

Aliens vs. Amphibians: new data on amphibians' ectoparasitic and endoparasitic leeches (Annelida: Hirudinea) of Vietnam and China

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ABSTRACT

Leeches are common parasites of amphibians and can affect them through blood loss, pathogen transmission, and predation, yet leech–amphibian interactions remain poorly documented in Indochina. This study provides the first documented cases of leech parasitism on amphibians from Vietnam and adjacent southwestern China. Amphibian-associated leeches appear to be widespread across the studied region, occurring across a broad range of habitats and elevations. Ectoparasitic associations were recorded for three hirudinean families (Haemadipsidae, Glossiphoniidae, and Salifidae) across northern and southern Vietnam, where leeches use at least 12 species (seven genera, five families, two orders) of amphibians as hosts. Most notably, we documented true endoparasitism by praobdellid leeches in the abdominal cavity of anurans, comprising 22 cases across five host species of the family Ranidae and two host species of the family Bufonidae from five localities in northern Vietnam and southwestern China. To our knowledge, such a case of endoparasitism has not been previously reported from the Asian region or for praobdellid leeches. Our findings substantially expand current knowledge of leech–amphibian interactions in Indochina, highlight a previously unrecognized life-history strategy in Praobdellidae, and provide a baseline for assessing potential impacts of leech parasitism on amphibian health and conservation in the region.

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INTRODUCTION

Several leech taxa (Annelida: Clitellata: Hirudinida Lamarck), including members of the families Haemadipsidae Blanchard, Glossiphoniidae Vaillant, Salifidae Johansson, and Praobdellidae Sawyer, are commonly known as ectoparasites (Phillips et al., 2020). Feeding on the blood of various vertebrates, the trophic ecology of leeches as vertebrate ectoparasites has not only conservation relevance (Nguyen et al., 2021; Wilting et al., 2022) but also public health importance (Barta & Dessler, 1989; Kikuchi et al., 2002; Kikuchi & Fukatsu, 2005; O'Dempsey, 2012). In particular, through parasitism, leeches are known as vectors for

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pathogen transmission (Barta, 1991; De Carle et al., 2017; Hamilton et al., 2005; Raffel et al., 2006; Siddall & Bowerman, 2006; Siddall & Dessler, 1990, 1992; Spodareva et al., 2018).

While the medical importance of leeches is well recognized in literature, the ecological and conservational relevance of leeches with respect to other organisms is relatively understudied, particularly through parasitism with amphibians. For leech-amphibian interactions, previous studies mainly focused on the predatory interactions (Ayres & Comesaña, 2010; Berven & Boltz, 2001; Burgin & Schell, 2005; Cotes-Perdomo et al., 2018; Elliott & Kutschera, 2011; Kutschera et al., 2010; Mann & Tyler, 1963; Schalk et al., 2002; Trauth & Neal, 2004), while only a few mentioned parasitism (Canazas-Teran et al., 2024).

Leeches often specialize on amphibians, which serve as convenient feeding hosts due to their relatively thin skin with dense vascular networks supporting cutaneous respiration (Phillips et al., 2020; Starzecka et al., 2020). Research on amphibian-parasitizing leeches is particularly important given that amphibians are currently the most threatened vertebrate group (Alroy, 2015; Rowley et al., 2010). Leech parasitism may negatively affect amphibian health through blood loss, tissue damage, and potential transmission of pathogens, which may ultimately influence host survival and population dynamics (e.g., Raffel et al., 2006; Siddall & Dessler, 1990). Such effects are of particular concern given the global decline of amphibian populations. In some regions, such as South America (Canazas-Teran et al., 2024), leech-amphibian interactions are well studied, and numerous cases have been documented across different countries. In contrast, only a few documented cases are available from the Asian region, most of which represent single records with rather fragmentary descriptions (Bae et al., 2018; Bilaskar & Walke, 2025; Delima-Baron et al., 2024; Kambayashi & Nakano, 2023; Lai et al., 2011; Maglangit et al., 2020; Nakano & Sung, 2014; Ngamprasertwong et al., 2007; Seo et al., 2013; Sim et al., 2012).

The Indochinese region represents one of the major global biodiversity hotspots for amphibians, harboring a high level of species richness and endemism. Within this region, northern Vietnam and southwestern China, particularly Yunnan Province, are among the most biodiverse areas in Asia, supporting a rich fauna of both amphibians (Frost, 2026; Poyarkov et al., 2021) and leeches (Bolotov et al., 2025; Nakano & Nguyen, 2015; Nakano et al., 2016; Yang, 1996). However, the leech-amphibian interactions remain poorly studied in both countries. For Vietnam, even fewer studies have been published regarding the leech-amphibian interactions, most of which concern only the taxonomy of leeches (Bolotov et al., 2025; Nakano & Nguyen, 2015; Nakano et al., 2016). In China, studies on leech-amphibian interactions remain superficial, existing only in monographs of leech taxonomy and agricultural literature. Several species of Glossiphoniidae leeches have been reported as ectoparasites on anuran species in China, including *Glossiphonia complanata* (Linnaeus) on *Rana chinensis* David (Ranidae) and *Strauchbufo raddei* (Strauch) (Bufonidae) (Yang, 1996; Zhou et al., 2021); *Batrachobdella* spp. on *Quasipaa spinosa* (David) and *Quasipaa boulengeri* (Günther) (Dicroglossidae) (Tan, 2008; Yang, 1996); *Parabdelia quadrioculata* (Moore) on *Amolops mantzorum* (David) and *Rana chinensis* (Ranidae) (Yang, 1996); *Placobdella okadai* (Oka) on *Pelophylax plancyi* (Lataste) (Ranidae) and *Q. boulengeri* (Dicroglossidae) (Yang,

1996); and *Oligobdella* spp. on *Nanorana quadranus* (Liu, Hu & Yang) (Dicroglossidae). Ji et al. (2022) reported common parasitism or predation of leeches on amphibians, detecting iDNA of 25 species of amphibians in terrestrial leeches in Yunnan, China. Similar studies from other regions of tropical Southeast Asia, such as Sumatra (Indonesia), have demonstrated that blood-feeding leeches retain host DNA that can be used to detect vertebrate species, including rare amphibians, further emphasizing the ecological significance of leech-vertebrate interactions across the region (Irawan & Eguchi, 2025). However, these documentations are biased taxonomically (i.e., towards parasitism on farmed amphibian species) and geographically (i.e., in northeastern and central China). As the subtropical and tropical regions in Southwest China, particularly in Yunnan Province, harbor a high proportion of China's biodiversity (Yang et al., 2004), such bias in past studies may neglect some important leech-amphibian interactions. For the only study that concerns leech-amphibian interactions in Yunnan, Ji et al. (2022) did not report the taxonomy of leeches nor observations of frog-leech interactions in the field. Because the leech-amphibian interactions, particularly parasitism, could impact the amphibian populations negatively and even spread other internal parasites (Yang, 1996), such interactions have important conservation implications and warrant better understanding. Despite increasing reports of leech-vertebrate associations from other regions, comprehensive field-based observations documenting host identity, parasitic behavior, and life-history strategies of leeches on amphibians remain scarce in Indochinese region.

Based on fieldwork conducted in 2023–2025, we collected numerous records of leech parasitism for amphibians from Vietnam and China. In addition to the expanded documentation of ectoparasitism on 12 species of amphibians by three leech families, most importantly, we report several cases of true leech endoparasitism in amphibians from both Vietnam and China, which appears to be the second observation in the Indo-Pacific Region since 1963 (Mann & Tyler, 1963). In particular, true endoparasitism of leeches in amphibians has rarely been documented worldwide and remains poorly understood in tropical Asia. This study reviews the taxonomic diversity, life history, and host relationships of the amphibian-associated leech of Vietnam and China.

MATERIALS AND METHODS

Sample collection and identification

Leeches and their hosts were collected between 2023 and 2025 from montane evergreen tropical forests at eight localities in Lang Son, Lao Cai, Son La, Thanh Hoa, and Tuyen Quang provinces (hereafter Provs.) as well as Hanoi City of northern Vietnam, and Lam Dong Province (hereafter Prov.) of southern Vietnam by Andrey M. Bragin and Nikolay A. Poyarkov, and in two localities in Yunnan Prov. of southwestern China by Kai Wang, Zhong-Bin Yu, and Jing Che. A summary (collection date, coordinates, elevation, collectors' names, and voucher numbers) of collected specimens of leeches and their hosts from Vietnam and southwestern China observed in this study is provided in Table 1.

Geographic coordinates and elevation were obtained using a Garmin Forerunner 955 Solar (Garmin International Inc., USA) and recorded in the WGS 84 datum. Specimens were

Table 1 Summary (localities, voucher information, and GenBank accession numbers) of leeches associated with amphibians of Vietnam and China observed in this study

Specimen ID	Field no.*	Taxon	Date	N	E	Alt.	Country	Locality	Host	ECP/ENP	GenBank ID(COI)	Leg.
VRTC BAM239	ZMMU NAP-16536–16540	Praobdellidae sp.	24.05.2024	21.84762	106.98690	1006	Vietnam	Mt. Mau Son, Cao Loc Dist., Lang Son Prov.	<i>Amolops cf. tonkinensis</i>	ENP	–	Bragin A.M.
VRTC BAM240	ZMMU NAP-16536–16540–	Praobdellidae sp.	24.05.2024	21.84762	106.98690	1006	Vietnam	Mt. Mau Son, Cao Loc Dist., Lang Son Prov.	<i>Amolops cf. tonkinensis</i>	ENP	–	Bragin A.M.
VRTC BAM241	ZMMU NAP-16536–16540–	Praobdellidae sp.	24.05.2024	21.84762	106.98690	1006	Vietnam	Mt. Mau Son, Cao Loc Dist., Lang Son Prov.	<i>Amolops cf. tonkinensis</i>	ENP	–	Bragin A.M.
VRTC BAM242	ZMMU NAP-16535	Praobdellidae sp.	24.05.2024	21.84762	106.98690	1006	Vietnam	Mt. Mau Son, Cao Loc Dist., Lang Son Prov.	<i>Amolops cf. tonkinensis</i>	ENP	–	Bragin A.M.
VRTC BAM243	ZMMU NAP-14515	Praobdellidae sp.	27.05.2023	22.49983	103.60176	2690	Vietnam	Mt. Ky Quan San, Bat Xat Dist., Lao Cai Prov.	<i>Bufo luchunnicus</i>	ENP	PX729570	Bragin A.M., Poyarkov N.A.
VRTC BAM330	ZMMU NAP-16626	Praobdellidae sp.	24.05.2024	21.84762	106.98690	1006	Vietnam	Mt. Mau Son, Cao Loc Dist., Lang Son Prov.	<i>Amolops cf. tonkinensis</i>	ENP	–	Bragin A.M.
VRTC BAM331	ZMMU NAP-13948	Praobdellidae sp.	16.04.2023	22.76240	104.83599	1040	Vietnam	Mt. Tay Con Linh, Cao Bo, Tuyen Quang Prov.	<i>Amolops cf. shihaitaoi</i>	ENP	–	Bragin A.M., Poyarkov N.A.
VRTC BAM244	ZMMU NAP-16629	Praobdellidae sp.	24.05.2024	21.84762	106.98690	1006	Vietnam	Mt. Mau Son, Cao Loc Dist., Lang Son Prov.	<i>Amolops cf. tonkinensis</i>	ENP	–	Bragin A.M.
VRTC BAM245	ZMMU NAP-13946	Praobdellidae sp.	16.04.2023	22.76240	104.83599	1040	Vietnam	Mt. Tay Con Linh, Cao Bo, Tuyen Quang Prov.	<i>Amolops cf. shihaitaoi</i>	ENP	PX729569	Bragin A.M., Poyarkov N.A.
VRTC BAM331	ZMMU NAP-13948	Praobdellidae sp.	16.04.2023	22.76240	104.83599	1040	Vietnam	Mt. Tay Con Linh, Cao Bo, Tuyen Quang Prov.	<i>Amolops cf. shihaitaoi</i>	ENP	–	Bragin A.M., Poyarkov N.A.
VRTC BAM245	ZMMU NAP-14079	Praobdellidae sp.	23.04.2023	22.79847	104.80890	2224	Vietnam	Mt. Tay Con Linh, Cao Bo, Tuyen Quang Prov.	<i>Amolops cf. daorum</i>	ENP	–	Bragin A.M., Poyarkov N.A.
VRTC BAM304	ZMMU NAP-18321–18322, ZMMU NAP-18359, ZMMU NAP-18361	Praobdellidae sp.	11.10.2025	21.321205	104.450372	2005	Vietnam	Mt. Sa Mu, Ta Xua NR, Bac Yen Dist., Son La Prov.	<i>Amolops cf. daorum</i>	ENP	–	Bragin A.M.
VRTC BAM305	ZMMU NAP-18334	Praobdellidae sp.	11.10.2025	21.329127	104.449819	2376	Vietnam	Mt. Sa Mu, Ta Xua NR, Bac Yen Dist., Son La Prov.	<i>Bufo cf. pageoti</i>	ENP	–	Bragin A.M.
VRTC BAM306	ZMMU NAP-18335	Praobdellidae sp.	11.10.2025	21.329127	104.449819	2376	Vietnam	Mt. Sa Mu, Ta Xua NR, Bac Yen Dist., Son La Prov.	<i>Bufo cf. pageoti</i>	ENP	–	Bragin A.M.
–	–	Praobdellidae sp.	15.07.2022	24.84854784	98.75857203	2095	China	Baoshan City, Longyang Dist., Yunnan Prov.	<i>Amolops viridimaculatus</i>	ENP	PX729571	Kai Wang, Zhongbin Yu, Jing Che
–	–	Praobdellidae sp.	26.05.2022	24.84854784	98.75857203	2095	China	Baoshan City, Longyang Dist., Yunnan Prov.	<i>Amolops viridimaculatus</i>	ENP	PX729572	Kai Wang, Zhongbin Yu

Specimen ID	Field no.*	Taxon	Date	N	E	Alt.	Country	Locality	Host	ECP/ENP	GenBank ID(COI)	Leg.
–	–	Praobdelidae sp.	26.05.2022	24.84854784	98.75857203	2095	China	Baoshan City, Longyang Dist., Yunnan Prov.	<i>Amolops viridimaculatus</i>	ENP	PX729573	Kai Wang, Zhongbin Yu
–	–	Praobdelidae sp.	26.05.2022	24.81770262	97.73726198	2072	China	Baoshan City, Longyang Dist., Yunnan Prov.	<i>Amolops viridimaculatus</i>	ENP	PX729574	Kai Wang, Zhongbin Yu
–	–	Praobdelidae sp.	27.10.2021	27.69160773	98.28229241	1294	China	Gongshan County, Nujiang Pref., Yunnan Prov.	<i>Amolops kaulbacki</i>	ENP	PX729575	Zhongbin Yu
–	–	Praobdelidae sp.	23.05.2023	27.68684157	98.27352495	1346	China	Gongshan County, Nujiang Pref., Yunnan Prov.	<i>Amolops kaulbacki</i>	ENP	–	Kai Wang
VRTC BAM249	ZMMU NAP-14528	Glossiphoniidae sp.	27.05.2023	22.49971	103.60070	2700	Vietnam	Mt. Ky Quan San, Bat Xat Dist., Lao Cai Prov.	<i>Boulengerophrys rubrimeris</i>	ECP	PX729580	Bragin A.M., Poyarkov N.A.
VRTC BAM259	ZMMU NAP-16631	Salifidae sp.	28.05.2024	21.862458	107.011681	901	Vietnam	Mt. Mau Son, Cao Loc Dist., Lang Son Prov.	<i>Tylostotriton vietnamensis</i>	ECP	PX729576	Bragin A.M.
VRTC BAM250	ZMMU NAP-15102	Haemadipsidae sp.	21.10.2023	19.97829	104.98335	960	Vietnam	Xuan Lien NR, Thuong Xuan Dist., Thanh Hoa Prov.	<i>Boulengerophrys palpebralespinosa</i>	ECP	PX729579	Bragin A.M.
VRTC BAM251	ZMMU NAP-15374	Haemadipsidae sp.	5.11.2023	11.85708	108.02942	1820	Vietnam	Mt. Ta Dung, Bich Khe Dist., Lam Dong Prov.	<i>Kurixalus gryllus</i>	ECP	–	Bragin A.M.
VRTC BAM258	ZMMU NAP-15375	Haemadipsidae sp.	5.11.2023	11.85708	108.02942	1820	Vietnam	Mt. Ta Dung, Bich Khe Dist., Lam Dong Prov.	<i>Kurixalus gryllus</i>	ECP	–	Bragin A.M.
VRTC BAM258	ZMMU NAP-15376	Haemadipsidae sp.	5.11.2023	11.85708	108.02942	1820	Vietnam	Mt. Ta Dung, Bich Khe Dist., Lam Dong Prov.	<i>Kurixalus gryllus</i>	ECP	–	Bragin A.M.
VRTC BAM258	ZMMU NAP-15377	Haemadipsidae sp.	5.11.2023	11.85708	108.02942	1820	Vietnam	Mt. Ta Dung, Bich Khe Dist., Lam Dong Prov.	<i>Kurixalus gryllus</i>	ECP	–	Bragin A.M.
VRTC BAM258	ZMMU NAP-15379	Haemadipsidae sp.	5.11.2023	11.85708	108.02942	1820	Vietnam	Mt. Ta Dung, Bich Khe Dist., Lam Dong Prov.	<i>Kurixalus gryllus</i>	ECP	–	Bragin A.M.
VRTC BAM258	ZMMU NAP-15380	Haemadipsidae sp.	5.11.2023	11.85708	108.02942	1820	Vietnam	Mt. Ta Dung, Bich Khe Dist., Lam Dong Prov.	<i>Kurixalus gryllus</i>	ECP	–	Bragin A.M.
VRTC BAM258	ZMMU NAP-15381	Haemadipsidae sp.	5.11.2023	11.85708	108.02942	1820	Vietnam	Mt. Ta Dung, Bich Khe Dist., Lam Dong Prov.	<i>Kurixalus gryllus</i>	ECP	–	Bragin A.M.
VRTC BAM258	ZMMU NAP-15402	Haemadipsidae sp.	7.11.2023	11.85708	108.02942	1820	Vietnam	Mt. Ta Dung, Bich Khe Dist., Lam Dong Prov.	<i>Kurixalus gryllus</i>	ECP	PX729578	Bragin A.M.
VRTC BAM260	ZMMU NAP-17406	Haemadipsidae sp.	15.06.2024	21.082274	105.382001	342	Vietnam	Mt. Ba Vi, Ha Noi Prov.	<i>Ophryophryne microstoma</i>	ECP	PX729577	Bragin A.M.
VRTC BAM233	ZMMU NAP-15103	Haemadipsidae sp.	26.10.2023	19.97148	104.98922	1260	Vietnam	Xuan Lien NR, Thuong Xuan Dist., Thanh Hoa Prov.	<i>Leptobrachium cf. guangxiense</i>	ECP	–	Bragin A.M.
VRTC BAM275	ZMMU NAP-17699	Haemadipsidae sp.	25.03.2025	12.09722	108.65800	2137	Vietnam	Mt. Bidoup, Lac Duong Dist., Lam Dong Prov.	<i>Kurixalus gryllus</i>	ECP	–	Bragin A.M.

Altitude is given in meters above sea level; ECP/ENP: ectoparasitic or endoparasitic leeches; –: information not available; leg.: collector initials. * – Field numbers correspond to the voucher numbers of host-amphibian specimens deposited in museum collections of VRTC and ZMMU.

collected by hand during the nighttime (1800h–2200h) from trees, the banks of rivers and streams, leaf litter, under fallen tree logs, and rocks. Video recording was carried out using an iPhone 15 Pro (Apple Inc., USA). Specimens were photographed in life using a Nikon D7200 camera equipped with a Nikon AF-S Micro NIKKOR 105 mm macro lens and a Nikon Speedlight SB-900 flash with a diffuser (Nikon Corporation, Japan). Air temperature and humidity data were obtained using an HTC-2 Thermo-Hygrometer (China).

The specimens of amphibians were photographed in life and euthanized using MS-222 solution (Sigma-Aldrich, USA) within 24 hours after capture; the samples of liver or muscle tissues were removed for the subsequent molecular analyses and stored in 95% ethanol. The specimens were subsequently fixed in 4% buffered formalin for 24 hours and later stored in 70% ethanol. The specimens of leeches were relaxed by the gradual addition of absolute ethanol to freshwater. For DNA extraction, muscle tissue samples were removed from the posterior part of the body and then stored in 95% ethanol. The remainder of the body was preserved in 70% ethanol.

Amphibian specimens were euthanized using MS-222 solution (Sigma-Aldrich, USA), following protocols approved by the Institutional Ethical Committee of the Joint Vietnam–Russia Tropical Science and Technology Research Center (VRTC), Hanoi, Vietnam, and the Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ), Kunming, China. Specimens of leeches were either dissected from the abdomen of amphibians after euthanasia of amphibians or collected from the cloaca of amphibians when they emerged voluntarily. Samples of liver or muscle tissues of amphibian hosts were taken and stored in 95% ethanol, while the specimens were subsequently fixed in 4% buffered formalin for 24 hours and later stored in 70% ethanol. For leeches, specimens were euthanized by ethanol solution, and muscle tissues were removed from the posterior part of the body and stored in 95% ethanol for DNA extraction. The remaining body of the leech was preserved in 70% ethanol for storage.

The collected amphibian specimens were subsequently deposited in the herpetological collections of the Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ); the Zoological Museum of Moscow University (ZMMU), Moscow, Russia; and the Joint Vietnam–Russia Tropical Science and Technology Research Centre (VRTC). The leech specimens collected in Vietnam have been deposited in the collection of the VRTC; no leech specimens were vouchered in China, where only tissue samples were obtained.

For anuran specimens, the following measurements (in mm) were taken (Ngam et al., 2022; Rowley et al., 2016; Tapley et al., 2017): Snout-vent length (SVL): from tip of snout to vent; thigh length (TL): distance from the middle of vent to knee when the thigh is held at a right angle to the body; shank length (SHL): distance between knee and the tibio-tarsal articulation; forelimb length (FLL): from elbow to tip of finger III. For urodele specimens (Decemson et al., 2023): snout-vent length (SVL): from tip of snout to vent; hindlimb length (HL): from groin to tip of toe III; forelimb length (FL): from axilla to tip of finger III; tail length (TAL): from posterior edge of vent to tip of tail. The sex of amphibian hosts was determined by direct observation of gonads. For leech specimens, following Nakano & Nguyen (2015), four measurements (in mm) were taken: body length (BL): from the anterior margin of the oral sucker to the posterior margin of the caudal sucker; maximum body width (BW); caudal sucker length (CL): from the anterior

to the posterior margin of the sucker; caudal sucker width (CW): from the right margin to the left margin of the sucker. Examination and dissection of the specimens were conducted using a Nikon C-Led binocular stereoscopic microscope (Nikon Corporation, Tokyo, Japan). Morphometric characters were measured to the nearest 0.1 mm using digital calipers X-PERT Xp150 (China).

Amphibian hosts were identified based on morphological diagnosis following relevant publications (e.g., Liu et al., 2025; Ohler, 2003; Orlov et al., 2012, 2024; Poyarkov et al., 2024; Qi et al., 2023; Tapley et al., 2017; Wang et al., 2022; Wu et al., 2025). Leeches were identified using molecular data at the family level. In some cases, when leeches were found in neighboring localities, we also assigned them to the same taxonomic grouping based on similarities in external morphology. Also, leeches from the families Haemadipsidae and Glossiphoniidae were identified using a combination of external characters according to the diagnoses provided by Lai et al. (2011) and Kambayashi & Nakano (2023). However, a comprehensive systematic revision of these lineages is beyond the scope of this work and is left to specialists on leech taxonomy.

DNA extraction, DNA amplification, and sequencing

Total genomic DNA was extracted from the muscle tissues and isolated using the standard phenol-chloroform-proteinase K extraction procedures with consequent isopropanol precipitation for a final concentration of about 1 mg/ml (protocols followed the works of Hillis et al. (1996) and Sambrook & Russell (2001)).

To maximize the identification ability and utilize the results from recent phylogenetic studies on Hirudinea leeches (Nakano, 2012; Nakano & Nguyen, 2015; Nakano & Prozorova, 2019), we sequenced a fragment of the cytochrome c oxidase subunit I (COI) gene. A total of 671 bp of COI was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). The following PCR protocol was followed: (1) initial denaturation step at 94°C for 5 min; (2) 40 cycles of denaturation at 94°C for 10 sec, annealing at 45°C for 20 sec, and extension at 72°C for 1 min 12 sec; (3) final extension at 72°C for 6 min; and (4) cooling step at 4°C for storage.

We purified the cycle sequencing products through ethanol precipitation. We carried out sequence data collection and visualization on an ABI 3730xl Automated Sequencer (Applied Biosystems, USA). PCR purification and cycle sequencing were done commercially through Evrogen (Moscow, Russia). We deposited the obtained sequences in GenBank under the accession numbers PX729569–PX729580.

Phylogenetic analyses

In addition to the newly obtained sequences, we included 141 hirudinean sequences representing the same mitochondrial markers from GenBank, comprising data for 72 nominal species in 29 genera and 11 families of leeches. Full details, including taxonomy, voucher, and GenBank accession numbers for all included sequences, are provided in Table 2. Nucleotide sequences were initially aligned in MAFFT v.6 (Kato et al., 2002) with default parameters, which were checked and adjusted manually in BioEdit v.7.2.5 (Hall, 1999). The optimum partitioning schemes were identified with PartitionFinder v.2.1.1 (Lanfear et al., 2012) using the greedy search algorithm under an AIC criterion. Phylogenies were

Table 2 Voucher information and GenBank accession numbers for all specimens used in molecular analyses in this study

	Species	Voucher or isolate	GenBank ID
1	Praobdellidae sp.	VRTC BAM245	PX729569
2	Praobdellidae sp.	VRTC BAM243	PX729570
3	Praobdellidae sp.	–	PX729571
4	Praobdellidae sp.	–	PX729572
5	Praobdellidae sp.	–	PX729573
6	Praobdellidae sp.	–	PX729574
7	Praobdellidae sp.	–	PX729575
8	Salifidae sp.	VRTC BAM259	PX729576
9	Haemadipsidae sp.	VRTC BAM260	PX729577
10	Haemadipsidae sp.	VRTC BAM258	PX729578
11	Haemadipsidae sp.	VRTC BAM250	PX729579
12	Glossiphoniidae sp.	VRTC BAM249	PX729580
13	<i>Americobdella valdiviana</i>	–	AY425443
14	<i>Barbronia arcana</i>	–	DQ235598
15	<i>Barbronia gwalagwalensis</i>	–	AY786455
16	<i>Barbronia</i> sp.	EB-2004	AY786457
17	<i>Barbronia weberi formosana</i>	–	AY786456
18	<i>Chtonobdella fallax</i>	–	EU100096
19	<i>Chtonobdella mediorubra</i>	LHI	HQ203184
20	<i>Chtonobdella tanae</i>	DCSP1	KT968380
21	<i>Chtonobdella tanae</i>	AU79	HQ203164
22	<i>Chtonobdella tanae</i>	AU81	KT968381
23	<i>Chtonobdella tanae</i>	AU4	KT968382
24	<i>Chtonobdella tanae</i>	AU11A	KT968383
25	<i>Chtonobdella tanae</i>	MHQU	KT968379
26	<i>Cylicobdella coccinea</i>	–	AY425444
27	<i>Dinobdella ferox</i>	CSU-MG-3	NC072217
28	<i>Dinobdella ferox</i>	CSUBar2023-50	OR578889
29	<i>Domanibdella palmyrae</i>	KUZJPN Z2046	LC414422
30	<i>Domanibdella palmyrae</i>	KUZJPN Z1636	LC414407
31	<i>Domanibdella palmyrae</i>	KUZJPN Z2053	LC414429
32	<i>Erpobdella buccera</i>	–	AF116024
33	<i>Erpobdella costata</i>	–	AY425460
34	<i>Erpobdella dubia</i>	–	AF116023
35	<i>Erpobdella japonica</i>	KUZJPN Z178	AB679654
36	<i>Erpobdella mexicana</i>	–	DQ235595
37	<i>Erpobdella montezuma</i>	–	GQ368760
38	<i>Erpobdella obscura</i>	–	AF003273
39	<i>Erpobdella ochoterenai</i>	–	DQ235603
40	<i>Erpobdella octoculata</i>	–	AF003274
41	<i>Erpobdella punctata</i>	–	HQ336346
42	<i>Erpobdella testacea</i>	–	AF116027
43	<i>Erpobdella triannulata</i>	–	DQ235602
44	<i>Gastrostomobdella monticola</i>	UNIMASMYS A3BH01/10	AB679656
45	<i>Haemadipsa</i> cf. <i>zeylanica</i>	EB-2010 PH09	HQ203189
–	<i>Haemadipsa interrupta</i>	–	EU100091
47	<i>Haemadipsa</i> sp.	WP894	MG933662
48	<i>Haemadipsa trimaculosa</i>	HAKY	HQ203172
49	<i>Haemadipsa trimaculosa</i>	CM13 3	MG933564
50	<i>Haemadipsa trimaculosa</i>	CM11 6	MG933562
51	<i>Haemadipsa trimaculosa</i>	CM11 9	MG933563
52	<i>Haemadipsa trimaculosa</i>	CM10 17	MG933604
53	<i>Haemadipsa trimaculosa</i>	CM13 4	MG933603
54	<i>Haemadipsa trimaculosa</i>	CM16 1	MG933576
55	<i>Haemadipsa trimaculosa</i>	CM11 5	MG933558
56	<i>Haemadipsa trimaculosa</i>	CM15 1	MG933556

	Species	Voucher or isolate	GenBank ID
57	<i>Haemadipsa trimaculosa</i>	CM13 1	MG933557
58	<i>Haemadipsa trimaculosa</i>	CM13 5	MG933555
59	<i>Haemadipsa zeylanica montivindicis</i>	HZAKI	HQ203180
60	<i>Hemiclepsis kasmiana</i>	RMBH Hir103/4	MN295423
61	<i>Hemiclepsis kasmiana</i>	RMBH Hir110/6	MN295430
62	<i>Hemiclepsis kasmiana</i>	RMBH Hir 433/1	ON841629
63	<i>Hemiclepsis khankiana</i>	RMBH Hir 498	ON841642
64	<i>Hemiclepsis khankiana</i>	RMBH Hir 496	ON841640
65	<i>Hemiclepsis khankiana</i>	RMBH Hir 495	ON841639
66	<i>Hemiclepsis khankiana</i>	RMBH Hir 494	ON841638
67	<i>Hemiclepsis khankiana</i>	RMBH Hir 493	ON841637
68	<i>Hirudinaria manillensis</i>	–	AY425449
69	<i>Hirudo medicinalis</i>	–	U74067
70	<i>Johanssonia arctica</i>	Jarct E	AY336012
71	<i>Johanssonia arctica</i>	–	DQ414320
72	<i>Limnatis nilotica</i>	–	MZ318072
73	<i>Limnoddella mexicana</i>	LMSON	GQ368756
74	<i>Limnoddella mexicana</i>	LMAL	GQ368757
75	<i>Limnoddella mexicana</i>	LMBC	GQ368759
76	<i>Linta be</i>	–	AY786460
77	<i>Macrobdella decora</i>	–	MH672574
78	<i>Macrobdella decora</i>	–	MH672573
79	<i>Macrobdella decora</i>	–	EU100095
80	<i>Macrobdella decora</i>	–	AF003271
81	<i>Macrobdella decora</i>	ROMIZ I10062	MK796994
82	<i>Macrobdella decora</i>	ROMIZ I10060	MK796993
83	<i>Macrobdella decora</i>	ROMIZ I10059	MK796992
84	<i>Macrobdella ditetra</i>	MDILA3 1	DQ097217
85	<i>Macrobdella ditetra</i>	MDILA2 1	DQ097216
86	<i>Macrobdella ditetra</i>	MDILA1 1	DQ097215
87	<i>Macrobdella ditetra</i>	–	AY425453
88	<i>Macrobdella mimicus</i>	USNM 1532307	MK796982
89	<i>Macrobdella mimicus</i>	USNM 1532306	MK796981
90	<i>Macrobdella mimicus</i>	USNM 1532261	MK796991
91	<i>Macrobdella mimicus</i>	USNM 1532309	MK796983
92	<i>Macrobdella mimicus</i>	USNM s.n.	MK796990
93	<i>Macrobdella mimicus</i>	CNHE 10262	MK796989
94	<i>Macrobdella mimicus</i>	USNM 1532254	MK796988
95	<i>Macrobdella mimicus</i>	USNM 1532253	MK796987
96	<i>Macrobdella mimicus</i>	USNM 1532255	MK796986
97	<i>Macrobdella mimicus</i>	USNM 1532305	MK796984
98	<i>Macrobdella mimicus</i>	USNM 1532304	MK796985
99	<i>Mimobdella japonica</i>	KUZJPN Z179	AB679658
100	<i>Mooreobdella melanostoma</i>	–	AF116025
101	<i>Myxobdella annandalei</i>	Myxo1	GU394014
102	<i>Myxobdella sinanensis</i>	KKshinano2	LC761929
103	<i>Myxobdella sinanensis</i>	KKshinano1	LC761928
104	<i>Myxobdella sinanensis</i>	KKshinano3	LC761930
105	<i>Myxobdella sinanensis</i>	KUZJPN Z1794	LC192132
106	<i>Myxobdella socotrensis</i>	–	MT648508
107	<i>Myzobdella lugubris</i>	–	DQ414323
108	<i>Myzobdella lugubris</i>	myz3 1	KY440059
109	<i>Odontobdella blanchardi</i>	KUZJPN Z180	AB938004
110	<i>Orobdella dolichopharynx</i>	KUZJPN Z120	AB679680
111	<i>Orobdella esulcata</i>	KUZJPN Z170	AB679666
112	<i>Orobdella esulcata</i>	KUZJPN Z29	AB679664
113	<i>Orobdella ghilarovi</i>	KUZJPN Z2068	LC431609

	Species	Voucher or isolate	GenBank ID
114	<i>Orobdella ibukifukuyamai</i>	KUZJPN Z4032	LC687621
115	<i>Orobdella ibukifukuyamai</i>	KUZJPN Z4031	LC687620
116	<i>Orobdella ijimai</i>	KUZJPN Z110	AB679672
117	<i>Orobdella kanaekoikeae</i>	KUZJPN Z1489	LC184527
118	<i>Orobdella kawakatsuorum</i>	KUZJPN Z167	AB679704
119	<i>Orobdella ketagalan</i>	KUZJPN Z208	AB704787
120	<i>Orobdella koikei</i>	KUZJPN Z156	AB679688
121	<i>Orobdella masaakikuroiwai</i>	KUZJPN Z699	AB938009
122	<i>Orobdella masaakikuroiwai</i>	KUZJPN Z696	AB938007
123	<i>Orobdella masaakikuroiwai</i>	KUZJPN Z694	AB938006
124	<i>Orobdella masaakikuroiwai</i>	KUZJPN Z694	AB938006
125	<i>Orobdella masaakikuroiwai</i>	KUZJPN Z689	AB938005
126	<i>Orobdella masaakikuroiwai</i>	KUZJPN Z687	AB938011
127	<i>Orobdella mononoke</i>	KUZJPN Z224	AB698866
128	<i>Orobdella octonaria</i>	KUZJPN Z181	AB679708
129	<i>Orobdella shimadae</i>	KUZJPN Z128	AB679676
130	<i>Orobdella tsushimensis</i>	KUZJPN Z625	AB828570
131	<i>Orobdella tsushimensis</i>	KUZJPN Z622	AB828569
132	<i>Orobdella tsushimensis</i>	KUZJPN Z616	AB828566
133	<i>Orobdella tsushimensis</i>	KUZJPN Z618	AB828567
134	<i>Orobdella tsushimensis</i>	KUZJPN Z134	AB679662
135	<i>Orobdella whitmani</i>	KUZJPN Z45	AB679668
136	<i>Orobdella yamaneae</i>	KUZJPN Z1678	LC106350
137	<i>Philobdella gracilis</i>	PGRLA1 1	DQ097218
138	<i>Pintobdella chiapasensis</i>	TrexMX	GU394015
139	<i>Piscicola cf. annae</i>	Pann X	AY336016
140	<i>Piscicolaria reducta</i>	–	DQ414339
141	<i>Piscicolidae</i> sp.	HLC-26546 1	MG423349
142	<i>Piscicolidae</i> sp.	HLC-30322 1	MG421319
143	<i>Poecilobdella javanica</i>	SGNC-D3f	KT693110
144	<i>Poecilobdella javanica</i>	SGNC-D4f	KT693111
145	<i>Salifa perspicax</i>	–	HQ336340
146	<i>Salifa perspicax</i>	–	HQ336341
147	<i>Salifa perspicax</i>	–	HQ336342
148	<i>Salifa perspicax</i>	–	HQ336343
149	<i>Salifa motokawai</i>	VNMN2015.63	LC029430
150	<i>Salifa motokawai</i>	VNMN2015.65	LC029431
151	<i>Salifa motokawai</i>	KUZJPN Z719	LC029429
152	<i>Salifa motokawai</i>	KUZJPN Z713	LC029428
153	<i>Tyrannobdella rex</i>	Tyra	GU394016
154	<i>Cosmoscarta bispecularis</i>	–	NC 040114

reconstructed via Maximum Likelihood (ML) and Bayesian inference (BI). We used IQ-TREE v.2.4.0 (Nguyen et al., 2015) to obtain ML phylogenies, whose confidence in tree topology was assessed by 10000 ultrafast bootstrap replications (UFBS). BI was performed in MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003) with four Metropolis-coupled Markov chain Monte Carlo (MCMCMC) runs made of one cold chain and three heated chains, iterated for one million generations and sampled every 1000 generations. Five independent MCMCMC runs were performed, and the first 1000 trees were discarded as burn-in. Nodal support in the BI was assessed by calculating posterior probabilities (BIPP). The best-fitting model for both BI and ML analyses suggested by the AIC was the GTR+I+G model. We considered tree nodes with BIPP values over 0.95 and UFBS values of 0.95 or greater as significantly supported, while BIPP values from

0.95 to 0.90 and ML *BS* values from 0.75 to 0.50 were interpreted with caution (as “tendencies”). Lower values indicate unsupported nodes (Minh et al., 2013). Both inter- and intraspecific uncorrected genetic *p*-distances were calculated in MEGA X (Kumar et al., 2018) using the complete deletion option to remove gaps and missing data from the alignment prior to analysis.

Other abbreviations: asl=above sea level; fam.=family; Mountain=Mt.; NP=National Park; NR=Nature Reserve; WS=Wildlife Sanctuary; Prov.=Province

RESULTS

Molecular analyses

The obtained ML tree had a topology identical to that of the BI tree (Figure 1). Our COI-based tree reconstructs 12 leech

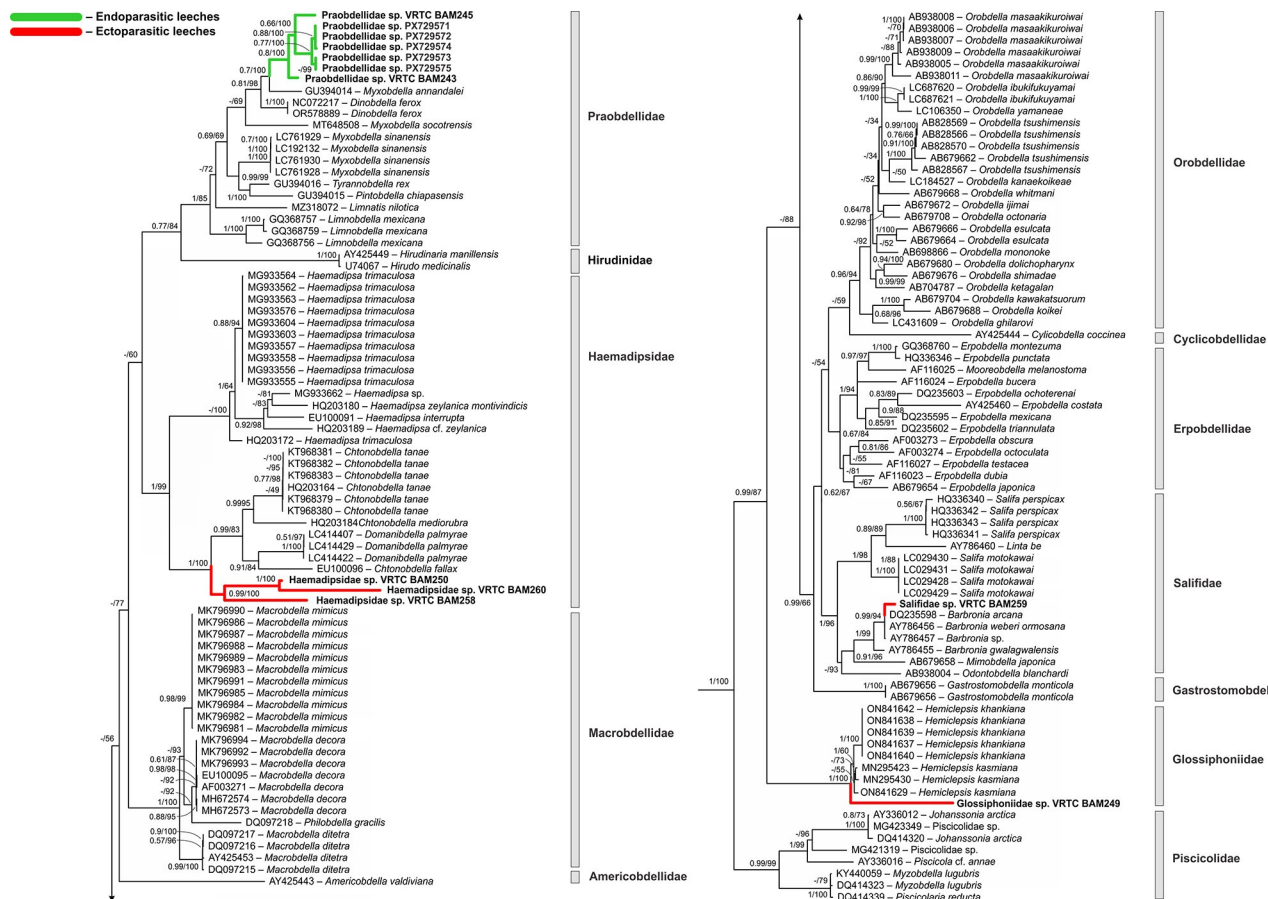


Figure 1 ML-tree showing the matrilineal genealogy of leeches (Hirudinea) based on 1 263 bp of COI gene

Nodal values correspond to ultrafast bootstrap supports (UBS) from ML analyses and Bayesian posterior probabilities (BPP) from BI analyses, respectively (UBS/BPP). Branches uniting populations of endoparasitic leeches are marked in green, while those of ectoparasitic leeches are marked in red.

families, with their monophyly being well supported and consistent with the current understanding of hirudinean systematics (Nakano, 2017; Zhu et al., 2023). Relationships among families at deeper nodes remain unresolved. Uncorrected inter- and intraspecific p -distances are presented in Table 2. Among Hirudinea species, interspecific p -distances based on the COI mtDNA gene ranged from 0.6% (between *Barbronia weberi* (Blanchard), *Barbronia arcana* (Richardson), *Barbronia* sp., and *Salifidae* sp.) to 35.6% (between *Pintobdella chiapasensis* (Caballero) and *Mimobdella japonica* Blanchard).

Our analyses revealed that all haplotypes of endoparasitic leeches detected in northern Vietnam and southwestern China form a single well-supported clade (100/0.8), sister to *Myxobdella annandalei* Oka (100/0.7) and *Dinobdella ferox* (Blanchard) (98/0.81), and nested within the radiation of the family Praobdellidae Sawyer. Genetic diversity among the studied populations of endoparasitic leeches from northern Vietnam and southwestern China was minimal, with uncorrected intraspecific p -distances of 0.02%. Interspecific p -distances based on the COI mtDNA gene ranged from 7.9% (to *Myxobdella annandalei*) to 30.9% (to *Mimobdella japonica*).

Single ectoparasitic leeches from anuran amphibians collected in the south (VRTC BAM258) and north (VRTC BAM250 and VRTC BAM258) Vietnam form a well-supported clade (100/0.99), sister to species of the genera *Domanibdella* Richardson and *Chtonobdella* Grube (100/1.0) within the family Haemadipsidae Blanchard. Genetic diversity among the

studied populations of Haemadipsidae leeches from Vietnam was relatively high, with uncorrected intraspecific p -distances of 18.5%. In contrast, interspecific p -distances ranged from 7.9% (to *Domanibdella palmyrae* [Richardson]) to 33.6% (to *Americobdella valdiviana* [Philippi]). Apparently, within the Haemadipsidae family, several genera and species parasitize amphibians in Vietnam.

Ectoparasitic leeches' mass-parasitizing Megophryidae Bonaparte frogs in northern Vietnam form a sister lineage to two species of the genus *Hemiclepsis* Vejdovsky (100/1.0) within the family Glossiphoniidae Vaillant. Interspecific p -distances ranged from 16.9% (to *Hemiclepsis kasmiana* Oka) to 31.2% (to *Chtonobdella tanae* Tessler, Barrio, Borda, Rood-Goldman, Hill & Siddall).

Ectoparasitic leeches parasitizing newts of the genus *Tylototriton* Anderson from northern Vietnam form a sister clade to the genus *Barbronia* Johansson (94/0.99) within the family Salifidae Johansson. Interspecific p -distances between the detected population and Asian species of the genus *Barbronia* (*B. arcana*, *B. weberi* (Richardson), *Barbronia* sp., *B. gwalagwalensis* Westergren & Siddall) were also minimal: 0.6% to 2.9%.

The monophyly of the families Praobdellidae (UFBS/BIPP=85/1.0), Haemadipsidae (99/1.0), Salifidae (96/1.0), and Glossiphoniidae (100/1.0), including the leech specimens examined in the present study, was strongly supported.

External morphology and identification

Based on the genetic evidence presented above, as well as general external morphological characters (Table 3) and patterns of distribution and ecology, we cautiously assign the leeches detected in this study to the following taxonomic groupings.

The medium-sized (BL~15 mm) ectoparasitic leeches (VRTC BAM233, VRTC BAM250–51, VRTC BAM258, VRTC BAM260, VRTC BAM275, see Table 1) with an expanded anterior end, large conspicuous eyes, and a tuberculate integument, dark green to dark brown and often mottled with longitudinal stripes or streaks (Figures 2A–C, 3A) from southern and northern Vietnam are herein assigned to the family Haemadipsidae.

The medium-sized (BL~23 mm), grayish-brown ectoparasitic leech (VRTC BAM259) with weakly expressed body segmentation (Figure 4A, B), recovered from the nasal cavity of *Tylotriton vietnamensis* Böhme, Schöttler, Nguyen & Köhler from northern Vietnam, is herein assigned to the family Salifidae.

The small (BL~0.2 mm), short (BW~0.1 mm), flattened, dark-green ectoparasitic leeches (VRTC BAM249) (Figure 2D) mass-parasitizing megophryid frogs in northern Vietnam are herein assigned to the family Glossiphoniidae.

The large (BL~38.9 mm), brick-red, endoparasitic leeches (VRTC BAM239–245, VRTC BAM304–306, VRTC BAM330–331, and Yunnan sampling [specimens were not collected; therefore; voucher numbers are not available]) with well-defined segmentation and a large posterior sucker (CL~5.0 mm, CW~5.5 mm) (Figures 5–11) from northern Vietnam and Yunnan Prov., China are herein assigned to the family Praobdellidae. The ecology of the endoparasitic Praobdellidae leeches detected in the present study is consistent with that of closely related species of the genus *Myxobdella* Oka: all of them inhabit unpolluted mountain streams and rapidly running waters (Nakano et al., 2017; Neseemann & Sharma, 2001).

Cases of leech parasitism on amphibians in Vietnam and China

Table 3 Morphometric characters of leech specimens

Voucher num.	BL	BW	CL	CW	Host	Hosts voucher num.
Praobdellidae leeches						
VRTC BAM331	22.0	7.1	5.0	5.5	<i>Amolops cf. shihaitaoi</i>	ZMMU NAP-13946
VRTC BAM245	51.6	10.9	5.8	6.4	<i>Amolops cf. shihaitaoi</i>	ZMMU NAP-13948
VRTC BAM242 (n1)	28.7	10.0	5.4	6.0	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16535
VRTC BAM242 (n2)	37.8	10.3	6.5	7.2	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16535
VRTC BAM239	59.7	14.0	5.8	6.4	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16536–16540
VRTC BAM240	23.5	3.6	3.0	3.3	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16536–16540
VRTC BAM241 (n1)	28.3	5.4	4.4	4.8	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16536
VRTC BAM241 (n2)	19.9	3.9	2.6	2.8	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16536–16540
VRTC BAM241 (n3)	27.7	3.5	3.4	3.7	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16538
VRTC BAM241 (n4)	18.9	3.9	2.8	3.0	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16536–16540
VRTC BAM241 (n5)	40.9	8.4	6.5	7.2	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16536–16540
VRTC BAM241 (n6)	67.1	12.3	8.5	9.5	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16536–16540
VRTC BAM241 (n7)	55.0	12.2	7.1	7.9	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16536–16540
VRTC BAM241 (n8)	60.1	10.5	6.3	7.0	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16536–16540
VRTC BAM330	41.1	13.5	6.6	7.3	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16626
VRTC BAM244	47.3	16.0	5.5	6.1	<i>Amolops cf. tonkinensis</i>	ZMMU NAP-16629
VRTC BAM304 (n1)	11.7	3.8	3.0	3.3	<i>Amolops cf. daorum</i>	ZMMU NAP-18321
VRTC BAM304 (n2)	23.6	7.2	3.4	3.7	<i>Amolops cf. daorum</i>	ZMMU NAP-18322
VRTC BAM243	38.2	10.7	5.7	6.3	<i>Bufo cf. luehmanni</i>	ZMMU NAP-14515
VRTC BAM305	43.4	9.5	3.5	3.8	<i>Bufo cf. pageoti</i>	ZMMU NAP-18334
VRTC BAM306	43.6	9.9	4.0	4.4	<i>Bufo cf. pageoti</i>	ZMMU NAP-18335
Haemadipsidae leeches						
VRTC BAM258	9.3	2.4	2.1	2.3	<i>Kurixalus gryllus</i>	ZMMU NAP-15402
VRTC BAM251 (n1)	21.6	3.1	3.7	2.7	<i>Kurixalus gryllus</i>	ZMMU NAP-15376
VRTC BAM251 (n2)	11.5	1.8	1.6	1.6	<i>Kurixalus gryllus</i>	ZMMU NAP-15374–15385
VRTC BAM251 (n3)	11.1	1.7	1.9	1.7	<i>Kurixalus gryllus</i>	ZMMU NAP-15374–15385
VRTC BAM251 (n4)	16.3	2.2	2.8	2.2	<i>Kurixalus gryllus</i>	ZMMU NAP-15374–15385
VRTC BAM251 (n5)	19.5	2.6	2.8	3.1	<i>Kurixalus gryllus</i>	ZMMU NAP-15374–15385
VRTC BAM251 (n6)	21.5	2.4	3.3	2.7	<i>Kurixalus gryllus</i>	ZMMU NAP-15374–15385
VRTC BAM251 (n7)	18.5	2.3	3.3	2.7	<i>Kurixalus gryllus</i>	ZMMU NAP-15374–15385
VRTC BAM275	14.1	2.3	3.0	3.3	<i>Kurixalus gryllus</i>	ZMMU NAP-17699
Salifidae leeches						
VRTC BAM259	23.1	2.1	0.8	0.7	<i>Tylotriton vietnamensis</i>	ZMMU NAP-16631
Glossiphoniidae leeches						
VRTC BAM249	0.2	0.1	–	–	<i>Boulengeriella rubrimera</i>	ZMMU NAP-14528

All metric measurements are in mm. Abbreviations of morphological characters are provided in the Materials and Methods section. –: information not available.

Below, we provide a list (Table 1) and a detailed description of the observed cases of leech parasitism in Vietnam and China. Available measurements of leeches and their hosts are provided in Tables 3 and 4.

Cases of leech ectoparasitism on amphibians in Vietnam

Cases of leech feeding on amphibians were recorded in northern and southern Vietnam for three leech families: Haemadipsidae, Salifidae, and Glossiphoniidae.

1. Ectoparasitic Haemadipsidae leeches were detected in Ha Noi City, Thanh Hoa, and Lam Dong provinces.

In Ha Noi City, ectoparasitic Haemadipsidae leech (VRTC BAM260) was detected in June 2024 on an adult male of *Ophryophryne microstoma* Boulenger (ZMMU NAP-17406) (fam. Megophryidae), collected in the vicinity of Mt. Ba Vi. The frogs were collected in a rocky stream in a montane forest dominated by Fagaceae and Magnoliaceae on north-facing slopes of Mt. Ba Vi at elevation of 342 m asl. In total, a series of 6 individuals (6 males) was collected, one of which was afflicted with an ectoparasitic leech. The leech was located in the axillary region of the frog. The male of *Ophryophryne microstoma* was not emaciated and did not differ in behavior from the other individuals. The male was actively calling.

In Thanh Hoa Prov., ectoparasitic Haemadipsidae leeches (VRTC BAM233, VRTC BAM250) were detected in October 2023 on an adult male of *Boulenophrys palpebralespinosa* (Bourret) (ZMMU NAP-15102) (Figure 2A, E) and on an adult male of *Leptobrachium cf. guangxiense* Fei, Mo, Ye, & Jiang (ZMMU NAP-15103) (Figure 2B, F) (fam. Megophryidae), collected in the vicinity of Ban Vin village. The frogs were collected on the bank of a small forest stream with a stony bottom and steep clay bank in secondary montane evergreen forest dominated by Cupressaceae Gray, Fagaceae,

Hamamelidaceae R.Br., Juglandaceae DC. ex Perleb, Lauraceae, Magnoliaceae, Meliaceae Juss., Primulaceae Batsch ex Borkh., Rhizophoraceae Pers., Styracaceae DC. & Spreng., and Urticaceae Juss. at elevation of 960–1 260 m asl. In *Boulenophrys palpebralespinosa*, one small leech was feeding in the axillary region. In *Leptobrachium cf. guangxiense*, one small leech was feeding on the posterior flank, close to the thigh. The frogs were not emaciated. The male *Boulenophrys palpebralespinosa* was actively calling. In Lam Dong Prov., ectoparasitic Haemadipsidae leeches (VRTC BAM251, VRTC BAM258, VRTC BAM275) were detected in November 2023 and in March 2025 on adult individuals of *Kurixalus gryllus* Smith (ZMMU NAP-15374–15377, ZMMU NAP-15379–15381, ZMMU NAP-17699) (fam. Rhacophoridae) (Figures 2C, G, 3), collected on the top of Mt. Ta Dung and Mt. Bidoup. The frogs were collected from tree trunks and tree hollows in montane cloud elfin forests dominated by Fagaceae Dumort., Elaeocarpaceae Juss., Lauraceae Juss., Magnoliaceae Juss., Fabaceae Lindl., Melastomataceae Juss., Illiciaceae A.C.Sm., Aquifoliaceae Bercht. & J.Presl., Rutaceae Juss., and Rubiaceae Juss. at elevation of 1820 m asl on Mt. Ta Dung and at elevation of 2 137 m asl on Mt. Bidoup. If the observation on Mt. Bidoup was a single case (one male), then on Mt. Ta Dung a total series of 12 individuals (seven females, one male, and four juveniles) was collected, seven of which (all females) were infested with ectoparasitic leeches. Most individuals had two, and sometimes three, large leeches attached to the dorsum and flanks. The frogs were not emaciated and were resting on branches, leaves, and tree hollows.

2. Ectoparasitic Glossiphoniidae leeches (VRTC BAM249) were detected in May 2023 in Lao Cai Prov. on an adult male of *Boulenophrys rubrimera* (Tapley, Cutajar, Mahony, Nguyen,

Table 4 Morphometric characters of amphibian (host) specimens

Voucher num.	Species	SVL	TL	SHL	FLL	Sex	Leeches voucher num.
Praobdellidae hosts							
ZMMU NAP-13946	<i>Amolops cf. shihaitaoi</i>	32.3	18.5	20.6	16.5	F	VRTC BAM245
ZMMU NAP-13948	<i>Amolops cf. shihaitaoi</i>	45.1	26.9	26.6	23.6	M	VRTC BAM331
ZMMU NAP-16536	<i>Amolops cf. tonkinensis</i>	50.8	26.3	28.0	24.1	F	VRTC BAM241 (n1)
ZMMU NAP-16538	<i>Amolops cf. tonkinensis</i>	52.9	25.9	26.1	23.2	F	VRTC BAM241 (n3)
ZMMU NAP-16626	<i>Amolops cf. tonkinensis</i>	47.3	24.5	24.9	20.6	F	VRTC BAM330
ZMMU NAP-16629	<i>Amolops cf. tonkinensis</i>	46.5	25.1	24.4	19.9	F	VRTC BAM244
ZMMU NAP-18321	<i>Amolops cf. daorum</i>	41.8	25.1	29.1	23.2	F	VRTC BAM304 (n1)
ZMMU NAP-18322	<i>Amolops cf. daorum</i>	37.9	21.8	25.7	20.8	F	VRTC BAM304 (n2)
ZMMU NAP-18359	<i>Amolops cf. daorum</i>	38.5	22.5	26.9	20.0	M	–
ZMMU NAP-18361	<i>Amolops cf. daorum</i>	35.8	20.8	22.9	19.6	M	–
ZMMU NAP-14515	<i>Bufo cf. luchunnicus</i>	69.5	23.3	24.7	26.1	M	VRTC BAM243
ZMMU NAP-18334	<i>Bufo cf. pageoti</i>	41.6	17.9	19.0	23.2	F	VRTC BAM305
ZMMU NAP-18335	<i>Bufo cf. pageoti</i>	63.2	29.5	26.1	34.1	F	VRTC BAM306
Haemadipsidae hosts							
ZMMU NAP-15376	<i>Kurixalus gryllus</i>	31.4	14.0	15.9	16.5	F	VRTC BAM251 (n1)
ZMMU NAP-17699	<i>Kurixalus gryllus</i>	29.8	14.3	14.5	15.2	M	VRTC BAM275
ZMMU NAP-15102	<i>Boulenophrys palpebralespinosa</i>	32.4	20.5	21.1	22	M	VRTC BAM250
ZMMU NAP-15103	<i>Leptobrachium cf. guangxiense</i>	60.3	25.5	21.2	30.3	M	VRTC BAM233
ZMMU NAP-17406	<i>Ophryophryne microstoma</i>	38.9	16.3	17.3	19.5	M	VRTC BAM260
Glossiphoniidae hosts							
ZMMU NAP-14528	<i>Boulenophrys rubrimera</i>	20.1	8.1	8.4	9.0	M	VRTC BAM249
Salifidae hosts							
ZMMU NAP-16631	<i>Tylototriton vietnamensis</i>	64.0	19.7	17.8	63.6	M	VRTC BAM259

All metric measurements are in mm. Abbreviations of morphological characters are provided in the Materials and Methods section.

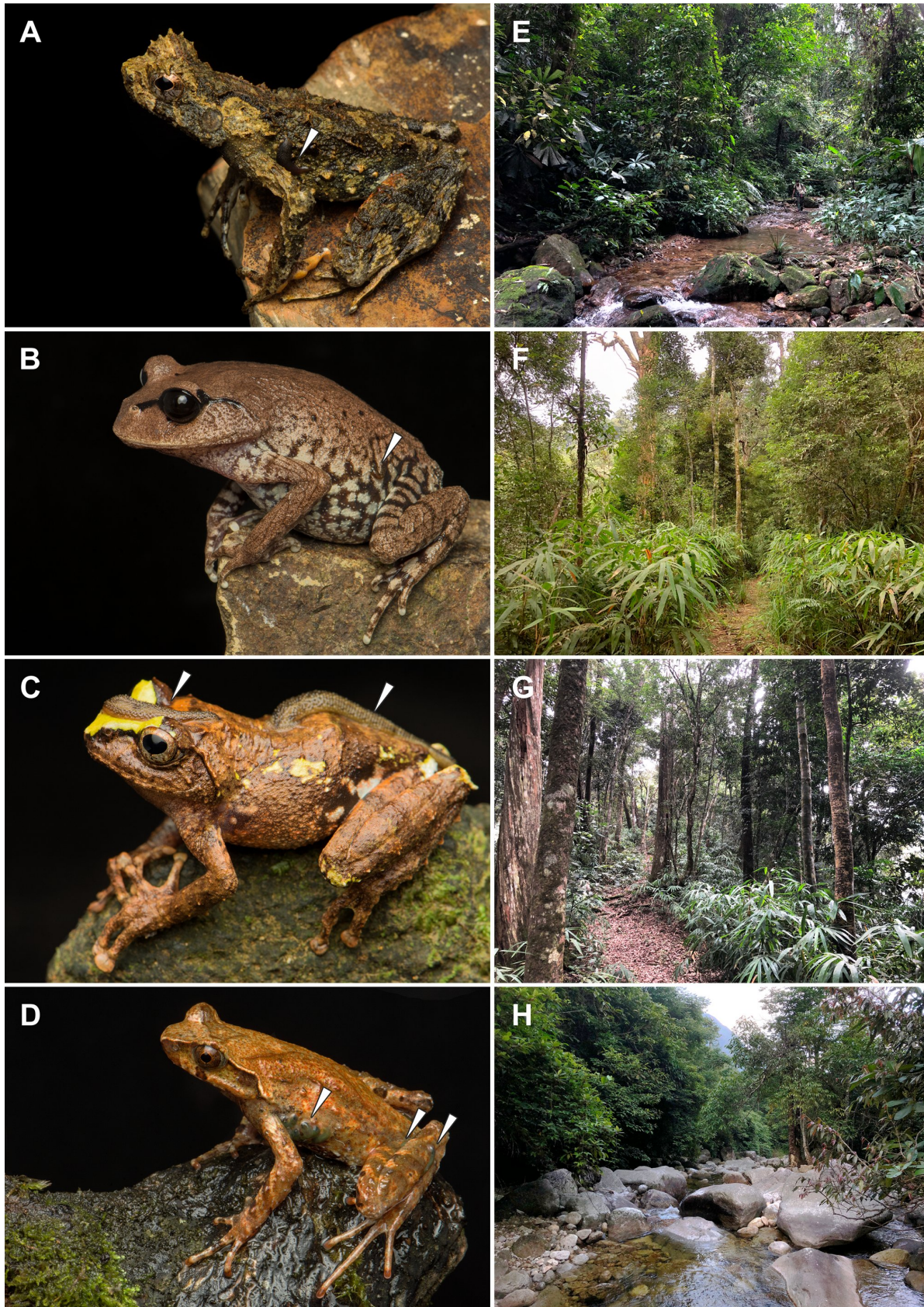


Figure 2 Ectoparasitic leeches (indicated by arrows) and their anuran hosts in Vietnam

A: Haemadipsidae leech (VRTC BAM250) on male *Boulenophrys palpebralespinosa* from Xuan Lien NR, Thanh Hoa Prov., northern Vietnam (E). B: Haemadipsidae leech (VRTC BAM233) on male *Leptobrachium* cf. *guangxiense* from Xuan Lien NR, Thanh Hoa Prov., northern Vietnam (F). C: Haemadipsidae leeches (VRTC BAM251) on female *Kurixalus gryllus* from Mt. Ta Dung, Lam Dong Prov., southern Vietnam (G). D: Glossiphoniidae leeches (VRTC BAM249) on male *Boulenophrys rubrimera* from Mt. Ky Quan San, Lao Cai Prov., northern Vietnam (H). Photos by A. M. Bragin.

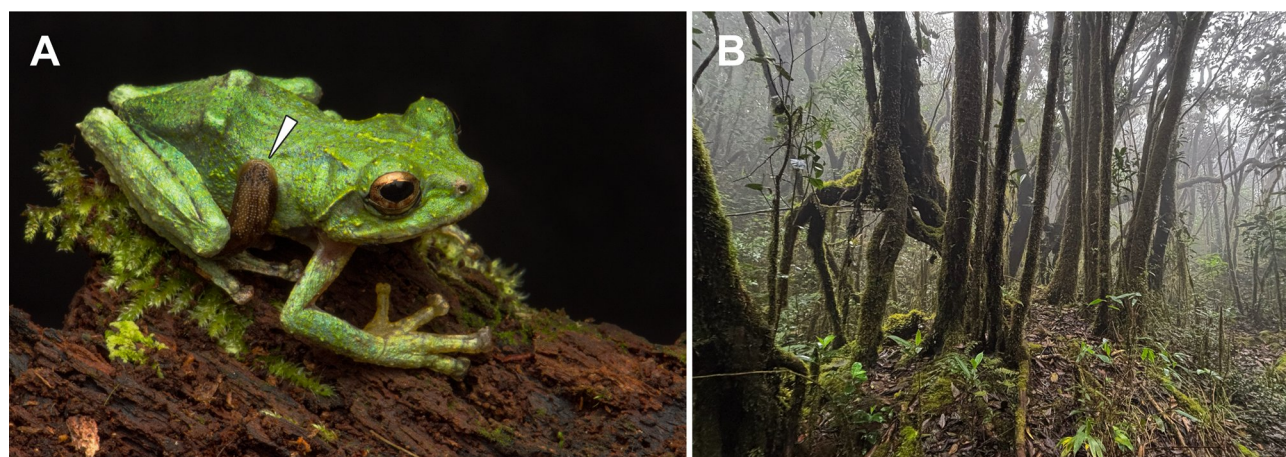


Figure 3 Ectoparasitic Haemadipsidae leech (VRTC BAM259) (indicated by arrow) on male *Kurixalus gryllus* (A) from Mt. Bidoup, Lam Dong Prov., southern Vietnam (B)

Photos by A. M. Bragin.

Dau, Nguyen, Luong & Rowley) (ZMMU NAP-14528) (fam. Megophryidae) (Figure 2D, H), collected in the vicinity of Mt. Ky Quan San. The frogs were collected in a small rocky stream in a stunted high-elevation montane forest dominated by Rubiaceae Juss., Aquifoliaceae Bercht. & J.Presl, and Fagaceae on east-facing slopes of Mt. Ky Quan San at elevation of 2700 m asl. In total, a series of seven individuals (six males, one female) was collected, one of which (one male) was afflicted with ectoparasitic leeches. The individual was not emaciated and did not differ in behavior from the other individuals. The male was actively calling. Numerous (ca. 20) small Glossiphoniidae leeches were located in the axillary region and on the posterior part of the thighs of male *Boulenophrys rubrimera*.

3. Ectoparasitic Salifidae leech (VRTC BAM259) was detected in May 2024 in Lang Son Prov. on an adult male of *Tylotriton vietnamensis* (ZMMU NAP-16631) (fam. Salamandridae) (Figure 4), collected in the vicinity of Mt. Mau Son. The newts were collected in the flooded reaches of a slow-flowing stream with stands of sedges and grasses within a polydominant evergreen mid-elevation montane forest dominated by Fagaceae, Lauraceae, and Magnoliaceae on steep north-facing slopes of Mt. Mau Son at elevation of 901 m asl. In total, a series of six individuals (three males, three females) was collected, one of which (one male) was with an ectoparasitic leech. The individual was not emaciated and did not differ in behavior from the other individuals. The Salifidae leech was located in the nasal cavity of a male newt, with the anterior third of its body protruding from the nostril.

Cases of leech endoparasitism on amphibians in northern Vietnam and southwestern China

All endoparasitic leeches detected in this study are genetically and morphologically similar and are assigned to the family Praobdellidae (Figures 1, 9). Cases of Praobdellidae endoparasitism on amphibians were detected in Lao Cai, Son La, Lang Son, and Tuyen Quang Provinces of northern Vietnam and in Yunnan Prov. of southwestern China (Figures 5–11; Supplementary Material Videos 1, 2).

1. In Lao Cai Prov., northwestern Vietnam, an endoparasitic leech was detected in May 2023 during dissections of adult female *Bufo* cf. *luchunnicus* (Yang & Rao) (ZMMU NAP-14515) (Figures 5D, H, 8E, F), collected in the vicinity of Mt. Ky Quan San. The toad was collected in an open pasture site

amid shrub thickets of Rosaceae Juss., Ericaceae Juss., and Melastomataceae Juss., and ferns of Blechnaceae Newman, Polypodiaceae J.Presl & C.Presl, and Adiantaceae Newman, on the eastern slope of Mt. Ky Quan San at elevation of 2690 m asl.

At the moment of encounter, the toad was inactive and perched on the top of a ca. 0.5 m tall ericaceous shrub. The infected individual contained a single large leech. Leech left the host body during dissection.

In addition to *Bufo* cf. *luchunnicus* (fam. Bufonidae), 28 amphibian species were recorded and examined in the same locality (Mt. Ky Quan San, Lao Cai Prov.): *Bombina microdeladigitata* (fam. Bombinatoridae); *Nanorana yunnanensis* (Anderson), *Quasipaa delacouri* (Angel) (fam. Dicroglossidae); *Hyla annectans* (Jerdon) (fam. Hylidae Rafinesque); *Boulenophrys* cf. *frigida* (Tapley, Cutajar, Nguyen, Portway, Mahony, Nguyen, Harding, Luong & Rowley), *Boulenophrys* cf. *hoanglienensis* (Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley), *Boulenophrys* cf. *rubrimera* (Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley), *Boulenophrys* sp., *Leptobranchium ailaonicum* (Yang, Chen & Ma), *Leptobranchella* cf. *bourreti* (Dubois), *Leptobranchella* cf. *graminicola* Nguyen, Tapley, Nguyen, Luong & Rowley, *Leptobranchella* cf. *petrops* (Rowley, Dau, Hoang, Le, Cutajar & Nguyen), *Leptobranchella* cf. *ventripunctata*, *Leptobranchella* sp. (fam. Megophryidae); *Microhyla heymonsi* (fam. Microhylidae); *Amolops* cf. *daorum*, *Amolops splendidissimus* (Orlov & Ho), *Amolops* sp., *Odorrana* sp. (fam. Ranidae); *Gracixalus gracilipes*, *Gracixalus sapaensis* (Matsui, Ohler, Eto & Nguyen), *Kurixalus odontotarsus*, *Polypedates impresus* (Yang), *Raorchestes* sp., *Theloderma bicolor* (Bourret), *Zhangixalus dorsovireidis* (Bourre), *Zhangixalus duboisi*, *Zhangixalus feae* (Boulenger), *Zhangixalus* sp. (fam. Rhacophoridae).

2. In Son La Prov., northwestern Vietnam, endoparasitic leeches were detected in October 2025 in five adult individuals of *Amolops* cf. *daorum* (ZMMU NAP-18321–18323, ZMMU NAP-18359, ZMMU NAP-18361), collected in the vicinity of Mt. Sa Mu. The amphibians were collected in a rocky riverbed with cascades in an evergreen montane forest dominated by Lauraceae, Fagaceae, Meliaceae, and Magnoliaceae at elevation of 2005 m asl. Frogs with endoparasitic leeches were active and did not differ from uninfected individuals. At the



Figure 4 Ectoparasitic Salifidae leech (VRTC BAM259) (indicated by arrow) in the nasal cavity of male *Tylotriton vietnamensis* Ectoparasitic Salifidae leech (VRTC BAM259) (indicated by arrow) in the nasal cavity of male *T. vietnamensis* (A–B) from Mt. Mau Son, Lang Son Prov., northern Vietnam (C)

Scale bar=10 mm. Photos by A. M. Bragin (A, B) and Le Xuan Duc (C).

time of discovery, infected males were actively calling. In total, a series of 8 individuals (three males, five females) was collected, five of which (two males and three females) contained endoparasitic leeches.

In the same region, an endoparasitic leech was also inside two females of *Bufo* cf. *pageoti* Bourret (ZMMU NAP-18334–35) (Figure 6). The toads were found actively moving through the evergreen high montane forest dominated by Lauraceae, Fagaceae, Meliaceae, and Magnoliaceae at elevation of 2376 m asl. One of the infected individuals (ZMMU NAP-18334) contained two large leeches, whereas the other contained only one. The leeches left the host body during handling.

In addition to *Amolops* cf. *daorum* (fam. Ranidae) and *Bufo* cf. *pageoti* (fam. Bufonidae), 30 amphibian species were recorded and examined in the same locality (Mt. Sa Mu, Son La Province): *Duttaphrynus* cf. *gymnauchen* (Bleeker) (fam. Bufonidae); *Fejervarya limnocharis*, *Limnonectes* cf. *limborgi* (Sclater), *Limnonectes nguyenorum*, *Quasipaa* sp. (fam. Dicroglossidae); *Brachytarsophrys* cf. *feae* (Boulenger), *Boulenophrys palpebralespinosa* (Bourret), *Boulenophrys* sp., *Leptobranchium ailaonicum*, *Leptobranchium* cf. *chapaense* (Bourret), *Leptobranchella* cf. *nyx*, *Leptobranchella ventripunctata*, *Leptobranchella* sp., *Ophryophryne microstoma*, *Xenophrys langcangica* Lyu, Wang & Wang (fam. Megophryidae); *Microhyla butleri*, *Microhyla* cf. *heymonsii* (fam. Microhylidae); *Amolops* sp., *Odorrana nasica* (Boulenger), *Hylarana* cf. *menglaensis* Fei, Ye & Xie, *Hylarana*

guentheri (fam. Ranidae); *Gracixalus gracilipes*, *Gracixalus* cf. *jinxuensis* (Hu), *K. odontotarsus*, *Polypedates* cf. *impresus*, *Raorchestes* sp., *Rhacophorus* cf. *orlovi*, *Theloderma albopunctatum*, *Theloderma corticale* (Boulenger), *Zhangixalus duboisi* (fam. Rhacophoridae).

3. In Lang Son Province, northeastern Vietnam, endoparasitic leeches were detected in May 2024 in ten adult individuals of *Amolops* cf. *tonkinensis* Ahl (ZMMU NAP-16481–16483, ZMMU NAP-16535–16540, ZMMU NAP-16626) (Figures 5B, F, 9–11; Supplementary Material Video 2), collected in the vicinity of Mt. Mau Son. The amphibians were collected in rocky streams with cascades and small waterfalls within a polydominant evergreen mid-elevation montane forest dominated by Fagaceae, Lauraceae, and Magnoliaceae on steep north-facing slopes of Mt. Mau Son at elevation from 917–1006 m asl.

In total, a series of 14 individuals (nine males, five females) was collected, ten of which (six males, four females) contained endoparasitic leeches. The behavior of amphibians with leeches differed markedly from that of uninfected individuals (see below). Some individuals contained two large leeches. Leeches left the host body during handling or transportation.

In addition to *Amolops* cf. *tonkinensis* (fam. Ranidae), 16 amphibian species were recorded and examined in the same locality (Mt. Mau Son, Lang Son Prov.): *Duttaphrynus* cf. *gymnauchen* (fam. Bufonidae); *Fejervarya limnocharis*, *Hoplobatrachus chinensis* (Osbeck), *Limnonectes* cf. *nguyenorum* (fam. Dicroglossidae); *Leptobranchium*

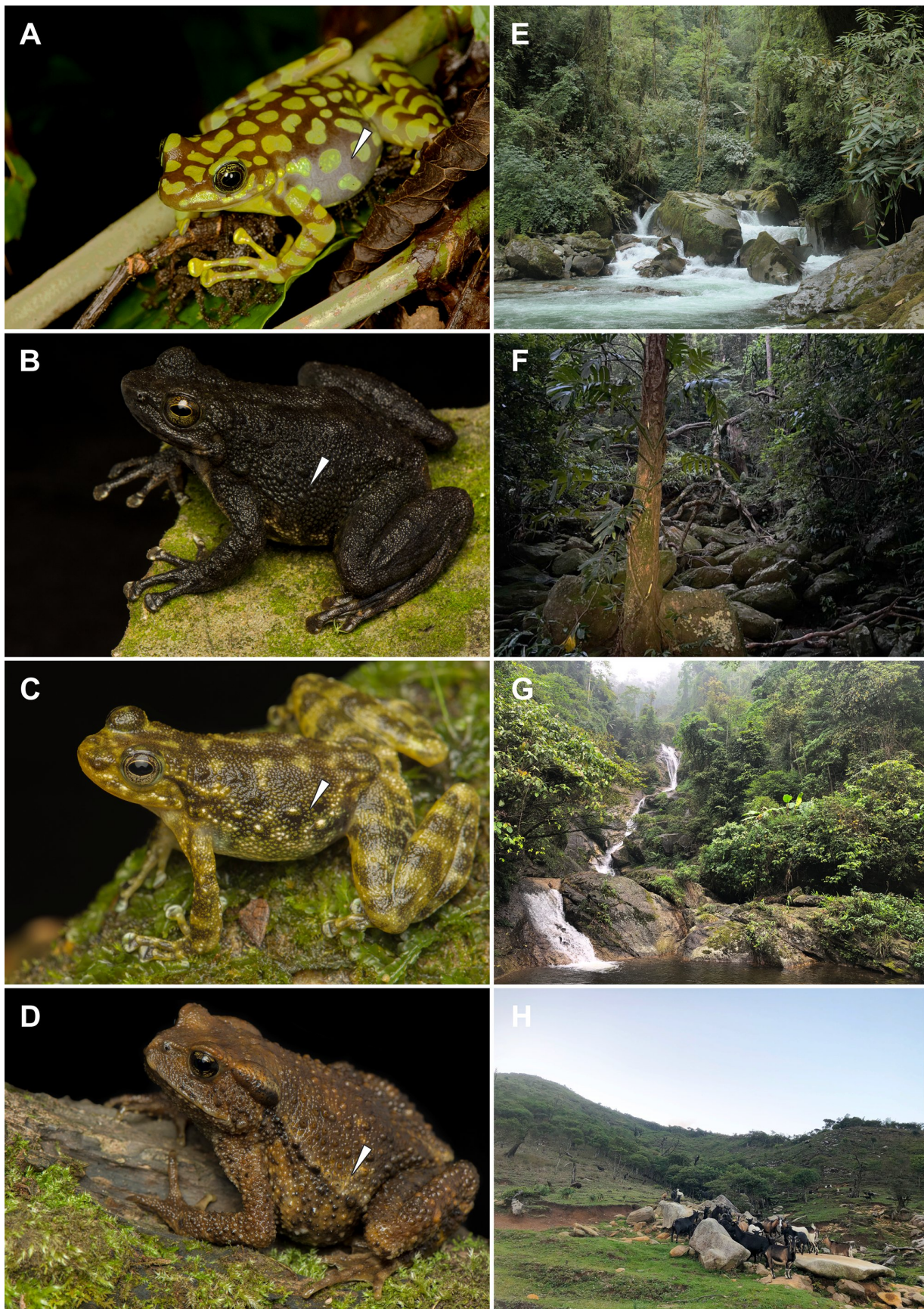


Figure 5 Endoparasitic Praobdellidae leeches inside their hosts (indicated by arrows) and their anuran hosts in northern Vietnam and southwestern China

A: female *Amolops viridimaculatus* from Yunnan Prov., southwestern China (E); B: female *Amolops* cf. *tonkinensis* from Mt. Mau Son, Lang Son Prov., northern Vietnam (F); C: male *Amolops* cf. *shihaitaoi* from Mt. Tay Con Linh, Tuyen Quang Prov., northern Vietnam (G); D: female *Bufo* cf. *luchunnicus* from Mt. Ky Quan San, Lao Cai Prov., northern Vietnam (H). Photos by Kai Wang (A, E) and A. M. Bragin (B–D, F–H).



Figure 6 Praobdellidae leech (indicated by arrow) emerging from the body cavity of a female *Bufo* cf. *pageoti* (A) from Mt. Sa Mu, Son La Prov., northern Vietnam (B)

Photos by A. M. Bragin.

guangxiense Fei, Mo, Ye & Jiang, *Leptobrachella* cf. *ventripunctata*, *Leptobrachella* sp., *Ophryophryne microstoma*, *Xenophrys maosonensis* (fam. Megophryidae); *Microhyla butleri*, *Microhyla fissipes* Boulenger, *Microhyla heymonsi*, *Microhyla pulchra* (fam. Microhylidae); *Odorrana* cf. *graminea* (Boulenger), *Hylarana guentheri* (fam. Ranidae); *Kurixalus hainanus* Zhao, Wang & Shi, *Polypedates* cf. *megacephalus*, *Polypedates impresus* (fam. Rhacophoridae); *Tylotriton vietnamensis* (fam. Salamandridae).

4. In Tuyen Quang Prov., northeastern Vietnam, endoparasitic leeches were detected in April 2023 during dissections of two adult individuals of *Amolops* cf. *shihaitaoi* Wang, Li, Du, Hou & Yu (ZMMU NAP-13948, ZMMU NAP-13946) (Figures 5C, G, 8A, B), collected in the vicinity of Mt. Tay Con Linh. The amphibians were collected in a rocky riverbed with cascades and sparse stands of *Bambusa Schreber* along the banks within the Cao Bo Village area at elevation of 1040 m asl. Frogs with endoparasitic leeches were active and did not differ from uninfected individuals. In total, a series of 16 individuals (11 males, five females) was collected, two of which (one male and one female) contained endoparasitic leeches.

In the same region, an endoparasitic leech was also found during dissection in the body cavity of a male *A. daorum* Bain, Lathrop, Murphy, Orlov & Ho (ZMMU NAP-14079) (Figure 8C, D). The frog was found in a slowly flowing mountain stream near the top of Mt. Tay Con Linh in evergreen montane forest dominated by Lauraceae Juss., Fagaceae Dumort., Theaceae Mirb., Meliaceae Juss., and Magnoliaceae at elevation of 2224 m asl. Each infected individual contained a single large leech. Leeches left the host body during dissection (Figure 8).

In addition to *A. daorum* and *A. cf. shihaitaoi* (fam. Ranidae), 31 amphibian species were recorded and examined in the same locality (Mt. Tay Con Linh, Tuyen Quang Province): *Bombina microdeladigitata* Liu, Hu & Yang (fam. Bombinatoridae Gray); *Fejervarya limnocharis* (Gravenhorst), *Limnonectes nguyenorum* McLeod, Kurlbaum & Hoang, *Quasipaa* sp. (fam. Dicoglossidae Anderson); *Boulengerophrys* sp., *Leptobrachium* sp., *Leptobrachella nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois), *Leptobrachella ventripunctata* (Fei, Ye & Li), *Leptobrachella* sp., *Ophryophryne microstoma*, *Xenophrys maosonensis* (Bourret) (fam. Megophryidae Bonaparte); *Microhyla butleri* Boulenger, *Microhyla heymonsi* Vogt, *Microhyla pulchra*

(Hallowell) (fam. Microhylidae Günther); *Odorrana* sp., *Hylarana maosonensis* (Bourret) (fam. Ranidae Batsch); *Gracixalus* cf. *yunnanensis* Yu, Hui, Wang, Rao, Wu & Yang, *Gracixalus gracilipes* (Bourret), *K. cf. odontotarsus* (Ye & Fei), *Polypedates megacephalus* Hallowell, *Polypedates* sp., *Raorchestes* sp., *Rhacophorus* cf. *orlovi* Ziegler & Köhler, *Theloderma albopunctatum* (Liu & Hu), *Theloderma hekouense* Du, Wang, Liu & Yu, *Zhangixalus duboisi* (Ohler, Marquis, Swan & Grosjean), *Z. puerensis* (He) (fam. Rhacophoridae Hoffman); *Tylotriton zieglerei* Nishikawa, Matsui & Nguyen (fam. Salamandridae Goldfuss).

5. In Dulongjiang, Gongshan County, Yunnan Prov., southwestern China, endoparasitic leeches were detected during two independent sampling events in October 2021 and May 2023. In October 2021, endoparasitic Praobdellidae leeches were found in one of two examined individuals of *Amolops kaulbacki* (Smith) (fam. Ranidae). In May 2023, endoparasitic Praobdellidae leeches were found in four of seven examined individuals of *A. kaulbacki*.

All infected individuals appeared externally healthy at the time of capture. Leeches became externally visible only when frogs were dying. In some cases, multiple leeches (one or two individuals) were present within a single host. Most leeches were located in the abdominal cavity; in one case (host voucher KIZ 51803), a leech was found beneath the skin on the dorsum. For one infected *A. kaulbacki* (KIZ 51809), the body mass of the frog and the two leeches was measured. The combined leech mass was 1.56 g, and the frog mass was 3.21 g, giving a leech-to-host mass ratio of ~0.49.

Although three other species of amphibians (i.e., *Amolops putaoensis* Gan, Qin, Lwin, Li, Quan, Liu & Yu; *Odorrana dulongensis* Liu, He, Wang, Beukema, Hou, Li, Che & Yuan; and *Xenophrys periosa* [Mahony, Kamei, Teeling & Biju]) were collected from the same locality at the same time, none of them were infected.

6. In Nankang Village, Baoshan City, Yunnan Prov., southwestern China, endoparasitic leeches were detected during two independent sampling events in May and July 2023. Endoparasitic Praobdellidae leeches were found in an adult female *Amolops viridimaculatus* (Jiang) (fam. Ranidae). For the case in July 2023, we documented a video of the case. The infected *A. viridimaculatus* individual appeared healthy and notably bulky at the time of capture. Leeches began moving rapidly within the abdominal cavity when the



Figure 7 Emergence of Praobdellidae leeches (indicated by arrow) from the cloaca of a female of *Amolops viridimaculatus* in the Gaoligong Mt. Range in Baoshan County, Yunnan Prov., China
Photo by Kai Wang.

frog was dying due to accidental suffocation, which was visible externally (see Supplementary Material Video 1). After the frog eventually deceased, leeches ultimately emerged from the cloaca one by one. When existing, leeches appeared very cautious, extending only a short portion of the anterior tip to explore the surroundings. If disturbed, it would retract back to the body cavity through the cloaca immediately. In total, four large leeches emerged from the cloaca of this host, and the abdomen of the host shrunk substantially after the leeches left.

Two other species of amphibian were collected from the same locality at the same time (e.g., *Odorrana grahami* [Boulenger, 1917] and *N. yunnanensis* [Anderson]), but none of them were infected either.

For the observation in Lang Son Province (see point 3 above), we provide the most detailed description of the behavior of Praobdellidae leeches and their hosts.

Some *Amolops* cf. *tonkinensis* individuals that were later found to be infected behaved normally: they perched in exposed places on rocks in the streambed or along the banks and, when approached, attempted to escape by making long jumps into the current, showing no obvious behavioral differences from uninfected amphibians. In contrast, several individuals differed markedly in behavior: they typically remained in moist crevices or under rock ledges overhanging seepage water; they pressed the limbs and head against the substrate, as if flattened against the surface. The body of such individuals was conspicuously distended and felt firm (Figure 5A, B). These frogs allowed a close approach and resisted only at the moment of capture (see Supplementary Material Video 2).

Almost immediately after capture (00:57 min), the “distended” body collapsed, and active movements of leeches became visible beneath the skin along the flanks and in the dorsal part of the trunk. If handling of the frog continued (4:37 min), the leeches oriented toward the cloaca, bracing against the wall of the urinary bladder and initiating peristaltic movements to force themselves outward. During this process, the leeches used the anterior end to stretch and rupture the host’s urinary bladder (6:28 min). After approximately 20 s, the

leech completely exited the host body and then moved away independently in an apparently random direction. In some cases (Figure 11), during emergence through the cloaca, the leech pulled out the tip of the frog’s lung. Also, Figure 11 clearly shows how markedly the body collapses after the leech emerges. In some *Amolops* cf. *tonkinensis* individuals, two Praobdellidae leeches were present, but no cases of infection by more than two parasites were observed. Leeches exited sequentially with a 20–30 s interval (see Supplementary Material Video 2, at 4:50 min, movements of the second leech are still visible within the body cavity). All events described above occurred only under host manipulation: holding the frog by the hind limbs or slight compression during capture. After leech emergence, the frogs were disoriented for 20–30 s; however, when approached again, they were able to escape, albeit with less coordinated jumps.

If host manipulation was stopped at the moment of leech emergence but before the leech finally left the host body, and the frog was left undisturbed (7:00 min), then after 30 s the leech turned its anterior end toward the cloaca along its body and, after fully reorienting, began to burrow back into the host’s body cavity by peristaltic movements. Contractions of the cloacal musculature and the abdominal body wall were observed as an apparent attempt to resist, although this response could not be considered active. The amphibian could slowly walk away or make short hops; however, this did not prevent the leech from maintaining attachment and returning into the body cavity. After 20 s, the leech completely disappeared into the host’s cloaca. Active movements within the body cavity remained visible for 5–7 min, after which the leech apparently returned to its original position. However, after complete emergence, the leech never attempted to return, neither in *Amolops* cf. *tonkinensis* nor in any of the other observed cases.

Within the abdominal cavity (Figure 10), the leech occupied the same position in all observed cases: it lay laterally, crescent-shaped (the left side oriented toward the dorsal body wall of the host and the right side toward the ventral body wall; the dorsal surface of the leech oriented toward the cloaca). The anterior end was inserted into the space posterior to the

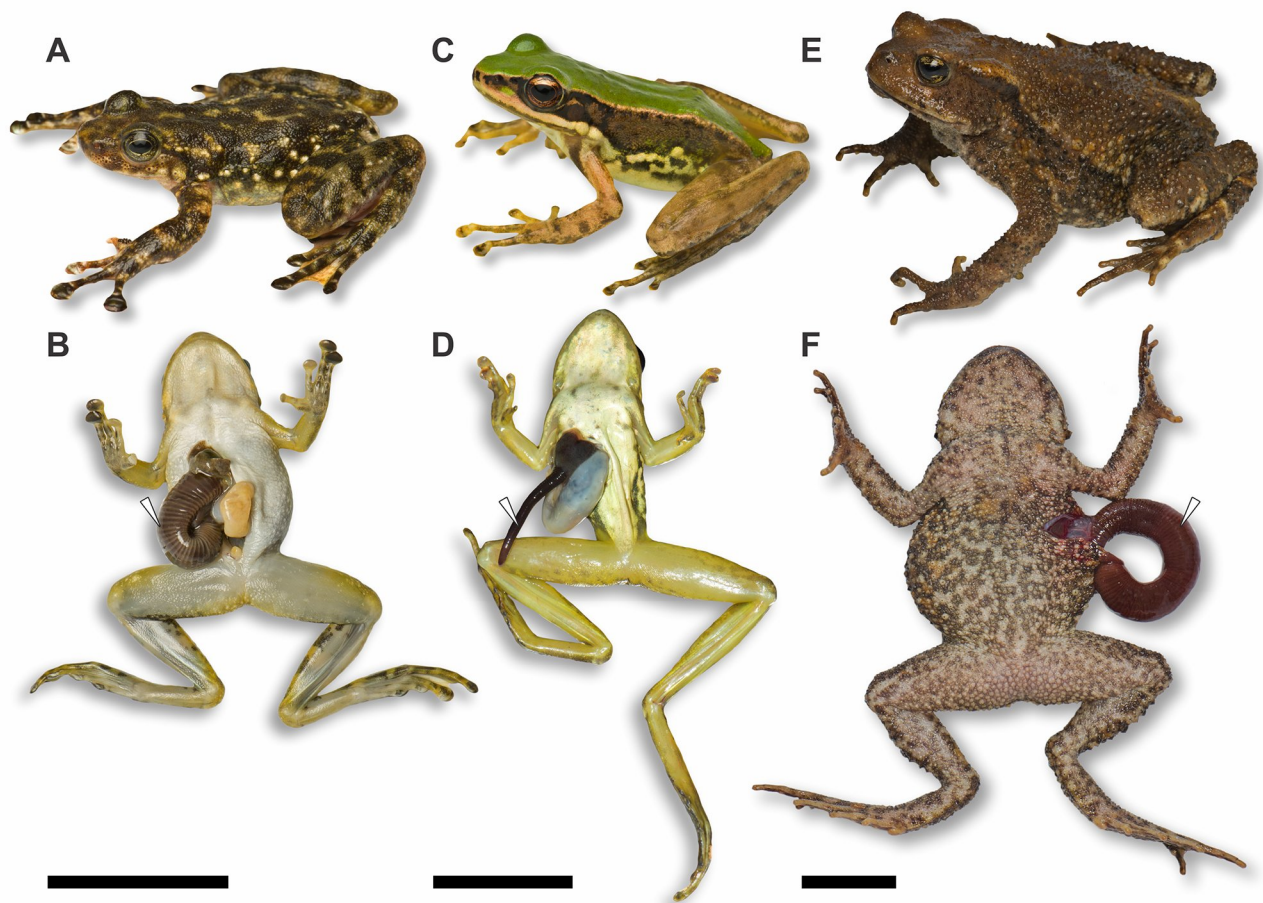


Figure 8 Amphibian hosts and associated *Praobdellidae* leeches (indicated by arrows) from northern Vietnam, shown at the time of collection (A, C, E) and during dissection (B, D, F), with the abdominal cavity opened

A–B: male *Amolops* cf. *tonkinensis* (ZMMU NAP-13948) from the vicinity of Mt. Tay Con Linh, Tuyen Quang Prov.; C–D: male *Amolops* cf. *daorum* (ZMMU NAP-14079) from the vicinity of Mt. Tay Con Linh, Tuyen Quang Prov.; E–F: male *Bufo* cf. *luchunnicus* (ZMMU NAP-14515) from the vicinity of Mt. Ky Quan San, Lao Cai Prov. Scale bar=20 mm. Photos by A. M. Bragin.

liver, in the region of the vena cava, on which the leech could presumably feed. Internal organs were slightly deformed and compressed but showed no signs of injury or emaciation. The leech pressed the gonads, fat bodies, rectum, kidneys, and lungs against the dorsal body wall; the intestine and stomach were displaced cranially. Dorsally, the leech was covered by the hepatic lobes of the host. In all cases, the leech positioned itself within a “loop” formed by the liver and lung: the lung partially embraced the leech from below and laterally, whereas the liver covered it from above. The urinary bladder was always damaged, sometimes represented only by separate flaps. It is worth noting that in nearly all observed cases, especially in species of the genus *Bufo* Garsault, the stomach was filled with prey, suggesting that leech infection does not prevent amphibians from foraging, at least.

DISCUSSION

In the study, we report cases of leech parasitism for amphibians from Asia, including the first documentation of true endoparasitism of leeches in frogs since 1963 (Mann & Tyler, 1963).

General patterns of leech parasitism: diversity and distribution

We found that leech parasitism on/in frogs is widespread across Vietnam and southwestern China, occurring across a

vast range of vegetation communities (from anthropogenic habitats to primary high-elevation forests) and altitudes (elevation ranging from 350 to 2700 m asl). Based on our observations, leech parasitism of amphibians, both external and internal, may be more widespread in subtropical and tropical Asia than currently documented, but additional studies across Asian countries are needed to confirm its broader distribution. Future studies across Asian countries are needed to better document this type of unique parasite-host interaction.

Our data indicate that at least four leech families are associated with amphibians: Haemadipsidae, Glossiphoniidae, Salifidae, and Praobdellidae. In total, amphibians served as hosts for at least 12 species belonging to 7 genera, 5 families, and 2 orders (Table 1). Haemadipsidae, Glossiphoniidae, and Salifidae were ectoparasites, using amphibians of the families Rhacophoridae, Megophryidae, and Salamandridae as hosts. Leech use of amphibians as hosts for feeding may be driven both by their availability (thin, highly vascularized skin with numerous capillaries and the high abundance of amphibians in Vietnam’s tropical forests, as noted previously) and by the low availability of mammals in high-montane forests where these leeches occur (Schenkova et al., 2021). For example, during many years of regular, year-round surveys in lowland forests of southern Vietnam (Vassilieva et al., 2016), where large and diverse mammal populations occur, no cases of

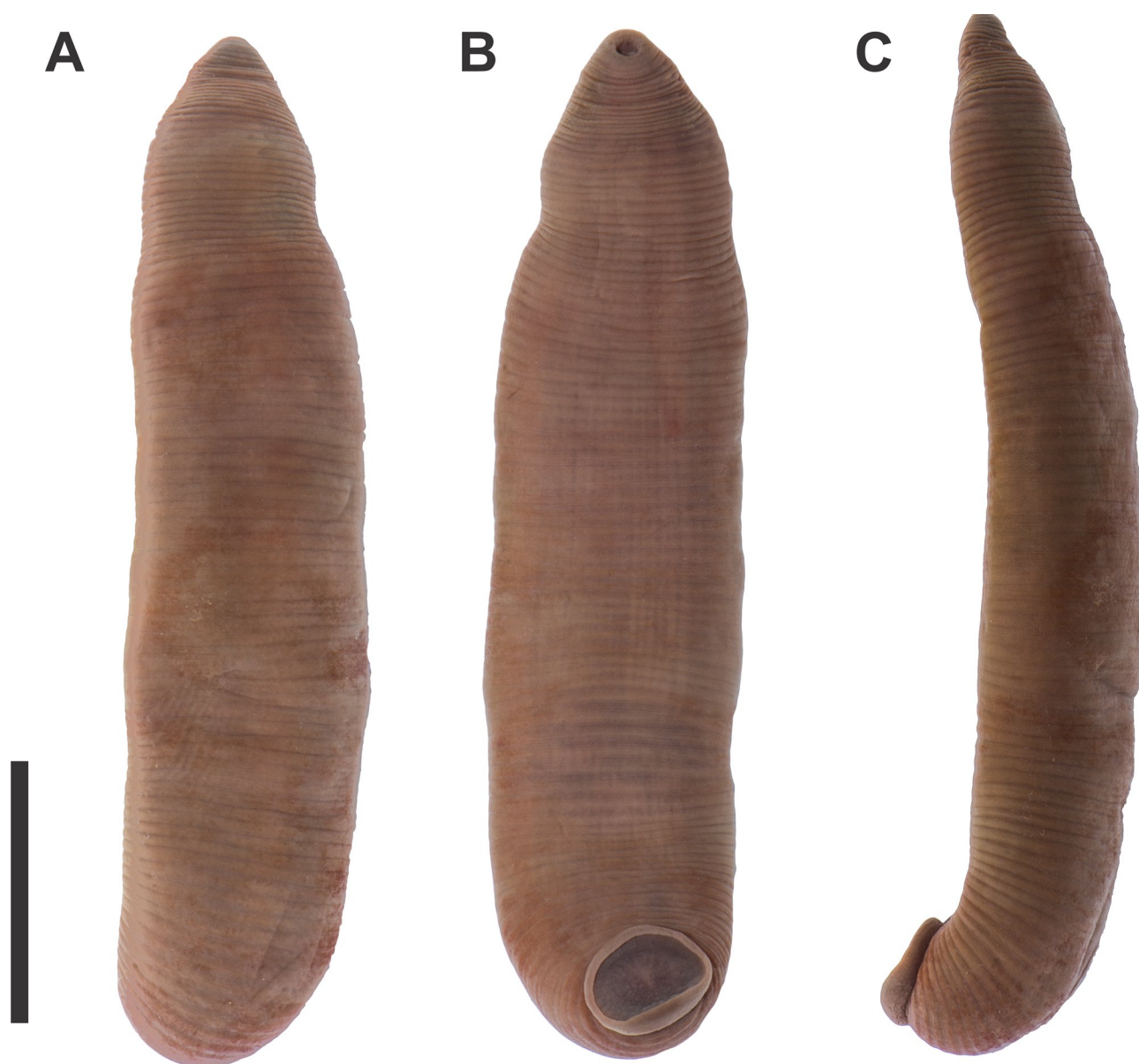


Figure 9 Endoparasitic Praobdellidae leech (VRTC BAM239) from the body cavity of *Amolops cf. tonkinensis* from Mt. Mau Son, Lang Son Prov., northern Vietnam

Dorsal (A), ventral (B), and left lateral (C) views. Scale bar=20 mm. Photos by A. M. Bragin.

leech ectoparasitism on amphibians were recorded. In some cases, infestation of amphibians by ectoparasitic leeches was common and did not appear accidental, which may indicate a certain degree of specialization.

True leech endoparasitism: new evidences, host specificity, and host entry-exit mechanisms

We reported cases of true endoparasitism by Praobdellidae leeches in amphibians of the families Ranidae and Bufonidae since 1963. Prior to us, only two studies reported usually free-living ectoparasitic leeches (i.e., *Desserobdella picta* [Verill] in the families Glossiphoniidae and *Philaemon grandidieri* [Blanchard] in the family Haemadipsidae) to be found inside amphibian bodies (Mann & Tyler, 1963; Richardson, 1949). In Richardson (1949), the Glossiphoniidae leeches were found beneath the skin of *Lithobates catesbeianus* (Shaw) in only two of a few hundred cases. This appears to be incidental rather than true endoparasitism, likely resulting from accidental entry through pre-existing damage of the integument. The only true case of leech endoparasitism in

amphibians was reported by Mann & Tyler (1963), where the Haemadipsidae leeches were found inside the dorsal lymph cavities and in the abdominal cavity of *Papurana grisea* (Van Kampen) (fam. Ranidae); *Litoria angiana* (Boulenger); *L. becki* (Loveridge); *Nyctimystes kubori* Zweifel; and *N. narinusus* Zweifel (fam. Hylidae) in Papua New Guinea. Since then, no similar observations have been documented. Six decades later, we not only reported the true leech endoparasitism in the abdominal cavity of vertebrates for the first time, but we also expanded the taxonomic coverage of both hosts and parasites and the geographic range of such parasitism, which is now documented in 22 cases across seven anuran species in two families at five localities in northern Vietnam and southwestern China. To the best of our knowledge, leech endoparasitism has not previously been reported from Asia, and endoparasitism is also reported here for the first time for praobdellid leeches.

Parasitic leeches of the family Praobdellidae have developed a highly efficient feeding strategy by entering

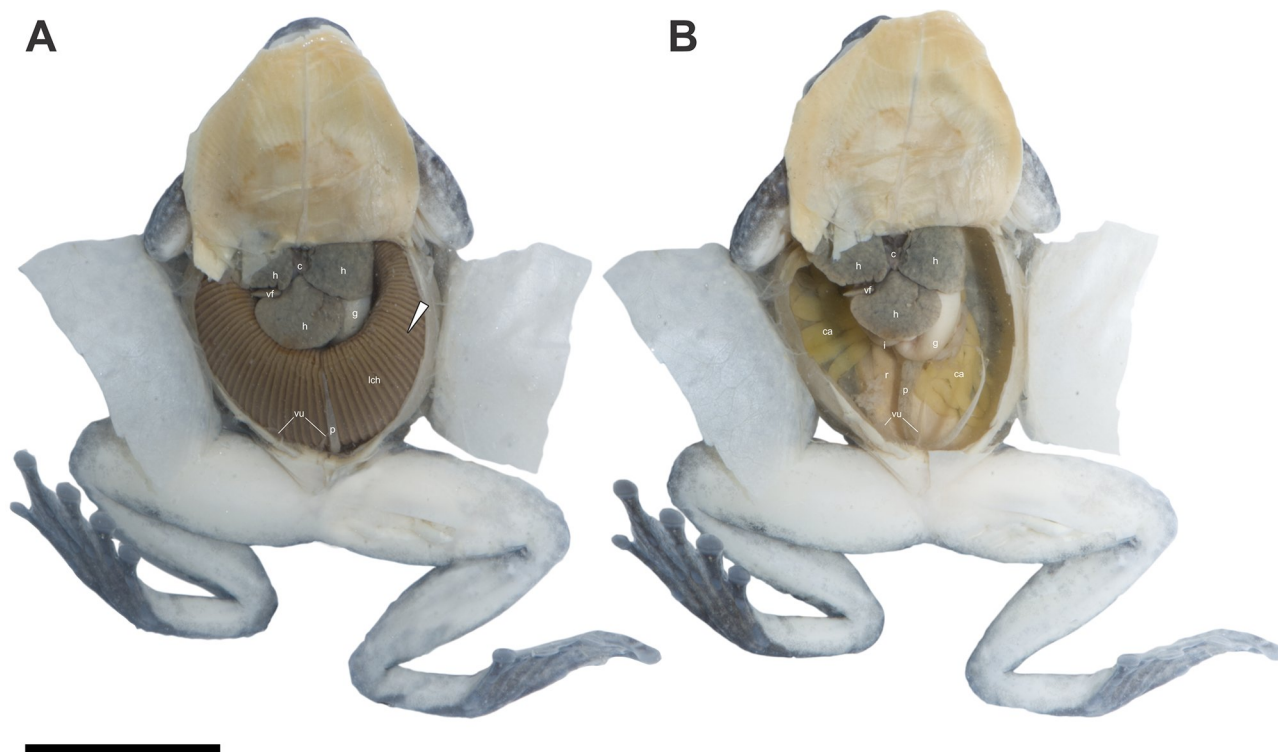


Figure 10 Position of Praobdellidae leech (VRTC BAM330) (A, leech [Ich] indicated by arrow) within the body cavity of female *Amolops* cf. *tonkinensis* (ZMMU NAP-16626) (A, B)

Abbreviations: Ich–leech, ca–fat body, c–heart, g–stomach, h–liver, i–intestine, p–lung, r–kidney, vf–gallbladder, vu–urinary bladder. The arrow indicates the position of the leech. Scale bar=20 mm. Photos by A. M. Bragin.

orifices and infesting mucous membranes. Praobdellid leeches burrow into the nasopharyngeal cavities, throats, and ocular orbits of birds (Forbes, 1903; Siddall et al., 2013; Swinhoe, 1863) and mammals (Caballero, 1931; Chen et al., 2010; Lai, 2019; Phillips et al., 2010; Saki et al., 2009; Sawyer, 1986) and also insert into the intersegmental membrane beneath the carapace of Potamonidae Ortmann crabs (Mitra & Dev Roy, 2018; Nakano et al., 2017; Oka, 1917; Schenková et al., 2021). Thus, the praobdellid leeches gain easy access to blood and use the body cavity as a shelter, which may prevent hosts from removing the parasites. According to Phillips et al. (2010), the capacity for feeding on mucous membranes can be considered one of the synapomorphies of Praobdellidae. The multiple cases we documented of praobdellid leeches infesting the body cavity of anurans fit well within this behavioral pattern. The scenario we observed may indicate a shift toward a deeper form of host–parasite interaction, namely a transition from ectoparasitism to facultative endoparasitism. This transition is also consistent with a previously noted pattern: whereas most blood-feeding leeches feed as ectoparasites for short periods of time, those that feed on mucous membranes (in most cases, the Praobdellidae family) have been known to remain in an orifice for days or even weeks (Cundall et al., 1986; Harding & Moore, 1927; Phillips et al., 2010). Within a single life stage, leeches may move between the host's body cavity and the external environment, apparently not only to facilitate feeding and dispersal (Borda & Siddall, 2011; Schenková et al., 2021; Siddall et al., 2013) but also to avoid predators by using the host as a temporary refuge—a “fortress” capable of rapidly escaping by jumping or swimming away and providing strong chemical protection.

In this context, the choice of host by the endoparasitic leeches is particularly noteworthy. Among all amphibian taxa recorded within each of the surveyed sites in the studied provinces (29 species in Lao Cai Prov., 32 species in Son La Prov., 17 species in Lang Son Prov., 33 species in Tuyen Quang Prov., three species in Gongshan and two species in Baoshan in Yunnan Prov.; for details, see the results section), leeches were found only in species of the genus *Amolops* (family Ranidae) and *Bufo* (family Bufonidae) in Vietnam and only *Amolops* in China. Several other sympatric amphibian taxa (i.e., of the families Ranidae, Dicroglossidae, and Megophryidae) all have comparable body size and similar ecology to the infected species, but none of them were infected in either China or Vietnam. This host choice appears far from random. It is well known that species of *Amolops* (Guo et al., 2020; Zhang et al., 2018) and *Bufo* (Kowalski et al., 2018; Rodríguez et al., 2017) possess numerous cutaneous glands that produce various bioactive chemical compounds. Many of these chemical compounds, such as bradykinin-related peptides and bufadienolides, play a critical role in predatory defense and are toxic to other species (Thompson et al., 2006). Apparently, these compounds are not repellents for leeches, as evidenced by reports of ectoparasitic leeches of several families on Bufonidae (Ben Hassine & Escoriza, 2014; Loebmann et al., 2008; Masetti et al., 2015; Ocegüera-Figueroa, 2006; Soler et al., 2014; Starzecka et al., 2020; Velasco et al., 2016) and Bombinatoridae (Zimić, 2015). On the contrary, leeches may be able to selectively recognize these chemical compounds when searching and selecting hosts, subsequently using the amphibian as a “living spacesuit” that not only provides nourishment but also offers reliable protection.



Figure 11 Female *Amolops cf. tonkinensis* (ZMMU NAP-16629) (A) after the emergence of Praobdellidae leech (VRTC BAM-244) (B-C). B: dorsal view of Praobdellidae leech; C: ventral view of Praobdellidae leech. The everted tip of the lung, protruding through the cloaca, is indicated by the arrow

Scale bar=10 mm. Photos by A. M. Bragin.

Another point of interest is how the leech enters and exits the host. A careful examination of all inspected amphibians revealed neither internal damage to the gastrointestinal tract nor lesions of the integument. Therefore, leech entry may occur at early developmental stages of the leech. It is difficult to imagine an adult leech actively burrowing into a host while the amphibian allows this without resistance. Mann & Tyler (1963) also reported that “none of the frogs shows a scar marking the point of entry” for their cases of Haemadipsidae endoparasitism, and they suggested that “the leeches entered when they (leeches) were very small and had lived and grown for a considerable period within the host.” While the question on how the leech enters the host body remains open, we found evidence on how they leave the frog bodies. All examined host amphibians had a ruptured urinary bladder, but the intestine was not damaged. Given the thickness of the digestive system, exit through the intestinal wall is presumably mechanically difficult for the leech. Therefore, we assume the leech stretched and tore the thin bladder wall when leaving the frog (6:28 min in Supplementary Material Video 2).

The trigger for emergence appears to be either a physical signal of danger to the host by a potential predator (i.e., compression and vigorous handling of the frog host) or the death of the host. Ultimately, the direct cues used by leeches may be stress-induced physiological changes in the amphibian, such as stress hormones or other internal chemical cues. The complexity of the leech’s behavior is further suggested by how rapidly it orients within the

amphibian body cavity. Only one-minute elapses between the disturbance of the frog and the leech emergence.

Conservation and ecological implications.

Our discoveries of leech ecto- and endoparasitism in amphibians also raised a series of questions that are ecologically relevant and waiting for future studies. Particularly, from a conservation perspective, how these types of parasitism affect amphibian species, both at the individual level and population level, remains unknown.

For example, the substantial relative size of the ectoparasitic leeches (BL/SVL ~58.1% for Haemadipsidae and ~36.1% for Salifidae; Tables 3, 4) and the common nature of infestation in some cases (for Haemadipsidae and Glossiphoniidae) suggest potentially significant blood loss in their hosts. Such infestations may impose energetic stress, and, in some extreme cases, may cause morbidity or mortality (Brockelman, 1969; Burgin & Schell, 2005; Merilä & Sterner, 2002). Furthermore, such external parasitism may further elevate vulnerability to pathogens and predators via behavioral impairment or wound-mediated exposure pathways (Phangurha et al., 2024; Phillips et al., 2020), which can lead to increased disease transmission and higher predation rates among infested hosts.

Our observations in cases of leech endoparasitism indicate that the infected adult amphibians generally remain functional and appear healthy superficially, but given the substantial size of the leeches (average BL/SVL~74.9%, leech-to-host body

mass ratio ~48.5%), such parasitism will certainly affect the host's health negatively. Detailed physiological conditions after infection, particularly how the infection affects longevity and reproduction success, would require further detailed studies.

From the population ecology perspective, the epidemiology of leech parasitism and the impact on the population dynamics of amphibians remain to be examined. Our limited samplings showed a high prevalence of infection in individual sites (Table 1) and sometimes a high infectious load in individual frogs (i.e., four leeches in a single individual from China), but long-term monitoring surveys are needed to assess the true epidemiology. As many of the potential host species are microendemic and threatened (i.e., *Amolops loloensis* [Liu], *Amolops tuberodepressus* Liu & Yang, *Amolops vitreus* [Bain, Stuart & Orlov], *Amolops minutus* Orlov & Ho, and *Bufo luehmanni* [IUCN, 2026; Poyarkov et al., 2021]), widespread leech parasitism may pose additional threats to these vulnerable species and warrant attention.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Fieldwork in Vietnam, including collection of animals in the field and specimen exportation, was funded by VRTC and was conducted under permission of the Peoples' Committees of Lam Dong Province of Vietnam for work in Ta Dung NP (permit numbers: No. 209/UBND-NGV of January 13, 2021 and No. 6587/UBND-NGV of October 31, 2023), of the Forestry Department and the People's Committee of Thanh Hoa Province of Vietnam for work in Xuan Lien NR (permit numbers: No. 5179/SNN&PTNT-CCKL of October 5, 2023), by the Department of Forestry, Ministry of Agriculture and Rural Development of Vietnam, and by the Forest Protection Departments of the Peoples' Committees of Tuyen Quang Province of Vietnam for work on Mt. Tay Con Linh (permit numbers: No. 182/SNgV-LS of 3.04.2023, No. 574/SNN-KL of 6.04.2023), of the Forestry Department and the People's Committee of Lang Son Province of Vietnam for work in Mau Son NR (permit numbers: No. 513/SNgV-LSHTTQT of May 20, 2024), of the Forestry Department and the People's Committee of Son La Province of Vietnam for work in Ta Xua NR (permit numbers: No. 5861/UBND-KGVX of October 9, 2025), of the Forestry Department and the People's Committee of Lao Cai Province of Vietnam for work in Bat Xat NR (permit numbers: No. 789/SNN-KL of April 27, 2023), and of the People's Committee of Lam Dong Province of Vietnam for work in Bidoup-Nui Ba NP (permit numbers: No. 54/VP-NV4 of January 21, 2025). Survey permission in China was granted by the Yunnan Department of Forestry and Grassland (2022GF258D-10) and the Gaoligong National Nature Reserve.

DATA AVAILABILITY

Assembled mitochondrial genome sequences were deposited in the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov>) under GenBank accession numbers PX729569–PX729580.

SUPPLEMENTARY DATA

Supplementary data related to this article are available online and can also be downloaded from the following links:

Video 1: Emergence of Praobdellidae leeches from the cloaca of a female *Amolops viridimaculatus* (should be in *Italic*) in southwestern China. Two leeches are visible emerging from the cloaca. Filmed by Kai Wang: <https://www.scidb.cn/en/s/ArE3I3>

Video 2 Emergence (00:00–06:50 min) and return (07:00–07:25 min) of a Praobdellidae leech from the cloaca of a female *Amolops* cf. *tonkinensis* (should be in *Italic*) in northern Vietnam. Filmed by A. M. Bragin: <https://www.scidb.cn/en/s/lymmAj2>

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

A.M.B., K.W., and N.A.P. designed the study; A.M.B., N.A.P., D.X.L. and S.X.L. collected materials for the study in Vietnam; K.W. Z.Y., J.C. and N.Z. collected materials for the study in China; K.W. and A.V.T. performed molecular analyses; A.M.B. performed morphological and phylogenetic analyses; A.M.B. and K.W. prepared the manuscript; J.C. and N.A.P. edited and revised the manuscript. All authors read and approved the final version of the manuscript.

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