Journal of Threatened Taxa

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ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

SHORT COMMUNICATION

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26 June 2020 | Vol. 12 | No. 9 | Pages: 16136–16142
DOI: 10.11609/jott.6031.12.9.16136-16142

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 June 2020 | 12(9): 16136–16142
ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)
DOI: https://doi.org/10.11609/jott.6031.12.9.16136-16142
#6031 | Received 21 April 2020 | Final received 24 May 2020 | Finally accepted 11 June 2020

SHORT COMMUNICATION

Osteological description of Indian Skipper Frog *Euphlyctis cyanophlyctis* (Anura: Dicroglossidae) from the Western Ghats of India

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Abstract: The present study provides description of the osteology of Skipper Frog *Euphlyctis cyanophlyctis*. Seven adult specimens of *E. cyanophlyctis* from northern Western Ghats of India were cleared and double stained for studying osteological characteristics. The baseline description of osteological characters of cranial and post-cranial elements (paired nasals, tubular sphenethmoid, well-developed vomerine teeth, arciferal pectoral girdle, fan-shaped omosternum, cartilaginous W-shaped xiphisternum, hind limb with longest cylindrical humerus, V-shaped pectoral girdle and phalangeal appendages) provided in present study will help in further taxonomic investigations of the genus *Euphlyctis*. Further, the baseline information on osteology of Skipper frog will serve as a reference material for investigations related to malformations, either in this or related species. We also provide first observation on sacro-pelvic malformation in one of the studied specimens.

Keywords: Skeletal morphology, amphibian decline, anthropogenic stressors, malformation, conservation.

Amphibians are declining globally with highest number of species at risk of extinction than any other vertebrate group (Stuart et al. 2004). Limited information on the basic biology, population status, distribution, life-history and potential threats to the anurans is one of the major hurdles in amphibian conservation (Dinesh & Radhakrishnan 2011; Dahanukar et al. 2013). In addition to population declines, amphibian malformations has become a major conservation concern and studies reporting and analyzing amphibian deformities have gained momentum (Johnson et al. 1999; Schoff et al. 2003; Peloso 2016). Investigations into the basic biology, including the osteology of a species, will not only help in understanding its taxonomy, but also form a baseline for comparative osteology of malformed individuals. Therefore, the fundamental objective of the present

Editor: Anonymity requested. **Date of publication:** 26 June 2020 (online & print)

Citation: Gorule, P.A, S.M. Gosavi, S.S. Kharat & C.R. Verma (2020). Osteological description of Indian Skipper Frog *Euphlyctis cyanophlyctis* (Anura: Dicroglossidae) from the Western Ghats of India. *Journal of Threatened Taxa* 12(9): 16136–16142. https://doi.org/10.11609/jott.6031.12.9.16136-16142

Funding: Department of Science and Technology (DST), Government of India, under DST-FIST scheme.

Competing interests: The authors declare no competing interests.

Acknowledgements: We would like to thank anonymous reviewers for their valuable comments and inputs on the earlier version of the manuscript. We also thank Neelesh Dahanukar, Indian Institute of Science Education and Research (IISER), Pune for his constant support, encouragement and help in specimen identification. We thank Nitin Sawant, Priyanka Gore, Manoj Pise, and Pradeep Kumkar for field assistance. Authors are grateful to Deepak Apte and Rahul Khot for their support in the registration of specimens in BNHS, Mumbai.

study was to describe detailed osteology of the Indian Skipper Frog *Euphlyctis cyanophlyctis* (Schneider, 1799) to serve as a baseline data for further investigations.

Adult specimens (n = 7; SVL= 25.50–55.67 mm) of *E. cyanophlyctis* consists of both male and female individuals were collected from temporary pool situated at Sangrun (18.404°N & 73.687°E) Haveli, Pune and Nere (18.619°N & 73.702°E) Mulshi, Pune, Maharashtra, India on 23 July 2017 and 2 August 2017, respectively. The specimens were cleared and double stained differentially for bone and cartilage as per Potthoff (1984). The osteological terminology follows Trueb (1973), Duellman & Trueb (1986) and Pugener & Maglia (1997). Cleared and double stained specimens are deposited in the museum collection of Bombay Natural History Society (BNHS) under the accession numbers BNHS 6031–BNHS 6037.

Irrespective of the sex, no significant differences were observed in the osteology of seven specimens. The osteological representation of *E. cyanophlyctis* is shown in Figure 1. The detailed description of the osteology is provided below.

Cranium (Figure 1A & B)

It is well-developed, triangular and dorso-ventrally flat structure. The frontoparietals are paired, fully ossified and flat structure forming the roof of the skull. They are separated throughout their length, cover the sphenethmoid anteriorly and extend till the exoccipitals posteriorly. They fail to articulate with nasals anteriorly but firmly attached with the prootic and exoccipitals posteriorly. Sphenethmoid is located anterior to the cranial cavity and forms the posterior boundary of the olfactory chambers. It is a tubular-shaped bone formed by a combination of anterior ethmoidal and posterior sphenoidal regions separated from each other by a transverse partition. Ethmoidal region separates into the right and left halves by longitudinal partition, which further encloses the olfactory sac. A small portion of the bone is visible particularly at the posterior boundary (sphenoidal region), as it is covered ventrally by parasphenoid and dorsally by nasals and frontoparietals bones. Parasphenoid is single, elongated, large dagger-like or inverted 'T' shaped bone forming the cranial floor. It shows a pointed long shaft directed anteriorly and its cross piece handle lies across the auditory capsule. The occipitals form posterior-most boundary of the cranium. Presence of a large hole, foramen magnum is noticeable, which serves as the entry point for the spinal cord. Postero-laterally, the foramen is surrounded by two roughly oval-shaped exoccipitals. The exoccipitals posteriorly have cartilaginous occipital condyles, which further articulates with the first vertebra (atlas) anteriorly. The outer margin of each exoccipital is firmly attached to the cartilaginous auditory capsule. The anterior wall and partly the roof and floor of each capsule are formed by a fully ossified, roughly rectangular prootic. It is partly covered by squamosal on its dorsal side. The occipital region is enclosed by frontoparietals, while the floor is occupied by a dagger-shaped parasphenoid. Supraoccipital and basioccipital are absent.

Sense capsule, consisting of an auditory capsule, an olfactory capsule and an optical capsule, encloses the organs of the hearing, the organs of the smell and the eye, respectively. The auditory and olfactory capsules are firmly attached to the cranium, while the eyes are not fused with the skull. Nasals are paired, fully ossified bones that form the roof of the olfactory capsule covering the anterior dorsal region of the skull and are not medially fused. Their anterior ends extend to the dorsal processes of the premaxillae and thus partially form the boundary of external nares. Septomaxillaries are small, irregular-shaped bones present close to the anterior part of each nasal bone. Vomers are paired, completely ossified, roughly triangular bones, located ventrally to the nasal capsule floor. The presence of a row of vomerine teeth on the posterolateral margin is noticeable. Anterolateral and lateral margin bears pointed projections.

Upper jaw (Figure 1A & B)

Premaxillae are paired bones, fuse medially, well-ossified and form the anterior boundary of the maxillary arch. Each premaxilla has two rows (16 + 14) of teeth. On the outer side, each premaxilla articulates a maxilla of its respective side. Maxillae are quite long, sharply curved bone and bears numerous, sharply pointed teeth arranged in two rows (34 + 51). At the middle of its length, it is articulated with the palatine and pterygoid. The maxilla is connected to the quadratojugal posteriorly. Quadratojugals are paired, short rod-like bones, completely ossified. The posterior portion of the quadratojugal underlies the margin of the squamosal. Squamosals are irregular shaped-bones located just above the pterygoid. Anteriorly it remains free, whereas posteriorly it forms a connection to the auditory and prootic capsules. Pterygoids are paired, fully ossified, 'Y'-shaped bone with three limbs, laterally positioned on either side of cranium and ventrally positioned to the squamosal. The anterior limb is linked to the maxilla. The inner limb is connected to the pterygoid and the

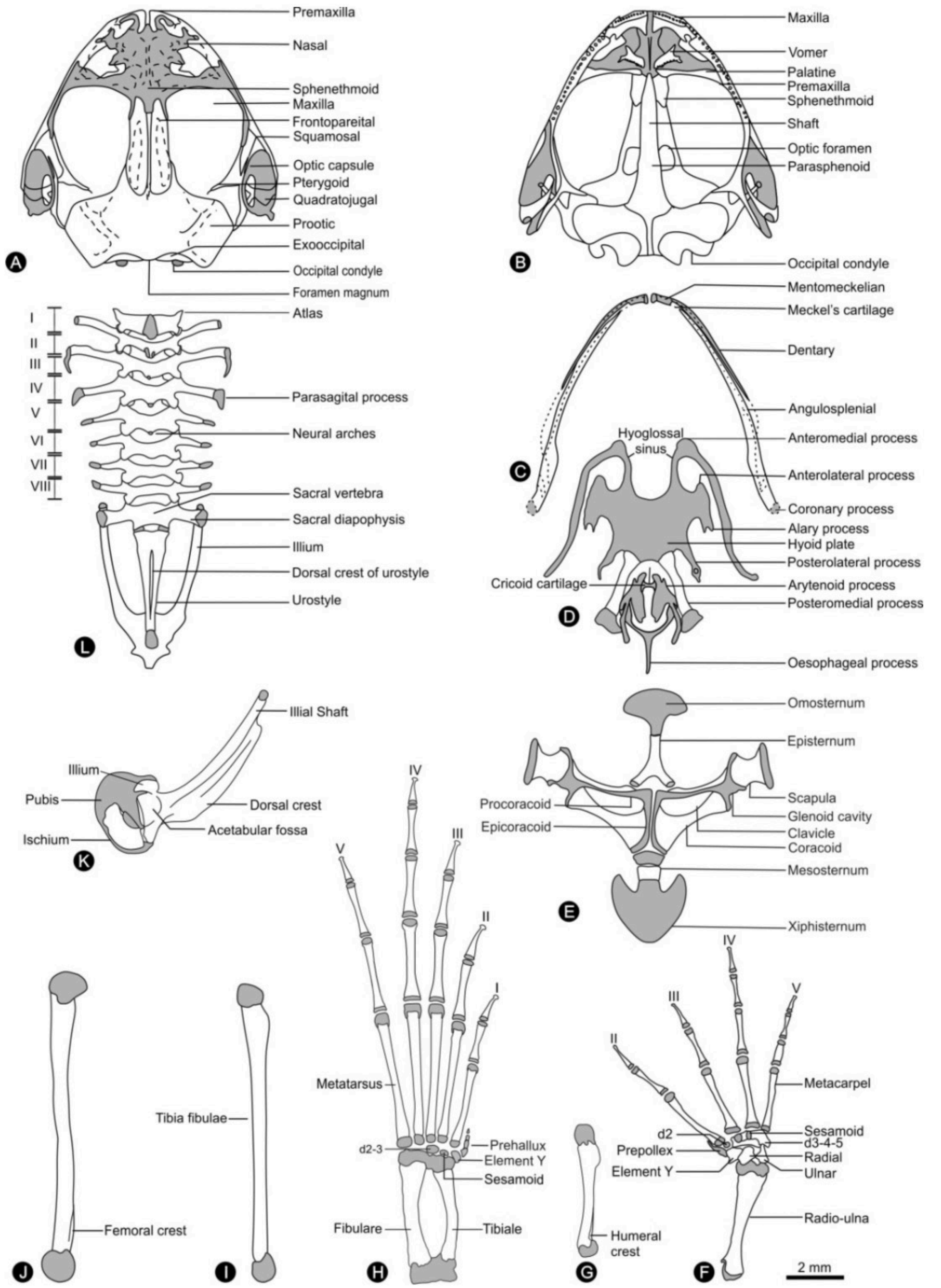

Figure 1. Osteology of *Euphlyctis cyanophlyctis*, BNHS 6032: A—cranium, dorsal view | B—cranium, ventral view | C—lower jaw | D—hyoid skeleton | E—pectoral girdle | F—forelimb | G—humerus | H—hindlimb | I—tibia and fibula | J—femur | K—pelvic girdle, lateral view | L—pelvic girdle, front view and axial skeleton.

auditory capsule, whereas the posterior limb joins with the quadratojugal. Pterygoids also contribute for the formation of the posteroventral margin of the orbit of respective side. Palatines are paired, elongated, rod-shaped bones that are located ventrally and form the anterior side of each orbit. They serve as a transverse link between the anterior side of the cranium and the maxilla.

Lower jaw or mandible (Figure 1C)

It is semi-circular and consists of two halves or rami which are connected to each other by a ligament at the anterior end. Teeth are absent. Each ramus consists of a core of Meckel's cartilage, surrounded by mento-meckelian, angulosplenial and dentary. Mento-meckelian is small, entirely cartilaginous and located at the extreme anterior end of the Meckel's cartilage, where both rami unite together. Angulosplenial is a long, strongly curved prominent bone forming most of the inner and posterior portion of each ramus of the mandible. Its anterior end is tapering, whereas the posterior end has articulating process/condyle for the quadrate cartilage of the skull. Just close to the articulating condyle, it also has a small knob-like coronary process. Dentary is an elongated rod-like bone covering almost 50% of the outer surface of the anterior portion of Meckel's cartilage. Anteriorly, it extends up to the Mento-meckelian, whereas posteriorly up to the outer side of the coronary process.

Hyoid skeleton (Figure 1D)

It consists of thin cartilaginous shield-shaped hyoid plate (=corpus), U-shaped hyoglossal sinus, anterolateral (cartilaginous), anteromedial (longest), posterolateral processes (cartilaginous and almost the same size as anterolateral), posteromedial (fully ossified) processes and cartilaginous, thin, long, curved hyales allowing the hyoid apparatus to be attached to the cranium. A small, thin, rounded medial element is present on the anterior process of the hyale. Stalk of anterolateral processes is comparatively thicker than its distal portion, which bears the alary process. Hyoglossal sinus is deeply grooved. Posterolateral processes are long and nearly 50% of the total length of the posteromedial process.

Vertebral column (Figure 1L)

The presacral region consists of eight vertebrae, a sacral region with one sacral vertebra and a caudal region with the urostyle. The atlas (1^{st} vertebra) anteriorly articulates with the posterior-most regions of exoccipitals. Centrum of the atlas is comparatively larger than other presacral vertebrae (visible from the ventral view). The presacral II–VII are procoelous, while the presacral VIII is amphicoelous. All vertebrae connected to each other through post-zygapophyses of one vertebra with the pre-zygapophyses of the next vertebra. The neural arches of each vertebra have a well-developed dorsal ridge and a pair of transverse processes extending laterally with terminal parasagital processes. The first four pairs of transverse processes (presacrals II–IV) are relatively more robust than those of last four pairs (presacrals V–VIII). The neural arches of I–V vertebrae are imbricate. The relative lengths of transverse processes are as follows: III > IV > V > VI=VII=VIII < II. The transverse process of vertebrae IV, V and VI are directed posteriorly, the VIII vertebra is directed anteriorly, whereas III and VII are approximately perpendicular to the notochordal axis. Sacral diapophyses are laterally oriented, positioned perpendicular to the notochordal axis and are of similar size to transverse processes of VI, VII, VIII vertebrae. Urostyle is slender, shorter than the presacral length and articulates the sacrum through a bicondylar articulation. It has a prominent dorsal crest most of its length.

Pectoral girdle (Figure 1E)

It is arciferal in structure. Suprascapulae are paired, completly ossified, with the larger partion forming the distal edge of the pectoral girdle and articulates with scapula medially. Cleithrum is cartilaginous and covers most of the suprascapula. Scapula is rectangular and articulates with the clavicle anterolaterally and coracoid medially at the glenoid cavity. Laterally it joins with supra scapula and the cleithrum. Clavicle is slender, completely ossified, slightly curled, with a shape of bow that forms the anterior part of the pectoral girdle. Procoracoids are present, separated medially, extend along the dorsal posterior of clavicles, and articulate with the scapula at the distal end. The coracoids are rectangular shaped, fully ossified and distally expanded extremities which articulate with the epicoracoid medially and scapula ventrolaterally. Epicorocoids is cartilaginous, separated throughout their length and forms arciferal arrangement. Omosternum is cartilaginous, fan-shaped expanded distal end and comparatively smaller than 'W' like shaped cartilaginous xiphisternum. Episternum is fully ossified, inverted 'Y' shaped structure, articulates with omosternum anteriorly and procoracoids posteriorly. Mesosternum is ossified with the anterior cartilaginous end and comparatively wider than the episternum.

Forelimbs (Figure 1F and 1G)

Humerus is the longest bone of forelimb with well-defined humeral crest, cylindrical in appearance, articulates into glenoid cavity proximally and radio-ulna distally. Six carpel elements (ulnare, distal carpel 3–4–5, distal carpel 2, element 'Y', radial, and prepollex) are present, representing 'Type-C' morphology of Fabrezi (1992). The sesamoid is cartilaginous, rounded and positioned above the radial. The relative length of metacarpals is as follows: II=IV>III>V. The phalangeal formula is 2–2–3–3.

Pelvic girdle (Figure 1K and 1L)

It is 'V' shaped and consists of pair of ilia, ischia and pubis. The ilial shafts are round in cross-section and articulate with the sacral diapophysis. Ischia and pubis are fused together, forming the acetabulum.

Hind limbs (Figure 1H, 1I, 1J)

Femur is the longest bone (slightly larger than tibia-fibulae) of the body, articulates with the acetabulum proximally and with tibia-fibula distally. Tibia-fibulae distally articulate with tibiale and fibulare, which are separated throughout their length, except for proximal and distal ends. Tibiale and fibulare are about half of the length of tibia-fibula. A sesamoid is positioned proximally to tibiale. Pes consists of fused distal tarsals 2–3, element 'Y' prehallical elements (cartilaginous elements; I, II, III and IV), metatarsals and phalanges. The relative length of the metatarsals is as follows: V=IV=III>II>I. The phalangeal formula is 2–2–3–4–3.

Sacro-pelvic malformation

In one of the specimens collected from Sangrun, we observed two sacral vertebrae with transverse processes (Image 1A; malformed individual) in contrast to the one sacral vertebra (Image 1B; normal individual).

Knowledge of the basic osteology of anurans is critically important in taxonomic investigations (Lynch 1971). At present, the genus *Euphlyctis* is represented by eight extant species (Priti et al. 2016). For the first time, using skeletal characters, Deckert (1938) provided brief generic description for *Dicroglossus* Günther (1860) and placed *cyanophlyctis* under *Dicroglossus* (= *Euphlyctis* Fitzinger 1843 according to Dubois 1980) other than *Rana*. However, detailed osteological characters for any species of the genus *Euphlyctis* is not available, which limits comparative analysis. Therefore, the osteology of *E. cyanophlyctis* was compared with the *Quasipaa robertingeri* (= *Quasipaa boulengeri*) and *Nannophrys marmorata* which belongs to the family Dicroglossidae (Senevirathne & Meegaskumbura 2015; Zhang et al. 2016). The comparative descriptions of the prominent and distinctive osteological characters between *E. cyanophlyctis*, *Q. robertingeri* and *N. marmorata* are given in Table 1. Although the characters presented in this study are generic rather than species-specific, they could be used as a reference data for more thorough species-specific osteological investigations of the genus *Euphlyctis*.

Several stressors (environmental pollution, greater exposure to the UV–B radiation, and parasitic overload) contribute to the development of abnormal or bizarre morphological features, especially affecting the limbs and vertebrae of amphibians, which are referred to as 'malformation' (Silva et al. 2019). The incidence of occurrence of malformed anurans is increasing (Blaustein & Johnson 2003; Pelaso 2016). Therefore, the osteological description provided in present study will also serve as a baseline data of normal form which

Table 1. Comparative analysis of osteological characters of three species belongs to the family Dicroglossidae representing three different genera.

Characters	*Euphlyctis cyanophlyctis*	*Quasipaa robertingeri*	*Nannophrys marmorata*
Frontoparietal	Thin and long	Rigid and broad	Rigid and broad
Episternum	Y-shaped	Funnel shaped	Cylindrical
Xiphisternum	W-shaped	W-shaped	Fan-shaped
Omosternum	Fan-shaped expanded distally	Fan-shaped expanded distally	Tube like fused with the cartilaginous epicoracoids
Metacarpal length	II=IV>III>V	IV>V> II>III	IV>III>V>II
Phalanges (Palm)	2–2–3–3	2–2–3–3	3–3–4–4
Metatarsal length	V=IV=III>II>I	V>III >V >II >I	IV>V>III>II>I
Phalanges (Toes)	2–2–3–4–3	2–2–3–4–3	3–3–4–5–4
Reference	Present study	Zhang et al. (2016)	Senevirathne & Meegaskumbura (2015)

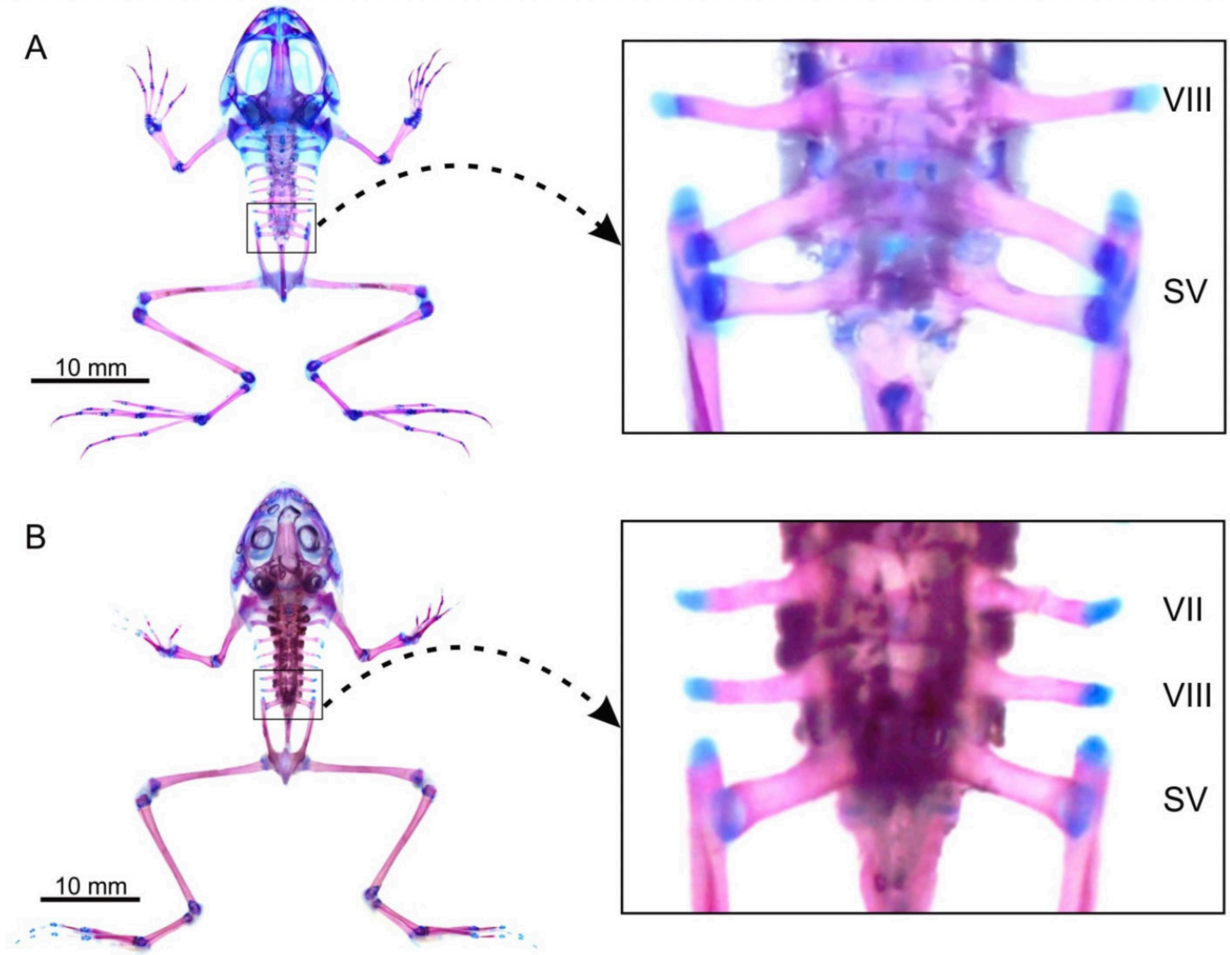

Image 1. Cleared and double stained specimens of *Euphlyctis cyanophlyctis*, A—malformed individual and its enlarged view with VIIIth pre-sacral vertebra followed by double sacral vetebrae and | B—normal individual and its enlarged view with VIIth, VIIIth pre-sacral vertebrae followed by single sacral vertebra. SV—sacral vertebra. © Chandani Verma.

could be effectively use to differentiate malformed individuals from normal onces. At present it is difficult to pinpoint the exact cause/s of observed malformation in *E. cyanophlyctis*, however, the malformed individuals are often easy target for the predators, failed to reproduce and thereby compromise with their survival and fitness (Sower et al. 2000; Blaustein & Johnson 2003; Bowerman et al. 2010). Owing to the global amphibian decline and ongoing controversy over the types of amphibian malformations caused by various factors, the information presented in this study not only have implications for the continued investigation of amphibian malformations but also has a conservation implication.

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Journal of Threatened Taxa

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ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

June 2020 | Vol. 12 | No. 9 | Pages: 15967–16194
Date of Publication: 26 June 2020 (Online & Print)
DOI: 10.11609/jott.2020.12.9.15967-16194

Communications

Short Communications

Notes

Corrigendum

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