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Article



# Small-sized dicroglossids from India, with the description of a new species from West Bengal, India

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## Abstract

A new small-sized species of dicroglossid frog from West Bengal is described as *Minervarya chilapata* **sp. nov.** and compared to *Minervarya sahyadris*. It differs from all *Fejervarya* species by its smaller size, by the presence of a distinct white band on upper lip and by the presence of a rictal gland. The new species is separable from its congener in showing a more pointed snout, smaller tympanum and more developed webbing. Its advertisement call is described and compared to that of *M. sahyadris*. Generic allocation is discussed. In the *Fejervarya* lineage, a trend towards small-sized species may exist. The species allocated to *Minervarya* show an important morphological shift, presumably reflecting occupation of a different adaptive niche which might indicate generic distinctiveness.

Key words: Anura, Dicroglossidae, Minervarya chilapata sp. nov., morphometry

## Introduction

Amphibian species described recently show decrease in genetic divergence between taxa compared to those described until 1991, but a significant increase if considering species described from 1992 to 2004 (Köhler *et al.* 2005). Size seems to be decreasing since the early days of taxonomy: the first 440 frogs described in 18<sup>th</sup> and early 19<sup>th</sup> century measured 10 to 150 mm with a mean of 68.4 mm whereas the last 440 species described before 2000 measured 9.6 to 132 mm with a mean of 35.8 mm (Alain Dubois, unpublished data).

Discovery of new frog species is an ongoing activity. In fact new methods and concepts, explorations of new territories, closed for scientists previously, open the door to new discoveries. Nevertheless, species are also discovered in relatively well explored places, such as West Bengal State, in eastern India. This state is one of the first explored on the Indian subcontinent by the British. Though the north-eastern part of India is considered one of the earth's biodiversity hotspots (Myers *et al.* 2000), and underexplored, holding numerous discoveries, the Brahmaputra and Ganges plain is not considered of particular interest for biodiversity.

South-western India is a region of high endemism in frogs holding several unique ranoid lineages due to a considerable period of isolation (Bossuyt *et al.* 2006). Its contact with Laurasia is considered secondary. Distribution pattern of many Indian taxa are either of south-western or of north-eastern type. It is mostly

common species of frogs that have large distribution including all India. The monospecific genus *Minervarya* Dubois, Ohler & Biju, 2001 was considered to be an endemic of south-west India (Dubois *et al.* 2001).

When intensive sampling for amphibians was conducted in the Chilapata Reserve Forest of West Bengal by one of us (SP), a series of 18 anuran species could be inventoried. Among these species was a particularly small-sized form, first only discovered by adult males which were calling. These small frogs could easily have been unnoticed because of their size, cryptic coloration and their habit to hide under vegetation.

The small frog described from south-western India (Dubois *et al.* 2001), *Minervarya sahyadris* has a series of exceptional characters among the members of Dicroglossidae. A new genus was erected for this species based on its unique character combination among ranoid frogs having forked omosternum, a rictal gland, rudimentary webbing, dorsal skin showing longitudinal folds, "Fejervaryan" line on side of belly and upper lip with a white horizontal line (Dubois *et al.* 2001). A recent molecular study placed *Minervarya* with low support among the *Fejervarya* radiation (Kuramoto *et al.* 2007), and it was considered a synonym of this genus. However, *Minervarya* is distinctly smaller than all other species of *Fejervarya* described (Kuramoto *et al.* 2007). Multivariate principal component analysis among taxa of Dicroglossidae showed an important discrimination of *Minervarya* specimens from *Fejervarya* specimens (Dubois *et al.* 2001). Various *Fejervarya* species formed a unique cluster, as do species of *Sphaerotheca*. Morphological differentiation of *Minervarya* (Dubois *et al.* 2001: Fig. 2).

In the following paper the new species is described, the generic allocation of the new species is assessed and the validity of *Minervarya* is discussed. The significance of small size in dicroglossid frogs is discussed and a biogeographic interpretation of the new distribution pattern is given.

#### Material and methods

**Specimens studied.** *Minervarya sahyadris*: **India**, Karnataka, Gundia: MNHN 2000.3026-35; Kerala, Thiruvanpady area: MNHN 2000.3036.

Minervarya sp. nov.: ZSI A 10784-A 10793.

Abbreviations for measurements. EL—eye length; EN—distance from front of eye to nostril; FFTF distance from maximum incurvation of web between fourth and fifth toe to tip of fourth toe; FL-femur length (from vent to knee); FLL-forelimb length (from elbow to base of outer tubercle); FOL-foot length (from base of inner metatarsal tubercle to tip of toe); FTL-fourth toe length (from base of first subarticular tubercle); HAL—hand length (from base of outer palmar tubercle to tip of longest finger); HL—head length (from back of mandible to tip of snout); HW—head width; IBE—distance between back of eyes; IFEdistance between front of eyes; IMT-length of inner metatarsal tubercle; IN-internasal space; ITL-inner toe length; IUE—minimum distance between upper evelids; MBE—distance from back of mandible to back of eye; MFE—distance from back of mandible to front of eye; MN—distance from back of mandible to nostril; MTFF-distance from distal edge of metatarsal tubercle to maximum incurvation of web between fourth and fifth toe; MTTF---distance from distal edge of metatarsal tubercle to maximum incurvation of web between third and fourth toe; NS-distance from nostril to tip of snout; SL-distance from front of eye to tip of snout; SN-distance from nostril to tip of snout; SVL-snout-vent length; TFL-third finger length (from base of first subarticular tubercle); TFOL-distance from base of tarsus to tip of fourth toe; TFTF-distance from maximum incurvation of web between third and fourth toe to tip of fourth toe; TL-tibia length; TWmaximum width of shank; TYD-greatest tympanum diameter; TYE-distance from tympanum to back of eye; UEW-maximum width of inter upper eyelid.

**Measurements.** Measurements have been taken with slide-callipers to the precision of 0.1 mm or with an ocular micrometer in a binocular microscope. These measurements were transferred to mm with a precision of 0.01 mm. Choice of tool depended on size and kind of distance measured.

**Morphometric analysis and statistical test.** All measurements were transformed to their ratio to SVL and given as per thousands. These transformed measurements are indicated by an R apposed to their abbreviation as given above.

Morphometrical analysis and graphs were made using the SPSS statistical programs for personal computers (Norusis 1992). Non-parametric tests were performed as sample size is small and these tests are more robust in such case. Mann-Whitney U tests were performed to show significant differences between single measurements or ratios. We used principal component analysis on ratios of measurements of adult male specimens to show morphological distinctiveness of the new species from *Minervarya sahyadris*. Principal components resulting from this analysis were submitted to multivariate ANOVA to test for discrimination potential in relation to species allocation. Significance levels for statistical tests were adapted to small sample size as follows: \*\*\* -  $p \le 0.001$ ; \*\* - 0.001 <  $p \le 0.01$ ; \*\* - 0.01 <  $p \le 0.1$ ; n.s. – Not significant.

**Call recording.** The advertisement call of one specimen (ZSI A 10787; SVL 19.6 mm) was recorded in the Chilapata Reserve Forest, on the 29<sup>th</sup> of July, 2007. Recording was made using an AIWA JS-195 audio cassette recorder and a hand-held AIWA stereophonic microphone. The recorded call was analysed using Canary 1.2.4 sound analysis software from the Cornell Laboratory of Ornithology (Charif *et al.* 1995) on a Power Mac G4 computer. The sampling rate used to convert the signals to digital format was 22.05 KHz with 16-bit precision. Filter bandwidths of 349.70 Hz and frame length of 256 points were used for spectrogram building.

**Nomenclatural terms.** *Holophoront*, a unique name bearing type (holotype); *onymotope*, geographical place of origin of holophoront (type locality); *paratopophoronts*, specimens originating from same place and population as holophoront and considered conspecific to the holophoront, but without nomenclatural function (paratopotypes).

## Description of the new species

#### Minervarya chilapata sp. nov.

**Holophoront.** ZSI A 10784, adult male (SVL 20.3 mm), collected on 29 July 2007 by Subhadip Paul and Kaushik Deuti.

**Onymotope.** Beside rainwater pools alongside forest road inside Mendabari Beat of Chilapata Reserve Forest (26°36'N, 89°24'E), Jalpaiguri District, West Bengal State, India.

**Paratopophoronts.** ZSI A 10785–A 10793, 7 adult males and 2 adult females, collected by Anand Kumar Ayyaswamy and Kaushik Deuti at the same locality as the holophoront.

Description of holophoront. ZSI A 10784, adult male (Fig. 1).

(A) Size and general aspect. (1) Specimen of small size (SVL 20.3 mm), body moderately slender.

(B) Head. (2) Head of moderate size, longer (HL 7.9 mm) than wide (HW 6.5 mm; MN 7.24 mm; MFE 5.79 mm; MBE 3.16 mm), convex. (3) Snout pointed, protruding, its length (SL 3.44 mm) longer than horizontal diameter of eye (EL 2.92 mm). (4) Canthus rostralis rounded, loreal region concave, angle to upper surface of snout rather vertical. (5) Interorbital space convex, larger (IUE 1.94 mm) than upper eyelid (UEW 1.88 mm) and narrower than internarial distance (IN 2.01 mm); distance between front of eyes (IFE 4.21 mm) three quarter of distance between back of eyes (IBE 5.64 mm). (6) Nostrils rounded, with flap of skin laterally, closer to eye (EN 1.56 mm) than to tip of snout (NS 1.62 mm). (7) Pupil indistinct. (8) Tympanum (TYD 1.36 mm) poorly distinct, rounded; less than half of eye diameter, tympanum-eye distance (TYE 0.71 mm) half its diameter. (9) Pineal ocellus present, between anterior borders of eyes. (10) Vomerine ridge absent. (11) Tongue rather large, cordate, emarginate; median lingual process absent; tooth like projections on maxilla absent.

 IV < III. (15) Tips of fingers bluntly rounded, not enlarged. (16) Fingers without dermal fringe; webbing absent. (17) Subarticular tubercles prominent, rounded, single, all present. (18) Prepollex oval, distinct; a single round, indistinct palmar tubercle; supernumerary tubercles absent.

(**D**) **Hindlimbs.** (19) Shanks three times longer (TL 9.9 mm) than wide (TW 3.05 mm), longer than thigh (FL 8.5 mm), but shorter than distance from base of internal metatarsal tubercle to tip of toe IV (FOL 11.84 mm). (20) Toes long, thin; toe IV long (FTL 6.84 mm) more than one third of distance from base of tarsus to tip of toe IV (TFOL 15.5 mm). (21) Relative length of toes, shortest to longest: I < II < V < III < IV. (22) Tips of toes rounded, not enlarged. (23) Webbing small: I - 2 II - 2 1/2 III - 2 I/2 III - 2 IV 3 - 1 V (MTTF 5.44 mm; MTFF 5.51 mm; TFTF 5.25 mm; FFTF 5.51 mm). (24) Dermal fringe along toe V absent. (25) Subarticular tubercles prominent, oval, simple, all present. (26) Inner metatarsal tubercle short, prominent; its length (IMT 0.78 mm) 2.72 times in length of toe I (ITL 2.12 mm). (27) Inner tarsal ridge present, flat. (28) Outer metatarsal tubercle present, small, rounded; supernumerary tubercles absent; tarsal tubercle absent.

(E) Skin. (29) Dorsal and lateral parts of head and body: snout, between eyes, side of head, anterior part of back and flanks smooth; posterior part of back with indistinct, glandular warts. (30) Latero-dorsal folds absent (light latero-dorsal bands in same position); lateral line system absent; "Fejervaryan" line present; supratympanic fold distinct, from eye to above shoulder. (31) Dorsal parts of limbs: forelimbs and tarsus smooth; thigh shagreened; shank with flat glandular warts. (32) Ventral parts of head, body and limbs: throat with small, dense glandular warts; chest, belly and thigh shagreened. (33) Rictal gland present, two small glands posterior to mouth; other macroglands absent.

(F) Coloration in alcohol. (34) Dorsal and lateral parts of head and body: dorsal parts of head and dorsum brown with light mid-dorsal line and darker indistinct spots, a darker line on each side of posterior back; a dark band from eye to groin; lower flank light grey; loreal region, tympanic region, supratympanic fold and tympanum dark brown; upper lip whitish; rictal gland white. (35) Dorsal parts of limbs: forelimbs light grey with indistinct darker bands; thigh, shank and foot light grey with grey bands; posterior part of thigh with white and blackish longitudinal bands. (36) Ventral parts of head, body and limbs: throat creamy white; margin of throat light grey without spots or bands; chest creamy white; belly and thigh creamy white with greyish spots. Webbing dark grey.

(G) Male secondary sexual characters. (37) Nuptial spines present, one single patch on prepollex and finger I up to subarticular tubercle: numerous, very small, whitish, transparent spines. (38) Vocal sacs present, unique subgular pouch; pair rounded openings in posterior part of mouth floor. (39) No other male secondary characters.

**Intrapopulational variation.** Morphometric variation is shown in table 1. Coloration is very similar throughout the sample. In some specimens the middorsal line is almost indistinct on the head, whereas in others it is a fine neat line. The colour in life of middorsal line is usually light orange, but can be light yellow or beige.

**Coloration in life.** Dorsal parts of head and body greyish beige with light orange middorsal line and darker greyish beige longitudinal lines. Upper flank greyish beige, lower part greyish brown; loreal region dark greyish brown with fine blackish canthal stripe; tympanic region and tympanum dark greyish brown with fine blackish stripe along tympanic fold; upper lip golden white. Forearm and dorsal part of thigh light orange with indistinct grey brown bands; dorsal part of shank and foot beige with grey brown bands; hind part of thigh black with yellowish longitudinal stripes. Throat and margin of throat light grey with some golden spots; vocal sacs denser grey; chest and belly whitish with golden shine; "Fejervaryan" line distinct, dark grey; ventral part of thigh greyish. Web greyish brown. Nuptial spines light grey.

**Natural history.** Chilapata Reserve Forest is a small (41 km<sup>2</sup>) moist deciduous forest between Jaldapara Wildlife Sanctuary and Buxa Tiger Reserve in Jalpaiguri District of West Bengal State, India. The forest is flanked by the Torsa and Kaljani rivers flowing down from Bhutan into the '*Duars*' area of Bengal. It is divided into three beats – Chilapata, Bania and Mendabari. Recently 18 species of amphibians have been recorded from this forest.

	<i>Minervarya chilapata</i> male N=8	<i>Minervarya</i> sahyadris male N=10	Mann-Whitney U test	<i>Minervarya</i> <i>chilapata</i> female N=2	<i>Minervarya sahyadris</i> female N=4	Mann-Whitney U test
svl	$20.0 \pm 6.95$ 18.6–20.9	$18.4 \pm 6.01$ 17.2–19.2	U = 4.0 p = 0.000 ***	23.8–25.1	22.1 ± 9.16 20.6–23.0	U = 0.0 p = 0.095 *
hw	$306 \pm 11.52$ 290–325	$342 \pm 15.78$ 317–369	U = 5.0 p = 0.000 ***	277–295	$326 \pm 6.09$ 316–331	U = 0.0 p = 0.095 *
hl	375 ± 15.36 362–411	$392 \pm 22.82$ 365-448	U = 30.0 p = 0.019 *	357–406	$366 \pm 16.62$ 345-383	U = 3.0 p = 0.571 n.s.
mn	364 ± 14.93 330–384	366 ± 33.13 331–428	U = 23.0 p = 0.448 n.s.	354–362	$328 \pm 24.54$ 302-352	U = 0.0 p = 0.133 n.s.
mfe	$272 \pm 10.52$ 251-285	$297 \pm 32.03$ 266–359	U = 8.0 p = 0.017 *	271–275	$266 \pm 16.73$ 242–281	U = 2.0 p = 0.533 n.s.
mbe	$157 \pm 15.16$ 128–174	$172 \pm 21.07$ 147–206	U = 20.0 p = 0.278 n.s.	155–168	$160 \pm 10.21$ 147–169	U = 4.0 p = 1.000 n.s.
ife	$183 \pm 14.44$ 157–207	$180 \pm 16.95$ 155–211	U = 65.0 p = 0.796 n.s.	150–177	$173 \pm 9.88$ 160–184	U = p = 0.571  n.s.
ribe	$261 \pm 11.00$ 244–278	$278 \pm 12.22$ 258–302	U = 22.0 p = 0.004 **	232–245	$262 \pm 10.18$ 250–273	U = 0.0 p = 0.095 *
rfll	$211 \pm 9.68$ 189–225	$194 \pm 22.88$ 161–234	U = 35.0 p = 0.042 *	202–204	$194 \pm 12.46$ 177–210	U = 2.0 p = 0.381 n.s.
rhal	$\begin{array}{c} 235\pm8.96\\ 222247\end{array}$	$226 \pm 19.36$ 191–264	U = 48.0 p = 0.212 n.s.	239–245	$218 \pm 14.15$ 201–240	U = 1.0 p = 0.190 n.s.
tfl	$140 \pm 7.49$ 125–153	126 ± 17.64 96–159	U = 32.0 p = 0.026 *	136–139	$130 \pm 3.18$ 126–134	U = 0.0 p = 0.095 *
tl	$473 \pm 13.70$ 444 - 489	$454 \pm 24.34$ 408-497	U = 32.0 p = 0.022 *	458–466	$427 \pm 16.75$ 403-443	U = 0.0 p = 0.095 *
fol	$544 \pm 22.93$ 517–583	$531 \pm 40.21$ 455–585	U = 60.0 p = 0.585 n.s.	553–587	$492 \pm 25.00$ 456-511	U = 0.0 p = 0.095 *
ftl	$335 \pm 13.23$ 311 - 353	$308 \pm 26.71$ 263–335	U = 8.0 p = 0.016 *	337–367	$311 \pm 15.82$ 293–328	U = 0.0 p = 0.133 n.s.
rin	$97 \pm 4.23$ 91-105	$100 \pm 5.32$ 92–106	U = 20.0 p = 0.313 n.s.	93–95	$96 \pm 3.36$ 93–100	U = 3.0 p = 0.800 n.s.
en	$\begin{array}{c} 81 \pm 5.24 \\ 72 - 88 \end{array}$	$77 \pm 7.86$ 68–92	U = 16.0 p = 0.147 n.s.	76–80	$\begin{array}{c} 76\pm5.42\\ 6880\end{array}$	U = 2.0 p = 0.533 n.s.
el	$134 \pm 7.72$ 122–149	$132 \pm 9.15$ 110–143	U = 62.0 p = 0.666 n.s.	125–132	$131 \pm 2.55$ 127–133	U = 3.0 p = 0.571 n.s.
tyd	51 ± 7.68 42–67	$64 \pm 5.21$ 55–72	U = 15.0 p = 0.001 ***	47–49	66 ± 6.11 62–76	U = 0.0 p = 0.095 *
tye	$38 \pm 8.00$ 22-52	$23 \pm 3.80$ 20–29	U = 3.0 p = 0.002 **	44–54	$22 \pm 4.37$ 17–26	U = 0.0 p = 0.133 n.s.
imt	$35 \pm 3.57$ 30-40	$37 \pm 6.52$ 25-48	U = 46.0 p = 0.172 n.s.	33–42	$37 \pm 4.97$ 30-42	U = 5.0 p = 1.0 n.s.

**TABLE 1.** Mean values of morphometric data of *Minervarya chilapata* **sp. nov.** and *Minervrya sahyadris*  $\pm$  standard deviation and range. Non-parametric comparison of *Minervarya chilapata* **sp. nov.** with *Minervarya sahyadris* based on Mann-Whitney U test. Abbreviation of measurements as given above; N—number; U—Mann-Whitney U; p— probability. Significance level: \*\*\* —p < 0.001: \*\*—0.001 <p< 0.01: \*-—0.01 <p< 0.1: n.s.—not significant.

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TABLE 1. (continued)

	<i>Minervarya</i> <i>chilapata</i> male N=8	<i>Minervarya</i> sahyadris male N=10	Mann-Whitney U test	<i>Minervarya</i> <i>chilapata</i> female N=2	<i>Minervarya</i> sahyadris female N=4	Mann-Whitney U test
ritl	$109 \pm 8.35$ 91–121	$92 \pm 10.63$ 75–117	U = 15.0 p = 0.001 ***	114–120	95 ± 7.49 83–100	U = 0.0 p = 0.095 *
rmttf	261 ± 11.31 246–280	$186 \pm 10.15$ 173–202	U = 0.0 p = 0.000 ***	254–273	$178 \pm 11.65$ 164–192	U = 0.0 p = 0.133 n.s.
rmtff	273 ± 18.60 248–292	$188 \pm 9.03$ 176–202	U = 0.0 p = 0.000 ***	266–283	185 ± 7.98 176–195	U = 0.0 p = 0.133 n.s.
rtftf	$256 \pm 16.96$ 220–283	$299 \pm 22.20$ 263–331	U = 3.0 p = 0.002 **	249 - 283	$302 \pm 15.10$ 283–320	U = 1.0 p = 0.267 n.s.
rfftf	$264 \pm 17.09$ 227–290	311 ± 19.10 277–334	U = 2.0 p = 0.001 ***	254 - 294	$308 \pm 8.76$ 299–320	U = 0.0 p = 0.133 n.s.

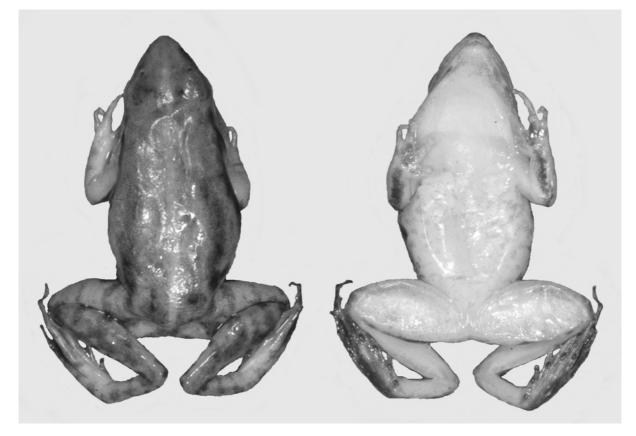


FIGURE 1. Minervarya chilapata. Holophoront, adult male, ZSI A 10784, SVL 20.3 mm. Dorsal and ventral view.

Specimens have been observed sitting on the ground or under low shrubs near ditches. Calling males were about 10 to 30 cm from the water (Fig. 2). The two adult females were found at a distance of 1-2 m from the ditch, in the grassy band near the road. Some male specimens were sitting on ferns and leaves about 20 cm above ground.

Advertisement call. A part of a call (four notes) of specimen ZSI A 10787 was recorded on 29.07.2007, air temperature was  $33^{\circ}$ C and water temperature was  $31^{\circ}$ C. It consists of a succession of long trilled notes. The sequence comprising these four notes last 13.57 s. Note repetition rate is 0.295 note/s. The duration of the notes and the duration of the inter-note intervals between notes increase during the call from 0.858 s to 1.913 s (mean 1.424 s, n = 4) for the note duration and from 2.219 s to 3.032 s (mean 2.551 s, n = 3) for the silent

duration. The notes are composed of groups of ill-defined impulsions resulting in a trilled note (Fig. 3A). The number of groups of impulsions in each note also increases during the call from 11 to 27 (mean 19.5, n = 4). On the contrary, these groups of impulsions are of constant duration within each note and between notes (about 70.0 ms with a range from 65.0 ms to 75.0 ms). Each group of impulsions consists of a big part which begins abruptly immediately followed by a small part, both parts being composed of several ill-defined and totally irregular impulsions (Fig. 3B). The dominant frequency, which is also the fundamental, lies between 2810 and 3870 Hz with a peak at about 3500 Hz. Two harmonic bands are visible, the first one at about 7000 Hz and the second one at about 10500 Hz. The second harmonic band appears to be more emphasized than the first one.

**Etymology.** Scientific name is derived from "Chilapata", the name of the onymotope, as a noun in apposition, invariable.



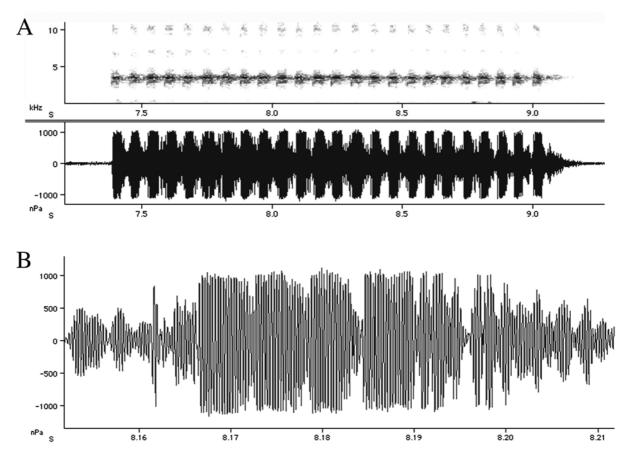
**FIGURE 2.** *Minervarya chilapata*. Left: Specimen in life, 29 July 2007; right: Specimen in natural position in the field, June 2008.

Comparison with Minervarya sahyadris. There is a series of morphometrical and morphological differences between the new species and Minervarya sahyadris. Comparison of males with Mann-Whitney U test shows 16 statistically significant differences in 25 measurements (Table 1). M. chilapata is significantly larger than M. sahyadris, but it has smaller head and smaller tympanum size. M. chilapata has shorter forelimb length, but longer tibia length, as well as longer finger and toe length. The webbing of this species is significantly larger than the webbing of *M. sahyadris*. Principal component analysis separates specimens of both species (Fig. 4, Table 2). Six principal component factors show loadings higher than 1 and explain 89.2 % of the overall variance. Principal components obtained were tested for their discriminant potential by ANOVA analysis and only principal component factor 1 shows significant discrimination in relation to taxonomic group. This component describes 40.9 % of overall variation. Head width (HW, IBE), tympanum diameter (TYD) and incurvation of webbing (TFTF, FFTF) show high positive loading whereas size (SVL), tympanums eye distance (TYE), length of inner toe (ITL) and extension of webbing (MTTF, TFTF) show high negative loadings for factor 1 and thus participate on discrimination of the two species. Vomerine teeth are absent in *M. chilapata*, but present in *M. sahyadris*. On the opposite, pineal ocellus is present in *M.* chilipata, but absent in M. sahyadris. The snout is more pointed in M. chilapata then in M. sahyadris. The tympanum is smaller in *M. chilapata* than in *M. sahyadris*. Differences exist in relative length of fingers: IV is shortest in *M. sahyadris*, whereas in *M. chilapata*, finger I is shortest; a pair of palmar tubercles is present in *M. sahyadris*, but a single tubercle, less distinct, in *M. chilapata*; the webbing is much larger in *M. chilapata* 

than in *M. sahyadris* (Fig. 5) leaving only three phalanges free in *M. chilapata* but four in *M. sahyadris*. The stripe on the upper lip is white in *M. sahyadris* and golden white in *M. chilapata*. The middorsal coloration is much more vivid, described as reddish, reddish brown, etc., in *M. sahyadris*, but greyish beige in *M. chilapata*. The middorsal line is creamish, golden yellowish or reddish in *M. sahyadris*, light orange or light yellow in *M. chilapata*.

Both species show a single nuptial pad which goes up to half penultimate phalange in *M. sahyadris*, but only to subarticular tubercle in *M. chilapata*.

The numerous differences in morphology, morphometrics and coloration give support to recognizing the specimens collected in West Bengal as a new species.



**FIGURE 3.** Sonagram and oscillogram of the advertisement call of *Minervarya chilapata*, ZSI A 10787, SVL 19.6 mm. (A). Third note showing its trilled appearance (B). Detail of one group of impulsions from the middle of the third note showing the two parts and the irregular impulsions which compose them.

## Discussion

**Generic classification and allocation.** Kuramoto *et al.* (2007) proposed *Minervarya* as a synonym of *Fejervarya* based on molecular data. In fact they published a phylogenetic tree for six species of *Fejervaya* from the Western Ghats which is redrawn from Kurabayashi *et al.* (2005) with modifications on the basis of additional unpublished data. The figure 4 of Kurabayashi *et al.* (2005) includes, for the South Asian clade of *Fejervarya*, not only species from southern India but also from Sri Lanka and does not include *M. sahyadris*. The nodes of this phylogenetic tree show a low support and the groupings proposed are different from those of the redrawn tree for which no information on statistical support is given. As the molecular phylogenetic placement of the small species within *Fejervarya* is not well supported and the two small species have several very distinct characters and likely form a monophyletic group, we think that the most appropriate solution to

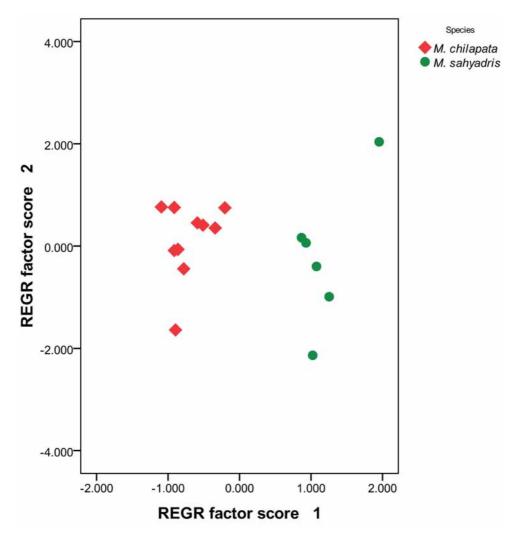
leave *Minervarya* as valid genus until the phylogeny of the whole *Fejervarya/Minervarya* lineage is reliably solved by molecular data.

Total Variance Explained							
Componen Initial Eigenvalues				Extraction Sums of Squared Loadings			
t	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	
1	10.226	40.904	40.904	10.226	40.904	40.904	
2	4.597	18.390	59.294	4.597	18.390	59.294	
3	2.742	10.966	70.260	2.742	10.966	70.260	
4	2.422	9.689	79.949	2.422	9.689	79.949	
5	1.260	5.041	84.991	1.260	5.041	84.991	
6	1.043	4.172	89.162	1.043	4.172	89.162	

TABLE 2. Orthogonal score weights from PCA of morphometric data for 16 adult males of Minervarya.

## **Component Matrix**<sup>a</sup>

	PC1	PC2	PC3	PC4	PC5	PC6
svl	-0.784	-0.252	0.275	0.442	-0.080	0.046
rhw	0.852	0.321	0.282	0.079	0.043	-0.101
rhl	0.658	0.273	-0.429	0.441	-0.074	0.046
rmn	0.269	0.601	-0.526	0.309	-0.225	-0.261
rmfe	0.680	0.511	-0.267	-0.073	-0.394	-0.076
rmbe	0.470	0.416	-0.263	-0.506	-0.469	0.170
rife	0.463	0.593	0.333	0.254	0.146	0.058
ribe	0.736	0.515	0.311	0.141	0.158	0.000
rfll	0.167	0.754	0.108	-0.197	-0.194	0.255
rhal	-0.438	0.697	0.256	-0.357	0.002	-0.313
rtfl	-0.620	0.507	-0.026	-0.075	0.226	-0.277
rtl	-0.597	0.692	0.114	0.048	0.254	-0.143
rfol	-0.624	0.371	0.430	0.179	-0.093	0.489
rftl	-0.598	0.386	0.582	-0.105	-0.162	0.057
rin	0.384	0.462	-0.288	0.523	0.347	0.163
ren	-0.287	0.400	-0.587	-0.174	0.355	0.431
rel	0.304	0.335	0.074	0.631	-0.100	-0.205
rtyd	0.762	-0.087	0.432	0.236	-0.187	0.311
rtye	-0.764	0.345	-0.315	-0.208	0.256	0.042
rimt	0.682	0.097	-0.127	-0.400	0.256	0.204
ritl	-0.734	0.396	0.003	-0.344	-0.235	-0.041
rmttf	-0.908	0.238	0.071	0.252	-0.055	0.047
rmtff	-0.904	0.144	0.095	0.283	-0.124	0.088
rtftf	0.716	0.083	0.480	-0.283	0.219	-0.053
rfftf	0.767	0.047	0.427	-0.253	0.202	-0.106



**FIGURE 4.** Results of principal components analysis (PCA) of 25 morphometric variables for 16 males of *Minervarya*, showing scores for individual specimens on PC1 and PC2. PC1 separates *Minervarya* specimens based largely on the different size, head width, tympanum size, and webbing.

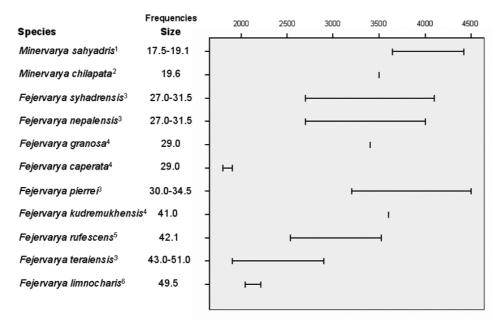
The distinctiveness of the new species form *Fejervarya* species is shown by various characters. The new species shows a white line on upper lip and a rictal gland which are absent in all species of *Fejervarya*. The typical spots on side of throat present in *Fejervarya* are absent in all species of the new species and in *Minervarya sahyadris*. The new species is smaller than all known species of *Fejervarya* but *Minervarya sahyadris*.

**Morphological shift due to miniaturisation.** Considering figure 4 of Kurabayashi *et al.* (2005), the species of *Fejervarya* studied show an evolutionary tendency to get smaller. The basal species (*F. cancrivora* and *F. vittigera*) of the clade are of large size (adult males SVL 46.1-88.1 mm and 37.3-66.6 mm, respectively; Inger 1954). The South-east Asian clade holds species of moderate size (Fig. 6) whereas the South Asian clade, which is the terminal clade, holds both moderate-sized species and small-sized species.

If the phylogenetic position of *Minervarya* proposed by Kuramoto *et al.* (2007) is confirmed, then this very small-sized frog is in terminal position in the phylogenetic tree thus showing an extreme diminution of body size. Morphological comparison of small- and moderate-sized frogs of *Fejervarya* does not show major morphological discrimination (Ohler & Dubois 1999; Veith *et al.* 2001). They show similar body and foot shape. Skin structures like foldings and position of macroglands are consistent. Colour patterns vary in a limited way as patterns like middorsal lines and spots on dorsum and throat which are present in all species allocated to *Fejervarya*, one reason why differentiation of species is so difficult in this genus.



FIGURE 5. Ventral view of hand and foot of (left) *Minervarya sahyadris*, MNHN 2000.3036, and (right) *M. chilapata*, ZSI A 10790.



**FIGURE 6.** Relationship of size (SVL in mm) and dominant frequency (in Hz) of *Minervarya* and *Fejervarya* species showing a general tendency of decreasing frequency with increasing size. Size is given as mean, except for *M. chilapata* and *F. limnocharis* from Bali where it is based on a single observation. Exponent number following species name refer to bibliographic references: 1, Kadadevaru *et al.* (2002); 2, this paper; 3, Dubois (1975, 1984); 4, Kuramoto *et al.* (2007); 5, Kadadevaru *et al.* (2000); 6, Márquez & Eekhout (2006). Only the commonest call of *F. limnocharis* from Bali, Indonesia (reference 6) was retained here.

Miniaturization is the evolution of extremely small body size within a lineage (Hanken & Wake 1993). In fact the size reduction does not lead to smaller size only but to other modifications. This definition corresponds to the situation in *Minervarya*. The species are smaller than all *Fejervarya* species known, but also show a set of characters that are distinct from all other *Fejervarya* such as white supralabial line, rictal gland and absence of spots on the border of the throat. These character states might not be due to reduction and simplification which is one of the consequences of miniaturization (Hanken & Wake 1993). In fact the rictal gland is a structure common in Ranidae frogs but absent in most Dicroglossidae species. In *Minervarya* it is clearly an apomorphic character, as it is absent in all other members of the *Fejervarya* lineage. White supralabial line is not only due to absence of spots, but it is clearly a new different colour pattern for Dicroglossidae which have usually upper lip of the same colour in continuation of loreal and tympanic region. Size is not explanation enough for the absence of spots on border of throat, as in post-metamorph imagos of *Fejervarya* which are less than 10 mm SVL such spots are already clearly distinct (Ohler unpublished data).

In the *Fejervarya-Minervarya* complex, miniaturization leads to modification of body proportions (see figure 1 of Dubois *et al.* 2001). This modification is the manifestation of shift to a different adaptive niche. This also is argument for distinct generic allocation.

Small species might be overlooked due to their small body size, the choice of their habitat, their cryptic coloration or behaviour (Clarke 1989). They might be mistaken for young of larger common species (Clarke 1989). Even though some might be quite rare, others might be much commoner than thought. *Minervarya sahyadris* was misidentified as *Fejervarya syhadrensis*, a sympatric small sized species (Daniels 2005; Kuramoto & Joshy 2001; Kadadevaru *et al.* 2002). *Minervarya* specimens might be present in other collections from all over India and misidentified as young of *Fejervarya*. Researchers should be attentive when doing field research as many small species of frogs might still be awaiting discovery.

Advertisement call. The advertisement call of *Minervarya chilapata* is composed of trilled notes. The advertisement call of the congeneric Minervarya sahyadris has been reported from Mangalore and Western Ghats, India (Kuramoto & Joshy 2001; Kadadevaru et al. 2002; Kuramoto et al. 2007; in the two first references as Limnonectes syhadrensis, Kuramoto et al. 2007). Kadadevaru et al. (2002) reported a call of the same general structure than that of M. chilapata for M. sahyadris though the notes of this latter species are composed of well-defined pulses (whereas this is not the case in *M. chilapata*). Indeed, the groups of impulsions that compose the notes of *M. chilapata* last about 70 ms and are not produced by a single impulsion as a pulse could be. Other differences occur between the calls of the two Minervarya species: in the call of *M. sahyadris*, the longest note is the first whereas it is the last in *M. chilapata* and the notes of *M.* chilapata are much longer. Three energy bands are present in both species but M. sahyadris has a higher dominant frequency than M. chilapata (3642-4420 Hz vs. 2810-3870 Hz). In M. chilapata the dominant frequency is the fundamental one whereas in *M. sahyadris* the dominant frequency is the second harmonic. The two species share a similar number of groups of impulsions by notes (7-28 in M. sahyadris and 11-27 in *M. chilapata*) and no silent intervals between these groups. The call of *M. sahyadris* reported by Kuramoto and Joshy (2001) differ from the call described by Kadadevaru et al. (2002) in its structure and in the structure of the notes. In these calls, the notes have a tendency to stretch in length and the number of pulses that compose the notes to increase. Furthermore, two call types were recorded. The call described by Kuramoto et al. (2007) is what we call a note in this paper and seems to be quite different from the other calls reported by the two previous studies. Although the groups of impulsions that compose this note are clearly separated from each other, the duration of this note is quite similar to that of the call of *M. chilapata*, as well as the repetition rate of the elements that compose it and the dominant frequency which lies at about 3700 Hz.

The few data available for *M. chilapata* do not allow knowing if it possesses two call types or a great increase in note length and number of groups of impulsions during call emission as reported for *M. sahyadris* (Kuramoto & Joshy 2001).

As the genus *Minervarya* has been recently synonymized with *Fejervarya* (Kuramoto *et al.* 2007), it is interesting to see if there is a common pattern in the calls of these two close genera and if the observed variations could be linked to decreasing size and eventually to miniaturization. Several works reported the

advertisement call of species of the genus *Fejervarya* (Heyer 1971; Dubois 1975; Roy & Elepfandt 1993; Kanamadi *et al.* 1995; Kadadevaru *et al.* 2000; Márquez & Eekhout, 2006; Kuramoto *et al.* 2007). The calls of these two genera have in common the structure of the notes, which are pulse groups or composed of larger groups of impulsions as observed in *M. chilapata*. However, there is no evident gradient in note length, number of pulse par note or other temporal characters linked to the size of the males of the different species whose advertisement call is known. It is also generally accepted that larger individuals have a call with a lower frequency. This is confirmed in the case of species of *Fejervarya* and *Minervarya*, where a general tendency of decreasing dominant frequency band of their call with increasing size of the males of the different species can be observed (Fig. 6).

**Distribution.** *Minervarya* was described as an endemic to South-western India. The description of a new species of this genus changes the biogeography of this group. The occurrence of the genus in the Himalayan foot plains and in the south-western region is a witness of a continuous habitat that could harbour these species. Humid forests and peat may have been widespread in India at the Early Eocene, getting more seasonal in Early Miocene. In particular, the change to a more ever-wet climate in the Middle Miocene resulted not only in the widespread development of evergreen rain forests across the region, but allowed also the extension of the occurrence of many amphibian species linked to such a humid habitat. Only in Late Miocene and Pliocene the forest disappeared, when the climates got more seasonal and dry thus restricting the forest to the refuges in the Western Ghats (Morley 1999). The timescale for evolution of different ranid groups proposed by Bossuyt et al. (2006) would correspond with the timing of climatic changes in the region as a high number of differentiations in the family-group taxa took place in Late Eocene to Miocene when the suitable habitat of forest frogs such as *Minervarya* was widespread. Subsequent restriction of this habitat might have led to speciation by habitat fragmentation.

No such small dicroglossid species is known from South-East Asia which is in agreement with our hypothesis on an evolutionary trend of the *Fejervarya* lineage to size reduction based on known data on their phylogeny and biogeography. *Minervarya* is phylogenetically close to the Indian clade of *Fejervarya* (Kuramoto *et al.* 2007) which shows a tendency of size reduction. This tendency is less developed in the South-East Asian clade.

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## Literature cited

- Bossuyt, F., Brown, R. F., Hillis, D. M., Cannatella, D. C. & Milinkovitch, M. C. (2006) Phylogeny and biogeography of a cosmopolitan frog radiation: late Cretaceous diversification resulted in continent-scale endemism in the family Ranidae. *Systematic Biology*, 55, 579–594.
- Charif, R. A., Mitchell, S. & Clarck, C. W. (1995) *Canary 1.2 User's Manual*. Cornell Laboratory of Ornithology, Ithaca, New York.

- Clarke, B. T. (1989) Real vs apparent distribution of dwarf amphibians: *Bufo lindneri* Mertens 1955 a case in point. *Amphibia-Reptilia*, 10, 297–306.
- Daniels, R. J. R. (2005) Amphibians of Peninsular India. Universities Press (India) Private Ltd., Hyderabad, 1-268.
- Dubois, A. (1975) Un nouveau complexe d'espèces jumelles distinguées par le chant : les Grenouilles du Népal voisines de *Rana limnocharis* Boie (Amphibiens, Anoures). *Comptes rendus de l'Académie des Sciences*, série D, 281, 1717–1720.
- Dubois, A. (1984) Note préliminaire sur le groupe de *Rana limnocharis* Gravenhorst, 1829 (Amphibiens, Anoures). *Alytes*, 3, 143–159.
- Dubois, A., Ohler, A. & Biju, S. D. (2001) A new genus and species of Ranidae (Amphibia, Anura) from south-western India. *Alytes*, 19 (2-4), 53–79.
- Hanken, J. & Wake, D. B. (1993) Miniaturization of body-size organismal consequences and evolutionary significance. *Annual Review of Ecology and Systematics*, 24, 501–519.
- Heyer, W. R. (1971) Mating calls of some frogs from Thailand. Fieldiana Zoology, 58, 61-82.
- Inger, R. F. (1954) Systematics and zoogeography of Philippine Amphibia. Fieldiana: Zoology, 33, 181-531.
- Kadadevaru, G. G., Kanamadi, R. D. & Schneider, H. (2000) Advertisement call of two Indian ranids, *Indirana beddomii* and *Tomopterna rufescens*. *Amphibia-Reptilia*, 21, 242–246.
- Kadadevaru, G. G., Kanamadi, R. D. & Schneider, H. (2002) Advertisement call, courtship and mating behaviour of the frog, *Limnonectes syhadrensis* from Western Ghats, India. *Current Science*, 82, 503–505.
- Kanamadi, R. D., Hiremath, C.R. & Schneider, H. (1995) Vocalization and territoriality of the Indian frog *Rana limnocharis. Proceedings of the Indian National Science Academy*, 61, 9–14.
- Köhler, J., Vieites, D. R., Bonett, R. M., Hita Garcia, F., Glaw, F., Steinke, D. & Vences, M. (2005) New amphibians and global conservation: a boost in species discoveries in a highly endangered vertebrate group. *BioScience*, 55, 693– 696.
- Kurabayashi, A., Kuramoto, M., Joshy, H. & Sumida, M. (2005) Molecular phylogeny of the ranid frogs from Southwest India based on the mitochondrial ribosomal RNA gene sequences. *Zoological Science*, 22 (5), 525–534.
- Kuramoto, M. & Joshy, S. H. (2001) Advertisement call structures of frogs from Southwestern India, with some ecological and taxonomic notes. *Current Herpetology*, 20, 85–95.
- Kuramoto, M., Joshy, S. H., Kurabayashi, A. & Sumida, M. (2007) The genus *Fejervarya* (Anura: Ranidae) in central Western Ghats, India, with descriptions of four new cryptic species. *Current Herpetology*, 26(2), 81–105.
- Márquez, R. & Eekhout, X. R. (2006) Advertisement calls of six species of anurans from Bali, Republic of Indonesia. *Journal of Natural History*, 40, 571–588.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. & Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858.
- Morley, R. J. (1999) Origin and evolution of tropical rainforests. Wiley, New York, i-xv + 1-362.
- Norusis, M. J. (1992) SPSS for Windows. Professional Statistics. Release 5. SPSS Inc., Chicago, (i-xii) + 1-348.
- Ohler, A. & Dubois, A. (1999) The identity of *Elachyglossa gyldenstolpei* Andersson, 1916 (Amphibia, Ranidae) with a discussion of some aspects of statistical support to taxonomy. *Zoologica Scripta*, 28, 269–279.
- Roy, D. & Elepfandt, A. (1993) Bioacoustic analysis of frog calls from northeast India. *Journal of Biosciences*, 18, 381–393.
- Veith, M., Kosuch, J., Ohler, A. & Dubois, A. (2001) Systematics of *Fejervarya limnocharis* (Gravenhorst, 1829) (Amphibia, Anura, Ranidae) and related species. 2. Morphological and molecular variation from the Great Sunda Islands (Sumatra, Java, Borneo) with definition of two species. *Alytes*, 19, 5–28.