitat health. In general, this relatively widespread species was found distributed only in non-overlapping small stretches, which indirectly indicates the fragmentation of the evergreen to moist deciduous forests that characterize the Western Ghats. Thus the discovery of the new rhacophorid species described here not only further reinforces the significance of the Western Ghats as a major hotspot of amphibian biodiversity, but also brings into focus the deterioration of forest habitats in the region and the need for prioritization of their conservation.

Key words: amphibian biodiversity, conservation, shrub frog, Western Ghats, habitat, rDNA diversity

INTRODUCTION

The Western Ghats, a chain of hills of varied width and height running parallel to the western coast of India, is a hotspot of biological diversity (Myers *et al.,* 2000). This region harbors a high proportion of endemic species, especially in lower-vertebrate group such as amphibians, reptiles and fishes (Daniels, 2001; Dahanukar *et al.,* 2004); this endemism has been attributed to the prevailing geographical, climatic and phenological conditions providing the necessary humid environment and habitat (Roelants *et al.,* 2004). Amphibians form an important faunal group of this region, but are incompletely documented (Bossuyt, 2002); some represent disjunct populations that necessitate the integrated morphological and molecular analyses to resolve their phylogeography (Karanth, 2003).

It is quite evident that the Western Ghats, as a part of the Old World region, represent a Cenozoic refugium for old lineages and a unique reservoir of ancient endemic anurans (Duellman, 1999; Roelants *et al.,* 2004). In recent years, there has been increasing interest worldwide in understanding the biogeography and evolutionary lineages of amphibians of the Western Ghats, especially in relation to their links with Madagascar's fauna and to patterns of amphibian dispersal in the Indian Ocean region (Vences *et al*., 2003). With the discovery of a new primitive frog, it was established that India had an ancient biogeographical link with the Seychelles, and that amphibian endemism in the region dates back to 150–195 Mya (Biju and Bossuyt, 2003; Dutta *et al.,* 2004). Several lineages may have originated on the Indian subcontinent during the trans-Tethys drift (Bossuyt and Milinkovitch, 2001).

Approximately 500 species of ranids have been recorded in the Oriental realm. To date about 135 species have been recorded from the Western Ghats (Gururaja,

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2004), of which over 100 (nearly 75%) are endemic to the region. The order Anura is represented by 109 species, including members of the Rhacophoridae. Species of the genus *Philautus* in the family Rhacophoridae form a unique group because they undergo direct development, wherein the tadpole stage is avoided (Marmayou *et al.,* 2000). Since the recent revision of the genus *Philautus* (Bossuyt and Dubois, 2001), several new *Philautus* species have been described from the Western Ghats (Kuramoto and Joshy, 2003; Bossuyt, 2002; Biju and Bossuyt, 2005a, b), which strongly reinforces that this region is a center of amphibian diversity, where many more new species await description (Aravind *et al.,* 2004; Gower *et al.,* 2004). However, this pristine biogeographic reservoir of evolutionary history in the Western Ghats is now threatened by heavy human demographic pressure and interference (Aggarwal, 2004; Dutta *et al.,* 2004), warranting urgent protective measures and a preemptive conservation strategy. We here describe a new species of *Philautus* and analyze its phylogenetic relationships. Our results further highlight the significance of the Western Ghats as hotspot of amphibian diversity and the need for prioritization of its conservation.

MATERIALS AND METHODS

Study area

The Sharavathi River basin is situated in the central part of the Western Ghats (Fig. 1). The Sharavathi River originates at Ambuthirtha and flows towards west for about 132 km before joining the Arabian Sea at Honnavar. The type specimens of the new frog species described in this study were collected during stratified systematic sampling (Heyer *et al.,* 1994) with time-constrained and search-all methods (Vasudevan *et al.,* 2001). The type locality is Arodi, Sagar Taluk, Shimoga District, Karnataka state (14°08'25"N, 74°47'44"E), 534 m asl (meters above sea level), a moist deciduous forest patch. The region has an undulating terrain, with forests of evergreen and moist-deciduous types. Relatively flat areas within this terrain form a freshwater habitat known as *Myristica* swamps, dominated by members of plant family Myristicaceae. Localities across the study area where the new species was found are listed in Table 1.

Sampling

The new frog species was recorded in the study area (Fig. 1) over a period of 4 years since 2001. Although more than 150 specimens were enumerated during multiple field explorations, only nine individuals of the new species (including holotype and paratypes)

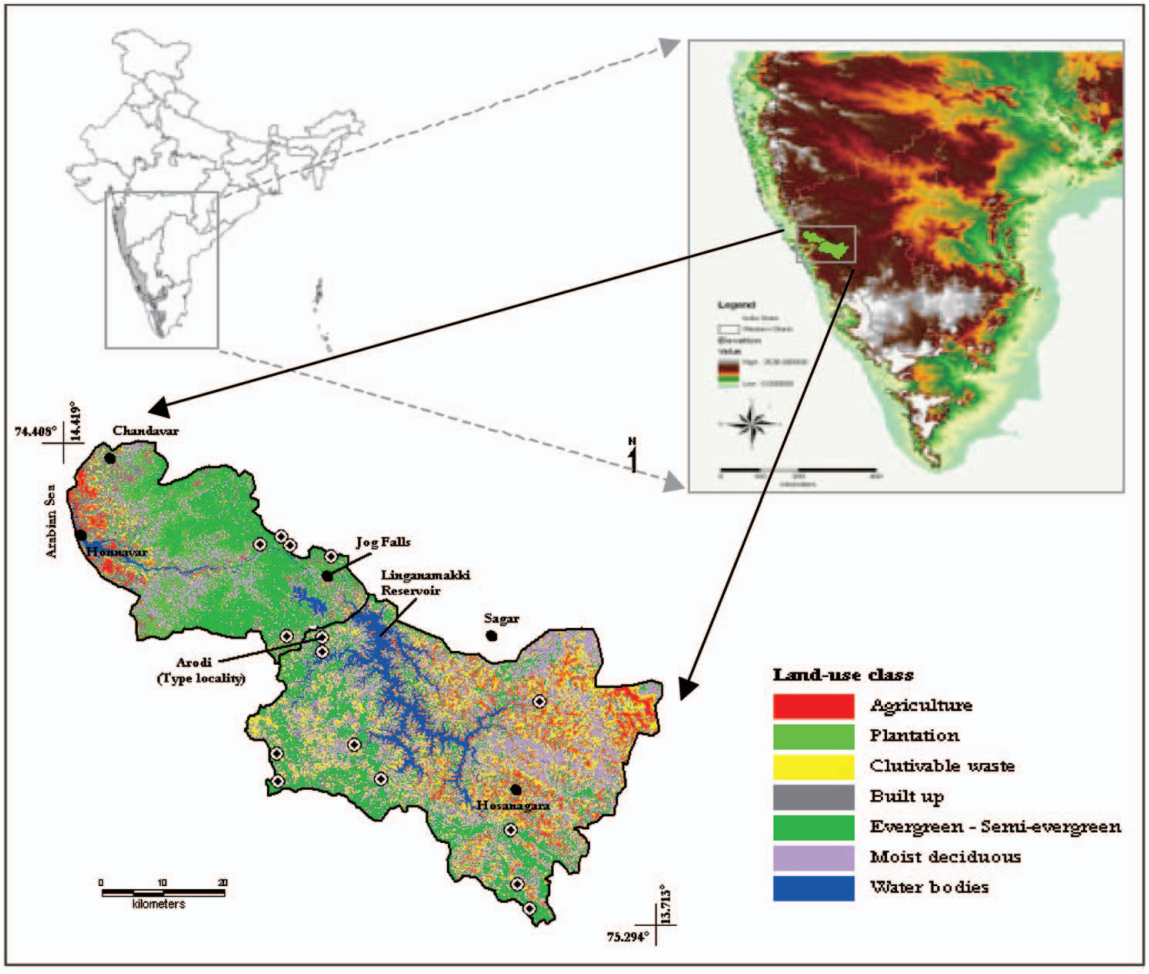

Fig. 1. Sharavathi River basin and the type localities of the new *Philautus* species.

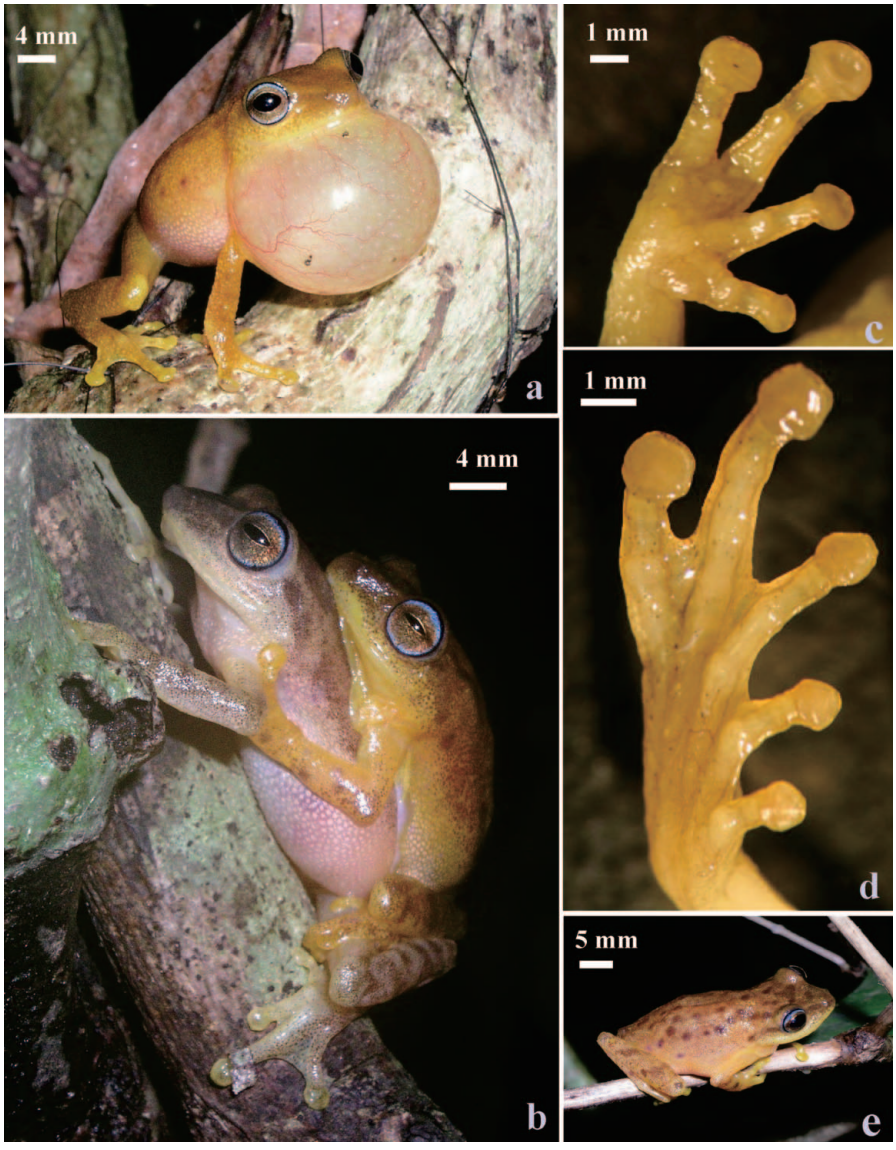

Fig. 2. Characteristic features of *P. neelanethrus* sp.nov. **(a)** Male while calling (SVL 29.8 mm, collected from the Nandiholé locality); **(b)** amplected pair (specimens not collected); **(c)** ventral view of forelimb; **(d)** ventral view of hindlimb; **(e)** dorsolateral view of an adult male.

Table 1. Localities across the central Western Ghats, India, where *Philautus neelanethrus* sp. nov. was recorded.

Area	Altitude (m asl)	Habitat
Kathalekan	619	*Myristica* swamp
Niluvase	692	Evergreen
Malemane1	603	Evergreen
Malemane2	615	Evergreen
Hilkunjiholé	599	Evergreen
Karni	598	Evergreen
Mavingundi	583	Evergreen
Nagodiholé	580	Evergreen
Dabbefall	566	Evergreen
Yenneholé	563	Evergreen
Hurliholé	598	Moist deciduous
Sharavathi	586	Moist deciduous
Muppane	571	Moist deciduous
Nandiholé	557	Moist deciduous
Arodi	534	Moist deciduous

were collected, on different dates by KVG, NAA and SA. The specimens were used for detailed morphometric description, as well as for molecular analysis to resolve its taxonomic status. Adult specimens of the new frog species were deposited in the Bombay Natural History Museum (BNHS), Mumbai (Holotype, BNHS-4510; Paratype, BNHS-4511) and in the museum of the Zoological Survey of India (ZSI), Kolkata, India (Paratype ZSI-A9866). Specimens were collected from the type locality, photographed, euthanized, and preserved in salt saturated 20% DMSO (dimethyl sulfoxide) solution and/or 80% ethanol. The preserved specimens were used for morphometric studies, and soft tissues taken from the same specimens were used to extract genomic DNA for molecular analysis.

Type specimens

Holotype (BNHS-4510): adult male, SVL 29.9 mm, collected at Arodi in the Sharavathi River basin on 7 July 2005 by KVG. Paratypes, two adult males: SVL 23.4 mm (ZSI-A9866) collected at Niluvase (13°44'18"N, 75°06'30"E; 692 m asl) on 6 November 2003 by KVG; SVL 28.7 mm (BNHS-4511) collected at Arodi (14°08'25"N,

Table 2. Morphometric measurements of *Philautus neelanethrus* sp. nov. (values are in millimeters, n=9, all males).

Parameter*	Mean±SD (n=9)	Range	Holotype (BNHS–4510)	Paratype (BNHS–4511)
SVL**	25.41±3.403	21.4–29.9	29.9	28.7
EL**	3.90±0.301	3.5– 4.4	4.1	4.1
EN**	2.66±0.205	2.5– 3.1	2.8	2.5
HL**	8.00±0.948	6.8– 9.1	9.0	9.1
HW**	9.54±1.334	7.8–10.8	10.8	10.7
IBE	8.81±1.108	7.6–10.2	10.0	10.2
IFE	4.94±0.777	4.0– 6.1	6.1	6.0
IN**	2.29±0.393	1.6– 2.7	2.4	2.7
IUE**	3.43±0.285	2.9– 3.8	3.8	3.6
MBE	1.78±0.285	1.3– 2.2	2.1	1.7
MFE	4.78±0.591	4.2– 5.7	5.7	5.5
MN	6.88±1.003	5.0– 8.2	8.2	8.0
NS**	1.30±0.247	1.0– 1.7	1.4	1.5
SL	3.66±0.403	3.1– 4.2	4.1	3.7
TYD**	No value	–	Absent	Absent
TYE	No value	–	Absent	Absent
UEW**	1.83±0.389	1.2– 2.8	2.2	2.4
fd_3	1.78±0.140	1.6– 1.9	1.8	1.9
FLL	6.35±0.833	4.8– 7.2	7.1	6.8
fw_3	0.77±0.163	0.7– 1.0	0.7	1.0
HAL**	7.91±0.442	7.4– 8.2	8.2	8.2
TFL**	5.65±0.980	4.6– 7.4	4.7	4.9
FFTF	4.97±0.203	4.8– 5.2	5.2	4.8
FL**	12.93±1.509	11.0–15.1	15.1	14.4
FOL**	9.80±1.581	7.7–12.0	12.0	11.4
FTL**	7.45±0.627	6.6– 8.1	7.0	6.7
IMT**	1.01±0.089	0.9– 1.1	0.9	1.1
ITL**	2.34±0.364	2.0– 3.1	2.0	2.3
MTFF	6.46±0.476	5.9– 6.7	6.7	6.7
MTTF	6.03±0.421	5.6– 6.4	6.4	6.1
td_4	1.61±0.110	1.5– 1.7	1.7	1.6
TFOL**	16.74±2.420	13.5–19.8	19.8	19.6
TFTF	5.14±0.104	5.1– 5.3	5.3	5.1
TL**	12.87±1.491	10.6–15.3	14.3	13.5
TW	2.24±0.566	1.4– 3.1	3.1	3.1
tw_4	0.92±0.185	0.7– 1.0	1.0	1.0

* See Supplemental Table 1 for explanation of abbreviations for various parameters.

** Parameters used for morphometric comparisons with congeners; for morphometric and meristic data on congeners, see Supplemental Tables 2 and 4.

74°47'44"E; 534 m asl) on 7 July 2005 by KVG.

Morphometric analysis

Nine individuals of new species were used for morphometric measurements. Thirty-five morphometric measurements were taken to the nearest 0.1 mm with digital slide calipers (Mitutoyo Corporation, Japan, CD-6BS) for each of the specimens (Table 2). Terminology (Supplemental Table 1) used in the description is based on Bossuyt and Dubois (2001).

A cluster analysis based on unweighted pair-group averages (UPGA) was used to understand the relationship of the new species to other, known congeners (Supplemental Tables 2, 3), and included data on 19 morphometric and three meristic characters (Table 2, Supplemental Table 4). The analysis was carried out using the software package STATISTICA (StatSoft Inc.).

Advertisement call analysis

Advertisement calls of the new species were recorded with a digital voice recorder (W-10, Olympus, Japan). A total of 16 calls from seven individuals were recorded at five different localities in the study area. Spectral features of the advertisement calls were analyzed using Sigview (version 1.91) acoustical software (SignalLab, Goran Obradovic).

Call-pattern characteristics of *P. neelanethrus* sp. nov. (n=16) were also compared with those of one of its closest congeners, *P. luteolus*, using Bartlett's test for homogeneity and significance of variances (Snedecor and Cochran, 1989). The data for *P. luteolus*, comprising six acoustic parameters based on 51 call samples, were taken from Kuramoto and Joshy (2001), where *P. luteolus* was referred to as *P.* cf. *travancoricus*. Parameters considered were total call duration, call duration in the fast and slow phases, number of pulses in the fast and slow phases, and frequency range.

DNA extraction and rDNA sequencing

Ribosomal typing was carried out to establish the species status of the new frog taxon. Total genomic DNA was extracted from muscle tissues, taken from preserved specimens collected from different localities, by the proteinase K, phenol-chloroform-isoamyl alcohol method (Shanker *et al.,* 2004). The DNA samples were used to determine molecular diversity across the 12S and 16S mitochondrial rRNA genes, in order to ascertain the species uniqueness and phylogenetic position of the new taxon. Parts of the 16S (~575 bp) and 12S rDNA (~435 bp) genes were amplified and sequenced as described by Dutta *et al.* (2004). Each sample was sequenced three times for both strands to confirm the sequence data. Sequences have been deposited in GenBank under accession numbers AY763797 (12S rDNA, 415 bp) and AY753560 (16S rDNA, 546 bp).

Phylogenetic analysis

The 12S and 16S rDNA sequences of the new frog taxon were used in similarity-based BLAST searches of the NCBI-GenBank database (National Center for Biotechnology Information, USA; http://www.ncbi.nlm.nih.gov) to identify related reference anuran species. Initially, corresponding sequences of >100 different reference anuran species were retrieved from the database. The final phylogenetic analysis included reference sequences for only 35 taxa, mainly of different *Philautus* species (Table 3), to ascertain the phylogenetic position of the new species, and also for possible molecular dating.

All sequences were aligned using the CLUSTAL-X program and then checked for large gaps. The aligned sequences were terminated flush at the ends to avoid missing data for any of the compared reference entries. Three aligned sequence sets, one each for 12S and 16S rDNA and one for combined 12S+16S, were used separately to derive corrected Kimura two-parameter distance estimates (Kimura, 1980) and to infer the phylogenetic position of the new taxon. Neighbor-joining trees were constructed with analytical routines available in the software packages PHYLIP 3.6 (http://evolution.genetics.washington.edu/phylip.html) and MEGA 2.1 (http://www.megasoftware.net). Character state-based maximum likelihood (ML) and maximum parsimony (MP) phylogenetic trees were also constructed using PhyloWin (http://pbil.univ-lyon1.fr/software/phylowin.html). In order to test for earliest branching patterns, all substitutions were considered, and separate analyses were conducted for assumed transition/transversion rate ratios (k) of 2 and 4. Support for nodes on the shortest tree and estimates of divergence time were derived using 1,000 bootstrap pseudoreplicates. The relative-rate test was performed to test the molecular clock hypothesis with MEGA 2 using Tajima's algorithm for clock hypotheses. In the final analysis, the phylogenetic trees were rooted using a representative species from each of the families, Dicroglossidae, Nyctibatrachidae, and Ranidae (Table 3).

Table 3. Details of the 12S and 16S rDNA sequences of the reference anuran taxa used in the final phylogenetic analysis (all sequences were retrieved from the NCBI-GenBank database).

Family	Sub-family	Genus	Species	16S rDNA	12S rDNA	Distribution
New frog species sequenced in the present study						
Rhacophoridae	Rhacophorinae	*Philautus*	*neelanethrus* sp. nov.	AY753560	AY763797	India
Ingroup Reference sequences						
Rhacophoridae	Rhacophorinae	*Philautus*	*luteolus*	AB167932	AB167904	India
Rhacophoridae	Rhacophorinae	*Philautus*	*wynaadensis*	AF249059	AF141796	India
Rhacophoridae	Rhacophorinae	*Philautus*	*microtympanum*	AF249046	AF249030	Sri Lanka
Rhacophoridae	Rhacophorinae	*Philautus*	*femoralis*	AY141833	AY141787	Sri Lanka
Rhacophoridae	Rhacophorinae	*Philautus*	*signatus*	AY141841	AY141795	India
Rhacophoridae	Rhacophorinae	*Philautus*	*charius*	AF249062	AY141794	India
Rhacophoridae	Rhacophorinae	*Philautus*	*aurifasciatus*	AY141851	AY141805	Java
Rhacophoridae	Rhacophorinae	*Philautus*	*petersi*	AF026366	AF026349	Malaya
Rhacophoridae	Rhacophorinae	*Philautus*	Sp. TBGRI*A*	AY880510	AY880596	India
Rhacophoridae	Rhacophorinae	*Philautus*	Sp. TBGRI*B*	AY880506	AY880592	India
Rhacophoridae	Rhacophorinae	*Philautus*	*griet*	AF536203	AY706108	India
Rhacophoridae	Rhacophorinae	*Philautus*	*schmarda*	AY880530	AY880617	Sri Lanka
Rhacophoridae	Rhacophorinae	*Philautus*	Sp. WHT3420	AY880515	AY880601	Sri Lanka
Rhacophoridae	Rhacophorinae	*Philautus*	*ingeri*	AY880496	AY880581	Malaya
Rhacophoridae	Rhacophorinae	*Philautus*	Sp. Java	AY880509	AY880595	Java
Rhacophoridae	Rhacophorinae	*Philautus*	*mjobergi*	AF026365	AF026348	Malaya
Rhacophoridae	Rhacophorinae	*Philautus*	*acutirostris*	AY326059	AY326059	Philippines
Rhacophoridae	Rhacophorinae	*Rhacophorus*	*pardalis*	AF215363	AF215189	Malaya
Rhacophoridae	Rhacophorinae	*Rhacophorus*	*malabaricus*	AF249050	AF249029	India
Rhacophoridae	Rhacophorinae	*Rhacophorus*	*arboreus*	AF458142	AF118476	Japan
Rhacophoridae	Rhacophorinae	*Polypedates*	*maculatus*	AF215358	AF215184	India
Rhacophoridae	Rhacophorinae	*Polypedates*	*cruciger*	AF215357	AY141799	Sri Lanka.
Rhacophoridae	Rhacophorinae	*Polypedates*	*leucomystax*	AF215343	AF161037	India
Rhacophoridae	Rhacophorinae	*Aglyptodactylus*	*madagascariensis*	AF458119	AF026341	Madagascar
Rhacophoridae	Rhacophorinae	*Boophis*	*erythrodactylus*	AF215339	AF026343	Madagascar
Rhacophoridae	Rhacophorinae	*Chirixalus*	*eiffingeri*	AF026363	AF026346	China
Rhacophoridae	Rhacophorinae	*Chirixalus*	*idiootocus*	AY141852	AY141806	China
Rhacophoridae	Rhacophorinae	*Chirixalus*	*palpebralis*	AF458130	AF458130	China
Rhacophoridae	Rhacophorinae	*Chirixalus*	*vittatus*	AF458131	AF161042	Myanmar
Rhacophoridae	Rhacophorinae	*Theloderma*	*corticale*	AF268256	AF268254	Vietnam
Rhacophoridae	Rhacophorinae	*Mantella*	*betsileo*	AF215282	AF215174	Madagascar
Rhacophoridae	Rhacophorinae	*Mantidactylus*	*boulengeri*	AF215318	AF215152	Madagascar
Reference sequences used as outgroup in the final analysis						
Ranidae		*Rana*	*temporalis*	AF249054	AF249022	India
Dicroglossidae		*Euphlyctis*	*cyanophlyctis*	AY014366	AF249015	India
Nyctibatrachidae		*Nyctibatrachus*	*major*	AF249052	AF249017	India

RESULTS

Taxonomy

Philautus neelanethrus sp. nov.

Diagnosis. A small sized frog, described as *Philautus* (Male: 23.2–29.9 mm SVL) based on small size, all digits with well-differentiated disks, predominantly inhabiting in shrubs, and presumably having direct development, is distinguished from all known congeners in the Western Ghats by the combination of absence of tympanum and supratympanic fold, both dorsal and ventral surfaces granular, unpigmented vocal sac (Fig. 2a, e), yellow to cream body coloration with minute brown dots and larger brown patches on the back, and a complete blue ring on the outer margins of the golden pupil.

Etymology. The species name *neelanethrus* is derived from Sanskrit meaning 'blue eye', and is a nominative singular noun standing in apposition to the generic name.

Description of the holotype (Male, BNHS-4510). A small-sized bush frog (SVL=29.9 mm), width of head broader than head length (HW=10.8 mm; HL=9.0 mm), flat dorsally, snout pointed in total profile, protruded slightly beyond mouth. Snout length is equal or subequal to diameter of eye (SL=4.1 mm; EL=4.1 mm). Canthus rostralis angular, loreal region slightly concave. Inter orbital distance (IUE=3.8 mm) flat and broader than upper eyelid (UEW=2.2 mm), wider than internarial distance (IN=2.4 mm). Internal distance between posterior margins of the eyes 1.64 times that of anterior margins (IFE=6.1 mm, IBE=10.0 mm). Nostrils oval, nearer tip of snout (NS=1.4 mm) than eye (EN=2.8 mm). Pineal ocellus absent. Vomerine ridge absent. Eyes protruding, prominent, pupil rounded, horizontal, with blue

ring on the outer margin. Tympanum indistinct. Tongue bifid, without papilla, Supratympanic fold obscure/absent (intense brown dots indicate the fold), unpigmented single vocal sac present. In alcohol-preserved specimens, the blue ring on the eye turns dark blue in color.

Forearm (FLL=7.1 mm) less than hand (HAL=8.2 mm). Relative length of fingers I<II<IV<III. Finger tips with well-developed disks (fd_1=1.0 mm; fd_2=1.3 mm; fd_3=1.8 mm; fd_4=1.7 mm; fw_1=0.7 mm; fw_2=0.7 mm; fw_3=0.7 mm; fw_4=0.8 mm), with distinct circum-marginal grooves, fingers with dermal fringes on both edges. Webbing in hand absent, subarticular tubercles prominent, rounded and single, pre-pollex tubercle oval, distinct (Fig. 2c).

Hindlimbs long, heels do not overlap when folded at right angles to the body, tibia 4.6 times longer than wide (TL=14.3 mm, TW=3.1 mm). Tibia shorter than femur (FL=15.2 mm). Tibia longer than foot (FOL=12.0 mm). Heel to tip of fourth toe (TFOL=19.8 mm) 2.8 times length of fourth toe (LT_4=7.0 mm). Relative toe lengths I<II<III<V<IV. Toe disk width and toe width are: td_1=1.0 mm, td_2=1.0 mm, td_3=1.1 mm, td_4=1.7 mm, td_5=1.5 mm, tw_1=0.7 mm, tw_2=0.7 mm, tw_3=0.9 mm, tw_4=1.0 mm, tw_5=1.0 mm. Webbing distinct and medium (MTTF=6.4 mm, MTFF=6.7 mm, TFTF=5.3 mm, FFTF=5.2 mm). Tibiotarsal articulation reaches anterior border of eye. Inner metatarsal tubercle present (IMT=0.9 mm), nearly 2.6 times length of first toe (ITL=2.0 mm) (Fig. 2d).

Overall coloration of the male of *P. neelanethrus* sp. nov. (live specimen) yellowish (during breeding season) to creamish white (non-breeding season). Abdominal region turned pink during and after advertisement-call bouts. Dorsum with varied intensity of brown granulation. Skin on dorsal as well as on ventral surface granular. Granulation on ventral surface round and white, on dorsum brownish. Feeble cross bars present on forelimbs and hindlimbs. Circular brown patches (region with more brown granules) on head (4–5 in a line) and nearer to vent (1–2). In alcohol-preserved specimens, the overall yellow coloration turned to cream and the blue-colored ring around the eye turned to dark blue/black, but there were no changes in the brown pigmentation. The morphological measurements were based on nine specimens, with ranges, means, and standard deviations detailed in Table 2.

During one of the field surveys, an amplected pair was spotted wherein the female was larger than the male (Fig. 2b). The female was cream colored with brownish black granulation on the body. We observed this pair for more than 9 hours (from 21:30 to 6:30 h), during which time the amplected pair descended from a shrub and entered a leaf-litter heap, making their way into a cavity inside the wet foliar litter.

Advertisement calls. The mating call of *P. neelanethrus* sp. nov. starts with a shrill 'treeek' note followed by repeated 'tink' notes (treeek – tink-tink-tink-tink-……..-tink). Variation was observed in the duration and pattern of calling, even though call notes and peak frequencies remained same. Calls were in the region of 2.35–2.41 kHz, and peak frequency was 2.35 kHz. The spectrogram of a call lasting for 6.6 sec, generated from a single call of a 29 mm *P. neelanethrus* sp. nov. male at 20:30 h on 18 June 2004, at 26.8°C (97% relative humidity), within 50 cm from the species and approximately 2 m above the ground, is shown in Fig. 3. Calling patterns analyzed using 16 calls (total duration of each call [mean±SD] 3.86±0.312 sec, range 1.93–

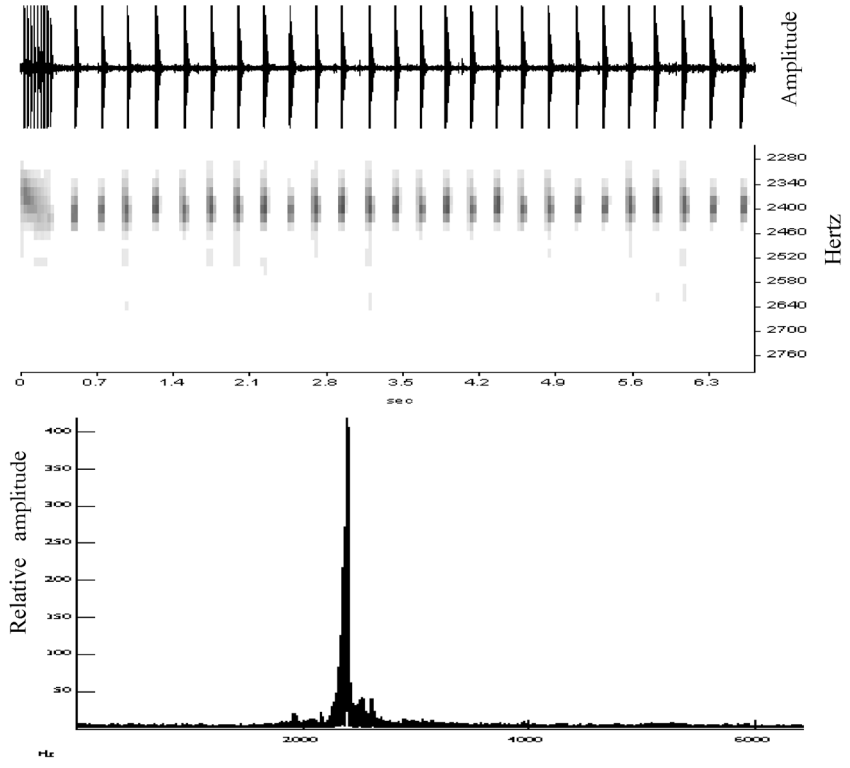

Fig. 3. Advertisement call spectrogram of *P. neelanethrus* sp.nov.

14.35 sec) revealed two types of calls, one with repeated short-duration calls (call duration 0.33±0.04 sec, range 0.27–0.42; number of pulses 10.88±1.654, range 9–15) and another with long-duration calls (call duration 3.34±3.029 sec, range 1.42–13.84; number of pulses 13.6±13.3, range 6–60). Two exceptionally long-duration calls examined (not included in the analysis) were 39.77 sec and 71.92 sec in duration, with 101–191 tinking notes.

The Bartlett test of homogeneity of variances revealed significant differences in many call characteristics of *P. neelanethrus* sp. nov. from *P. luteolus*, as evident from the χ^2 values for peak frequency, total duration, slow-phase duration, and number of pulses.

Comparison with congeners. As many as 118 valid species names are recognized in *Philautus* (Manamendra-Arachchi and Pethiyagoda, 2005), and we examined the nomenclature of all of them. For morphometric comparisons, data from 14 congeners (Supplemental Table 2) among the 20 available names (Supplemental Table 3) endemic to the Western Ghats, and *P. longicrus* (a species described from Borneo and Philippines, but included in the Indian frog fauna by some workers; Rao, 1937), were included in an unweighted pair-group average cluster analysis. The data on 19 morphometric and three meristic characters for the 15 reference taxa used for the cluster analysis are shown in Supplemental Table 4. As systematic morphometric information was not available for six congeneric species (Supplemental Table 3), these were not included in the cluster analysis; nonetheless, their known morphological features such as pointed snout, supratympanic fold, tympanum, granulation on dorsum and belly, and dorsum coloration, were used for comparisons to distinguish *Philautus neelanethrus* sp. nov. from each of them. *Philautus* taxa from Sri Lanka were not used for comparisons, as advocated by Manamendra-Arachchi and Pethiyagoda (2005).

With relatively small size (21.4–29.9 mm), yellow to cream coloration on the body, lack of tympanum, indistinct supratympanic fold, granular dorsum and ventral region, blue-colored outer margins of the eye, and distinct calling pattern, the new species clearly differs from all congeners. *Philautus neelanethrus* sp. nov is distinct from *P. beddomi, P. bombayensis, P. chalazodes, P. femoralis,* and *P. travancoricus* in having a pointed snout. It is distinct from *P. temporalis* in the absence of a tympanum and a distinct supratympanic fold. Apart from these, the dorsal coloration in *P. beddomi*, *P. chalazodes, P. femoralis*, and *P. temporalis* varies from green to brown, whereas in *P. neelanethrus* sp. nov. it is yellowish. Moreover, while the dorsum is smooth in *P. beddomi, P. chalazodes, P. femoralis, P. travancoricus*, and *P. temporalis*, whereas it is distinctly granular in *P. neelanethrus* sp. nov.

The UPGMA cluster analysis, based on 19 morphometric and three meristic characters for the other 15 congeners and reference species, revealed *P. neelanethrus* sp. nov. as a distinct species and closest to *P. luteolus* (Fig. 4). The distinction of *P. neelanethrus* sp. nov. from *P. luteolus* was even clearer when the clustering was done using only morphometric characters and excluding meristic characters, which are constrained by being subjective in their comparative weighting (data not shown). The relationship observed between *P. neelanethrus* sp. nov. and *P. luteolus* is also apparent in morphological features, many of which distinguish between them, though many others are similar between them. Distinctive features of *P. neelanethrus* sp. nov. include lack (indistinct) of tympanum and supratympanic fold, snout length equal/subequal to eye diameter, and a distinct blue ring on the outer margin of the eye. In contrast, *P. luteolus* has a distinct supratympanic fold seen as

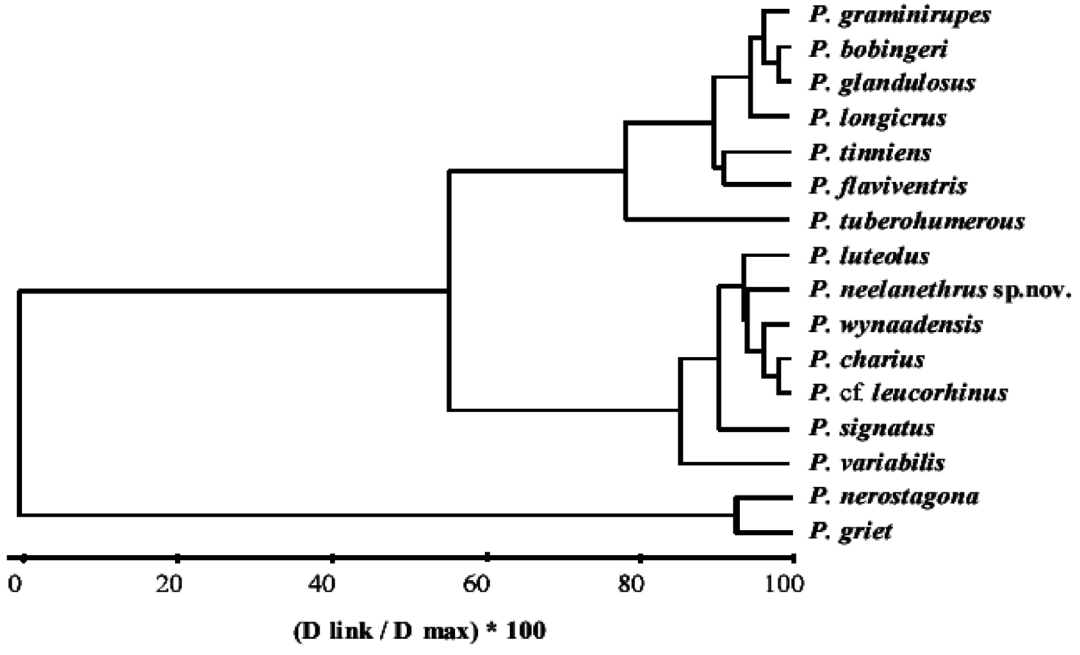

Fig. 4. UPGA cluster analysis of 16 *Philautus* species based on 19 morphometric and three meristic characters (see Table 2, and for reference data, Supplemental Table 4).

a strong, arch-shaped skin fold extending from behind eyes to the shoulder, and a longer snout compared to eye diameter (Kuramoto and Joshy, 2003). In their description of *P. luteolus*, Kuramoto and Joshy (2003) did not mention a distinct blue ring around the iris, which is also not visible in the preserved type specimen. The two species also differ in advertisement call pattern, and emerged as distinct in the phylogentic analysis. Advertisement call characteristics differed significantly, with a peak frequency of 2.39 kHz (range 2.35–2.41 kHz) in *P. neelanethrus* sp. nov. compared to 2.70 kHz (range 2.45–2.87 kHz) in *P. luteolus*. There were significant differences in peak frequency, total call duration, slow-phase duration, and number of pulses.

rDNA phylogenetic analysis

The sequenced rDNA fragments were identical for individuals of the new taxon collected from different localities. Phylogenetic analysis of the 12S and 16S sequences with a large number (>100) of reference amphibian taxa representing families Nyctibatrachidae, Dicroglossidae, and Ranidae revealed the new taxon to be a member of the family Rhacophoridae of Rhacophoroidea, and closest to the *Philautus* species (data not shown). A subsequent analysis done to resolve the exact taxonomic status of the new taxon, using a reduced number of reference taxa belonging mainly to the Rhacophoridae and including three taxa from the related families Ranidae and Nyctibatrachidae (Table 2) as outgroup species, revealed *P. neelanethrus* to be a new, distinct *Philautus* species most closely related to *P. luteolus* (Fig. 5). Moreover, *P. neelanethrus* sp. nov. was revealed to be a distinct and relatively early member of the sub-clade/lineage including other *Philautus* species described from the Western Ghats, and overall as a member of a broader clade distributed in the Western Ghats and Sri Lanka.

Distribution

Philautus neelanethrus sp. nov. was widespread across the study area (Fig. 1, Table 1), though its abundance varied. There were 6–8 individuals/mhs (man-hours of search) in the *Myristica* swamps, where densities were relatively

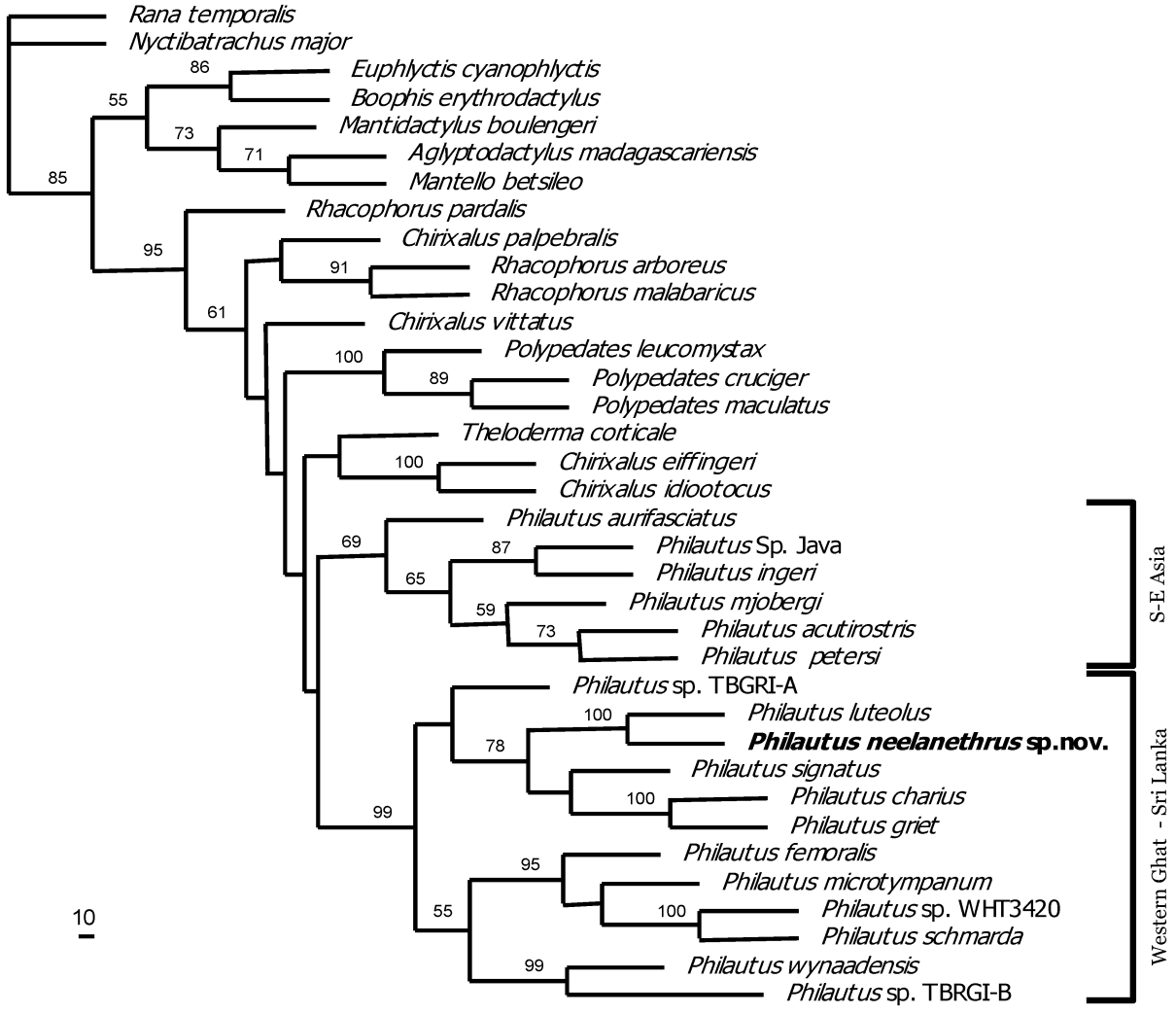

Fig. 5. NJ phylogram (gamma-corrected Kimura two-parameter consensus tree with Tr/Tv=4) based on the combined 12S+16S rDNA data set (alignment 788 bp long, of which 575 sites were complete (without any gaps) and 256 were phylogenetically informative) showing the phylogenetic position of the new frog taxon, *P. neelanethrus* sp. nov. Values at the nodes are bootstrap values.

high compared to the densities of 2–4 individuals/mhs observed in other locations in the Western Ghats (Table 1). Moreover, different localities where the frog was spotted were far from each other, with long stretches of the Ghats without any sign of the frog. Importantly, these stretches were characterized by various ecological (*e.g*., rock outcrops, forest fires) and/or anthropogenic disturbances (*e.g*., open/barren lands, agricultural fields, plantations, and built-up areas).

DISCUSSION

In recent years a number of reports on new descriptions and ancient lineages have suggested that the Western Ghats represent a major hotspot of amphibian diversity and probably a relict habitat (Dutta *et al.,* 2004). In the last five years, 13 new species of amphibians have been discovered from the Western Ghats. Of these, nine are anurans (Dubois *et al.,* 2001; Krishnamurthy *et al.,* 2001; Bossuyt, 2002; Biju and Bossuyt, 2003; Kuramoto and Joshy, 2003; Biju and Bossuyt, 2005a, b; Das and Kunte, 2005) and four are caecilians (Ravichandran *et al.,* 2003; Giri *et al.,* 2003; Bhatta and Prashath, 2004; Bhatta and Srinivas, 2004). Our description of a new species based on morphogenetic analysis from the same biodiversity hotspot adds to the growing list of amphibians from the region, clearly indicates the hotspot status of the region, and reinforces the current notion that there are several new species yet to be discovered (Aravind *et al.,* 2004) requiring proper methods for describing new species.

We described the new species based on morphometry, molecular analysis, and acoustics, which complemented the taxonomic description of the species. Also, the observation that the amplected pair of *P. neelanethrus* sp. nov. descended to the ground without any water body nearby was probably indicative of ground nesting and direct development to a froglet, which are characteristic of the genus (Marmayou *et al.,* 2000).

The study also revealed that although traditional approaches based on morphometric comparisons and acoustics provided an initial indication that *P. neelanethrus* was a new species, its identity and overall taxonomic relationships could most reliably be inferred based on molecular analysis. Furthermore, it is important to note that the specimens of the putative new species from different localities carried identical rDNA sequences, which strongly suggests that the isolated, disjunct small populations spread over a considerable part of the central Western Ghats were indeed *P. neelanethrus* sp. nov., which is expected to be a very poor disperser.

Philautus neelanethrus sp. nov. was found mainly in the mid-altitudinal range (500–700 m asl) characterized by evergreen/semi-evergreen/moist deciduous forest patches in the central Western Ghats, and most importantly in *Myristica* swamps, which are considered to be living fossils among the vegetation types prevailing in the region (Chandran and Divakar, 2001). The phylogenetic and molecular-dating analysis suggests that *P. neelanethrus* sp nov. is a relatively old taxon among other species of *Philautus* endemic to the Western Ghats. Systematic sampling carried out in the Sharavathi River basin shows that forest patches (as mentioned above) are a prerequisite for this species to survive; these patches are not found in many parts of the study area due to multiple anthropogenic disturbances. These unique features, the relatively older origin of the taxon but presence of its extant population in restricted, non-overlapping and non-contiguous patches, suggests that there had been significant habitat fragmentation in the Western Ghats leading to the present day disjunct populations. The species thus appears to be a useful, indirect bioindicator of the ecological health of the Western Ghats, where the remaining evergreen/semi-evergreen/moist deciduous forests are becoming patchy and insularized.

A number of recent studies have documented habitat fragmentation in the Western Ghats due to various anthropogenic activities, *viz*, construction of dams for hydropower, extension of agricultural fields into forested areas, and urbanization (Vasudevan *et al.,* 2001; Gururaja *et al.,* 2003; Aggarwal, 2004). We emphasize here that such fragmentation of natural forest habitats has led to the formation of ecological barriers. These barriers have curtailed poor dispersers like *P. neelanethrus* sp. nov. from dispersing into adjoining similar habitats, leading to the formation of metapopulations. Such metapopulations are always at high risk of extinction due to progressively decreasing native habitats, inbreeding stress, invasion by introduced species, etc. Thus the new species is clearly an indicator of forest fragmentation, at the same time warning of the consequences of fragmentation of the remaining biodiversity in the region and calling for immediate conservation measures to be initiated.

The supplementary data for this article can be found online at http://dx.doi.org/10.2108/zsj.24.525.

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