



A new species of *Polypedates* Tschudi, 1838 (Amphibia: Anura: Rhacophoridae) from West Bengal State, Eastern India

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Abstract

A new species of frog belonging to the genus *Polypedates* Tschudi is described from the state of West Bengal, Eastern India. A mid-sized frog, SVL ranges from 47.9–53.6 mm in males and 72.0 mm in the single female. The species is diagnosable in showing the following suite of characters: digits lack webbing, inner and outer metacarpal tubercles present; no dermal fold on forearm; toes webbed, webbing formula I₁₋₁ II_{0.5-2} III₁₋₂ IV_{2-0.5} V; an inner metatarsal tubercle present; tibio-tarsal articulation reaches between eye and nostril; and skin on forehead co-ossified to cranium. Additionally, males possess paired vocal sacs. The new species is compared with known species of the genus *Polypedates*.

Key words: West Bengal, India, *Polypedates*, new species, 16s rRNA

Introduction

The genus *Polypedates* Tschudi, 1838 (Amphibia: Rhacophoridae) currently includes twenty-four nominal species (Frost 2019) and has a distribution from southern Asia to south-east Asia. Using a phenetic approach and using exclusively morphological characters, Dubois (1987 “1986”) treated the genus as a synonym of the subgenus *Rhacophorus*, within the genus *Rhacophorus*. However, Jiang *et al.* (1987) treated the two as distinct, and further, Channing (1989) did not consider *Rhacophorus* to be its phylogenetically closest relative. Frost *et al.* (2006) discovered a sister taxon relationship between *Polypedates* and the East African genus *Chiromantis*, based on molecular data, although Grosjean *et al.* (2008), on the basis of larval and molecular characters, reported a sister relationship with *Rhacophorus*. Li *et al.* (2008, 2013) on the basis of another molecular dataset, discovered *Polypedates* (including the Sri Lankan *Taruga*) to be the sister taxon of *Feihyla* + *Rhacophorus*, as also confirmed by Pyron & Wiens (2011). Again, Chan *et al.* (2018) placed *Polypedates* + *Taruga* + *Chiromantis* as a sister taxon to *Feihyla* + *Rhacophorus*.

Recently, Gogoi & Sengupta (2017) revived *P. himalayensis* (Annandale), which was synonymised with both *P. leucomystax* and *P. maculatus* (Gray) (see Frost 2019), making it the twenty-fifth recognised species. The genus includes eleven species from India, namely *P. assamensis* Mathew & Sen, *P. himalayensis* (Annandale), *P. insularis* Das, *P. leucomystax* (Gravenhorst), *P. maculatus* (Gray), *P. megacephalus* Hallowell, *P. occidentalis* Das & Dutta, *P. pseudocruciger* Das & Ravichandran, *P. subansiriensis* Mathew & Sen, *P. taeniatus* (Boulenger) and *P. teraiensis* (Dubois). In India, the occurrence of *P. leucomystax* sensu stricto is the subject of debate, and a recent study by Gogoi & Sengupta (2017) suggested that the north-eastern population of the complex (Matsui *et al.* 1986, Kuraishi *et al.* 2013) be treated as *P. teraiensis*.

In this paper, we describe a new species of the genus *Polypedates* from West Bengal state of eastern India. The new species herein is compared with its congeners.

Materials and Methods

Eight specimens were collected from from Khordanahala (22.25°N, 88.14°E) in South 24 Parganas District and Madhyamgram (22.69°N, 88.50°E) in North 24 Parganas District (Fig. 1), in the state of West Bengal, eastern India. They were euthanised following standard protocol, preserved in 70% unbuffered ethanol and deposited in the National Zoological Collection, maintained by the North Eastern Regional Centre, Zoological Survey of India, Shillong (V/A/NERC/1448- V/A/NERC/1455). Measurement data were taken from preserved specimens with a dial caliper measured to the nearest 0.02 mm and rounded to the nearest 0.1 mm. Abbreviations used for morphometric analysis and their definition are as follows: snout-vent length (SVL), measured from tip of snout to vent; head width (HW), measured across angle of jaws; head length (HL), distance between angle of jaws and snout tip; head depth (HD), distance between the apex of eye and the bottom of upper jaw; upper eyelid length (UE), the greatest length of upper eyelid; interorbital space (IOS), least distance between the upper margins of orbits; nostril diameter, (ND), greatest diameter of the nostril; nostril to snout (NS), distance between anterior edge of nostril to snout tip; eye to nostril (EN), distance between anterior edge of the eye to the posterior corner of the nostril; vertical eye diameter (EDV), the greatest vertical diameter of eye; horizontal eye diameter (EDH), the greatest horizontal diameter of eye; vertical tympanum diameter (TDV), the greatest vertical diameter of tympanum; horizontal tympanum diameter (TDH), the greatest horizontal diameter of tympanum; tympanum to eye (TE), the distance between anterior edge of tympanum to the posterior corner of eye; forelimb length (FLL), distance between insertion of arm and the tip of the third finger; femur length (FL), distance between the insertion point of hind limb to the knee; tibia length (TBL), distance between knee and the heel; foot length (FTL), the distance between heel and the tip of the fourth finger; hand length (HDL): the distance between carpal region and the tip of the third finger; arm length (ARL): distance between elbow and the tip of the third finger; humerus length (HRL), distance between elbow and wrist; hindlimb length (HLL), distance between vent to the tip of the fourth toe; third finger disk width (3FDW), the diameter of the disk on the third finger; fourth finger disk width (4TDW), the diameter of the disk on the fourth toe; inner metacarpal tubercle length (IMC), the greatest length of the inner metacarpal tubercle; outer metacarpal tubercle length (OMC), the greatest length of the outer metacarpal tubercle; inner metatarsal tubercle length (IMT), the greatest length of the inner metatarsal tubercle; length of first finger (F1): the length between the point of insertion of first finger and its tip; length of second finger (F2): the length between the point of insertion of second finger and its tip; length of third finger (F3): the length between the point of insertion of third finger and its tip; length of fourth finger (F4): the length between the point of insertion of fourth finger and its tip; length of first toe (T1): the length between the point of insertion of first toe and its tip; length of second toe (T2): the length between the point of insertion of second toe and its tip; length of third toe (T3): the length between the point of insertion of third toe and its tip; length of fourth toe (T4): the length between the point of insertion of fourth toe and its tip; length of fifth toe (T5): the length between the point of insertion of fifth toe and its tip.

Call recordings of the new species were made in the field using a Nikon D7000 camera body in video mode (ambient temperature 34°C). Signal was extracted using the video editing software Wondershare's Filmora 8.5 and calls analyzed with Audacity™ and Sound Ruler™. For call recordings and analysis of *P. leucomystax*, the same devices and softwares were used. Call analysis was done following Köhler *et al.* (2017).

Comparison of the species was done using data from material examined (Appendix I), as well as literature (including Blyth 1852; Boulenger 1890, 1891, 1893, 1906; Das & Ravichandran 1998 “1997” Das 1995, 2005; Das & Dutta 2006; Dubois 1987 “1986”; Gray 1830; Hallowell 1861 “1860”; Kuraishi *et al.* 2011; Mathew & Sen 2009; Matsui *et al.* 2014; Pan *et al.* 2013; Peters 1863; Smith 1940; Riyanto *et al.* 2011; Rujirawan *et al.* 2013; Vogt 1911; Wickramasinghe *et al.* 2012; Yang 2008). Museum abbreviations follow Sabaj (2016).

Molecular work. Tissue samples were preserved in absolute alcohol. DNA isolation was carried out with Phenol:Chloroform:Isoamyl alcohol (25:24:1) method. The isolated DNA was subjected to 0.8% agarose gel electrophoresis to check the yield and presence of isolated DNA. PCR amplification of a fragment of the mitochondrial gene for 16S rRNA was carried out.

For isolation of DNA, tissues (liver), around 25mg was taken in a vial and cut into small pieces; 150µl of TE (tris EDTA buffer) was added to the vial, 150µl of Guanidine-HCl was next added to it. Further, 5µl of proteinase K was added, and the vials kept at 56°C in a hot water bath, till tissues lysed. In case the tissues were kept overnight, 1% SDS was added after incubation overnight for further lysis. 250µl of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added and centrifuged at 1200rpm for 10 min, the aqueous layer pipetted from top and poured in different vials. 250µl of chloroform was added to vials and centrifuged at 12000rpm for 5–10min, the aqueous layer

was again pipetted out and poured in a separate vial, 100% ethanol 250µl was added and centrifuged at 12000rpm for 5 min. The supernatant was discarded and to the pellet again 250µl cold 70% ethanol was added and the samples were centrifuged at 12000rpm for 5min. The supernatant was discarded and pellets dried and 40µl molecular biology grade water was added to the pellet. Bands were observed by 0.8% Agarose Gel electrophoresis.

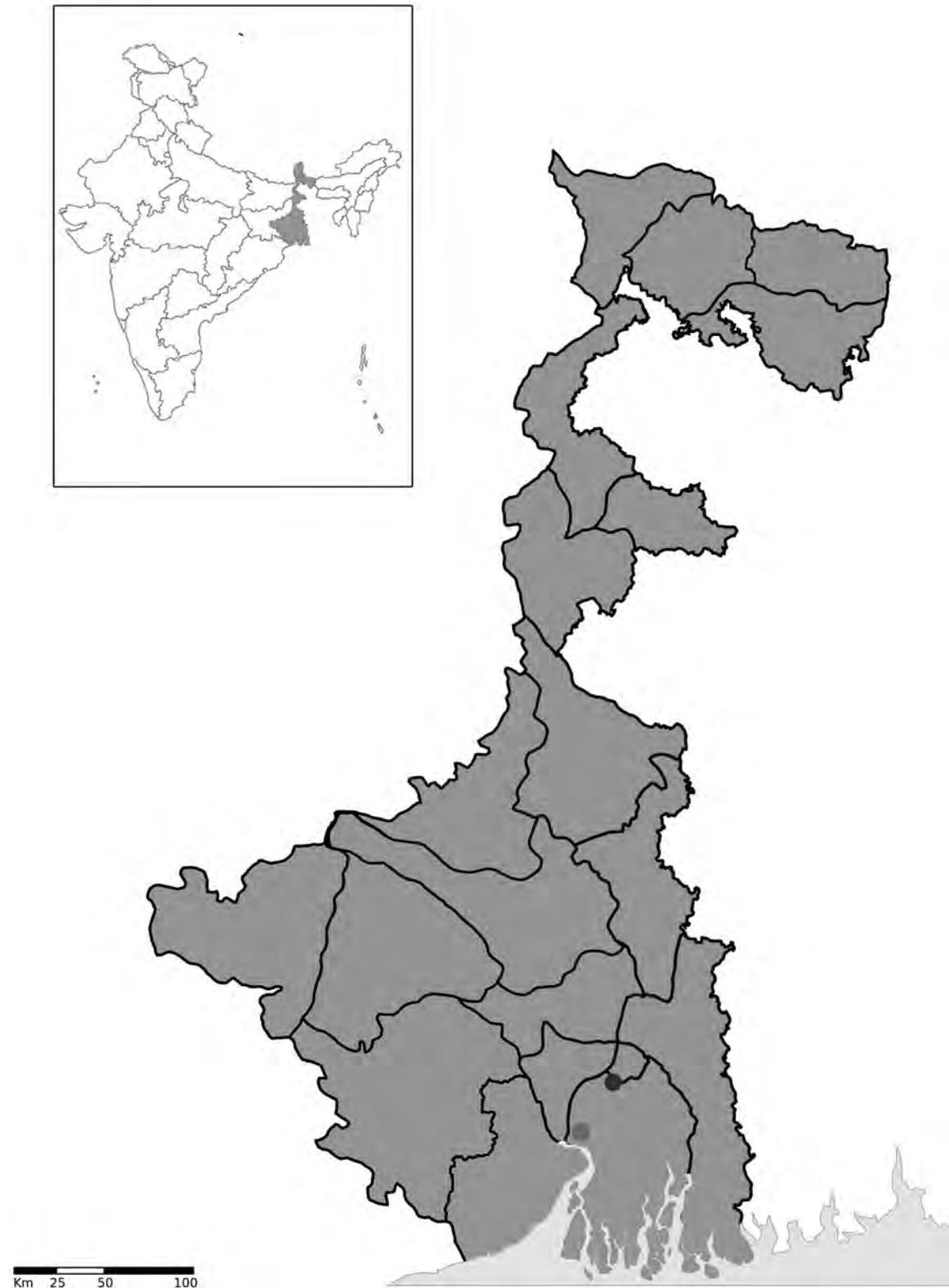


FIGURE 1. Map showing the known distribution of *Polypedates bengalensis* **sp. nov.** Grey circle: Khordanahala, District: 24 Parganas South; black circle: Madhyamgram, District: 24 Parganas North.

TABLE 2. Sequences of members of the genus *Polypedates* used for analysis in this study (*: specimens sequenced in this study).

GenBank Accession no.	Species	Locality
MK490922	<i>P. bengalensis</i> sp. nov.*	Khordanahala, West Bengal
MK490923	<i>P. bengalensis</i> sp. nov.*	Khordanahala, West Bengal
MK490924	<i>P. teraiensis</i> *	Guwahati, Assam
MK490925	<i>P. teraiensis</i> *	Guwahati, Assam
AB530517	<i>P. teraiensis</i>	Gazipur, Bangladesh
AB530515	<i>P. teraiensis</i>	Gazipur, Bangladesh
MG935986	<i>P. teraiensis</i>	Bago, Myanmar
AB728168	<i>P. leucomystax</i>	Chatthin, Myanmar
AB728167	<i>P. leucomystax</i>	Chatthin, Myanmar
AB728152	<i>P. leucomystax</i>	Sarawak, Malaysia
AB728135	<i>P. leucomystax</i>	Java, Indonesia
AB728129	<i>P. leucomystax</i>	Sumatra, Indonesia
AB728126	<i>P. leucomystax</i>	Johor, Malaysia
AB728116	<i>P. leucomystax</i>	Kuala Lumpur, Malaysia
AB728098	<i>P. leucomystax</i>	Trang, Thailand
AB728094	<i>P. leucomystax</i>	Surat Thani, Thailand
HM359105	<i>P. leucomystax</i>	Singapore
AB728136	<i>P. leucomystax</i>	Java, Indonesia
AB728017	<i>P. mutus</i>	Guangxi, China
KR828027	<i>P. mutus</i>	VinhPhuc, Viet Nam
HM770139	<i>P. macrotis</i>	Tawi-tawi Island, Philippines
HM770138	<i>P. macrotis</i>	Tawi-tawi Island, Philippines
AB728016	<i>P. braueri</i>	Yunnan, China
AB728009	<i>P. braueri</i>	Guangxi, China
AB564281	<i>P. megacephalus</i>	Taipei, Taiwan
KC465836	<i>P. megacephalus</i>	Motuo, China
KF303467	<i>P. discantus</i>	Songkhla, Thailand
AB728189	<i>P. colletti</i>	Sumatra, Indonesia
HM770129	<i>P. colletti</i>	Malaysia
KR827999	<i>P. impresus</i>	Louangphrabang, Laos
AB907717	<i>P. otilophus</i>	Borneo, Malaysia
AB167934	<i>P. maculatus</i>	Mangalore, India
AB728188	<i>P. maculatus</i>	Bihar, India
JF832394	<i>P. maculatus</i>	Thekkatte, India
GQ204694	<i>P. maculatus</i>	Sri Lanka
AB530551	<i>P. pseudocruciger</i>	Bajipe, India
AB530552	<i>P. pseudocruciger</i>	Bajipe, India
KR828078	<i>R. rhodopus</i>	Phitsanulok, Thailand

For polymerase chain reaction of the 16S rRNA gene, we used the following primers: AH-16S_S (5'-CGC CTG TTT ACC AAA AAC ATC GCC T-3'); AH-16S_R (5'-TGC GCT GTT ATC CCY RGG GTA ACT-3'). Primers were obtained commercially, according to Grosjean et al. (2015). For amplification, a total of 0.2 µM of the primer sets, 0.2 mM dNTP mix, 1–2 µg of template DNA were used in a 30 µl PCR reaction mixture. The amplification was carried out using DyNAzyme II DNA polymerase. Polymerase Chain Reaction (PCR) was performed in Prima 96 Thermal cycler (Himedia) as follows: One cycle of 95°C for 3mins; 35 cycles of 95°C for 30 sec, 56.3°C for 30 sec,

72°C for 2 min; and final extension of 72°C for 5 min and 4°C for 10 min. The amplified DNA was electrophoresed on 0.8% agarose gel and visualized under UV light.

Alignment of sequences and phylogenetic analyses. We have sequenced two specimens of each *Polypedates bengalensis* sp. nov. (MK490922 and MK49092) and *P. teraiensis* (MK490924 and MK49092). The other compared specimens were from NCBI GenBank (Table 2). The sequences were aligned with Mega 5.2.2 using the Muscle algorithm with default parameter settings (Kumar *et al.* 2016, Tamura & Nei 1993). Phylogenetic relationships were reconstructed using Maximum Likelihood (ML) using RaxmlGUI v1.3 (Silvestro & Michalak 2012) using GTRGAMMA with 1000 rapid bootstraps. *Rhacophorus rhodopus* was assigned as the outgroup, *Rhacophorus* being the closest relative (sister taxa) to *Polypedates*. Uncorrected percent pairwise sequence divergence was calculated in MEGA 5.2.2 (Tamura *et al.* 2011).

Systematics

Polypedates bengalensis sp. nov.

Figs. 2–6



FIGURE 2. Holotype of *Polypedates bengalensis* sp. nov. (V/A/NERC/1448) from 24 Parganas, West Bengal, India.

Holotype. V/A/NERC/1448, mature male, SVL 53.6 mm (Fig. 2). Khordanahala, South 24 Parganas District (22.2 47719°N, 88.144078°E; WGS84), elevation 7 m above sea level, collected on 13 June 2018, by Kingshuk Mondal.

Paratypes. (V/A/NERC/1449, mature male, SVL 52.1 mm; V/A/NERC/1450, mature male, SVL 47.9 mm; collector and date as in holotype. V/A/NERC/1451, mature male, SVL 50.9 mm; V/A/NERC/1452, mature female, SVL 72.0 mm; V/A/NERC/1453, mature male, SVL 51.5 mm; V/A/NERC/1454, mature male, SVL 48.2 mm and V/A/NERC/1455, mature male, SVL 48.1 mm were collected by Shibajee Mitra on 21 June 2018 from Badu, North 24 Parganas District (22.692860°N, 88.505926°E), elevation 13 m above sea level.

Diagnosis. A mid-sized *Polypedates*, SVL range from 47.9–53.6 mm (mean: 50.3 mm) in males and 72.0 mm in the single female specimen. Tympanum large, about three fourth of the size of orbit (Fig. 3). Digits without webbing (rudimentary webbing can be seen between the first and second finger, Fig. 4). Inner and outer metacarpal tubercles present, outer one about 65% in length of the inner one. Toe webbed with webbing formula $I_{1-1} II_{0.5-2} III_{1-2} IV_{2-0.5} V$ (Fig. 4). An inner metatarsal tubercle present. Tibio-tarsal articulation reaches between eye and nostril. Canthus

rostralis steeply concave. A straight angular supratympanic fold runs from behind the eyes to the angle of the jaw. The skin on the forehead co-ossified to the skull, vomerine teeth oblique between the choanae (Fig 5). No dermal fold present on the forearm; thigh with white spots forming a reticulated pattern.



FIGURE 3. Paratype of *Polypedates bengalensis* sp. nov.(V/A/NERC/1449) showing (top) lateral, (bottom left) dorsal and (bottom right) ventral aspects.

Description of holotype. Body elongate (SVL/HW 3.1); head slightly longer than broad (HW/HL 0.9), relatively deep (HD/HL 0.7). Canthus rostralis concave. Interorbital space flat and wider than upper eyelid (UE/IOS 0.7). The skin on the forehead co-ossified to cranium. An angular supratympanic fold runs from behind the eyes to the angle of the jaw. Nostrils small (ND/EHD 0.2), laterally placed, closer to snout (NS/EN 0.6) than to the eye. Eye horizontally elliptical (EVD/EHD 0.7); tympanum horizontally elliptical (TVD/THD 0.8), more than $4/5^{\text{th}}$ of the size of the eye, tympanum separated from eye by a small gap (TE 1.2 mm). A straight angular supratympanic fold runs from behind the eyes to the angle of the jaw. Vomerine teeth oblique (30 degrees to body axis) between the choanae, seven in number separated broadly from each other. Tongue lanceolate with bifid tip. Forelimb relatively long (FLL/SVL 0.5), No dermal fold present on forearm; relative length of the fingers are F1 (6.3) < F2 (7.1) < F3 (8.4) < F4 (11.3). Tip of the digits with prominent discs. Inner and outer metacarpal tubercles present, outer ca. 64.5% in greatest length of inner. The nuptial pad is elongated and is on dorsal surface of first finger. Hind limb

long (x 1.5 SVL), tibia longer than femur (FL/TBL 0.9), tibio-tarsal articulation reaches between eye and nostril, subarticular tubercle and toe disk distinct, relative toe length: T1 (4.2) < T2 (5.2) < T3 (8.5) < T5 (10.2) < T4 (14.7). Webbing formula of toe I₁₋₁II_{0.5-2}III₁₋₂IV_{2-0.5}V. Large, spade-shaped outer metatarsal tubercle present.



FIGURE 4. Manus and pes of *Polypedates bengalensis* sp. nov. (V/A/NERC/1448).



FIGURE 5. The orientation of vomerine ridge of *Polypedates bengalensis* sp. nov. (V/A/NERC/1448).

Dorsum smooth, lacking tubercles, dorsal region of forelimbs and hindlimbs smooth. Ventral surface of the body granular with enlarged tubercles present in the abdominal region extending up to vent. Ventral surface of the femoral region granulated. Ventral surface of forelimb, tibia and tarsus smooth.

Colour in life (based on type series). Ground colour of dorsum yellowish brown to greenish-brown. A continuous series of 6–9 dark brown blotches extend laterally from behind the eye to vent (Fig. 3). The blotches vary in shape, but are mostly elongate and run parallel to body axis. A maximum of three rows of such blotches can be seen around midbody, and are largest medially. Typically, two triangular shaped blotches in interorbital region, each in

contact with upper eyelid (Fig. 3). A dark brown streak runs from behind the nostril to the eye and continues from behind the eye to angle of jaws. Iris golden brown, tympanum dark brown (Fig. 3). Upper lip cream coloured. Dorsal surface in forelimb and hindlimb is lighter compared to colouration of dorsum, dorsum with distinct broad bands of dark brown colour. Back of thigh with yellowish brown blotches, forming a reticulate pattern (Fig. 6). Similar reticulated pattern are present on region between axilla and groin. A narrow dark brown line runs along outer edge of forearm and tarsus. Venter cream-coloured. Gular region yellowish-white, sometimes with a light reticulate pattern.

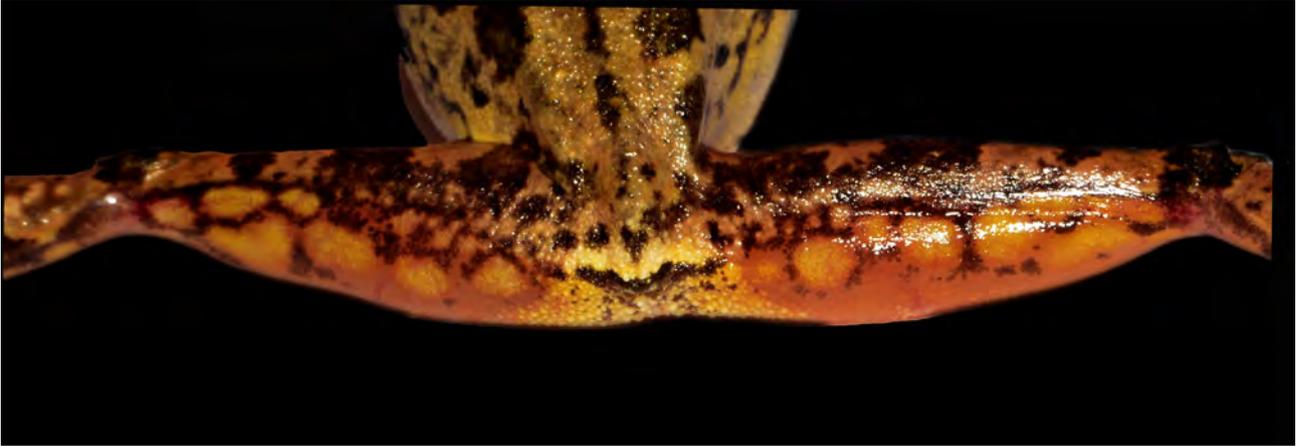


FIGURE 6. Coloration of posterior of the thigh of *Polypedates bengalensis* sp. nov. (V/A/NERC/1448).

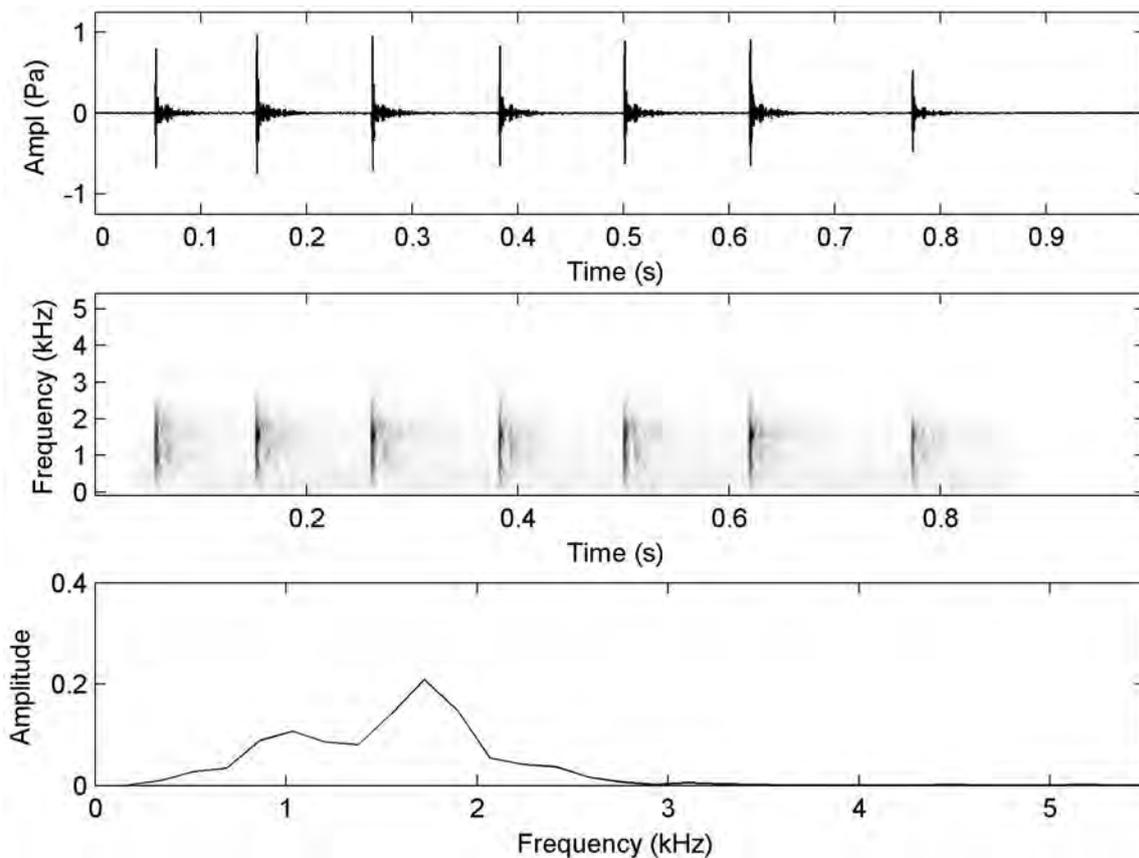


FIGURE 7. Top: Oscillogram, Middle: spectrogram and Bottom: amplitude graph of the advertisement call of *Polypedates bengalensis* sp. nov. (V/A/NERC/1448).

Colour in preservative. Body colour has turned into a lighter shade compared to the live specimen. The yellow colour of the spots behind the thigh and between axilla and groin completely faded, producing a pale off-white appearance. The blotches and stripes are dull in comparison to live specimens.



FIGURE 8. Axillary amplexus shown by a pair of *Polypedates bengalensis* sp. nov. (unpreserved) in Khordanahala, 24 Parganas, West Bengal, India.

Etymology. The specific epithet *bengalensis* is proposed, referring on to the type locality of the species located in the West Bengal State of India.

Suggested vernacular name. The vernacular names suggested for the new species are ‘Khoyeri Daag Gecho Bang’ and ‘Brown Blotched Bengal Tree Frog’, in Bengali and English, respectively.

Bioacoustics. The advertisement call was recorded at an ambient temperature of 34°C (<https://doi.org/10.6084/m9.figshare.9768059>). A typical advertisement call consists of five to eight notes with an average note length of 10.3 ms (max: 14ms, min: 7ms, σ : 2.5) and inter-note interval of 107 ms. (max: 86ms, min: 137ms, σ : 15.3). The call we worked with has nineteen numbers of repetition in a minute. The portion of the call we analyzed consisted of seven notes. Each note comprised of six pulses. Approximate prevalent bandwidth of the calls ranged from 0.5 kHz to 2.5 kHz with a dominant frequency of 1464.25 Hz. The call has only one peak of amplitude (Fig. 7).

Distribution. The new species is known from Khordanahala, South 24 Parganas and Madhyamgram, North 24 Parganas in the State of West Bengal, in eastern India (Fig. 1).

Natural history. All frogs were encountered around residential areas. Calls were heard after sunset and continued till after midnight. The males were seen perched on vegetation, including bamboo, banana and taro leaves, and were calling from a height of ca. 1.2–1.8 m above ground, over stagnant waters bodies (mostly rainwater pools), and amplexus is axillary (Fig. 8).

Comparisons. *Polypedates bengalensis* sp. nov. genetically differs (16s rRNA) from congeners (0.094–0.147 uncorrected genetic distance; Table 1); and no sister-group relationship could be uncovered with any single of the congeners with certainty, rather the new taxon is sister-group to a clade containing several species (Fig. 9). *Polypedates bengalensis* sp. nov. shows skin on the forehead co-ossified to the skull (free in *P. assamensis*, *P. chlorophthalmus*, *P. colletti*, *P. cruciger*, *P. discantus*, *P. himalayensis*, *P. maculatus*, *P. occidentalis*, *P. subansiriensis*,

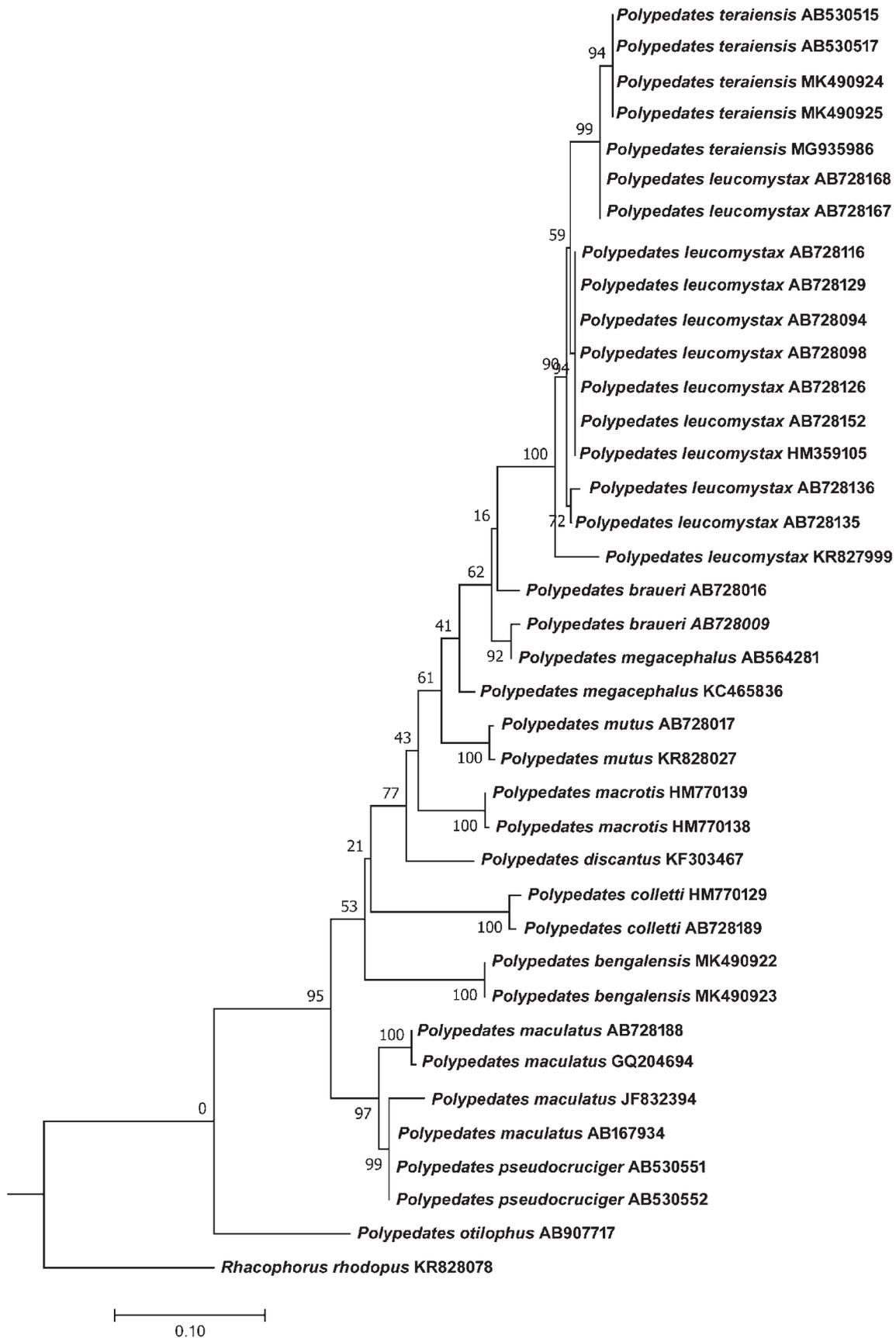


FIGURE 9. Maximum likelihood phylogeny based on DNA sequences of the mitochondrial gene for 16s rRNA, showing the relationship of *Polypedates bengalensis* **sp. nov.** and its congeners, with *Rhacophorus rhodopus* as the outgroup. The values at nodes are bootstrap support values.



FIGURE 10. Comparative structure of pes of *Polypedates bengalensis* sp. nov. (left) and *Polypedates leucomystax* (right).

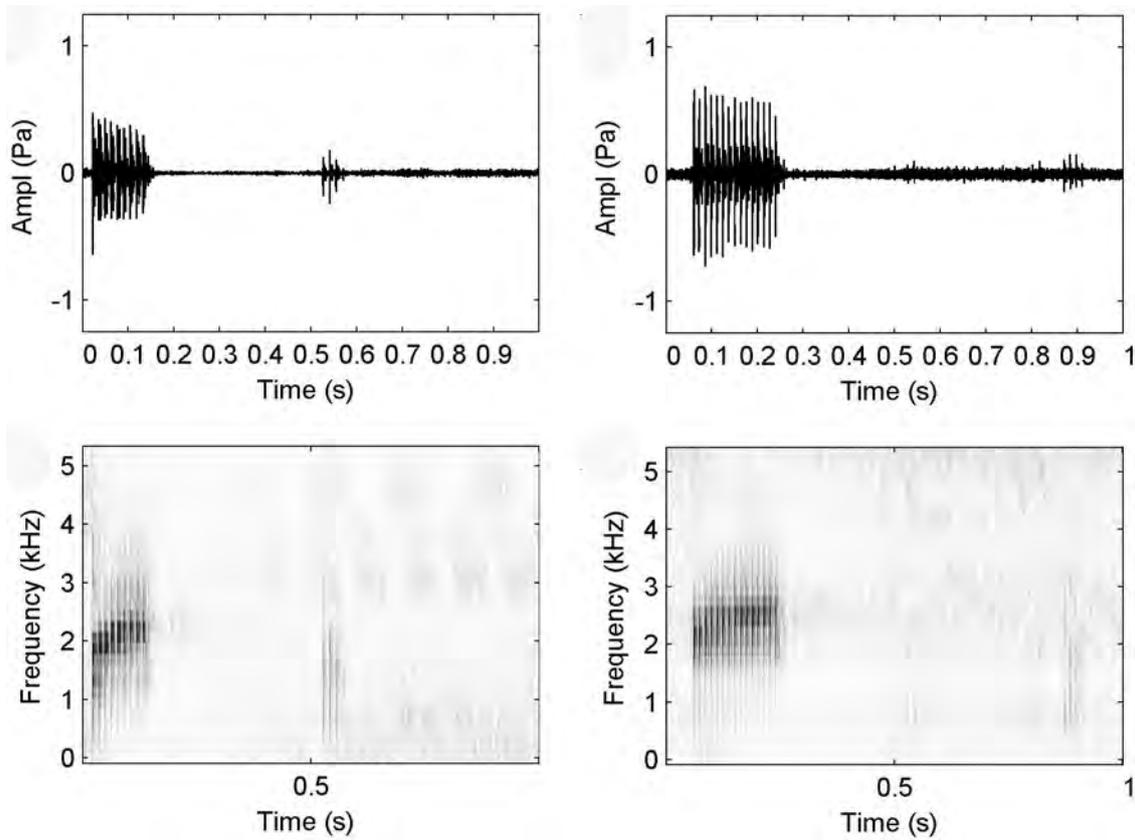


FIGURE 11. Oscillogram (top), spectrogram (bottom) of call of *Polypedates leucomystax* from Assam, India (left) and Kedah, Malaysia (right).

TABLE 1. Uncorrected pairwise distances (%) of the 16s rRNA sequences of *Polypedates* (*P.*) and *Rhacophorus* (*R.*) used in this study.

	<i>P. bengalensis</i> sp. nov.	<i>P. teraiensis</i>	<i>P. leucomystax</i>	<i>P. mutus</i>	<i>P. macrotis</i>	<i>P. braueri</i>	<i>P. megacephalus</i>	<i>P. discantus</i>	<i>P. colletti</i>	<i>P. impresus</i>	<i>P. ottilophus</i>	<i>P. maculatus</i>	<i>P. pseudocruciger</i>
<i>P. bengalensis</i> sp. nov. (N=2)	0												
<i>P. teraiensis</i> (N=5)	0.131- 0.134	0.0- 0.008											
<i>P. leucomystax</i> (N=11)	0.123- 0.131	0.008- 0.034	0- 0.026										
<i>P. mutus</i> (N=2)	0.94	0.073- 0.079	0.071- 0.076	0.005									
<i>P. macrotis</i> (N=2)	0.105- 0.107	0.089- 0.092	0.086- 0.089	0.065- 0.068	0.003								
<i>P. braueri</i> (N=2)	0.107- 0.110	0.065- 0.071	0.063- 0.068	0.052- 0.058	0.071- 0.079	0.024							
<i>P. megacephalus</i> (N=2)	0.097- 0.113	0.065- 0.068	0.063- 0.065	0.45- 0.50	0.060- 0.081	0.026- 0.045	0.039						
<i>P. discantus</i> (N=1)	0.102	0.084	0.086	0.076	0.071	0.058	0.076	0					
<i>P. colletti</i> (N=2)	0.107- 0.110	0.128- 0.131	0.123- 0.126	0.113- 0.115	0.105- 0.107	0.097- 0.099	0.105- 0.107	0.097- 0.099	0.01				
<i>P. impresus</i> (N=1)	0.131	0.05	0.045	0.079	0.097	0.065	0.065	0.081	0.102	0			
<i>P. ottilophus</i> (N=1)	0.147	0.157	0.157	0.149	0.128	0.141	0.141	0.139	0.136	0.154	0		
<i>P. maculatus</i> (N=4)	0.099- 0.107	0.099- 0.110	0.097- 0.107	0.089- 0.097	0.084- 0.094	0.089- 0.097	0.086- 0.102	0.092- 0.102	0.102- 0.110	0.115- 0.123	0.115- 0.123	0.008- 0.026	
<i>P. pseudocruciger</i> (N=2)	0.099	0.099	0.097	0.089	0.086	0.089	0.086	0.094	0.102	0.115	0.115	0	0
<i>R. rhodopus</i> (N=1)	0.173	0.178	0.181	0.178	0.162	0.183	0.181	0.17	0.162	0.181	0.149	0.165	0.165

P. taeniatus and *P. zed*); lack of hour glass pattern on dorsal surface (hour glass pattern on dorsal surface present in *P. insularis*, *P. megacephalus*, *P. pseudotilophus*, *P. subansiriensis*); lack of spiny projection from the angle of jaw (a spine at the angle of jaw is present in *P. ottilophus* and *P. ranwellai*), iris golden yellow (iris bright green in *P. chlorophthalmus*), lack of postcloacal tubercles (2–4 post cloacal tubercle present in *P. macrotis*), body with elongated blotches (body without spots in *P. cruciger*), palmar tubercles two in number (three palmer tubercles present in *P. impresus*, *P. leucomystax* and *P. teraiensis*), males with vocal sacs (vocal sac absent in *P. macrotis*, *P. mutus* and *P. hecticus*). Toes are less webbed in comparison to *P. iskandari* (web formula for *P. iskandari* is I₁₋₁II₁₋₂III₁₋₂IV₂₋₁V).

Discussion

Polypedates bengalensis sp. nov. shows molecular affinity to *P. mutus* (uncorrected p-distance 0.094) and to *P. maculatus* (0.099–0.107). Morphologically *Polypedates bengalensis* sp. nov. shows affinity to the *P. leucomystax* complex (sensu Matsui et al. 1986), but can be distinguished from the latter by its dorsal pattern (with pattern of distinct broken blotches in *P. bengalensis* vs distinct or faint lines running from snout to vent region in *P. leuco-*

mystax), reduced toe webbings (I₁₋₁ II_{0.5-2} III₁₋₂ IV_{2-0.5} V in *P. bengalensis* vs I₁₋₁ II_{1.5-2} III₁₋₂ IV_{2-0.5} V in *P. leucomystax*, Fig. 10), palmar tubercles two in number (vs three palmar tubercles in *P. leucomystax*), steeply concave canthus rostralis (vs vertical canthus rostralis in *P. leucomystax*, see Das (2005)), a more granulated venter (vs smooth venter in *P. leucomystax*), larger subarticular tubercles (vs relatively smaller subarticular tubercles, Fig 10), and call pattern (Fig. 7). *Polypedates bengalensis* sp. nov. differs from *P. leucomystax* by a strong genetic divergence (uncorrected p-distance of 0.123–0.131).

Morphologically, species within *P. leucomystax* complex provide a challenge to separate from *P. leucomystax* sensu stricto (fide Kuraishi *et al.* 2013). Advertisement calls are a mechanism of segregation of such sibling species while dealing with morphologically similar group of frogs (Grosjean & Dubois 2011). Frost (2019) doubted the validity of one such member of the complex, i.e., *P. teraiensis*, and stated that the species may well be a junior synonym of *P. himalayensis*. Recently, Gogoi & Sengupta (2017) revalidated the nomen *P. himalayensis* and in their same work, referred to the north-east Indian population of the *P. leucomystax* complex to *P. teraiensis*. According to these authors, *P. teraiensis* of north-eastern India differs from *P. leucomystax* of Borneo in having larger SVL, longer snout and smaller eyes. They also stated the tibia was longer in *P. teraiensis* from north-eastern India but perhaps their mention that TTA of *P. teraiensis* reaches anterior corner of the eye, whereas it reaches snout in case of *P. leucomystax* may be a lapsus calami. Additionally, *P. leucomystax* differs genetically from *P. teraiensis* by an uncorrected p-distance of 0.123–0.131 (Table 1). Furthermore, the call pattern of *P. leucomystax* occurring in Malaysia Population (MP) in Kedah (<https://doi.org/10.6084/m9.figshare.9768062>) is similar to that of Assam (AP), India (<https://doi.org/10.6084/m9.figshare.9768065>). The typical advertisement call in both cases consists of two notes (that can be syllabilized as “Prak-pok”). The length and number of notes of the first call is 0.187s and 15 in MP and 0.130s and 9 in AP with a mean inter-node period of 0.124 s in MP and 0.144 s in AP. The length of the second call is 0.040 in both cases consisting of three notes. The dominant frequency of the first note was 2174 in MP and 1898 in AP (Fig. 11). Thus, pending further research including molecular and bioacoustics data, we propose to retain *P. leucomystax* as a member of the north-east Indian amphibian fauna.

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APPENDIX I. List of Comparative Material Examined

- Polypedates assamensis* Not preserved (Gibbon Wildlife Sanctuary, Assam, India).
- Polypedates chlorophthalmus* ID 8017 (“Unnamed stream, near Samling Camp at Ravenscourt, 04.05.14.1N; 115.28.42.7E, Lawas, Sarawak; Malaysia; 1,351 m ASL”; holotype).
- Polypedates colletti*. ID 7932 (Gunung Santubong, Sarawak, Malaysia); ID 8094 (Loagan Bunut National Park, Sarawak, Malaysia); ID 8119 (Loagan Bunut National Park, Sarawak, Malaysia); ID 8222 (Gunung Gading National Park, Sarawak, Malaysia); ID 8792 (Camp 1, Gunung Mulu National Park, Sarawak, Malaysia); ID 8769 (Moonmilk Cave trail, Gunung Mulu National Park, Sarawak, Malaysia); DWNP A.0996 (Interpretive Trail, Sungai Relau, Taman Negara, Pahang, Malaysia).
- Polypedates cruciger*: CM 67582, CM 67687 (Sri Lanka); MCZ 20870–72 (Queenswood Estate, Dimbulla, Sri Lanka); ZSI 10016 (Colombo, Sri Lanka); ZSI 10176–79 (four syntypes); type locality not specified in the original description; museum register gives the type locality as Ceylon (= Sri Lanka).
- Polypedates himalayensis* ZSI 16944 and ZSI 16969 (two syntypes of *Rhacophorus maculatus himalayensis* Annandale, 1912), from “Kobo, alt. 4000 ft, Abor Hills” (in Arunachal Pradesh, north-eastern India); ID 9337 (Cherrapunjee Resort, Meghalaya, India); ID 9367 (Nongkhylllem WS, Meghalaya, India)
- Polypedates insularis*. ZSI A8731 (holotype), ZSI A8732–34 (three paratopotypes), (“circa 2 km E mouth of Galathea River, Galathea National Park, Great Nicobar, India”); ZSI A8575 (“Campbell Bay, Great Nicobar, India”); ZSI A8735–36 (two paratypes), (“Shompen Hut, Great Nicobar, India”); ZSI A8737–40 (four paratypes), (“circa 2 km E Kopen Heat (41 km point on the East-West Road), Great Nicobar, India”).
- Polypedates leucomystax* DWNP A0978–80 (Sungai Relau, Taman Negara, Pahang, Malaysia); ID 7599 (Gunung Gading National Park, Sarawak, Malaysia); ID 8096 (Loagan Bunut National Park, Sarawak, Malaysia); ID 8187, ID 8478–79, ID 8853 (Summit trail, > 300 m asl, Kubah National Park, Matang, Sarawak, Malaysia); ID 8786 (Headquarters, Gunung Mulu National Park, Sarawak, Malaysia); ID 8702 (Below Rajawali Lodge 2, Poring, Kinabalu National Park, Sabah, Malaysia); ID 8338–39 (Near Bombalai Chalet, Park HQ, Tawau Hills Park, Sabah, Malaysia).
- Polypedates macrotis* ID 7588 (Gunung Gading National Park, Sarawak, Malaysia); ID 8185–86; 8477 (Kubah National Park,

Matang, Sarawak, Malaysia); ID 8709 (Below Rajawali 1, Poring, Kinabalu National Park, Sabah, Malaysia); ID 8318; ID8353 (Sungei Tawau, Tawau Hills Park, Sabah, Malaysia).

Polypedates maculatus MCZ 451:1–2 (Ambala, Punjab, India); ZSI/SRS VA 257 (Sitteri, Mamarati, Odai, Kalakad Tiger Reserve, Tamil Nadu, India); ZSI/SRS VA 280 (Vayalagam, Pudukkottai, Tamil Nadu, India); USNM 122268 (Asansol, West Bengal, India); MCZ 10158 (Sri Lanka).

Polypedates occidentalis CM 121991 (Charpa Forest Range, Kerala, India; holotype).

Polypedates otlophus ID 8106 (Loagan Bunut National Park, Sarawak, Malaysia); ID 8176 (Kubah National Park, Matang, Sarawak, Malaysia); ID 8177 (Kubah National Park, Matang, Sarawak, Malaysia); ID 8178 (Kubah National Park, Matang, Sarawak, Malaysia); ID 8265 (Matang, Sarawak, Malaysia); ID 8710 (Below Rajawali 1, Poring, Kinabalu National Park, Sabah, Malaysia).

Polypedates pseudocruciger ZSI/SRS VA 1077 (Glenbeck Estates, nr.Nagercoil, Tamil Nadu, India; holotype); BNHM 2863 (Maramai, Tamil Nadu, India; paratype); BNHM 2887 (Coondapur, Karnataka, India; paratype).