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ARTICLE

Indirana chiravasi, a new species of Leaping Frog (Anura: Ranixalidae) from Western Ghats of India

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Abstract: *Indirana chiravasi*, a new species of leaping frog is described from the northern Western Ghats around Amboli, Sindhudurg District, Maharashtra, India. It differs from all its congeners based on a combination of characters including presence of median single internal vocal sac, head longer than wide, distinct canthus rostralis, tympanum 2/3rd to 3/4th the diameter of eye, vomerine teeth in two oblique series at the posterior border of choanae, long midventral lingual papilla, first finger longer than or equal to second, presence of double outer palmer tubercle, thin and elongated inner metatarsal tubercle, absence of outer metatarsal tubercle, webbing moderate, dorsal skin with glandular folds but without warts, ventral skin smooth without mottling and palms and soles dark brown. Molecular analysis based on mitochondrial 12S and 16S genes and nuclear rhodopsin and rag1 genes suggests that the species is genetically distinct from other species for which genetic data is available. Preliminary observations on the development of the species are also provided. We also provide genetic data and images for *Indirana gundia* collected from the type locality.

Keywords: Endemic frogs, *Indirana gundia*, molecular phylogeny, new species, taxonomy.

IUCN RED LIST Guiding Conservation for 50 Years

NOT EVALUATED NE

DATA DEFICIENT DD | LEAST CONCERN LC | NEAR THREATENED NT | VULNERABLE VU | ENDANGERED EN | CRITICALLY ENDANGERED CR | EXTINCT IN THE WILD EW | EXTINCT EX

Indirana chiravasi
Amboli Leaping Frog

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For **Author Details** and **Author Contribution** see end of this article.

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INTRODUCTION

The genus *Indirana* Laurent, 1986, is one of the 10 endemic anuran genera of the Western Ghats of India and the sole member of the family Ranixalidae Dubois, 1987. Genus *Indirana*, which has terrestrial tadpoles, is characterized by the presence of vomerine teeth, mid-ventral lingual papilla, keratodont formula of 3–5/3–4, T or Y-shaped terminal phalanges and absence of terminal intercalary cartilage (Laurent 1986; Frost et al. 2006). Currently, genus *Indirana* comprises of 10 valid species, namely *Indirana beddomii* (Günther, 1876), *I. brachytarsus* (Günther, 1876), *I. diplosticta* (Günther, 1876), *I. leptodactyla* (Boulenger, 1882), *I. phrynoderma* (Boulenger, 1882), *I. semipalmata* (Boulenger, 1882), *I. leithii* (Boulenger, 1888), *I. longicrus* (Rao, 1937), *I. tenuilingua* (Rao, 1937) and *I. gundia* (Dubois, 1986).

The true species diversity of *Indirana* within the Western Ghats, however, is poorly understood as several species are suggested to be in species complexes (Nair et al. 2012a,b; Modak et al. 2014). With the presence of undescribed species (Nair et al. 2012a,b; Modak et al. 2014) and poor knowledge on the distribution of known species (Modak et al. 2014), the knowledge on *Indirana* is plagued by both Linnean and Wallacean shortfalls (Bini et al. 2006). Because several species of *Indirana* are under threatened category (Stuart et al. 2008), taxonomic studies on this group are of immediate concern.

While studying the diversity and distribution of species under the genus *Indirana*, we came across a population showing marked difference in morphology from its congeners and genetically distinct from species for which genetic data is available. This population is described as a new species of *Indirana* from Amboli, in Western Ghats of southern Maharashtra, India.

MATERIALS AND METHODS

Study site and specimen collection

Specimens of the new species were collected from Amboli (15.956^{0}N & 73.997^{0}E; 744m), Sindhudurg District, Maharashtra, India. Five male and two female specimens were collected and preserved in absolute alcohol for further analysis. Two specimen of *Indirana gundia* were collected from non-protected area of Gundia, Karnataka, for genetic analysis. Collection of specimens was kept at the minimum and all collections were made following the IUCN (2008) guidelines for use of threatened species in research.

Museum details

Specimens studied in this paper are deposited in the museum collection of Natural History Museum, London (BMNH); Muséum National de histoire Naturelle, Paris (MNHN), Bombay Natural History Society, Mumbai (BNHS); Wildlife information Liaison Development Society, Coimbatore (WILD) and Zoological Survey of India, Western Regional Center, Pune (ZSI-WRC). Inger et al. (1984) designated BMNH 1947.2.27.92 as a lectotype of *Indirana brachytarsus.* Therefore, we have considered the specimen BMNH 1947.2.2.85, currently a syntype of *I. brachytarsus*, as paralectotype.

Morphometry

Morphometry was done with the help of a digital caliper (Ocean Premium measuring instruments) to the nearest 0.1mm. Totally, 27 characters were selected following Biju et al. (2011) and Dubois & Ohler (1999) with some modifications for morphometry, viz.—SUL (Length of specimen from snout to the visible tip of urostyle); HL (head length: from the posterior border of tympanum to the tip of snout); HW (head width: width of head between to posterior borders of tympanum); SL (Snout Length: from the anterior orbital border to the tip of snout); EL (Eye Length: length of eye between two orbital borders); TYL (maximum tympanum length); UEW (upper eyelid width); SNL (snout to nostril distance); ENL (eye to nostril distance); INL (inter-narial distance); IOL (inter-orbital distance: minimum distance between two eyelids); UAL (Upper arm length); FoAL (Fore-arm Length); F1 to F4 (Finger 1 to Finger 4 length from the base of the sub-articular tubercle); F3D (finger three disc width); F3W (finger three width at the base of disc); THL (thigh length); TL (Tibia/shank length); ACL (Astragalo-calcaneal length); FOL (Foot length: from the base of the inner metatarsal tubercle to the tip of the fourth toe); TFOL (Total foot length: from the tibio-tarsal articulation to the tip of fourth toe) and T1 to T5 (Toe1 to Toe5 length from the base of the respective sub-articular tubercle). Webbing formula was determined following the method provided by Savage & Heyer (1967) with modifications by Myers & Duellman (1982).

Statistical analysis

Statistical analysis of the morphometric data was performed on size adjusted measurements by taking all measurements as percent of SUL. The null hypothesis that the data is multivariate normal was checked using Doornik & Hansen (2008) omnibus. MANOVA/CVA was performed to understand whether related species of *Indirana* form significantly different clusters (Huberty

Table 1. Primers used for molecular study along with melting temperature (Tm) and annealing temperatures (Ta).

Primer	Sequence (5'→ 3')	Tm (°C)	Ta (°C)	Reference
12SF	AAACTGGGATTAGATACCCCACTAT	55.1	56	Simon et al. (1994)
12SR	GAGGGTGACGGGCGGTGTGT	64.8		
16SF	CGCCTGTTTATCAAAAACAT	49.2	50	Palumbi et al. (1991)
16SR	CCGGTCTGAACTCAGATCACGT	58.6		
RhoF	ACCATGAACGGAACAGAAGGYCC	60.4	56	Bossuyt & Milinkovitch (2000)
RhoR	GTAGCGAAGAARCCTTCAAMGTA	54.9		
Rag1F	ATGGGAGATGTGAGTGARAARCA	56.2	54	Biju & Bossuyt 2003
Rag1R	TCCGCTGCATTTCCRATGTCRCA	60.2		

& Olejnik 2006). Pillai's trace statistic was performed to find the significant difference between the clusters (Harris 2001). Statistical analysis was performed in PAST (Hammer et al. 2001).

Molecular analysis

Two specimens of the new species (BNHS 5890 and WILD-14-AMP-489) and two specimens of topotypic *Indirana gundia* (WILD-14-AMP-499 and WILD-14-AMP-500) were used for molecular work. Genomic DNA was extracted from thigh muscle tissue. The tissue was digested at 55^{0}C using STE buffer (50 mM Tris-HCl, 20 mM EDTA and 50µl of 10%SDS) with 10µl of 20mg/ml Proteinase K. RNase treatment was given for two hours at 37^{0}C. Final extraction process was done using phenol-chloroform method. Polymerase Chain Reaction was performed for amplification of two mitochondrial genes (12S and 16S) and two nuclear genes (rho and rag 1) (Table 1). In addition to the DNA extracted in the current study, we also used DNA extracted in a previous study (Modak et al. 2014) for amplification of Rag1 gene from two topotypes of *Indirana leithii* BNHS 5590 and BNHS 5591. PCR reaction was performed in a 25µl reaction volume containing 5µl of template DNA (~200ng), 2.5µl of 10X reaction buffer (100 mM Tris pH 9.0, 500 mM KCl, 15 mM MgCl2, 0.1% Gelatin), 2µl of 25 mM MgCl2, 1µl of 10 mM dNTPs, 1µl of each primer, 1µl Taq polymerase and 16.5µl nuclease free water. The thermal profile was 10 minutes at 95^{0}C, and 35 cycles of 1 minute at 94^{0}C, 1 minute at respective annealing temperature for 12S, 16S, rho and rag1 primers (Table 1) and 2 minutes at 72^{0}C, followed by extension of 10 minutes at 72^{0}C. Amplified DNA fragments were purified using the 'Promega Wizard Gel and PCR clean up' system and sequenced. The purified PCR products were sequenced using ABI prism 3730 sequencer (Applied Biosystems, USA) and Big dye terminator sequencing kit (ABI Prism, USA). Sequences were analyzed by BLAST tool (Altschul et al. 1990). These sequences have been deposited in GenBank (Accession numbers KM386526–KM386543).

Additional sequences of related species were retrieved from NCBI GenBank database (http://www.ncbi.nlm.nih.gov/). GenBank accession numbers for the sequences used for the analysis are provided in Appendix A. Gene sequences were aligned separately using MUSCLE (Edgar 2004) implemented in MEGA 6 (Tamura et al. 2013) and were concatenated to make a combined matrix of 1342 nucleotides. Best fit model for nucleotide substitution was selected in TOPALi v2.5 (Milne et al. 2004) based on minimum Akaike Information Criterion (AIC) value (Posada & Crandall 2001). The phylogenetic relationships were inferred by maximum likelihood analysis using RAxML (Stamatakis 2006) implemented in TOPALi v2.5 (Milne et al. 2004). Reliability of the phylogenetic tree was estimated using bootstrap values run for 1000 iterations.

RESULTS

Indirana chiravasi sp. nov.
(Images 1, 2, 3a–c, 4a–b, 5, 6, 8, 9, 10)

urn:lsid:zoobank.org:act:21DA778F-2F2A-4FD8-9551-85D734A5BB45

Holotype

BNHS 5888, male, 11.vi.2013, 27.3mm SUL, Amboli, Sindhudurg District, Maharashtra, India, coll. Nikhil Modak, Neelesh Dahanukar, Keerthi Krutha and Unmesh Katwate.

Paratypes

BNHS 5889, female, 9.vi.2014, 39.2mm SUL, Amboli, Sindhudurg District, Maharashtra, India, coll. Nikhil Modak; BNHS 5890, male, 11.vi.2013, 25.0mm SUL, Amboli, Sindhudurg District, Maharashtra, India, coll.

Image 1. Holotype (male) of *Indirana chiravasi* sp. nov. (BNHS 5888).

Nikhil Modak, Neelesh Dahanukar, Keerthi Krutha and Unmesh Katwate; WILD-14-AMP-489, male, 11.vi.2013, 24.7mm SUL, Amboli, Sindhudurg District, Maharashtra, India, coll. Nikhil Modak, Neelesh Dahanukar, Keerthi Krutha and Unmesh Katwate; WILD-14-AMP-490, 31.7mm SUL, female, 9.vi.2014, Amboli, Sindhudurg District, Maharashtra, India, coll. Nikhil Modak; WILD-14-AMP-491, male, 19.vi.2013, 25.6mm SUL, Amboli, Sindhudurg District, Maharashtra, India, coll. Nikhil Modak, Neelesh Dahanukar, Keerthi Krutha and Unmesh Katwate; ZSI-WRC A/1541, male, 11.vi.2013, 25.2mm SUL, Amboli, Sindhudurg District, Maharashtra, India, coll. Nikhil Modak, Neelesh Dahanukar, Keerthi Krutha and Unmesh Katwate.

Diagnosis

Indirana chiravasi sp. nov. differs from all other congeners based on the following combination of characters: medium-sized (24.7–39.2 mm SUL) frog, with median single internal vocal sac, head longer than wide, distinct canthus rostralis, tympanum 3/4th the diameter of eye in males and 2/3rd in females, vomerine teeth in two oblique series at the posterior border of choanae, long midventral lingual papilla, first finger longer than or equal to second, presence of double outer palmer tubercle, thin and elongated inner metatarsal tubercle present, outer metatarsal tubercle absent, webbing moderate and differs in male (I1-2II1-3III1¼-3IV3-1¼V) and female (I1-2II1-2½III1¼-3IV3-1¼V) by having reduced webbing on the third toe in males as compared to females, dorsally skin with glandular folds but without warts, ventrally skin smooth without mottling and palms and soles dark brown.

Description

General appearance of holotype as in Image 1 and of female paratype as in Image 2. Morphometric details as in Table 2.

Image 2. Female paratype of *Indirana chiravasi* sp. nov. (BNHS 5889).

Table 2. Morphometric data (mm) of *Indirana chiravasi* sp. nov.

Voucher number	Type status	Gender	SUL	HL	HW	SL	EL	TYL	UEW	SNL	ENL	INL	IOL	UAL	FoAL	F1
BNHS 5888	Holotype	Male	27.3	11.4	9.8	4.8	3.7	2.8	2.0	2.0	2.7	2.6	2.6	5.3	6.0	2.4
WILD-14-AMP-489*	Paratype	Male	24.7	10.5	8.9	4.8	3.3	2.5	2.1	1.6	2.9	2.5	3.1	3.7	5.7	1.9
BNHS 5890*	Paratype	Male	25.0	10.8	9.0	4.7	3.1	2.3	1.8	1.7	2.7	2.0	2.9	3.3	5.4	1.9
ZSI-WRC A/1541	Paratype	Male	25.2	11.0	9.2	4.8	3.2	2.4	2.1	1.9	2.9	2.4	2.5	4.7	5.5	2.0
WILD-14-AMP-491	Paratype	Male	25.6	11.5	9.3	5.0	3.1	2.6	1.7	1.7	2.7	2.7	2.9	4.0	5.3	2.0
BNHS 5889	Paratype	Female	39.2	14.9	13.9	6.5	5.0	3.1	2.9	2.6	3.9	4.1	4.2	7.1	8.4	3.5
WILD-14-AMP-490	Paratype	Female	31.7	12.7	11.1	5.3	3.3	3.0	1.8	2.0	3.3	3.6	3.2	4.7	6.6	2.2

Voucher number	Type status	Gender	F2	F3	F4	THL	TL	ACL	FOL	TFOL	T1	T2	T3	T4	T5
BNHS 5888	Holotype	Male	1.9	3.5	2.6	13.4	13.9	6.4	12.4	18.0	1.6	2.7	4.2	6.5	4.2
WILD-14-AMP-489*	Paratype	Male	1.8	3.1	2.3	12.0	13.3	5.9	12.1	16.8	1.9	2.3	3.8	6.1	3.2
BNHS 5890*	Paratype	Male	1.9	3.3	1.9	12.6	13.0	5.9	12.2	18.2	1.8	2.1	4.3	6.8	3.7
ZSI-WRC A/1541	Paratype	Male	2.0	3.6	2.6	11.2	13.3	6.3	12.5	17.2	2.1	2.9	4.5	7.3	4.3
WILD-14-AMP-491	Paratype	Male	1.8	3.4	2.6	13.5	15.0	6.4	13.5	20.1	2.1	2.7	4.3	7.5	4.4
BNHS 5889	Paratype	Female	3.1	5.6	4.1	20.0	23.2	11.4	20.7	30.7	2.9	4.7	7.4	12.8	7.4
WILD-14-AMP-490	Paratype	Female	2.2	4.1	3.7	17.0	17.6	9.6	15.2	21.1	1.7	4.6	5.4	8.6	5.5

* used for genetic analysis.

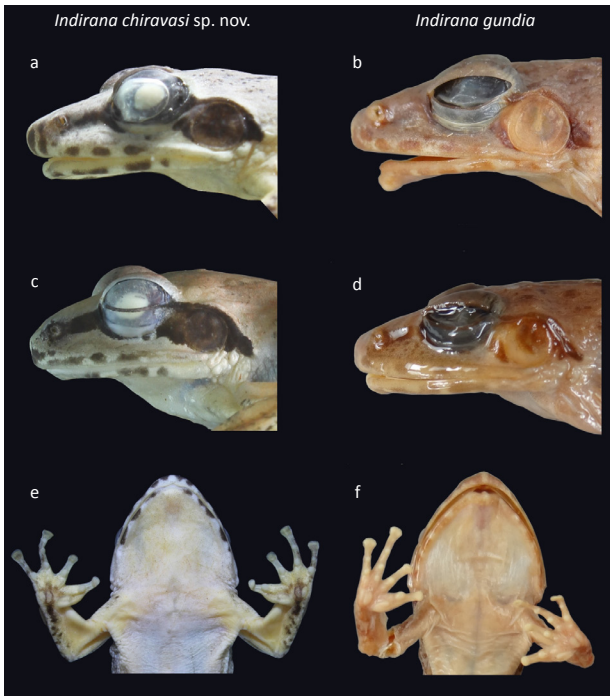

Image 3. Lateral and ventral view of head of *Indirana chiravasi* sp. nov.: (a) - holotype (BNHS 5888), (b) - female paratype (BNHS 5889), (c) - holotype (BNHS 5888); *Indirana gundia*: (d) - (male) holotype (MNHN 1985.0633), (e) - (female) paratype (MNHN 1985.0622), (f) - (male) holotype (MNHN 1985.0633). Photo credit: (a-c) Neelesh Dahanukar and (d-f) Nikhil Modak.

Description of the holotype (BNHS 5888; male) (all measurements in mm)

Medium-sized (SUL 27.3); head longer than wide (HL 11.4 > HW 9.8); snout longer than horizontal diameter of eye (SL 4.8 > EL 3.7); pupil horizontal; outline of snout rounded in shape dorsally (Image 1a), truncated laterally (Image 3a); ventrally snout protruding beyond the mouth (Image 3c); nostrils nearer to snout than to the eye (SNL 2.0 < ENL 2.7); tympanum about 3/4th the diameter of eye, very close to eye; supra-tympanic fold distinct; upper eyelid width slightly more than half the horizontal diameter of eye; upper eyelids bear very few granulations; inter-narial distance almost equal to the inter-orbital distance (INL 2.6 ≈ IOL 2.6); canthus rostralis obtuse; loreal region slightly concave and oblique; vomerine teeth in two slightly oblique rows at the posterior border of choanae; tongue thin, bifid; bear a long mid ventral papilla.

Upper arm shorter than fore arm (UAL 5.3 < FoAL 6.0); hand long (PAL 6.7); finger lengths from shortest to longest – F2 (1.9) < F1 (2.4) < F4 (2.6) < F3 (3.5); palmer tubercles present, outer palmer tubercle double, subarticular tubercles moderate, supernumerary tubercles present, single; nuptial pads on the sides of first finger, distinct, flat, granular; finger discs moderate

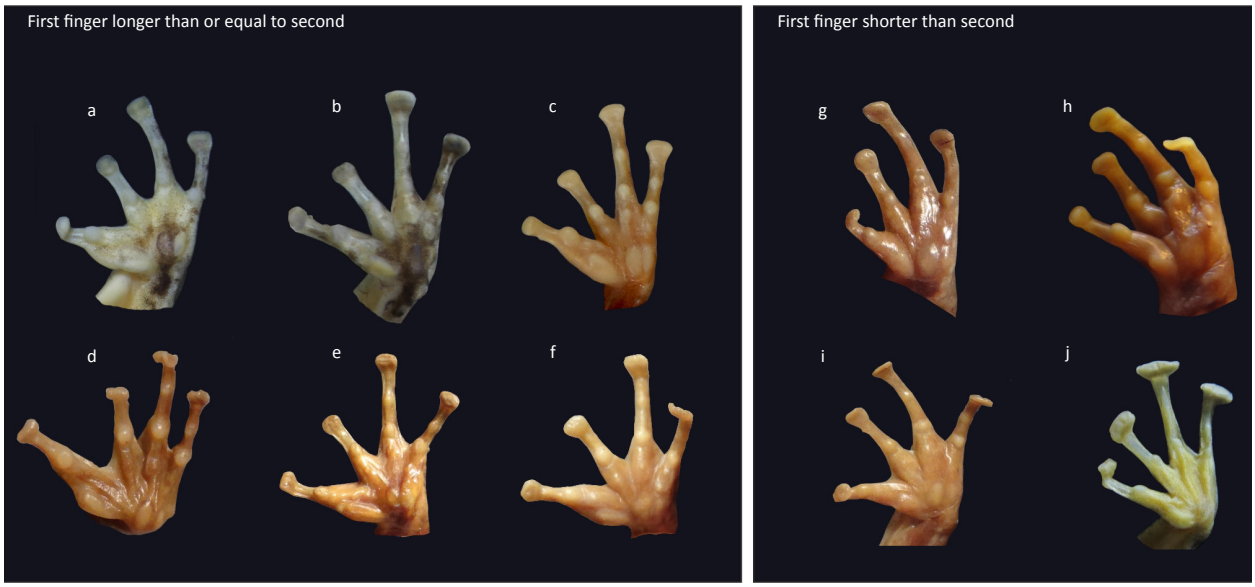

Image 4. Palms of different species of *Indirana* showing the distinction between first finger longer than or equal to second and first finger shorter than second. (a) *Indirana chiravasi* sp. nov. holotype, (b) *I. chiravasi* sp. nov. female paratype, (c) *I. gundia* Holotype (MNHN 1985.0633), (d) *I. semipalamata* syntype (BMNH 1947.2.29.50), (e) *I. beddomii* syntype (BMNH 1947.2.27.72), (f) *I. brachytarsus* Paralectotype (BMNH 1947.2.2.85), (g) *I. diplosticta* Syntype (BMNH 1947.2.2.21), (h) *I. phrynoderma* Syntype (BMNH 1947.2.3.8), (i) *I. leptodactyla* syntype (BMNH 1947.2.29.40) and (J) *I. leithii* topotype (uncataloged). Photo credit: (a-b) Neelesh Dahanukar, (c-i) Nikhil Modak and (j) Anand Padhye.

Image 5. Foot of (a) *Indirana chiravasi* sp. nov. holotype and (b) *I. chiravasi* sp. nov. female paratype.

in shape, broad, truncate, bearing semicircular groove; fingers without web or fringe of skin (Image 4a).

Hindlimbs long; thigh shorter than shank (tibia) (THL 13.4 < TL 13.9); thigh bearing distinct femoral glands, occupying posterior-ventral side of thigh; astragalus-calcaneum about half the length of foot; total foot length (including astragalus-calcaneum) longer than tibia (TFOL 18.0); toe lengths from shortest to longest are- T1 (1.6) < T2 (2.7) < T5 (4.2) ≤ T3 (4.2) < T4 (6.5); toe discs moderate; bear semicircular groove; inner metatarsal tubercle thin, elongated and shovel shaped; outer metatarsal tubercle absent; supernumerary tubercles absent; subarticular tubercles moderate; tarsal fold and outer phalangeal fringe absent; webbing formula I1-2II1-3III1¼-3IV3-1¼V (Image 5a).

Dorsal and ventral skin smooth; few longitudinal folds on dorsal side; lateral side granular.

Description of female paratype (BNHS 5889) (all measurements in mm)

Medium-sized (SUL 39.2); with head longer than wide (HL 14.9 > HW 13.9); snout longer than eye (SL 6.5 > EL 5.0); outline of snout rounded in shape dorsally (Image 2a); truncated laterally (Image 3b); ventrally protruding beyond the mouth (Image 2b); nostrils nearer to snout than to the eye (SNL 2.6 < ENL 3.9); tympanum about 2/3rd the diameter of eye, slightly more apart from eye than in male; supra-tympanic fold distinct; upper eyelid width slightly more than half the horizontal diameter of eye; upper eyelid bearing very few granulations; inter-narial width slightly narrower than inter-orbital distance (INL 4.1 < IOL 4.2); canthus rostralis obtuse; loreal region slightly concave and oblique; vomerine teeth in two slightly oblique rows at the posterior border of choanae; tongue thin, bifid; bearing a long mid-ventral papilla.

Upper arm shorter than fore arm (UAL 7.1 < FoAL 8.4); hand about 1/5th of SUL; finger lengths from shortest to longest - F2 (3.1) < F1 (3.5) < F4 (4.1) < F3 (5.6); palmer tubercles present, outer palmar tubercle double; subarticular tubercles moderate; all supernumerary tubercles present, single; finger discs moderate in shape, broad, truncate, bearing semicircular groove; fingers without web or fringe of skin (Image 4b).

Hindlimb long, thigh shorter than tibia (THL 20.0 < TL 23.2); astragalus-calcaneum about half the length of foot; total foot length (including astragalus-calcaneum) longer than tibia; toe lengths in order of T1 (2.9) < T2 (4.7) < T5 (7.4) ≤ T3 (7.4) < T4 (12.8); toe discs moderate; bear semicircular groove; inner metatarsal tubercle thin, long; outer metatarsal tubercle absent; supernumerary tubercles absent; subarticular tubercles moderate; tarsal fold and outer phalangeal fringe absent; webbing formula I1-2II1-2½III1¼-3IV3-1¼V (Image 5b).

Dorsal and ventral skin smooth; few longitudinal folds on dorsal side; lateral side granular.

Colouration

Male: Dorsal colour grey to brown in preservation (Image 1) and olive brown with scattered yellow markings in living specimen (Image 6) with W-shaped marking comprising of densely organized black spots; black strip running from tip of snout to shoulder through eye and tympanum; creamy white band across head between upper eyelids usually present followed posteriorly by a dark band; in life, olive green to brown band running from above shoulder to groin (Image 6); forelimb bearing transverse bands also on fingers; palm dark brown in colour at least in live condition; brown bars on thigh, tibia, outer side of foot and dorsal surface of toes; ventral and inner side of foot dark brown in colour up to tibiotarsal articulation; ventrally white with few melanophores visible only under magnification.

Female: General appearance as in male. Dorsally more pale than male without W-shaped marking (Image 2); darker flanks; limbs with dark cross bars in life, faded in preservation.

Sexual Dimorphism

Tympanum about 3/4th the diameter of eye in male and 2/3rd the diameter of eye in female. Tympanum very close to eye in male and slightly farther apart in female

Image 6. *Indirana chiravasi* sp. nov. in life (a) paratype (BNHS 5890) and (b) paratype (WILD-14-AMP-491).

(Image 3). Inner metatarsal tubercle thin and shovel-shaped in male while slightly thicker and long in female (Image 5). Webbing formula I1-2II1-3III1¼-3IV3-1¼V in male and I1-2II1-2½III1¼-3IV3-1¼V in female. Breeding males show single internal vocal sac that is visible only during calling (Appendix B); bearing nuptial pad on the outer side of first finger and femoral glands on thighs; mature eggs visible from transparent latero-ventral skin of breeding females.

Etymology

The specific epithet, a combination of words 'chir' (singular) or 'chira' (plural) which means crevice or crevices in Marathi and 'vasi' in Sanskrit means 'inhabitant of', which emphasizes crevice dwelling habit of this species. The specific name is noun in apposition.

Distribution

The species is currently known only from its type locality at Amboli (15.956^{0}N & 73.997^{0}E; 744m), which is a small hill station in the southwestern Maharashtra, India (Fig. 1).

Habitat

The species occupies lateritic rocky outcrops (Image 7a). It is often found in the crevices of the laterite boulders (Image 7b). Males were mostly seen while calling from the wet rocks or boulders covered with mosses. Females were collected from under the log in the forest and from under the roadside stone.

Natural history and description of tadpoles

Eggs were seen laid under the mosses on lateritic wet rocks and boulders (Image 8). Hatchlings remain at the egg laying site (Image 9a). Embryos, hatchlings and tadpoles of two different stages were observed in the same habitat. Unhatched eggs (Images 8a,b) show the embryos with external gills, parallel to stage 20 (Gosner 1960). Tadpoles were seen on wet boulders feeding on algal matter (Image 9). Image 9a shows hatchlings, parallel to stage 25 (Gosner 1960). Image 9b shows tadpole in its terrestrial habitat with fully developed hind limbs (without forelimb), parallel to stage 40 (Gosner 1960). Image 9c shows terrestrial tadpoles with long, finless tail which is not under resorption, oral apparatus and fully developed forelimbs as well as hind limbs, parallel to stage 42 (Gosner 1960). Image 9d shows stage 44 (Gosner 1960) with mouth beneath eye and greatly reduced tail, while image 9e shows stage 46 (Gosner 1960) - a completely metamorphosed froglet.

Tadpoles showed semi-condensed individual keratodont formula (Dubois 1994) as $4[A_1\text{-}A_4]/4[P_1\text{-}P_2]$. The oral apparatus is divided into two lateral parts by large horny beak. The first anterior keratodont ridge A_1 is divided while three succeeding anterior keratodont ridges A_2–A_4 are placed lateral to the horny beak. On the posterior labia first keratodont ridge P_1 is marginal, present on the either sides, while the second one - P_2 is placed lateral to the horny beak. Third and fourth keratodont ridges - P_3 and P_4 are continuous. Although, the ridge P_3 appears to be divided into four subunits the keratodont rows are continuous on it (Image 10).

Common name

Amboli Leaping Frog.

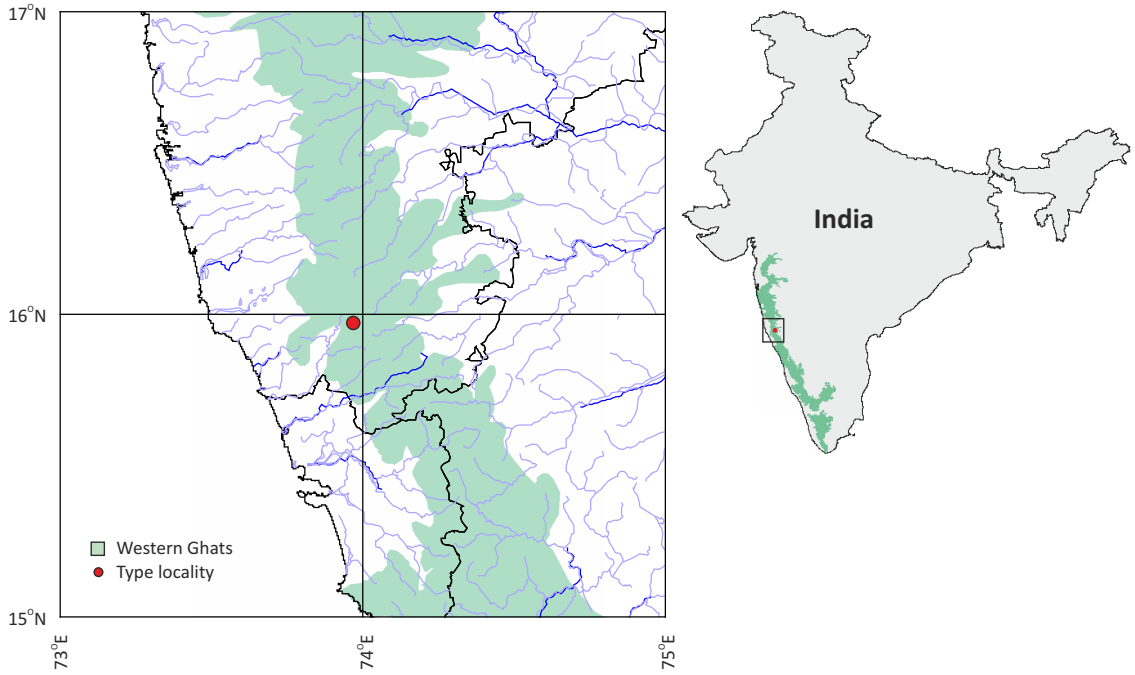

Figure 1. Type locality of *Indirana chiravasi* sp. nov.

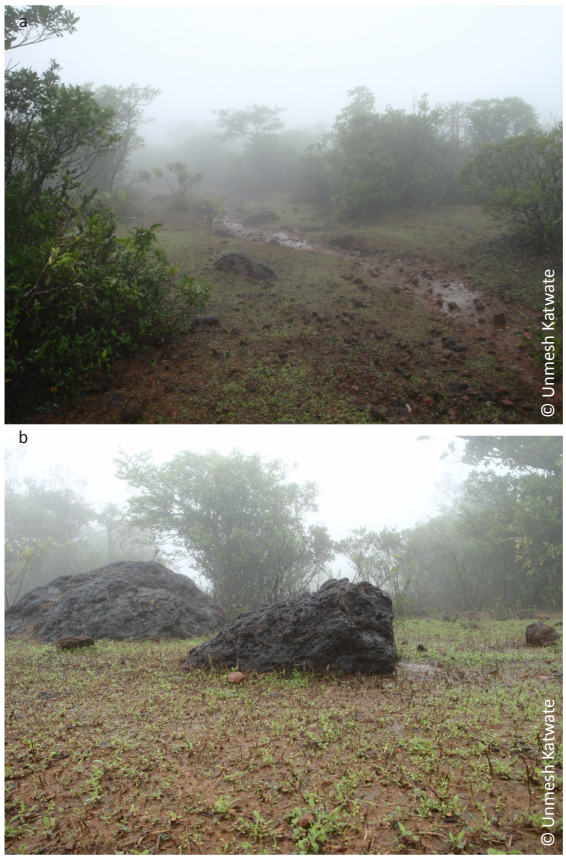

Image 7. Habitat at type locality of *Indirana chiravasi* sp. nov. during monsoon season. (a) General habitat on lateritic plateau and (b) lateritic rocks where adults, eggs and tadpoles are found in crevices and moss covered surfaces.

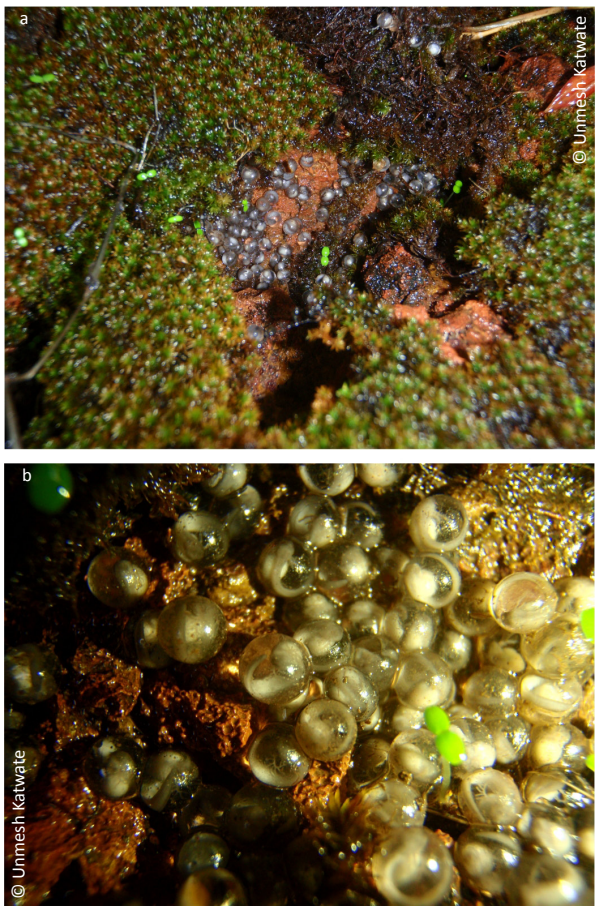

Image 8. Eggs of *Indirana chiravasi* sp. nov. laid in the moss on a lateritic rock.

Image 9. *Indirana chiravasi* sp. nov. hatchlings and egg (a), early phase tadpoles without forelimb (b), late phase tadpoles with fore limbs (c) and different stages in tail regression and completion of metamorphosis (d–e). Photo credit: (a, b) Abhijeet Bayani and (c–e) Avishkar Munje.

Statistical analysis

Size corrected morphometric data was not significantly different from multivariate normal (Doornik & Hansen omnibus, Ep = 67.14, P = 0.0561). MANOVA suggested that all species formed significantly distinct clusters (Pillai's trace = 5.001, $F_{208,216}$ = 1.86, P < 0.0001). First three canonical axes explained 86.81% of the total variation in the data where the first axis explained 43.89%, second axis explained 25.39% and third axis explained 17.54% of the total variation. First two canonical axes separated *Indirana chiravasi* sp. nov. from *I. beddomii*, *I. diplosticta*, *I. leithii*, *I. leptodactyla*

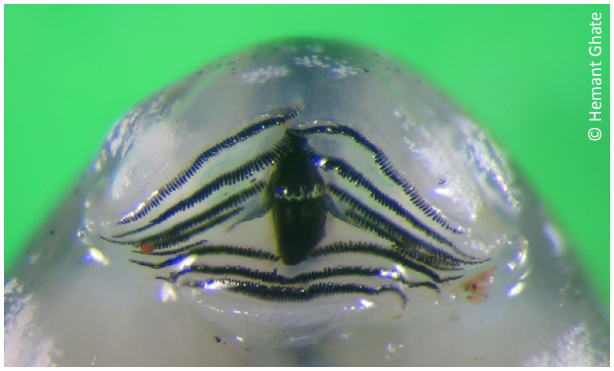

Image 10. Oral disc of *Indiana chiravasi* sp. nov. tadpole.

and *I. phrynoderma* (Fig. 2a). *Indirana chiravasi* sp. nov. was separated from *I. brachytarsus*, *I. gundia* and *I. semipalmata* on the third canonical axis (Fig. 2b). CVA loadings of morphometric characters on the first three canonical axes are shown in Table 3. Characters such as ENL, INL, F3, F4, TL, ACL, FOL, T1 and T2 separated *Indirana chiravasi* sp. nov. from other related species.

Table 3. CVA loadings of morphometric characters on the first three canonical axis.

	Axis 1	Axis 2	Axis 3
HL	-0.030	-0.320	-0.330
HW	-0.220	-0.120	-0.190
SL	0.031	-0.090	-0.190
EL	-0.040	-0.200	-0.040
TYL	0.122	-0.400	-0.180
UEW	-0.050	0.042	0.042
SNL	-0.100	-0.030	0.072
ENL	0.069	-0.030	-0.200
INL	-0.200	0.017	0.079
IOL	-0.120	-0.060	-0.180
UAL	-0.350	0.181	-0.020
FoAL	-0.100	0.049	-0.210
F1	0.167	0.144	-0.110
F2	0.095	0.313	0.168
F3	0.135	0.345	-0.090
F4	0.109	0.331	0.061
THL	-0.360	1.076	-0.410
TL	-0.430	1.351	-0.470
ACL	-0.330	0.680	-0.280
FOL	-0.150	1.387	-0.540
TFOL	-0.460	2.016	-0.810
T1	0.132	0.222	-0.020
T2	0.033	0.292	-0.150
T3	-0.010	0.521	-0.180
T4	0.017	0.914	-0.380
T5	0.178	0.543	-0.150

Genetic analysis

Concatenated genetic sequences of mitochondrial 12S and 16S genes and nuclear rho and rag1 genes had total 1342 bases. Best fit model for the nucleotide substitution was general time reversal model with gamma distribution and invariant sites (GTR+G+I, AIC = 10516.66, lnL = -5157.33, G = 0.39, I = 0.34, *df* = 101, n = 1342). Maximum likelihood analysis of the genetic data (Fig. 3) suggested that *Indirana chiravasi* sp. nov. formed a monophyletic group genetically distinct from the other *Indirana* species for which genetic data are available.

Comparison with other species of *Indirana*

Indirana chiravasi differs from *I. diplosticta*, *I. leithii*, *I. leptodactyla*, *I. longicrus* and *I. phrynoderma* in having first finger equal to or longer than second finger (vs. first finger shorter than second) (Image 4). Furthermore, *I. chiravasi* has moderate webbing (I1-2II1-3III1¼-3IV3-1¼V*) vs. reduced webbing (I2-2II2-3III2½-4IV4-2½V in *I. diplosticta*; I1-2II2-3III3-4IV4-3V in *I. phrynoderma* and I1-2¼II2-3III3-4IV4-3V in *I. leptodactyla*); distinct canthus rostralis (vs. indistinct canthus rostralis in *I. phrynoderma*); smooth skin with glandular folds (vs. warty skin in *I. phrynoderma*), presence of double outer palmer tubercle (vs. single outer palmer tubercle in *I. leithii*); vomerine teeth in two oblique series at the posterior border of choanae and long midventral lingual papilla (vs. vomerine teeth none and tongue without papilla in *I. longicrus*) and tympanum 2/3rd (in female) to 3/4th (in male) the diameter of eye (vs. tympanum half the diameter of eye in *I. longicrus*). Raw genetic distance between *I. chiravasi* and *I. leptodactyla* (as identified by Nair et al. 2012b) is 23.9% for 16S gene and 12.3–12.7 % for all the genes together; between *I. chiravasi* and *I. diplosticta* (as identified by Nair et al. 2012b) is 9.1–11.0 % for 16S gene and 4.2–4.7 % for all the genes together; and between *I. chiravasi* and *I. leithii* (topotypic material from Modak et al. 2014) is 12.5–12.8 % for 16S gene and 6.9–7.3 % for all the genes together.

The whereabouts of the type specimen of *Indirana tenuilingua* described by are not known and is suggested to be lost (Dubois 1984). Therefore, for the comparison of the new species with *I. tenuilingua*, we have relied on the original description by Rao (1937). *Indirana chiravasi* differs from *I. tenuilingua* in having head longer than broad (vs. head slightly wider than long), inter-orbital

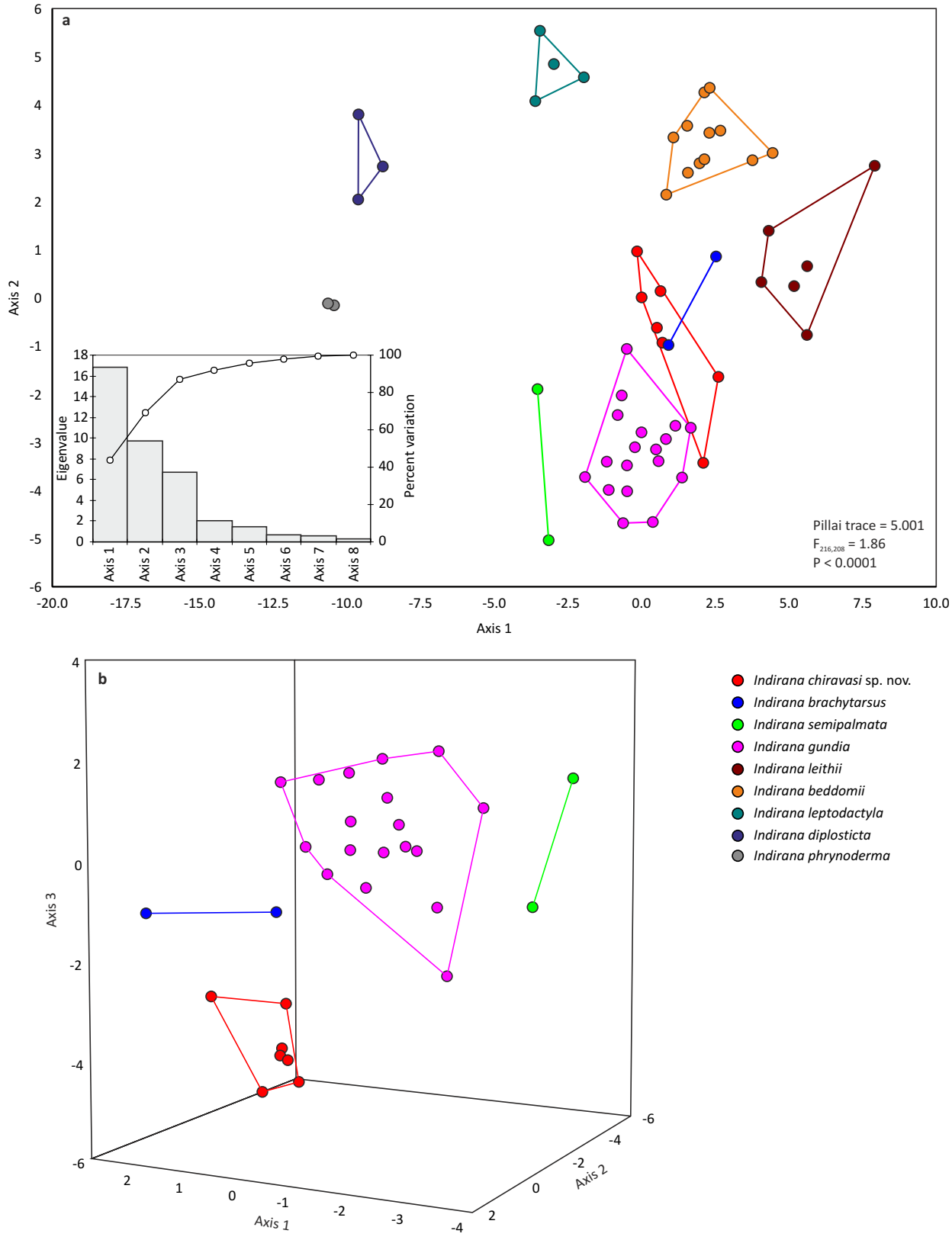

Figure 2. Multivariate Analysis of Variance – Canonical Variates Analysis (MANOVA-CVA) of morphometric data of *Indirana* species. (a) CVA on first two canonical axes, scree plot is shown in the inset, and (b) CVA on first three canonical axes for morphometrically closely related species to *Indirana chiravasi* sp. nov.

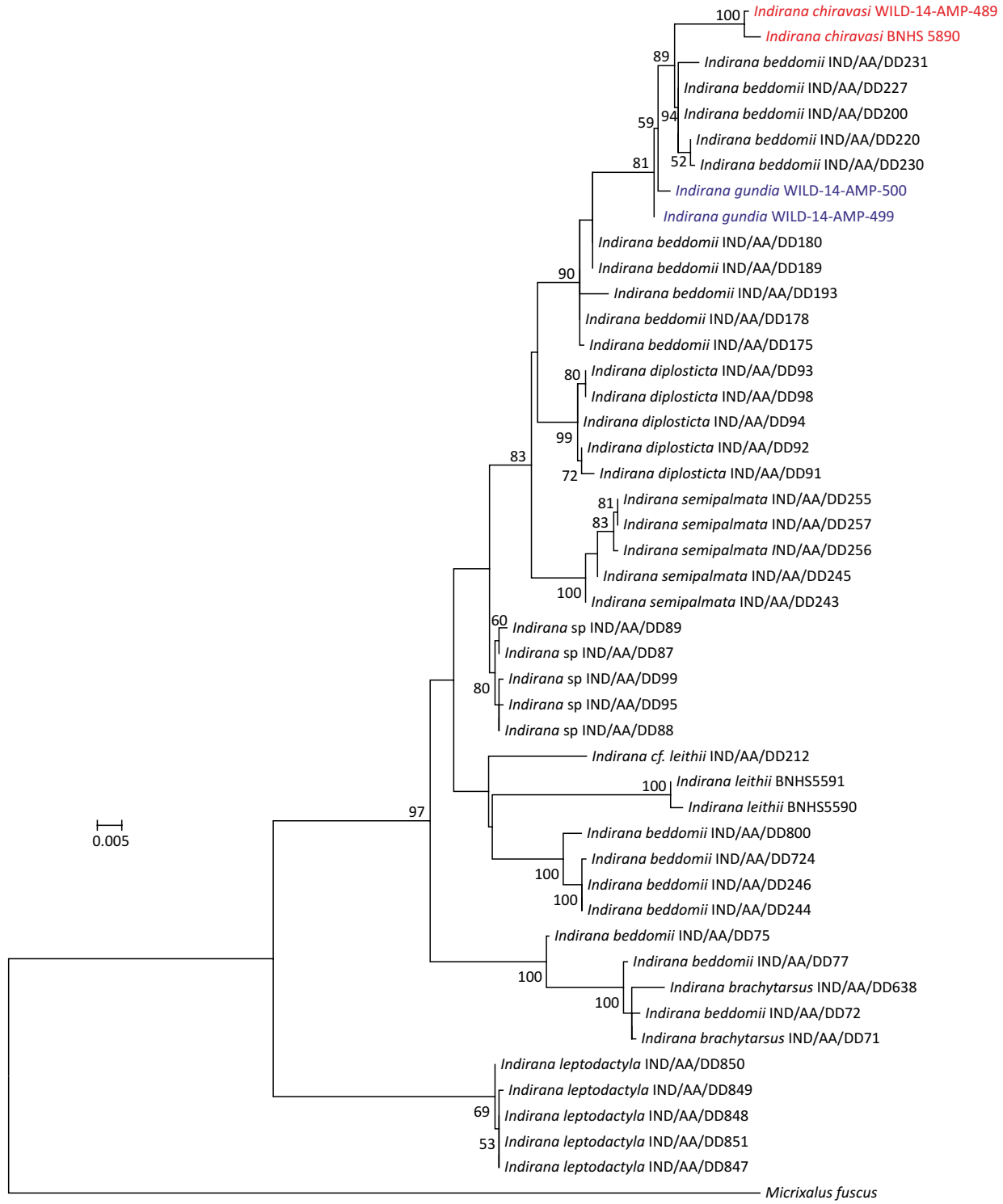

Figure 3. Maximum likelihood phylogenetic analysis of *Indirana* based on mitochondrial 12S and 16S genes and nuclear rho and rag1 genes. Best fit model for the nucleotide substitution was general time reversal model with gamma distribution and invariant sites (GTR+G+I, AIC = 10516.66, lnL = -5157.33, G = 0.385, I = 0.340). There were total 1342 bases in final matrix. *Micrixalus fuscus* is used as an outgroup. Numbers at the node are percent bootstrap values for 1000 iterations.

distance equal to or wider than inter-narial distance (vs. interorbital width more than twice the distance between the nostrils) and toes and fingers with deep semicircular groove (vs. semicircular groove in front of the toes and fingers absent, faint or indistinct).

Indirana chiravasi differs from *I. semipalmata* in having broader head (35.2–36.4 % SUL vs. 33.6–33.7 % SUL), tympanum 2/3rd (in female) to 3/4th (in male) the diameter of eye (vs. tympanum equal to or larger than eye in male and slightly smaller in female) and moderately webbed toes with the webbing formula I1-2II1-3III1¼-3IV3-1¼V in male and I1-2II1-2½III1¼-3IV3-1¼V in female (vs. half webbed toes with the webbing formula I2-2II2-3III2-3¼IV3¼-2V I both the sexes). Raw genetic distance between *I. chiravasi* and *I. semipalmata* (as identified by Nair et al. 2012b) is 7.4–8.4 % for 16S gene and 4.1–4.3 % for all the genes together.

Indirana chiravasi differs from *I. beddomii* in having smaller finger 2 length (7.0–7.9 % SUL vs. 8.3–10.5 % SUL), webbing formula I1-2II1-3III1¼-3IV3-1¼V in male and I1-2II1-2½III1¼-3IV3-1¼V in female (vs. webbing formula, I1-2II1-2III1-3IV3-1V, in both males and females) and long, thin, shovel-shaped inner metatarsal tubercle (vs. long and stout inner metatarsal tubercle). Genetic difference between *I. chiravasi* and *I. beddomii* cannot be determined as Nair et al. (2012b) have considered several genetically distinct populations as members of *I. beddomii* species complex.

Indirana chiravasi can be distinguished from *I. brachytarsus* in having few longitudinal folds on dorsal side (vs. numerous longitudinal folds on dorsal side), thin, shovel-shaped inner metatarsal tubercle (vs. long and stout inner metatarsal tubercle) moderate webbing (vs. extensive webbing, webbing formula, I1-2II1-21/2III1-3IV3-1V) and having broader head (35.2-36.4% SUL vs. 33.1-34.6% SUL). Furthermore, if the identification of *I. brachytarsus* by Nair et al. (2012b) is correct then the two species are also genetically quite distinct (Fig. 3). Raw genetic distance between *I. chiravasi* sp. nov. and *I. brachytarsus* (as identified by Nair et al. 2012b) is 11.6% for 16S gene and 8.0–8.3 % for all the genes together.

Indirana chiravasi sp. nov. differs from *I. gundia* in the most prominent feature of having a single internal vocal sac (vs. bilateral vocal sacs). The presence of bilateral vocal sac in *I. gundia* not only appears in the original description (Dubois 1986) but they can also be seen in two patches on the ventral side of the head (Image 3f). Furthermore, *I. chiravasi* sp. nov. differs from *I. gundia* in having the webbing formula I1-2II1-3III1¼-3IV3-1¼V in male and I1-2II1-2½III1¼-3IV3-1¼V in female (vs. webbing formula, I1-2II1-2½2III1-3IV3-1V, in both males and females) and inner metatarsal tubercle thin shovel-shaped (vs. distinct and stout). Morphometrically (Fig. 2) both the species form significantly distinct clusters (Fisher's distance = 3.142, P = 0.004). Raw genetic distance between *I. chiravasi* sp. nov. and *I. gundia* is 5.2–5.5 % for 16S gene and 2.8–3.1% for all the genes together.

DISCUSSION

Indirana chiravasi sp. nov. forms the eleventh species in the Western Ghats endemic genera *Indirana* and monotypic family Ranixalidae. Phylogenetic analysis of *Indirana* species based on two mitochondrial and two nuclear genes suggests that, *I. chiravasi* forms a monophyletic group with topotypic *I. gundia* and five specimens (IND/AA/DD/CC 200, 220, 227, 230 and 231) of *I. beddomii* species complex (as identified by Nair et al. 2012b).

Both morphologically and genetically, *Indirana gundia* is one of the close congeners of *I. chiravasi*. However, apart from the morphological variations, there was a significant difference in the multivariate morphometric analysis of the two species. Further, the two species are separated by a genetic difference of 5.2–5.5 % for 16S gene. Based on the suggestions of Vences et al. (2005) the high genetic divergence in 16S gene validates that *I. chiravasi* is a distinct species. Further, based on the current distribution records, these two species are separated by a geographical distance of more than 400km. Genetic data for *I. gundia* is provided for the first time in the current study. This data is based on topotypic material (Image 11), for which the species identity is confirmed by its morphological comparison with type series as well as morphometric analysis. The two specimens of *I. gundia* did not form a monophyletic group, although there was only 0.3% genetic distance considering all genes together and 0.2% genetic distance in 16S gene. This could be attributed to small sample size (only two specimens) in the current study and further genetic analysis with additional samples may reveal within species variation in *I. gundia*.

Based on the phylogenetic analysis (Fig. 3) five specimens (IND/AA/DD/CC 200, 220, 227, 230 and 231) of *Indirana beddomii* species complex (as identified by Nair et al. 2012b) form a monophyletic group and fall between *I. chiravasi* and *I. gundia*. This cluster is separated from *I. chiravasi* by raw genetic distance of 3.5–3.9 % and from *I. gundia* by 3.6–3.9 % in 16S gene. However, because of the lack of information

Image 11. Topotypic *Indirana gundia* male (a-e) and female (f-j) in life. WILD-14-AMP-499 (Male) and WILD-14-AMP-500 (female), Gundia, Karnataka.

on the collection locality as well as details of museum deposition, we could not compare these specimens with *I. chiravasi*. Nevertheless, it should be noted that both *I. chiravasi* and *I. gundia* are morphologically distinct from any of the syntypes of *I. beddomii*.

Despite of the above mentioned lacunae, Nair et al. (2012b) have provided genetic data for a wide distribution of *Indirana* populations in southern Karnataka, Kerala and Tamil Nadu. Further, Modak et al. (2014) have provided details for *Indirana leithii* based on topotypic material and a wide distribution in the northern Western Ghats. As a result genetic data from Nair et al. (2012b) and Modak et al. (2014) forms a good comparative material for assessing the affinities between *I. chiravasi* and other populations of *Indirana* sp. nov. from a wide geographic range within the Western Ghats.

Recent documentation of primitive breeding in *Indirana* species from Amboli by Gaitonde & Giri (2014) actually refers to the breeding biology of *I. chiravasi* based on the photographs provided by Gaitonde & Giri (2014).

Further, according to our extensive survey records, there is no other species of *Indirana* present in Amboli. Gaitonde & Giri (2014) have provided a detailed account of courtship and breeding bahaviour of the species along with the fertilization success. However, they do not provide the information on developmental stages.

Our observations, therefore, complete the information on breeding and developmental cycle of the species. Although we have provided developmental stages of this species parallel to the stages suggested by Gosner (1960), it is essential to note that the development and metamorphosis of the genus *Indirana* is different from the general ranid pattern, as the tadpoles do not have tail fins and are semi-terrestrial (Dubois 1986; Gaitonde & Giri 2014; Modak et al. 2014). The developmental stages of *Indirana chiravasi* sp. nov. differs from the stages in Gosner (1960) as follows. The unhatched eggs with embryo of *I. chiravasi* sp. nov. showed presence of external gills. This stage is parallel to stage 20 of Gosner (1960). However, hatching does not occur at this stage. It should be noted that in *I. chiravasi*, hatching does not occur until the development of operculum, which is a stage parallel to stage 25 of Gosner (1960). Further, the semi terrestrial tadpole with oral apparatus, full tail without any regression and fully developed forelimb (stage 42 of Gosner 1960) persists for a long duration. In *I. chiravasi*, tadpole of this stage continues to feed in its terrestrial habitat for around a month after which metamorphosis completes. This is unlike the classical metamorphosis described by Gosner (1960) where in on complete development of fore limb there is onset of metamorphosis with changes such as the beginning of tail regression, oral apparatus starts disappearing leading to formation of adult like mouth and metamorphosis is essentially completed within a short duration. These subtle differences in the development of *I. chiravasi* from the pattern provided by Gosner (1960), calls for the detailed study of developmental patterns in Western Ghats endemic genera such as *Indirana*.

Description of the new species *Indirana chiravasi*, and previous suggestions that there are species complexes and undescribed species (Nair et al. 2012a, 2012b; Modak et al. 2014), suggests that especially for an endemic and monotypic family such as Ranixalidae, the Western Ghats are subject to Linnean shortfall (Bini et al. 2006). Out of the 10 known species of this genus, *Indirana gundia* and *I. phrynoderma* are Critically Endangered; *I. brachytarsus*, *I. leptodactyla* and *I. diplosticta* are Endangered; *I. leithii* is Vulnerable; *I. longicrus* and *I. tenuilingua* are Data Deficient; and *I. beddomii* and *I. semipalmata* are Least Concern (IUCN 2014). With high proportion of threatened species within this endemic family, there is immediate concern for conservation initiatives. Therefore, further studies to resolve taxonomic status of species complexes and understanding their distribution patters are essential. Moreover, additional information on ecology and natural history would help in designing conservation strategies.

Comparative Material

Indirana beddomii: Syntype, BMNH 1947.2.27.72 (Female), Syntype, BMNH 1947.2.27.82 (female), Syntype, BMNH 1947.2.27.83 (Male), Syntype, BMNH 1947.2.27.85 (female), 4 exs., Malabar, coll. Col. Beddome; Syntypes, BMNH 1947.2.27.89–91 (females), 3 exs. Anamallays (=Annamalai), coll. Col. Beddome; Syntype, BMNH 1947.2.4.86 and 87 (females), BMNH 1947.2.4.88 (Male), 3 exs., Sevagherry (=Sivagiri, Tamil Nadu), coll. Col. Beddome; Syntype, BMNH 1947.2.27.87 (female), Syntype BMNH 1947.2.27.88 (Male), 2 exs., Travancore, coll. Col. Beddome.

Indirana brachytarsus: Lectotype, BMNH 1947.2.27.92 (Female), 1 ex., Anamallays (=Annamalai), coll. Col. Beddome; Paralectotype, BMNH 1947.2.2.85 (female), 1 ex., Sevagherry (=Sivagiri, Tamil Nadu), coll. Col. Beddome.

Indirana diplosticta: Syntypes, BMNH 1947.2.2.21 and 23 (females), 2 exs., Malabar, coll. Col. Beddome;

Syntype, BMNH 1947.2.2.22 (male), 1 ex., Malabar, coll. Col. Beddome.

Indirana gundia: Holotype, MNHN 1985.0633 (Male), 26.vii.1984, 1 ex., Gundia, forêt de Kemphole, à l'ouest de Sakleshpur, Karnataka, Inde (Gundia, Kemphole forest, west of Sakleshpur, Karnataka, India), coll. A. Dubois; Paratypes, MNHN 1985.0596 (male), 24.vii.1984, 1 ex., MNHN 1985.0599, MNHN 1985.0603, MNHN 1985.0605, MNHN 1985.0608, 1985.0610 and MNHN 1985.0628 (males), 26.vii.1984, 6 exs.; MNHN 1985.0637-0638 (females), 26.vii.1984, 2 exs., MNHN 1985.0611, MNHN 1985.0617–0620 and MNHN 1985.0622 (females), 27.vii.1984, 6 exs., Gundia, forêt de Kemphole, à l'ouest de Sakleshpur, Karnataka, Inde (Gundia, Kemphole forest, west of Sakleshpur, Karnataka, India), coll. A. Dubois. Topotype, WILD-14-AMP-499 (Male), 1 ex., 29.vii.2014, Gundia, Karnataka (12.825^{0}N & 75.569^{0}E, 128m), coll. A. Padhye, N. Modak and S. Sulakhe; Topotype WILD-14-AMP-500 (female), 1 ex., 29.vii.2014, Gundia, Karnataka (12.829^{0}N & 75.607^{0}E, 224m), coll. A. Padhye, N. Modak and S. Sulakhe.

Indirana leithii: Topotypes, BNHS 2830–31, BNHS 2833, BNHS 2838–39 (females), 8.viii.1991, 5 exs., Matheran, Mumbai, India, coll. A.G. Sekar and V. Hegde; Topotype, BNHS 5590 (Female), 30.ix.12, 1 ex., Matheran, Mumbai, India, coll. N. Modak and A. Bayani.

Indirana leptodactyla: Syntype, BMNH 1947.2.29.39-40 (females), 2 exs., Malabar, coll. Col. Beddome; Syntype BMNH 1947.2.29.41 (Male), 1 ex., Malabar, coll. Col. Beddome; Non-Type, BMNH 1897.1.10.11 (female), 1 ex. Devicolum, Travancore, 1,219–2,133 m, coll. Fergusson.

Indirana phrynoderma: Syntypes, BMNH 1947.2.3.8–9 (males), 2 exs., Anamallays (=Annamalai), coll. Col. Beddome.

Indirana semipalmata: Syntype, BMNH 1947.2.29.50 (female), 1 ex., Malabar, coll. Col. Beddome; Syntype, BMNH 1947.2.29.51 (male), 1 ex., Malabar, coll. Col. Beddome.

Data for *I. longicrus* and *I. tenuilingua* from Rao (1937) as the type specimens are missing and are suggested to be lost (Dubois 1984)

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Author Contribution: ADP diagnosed the species. ADP, NM and ND collected specimens. NM studied the type and comparative material. NM and ND performed molecular analysis. ND performed statistical analysis. ADP, NM and ND wrote the paper.

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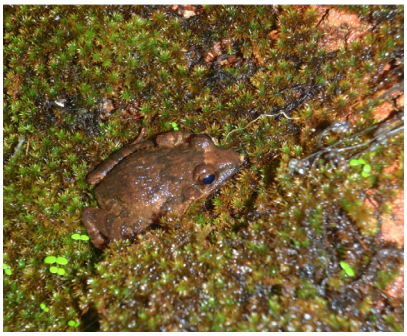
Appendix A. Voucher numbers and GenBank accession numbers for genetic data used for phylogenetic analysis.

Species	Voucher				GenBank Accession number			
	12S	16S	Rho	Rag1	12S	16S	Rho	Rag1
Indirana chiravasi sp. nov.	BNHS 5890				KM386527	KM386531	KM386539	KM386535
Indirana chiravasi sp. nov.	WILD-14-AMP-489				KM386526	KM386530	KM386538	KM386534
Indirana gundia	WILD-14-AMP-499				KM386528	KM386532	KM386540	KM386536
Indirana gundia	WILD-14-AMP-500				KM386529	KM386533	KM386541	KM386537
Indirana leithii	BNHS 5590				KF590627	KF590637	KF590647	KM386542
Indirana leithii	BNHS 5591				KF590628	KF590638	KF590648	KM386543
Indirana cf. *leithii*	IND212	AA212	DD212	CC212	JQ596717	JQ596673	JQ596778	JQ596740
Indirana semipalmata	IND256	AA256	DD256	CC256	JQ596715	JQ596671	JQ596787	JQ596748
Indirana semipalmata	IND245	AA245	DD245	CC245	JQ596713	JQ596669	JQ596785	JQ596745
Indirana semipalmata	IND257	AA257	DD257	CC257	JQ596716	JQ596672	JQ596788	JQ596749
Indirana semipalmata	IND255	AA255	DD255	CC255	JQ596714	JQ596670	JQ596786	JQ596747
Indirana semipalmata	IND243	AA243	DD243	CC243	JQ596712	JQ596668	JQ596784	JQ596746
Indirana leptodactyla	IND850	AA850	DD850	CC850	JQ596719	JQ596682	JQ596805	JQ596761
Indirana leptodactyla	IND848	AA848	DD848	CC848	JQ596721	JQ596684	JQ596803	JQ596759
Indirana leptodactyla	IND851	AA851	DD851	CC851	JQ596718	JQ596685	JQ596806	JQ596762
Indirana leptodactyla	IND849	AA849	DD849	CC849	JQ596720	JQ596681	JQ596804	JQ596760
Indirana leptodactyla	IND847	AA847	DD847	CC847	JQ596722	JQ596683	JQ596802	JQ596758
Indirana brachytarsus	IND71	AA71	DD71	CC71	JQ596690	JQ596646	JQ596800	JQ596751
Indirana brachytarsus	IND638	AA638	DD638	CC638	JQ596691	JQ596647	JQ596799	JQ596750
Indirana diplosticta	IND92	AA92	DD92	CC92	JQ596698	JQ596654	JQ596813	JQ596768
Indirana diplosticta	IND94	AA94	DD94	CC94	JQ596700	JQ596656	JQ596815	JQ596771
Indirana diplosticta	IND91	AA91	DD91	CC91	JQ596697	JQ596653	JQ596812	JQ596769
Indirana diplosticta	IND93	AA93	DD93	CC93	JQ596699	JQ596655	JQ596814	JQ596770
Indirana diplosticta	IND98	AA98	DD98	CC98	JQ596701	JQ596657	JQ596816	JQ596772
Indirana beddomii	IND77	AA77	DD77	CC77	JQ596688	JQ596644	JQ596795	JQ596754
Indirana beddomii	IND175	AA175	DD175	CC175	JQ596692	JQ596648	JQ596773	JQ596730
Indirana beddomii	IND180	AA180	DD180	CC180	JQ596694	JQ596650	JQ596775	JQ596732
Indirana beddomii	IND193	AA193	DD193	CC193	JQ596696	JQ596652	JQ596777	JQ596734
Indirana beddomii	IND220	AA220	DD220	CC220	JQ596708	JQ596664	JQ596779	JQ596736
Indirana beddomii	IND230	AA230	DD230	CC230	JQ596710	JQ596666	JQ596782	JQ596738
Indirana beddomii	IND244	AA244	DD244	CC244	JQ596729	JQ596674	JQ596789	JQ596742
Indirana beddomii	IND724	AA724	DD724	CC724	JQ596726	JQ596676	JQ596791	JQ596743
Indirana beddomii	IND246	AA246	DD246	CC246	JQ596728	JQ596675	JQ596790	JQ596741
Indirana beddomii	IND800	AA800	DD800	CC800	JQ596727	JQ596677	JQ596792	JQ596744
Indirana beddomii	IND178	AA178	DD178	CC178	JQ596693	JQ596649	JQ596774	JQ596731
Indirana beddomii	IND189	AA189	DD189	CC189	JQ596695	JQ596651	JQ596776	JQ596733
Indirana beddomii	IND200	AA200	DD200	CC200	JQ596707	JQ596663	JQ596780	JQ596735
Indirana beddomii	IND75	AA75	DD75	CC75	JQ596687	JQ596643	JQ596794	JQ596753
Indirana beddomii	IND227	AA227	DD227	CC227	JQ596709	JQ596665	JQ596781	JQ596737
Indirana beddomii	IND231	AA231	DD231	CC231	JQ596711	JQ596667	JQ596783	JQ596739
Indirana beddomii	IND72	AA72	DD72	CC72	JQ596686	JQ596642	JQ596793	JQ596752
Indirana sp.	IND88	AA88	DD88	CC88	JQ596703	JQ596659	JQ596809	JQ596765
Indirana sp.	IND95	AA95	DD95	CC95	JQ596705	JQ596661	JQ596808	JQ596764

Species	Voucher				GenBank Accession number			
	12S	16S	Rho	Rag1	12S	16S	Rho	Rag1
Indirana sp.	IND99	AA99	DD99	CC99	JQ596706	JQ596662	JQ596811	JQ596767
Indirana sp.	IND89	AA89	DD89	CC89	JQ596704	JQ596660	JQ596810	JQ596766
Indirana sp.	IND87	AA87	DD87	CC87	JQ596702	JQ596658	JQ596807	JQ596763
Micrixalus fuscus	MF5111	MF3006	NA	E224.1	GU143817	GU136106	AF249120	KF991333

NA = not available



Appendix B. Movie of *Indirana chiravasi* sp. nov. calling behavior showing single internal vocal sac.