Rec. zool. Surv. India: Vol 120(1)/ 49-54, 2020
DOI: 10.26515/rzsi/v120/i1/2020/138963

ISSN (Online) : 2581-8686
ISSN (Print) : 0375-1511

Description of *Nanorana conaensis* (Fei and Huang, 1981) (Amphibia: Anura: Dicroglossidae) reported from Arunachal Pradesh, India

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Abstract

The dicroglossid frog *Nanorana conaensis* was described from the high elevation mountain ranges of Tibet about three decades ago. Recently this species was reported from India as a first record for India. In the present account, detailed morphological data, genetic data and phylogenetic status has been provided with a photograph of live animal in order to facilitate easy identification of the species from India.

Keywords: *Nanoranna conaensis*, Morphology, Arunachal Pradesh, Phylogenitic Status

Introduction

Among the north-eastern states of India, Arunachal Pradesh is least explored in terms of amphibian diversity. However, over the years, there has been a gradual increase in the number of amphibian species from the State due to involvement of several scientists in exploring new species, finding new records and delineating species ranges (Sen, 2004; Sarkar and Ray, 2006; Mathew and Sen, 2007; Borah *et al.*, 2013; Mahony *et al.,* 2013; Kamei *et al.,* 2013; Saikia *et al.*, 2017a & b; Ohler *et al.*, 2018). Most of these studies have been carried out from the lower reaches of the southern slopes of the Eastern Himalayas, which encompasses almost the entire length of the State. Currently, there are 65 species of amphibians known from the State (Ohler *et al.*, 2018), which is set to increase further to 78 species (based on a recent study on the Amphibian fauna of the State by the first two authors; *manuscript communicated*).

Among the species reported from the higher altitudes of the Himalayas, the species under *Nanorana* genus is one of the most commonly seen groups of anurans. The previous populations of *Nanorana* in the State were attributed to *N. annandalii*, *N. liebigii* (Bordoloi, *et al.*, 2001; Saikia and Das, 2017) and *N. conaensis* (as *Paa conaensis* by Ohler *et al.*, 2018). However, there have been taxonomic ambiguities in the generic allocation of the dicroglossid frogs in general; the taxonomy followed here is after Frost (2018). During our routine explorations in the Arunachal Pradesh for amphibians, two specimens of *Nanorana* were collected from the outskirt of the Tawang Township in March, 2018, which were identified as *Nanorana conaensis* based on the morphological and molecular studies.

Nanorana conaensis (Fei and Huang, 1981) was described from Mama, Chuona County in Tibet, China (Huang, *et al.,* 1998). Since then, this species was only reported from the western side of Bhutan (Wangyal, 2013). Recently Ohler *et al.*, (2018) has reported this species from Tawang as a new record for the state of

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Article Received on: 26.11.2018 Accepted on: 20.06.2019

Arunachal Pradesh and India. However, there were no morphometric and genetic details available for this species from the country; herein we report the species with detailed morphometric diagnosis and phylogenetic relationship with the congeners with the available sequences.

Material and Methods

Two specimens (both female) of *Nanorana conaensis* (V/A/NERC/1412-1413) were collected from a domestic water tank at Khremteng village in the outskirt of the Tawang township (N: 27.587; E: 91.865; Alt. 2844 m ASL, ±5 m) in Arunachal Pradesh on 9th March, 2018. The specimens were dissected to ascertain their respective sexes, and the liver tissue was extracted and fixed in absolute alcohol (EMSURE(R), Merck KGaA, Germany) for molecular study, while the specimens were later fixed in 5% formalin solution and preserved in 70% alcohol.

All the morphometric measurements (in millimeters) were done with a Mitutoyo™ digital calliper. The measurements taken are as per the parameters used in measuring the types of *Nanorana conaensis* (Huang, *et al.,* 1998). These are SVL (Snout Vent length), HL (Head Length), HW (Head Width), INS (Inter-Narial Distance), EHD (Eye Horizontal Diameter), SL (Snout Length), IOS (Minimum distance between the upper eyelids),UEW (maximum width of the upper eyelid), FHAL (Forearm and Hand Length), FAW (Width of Forearm), HAL (Hand Length), HAW (Hand Width), TLL (Total Leg Length), TBL (Tibia Length), TBW (Tibia Width), TFL (Length of Tarsus and Foot), FOL (Foot Length), TTA (Tibio-Tarsal Articulation).

Genetic analysis including DNA extraction, PCR amplification and sequencing followed as methods described for the dicroglossid frogs in Dinesh *et al.,* (2015). Mitochondrial 16S rRNA was used for the genetic studies, with our sequences for *Nanorana conaensis* 16 species of *Nanorana* (Table 2) were used for phylogenetic reconstruction. Species of *Quasipaa* (earlier *Paa*) was used as out group (Pyron and Weins, 2011). Maximum Likelihood (ML) tree was generated using RaxML (Silvestro and Michalak, 2012) under GTR+GAMMA+I model by running 1000 thorough bootstraps and the final consensus tree was visualized by FigTree v1.4.0.

Results

Phylogenetic studies suggested no genetic differences from 16SrRNA sequences generated for *Nanorana conaensis* from our studies and the *N. conaensis* sequences from Mama, Cona, Xizang (GenBank number: EU979834.1) (Figure 1). The generated sequence of the specimen was submitted to the NCBI with accession numbers MK695682 and MK695683.

Morphological Diagnosis

The morphometric details of the Tawang specimens (Table 1) correspond with the measurements of types of *N. conaensis* (Hunag *et al.,* 1998). Medium to large sized frogs (SVL: 47.57-48.51), head wide, snout blunt and rounded, inter-narial region wider than inter-orbital space or upper eyelid. Tympanum indistinct, vomerine teeth in short rows, tongue large and notched deeply. Dorsum with warts, no dorsolateral folds; ventrum smooth. Forearm small, finger tips rounded, relative length of fingers 2<1<4<3, three distinct metacarpal tubercles. Hind limbs long and strong, TTA reaching anterior eye corner or nostril, tibia more than half of SVL. Inner metatarsal tubercle prominent, outer absent; toes fully webbed. Skin relatively smooth, elongated warts over dorsum. In life, dorsum olive brown in colour; faded towards the vent region. Black bars and spots scattered irregularly on the dorsum and forearms; hind limbs with black bands. Dark bars from the snout to the forearm joints, interrupted by the orbit; another dark bar between the eyes (Figure 2). Ventrally off-white in colour anteriorly; posterior portion yellowish up to the hind limbs.

Habitat

The collection of these specimens was done from a domestic water tank at Khremteng village on the outskirt of Tawang Township. The area is thickly populated but there is a large tract of forest (sacred forest) on the vicinity. Wangyal, (2013) reportedly collected a single specimen of this species from nearby a lake (Lake Tshenchulum) in Haa District of Bhutan. From the type locality, this species was reported to dwell in small streams, springs, or even puddles, but mostly hidden under rocks (similar to *N. liebigii; pers. obs.*), or fallen trees in shallow water, etc. (Huang *et al.,* 1998). Further, when they are scared, they immediately jump into water; an observation similar to this was noticed while collecting the Tawang specimens.

Distribution

N. conaensis is presently known from Chuona County in Tibet, China; western Bhutan and Tawang, in Arunachal Pradesh, India.

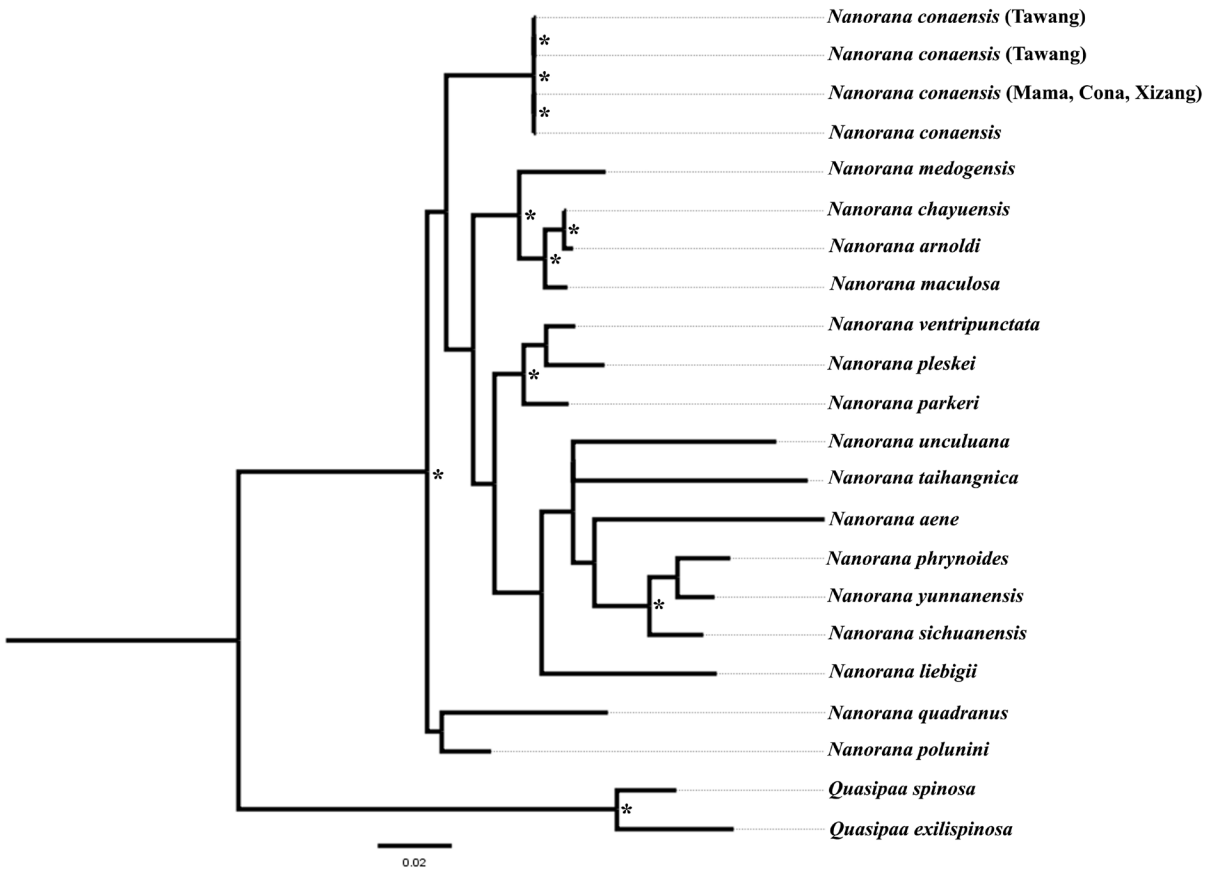

Figure 1. Maximum Likelihood (ML) tree for the species of *Nanorana* based on 570 bp of mitochondrial 16S rRNA (*represents the bootstrap values above 50%).

***Nanorana conaensis* (A) V/A/NERC/1412** ***Nanorana conaensis* (B) V/A/NERC/1413**

Figure 2. *Nanorana conaensis* from Tawang **(a)** V/A/NERC/1412 and **(b)** V/A/NERC/1413.

Table 1. Morphometric data for the two specimens from Tawang compared with the type series of *N. conaensis* (HT - Holotype; PT-Paratype) as per Huang *et al.* (1998)

Reg. No	V/A/NERC/1412	V/A/NERC/1413	770531 (HT)	20 exs (PT)	9 ex. (PT)
Sex	Female	Female	Male	Male	Female
SVL	47.5	48.5	57.6	43.5-69.0	46.1-68.4
HL	13.9	14.0	18.3	15.0 -22.0	15.2-21.0
HW	17.8	17.9	20.8	16-24	16.6-23.7
SL	6.8	6.6	8.3	6.4-9.3	6.9-9.0
INS	5.2	5.2	6.4	4.7-7.0	5.0 -6.5
IOS	3.1	3.2	4	3.6-5.0	3.5-4.2
UEW	4.2	4.3	4.7	3.3-5.4	4.0 -5.2
EHD	5.8	5.8	6.3	5.2-7.5	5.3-8.0
FHAL	23.4	23.5	26.2	21.2-33.5	21.7-27.6
FAW	5.9	4.4	9.5	5.2-12.3	4.3-6.5
HAL	14.6	13.6	NA	NA	NA
HAW	NA	NA	15.9	13.3-20.5	13.5-17.2
TLL	94.7	90.6	102	75.0 -132.0	80.0 -107.0
TBL	30.2	29.2	31.5	24.5-40.7	25.4-33.3
TBW	8.3	7.9	8.5	6.8-12.1	7.4-10.0
TFL	44.2	39.7	46.0	34.4-58.3	35.7-47.0
FOL	29.5	26.1	31.0	23.5-39.5	24.4-31.7
TTA	nostril	anterior eye	nostril	NA	NA

Table 2. Details of GenBank numbers used for the construction of Maximum-likelihood tree.

Sl. No	Species	GenBank number	Sl. No	Species	GenBank number
1	*Nanorana conaensis**	MK695682	12	*N. phrynoides*	KU140002.1
2	*N. conaensis**	MK695683	13	*N. pleskei*	DQ118505.1
3	*N. conaensis*	EU979834.1	14	*N. polunini*	KR827957.1
4	*N. conaensis*	DQ118513.1	15	*N. quadranus*	KU865244.1
5	*N. aene*	KR827954.1	16	*N. sichuanensis*	KU140064.1
6	*N. arnoldi*	EU979838.1	17	*N. taihangnica*	KU865298.1
7	*N. chayuensis*	DQ118510.1	18	*N. unculuana*	DQ118491.1
8	*N. liebigii*	DQ118500.1	19	*N. ventripunctata*	EU979839.1
9	*N. maculosa*	DQ118512.1	20	*N. yunnanensis*	KU140026.1
10	*N. medogensis*	DQ118507.1	21	*Quasipaa exilispinosa*	DQ283244.1
11	*N. parkeri*	DQ118497.1	22	*Q. spinosa*	JX989362.1

* Sequences generated from the present studies

Discussion

Nanorana conaensis (Fei and Huang, 1981) was described from Mama, Chuona County in Tibet, China (Huang, *et al.* 1998). Since then, this species was only reported from the western Bhutan (Wangyal, 2013). Recently Ohler *et al*. (2018) has reported this species from Tawang (stated to be 13000 m elevation which could be erroneous), a male specimen of SVL 47.8 mm (voucher number AR 38) deposited at IASST, Guwahati.

This species was first reported from India by Ohler *et al.,* (2018) after a span of three decades as a first record to India from its original description and the details provided in that report were scanty. To understand the morphological variations and for the easy identification of the species from the country detailed morphological data is provided here. To understand the genetic differences between the populations across the political boundaries, genetic data has been generated and this data is expected to understand the population genetics of the species across its range of distribution (China, Bhutan and India).

The specimens collected from Tawang, in Arunachal Pradesh now represent the eastern limit of this species, while the single specimen reported from western Bhutan represents the western limit. Being a predominantly high altitude species, *N. conaensis* was known to occur at an elevation of 2900-3400 m. The collection altitude of 2844 m in Tawang and the collection altitude of 4066 m in Haa district of Bhutan (Wangyal, 2013) further expand the altitudinal range of this species.

Acknowledgments

The authors are grateful to the Director, Zoological Survey of India, Kolkata for providing facilities. BS would like to acknowledge Mrs. I. J. Kharkongor, Scientist-D, ZSI, Shillong for her help and encouragement. KPD is thankful to the Officer-in-charge, ZSI, WRC, Pune for the support.

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