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Morphology and Morphometric Study of Adults and Tadpoles of *Nanorana minica* (Dubois, 1975) from Uttarakhand and threats to the Species due to Habitat Modification

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ABSTRACT: ***Nanorana minica*** **(Dubois, 1975) is a species of frog of family Dicroglossidae. The species is dedicated to Dominique Payen, a colleague of A. Dubois, whose shortened forename was Minique (Lat Dominica- Minica). Since the morphometric details are not available for the species thus based on collection present at Northern Regional Center, Zoological Survey of India, the detailed morphometry and morphology was given in the present study. Web formula was also provided. The species was collected from Gauri Ganga valley, Ratgiri, Madkot Reservoir Site, Musiari, Pithoragarh, Uttarakhand on 28th June 1993. Total specimens collected were 18, out of 18, 5 are adults (although short tail was attached) and 13 are tadpoles. The tadpoles of the species are also known to overwinter in the streams. The detailed morphological structure of the tadpoles and morphometry was discussed in the present study. The species is under threat due to check dams and habitat modification.**

Keywords: *Nanorana minica*, Uttarakhand, Morphometry, Morphology.

INTRODUCTION

There is a report that approximately one third of global amphibian species are now threatened with extinction (Stuart *et al.*, 2004). Beebee and Griffiths (2005) noted the main reasons of decline of amphibians and they are habitat loss, exploitation for food/medicine, disease and climate change. The genus *Nanorana* contains 33 species and 10 of them belong to the subgenus *Nanorana* are present in India (Dinesh *et al.*, 2024). The genus *Nanorana* contains 3 subgenera, namely *Nanorana, Paa* and *Chaparana* (Shangjing *et al.*, 2023). One new species from China was described by Shangjing *et al.* (2023) thus increased the number of species under the genus to 33. *Nanorana* Günther (1896) is endemic to Asia. It has a wide distribution from the Himalayan region of northern Pakistan; northern India, Nepal; and western China through Myanmar, Thailand, Laos, and northern Vietnam to montane central and southern China (Frost, 2013, 2023). The two subgenera *Paa* and *Chaparana* were earlier erected as two independent genera by Dubois (1975); Bourret (1938), respectively. On the basis of molecular data Roelants *et al.* (2004) mentioned that *Nanorana* is imbedded within *Paa*. Jiang *et al.* (2005) also provided molecular evidence for paraphyly of *Paa* with respect to *Nanorana* and the polyphyly of *Chaparana*. Chen *et al.* (2005) further placed *Chaparana* and *Paa* into *Nanorana* on the basis of a paraphyletic position of *Paa* with respect to *Nanorana* and *Chaparana.* Frost *et al*. (2006), placed *Chaparana* and *Paa* into the synonymy of *Nanorana* to resolve the paraphyly of *Paa* with respect to *Nanorana* (sensu stricto). *Nanorana minica* (Dubois, 1975) common names: Nepal paa frog, tiny frog, small paa frog is a species of frog of family Dicroglossidae. It is found in the West Nepal, India (Uttar Pradesh and Himachal Pradesh). The species is dedicated to Dominique Payen, a colleague of A. Dubois, whose shortened forename was Minique (Lat Dominica- Minica). The species is aquatic and resides in springs and small brooks surrounded with dense shrub and wood environment. It is known to be distributed in altitude between 1000m to 2000m. Their reproductive seasons are in April and May. The species is smallest among *Nanorana* species (Schleich and Kastle 2002). The dorsum of the species is covered with numerous elongate tubercles and many small warts. It is threatened by habitat loss through the localized clearance of forest (IUCN SSC Amphibian Specialist Group 2022). Tadpoles of *N. minica* have been reported to overwinter in the streams (Jithin, 2021). Stream flow management resulting in habitat modification by check dams is one of the threats the species is facing (IUCN SSC Amphibian Specialist Group 2022) and check dams have been found to influence the overwintering tadpoles' behavior, habitat use patterns and morphometric traits (Jithin, 2022a, 2022b). The species is currently listed as the least concerned species (IUCN SSC Amphibian Specialist Group 2022). The species was listed as vulnerable species in previous assessment

conducted in 2004 but at present it is Least Concern (IUCN).

MATERIALS AND METHODS

18 specimens collected by Arun Kumar and team from Gauri Ganga, Musiari, Pithoragarh, Uttarakhand (Latitude 29.582861° N, Longitude 80.218185°E, altitude 2,200 m) on 28th June 1993.All specimens were examined for various morphometric parameters by using electronic digital calipers to the nearest 0.1 mm. Current morphometry protocol given by Watters *et al.* (2016) was followed for the present study. Measurements included the following: snout–vent length (SVL); head length (HL); head width (HW); snout length (SL); internarial distance (IND); interorbital distance (IOD); eye diameter (ED); nostril–eye distance (DNE); tympanum diameter (TD); forearm and hand length (FHL); tibia length (TL); foot length (FL).

RESULTS AND DISCUSSION

Amphibians are considered to be good bio indicator (Welsh and Ollivier 1998; Sheridan and Olson 2003) of the stream ecosystem. Some features of amphibians make them useful as indicator species especially the permeable skin and biphasic life cycle. They are very sensitive to environmental stress. Thus, their distribution indicates the health of stream ecosystem. Thus, the research on their distribution etc. is very useful for the State like Uttarakhand where mountain streams and rivers are facing rampant development due to dam construction (Jithin 2021, 2022a, 2022b). In India 454 species of amphibian have been listed and among them 10 species belong to genus *Nanorana*. The frog's habitat is fragmented and its distribution is limited to less than 20,000 sq km. The main threats to the frog are habitat loss due to forest clearance and dam building, and pollution (Jithin 2021, 2022a, 2022b).

After examining all specimens for morphometric and morphology it was concluded that all specimens belong to species *Nanorana minica*. *Nanorana minica* are small frogs with SVL of around 27.0-36.5 mm. The species is smallest species of the genus with an oval body shape in dorsal view. The head is minimally wider than long and the snout is pointed in both lateral and dorsal views and justs distinctly over the lower jaw (Figs 1-3). The nares are midway between the snout tip and the anterior corners of the eyes. The canthus rostralis is weakly developed and blunt. The present study indicates the SVL of 17mm to 23mm (Table 1) which is the smallest so far reported. Interorbital distance is 3.5 to 4 mm and head length and head width is 5 and 4 mm respectively. The foot length is 10-14 mm. The forearm length varies between 8 mm to 9 mm (Table 1). The tympanum is distinct and prominent and reaches from the posterior corners of the eyes to the forelimb insertion. The morphometric studies done are the first study with 10 parameters from Uttarakhand.

The species differs morphologically from *Nanorana blandfordii* in presence of rounded tips in fingers (Fig. 5) and absence of black marks between eyes. The relative finger length is 1<2<4<3 with a minimal difference between the 1st and 2nd fingers. Only the proximal sub articular tubercles are well developed and have rounded conical form. There are two ovoid palmer tubercles and unelongated metacarpal tubercles (Fig. 4). The toe webbing is complete. The web formula of the species was provided for the first time here in the present study. The Web formula is I 1 + 1II 0-1 $^{1/2}$III 1-2IV2 $^{1/2}$-1V (Fig. 4) (Schleich and Kastle 2002).

Tadpoles of this *N. minica* have been reported to overwinter in the streams (Jithin 2021, 2022a,b). The management of stream and subsequent habitat modification by check dams is one of the threats (IUCN SSC Amphibian Specialist Group 2022*)*, the species is facing. This can affect the overwintering tadpoles' behavior, habitat use patterns and morphometric traits (Jithin 2022a, 2022b). Overwintering is delaying metamorphosis in low-temperature conditions by anuran larvae. Overwintering mechanism exhibited by tadpoles of the species is not much studied to understand the habitat ecology and behavioral aspects (Jithin 2022a, 2022b).

Jithin and Das (2022) collected samples of the dead tadpoles and deposited it at the Wildlife Institute of India Herpetofauna Collection (N=14, WIIAD T-175-188). As reported by them the size of these tadpoles ranged from 25.32 to 63.4 mm (total length) and stages from 26 to 36 (Gosner, 1960). The present study reported the tadpole sizes from 23 to 49 mm (total length), (Fig. 6) and these samples were collected from Gauri Gana, Madkot Reservoir Site, Musiari, Uttarakhand in 1993. Mouth shape with beaked jaw is given in Fig. 7 and the tail of the tadpole is given in Fig. 8.

Considering the large-scale killing of overwintering tadpoles in check dam pools during maintenance, as reported by Jithin and Das (2022), it is suggested that the complete draining of pools and river near barrage like in Assan barrage should be avoided to prevent the loss of refuge areas for the tadpoles and for other aquatic organisms. They also suggested that if complete draining operations are required during maintenance then aquatic organisms (including tadpoles, fish, crabs etc.) must be carefully transferred to nearby pools or man-made pools to avoid large-scale deaths due to drying of the beds of streams. The species is currently listed as the least concerned species (IUCN SSC Amphibian Specialist Group 2022).

Fig. 1. Developing stages of *Nanorana minica*.

Fig. 2. Dorsal view of adult *Nanorana minica.*

Fig. 3. Ventral view of adult *Nanorana minicai.*

Fig. 4. Hindlimb with web and rounded tips of toes. Web formula for the species I 1 + 1II 0-1 $^{1/2}$III 1-2IV2 $^{1/2}$-1V.

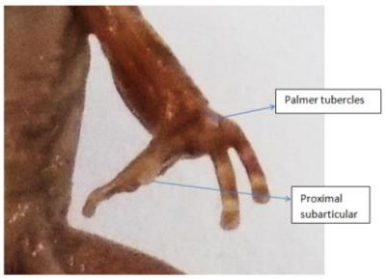

Fig. 5. Hand with positions of ovoid palmer tubercles proximal subarticular tubercles, fingers with round tips.

Morphology of tadpoles

Fig. 6. Tadpole of the species with growing hindlimbs.

Fig. 7. Mouth shape and structure of the tadpole with beaked jaw.

Fig. 8. Tail of the tadpole of the species with growing hindlimb.

Table 1: Morphometric measurements of the adult.

Specimen nos	HW	SVL	SL	IOD	HL	ED	IND	TD	DNE	TL	FL	FHL
18	**6**	**21**	**4**	**4**	**5**	**2.9**	**2**	**2**	**2.5**	**7**	**13**	**8**
17	**6**	**23**	**4.2**	**4**	**5**	**2.9**	**3**	**2**	**2.4**	**9**	**14**	**9**
16	**6**	**17**	**3.8**	**4**	**4**	**2**	**2**	**2**	**2**	**6**	**10**	**8**
15	**5**	**17**	**3.8**	**3.5**	**5**	**2**	**2**	**2**	**2**	**6**	**11**	**9**
14	**5**	**17**	**3.8**	**3.5**	**5**	**2**	**2**	**2**	**2**	**6**	**11**	**9**

Measurements included the following: head width (HW), snout–vent length (SVL), snout length (SL), inter orbital distance (IOD), head length (HL), eye diameter (ED), inter narial distance (IND), tympanum diameter (TD), nostril–eye distance (DNE), tibia length (TL), forearm and hand length (FHL); foot length (FL).

Table 2: Morphometry of tadpoles.

Tadpole no.	Total length	Remarks
1	23	early stage
2	31	mid stage
3	32	mid stage
4	34	mid stage
5	35	mid stage
6	36	mid stage
7	40	mid stage
8	42	mid stage
9	44	growing hind limb
10	46	growing hind limb
11	42	growing hind limb
12	47	growing hind limb
13	49	Well-developed hind limb

The species was listed as vulnerable species in previous assessment conducted in 2004. Further assessment is required for the species with restricted distribution in India and Nepal due to various threats and the species needs special conservation strategies and its implementation.

CONCLUSIONS

Distribution of *Nanorana minica* and other species of *Nanorana* in North Western India need to be studied as the species of the genus are known to be facing threat due to dam construction and climate change. The characteristic features of overwintering is present in the tadpoles of the species and making them unique to adapt to extreme of winters thus need to conserve the species and their habitat. They are useful as indicator of the health of a stream ecosystem. Nature of mountain streams and rivers in Uttarakhand is changing due to dam construction thus there is threat to many aquatic fauna including *Nanorana minica*. IUCN has now listed the species as Least Concern which was earlier categorized as VU, thus there is need to reassess the species.

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Conflict of Interest. None.

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