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Natural history notes on three sympatric frogs, *Amolops formosus* (Günther 1875), *Nanorana liebigii* (Günther 1860), and *Ombrana sikimensis* (Jerdon 1870), from Manaslu Conservation Area, Nepal

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***Abstract.*—Three stream dwelling mountain frogs, *Amolops formosus*, *Nanorana liebigii*, and *Ombrana sikimensis* are sympatric species, native to Asia and distributed much across Nepal. Here, a brief natural history account of the three species is provided that enhances the existing knowledge of these understudied frogs. Altogether 21 adults (eight *Amolops formosus*, six *Nanorana liebigii*, and seven *Ombrana sikimensis*) were collected from the streams of Sirdibas, Chumchet, and Bihi villages in April and May 2016 and in March 2017. Since the survey time coincided with breeding season, egg clutches and tadpoles of *Nanorana liebigii* were observed. Basic morphometric features of the adults (snout-vent length, head length, head width, femur length, and tibia length) and tadpoles (total length, body length, body width, and tail muscle width) were measured with a Mitutoyo digital Vernier caliper to the nearest 0.1 mm. Environmental parameters of the habitat were also noted, including air temperature, water temperature, relative humidity, and pH of the water body. A review of the conservation status of these sympatric frogs highlights the threats they face from unchecked harvesting in Manaslu and across the entire mountain villages of Nepal. Other potential threats include declining stream habitats through water use management decisions such as dams and diversions, pollution, and forest degradation. The field observation data collected will help to fill in the knowledge gaps for these species, in order to prioritize conservation action and aid future research.**

Keywords. Amphibia, Anura, Asia, habitat degradation, morphometrics, threats

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Introduction

Amolops formosus, *Nanorana liebigii*, and *Ombrana sikimensis* are sympatric species that are largely dependent upon mountain brooks and associated riparian habitats characterized by coniferous or oak forests (Schleich and Kästle 2002). They are native to Asia, found across many of the mountains of Nepal, and also recorded in India, China, Bangladesh, and Bhutan (Bordoloi et al. 2004; Liang et al. 2004). In Nepal, they are distributed within an altitudinal range of 1,190–3,360 m asl (Schleich and Kästle 2002). All three species were previously placed in the genus *Rana* (Boulenger 1920), but later revised into distinctive genera of *Amolops*, *Nanorana*, and *Ombrana* (Chen et al. 2005; Dubois 1974; Frost et al. 2006). The earliest first-hand records related to morphometrics, life history, and habitat notes of the three species (Boulenger 1882; Günther 1860; Jerdon 1870) are not readily accessible at the present time. While the recent publication of Schleich and Kästle (2002) is rather comprehensive, it is still unavailable to many readers due to the high price of the book (Zug 2004). Shah and Tiwari (2004) provided little information on the associated habitats of these sympatric amphibians, like surrounding vegetation and environmental parameters, but their report lacks data on egg deposition and tadpole stages. The IUCN Red List Assessment 2004 has further emphasized the need for research on the taxonomy, population size, distribution, trends, ecology, and life history of these frogs to prioritize conservation actions (Bordoloi et al. 2004; Liang et al. 2004). Therefore, any readily-available publication on the natural history of these frogs is of great importance to the scientific community, conservationists, natural resource managers, and decision makers.

Stream-dwelling frogs serve as good indicators of the stream ecosystem health, since they are philopatric

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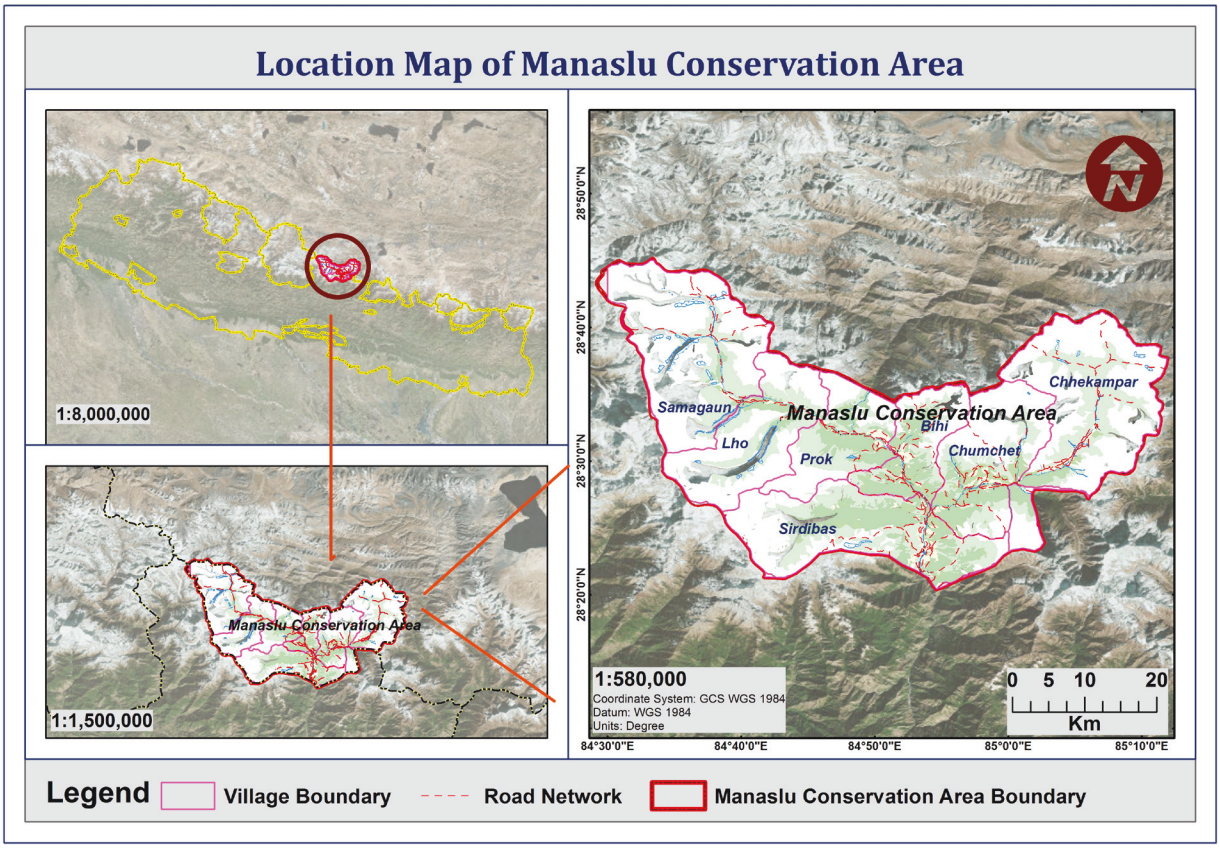

Fig. 1. Study sites in Manaslu Conservation Area, Nepal, with villages indicated in blue text.

in nature and found in steady populations (Welsh and Ollivier 1998). Studying such stream frogs with respect to morphology, life history, and habitat conditions will help to further understanding of their ecological niches (Ningombam 2009). This knowledge is vital for devising efficient conservation strategies when one-third of the total amphibian species of the world are being threatened with extinction (Baillie et al. 2004). This study presents the natural history notes of these three sympatric frogs. This information will help to aid in further research and monitoring, while providing background support for good decision making regarding their conservation in the future.

Materials and Methods

Surveys. Manaslu Conservation Area is one of the protected areas in Nepal, located at the upper north area of Gorkha district, province number 4 (Gandaki Pradesh). Surveys were conducted in five major villages of the Manaslu Conservation Area, namely Sirdibas, Bihi, Chumchet, Prok, and Samagaun, excluding Lho and Chhekampar (Fig. 1). The entire survey spanned 49 days during the day-time in April–May 2016 and March 2017.

A distance of 279 km was covered on foot throughout the survey and a transect of 200 m was walked in each site of the 14 streams (Table 1). Time-constrained searches were conducted for 2 h with two people at a time, for a total of four person-h per search. Live specimens of *Amolops formosus*, *Nanorana liebigii*, and *Ombrana sikimensis* were collected for morphological examination. They were released in-situ after recording observational notes and taking photographs with a Canon EOS 700D (18–135 mm) kit lens DSLR camera (Fig. 2). Egg clutches and tadpoles of different sizes were found in a few streams, and they were closely observed while causing minimal disturbance. The distribution of these sympatric frogs in Manaslu is restricted to Sirdibas, Bihi, and Chumchet (Fig. 3).

Measurements. The snout-vent length (SVL), head length (HL), head width (HW), femur length (FL), and tibia length (TL) were the morphological parameters measured following Fei et al. (2009) for the adult frogs. The morphometric keys for tadpoles were total length (TL), body length (BL), body width (BW), and tail muscle width (TMW), and followed Mitchell et al. (2012). All the measurements were taken using a Mitutoyo digital Vernier caliper to the nearest 0.1 mm.

Air and water temperature measurements were taken using a digital thermometer. The humidity was measured using a Hygrometer and the pH of the water was recorded with a digital pH meter. Geographic coordinates and altitude were recorded with a Garmin eTrex 10 GPS. Species identification and additional information followed Boulenger (1882, 1920), Chen et al. (2005), Frost et al. (2006), Günther (1860), Ningombam (2009), Schleich and Kästle (2002), and Shah and Tiwari (2004).

Results and Discussion

Morphometrics. The morphological notes of the three sympatric frogs correspond well with the earlier

Table 1. Sampling locations and observations of frogs (adults, egg clutches, and tadpoles) in the survey.

Sampling Site	Village	Location	Altitude (m asl)	Survey Time	Observations
1	Sirdibas	Yuwang Khola*	1,622	Day	*Amolops formosus* (2 ♀, 1 ♂); *Nanorana liebigii* (5 tadpoles, egg clutch)
2	Sirdibas	Ghatte Khola	2,425	Day	None
3	Sirdibas	Myarchwang Khola	1,629	Day	*Nanorana liebigii* (1 ♂); *Ombrana sikimensis* (7 individuals, unidentified sex)
4	Chumchet	Gyanak Khola	2,294	Dawn	*Amolops formosus* (3 ♀); *Nanorana liebigii* (1 ♂)
5	Chumchet	Sipchet Ripchet	2,473	Day	*Nanorana liebigii* (1 ♂)
6	Chumchet	Chumling	2,485	Dawn	*Nanorana liebigii* (1 ♀)
7	Chumchet	Gumlung Khola	2,482	Day	None
8	Chumchet	Sardi Khola	1,938	Dawn	*Nanorana liebigii* (tadpole with metamorphosed legs)
9	Chumchet	Lokpa	1,887	Day	*Nanorana liebigii* (egg clutch)
10	Chumchet	Phujung Khola	1,931	Day	*Nanorana liebigii* (1 ♂)
11	Samagaun	Birendra Tal	3,700	Day	None
12	Prok	Namrung Khola	2,462	Day	None
13	Bihi	Bihi Khola	2,189	Day	*Amolops formosus* (1 ♀); *Nanorana liebigii* (1 ♂)
14	Bihi	Dyang Khola	1,838	Night	*Amolops formosus* (1 ♀)

*Khola refers to stream

descriptions provided by Günther (1860, 1875) and Jerdon (1870) quoted in Schleich and Kästle (2002). *Nanorana liebigii* has the largest mean body size and body weight followed by *Ombrana sikimensis* and *Amolops formosus*. The body weight measurements of these frogs found in Nepal did not set new records. In Bhutan, Wangchuk (2017) documented the average weight of *Nanorana liebigii* as exceptionally higher (males 500–750 g and females 350–500 g) than the present findings for Nepal. The head lengths (HL) of *Amolops formosus* and *Nanorana liebigii* were smaller than the head widths (HW), however, *Ombrana sikimensis* was different, with HL greater than HW (Table 2). On the contrary, Boulenger (1920) has described broader HW than HL in *Ombrana sikimensis*.

Frogs in general tend to exhibit sexual dimorphism, with females mostly being larger in body size than males (Monnet and Cherry 2002). The results here agree for *Amolops formosus*, where females were larger in size than males; however, the males of *Nanorana liebigii* were larger in size than their female counterparts. The adult males of *Amolops formosus* had a nuptial pad on the 1st finger of the forelimb and also partly turquoise-colored hind limbs on the ventral side (Fig. 4A), as documented by Schleich and Kästle (2002). Likewise, males of the *Nanorana liebigii* had strongly hypertrophied forelimbs with a nuptial pad on the 1st finger. Further, numerous black horny spines were present on the 1st, 2nd, and 3rd fingers on both the arms and extending along the pectoral region (Fig. 4C). It was difficult to identify the sexes of *Ombrana sikimensis* based only on SVL measurements, since nuptial spines are not present in *Ombrana sikimensis* (Boulenger 1920).

Egg deposition and larval stages. Spring (March–May) is the season of breeding for *Nanorana liebigii* as egg clutches were found in slowly drifting Yuwang Khola in Sirdibas village to fast flowing streams in Lokpa, Chumchet village. The eggs were attached to the undersides of stones, totally submerged, and white in

Table 2. Morphological parameters (SVL, HL, HW, FL, and TL) of the three species of adult sympatric frogs (mm) and BW (g). Min = Minimum value, Max = Maximum value, M = Average value (Mean), SD = Standard Deviation, and n = number of individuals.

Morphometric keys	*Amolops formosus* (n = 8)			*Nanorana liebigii* (n = 6)			*Ombrana sikimensis* (n = 7)		
	Min	M ± SD	Max	Min	M ± SD	Max	Min	M ± SD	Max
Snout-vent length (SVL)	67.3	74.1 ± 3.8	81.5	78.8	87.7 ± 7.6	99.6	67.1	81.2 ± 11.6	92.1
Head length (HL)	23.7	24.7 ± 0.7	26.1	25.8	27.9 ± 2.0	31.5	21.6	24.6 ± 2.3	27.1
Head width (HW)	24.6	25.8 ± 1.0	27.9	27.4	29.1 ± 1.8	32.4	20.5	23.3 ± 2.2	25.8
Femur length (FL)	39.9	42.9 ± 1.9	46.1	43.2	50.6 ± 3.7	53.1	39.3	44.0 ± 3.9	47.9
Tibia length (TL)	45.2	47.5 ± 1.2	49.1	47.6	54.7 ± 3.9	58.7	42.3	46.4 ± 3.4	50.2
Body weight (BW)	35	46.9 ± 6.2	55	60	82.8 ± 14.8	100	50	70.1 ± 14.6	85

Fig. 2. Dorsal view of live adults: (**A**) *Ombrana sikimensis*, (**B**) *Amolops formosus*, and (**C**) *Nanorana liebigii*. (**D**) Dorso-lateral view of *Nanorana liebigii*. *Photos: Biraj Shrestha and Min Bahadur Gurung.*

color inside the gelatinous ball that had a honeycomb-like appearance (Fig. 5A–B). The clutch size consisted of about 80–140 eggs although no adults were seen nearby, which is noteworthy since males of *Nanorana liebigii* are reported to guard the eggs as a form of parental care (Rai 2003). Some gradually developing egg clutches were observed that had embryos with eyes and were surrounded by jelly of a "liver-like" color (Fig. 5C).

Five tadpoles of *Nanorana liebigii* were observed at the Yuwang Khola and one metamorphosed tadpole with hind limbs was evident in the shallow pools of a rapidly flowing stream in Sardi Khola. The metamorphosed tadpole had a well-developed oral disc (Fig. 5D), with the structure of the upper and lower lips forming an atrium feature (Kästle et al. 2013). The tails of the tadpoles were nearly twice the length of the body (Schleich and Kästle 2002), while the body lengths were significantly greater than widths (Table 3).

Tadpoles of *Nanorana liebigii* were found sympatric with the tadpoles of *Duttaphrynus himlayanus*. However, no egg clutches of *Amolops formosus* were observed during the study. Published information on egg deposition by *Amolops formosus* is limited, though Nidup et al. (2016) reported an egg clutch of *Amolops himalayanus* attached underneath of rocks and clear white, from a gentle flowing stream in Bhutan.

Habitat notes. The general habitat of all the three sympatric frogs studied here is mountain streams above 1,100 m asl elevation and of varied intensity. In addition, *Nanorana liebigii* was also found to inhabit other water bodies, such as the puddles in a bamboo forest and irrigation ditches near the cropland where *Karu* (a type of naked barley) was grown (Fig. 6). *Amolops formosus* preferred fast flowing streams, typically cascades, attaching themselves to the steep slopes of rocks and resting on fissures, partly covered in moss and ferns, while *Ombrana sikimensis* were typically hiding in clusters underneath rocks in shallow streams (Fig. 6C). The nearby riparian vegetation included Nepalese Alder (*Alnus nepalensis*), Broom Grass (*Thysanolaena maxima*), Himalayan Blue Bamboo (*Himalayacalamus hookerianus*), Himalayan Silver Birch (*Betula utilis*), Tree Rhododendron (*Rhododendron arboreum*), Chir Pine (*Pinus roxburghii*), Walnut (*Juglans*

Table 3. Morphological parameters (TL, BL, BW, and TMW) of the tadpoles of *Nanorana liebigii* (mm). Min = Minimum value, Max = Maximum value, M = Average value (Mean), SD = Standard Deviation, and n = number of individuals.

Morphometric keys	*Nanorana liebigii* Tadpole (n = 6)		
	Min	M ± SD	Max
Tail length (TL)	41.4	49.1 ± 6.7	57.7
Body length (BL)	13.3	19.3 ± 4.5	24.5
Body width (BW)	8.3	11.6 ± 2.5	14.7
Tail muscle width (TMW)	3.2	7.3 ± 3.3	11.5

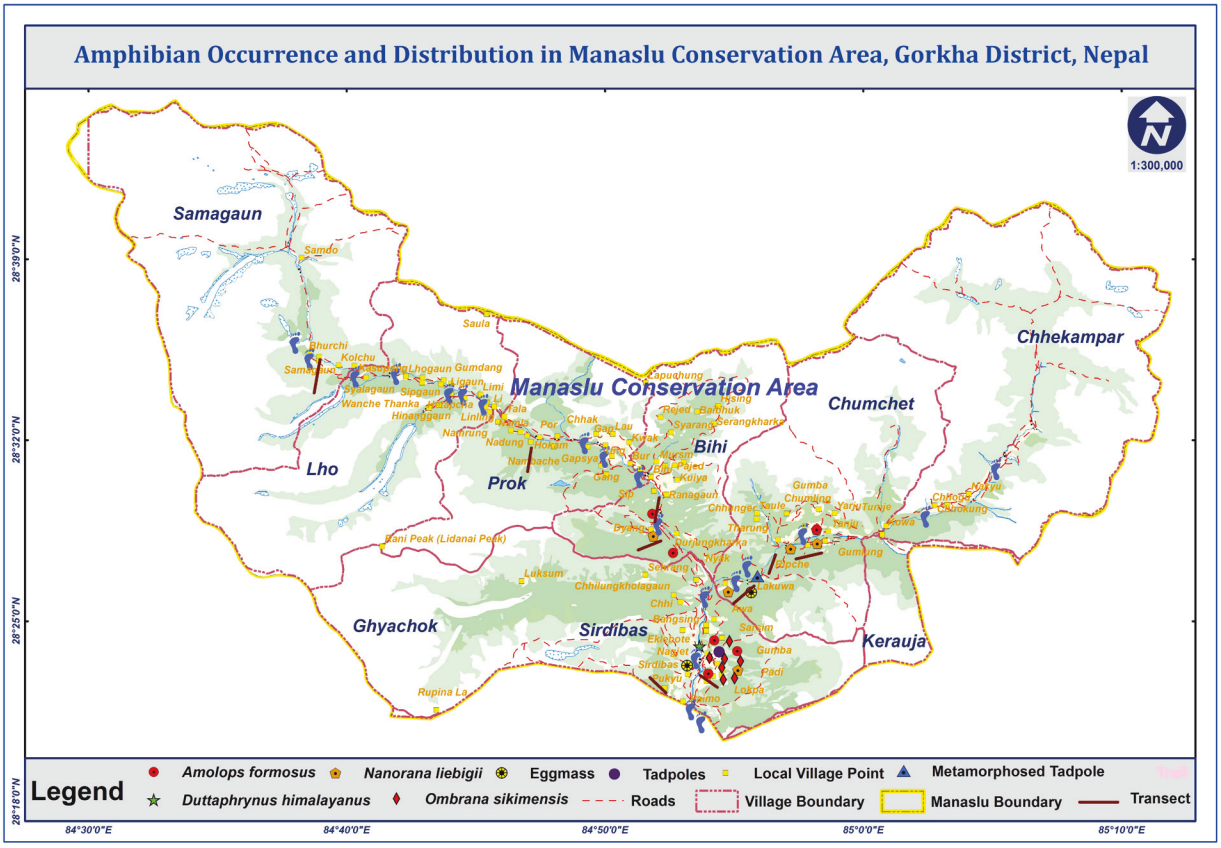

Fig. 3. Frog observation sites and distances travelled throughout the survey.

regia), and others.

Amphibians are often mentioned as the bio-indicators of water quality because of their permeable skin. Their eggs and larval stages are generally much more vulnerable to any type of pollution in the water bodies due to their lack of protective covering, and direct connection with the water for survival and growth. The optimal environmental conditions of their habitat are crucial to allow for metamorphosis and for habitat management strategies. Cold water is favorable for the growth of different life stages with neutral to slightly alkaline water pH. This range is desirable for much of the aquatic fauna, including stream dwelling frogs, as lower acidic pH conditions can impede amphibian growth and inhibit the development of eggs and embryos (Ningombam 2009).

Conservation status. All the three species of frogs are listed in the Least Concern (LC) category in the IUCN Red List Assessment from 2004 (15 years prior to this writing) based upon the presumption of their large populations, wider distributions, and with no prospects of immediate decline. However, a reassessment is desperately needed, as the current population trends for all three species are going down due to declining stream habitats from various causes, such as water diversion and dams to deforestation and pollution (Bordoloi et al. 2004; Liang et al. 2004). In India, all three of these species are protected under the national legislation, while no similar effort by the Nepalese government has been undertaken to provide any legal measures for amphibian conservation. This remains the case today (in 2019), despite the recommendation by the Biodiversity Profiles Project (BPP, 1995) for nine species of endemic Nepalese amphibians to be included in the Schedule I of National Parks and Wildlife Conservation (NPWC) Act 1973.

Anurans of genera *Amolops*, *Nanorana*, and *Ombrana* are called 'Paha' frogs in Nepal and have ethnozoological relationships with the communities living mostly in hills and mountains (Shah and Tiwari 2004; Shrestha 2018). People often harvest paha frogs as a delicacy and for their apparent therapeutic benefits. Every indigenous community in the mountains of Nepal either has experience in paha hunting or at least knows about its use. As a result, paha hunting is popular in villages from the east to western part, all across the nation. The hunting usually takes place at night during pre- and post-monsoon seasons, when the water flow is minimum. There is no limit for harvested quantities from the streams, and people usually collect as many as they can find during their searches. In Manaslu, Gurung communities in Sirdibas village typically collect 51–100 individuals on

Table 4. Physico-chemical characteristics of water quality in the survey sites and altitudinal range of the detected frogs. Min = Minimum value, Max = Maximum value, M = Average value (Mean), and SD = Standard Deviation.

Abiotic factors	Min	M ± SD	Max
Air Temperature (°C)	8	20.0 ± 6.5	26.5
Water Temperature (°C)	4	13.2 ± 2.8	16.3
Relative Humidity (%)	25	43.5 ± 11.7	55
pH	7	8.0 ± 0.3	8.6
Altitude (m asl)	1,591	1,880.5 ± 39.4	2,480

Fig. 4. Ventral views of live adults: (**A**) male of *Amolops formosus*, (**B**) female of *Amolops formosus*, (**C**) male of *Nanorana liebigii*, and (**D**) female of *Nanorana liebigii*. *Photos: Biraj Shrestha*.

average in one season and they trade locally in the price range of USD 0.45–2.26 (Shrestha and Gurung 2019). *Nanorana liebigii* is the most popular paha frog across the country, followed by *Ombrana sikimensis* which is highly sought after as its meat serves as a delicacy and nutritional purposes (Shah and Tiwari 2004). In addition, the meats of *Nanorana liebigii* and *Amolops formosus* are traditionally assumed to have medicinal properties that cure fever, cough, cold, dysentery, and stomach ache; while their skin secretions have antiseptic properties (Shrestha and Gurung 2019). In recent years, paha hunting is largely practiced to enjoy its meat and for recreational purposes in the villages, since the nutritional requirements are often met by poultry and livestock, and medical supplies are readily available thanks to improved road access for most villages these days. However, the continued paha hunting practice has depleted its numbers as reported by the local communities across the country, including Manaslu, and has led to the recommendation for some form of legal conservation protection.

The diminishing populations of *Amolops formosus*, *Nanorana liebigii*, and *Ombrana sikimensis* can be averted by developing species specific conservation priority plans. Habitat conservation planning, population study and monitoring, hunting regulation policies, and effective outreach programs are some of the key action steps. The brief natural history notes presented here on morphometrics, sexual dimorphism, egg deposition, larval stages, and habitat conditions will be helpful in this regard. But since the identification of congeneric amphibians can be tricky, the use of molecular phylogeny and call identification coupled with morphometrics is strongly recommended for accurate species identification.

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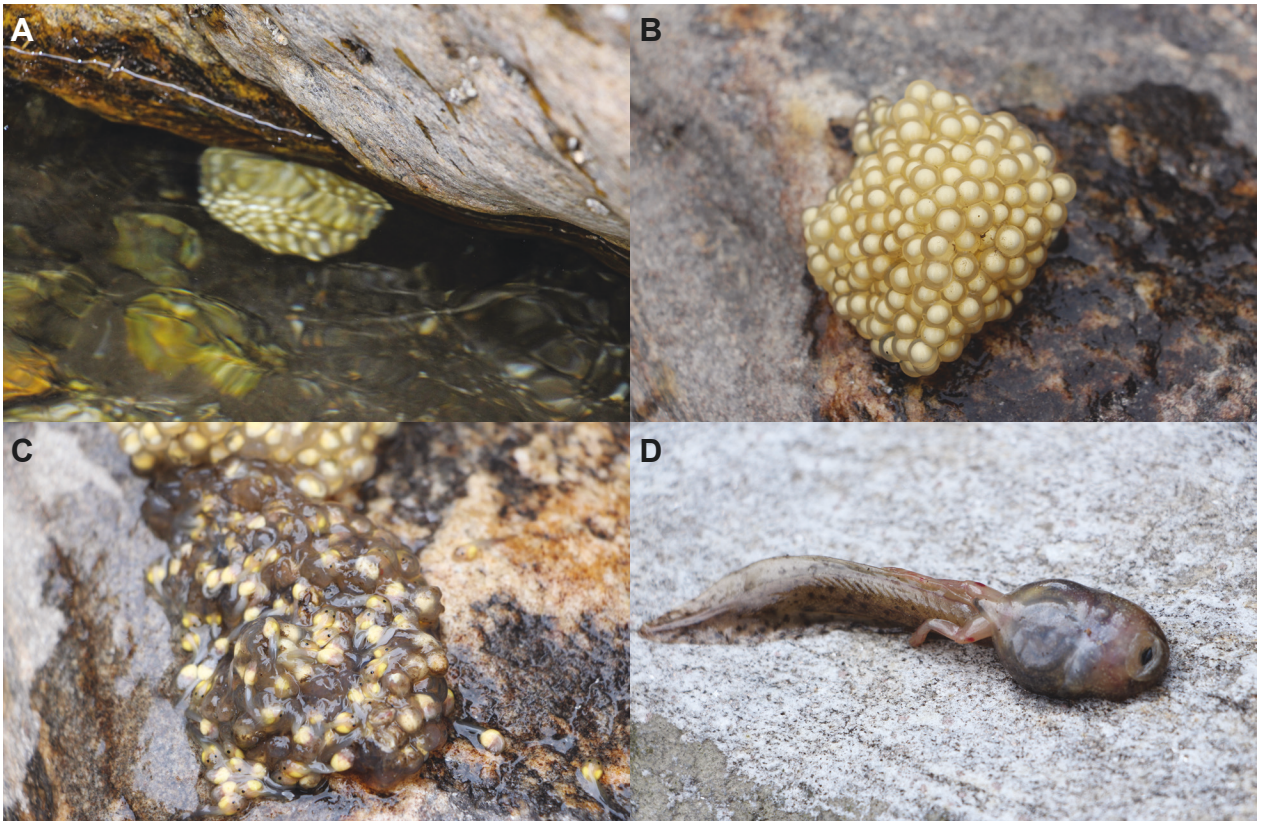

Fig. 5. Life stages of *Nanorana liebigii*: (**A**) eggs deposition underneath a stone, (**B**) egg clutch, (**C**) embryo development, and (**D**) tadpole with metamorphosed legs. *Photos: Biraj Shrestha*.

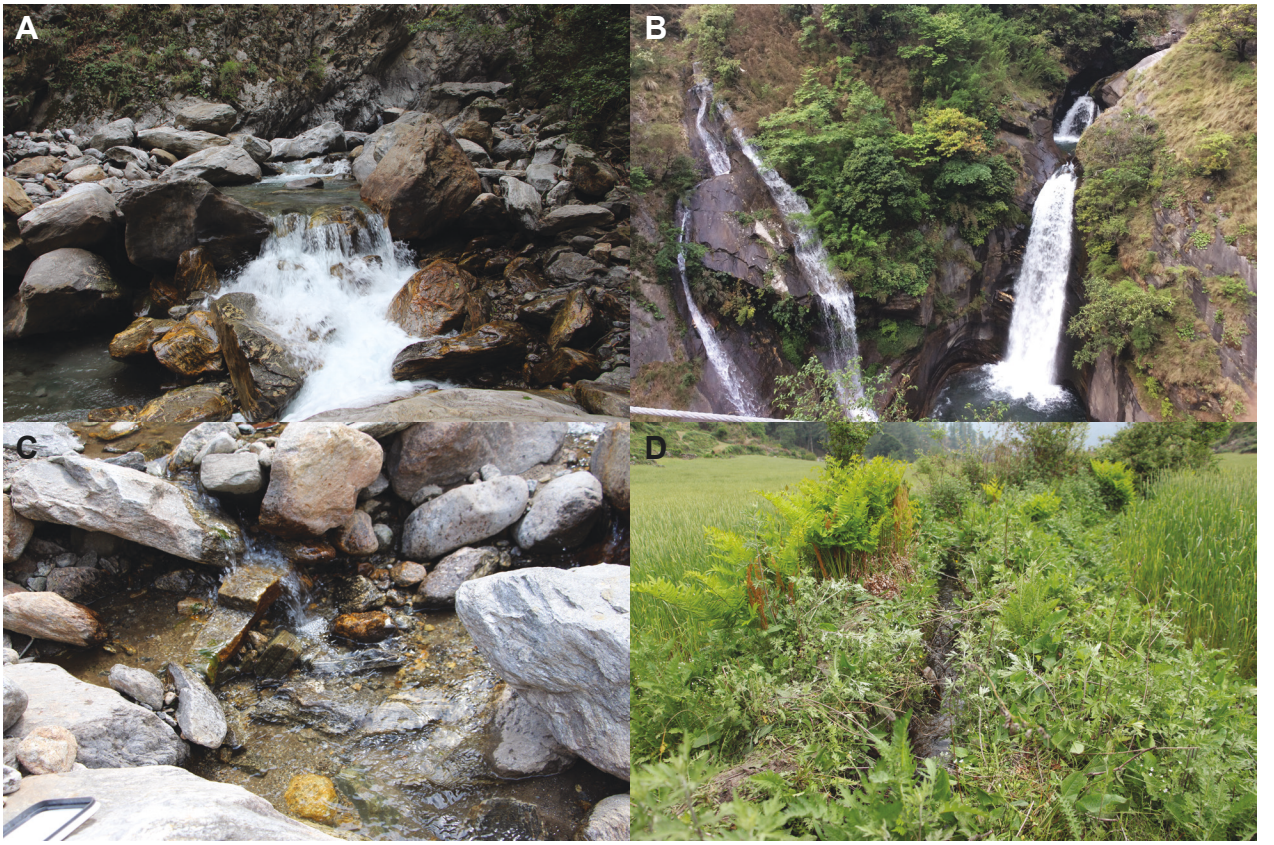

Fig. 6. Habitat varieties of the three sympatric frogs: (**A**) rapidly flowing stream, (**B**) series of waterfalls inhabited by *Amolops formosus*, (**C**) slow flowing shallow stream, and (**D**) irrigation ditch. *Photos: Biraj Shrestha*.

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