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Indirana salelkari, a new species of leaping frog (Anura: Ranixalidae) from Western Ghats of Goa, India

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Abstract: *Indirana salelkari*, a new species of leaping frog, is described from Netravali, Goa, India. The species can be distinguished from its congeners by a combination of morphological characters, viz., head longer than wide, narrow and deep buccal cavity, vomerine teeth large and acutely placed close to each other, oval choanae, distinct canthus rostralis, first finger longer than or equal to second, presence of double outer palmer tubercles, elongated inner metatarsal tubercle, moderate webbing, discs of fingers and toes with crescentic deep marginal grooves restricted only to the anterior side of the discs, dorsal skin with glandular folds but without warts, ventral skin granular with some mottling on throat and, palms and soles dark brown. *Indirana salelkari* differs from its sister taxa, *I. chiravasi*, in the placement and structure of vomerine teeth and choanae. The new species is genetically distinct from *I. chiravasi*, with a genetic distance of 3.8% for the 16S rRNA gene. We also provide phylogentic placement of *Indirana salelkari* based on mitochondrial 12S and 16S ribosomal genes and nuclear rhodopsin gene along with molecular clock analysis, which further confirms its genetic distinctness from other related taxa.

Keywords: Buccal cavity structure, molecular phylogeny, multivariate analysis, new species, tadpole oral apparatus structure, taxonomy.

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

Author Details: Nikhil Modak is a doctoral student interested in ecology, systematics and evolution of amphibians of the Western Ghats. Neelesh Dahanukar works in ecology and evolution with an emphasis on mathematical and statistical analysis. He is also interested in taxonomy, distribution patterns and molecular phylogeny of fish and frogs. Ninad Gosavi is an undergraduate student interested in amphibian ecology. Anand D. Padhye is an Associate Professor working on systematics, ecology, diversity, distribution and evolution of amphibians.

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INTRODUCTION

The genus *Indirana* Laurent, 1986, is characterized by small to moderate size, forked omosternum, vomerine teeth present behind the choanae, large nasal bones in contact with each other and fronto-parietals, clubbed metatarsus barely separated by webbing, presence of deeply notched tongue bearing a mid-ventral lingual papilla, Y-shaped terminal phalanges, and having specialized tadpoles adapted to terrestrial development (Laurent 1986). Currently, there are 11 known species in the genus, namely *I. 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), *I. gundia* (Dubois, 1986), and *I. chiravasi* Padhye et al. (2014). Recent species and distributional delimitation based on molecular studies (Nair et al. 2012; Modak et al. 2014) and description of a new species in the genus (Padhye et al. 2014) suggests that there are several undescribed species in the genus.

During the field surveys in Goa region of the Western Ghats, we came across a population of *Indirana* which was found to be morphologically and genetically different from other known species of the genus. The new species is described here.

MATERIALS AND METHODS

Study site and specimen collection

Specimens of the new species were collected from the Tanshikar Spice Farm at Netravali (Neturlim) in Sanguem Taluk of South Goa, India (15.095^{0}N & 74.211^{0}E; elevation 78m). Four male and four female specimens were collected and preserved in absolute alcohol for further analysis. Ten tadpoles of different stages were collected from the lateritic rocks near the same locality.

Museum details

Specimens studied in this paper are deposited in the museum of the Bombay Natural History Society (BNHS), Mumbai; the Wildlife information Liaison Development (WILD) Society, Coimbatore; the Zoological Survey of India, Western Regional Center (ZSI-WRC), Pune and Abasaheb Garware College, Zoology Research Laboratory (AGCZRL), Pune, India. Type specimens from the Natural History Museum (BMNH), London and the Muséum National d'histoire Naturelle (MNHN), Paris, were studied for comparison by the first author.

Morphometry

Morphometric measurements were carried out with the help of a digital caliper (Ocean Premium measuring instruments) to the nearest 0.1mm. A total of 27 characters were measured following Padhye et al. (2014), 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: Horizontal length of eye between 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); THL (thigh length from hip joint to joint between thigh and shank); TL (Tibia/shank length from joint between thigh and shank to joint between shank and tibiotarsal articulation); ACL (Astragalo-calcaneal length from joint between shank and tibiotarsal articulation to the base of the inner metatarsal tubercle); 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). We also measured the characters related to the roof of buccal cavity (Fig. 1a) using stage and ocular micrometer scale (least count 0.01mm) in Leica 58AP0 dissecting microscope. The depth of the buccal cavity (Fig. 1b) was determined by measuring the difference in the focal planes of upper lip and vomerine region of the buccal

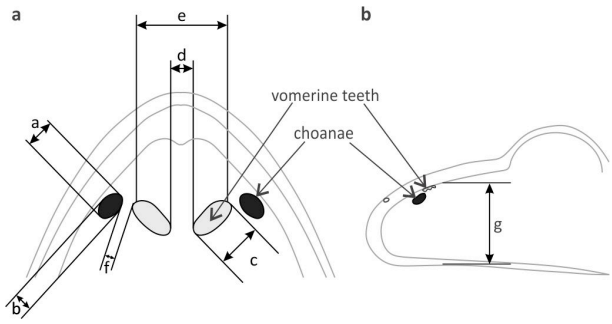

Figure 1. Diagrammatic illustration of the roof of buccal cavity indicating the measured distances. (a) ventral view of the roof and (b) sagittal section.

roof using scale (least count 0.002mm) on the fine focus knob of Zeiss Primostar compound microscope.

Statistical analysis

Statistical analysis of the morphometric data was performed on size adjusted measurements by taking all measurements as percent of SUL to remove the bias due to body size variation. Multivariate normality of the data was checked using Doornik & Hansen (2008) omnibus. Discriminant Analysis (DA) was performed to understand whether related species form significantly different clusters (Huberty & Olejnik 2006) in the genus *Indirana*. Pillai's trace statistic was used to test the null hypothesis that the mean vectors of different clusters are equal (Harris 2001). Mahalanobis distances (Harris 2001) between pair of individuals were calculated and were used for computing Fisher's distances (distance between the centroids of the clusters, divided by the sum of their standard deviations) between two clusters to check if the clusters were significantly different. Statistical analysis was performed in PAST 3.0 (Hammer et al. 2001).

Molecular analysis

Thigh muscles of the three specimens (BNHS 5931, WILD-15-AMP-551 and AGCZRL-Amphibia-210) were used for extracting DNA and conducting molecular analyses. Genomic DNA extraction, Polymerase Chain Reaction (PCR) for two mitochondrial (12S and 16S) and one nuclear (rho) genes, PCR product purification and sequencing was performed following the protocols detailed in Padhye et al. (2014). Sequences were checked by BLAST tool (Altschul et al. 1990) to identify the nearest congeners. These sequences have been deposited in GenBank (KP826821 to KP826829). Additional sequences of related species were retrieved from NCBI GenBank database (http://www.ncbi.nlm.nih.gov/). GenBank accession numbers of 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 921 nucleotides. Best fit model for nucleotide substitution was selected from 24 models using MEGA 6 (Tamura et al. 2013) based on minimum Bayesian Information Criterion (BIC) value (Schwarz 1978; Nei & Kumar 2000). This best fit model was also used for constructing the phylogenetic trees using maximum likelihood in MEGA 6 (Tamura et al. 2013). Reliability of the phylogenetic tree was estimated using bootstrap values run for 1000 iterations. Phylogenetic tree was edited in FigTree v1.4.2 (Morariu et al. 2009). Pairwise raw genetic distances using 16S rRNA gene and combined matrix of 12S, 16S and rho genes were calculated using p distances method in MEGA 6 (Tamura et al. 2013).

Molecular clock analysis

A subset of concatenated sequences, with monophyletic clades, were used for molecular clock analysis. Separation of Nasikabatrachidae (149.5 mya); separation of Nyctibatrachidae (91.6 mya) and separation of Micrixalidae (89.7 mya) were used as calibration points obtained from time tree (Hedges et al. 2006). Aligned sequences were used for finding the best fit model for nucleotide substitution using J Model test (Darriba et al. 2012). The best fit models for the three partitions (12S: TIM2ef + G; 16S: TIM2 + G; Rho: K80 + Inv) were used for molecular clock analysis using BEAST v.1.8.0 (Drummond et al. 2012). Phylogenetic tree was edited in FigTree v1.4.2 (Morariu et al. 2009). Time of taxa split are expressed as mean ± 95% Highest Posterior Density (HPD).

RESULTS

***Indirana salelkari* sp. nov.**

(Images 1, 2, 3a, 4, 5)

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Holotype: BNHS 5931, 11.x.2014, male, 27.7mm SUL, Tanshikar Spice Farm in Neturlim (15.095^{0}N & 74.211^{0}E; elevation 78m), Sanguem Taluk, South Goa District, Goa, India, coll. Nikhil Modak.

Paratypes (n = 7): BNHS 5933, female, 30.2mm SUL, locality same as holotype; BNHS 5932, male, 26.2mm SUL, locality same as holotype; WILD-15-AMP-551, female, 30.8mm SUL, locality same as holotype; WILD-15-AMP-552, male, 24.7mm SUL, locality same as holotype; ZSI-WRC A/1547, female, 30.0mm SUL, locality data as holotype; AGCZRL-amphibia-209, male, 26.0mm, Tanshikar Spice Farm in Neturlim (15.095^{0}N & 74.211^{0}E; 78m), Sanguem Taluk, South Goa District, Goa, India, collected on 6.ix.2014 by Nikhil Modak and Ninad Gosavi; AGCZRL-amphibia-210, female, 30.9mm SUL, Tanshikar Spice Farm in Neturlim (15.095^{0}N & 74.211^{0}E; 78m), Sanguem Taluk, South Goa District, Goa, India, collected on 1.vi.2014 by Ninad Gosavi.

Diagnosis: *Indirana salelkari* sp. nov. differs from all other congeners based on the following combination of characters: medium-sized frog (20.9–30.9 mm SUL), head longer than wide, distinct canthus rostralis, first finger longer than or equal to second, presence of double

Image 1. *Indirana salelkari* sp. nov. holotype male (BNHS 5931, 27.7mm SUL).

outer palmar tubercle, elongated inner metatarsal tubercle, webbing moderate (I1-2II1-2½III1¼-3IV3-1¼V), discs of fingers and toes with crescentic deep marginal grooves restricted only to the anterior side of the discs, buccal cavity narrow and deep, vomerine teeth large and acutely placed closer to each other with a distance less than the length of vomerine teeth series, oval choanae, dorsally skin with glandular folds but without warts, ventrally skin granular with some mottling on throat 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 1.

Description of the Holotype (BNHS 5931; male) (all measurements in mm)

Medium-sized frog (SUL 27.7); head longer than wide (HL 11.6 > HW 9.5); snout longer than horizontal diameter of eye (SL 4.8 > EL 3.4); pupil horizontal; outline of snout suboval dorsally, truncated laterally; ventrally snout slightly protruding beyond the mouth; nostrils nearer to snout than to the eye (SNL 2.0 < ENL 2.5); tympanum about 3/4th the diameter of eye (TYL = 2.7; EL = 3.4), very close to eye; supra-tympanic fold distinct; upper eyelid width 3/4th the horizontal diameter of eye; upper eyelids smooth; inter-orbital distance equal to inter-narial distance (IOL 2.6 = IOL 2.6); canthus rostralis obtuse; loreal region slightly concave and oblique; buccal cavity narrow, deep, vomerine teeth in two sharply oblique rows at the posterior border of choanae (Image 3); tongue thin, bifid, bearing a mid ventral papilla.

Upper arm shorter than fore arm (UAL 5.0 < FoAL 5.7); hand long (PAL 6.0); finger lengths from shortest to longest - F2 (2.1) < F1 (2.2) < F4 (2.3) < F3 (2.5); palmar tubercles present, outer palmar tubercle double, subarticular tubercles moderate, supernumerary tubercles present, single; finger discs moderate in shape, broad, truncate, bearing semicircular groove; fingers without web or fringe of skin.

Hind limbs long; thigh shorter than shank (tibia) (THL 12.4 < TL 15.2); total foot length (including astragalus-calcaneum) longer than tibia (TFOL 20.9); toe lengths from shortest to longest are - T1 (1.4) < T2 (1.8) < T3 (4.3) < T5 (4.8) < T4 (7.0); toe discs moderate; bear semicircular groove; inner metatarsal tubercle thin and elongated; outer metatarsal tubercle absent;

Image 2. *Indirana salelkari* sp. nov. paratype female (BNHS 5933, 30.2m SUL)

supernumerary tubercles absent; subarticular tubercles moderate; tarsal fold and outer phalangeal fringe absent; webbing formula I1-2II1-2½III1¼-3IV3-1¼V.

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

Description of Female (Paratype, BNHS 5933) (all measurements in mm)

Medium-sized frog (SUL 30.2); with head longer than wide (HL 11.8 > HW 11.2); snout longer than eye (SL 5.6 > EL 3.5); outline of snout suboval in shape dorsally; truncated laterally; ventrally slightly protruding beyond the mouth; nostrils slightly nearer to snout than to the eye (SNL 2.5 < ENL 2.8); tympanum about 3/4th the diameter of eye (TYL = 2.8; EL = 3.1); 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 wider than inter-orbital distance (INL 3.2 < IOL 2.8); canthus rostralis obtuse; loreal region slightly concave and oblique; buccal cavity narrow and deep, vomerine teeth in two sharply oblique rows at the posterior border of choanae; tongue thin, bifid; bearing a mid-ventral papilla.

Upper arm shorter than fore arm (UAL 7.3 < FoAL 6.8); hand (PAL 8.3) about 1/4th of SVL; finger lengths from shortest to longest - F2 (2.2) < F1 (2.8) < F4 (3.6) < F3 (4.4); palmar 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.

Hindlimb about double the SVL, thigh and tibia subequal (THL 17.0 < TL 17.4); total foot length (including astragalus-calcaneum) (22.7) longer than tibia; toe lengths in order of T1 (2.3) < T2 (3.2) < T3 (5.0) < T5 (5.5) < T4 (8.7); toe discs moderate; bear semicircular groove; inner metatarsal tubercle thin, long; outer metatarsal tubercle absent; supernumerary tubercles absent;

Table 1. Morphometry (in mm) of type specimens of *Indirana salelkari* sp. nov.

Character	Holotype	Paratypes						
	Male	Male			Female			
	BNHS 5931	BNHS 5932	WILD-15-AMP-552	AGCZRL-Amphibia-209	BNHS 5933	WILD-15-AMP-551	ZSI-WRC A/1457	AGCZRL-Amphibia-210
SUL	27.7	26.2	24.7	26.0	30.2	30.8	30.0	30.9
HL	11.6	11.6	10.7	10.5	11.8	12.9	12.7	12.3
HW	9.5	9.5	9.1	9.9	11.2	11.4	11.2	11.1
SL	4.8	5.0	4.6	4.9	5.6	5.7	5.3	5.4
EL	3.4	3.1	3.0	3.2	3.5	3.7	3.7	4.6
TYL	2.7	2.8	2.7	1.9	2.4	2.6	2.8	2.4
UEW	2.5	2.7	2.4	2.4	2.3	2.4	2.8	2.6
SNL	2.0	1.8	2.1	1.7	2.5	1.9	2.1	2.3
ENL	2.5	2.5	2.6	3.1	2.8	3.5	2.9	3.2
INL	2.6	2.6	2.1	2.4	3.2	3.4	3.0	3.3
IOL	2.6	2.7	2.9	3.0	2.8	3.1	3.0	3.2
UAL	5.0	5.8	6.1	4.5	7.3	5.5	6.4	5.3
FoAL	5.7	5.6	6.2	5.3	6.8	7.4	6.6	7.2
F1	2.2	2.1	2.0	2.0	2.8	3.3	2.5	3.0
F2	2.1	1.9	1.9	1.9	2.2	3.0	2.1	2.5
F3	2.5	3.6	3.2	3.9	4.4	4.7	4.2	4.0
F4	2.3	2.9	3.0	2.8	3.6	3.6	2.9	3.3
THL	12.4	14.2	12.9	14.4	17.0	16.4	17.8	15.6
TL	15.2	14.5	13.8	16.7	17.4	20.0	18.4	17.2
ACL	7.2	7.2	6.6	7.1	9.4	8.8	9.0	8.3
FOL	13.9	13.6	13.9	13.2	16.8	17.6	16.8	15.2
TFOL	20.9	20.3	19.0	22.0	25.4	25.1	25.8	22.4
T1	1.4	1.6	1.5	1.7	2.6	3.0	2.3	2.0
T2	1.8	2.2	1.9	3.0	3.7	3.5	3.3	3.0
T3	4.3	4.1	4.5	4.5	5.8	6.6	4.6	5.2
T4	7.0	7.1	7.5	7.8	9.4	9.5	9.3	9.0
T5	4.8	3.9	4.4	4.5	5.7	5.5	5.1	5.2

subarticular tubercles moderate; tarsal fold and outer phalangeal fringe absent; webbing formula I1-2II1-2½III1¼-3IV3-1¼V.

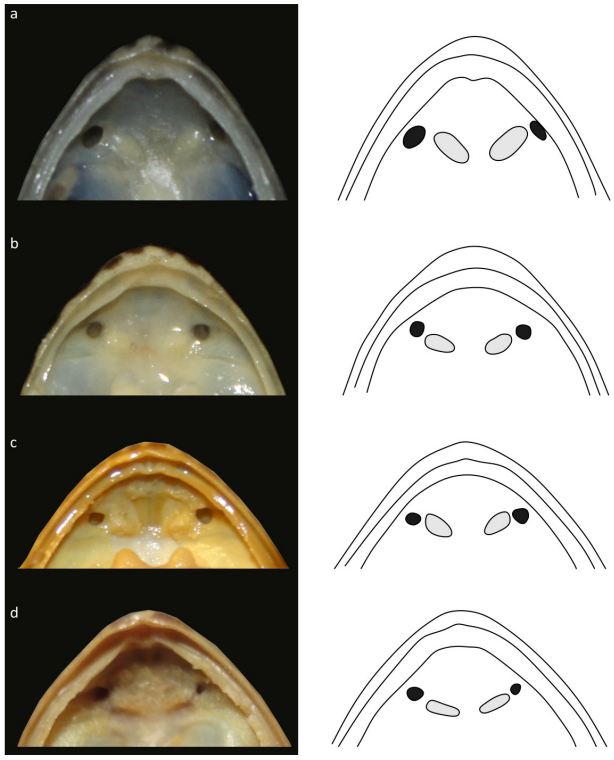
Dorsal and ventral skin smooth; few longitudinal folds on dorsal side; lateral and ventral side granular.

Coloration in life (Image 4)

Dorsum Pale to dark brown, some specimens were also observed with pinkish dorsum; dark band between the eyes which continues on the upper eyelid; interrupted W shaped mark on the back of the head may or may not be present; upper and lower mandible barred with brown stripes which are sometimes interrupted or absent on upper mandible; a dark brown stripe running from the tip of the snout to shoulder through the eye and tympanum; forelimbs and hind limbs bearing transverse bands which are also present on fingers and toes which may not be quite distinct in darker specimens (usually these bands are paler in females); lateral margin of forelimbs and hind limbs densely spotted with dark brown or black (fewer in females) which may continue on ventral side in forelimbs; palm dark brown in color; foot and soles dark brown; ventrally white, throat in some specimens mottled with brown.

Coloration in preservation (Image 1 and 2)

Dorsal pale to dark brown, dark band between the eyes which continues on the upper eyelid; interrupted W shaped mark on back of the head which may or may not be present; upper and lower mandible barred with



brown strips sometimes interrupted or absent on upper mandible; dark brown strip running from tip of snout to shoulder through eye and tympanum visible; few dark spots on the lateral side of abdomen; forelimbs and hindlimbs barred with dark brown strips which may not be quite distinct in darker specimens; lateral margin of forelimbs and hind limbs densely spotted with dark brown or black which may continue on ventral side in forelimbs; region near outer palmer tubercle darker; sole and foot dark brown; ventrally creamish to white; brown mottling on the throat of some specimens.

Image 3. Roof of buccal cavity showing position of vomerine teeth and choanae. (a) *Indirana salelkari* sp. nov. holotype (BNHS 5931), (b) *I. chiravasi* holotype (BNHS 5890), (c) *I. beddomeii* syntype (BMNH 1947.2.27.72), and (d) *I. brachytarsus* lectotype (BMNH 1947.2.27.92). Photo credit: (a–b) Neelesh Dahanukar; (c–d) Nikhil Modak.

Image 4. *Indirana salelkari* sp. nov. in life (female paratype, AGCZRL-amphibia-210, 30.9mm SUL).

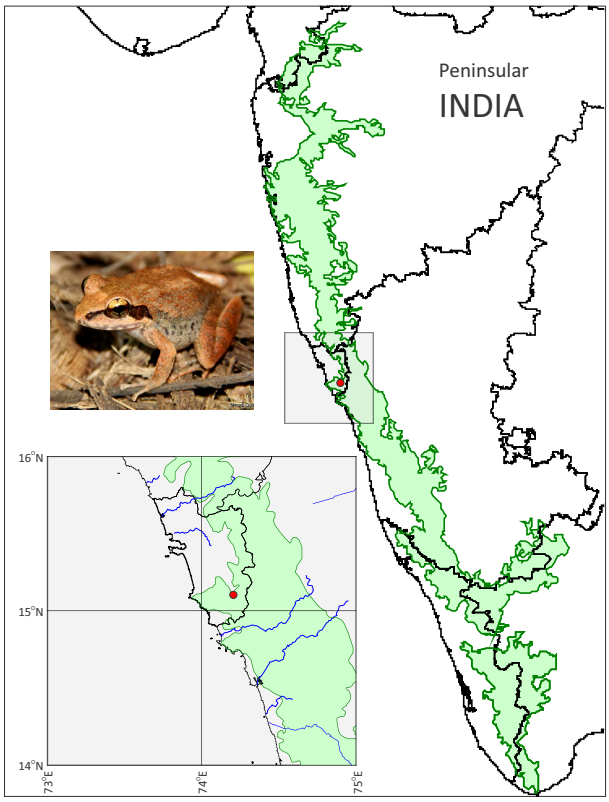


Image 5. Type locality of *Indirana salelkari* sp. nov. Photo credit: Vivek Kale

Image 6. Microhabitat at the type locality at Tanshikar Spice Farm.

Image 7. Metamorphic stage 45 of *Indirana salelkari* sp. nov.

Image 8. Oral apparatus of prometamorphic (stage 41) tadpole of *Indirana salelkari* sp. nov.

Sexual Dimorphism

In the breeding season males bear nuptial pad on the outer side of first finger and femoral glands on thighs.

Etymology

The species is named after Prakash Salelkar, Range Forest Officer, Netravali, Goa, to honor his dedicated work on the conservation of wildlife in Goa State and for his continual help since 2003 during field work in Goa.

Distribution

The species is currently known only from its type locality in Sanguem Taluk of South Goa, India (15.095^{0}N & 74.211^{0}E; 78m) (Image 5).

Habitat

General habitat at type locality is shown in Image 6. The specimens were collected from Tanshikar Spice Farm. The species was seen to occupy nearby riparian habitats. Some sub adults were seen under leaf litter. The tadpoles were collected from the exposed laterite in the vicinity of the type locality.

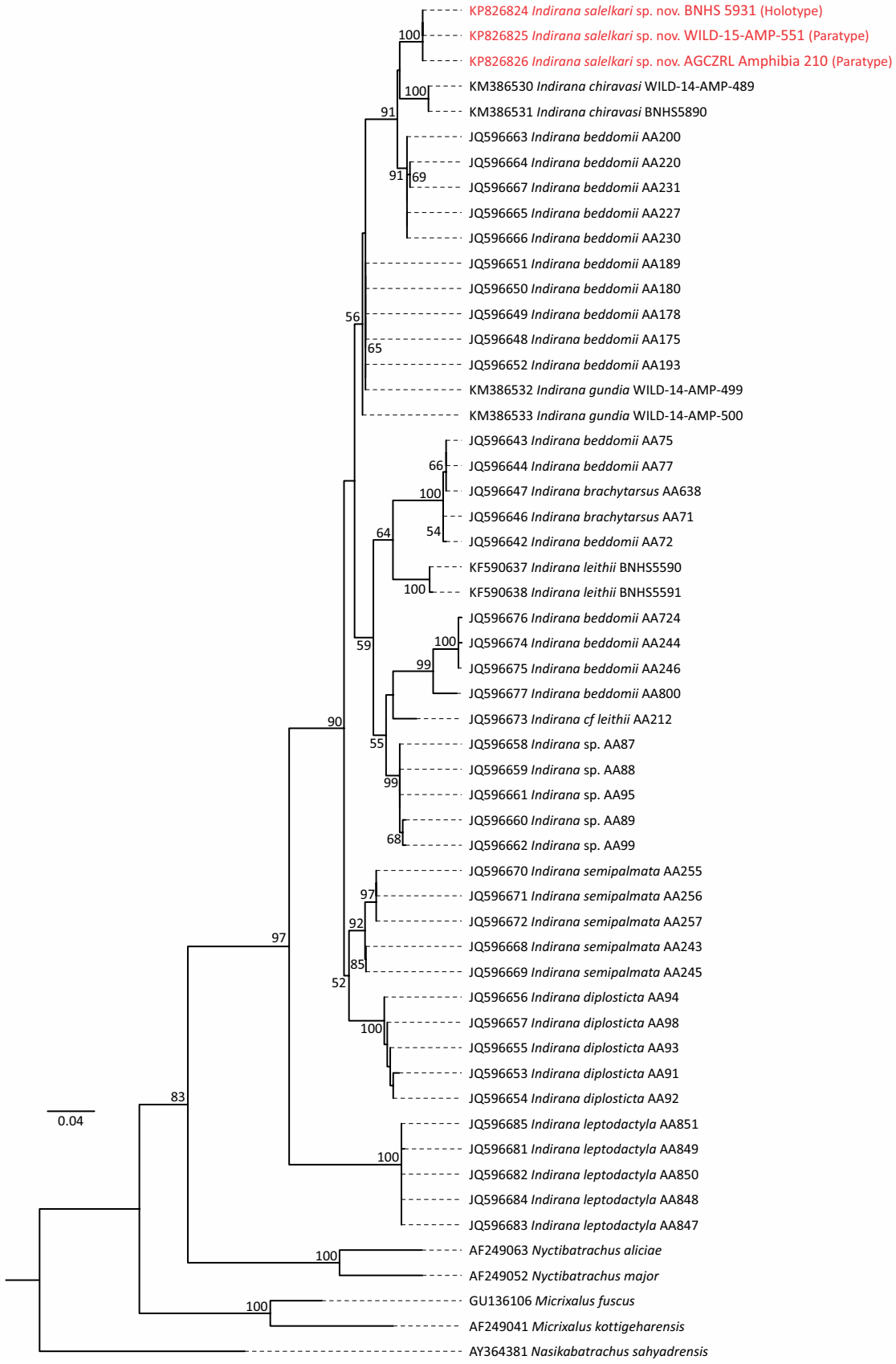


Figure 2. Maximum likelihood tree based on GTR+G (BIC = 6137.51, lnL = -2496.68, G = 0.37) nucleotide substitution model for 16s rRNA gene sequences. Values along the nodes are percent bootstraps for 1000 iterations. *Nasikabatrachus sahyadrensis* is used as an outgroup.

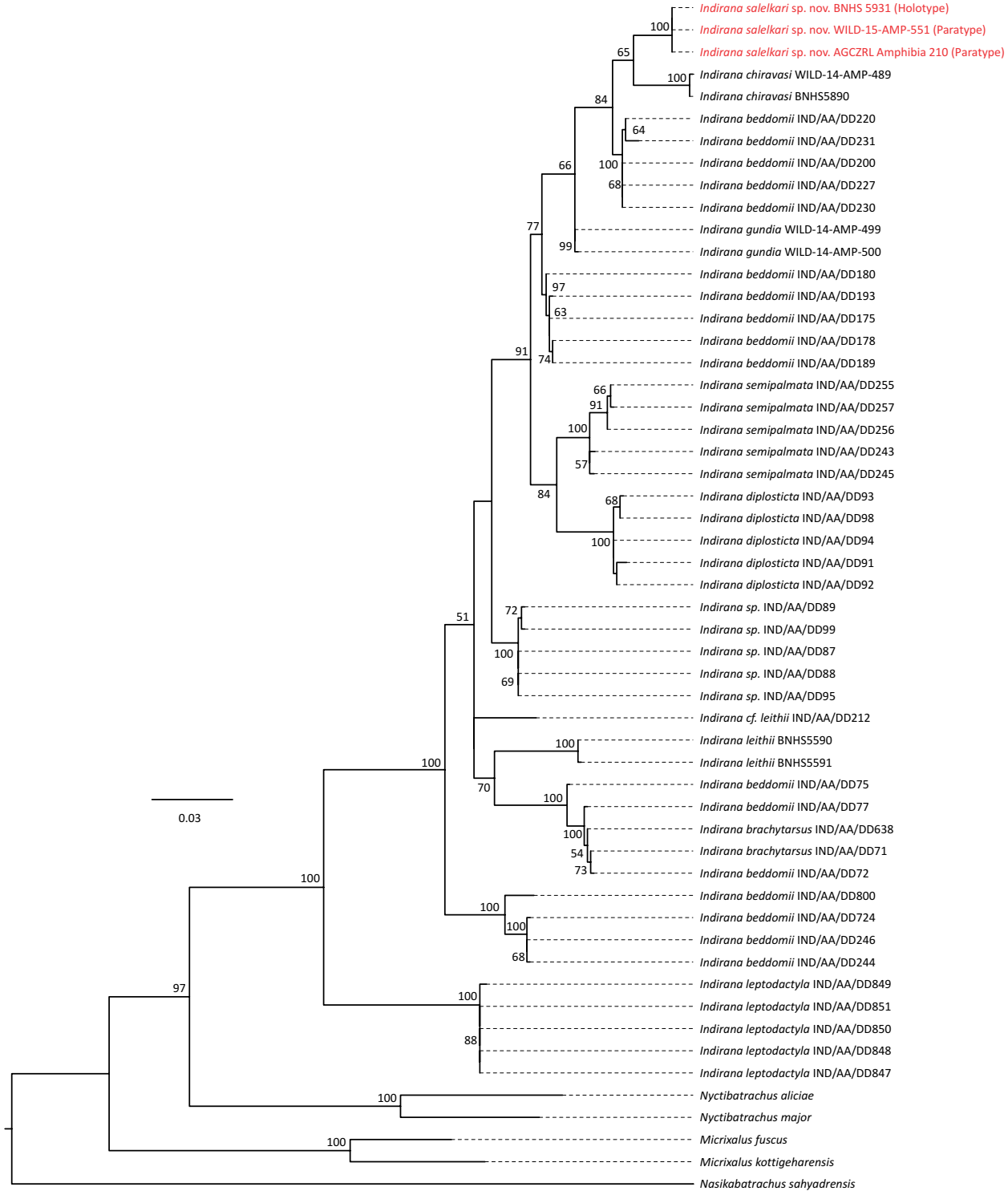


Figure 3. Maximum likelihood tree based on GTR+G+I (BIC = 10429.89, lnL = -4596.32, I = 0.32, G = 0.58) nucleotide substitution model for combined matrix of 12SrRNA, 16s rRNA and rhodopsin gene sequences. Values along the nodes are percent bootstraps for 1000 iterations. *Nasikabatrachus sahyadrensis* is used as an outgroup.

Natural history and description of tadpoles

Tadpoles of various stages in prometamorphic (Stage 36, stage 39 and stage 41) and metamorphic (stage 45, Image 7) stages (McDiarmid & Altig 1999) were collected from exposed laterite in the vicinity of type locality. Tadpoles of stage 41 showed semi-condensed

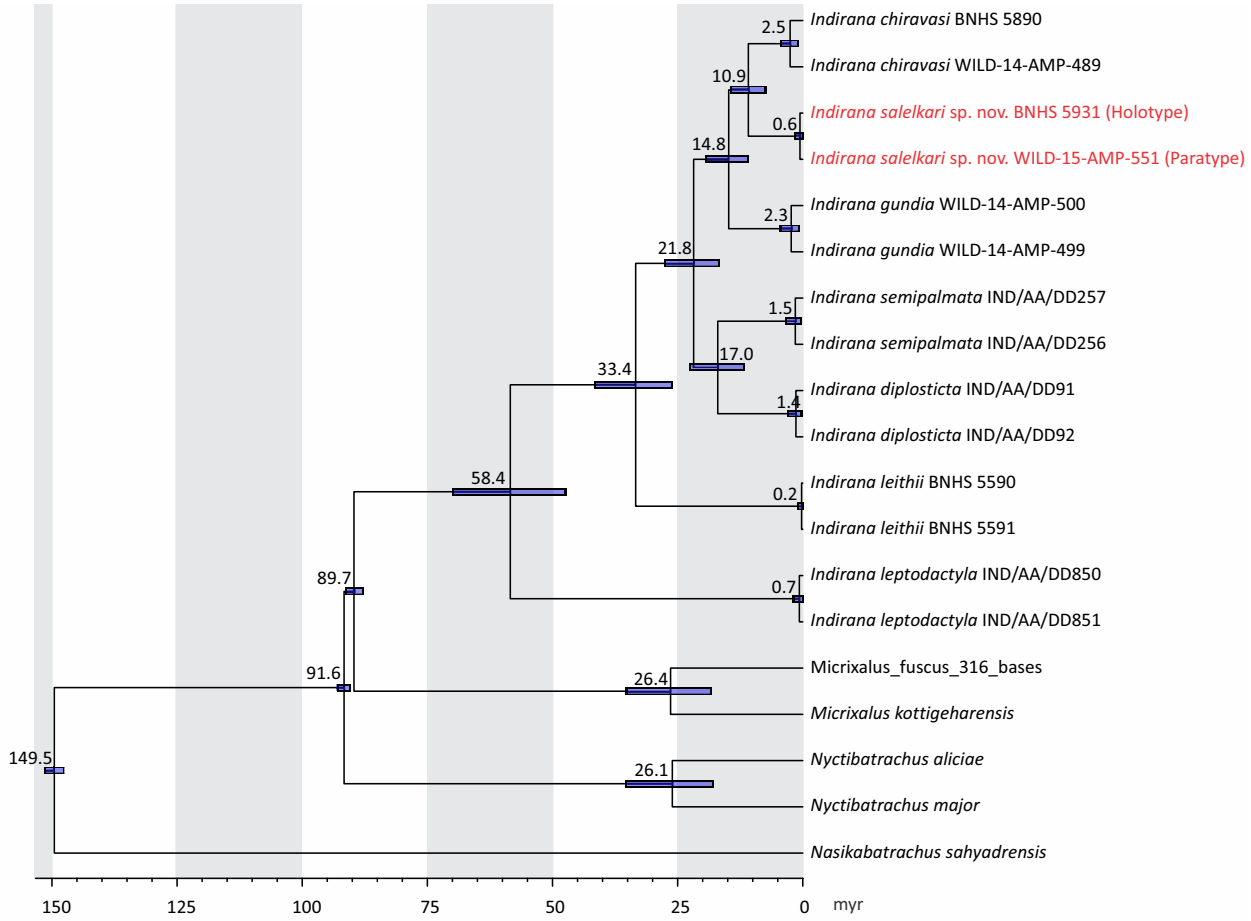


Figure 4. Molecular clock analysis based on concatenated sequences and separation of Nasikabatrachidae (149.5 mya), separation of Nyctibatrachidae (91.6 mya) and separation of Micrixalidae (89.7 mya) as calibration points. Blue bars are 95% HPD intervals.

individual keratodont formula (Dubois 1994) as 4[A_1-A_4]/4[P_1-P_2] (Image 8). 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 keratodont ridge P_1 is marginal and keratodont ridge P_2 is placed lateral to the horny beak and P_3 and P_4 are continuous.

Common name

Netravali Leaping Frog.

Genetic analysis

Best fit model for 16S rRNA barcoding gene was GTR+G (BIC = 6137.51, lnL = -2496.68, G = 0.37). The best fit model for the nucleotide substitution for cconcatenated genetic sequences (921 bases) of mitochondrial 12S and 16S rRNA genes and nuclear rho gene was GTR+G+I (BIC = 10429.89, lnL = -4596.32, I = 0.32, G = 0.58). Maximum likelihood analysis of the genetic data (Figs. 1 and 2) suggested that *Indirana salelkari* sp. nov. is a monophyletic group genetically distinct from the other *Indirana* species for which genetic data are available. The sister taxa for *I. salelkari* is *I. chiravasi* from which it differs with the raw distance of 3.8% in 16S rRNA gene and 3.1–3.2 % in concatenated sequences. Molecular clock analysis (Fig. 4) suggested that *Indirana salelkari* separated from *I. chiravasi* about 10.9 myr ago (95% HPD 14.5-7.4).

Statistical analysis

Size corrected morphometric data was not significantly different from multivariate normal (Doornik and Hansen omnibus, within group Ep = 74.91, P = 0.0512). MANOVA suggested that there were significantly distinct clusters among the species (Pillai's trace = 5.13, $F_{234,333}$ = 1.886, P < 0.0001). Discriminant Analysis extracted nine factors out of which first three canonical axes explained 86.77% of the total variation in the data where the first axis explained 40.13%, second

Table 2. Factor loadings for variables along the first three axes of Discriminant Analysis.

Character	Axis 1	Axis 2	Axis 3
HL	0.0291	0.2015	-0.4177
HW	0.2372	0.0233	-0.3140
SL	-0.0264	0.0156	-0.2285
EL	0.0325	0.2053	-0.0765
TYL	-0.1330	0.3433	-0.2537
UEW	0.0674	-0.0725	-0.0219
SNL	0.0999	0.0446	0.0450
ENL	-0.0697	-0.0111	-0.1793
INL	0.2012	0.0204	0.1129
IOL	0.1124	0.0376	-0.1514
UAL	0.3824	-0.2329	-0.1250
FoAL	0.1134	-0.0920	-0.1802
F1	-0.1631	-0.1691	-0.0858
F2	-0.0958	-0.2401	0.2541
F3	-0.1271	-0.3287	0.0325
F4	-0.0943	-0.3110	0.1022
THL	0.3980	-1.0742	-0.1056
TL	0.4816	-1.2721	0.0043
ACL	0.3564	-0.7046	-0.1310
FOL	0.1899	-1.4077	-0.1682
TFOL	0.5484	-2.1421	-0.4440
T1	-0.1351	-0.1986	0.0650
T2	-0.0337	-0.2738	0.0168
T3	0.0310	-0.5170	-0.0201
T4	0.0073	-0.8968	-0.0751
T5	-0.1643	-0.5400	0.0086

axis explained 26.26% and third axis explained 20.38% of the total variation (Fig. 3c). First two canonical axes readily separated *Indirana salelkari* sp. nov. from *I. diplosticta*, *I. leptodactyla* and *I. phrynoderma* (Fig. 3a). *Indirana salelkari* sp. nov. was separated from *I. leithii*, *I. beddomii*, *I. brachytarsus*, *I. chiravasi*, *I. gundia* and *I. semipalmata* on the third canonical axis (Fig. 3b). DA loadings of morphometric characters on the first three canonical axes are shown in Table 2. Relatively higher values of characters such as TFOL, FOL, TL, THL, T1 and ACL and lower values of TYL separated *Indirana salelkari* sp. nov. from other related species.

Comparison with other species of *Indirana*

Indirana salelkari sp. nov. differs from *I. diplosticta*, *I. leithii*, *I. leptodactyla*, *I. longicrus* and *I. phrynoderma* in having first finger equal to or longer than second finger

Table 3. Measurements (in mm) of buccal structures in *Indirana salelkari* and *I. chiravasi* males. Small letters in parenthesis refer to distances indicated in Figure 5.

Character	*Indirana salelkari* sp. nov.		*Indirana chiravasi*	
	BNHS 5931	BNHS 5932	BNHS 5888	BNHS 5890
	Holotype	Paratype	Holotype	Paratype
Snout vent length	27.7	26.2	27.3	25
Head length	11.6	11.6	11.4	10.8
Choanae maximum diameter (a)	0.8	0.7	0.7	0.6
Choanae minimum diameter (b)	0.4	0.4	0.6	0.6
Vomerine teeth series length (c)	1.3	1.2	0.9	0.7
Minumum distance between vomerine teeth (d)	0.7	0.7	1.5	1.1
Maximum distance between vomerine teeth (e)	2.7	2.8	3.7	2.7
Distance between vomerine teeth and choanae (f)	0.4	0.4	0.3	0.4
Depth of buccal cavity (g)	2.9	2.5	1.5	1.4

(vs. first finger shorter than second).

Indirana salelkari sp. nov. differs from *I. tenuilingua* in having head longer than broad (vs. head slightly wider than long), inter-orbital 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 crescentic deep marginal grooves restricted only to the anterior side of the discs (vs. semicircular groove in front of the toes and fingers absent, faint or indistinct).

Indirana salelkari sp. nov. differs from *I. semipalmata* in having broader head (34.3-38.1% SVL vs. 33.6-33.7% SVL) and moderately webbed toes with the webbing formula I1-2II1-2½III1¼-3IV3-1¼V (vs. half webbed toes with the webbing formula I2-2II2-3III2-3¼IV3¼-2V). Genetic distance between *I. salelkari* and *I. semipalmata* is 5.9–6.4% for 16S gene and 4.1-4.7% for concatenated sequences.

Indirana salelkari sp. nov. differs from *I. beddomii* in having narrow and deep buccal cavity (vs. broader and shallow buccal cavity); vomerine teeth close to each other (vs. vomerine teeth quite apart from each other) (Image 3); the webbing formula I1-2II1-2½III1¼-3IV3-1¼V (vs. webbing formula I1-2II1-2III1-3IV3-1V).

Indirana salelkari sp. nov. can be distinguished from *I. brachytarsus* in having moderate webbing I1-2II1-2½III1¼-3IV3-1¼V (vs. extensive webbing, webbing formula, I1-2II1-2½III1-3IV3-1V), longer upper arm (17.1-

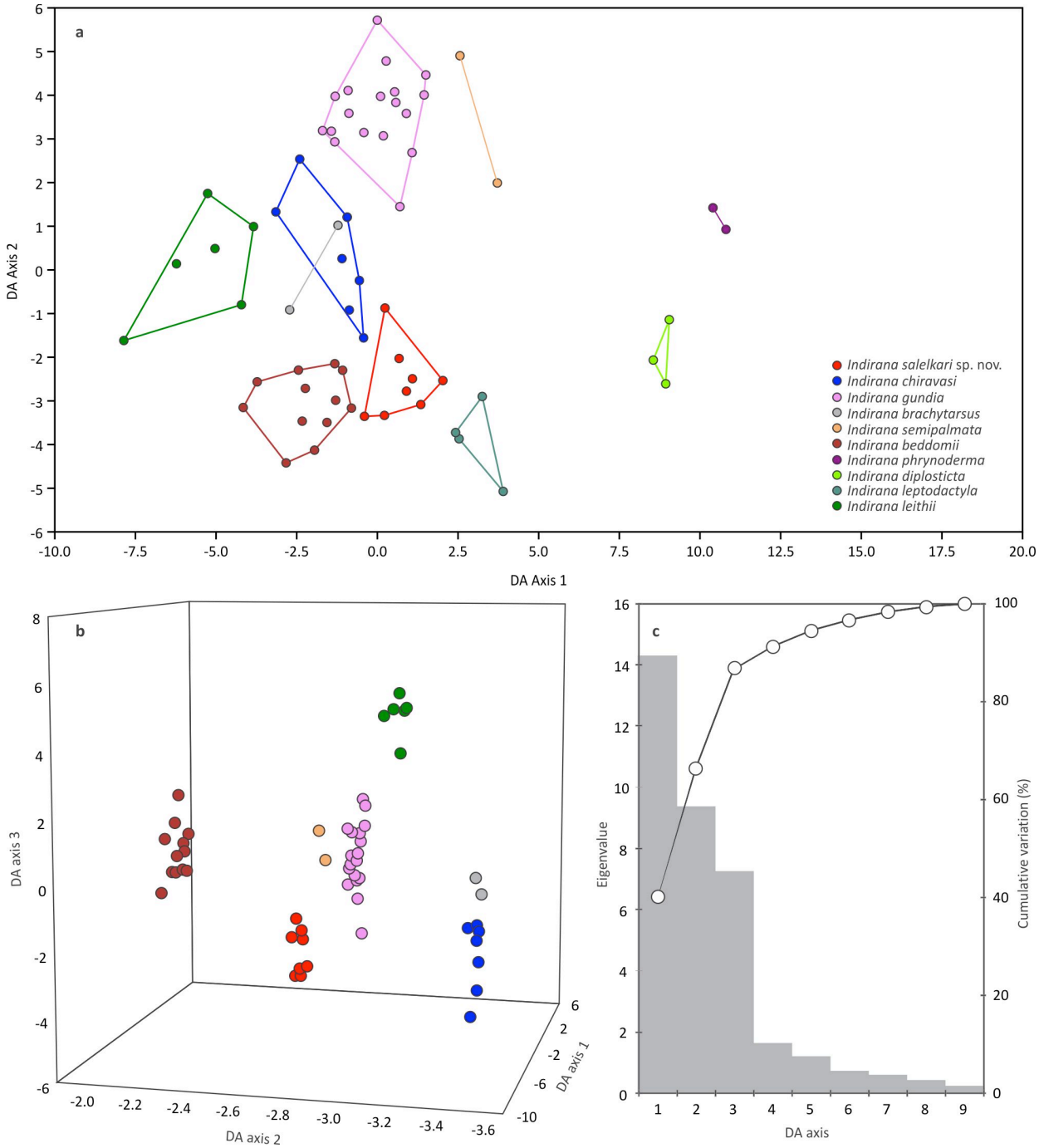


Figure 5. Discriminant analysis of size corrected morphometric data in first two axis for all the species (a) and first three axis for the taxa closely related to *Indirana salelkari* sp. nov. (b). Scree plot is given in (c).

24.9% SVL vs. 16.1% SVL), deep and narrow buccal cavity (vs. shallower and broader), large choanae (vs. small) and thick vomerines (vs. thin) (Image 3). Furthermore, genetic distance between *I. salelkari* and *I. brachytarsus* is 8.3% for 16S gene and 7.0% for concatenated sequences.

Indirana salelkari sp. nov. differs from *I. gundia* in having tympanum flushing with the lateral side of the head (vs. tympanum protruding out of the lateral side of the head) and discs have marginal groove (vs. discs of males and females have sub-marginal groove). Genetic distance between *I. salelkari* and *I. gundia* is 4.3–4.5 % for 16S gene and 3.2–3.3% for concatenated sequences.

Indirana salelkari sp. nov. is morphologically similar to

I. chiravasi, however it differs from *I. chiravasi* in having thin elongated metatarsal tubercle in males and females (vs. thin shovel shaped in males and thin elongated in females) and deep and narrow buccal cavity (vs. shallow and broad, Image 3, Table 3). Both the species also differ in their placement and structure of vomerine teeth in relation to choanae (Image 3, Table 3) where *I. salelkari* has a much longer and acutely placed vomerine teeth series, which are placed closer to each other with a distance less than the length of vomerine teeth series (vs. shorter vomerine teeth series placed at a distance larger than the length of vomerine teeth series) (Table 3). Further, *I. salelkari* has oval choanae that are more laterally placed in the buccal cavity as compared to *I. chiravasi* which has circular and dorsally placed choanae (Image 3, Table 3). *Indirana salelkari* is genetically different from *I. chiravasi* with a genetic distance 3.8% in 16S rRNA gene and 3.1-3.2% in concatenated sequences.

DISCUSSION

Indirana salelkari sp. nov. is the twelfth species of the monogeneric family Ranixalidae, endemic to the Western Ghats of India. *Indirana chiravasi*, the sister species of *I. salelkari*, is a widely distributed species in northern Western Ghats from 15.4–18.5 ^{0}N latitudes (Modak et al. in prep.). *Indirana salelkari* is known from just south of this range (15.1^{0}N), it is morphologically distinct species and forms a monophyletic group in genetic analysis. Although, the raw genetic distance between the two species is only 3.8%, Vences et al. (2005) have observed differentiation among species ranging from 1–16.5 %. Further, we are delineating the species based on morphology, by studying the available types of known species, and genetic evidence is just used as a support to bolster our claims. The other genetically closely related individuals are identified as *I. beddomii* by Nair et al. (2012) with voucher numbers IND/AA/DD-220, 231, 200, 227 and 230. However, our study of the type material of *I. beddomii* clearly suggests that *I. salelkari* is distinct from *I. beddomii*.

Oral apparatus structure in the tadpoles of *I. chiravasi*, *I. leithii* and *I. semipalmata* (Sekar 1992; Gopalan et al. 2012; Padhye et al. 2014) are similar to that of *I. salelkari*. Bonacci et al. (2008) have suggested that the oral apparatus structure can be related to feeding habits and dietary specializations. We have observed similar feeding habits in *I. leithii*, *I. chiravasi* and *I. salelkari*, where the semiterrestrial tadpoles occupy wet rocks and boulders to feed on the algal matter. Owing to similar food and feeding habits, the members of this genus might have developed similar oral apparatus. Unfortunately, very little information is available on the ecology of *Indirana* and more studies in this respect are essential.

In the IUCN redlist of threatened taxa (IUCN 2014), six species of *Indirana* are currently listed under threatened categories and include *I. gundia* and *I. phrynoderma* under Critically Endangered; *I. brachytarsus*, *I. diplosticta* and *I. leptodactyla* under Endangered; and *I. leithii* under Vulnerable. Two species, *I. gundia* and *I. phrynoderma*, are also Alliance for Zero Extinction (AZE) species (Parr et al. 2009). Further, there are reports of chytrid infection in *Indirana brachytarsus* and *I. leithii* (Nair et al. 2012, Dahanukar et al. 2013; Molur et al. 2015). The fact that half of the known species in this endemic family are threatened calls for immediate attention towards their conservation. Given that the taxonomy and distribution of several of these species is still ambiguous and there is also possibility of discovering cryptic species within the genus (Nair et al. 2011; Modak et al. 2014), further explorations and molecular studies are essential to reveal conservation status of this taxon.

Comparative Material

Indirana beddomii (n=12): Syntype, NHM 1947.2.27.72 (female), Syntype, NHM 1947.2.27.82 (Female), Syntype, NHM 1947.2.27.83 (Male), Syntype, NHM 1947.2.27.85 (female), 4 exs., Malabar, collected by Col. Beddomme; Syntypes, NHM 1947.2.27.89–91 (Females), 3 exs. Anamallays (=Annamalai), collected by Col. Beddomme; Syntype, NHM 1947.2.4.86 and 87 (females), NHM 1947.2.4.88 (male), 3 exs., Sevagherry (=Sivagiri, Tamil Nadu), collected by Col. Beddomme; Syntype, NHM 1947.2.27.87 (Female), Syntype NHM 1947.2.27.88 (male), 2 exs., Travancore, collected by Col. Beddomme.

Indirana brachytarsus (n=2): Lectotype, NHM 1947.2.27.92 (female), 1 exs., Anamallays (=Annamalai), collected by Col. Beddomme; Paralactotype, NHM 1947.2.2.85 (female), 1 exs., Sevagherry (=Sivagiri, Tamil Nadu), collected by Col. Beddomme.

Indirana diplosticta (n=3): Syntypes, NHM 1947.2.2.21 and 23 (females), 2 exs., Malabar, collected by Col. Beddomme; Syntype, NHM 1947.2.2.22 (Male), 1 ex., Malabar, collected by Col. Beddomme.

Indirana gundia (n=18): Holotype, MNHN 1985.0633 (Male), 26.vii.1984, 1 ex., Gundia, forêt de Kemphole, à l'ouest de Sakleshpur, Karnataka, Inde (Gundia, Kemphole, west of Sakleshpur, Karnataka, India), collected by 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, west of Sakleshpur, Karnataka, India), collected by A. Dubois. WILD-14-AMP-499 (Male), 1 ex., 29.vii.2014, Gundya, Karnataka (12.825^{0}N & 75.569^{0}E, 128m), collected by A. Padhye, N. Modak and S. Sulakhe; WILD-14-AMP-500 (female), 1 ex., 29.vii.2014, Gundya, Karnataka (12.829^{0}N & 75.607^{0}E, 224m), collected by A. Padhye, N. Modak and S. Sulakhe.

Indirana leithii (n=6): Topotype, BNHS 2830-31, BNHS 2833, BNHS 2838-39 (females), 8.viii.1991, 5 exs., Matheran, Mumbai, India, collected by A. G. Sekar and V. Hegde; Topotype, BNHS 5590 (female), 30.ix.12, 1 exs., Matheran, Mumbai, India, collected by N. Modak and A. Bayani.

Indirana leptodactyla (n=4): Syntype, NHM 1947.2.29.39-40 (females), 2 exs., Malabar, collected by Col. Beddomme; Syntype NHM 1947.2.29.41 (male), 1 exs., Malabar, collected by Col. Beddomme; Non-Type, NHM 1897.1.10.11 (female), 1 exs. Devicolum, Travancore, 1220–2130 m., collected by Fergusson.

Indirana phrynoderma (n=2): Syntypes, NHM 1947.2.3.8-9 (males), 2 exs., Anamallays (=Annamalai), collected by Col. Beddomme.

Indirana semipalamata (n=2): Syntype, NHM 1947.2.29.50 (female), 1 ex., Malabar, collected by Col. Beddomme; Syntype, NHM 1947.2.29.51 (male), 1 ex., Malabar, collected by Col. Beddomme.

Indirana chiravasi (n=7): Holotype, BNHS 5888, male, 27.3mm SVL, India: Maharashtra: Sindhudurg District: Amboli, collected on 11.vi.2013 by Nikhil Modak, Neelesh Dahanukar, Keerthi Krutha and Unmesh Katwate. Paratype, BNHS 5889, female, 39.2mm SVL, India: Maharashtra: Sindhudurg District: Amboli, collected on 9.vi.2014 by Nikhil Modak. Paratype, BNHS 5890, male, 25.0mm SVL, India: Maharashtra: Sindhudurg District: Amboli, collected on 11.vi.2013 by Nikhil Modak, Neelesh Dahanukar, Keerthi Krutha and Unmesh Katwate; Paratype, WILD-14-AMP-489, male, 24.7mm SVL, India: Maharashtra: Sindhudurg District: Amboli, collected on 11.vi.2013 by Nikhil Modak, Neelesh Dahanukar, Keerthi Krutha and Unmesh Katwate; Paratype, WILD-14-AMP-490, 31.7mm SVL, female, 39.2mm SVL, India: Maharashtra: Sindhudurg District: Amboli, collected on 9.vi.2014 by Nikhil Modak; Paratype, WILD-14-AMP-491, male, 25.6mm SVL, India: Maharashtra: Sindhudurg District: Amboli, collected on 19.vii.2013 by Nikhil Modak, Neelesh Dahanukar, Keerthi Krutha and Unmesh Katwate; Paratype, ZSI-WRC A/1541, male, 25.2mm SVL, India: Maharashtra: Sindhudurg District: Amboli, collected on 11.vi.2013 by Nikhil Modak, Neelesh Dahanukar, Keerthi Krutha and Unmesh Katwate.

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

Species	Voucher			GenBank Accession number		
	12S	16S	Rho	12S	16S	Rho
Indirana salelkari sp. nov.	BNHS 5931			KP826821	KP826824	KP826827
Indirana salelkari sp. nov.	WILD-15-AMP-551			KP826822	KP826825	KP826828
Indirana salelkari sp. nov.	AGCZRL Amphibia 210			KP826823	KP826826	KP826829
Indirana chiravasi	BNHS 5890			KM386527	KM386531	KM386539
Indirana chiravasi	WILD-14-AMP-489			KM386526	KM386530	KM386538
Indirana gundia	WILD-14-AMP-499			KM386528	KM386532	KM386540
Indirana gundia	WILD-14-AMP-500			KM386529	KM386533	KM386541
Indirana leithii	BNHS 5590			KF590627	KF590637	KF590647
Indirana leithii	BNHS 5591			KF590628	KF590638	KF590648
Indirana cf. *leithii*	IND212	AA212	DD212	JQ596717	JQ596673	JQ596778
Indirana semipalmata	IND256	AA256	DD256	JQ596715	JQ596671	JQ596787
Indirana semipalmata	IND245	AA245	DD245	JQ596713	JQ596669	JQ596785
Indirana semipalmata	IND257	AA257	DD257	JQ596716	JQ596672	JQ596788
Indirana semipalmata	IND255	AA255	DD255	JQ596714	JQ596670	JQ596786
Indirana semipalmata	IND243	AA243	DD243	JQ596712	JQ596668	JQ596784
Indirana leptodactyla	IND850	AA850	DD850	JQ596719	JQ596682	JQ596805
Indirana leptodactyla	IND848	AA848	DD848	JQ596721	JQ596684	JQ596803
Indirana leptodactyla	IND851	AA851	DD851	JQ596718	JQ596685	JQ596806
Indirana leptodactyla	IND849	AA849	DD849	JQ596720	JQ596681	JQ596804
Indirana leptodactyla	IND847	AA847	DD847	JQ596722	JQ596683	JQ596802
Indirana brachytarsus	IND71	AA71	DD71	JQ596690	JQ596646	JQ596800
Indirana brachytarsus	IND638	AA638	DD638	JQ596691	JQ596647	JQ596799
Indirana diplosticta	IND92	AA92	DD92	JQ596698	JQ596654	JQ596813
Indirana diplosticta	IND94	AA94	DD94	JQ596700	JQ596656	JQ596815
Indirana diplosticta	IND91	AA91	DD91	JQ596697	JQ596653	JQ596812
Indirana diplosticta	IND93	AA93	DD93	JQ596699	JQ596655	JQ596814
Indirana diplosticta	IND98	AA98	DD98	JQ596701	JQ596657	JQ596816
Indirana beddomii	IND77	AA77	DD77	JQ596688	JQ596644	JQ596795
Indirana beddomii	IND175	AA175	DD175	JQ596692	JQ596648	JQ596773
Indirana beddomii	IND180	AA180	DD180	JQ596694	JQ596650	JQ596775
Indirana beddomii	IND193	AA193	DD193	JQ596696	JQ596652	JQ596777
Indirana beddomii	IND220	AA220	DD220	JQ596708	JQ596664	JQ596779
Indirana beddomii	IND230	AA230	DD230	JQ596710	JQ596666	JQ596782
Indirana beddomii	IND244	AA244	DD244	JQ596729	JQ596674	JQ596789
Indirana beddomii	IND724	AA724	DD724	JQ596726	JQ596676	JQ596791
Indirana beddomii	IND246	AA246	DD246	JQ596728	JQ596675	JQ596790
Indirana beddomii	IND800	AA800	DD800	JQ596727	JQ596677	JQ596792
Indirana beddomii	IND178	AA178	DD178	JQ596693	JQ596649	JQ596774
Indirana beddomii	IND189	AA189	DD189	JQ596695	JQ596651	JQ596776
Indirana beddomii	IND200	AA200	DD200	JQ596707	JQ596663	JQ596780
Indirana beddomii	IND75	AA75	DD75	JQ596687	JQ596643	JQ596794
Indirana beddomii	IND227	AA227	DD227	JQ596709	JQ596665	JQ596781
Indirana beddomii	IND231	AA231	DD231	JQ596711	JQ596667	JQ596783

Appendix A. contd.

Species	Voucher			GenBank Accession number		
	12S	16S	Rho	12S	16S	Rho
Indirana beddomii	IND72	AA72	DD72	JQ596686	JQ596642	JQ596793
Indirana sp.	IND88	AA88	DD88	JQ596703	JQ596659	JQ596809
Indirana sp.	IND95	AA95	DD95	JQ596705	JQ596661	JQ596808
Indirana sp.	IND99	AA99	DD99	JQ596706	JQ596662	JQ596811
Indirana sp.	IND89	AA89	DD89	JQ596704	JQ596660	JQ596810
Indirana sp.	IND87	AA87	DD87	JQ596702	JQ596658	JQ596807
Micrixalus fuscus	MF5111	MF3006	NA	GU143817	GU136106	AF249120
Micrixalus kottigeharensis	NA	NA	NA	AF249025	AF249041	AF249121
Nyctibatrachus aliciae	NA	NA	NA	AF249018	AF249063	AF249114
Nyctibatrachus major	NA	NA	NA	AF249017	AF249052	AF249113
Nasikabatrachus sahyadrensis	BNHS 4202			AY364360	AY364381	AY364381

NA = not available

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