SALAMANDRA **60**(4): 283–296
15 November 2024 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Mystery solved: unravelling the life history of the enigmatic and ancient Philippine frog *Barbourula busuangensis* (Anura: Bombinatoridae)

Marta Miñarro[1], Patricia A. Burrowes[1,2], Claudia Lansac[1], Leticia Afuang[3] & Ignacio De la Riva[1]

[1] Museo Nacional de Ciencias Naturales-CSIC, C/ José Gutiérrez Abascal 2, 28006 Madrid, Spain
[2] Department of Biology, University of Puerto Rico, P.O. Box 23360, San Juan, PR 00931, USA
[3] Animal Biology Division, University of the Philippines, Los Baños, 4031 College Laguna, Philippines

Corresponding author: Marta Miñarro, email: marmiro@mncn.csic.es

Manuscript received: 23 September 2024
Accepted: 18 October 2024 by Jörn Köhler

Abstract. The biology of the poorly known Philippine frog *Barbourula busuangensis* has been a challenging mystery since the discovery of the species one hundred years ago. Based on extensive fieldwork and examination of scientific collections, we present novel information on its reproductive biology, including the discovery and morphological description of the tadpole. This species exhibits characteristics of a K-strategist, with large, unpigmented eggs in small number relative to body size (low Ovarian Size Factor, mean = 6.56). Field observations suggest a prolonged reproductive period, with gravid females present across seasons, and possible egg retention for at least up to two months. We describe for the first time to science their unique tadpoles, which are rheophilous, endotrophic, and nidicolous. These tadpoles lack keratinized mouthparts (rostrodonts and tooth rows) and have a large sucker-like oral disc used for adhesion within rock crevices in river cave systems, where they develop. Camera recordings at nest site revealed the presence of an adult with the larvae, suggesting parental care. Overall, our findings offer significant insights into the life history of this secretive species, and point to a new, undescribed reproductive mode for anurans, thus contributing to our knowledge on the evolution of reproductive strategies of tropical species in different ecological contexts.

Key words. Amphibia, ecology, endotrophic, nidicolous, reproductive mode, tadpole.

Introduction

Among other characteristics, amphibians are known for having multiple and sometimes astonishing reproductive behaviours, particularly remarkable in anurans (Duellman & Trueb 1986). Boulenger (1886) was a pioneer in categorizing this diversity, and identified ten reproductive variants. The multiplicity of modes in anuran reproduction accommodates along the traditional r–K continuum of life history strategies (MacArthur & Wilson 1967), which, in order to warrant the continuity of a given species' abundance, imposes a trade-off between quantity and quality of the offspring depending on the characteristics and stability of the environment. The commonly used term 'reproductive mode' was first introduced by Breder Jr. & Rosen (1966) in the context of fish reproductive biology, and was later redefined for anurans by Salthe & Duellman (1973) and Salthe & Mecham (1974). Accordingly, what determined a particular reproductive mode was a combination of features including, for example, oviposition site, characteristics of the eggs and the clutch, developmental rate and duration (admittedly, something difficult to study in natural conditions), size and stage of hatchlings, and absence/presence of parental care. Some further modifications of this scheme are those introduced by Crump (1974), Lamotte & Lescure (1977), and Brown & Alcala (1983). Duellman & Trueb (1986) defined 29 anuran reproductive modes, which were raised to 39 by Haddad & Prado (2005). New modes for anurans were described in subsequent years, reaching 71 modes in the latest compilation by Nunes de Almeida et al. (2021). These authors redefined the criteria used so far to characterize reproductive modes, and proposed a combination of eleven characters, namely: reproduction type, oviposition macrohabitat, spawning type, oviposition substrate, egg-surrounding medium, nest construction, oviposition microhabitat, embryonic development, embryonic nutrition, larval and new-born nutrition, and place of larval development. The combinations of these characters revealed unique adaptations among species. To the opposite side of the most "r-selected" reproductive modes (i.e., clutches of numerous, even thousands, small eggs that produce exotrophic larvae in absence of parental care) exist sophisticated, more "K-selected" modes with smaller clutches of large eggs producing either exotrophic or endotrophic larvae. Reproductive modes in the "K-strategy" end are extensively represented in various families of anurans, and include skipping metamorphosis with lecithotrophic embryos that hatch as froglets, presence of parental care, and, in most extreme cases, egg retention in the female's oviduct leading to viviparity (sensu Nunes de Almeida et al. 2021).

According to Liedtke et al. (2022), reproductive modes are known for most anuran species, and unknown for only 4.6% of them. This small figure likely reflects the assumption that the reported reproductive mode for a species in a given genus or clade is extensible to the rest of the species of such group. However, the reality is that life histories have been properly documented only for a small proportion of the 7752 species (Frost 2024) of extant frogs. For example, reproduction has been studied only for a handful of species in the most diverse anuran genus, *Pristimantis* Jiménez de la Espada, 1870 (610 species; Frost 2024) (Duellman & Lehr 2009). In addition to this, there are exceptions and many instances in which a particular species has developed a reproductive mode different from that of other congeners (e.g., the viviparous *Eleutherodactylus jasperi* Drewry & Jones, 1976). As a consequence of this lack of empirical knowledge, together with the growing number of anuran species being described, it is expectable that the diversity of reproductive modes within clades (and even within species; see Nunes de Almeida et al. 2021) will increase and, considering the scarce information available for entire groups, new modes might be described.

In this respect, an interesting case is that of the family Bombinatoridae, which consists of two genera, *Bombina* Oken, 1816 and *Barbourula* Taylor & Noble, 1924, containing seven and two species respectively (Frost 2024). The genus *Bombina*, known as yellow or fire-bellied toads, has a disjunct distribution across Eurasia (central and southern Europe, Turkey, western Russia, eastern Russia, China, Vietnam and Korea; Frost 2024). In contrast, the two species of *Barbourula*, or flat-headed frogs, are insular, where *B. busuangensis* Taylor & Noble, 1924 is endemic to the Philippine province of Palawan, and *B. kalimantanensis* Iskandar, 1978 is endemic to the Indonesian portion of Borneo (Frost 2024). Among these two genera there is a considerable difference in the amount of published information on their natural history: whereas several *Bombina* species (particularly those occurring in Europe) have been thoroughly studied by many authors (e.g., Rafińska 1991, Kaplan & King 1997, Hernandez & Espallargas 2020), *Barbourula* remains as one of the most enigmatic genera of frogs in the world. This lack of information is particularly problematic since this genus has a remarkably ancient origin and the two species also diverged long ago (estimated time of divergence from *Bombina*, 47.1 mya; that between *B. busuangensis* and *B. kalimantanensis*, 10.8 mya; Blackburn et al. 2010). Thus, it is not justified to assume that the reproductive mode of *Barbourula* would be necessarily similar to that of the other bombinatorid frogs in the genus *Bombina*. In addition to this, some observed features of *B. busuangensis,* as, for example, females having a low number of large, unpigmented eggs, point to a reproductive mode potentially different and not aligned with the common "r" strategy of many anurans. Nothing is known on the life history of *B. kalimantanensis*, and most information published on the species concerns its delicate conservation status (Rachmayuningtyas et al. 2011) and its bizarre, nearly lungless condition, unique among anurans (Bickford, et al. 2008, Blackburn et al. 2024).

Barbourula busuangensis is a nocturnal, aquatic frog that lives in rocky, fast-flowing streams within the rainforest; although the species can be locally common, individuals are difficult to observe and capture due to their secretive habits, hiding under large boulders or in rock holes and crevices (Alcala & Brown 1987). This explains why the information on its biology and ecology is remarkably anecdotal and fragmentary (Flores et al. 2024). In contrast, its insular endemicity, ancient origin and distinctness, has attracted considerable interest from zoologists, resulting in comprehensive studies on the species' musculoskeletal anatomy (Clarke 1987, Prikryl et al. 2009, Roček et al. 2016), urogenital system (Bhaduri & Mondal 1965), phylogenetic relationships (Blackburn et al. 2010), and vocalizations (Bosch et al. 2023).

The state of the art regarding the reproductive biology of *B. busuangensis* is easy to summarize: one hundred years after the description of the species by Taylor & Noble (1924), it remains a total mystery and no field researcher has been able to observe reproductive behaviour whatsoever. The first speculation about it was made by Inger (1954), who, based on observations of gravid females carrying few, large, unpigmented eggs, and on the fact that no tadpoles of this species had ever been seen, suggested that eggs might be deposited under stones in the water, where they could follow direct development, bypassing a free larval stage. This supposition was further assumed as a possibility by subsequent authors, as for example, Brown & Alcala (1983) in their review of the modes of reproduction of Philippine anurans. Later, Alcala & Brown (1987) reported on how they were unable to observe reproductive behaviour in the field or in captivity. After keeping 20 individuals in the lab and injecting them with hormones to trigger oviposition (without success), these individuals lived for more than a decade, without providing a single clue about their reproductive biology (Infante et al. 2002). The lack of observations on amplexus, larvae, or any indication of reproductive activity apart from finding gravid females has been repeatedly commented by other authors that have studied the species in the field (e.g., Afuang & Cielo 2010, Bosch et al. 2023, Flores et al. 2024).

To address the mystery of the reproductive biology of *B. busuangensis*, we examined preserved gravid females and carried out extensive fieldwork to study two populations in their natural habitat in the island of Busuanga, Province of Palawan, Philippines. We present the first detailed description of aspects of the reproduction mode and development of this ancient and elusive frog, whose breeding behaviour proved extremely difficult to be investigated.

Material and methods

Study area

Observations on the life history of *Barbourula busuangensis* were done as part of a broader capture-mark-recapture survey conducted for a total of ten months during three field sessions, two in 2022 (April–July and October–December) and one in 2023 (April–June), in the province of Palawan, Philippines. Fieldwork took place along small rocky rivers at two main study sites in the island of Busuanga: Malbato (Barangay Bintuan, 12°2'14" N, 120°5'49" E) and San Rafael (Barangay New Busuanga, 12°12'12" N, 119°55'9" E). Additionally, we made two short surveys at Simpocan (Puerto Princesa, 9°47'24" N, 118°33'16" E), in the island of Palawan. The climate of the region is characterized by a rainy season that extends from June to November and a drier season from December to May. During our field work the mean temperature at our sampling sites was of 28 °C (ranging from 26–31 °C), and the mean daily precipitation varied between 4.86 and 7.13 mm/day (data obtained from visualcrossing.com).

Preserved gravid females examined

We dissected nine gravid adult females of *Barbourula busuangensis* belonging to the herpetological collection of the Natural History Museum, the University of Kansas (Lawrence, Kansas). A small window-like incision was made on the right side of every female using dissection scissors and fine-tip tweezers (Fig. 1). The skin and muscles were lifted, and all the eggs observed on that side were extracted to be counted and measured. From the total number of eggs, ten were set aside to measure their diameter to the nearest 0.01 mm using a digital calliper. The eggs extracted were then placed in individual glass collection containers, with 70% ethanol and their corresponding identifying tag number.

The ovarian size factor (OSF) (Duellman & Crump 1974), as modified by Duellman (1978), was calculated for the species as an index of egg mass relative to body length: OC (OD) / SVL, where OC (ovarian complement) is the mean number of mature ovarian eggs, OD is the maximum ovum diameter, and SVL is the mean snout-to-vent length of gravid females. Because we only counted the eggs in the right ovary, calculations were made assuming this quantity to be half the total number of eggs, and thus multiplied by two for each individual to calculate the mean OC for the species.

Gravid females observed during field work

The gravid females considered hereafter belong to the mentioned capture-mark-recapture study, where individuals measuring more than 35 mm in snout-to-vent length (SVL, mm) were marked with an 8 mm mini-Passive Internal Transponder (PIT) magnetic tag (Trovan, ID 100A/1.4) (Miñarro et al. in prep.). Gravid females were easily identified in the field because large egg masses are visible through their ventral skin in both ovaries (Bosch et al. 2023: Fig. 3).

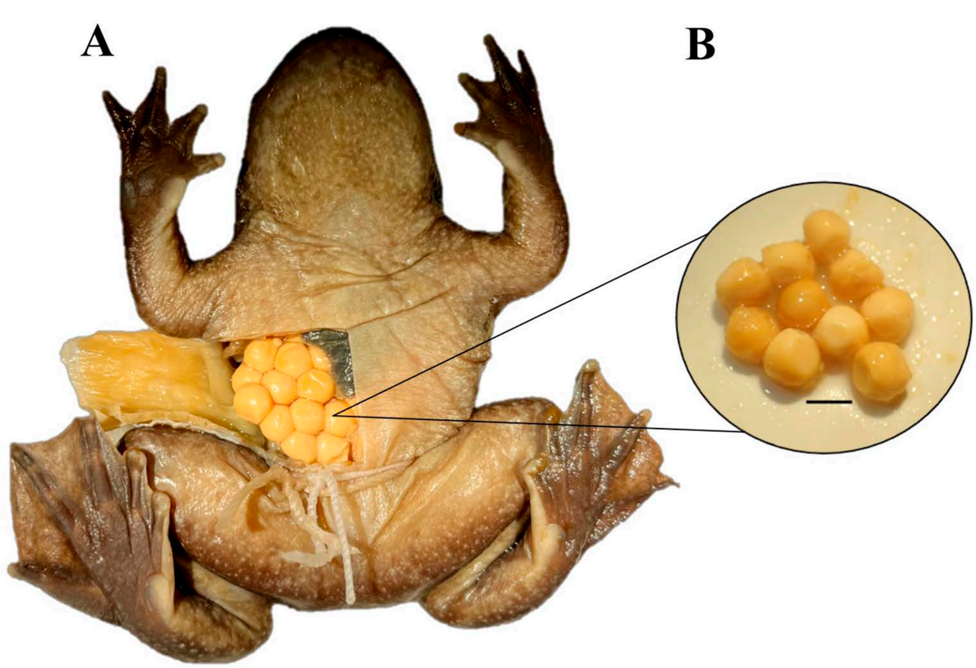

Figure 1. (A) Female *Barbourula busuangensis* from the island of Palawan (Palawan province, Philippines) examined at the herpetological collection of the Museum of Natural History, the University of Kansas (KU 309042) and (B) detailed close-up of the observed eggs. Scale bar 4 mm. Photos by MM.

Tadpole capture and description

To look for possible reproduction sites we used an endoscope camera (KZYEE KZ3000) introduced into crevices and holes within rocky habitat. We found two tadpole nests in the same rock wall. The first nest was discovered in November 2022, and the second one in April 2023. On each occasion, we collected a single tadpole. The tadpole description is based entirely on the individual collected in April 2023 because it was at an earlier Gosner stage (Gosner 1960) than the tadpole collected in November 2022, which was only used to observe the final phases of development until metamorphosis.

The extraction of the April 2023 tadpole from its nest was challenging due to the microhabitat characteristics (see below) and the precarious logistics at our field site. A plastic hose containing the endoscope camera was introduced trough the small crevice and directed towards the tadpole nest. Once the tadpoles were visible in the camera screen, the endoscope was gently removed leaving only the plastic hose. Then, suction was applied at our end of the hose, creating a negative pressure inside of the crevice leading to the discharge of water into a plastic container, which luckily carried one tadpole. This tadpole was collected at Gosner stage 36 and kept in captivity until stage 45, at which point it was released at the same capture site. During this time, we kept the tadpole in a plastic container (15 × 7 cm) with small rocks from the original capture site and no added food source (Fig. 2). The enclosure was kept in the dark and the tadpole was handled only three times, corresponding to the three Gosner stages described below.

Tadpole measurements and terminology follow Altig & McDiarmid (1999) and Pezzuti et al. (2021), and the development stages follow Gosner (1960) (see Fig. 3). During field work, total length (tip of snout to tip of tail; TL) was measured using a calliper to the nearest 0.1 mm. However, due to the lack of proper facilities at our field site and the additional complication of not having permits to collect, we limited manipulations to the minimum. All other measurements were obtained from photographs using ImageJ Version 1.54 (Schneider et al. 2012), using TL as a defined comparative scale for all measurements and stages. Measurements taken are as follows: body length (distance between the tip of snout and the end of body at the level of caudal muscle medial line, BL); body height (maximum distance between dorsal and ventral edges of the body, BH); body width (maximum distance between the lateral edges of the body, BW); tail length (distance between the beginning of the tail at the level of caudal muscle medial line and the tail tip, TAL); ventral fin height (maximum distance between external and internal edges of ventral fin, VFH); dorsal fin height (maximum distance between external and internal edges of dorsal fin, DFH); maximum tail height (maximum distance between external edges of ventral and dorsal fins, MTH); oral disc width (maximum distance between lateral margins of the closed oral disc, ODW); oral disc length (maximum distance between the posterior and anterior end of the closed oral disc, ODL); eye diameter (distance between eye edges, ED); interorbital distance (distance between centres of pupils, IOD); internostril distance (distance between the medial margin of nostrils, IND); hindlimb length (distance from the groin to the tip of the longest toe, HL); femur length (length of femur, F); tibia length (length of tibia, T); foot length (length of tarsus + metatarsus + longest toe, FoL); forelimb length (distance from the axilla to the tip of the longest finger,

Figure 2. *Barbourula busuangensis* tadpole enclosure, where an individual was kept for 18 days. Red arrow shows the tadpole at Gosner stage 43. Photo by MM.

FL); humerus length (length of humerus, H); radio–ulna length (length of radio–ulna, RU); and metacarpals length (length of the longest metacarpal, MC). The coloration of the tadpole was described on the basis of field observations and live photographs taken in the plastic enclosure or in a transparent bag.

Results

Preserved gravid females

The SVL of the nine examined gravid females of *B. busuangensis* ranged from 69.2 to 86.2 mm, with a mean SVL of 76.9 mm (Table 1, Fig. 1). The mean estimated ovarian complement was 108, with a maximum of 178 and a minimum of 50 eggs per ovary/female. The diameter of the 90 eggs measured (10 per female) ranged from 3.08 mm to 4.88 mm, with a mean diameter of 4.17 mm. The ovarian size factor (OSF) was low, ranging from 2.7 to 11.68, with a mean value of 6.56, which is herein considered the species

Table 1. Summary of reproductive data; snout-to-vent length (SVL, mm), estimated ovarian complement, range in egg diameter (mm), and ovarian size factor in preserved females of *Barbourula busuangensis* from the herpetological collection at the Museum of Natural History, the University of Kansas (Lawrence, Kansas); order from smallest to largest SVL.

Catalogue number	SVL (mm)	Estimated Ovarian Complement	Range in egg diameter (mm)	Ovarian size factor
KU326278	69.2	78	2.84–3.52	3.97
KU309092	71.6	68	4.00–4.63	4.40
KU324602	72.4	82	3.53–3.99	5.52
KU309042	76.0	90	4.27–4.89	5.79
KU309390	76.8	152	4.52–4.95	9.80
KU309243	78.6	148	4.15–5.23	9.84
KU309278	79.3	178	4.46–5.20	11.68
KU324601	82.2	50	3.92–4.44	2.70
KU324606	86.2	124	2.81–3.72	5.35

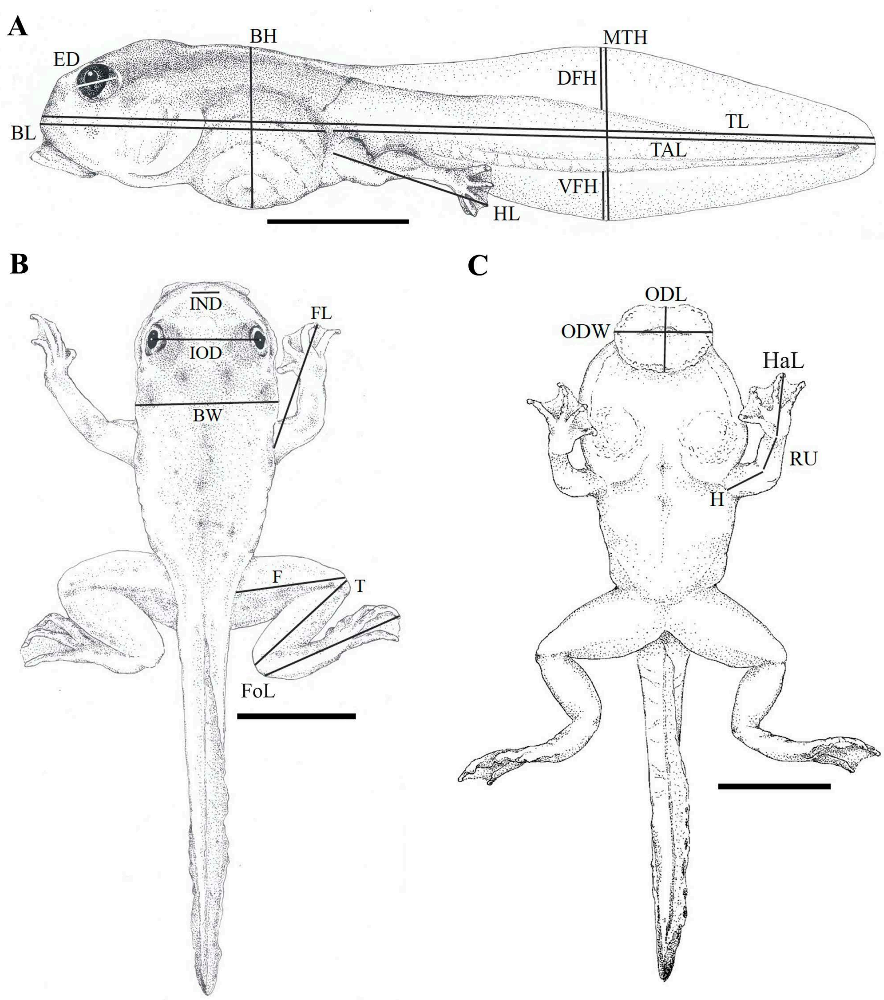

Figure 3. Measurements of *Barbourula busuangensis* tadpoles following Altig & McDiarmid (1999) and Pezzuti et al. (2021), in (A) lateral (Gosner stage 36), (B) dorsal, and (C) ventral views (Gosner stage 43). Scale bars 5 mm. Illustrations by CL.

OSF. The colouration of the eggs was uniformly pale yellow with no darker pigmentation (Fig. 1B). These females were collected in the months of January, March and July in years ranging from 1994 to 2007.

Gravid females observed during field work

A total of 27 gravid females were observed during our study (4 in Palawan and 23 in Busuanga), with SVLs ranging from 51.2 to 85.2 mm. These females were encountered in the following months: eleven in April, two in May, eight in June, four in July, and one in November of 2022; and one in April of 2023. Among the 23 gravid females from our recapture study in Busuanga, four were recaptured (one to three times) during the study, and presented changes in their reproductive condition (Table 2). Female #1 was captured and marked on 5 May 2022 showing no evidence of eggs, and was gravid when recaptured a month later. A year later, in April 2023, this same individual was recaptured showing no evidence of eggs. Female #2 was gravid when captured on 5 May 2022, and remained gravid upon recapture one month later. Female #3, captured and marked on 4 June 2022, was not gravid when found in November 2022 nor in April 2023. Finally, female #4 was gravid when captured on 5 May 2022, and remained gravid when recaptured on 6 July 2022. Also, two gravid females were observed in Palawan Island in March 2018 by PAB and IDlR.

Nesting, tadpole microhabitat and parental care

Two nests were found in a rocky wall that formed a small waterfall (Fig. 4) at our sampling site in San Rafael (see Material and methods for a description of the area). The rock wall measured approximately 3 m in height and was covered in mossy vegetation. On top of the wall there was a small man-made concrete dam-like structure, that would probably be used as a drainage outlet. Water flowed continuously from the upper part of the stream, forming a small pond that continued downstream into the fast-flowing river. The immediate lateral parts of the stream were covered by dense vegetation.

The first nest was encountered on 18 November 2022. Approximately 15 metamorphs were found inside a crevice within the rock wall (Fig. 5A white arrow, Video 1). The entrance of the crevice measured 18 cm in height and 7 cm in width. Inside the crevice the height increased to 27 cm and its bottom was composed of 9 cm of sandy floor partially covered by water. The furthest part from the entrance of the crevice had a small opening that connected with other crevices inside the rocky wall, probably forming a network of tunnels with running water. From this opening, an adult *B. busuangensis* was observed emerging several times (Fig. 5B). A group of metamorphs was found, all together and pressed against each other on the interface between the water and the rock walls of the crevice (Fig. 5C). These froglets had similar body size (two individuals collected

Table 2. Female *Barbourula busuangensis* captured in Busuanga (province of Palawan, Philippines), showing differences in their gravid condition during subsequent recapture events; dates in order of collection.

Females	Date (y/m/d)	Capture times	Gravid	Season
#1	2022-05-05	Marked	No	Transition
	2022-06-05	Recapture 1	Yes	Wet
	2023-04-20	Recapture 2	No	Dry
#2	2022-05-05	Marked	Yes	Transition
	2022-06-05	Recapture 1	Yes	Wet
#3	2022-06-04	Marked	Yes	Wet
	2022-06-05	Recapture 1	Yes	Wet
	2022-11-18	Recapture 2	No	Wet
	2023-04-20	Recapture 3	No	Dry
#4	2022-05-05	Marked	Yes	Transition
	2022-07-06	Recapture 1	Yes	Wet

Figure 4. Habitat of *Barbourula busuangensis* in San Rafael (New Busuanga, Palawan), showing a rock wall forming a small waterfall with a man-made concrete dam-like structure. The red circle indicates the exact location of both crevices where tadpoles were found. Photo by MM.

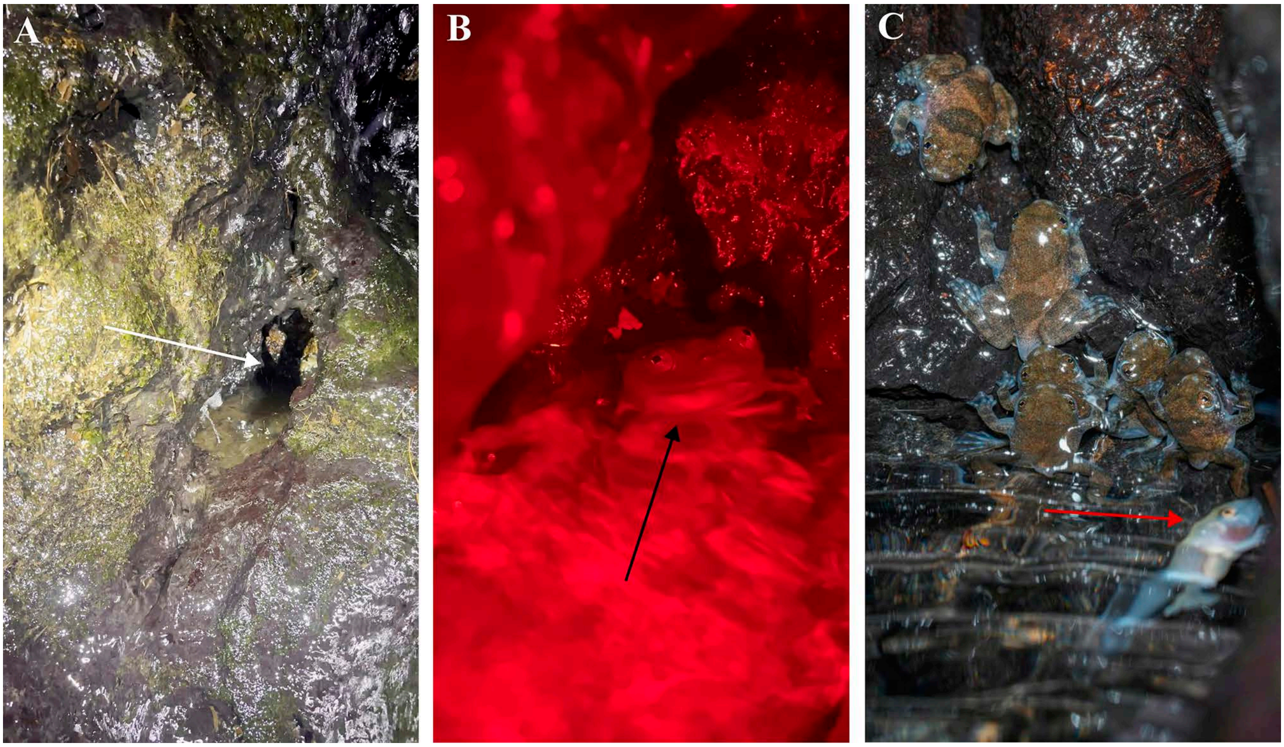

Figure 5. Nest of *Barbourula busuangensis* tadpoles found in November 2022 in San Rafael (New Busuanga, Palawan). (A) Entrance of the crevice showed by a white arrow, (B) adult *B. busuangensis* (black arrow) observed guarding the nest of tadpoles, and (C) metamorphs grouped together in the same developmental stage, except one tadpole (red arrow). Photos A and B by MM, photo C by Javier Aznar.

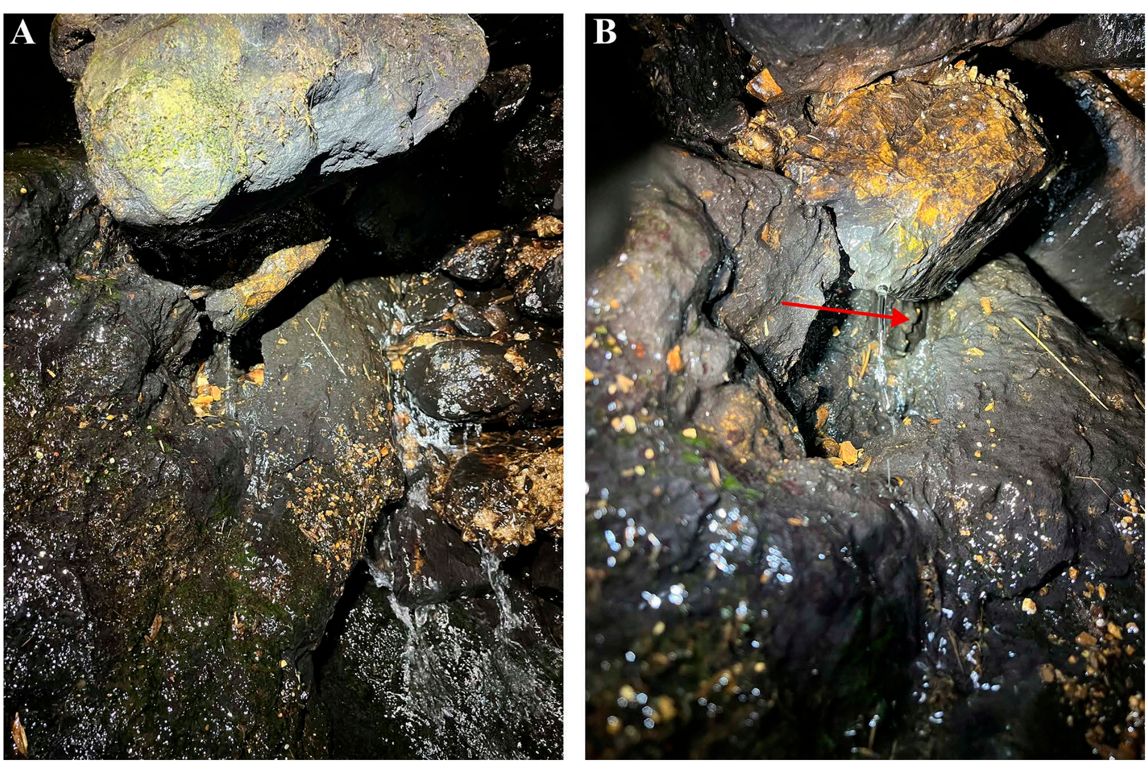

Figure 6. Nest of *Barbourula busuangensis* tadpoles found in a rock wall at San Rafael (New Busuanga, Palawan) in April 2023. (A) Exterior part of the rock wall and (B) entrance of the crevice showed by a red arrow, where the endoscope camera was introduced. Photos by MM.

and immediately released measured 10.3 mm and 13.7 mm SVL) and were at approximately the same developmental stage (Gosner stage 45), showing only the remains of the almost reabsorbed tail. The exception was one single individual showing less pigmentation and a long tail (Fig. 5C, red arrow), which was still submerged in the water and adhered to the rock wall. This individual, at Gosner stage 40–43 was collected to be described and photographed, and then released at the same point three days later.

We encountered a second nest on 18 April 2023 inside a different rock crevice within the same rock wall (Fig. 6). The exterior opening of this crevice was 6.5 cm in height and 7 cm in width, and there was a hole only 2 cm in diameter through which the endoscope camera was introduced (Fig. 6B, red arrow); the endoscope had been introduced about 38 cm when it reached a chamber where an adult *B. busuangensis* was found. This frog may have been defending the nest as it appeared to "attack" with abrupt movements the endoscope when it entered the chamber (Video 2). A group of 20–25 tadpoles at the same developmental stage (approximately Gosner stage 36) was found behind the adult, fully submerged in water and adhered to the rock wall. One tadpole from this group was collected (see Materials and methods). Eighteen days later, the tadpole completed its development and was released at the capture location, as a young metamorph. At the time we observed other smaller metamorphs near the entrance of the rock crevice, suggesting that their development had taken place in a similar time frame.

Description of the tadpole and development to metamorphosis

The larva belonging to the second nest found in April 2023, collected at Gosner stage 36, developed in captivity over 18 days until reaching stage 45.

Description at stage 36 (day 1) (see Table 3, first column; Fig. 7): Total length 29.4 mm; body length 11.7 mm; body wider than high (BW > BH), rounded and globular in dorsal view, depressed in lateral view. Snout rounded in dorsal view and truncated in lateral view. Large sucker-like oral disc, ellipsoidal in shape, measuring 2.52 mm in length and 3.84 mm in width; anterior margin of the oral disc surpassing the snout in dorsal view when adhered to a surface; marginal papillae present along the whole margin; keratinized mouthparts (rostrodonts, queratodonts) absent. Eyes directed dorsolaterally, with an interorbital distance of 4.47 mm. Nostrils rounded, dorsally positioned, with an internostril distance of 1.72 mm. Intestinal tube coiling circular and centred, with abundant yolk inside (Figs 7 A–C). Spiracle not evident. Tail long (17.74 mm), comprising 60% of total length; maximum tail height 5.95 mm, dorsal fin slightly lower than ventral fin (1.68 mm and 1.99 mm respectively); dorsal fin origin at the anterior third of the tail, ventral fin origin at the body/tail junction; dorsal fin insertion angle low; tail's external margins slightly convex; tail tip rounded. Hindlimbs present, measuring 5.6 mm, with no visible plantar or subarticular tubercles; evident, complete webbing between the toes. Forelimbs not protruding outside the body wall, but an evident bud present under the skin.

Description at stage 43 (day 10) (see Table 3, second column; Fig. 7): The total length remained the same, but the tail length had decreased by 2.34 mm while the body length had increased 2.7 mm. The snout, eyes, and nostrils remained unchanged. The sucker-like oral disc decreased in both length and width (3.24 mm and 6.89 mm respectively), becoming more oval; the marginal papillae were still present, while keratinized mouthparts remained absent (Fig. 7F). The intestinal tube became much less coiled, less visible, and the yolk was almost consumed. The hind limbs had experienced a major change, more than doubling their length (14.64 mm), and the forelimbs had emerged from the brachial chamber, measuring a total of 6.09 mm in length and showing extensive webbing between the fingers.

Table 3. Measurements (in mm) at each Gosner (1960) development stage of the tadpole of *Barbourula busuangensis*. Abbreviations: BH = body height, BL = body length, BW = body width, DFH = dorsal fin height, ED = eye diameter, F = femur length, FL = fore limb length, H = humerus length, HL = hind limb length, IND = internostril distance, IOD = interorbital distance, MT = metatarsals (longest finger), MC = metacarpals (longest finger), MTH = maximum tail height, ODL = oral disc length, ODW = oral disc width, RU = radioulna length, TL = tip of snout to tip of tail, T = tibia length, TAL = tail length, VFH = Ventral fin height. A "–" indicates no information on that measurement.

	Gosner stage		
Measurements	36	43	45
TL	29.4	29.8	13.17
BL	11.7	14.4	12.2
TAL	17.74	15.4	0.97
ODL	2.52	3.24	–
ODW	3.84	6.89	–
BH	5.6	–	–
BW	6.1	5.62	6.2
DFH	1.68	–	–
VFH	1.99	–	–
MTH	5.95	–	–
ED	1.37	1.49	1.44
IOD	4.47	4.44	3.86
IND	1.72	1.43	1.18
HL	5.6	14.64	14,04
F	2.24	4.73	4.23
T	2.23	3.8	5.03
MT	3.14	6.11	4.78
FL	–	6.09	5.89
H	–	1.72	1.63
RU	–	1.26	1.62
MC	–	3.11	2.64

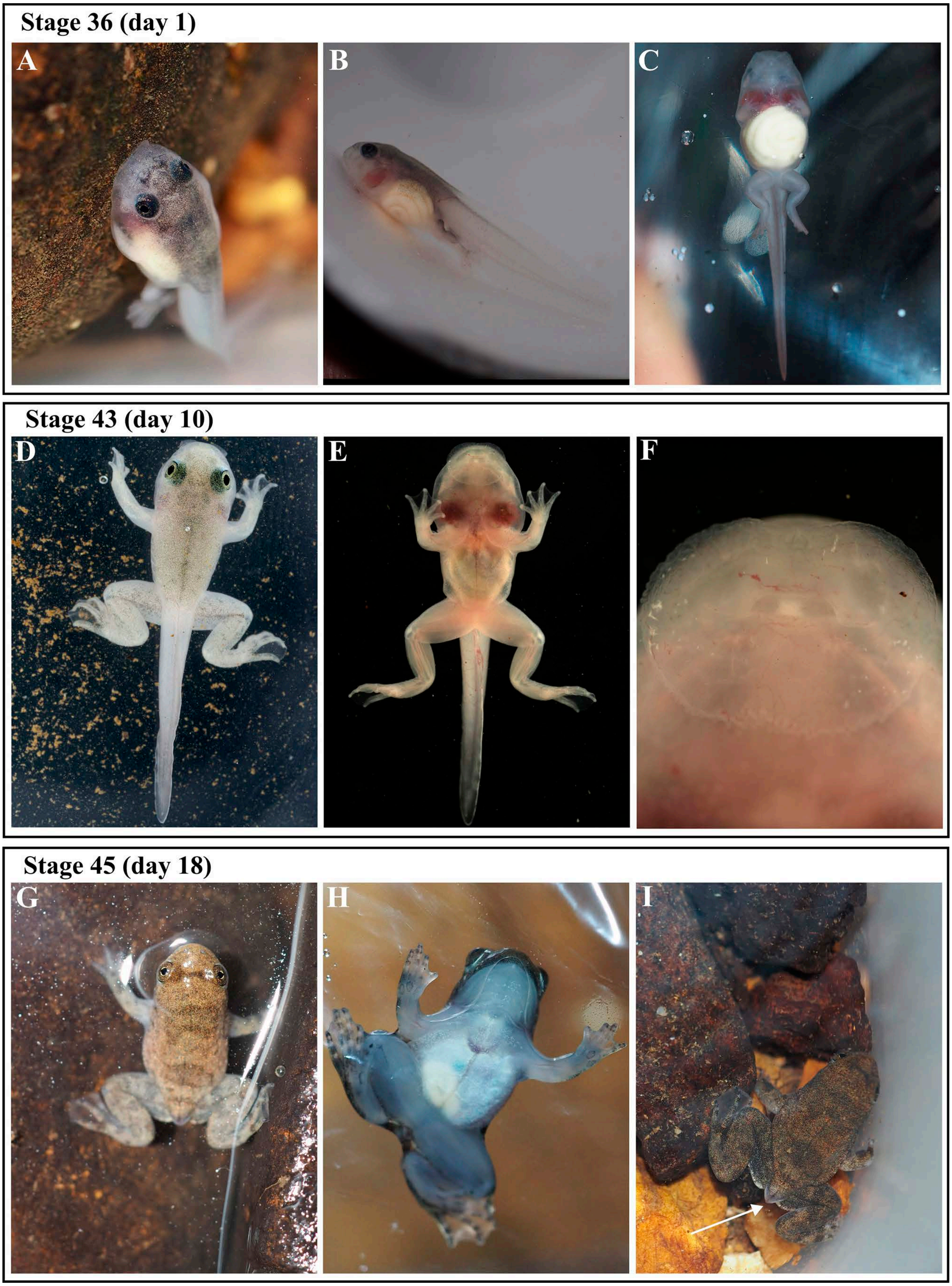

Figure 7. *Barbourula busuangensis* tadpole at different Gosner stages. Tadpole at stage 36 in (A) dorsal, (B) lateral, and (C) ventral view; stage 43 in (D) dorsal and (E) ventral view; (F) close-up of the large sucker-like oral disc at stage 43; stage 45 in (G) dorsal and (H) ventral view; (I) detail of the tail (white arrow) as a reduced triangular stub at the posterior end of the body at stage 45. Photos A, B, C, G, H and I by MM, photos D, E, F by Javier Aznar.

The coloration of the tadpole was identical for these two stages, contrasting between the venter and the dorsum (Fig. 7). Dorsally, the background of the body was whitish, nearly translucent, covered with scattered golden and grey chromatophores and irregular darker marbled spots; this pattern ranged from the snout to the base of the tail, including the dorsal part of the hindlimbs, and slightly fading away latero-ventrally. The tail was scarcely pigmented, with finely reticulated melanophores and a longitudinal short darker stripe. The iris had a pattern similar to the dorsal pigmentation, with a black background with densely speckled golden flecks and a golden pupil ring. The ventral region was smooth and only the abdominal region had scarce scattered golden specks, while the rest remained unpigmented, transparent, making the internal organs visible, with the coiled intestinal tube cream.

Description at stage 45 (day 18) (see Table 3, third column; Fig. 7): The individual resembled an adult in external morphology. The total length had decreased noticeably to 13.17 mm, remaining depressed, with the body wider than high (BW > BH) and appearing rounded in dorsal view. At this stage, the body length comprised most of the total length (93%), as the tail had been reduced to a triangular knob at the posterior body end (Fig. 7I). The snout became more oval and remained truncated in lateral view. The oral disc had completely disappeared, making way for a fully developed buccal cavity. The eyes were positioned laterally and markedly dorsal, with an interorbital distance slightly smaller than in previous stages (3.86 mm). The nostrils remained dorsally positioned and rounded in shape. The intestinal tube coiling was less evident. The hind- and forelimbs remained similar in size to those in stage 43 (14.04 mm and 5.89 mm respectively), both with extensive webbing. However, the hand now showed the first traces of palmar tubercles, and the foot showed an inner metatarsal tubercle.

The dorsal colour pattern of the metamorph was drastically more intense than that in previous stages, starting to resemble a typical juvenile *B. busuangensis* (Fig. 7G). The background of the body was dark brown, covered with scattered chromatophores with golden specks and characteristic darker, broad transversal stripes ranging from the interorbital area to the anterior part of the tail stub. This coloration extended all the way into the hind- and forelimbs. The coloration of the iris remained unchanged with respect to previous stages. The ventral region remained smooth, partially unpigmented, being the internal organs still visible, with scattered whitish specks only on the gular, chest and belly regions. The dorsal region of the hind and fore limbs also had darker specks, forming contrasting bands on the fingers and toes, as well as highlighting the palmar and metatarsal tubercles.

Discussion

This work unravels the one-century-old mystery of the reproductive mode of *Barbourula busuangensis*. Contrary to the original suspicion that it lays direct developing eggs, we discovered it has an aquatic larval stage with endotrophic tadpoles, as indicated by the absence of keratinized mouth parts, otherwise involved in active feeding, and the abundant yolk in their intestine. These tadpoles obtain all their developmental energy entirely from the yolk (as observed in our captive tadpole until it became a metamorph), and are nidicolous (sensu Altig & Johnston 1989), remaining in a nest with running water until metamorphosis. Endotrophic larvae are not common in anurans; our results represent a novel case to be added to the 226 cases so far reported among the 41% of frog species for which larvae are known (Vera Candioti et al. 2024). Females lay rather small clutches of large, unpigmented eggs, and one of the parents (either the male or the female, but we could not ascertain which one) remains with the tadpoles (and, presumably, with the eggs) during their development.

We found that the ovarian size factor (OSF) of *B. busuangensis* is low, with a mean value of 6.56. This indicates that the egg clutch size relative to the female's size is small, resulting in a lower number of eggs than might be expected in other frogs of similar proportions, thus leaning towards a K-reproductive strategy, characterized by fewer offspring and greater parental investment (Summers et al. 2006). Many anurans have remarkably lower OSF; examples are species within the family Dendrobatidae, such as *Ameerega picta* (Tschudi, 1838), which exhibits values ranging from 0.66 to 1.77 (Crump 1974), or members of Leptodactylidae like *Adenomera andreae* (Müller, 1923) and *A. hylaedactyla* (Cope, 1868) with values of 0.98 and 1.48 respectively (Crump 1974, De la Riva 1995). In the other extreme are typical "r" strategists such as large species of bufonids, with OSF values above 100 (Duellman 1978). The OSF values of *B. busuangensis* are similar to those found in other species with or without parental care and moderately low number of ovarian eggs, like, for example, *Dendropsophus bokermanni* (Goin, 1960), *Scarthyla goinorum* (Bokermann, 1962) and *Leptodactylus rhodonotus* (Günther, 1869) with OSF of 6.58, 6.54, 6.79, respectively (Duellman 2005); however, the low number of gravid females examined by us and the great variation observed in their respective OSF make these comparisons scarcely relevant.

Gravid females of *B. busuangensis* captured during field work were found across dry, transition, and wet seasons, from January to November. Thus, it appears that the species may reproduce all year-round (although it is important to note that field work was not conducted in August and September). Several females marked with pit-tags were recaptured showing changes in their reproductive condition. The gravid female marked on 5 May 2022 and recaptured two months later, on 6 July, still gravid, suggests that *B. busuangensis* can potentially retain their mature eggs for at least two months.

Unfortunately, we did not observe amplexus or other type of mating behaviour. The fact that there are no external structures present in the species, such as those found in the torrent genus *Ascaphus* Stejneger, 1899, where sperm transfer is done via an erectile intromittent organ adapted for internal fertilization (Stephenson & Verrel 2003), suggests that external fertilization is most likely present in *B. busuangensis*. Due to the lack of external sexual dimorphism in this species (Bosch et al. 2023), we were unable to confirm the sex of the adult providing parental care. Another important information that is missing from our work, is that we never observed egg clutches and thus, we do not know the oviposition substratum used by *B. busuangensis*, which is one of the key characters used to define reproductive modes by Nunes de Almeida et al. (2021). In the case of *B. busuangensis*, potential oviposition substratum alternatives inside the observed nests, would be: (1) above the water on the rock walls, (2) underwater, adhered to the rock wall, or (3) underwater, on the sandy bottom of the chamber. Another attractive possibility is that eggs might be swallowed by an adult, as in the extinct species of the Australian genus *Rheobatrachus* Liem, 1973 (Myobatrachidae). Given that tadpoles attributable to *B. busuangensis* had never been observed, and considering the ecological and morphological similarities between *Barbourula* and *Rheobatrachus*, we cannot rule out gastric brooding as a plausible hypothesis, resulting from convergent evolution (although, if this were the case, the adult *B. busuangensis* would expel larvae instead of froglets).

Anurans living in extreme habitats, such as torrential streams, require specialized morphologies and behaviours. Examples of some adaptations to these environments include ultrasonic calls reported in two species of southeast Asian ranids, *Odorrana tormota* (Wu, 1977) and *Huia cavitympanum* (Boulenger, 1893) (Feng et al. 2006, Arch et al. 2009), foot flagging in *Staurois* spp. (Grafe et al. 2012), or rheophilous tadpoles showing suctoriality. The tadpoles of *B. busuangensis* were seen using their oral sucker to adhere to the rock walls of the nest and to the artificial containers (small aquaria and plastic bags) where they were kept or transported.

The evolution, development, and utilization of a sucker-like apparatus has been reported in several groups of diverse and unrelated anuran larvae. Anuran sucker-like apparatus could be categorized into three main distinct types. The first, and most common, is the oral sucker, which for example, occurs in tadpoles of North American Ascaphidae, some hylids from Australia, e.g., *Ranoidea nannotis* (Andersson, 1916), *Ranoidea rheocola* (Liem, 1974) and *Ranoidea dayi* (Günther, 1897), and the Neotropical region, e.g., *Hyloscirtus armatus* (Boulenger, 1902), *Ololygon ariadne* (Bokermann, 1967) and *Ololygon pombali* (Lourenço, Carvalho, Baêta, Pezzuti, & Leite, 2013), Asian Bufonidae of the genus *Ansonia* (*A. longidigita* Inger, 1960, *A. minuta* Inger, 1960 and *A. platysoma* Inger, 1960), African bufonids of the genus *Werneria* [*W. bambutensis* (Amiet, 1972) and *W. tandyi* (Amiet, 1972)], South African Heleophrynidae, and Western African Odontobatrachidae torrent frogs (see respectively Brown 1990, Cadle & Altig 1991, Haas & Richards 1998, Haas et al. 2009, Hirschfeld et al. 2012, Conradie & Conradie 2015, Pezzuti et al. 2016, Doumbia et al. 2018). The second type consist in an abdominal sucker or a large belly adhesive disc; it occurs in tadpoles of the Neotropical bufonid genus *Atelopus* Duméril & Bibron, 1841, and in the Asian torrent-dwelling ranid frogs of the genus *Amolops* Cope, 1865 and *Meristogenys maryatiae* Matsui, Shimada & Sudin, 2010 (see respectively Lötters 2001, Matsui et al. 2010, Nokhbatolfoghahai et al. 2020); likewise, in this same category would be included all species of *Cycloramphus* Tschudi, 1838 and some of *Thoropa* Cope, 1865 (Cycloramphidae), which are semiterrestrial and have an abdominal flap to adhere to the rocky substrate where they live (Dias et al. 2021). Finally, recorded for the gastromyzophorous tadpoles of the ranid frogs *Huia cavitympanum* and *Meristogenys jerboa* (Günther, 1872) from Borneo, is the presence of both an abdominal and an oral sucker apparatus (Gan et al. 2016).

One feature that has been considered important among tadpoles inhabiting fast flowing streams is the presence of keratinized structures in sucker-like oral discs. Specifically, the possession of a tooth row at the tip of the anterior labium and/or having many rows of labial teeth is important because these structures, besides being used for feeding, may be advantageous for aiding in the anchoring and grip of the adhesive disc (Hora 1930, 1934). However, in the case of *B. busuangensis* rheophilous tadpole, the most remarkable feature is the combination of a large sucker-like oral disc with the absence of labial tooth rows or any keratinized mouth parts, contrasting with all previously discussed examples; this represents a novel characteristic and a potential autapomorphy for the species (some Microhylid species lack keratinized mouth parts but are not torrent forms and lack a sucker-like oral disc; Altig & McDiarmid 1999). It is therefore hypothesized that suction is the sole adhesive mechanism for the sucker-like oral disc of the tadpole of *B. busuangensis*, and its only function, preventing the tadpole from being swept away by the fast-flowing waters. The non-feeding condition of *B. busuangensis*' tadpoles makes keratinized structures unnecessary, and likely represents an adaptation to the microenvironment in which they develop, where the absence of light and the fast-running water would not facilitate the presence of any nourishment. We observed tadpoles only inside rock crevices within a network of cave systems with running water. The whitish coloration of the larvae is concordant with the absence of exposure to light and suggests that their entire larval development occurs within these caves. This microhabitat provides a protected environment for development, perhaps making difficult the access of potential predators, such as freshwater prawns (*Macrobrachium* spp.) and crabs (*Insulamon* spp.). In addition to this, the parental care provided likely constitute another defensive barrier for the tadpoles, as it has been documented that adult *B. busuangensis* feed on those crabs (Afuang & Cielo 2010).

The reproductive mode of *B. busuangensis* does not match any of those described by Duellman & Trueb (1986), indicating that such definitions turned out to be too vague to embrace the new findings of complex reproductive modes in anurans. Furthermore, the observed reproduction of this species does not match any of the reproductive modes proposed by Nunes de Almeida et al. (2021). The closest fit is reproductive mode 14, namely: "non-froth eggs laid in subaquatic chamber in lotic water. Offspring with indirect development, lecithotrophic nutrition, exotrophic, without parental feeding, and larvae develop in lotic water". Remarkably, this definition does not include endotrophic larvae, an obviously pivotal difference regarding *B. busuangensis*. Mode 14 differs from mode 18 only in that the chamber, on sandy bottom under rocks, is constructed (by the male) in the latter. This reproductive mode (18) is shown by the torrent frogs of the genus *Hylodes* Fitzinger, 1826 (Hylodidae) from southeastern Brazil, which lay small clutches of unpigmented eggs (Haddad & Giaretta 1999, Haddad & Prado 2005, Narvaes & Rodrigues 2005, De Sá et al. 2015). Although modes 14 and 18 are exclusively attributed to *Hylodes* frogs (Nunes de Almeida et al. 2021), no species in this genus has ever been observed using unconstructed nests; thus, mode 14 may not exist (C. F. B. Haddad & L. F. Toledo pers. comm.). In any case, subaquatic chambers like those mentioned above have not been observed being used by *B. busuangensis*.

In summary, in spite of the lack of observations of some important features of the reproductive behaviour of *B. busuangensis*, namely type of amplexus and fertilization, exact oviposition site, and sex of the parent taking care of the offspring, we can still consider that the combination of the remaining features observed qualifies potentially as a new reproductive mode, characterized by the following set of traits (sensu Nunes de Almeida et al. 2021; some traits, marked with an asterisk, have not been observed directly, but we take the risk to assume that they are present in the species and no bizarre deviations from them do exist): (1a) oviparous; (2a) clutch deposited in the environment; (3b) no foam or bubbles; (4a) eggs aquatic*; (5b) eggs lotic; (6b) adopted nest, not constructed or modified by parents; (7i) clutch laid on rock walls of the chamber, either above or below water*; (8a) indirect embryonic development; (9a) embryo lecitotrophic; (10a) tadpole endotrophic; (11b) tadpole lotic.

The reproductive mode described is extremely contrasting with that of *Barbourula*'s sister taxon, *Bombina*, in which unattended eggs are deposited in lentic waters and hatch into feeding, exotrophic benthic larvae (Rafinska 1991, Di Cerbo & Biancardi 2010). Such a strong intergeneric dissimilarity is not extraordinary given the ancient divergence between both genera and the remarkable differences in their ecology and purported selective pressures. It is interesting that, despite their generalist reproductive mode, *Bombina* species show a great inter- and intraspecific plasticity regarding egg size and clutch size (Rafinska 1991, Kaplan & King 1997), which might suggest a great potential for evolutionary shifts in reproductive modes in Bombinatoridae. We speculate that the other species of *Barbourula, B. kalimantanensis*, whose life history remains unknown, will likely have the same reproductive mode as its Philippine congener, but this must be confirmed with appropriate observations.

Studying the life history and ecology of under-researched species, like *B. busuangensis*, is crucial for advancing our understanding of evolutionary novelties, behavioural mechanisms, and ecological roles. This is nowadays especially true for amphibians, which are living an unprecedent crisis due to anthropogenic factors, driving them to be considered the most threatened vertebrate class (IUCN 2022). In the era of remote sensing and automatic data capture, only through dedicated fieldwork we can achieve a full understanding of the intricacies of species' ecology and life history (see Crump 2024) and, hence, of the potential threats they could confront as humans continue to degrade the environment. Our study has significantly advanced the understanding of the reproductive traits of one of the most enigmatic and understudied anurans in the World. While we have partially resolved a century-old mystery, additional field research is still required to address the numerous intriguing, unanswered questions that emerged during this investigation.

Acknowledgements

Research of IDlR, MM, CL, and PAB in Palawan and Busuanga was financed by projects CGL2014-56160-P and PGC2018-097421-B-I00 (PI: I. De la Riva), funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe", by the European Union. Research by PAB was also partly supported by NSF-IOS-2011281. MM was supported by a Ph.D. fellowship of the Spanish Ministry of Science and Innovation. We are deeply grateful to the University of the Philippines at Los Baños and to the Palawan Council for Sustainable Development for providing permits to conduct fieldwork in Busuanga (Wildlife Gratuitous Permit Nº 2022-11). We are deeply thankful to Rafe Brown for the opportunity to examine and dissect valuable gravid females of *B. busuangensis* hold in the herpetological collection of the University of Kansas. We also thank A. Sánchez-Vialas, J. Goyes, M. Guilbaud, R. Cueva and T. Aledón for their help in the field when they visited MM in Busuanga and J. Aznar for kindly providing some photographs used in this study. We are indebted to B. Zobel de Ayala, at the time consul of Spain in Palawan, for her many and generous ways to help us. We are especially grateful to Kuya Lito and Kuya Archie, our regular field assistants at Malbato and San Rafael respectively. Finally, we must acknowledge Manny Reyes and Tunggay Reyes (King Fisher Park) for facilitating our living quarters and boarding in Malbato.

References

Afuang, L. & K. L. S. Cielo (2010): Biology and conservation of the Philippine Flat-headed frog (*Barbourula busuangensis*) in the Calamian Islands. – Unpublished report.

Alcala A. C. & W. C. Brown (1987): Notes on the microhabitat of the Philippine discoglossid frog *Barbourula busuangensis*. – Silliman Journal, **34**: 12–17.

Altig, R., & G. F. Johnston (1989): Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. – Herpetological Monographs, **3**: 81–109.

Altig, R. & R. W. McDiarmid (1999): Body plan – development and morphology in tadpoles. – pp. 24–51 in: McDiarmid, R. W. & R. Altig (eds): Tadpoles: the biology of anuran larvae. – The University of Chicago Press, Chicago, Illinois.

Arch, V. S., T. U. Grafe, M. Gridi-Papp & P. M. Narins (2009): Pure ultrasonic communication in an endemic Bornean frog. – PLoS One, **4**: e5413.

Bhaduri, J. L. & A. Mondal (1965): A study of the urogenital system of Philippine Salientia, together with notes on *Cornufer novae-britannae* from New Guinea and a north Bornean *Rhacophorus otilopus*. – The Philippine Journal of Science, **94**: 469–517.

Bickford, D., D. Iskandar & A. Barlian (2008): A lungless frog discovered on Borneo. – Current Biology, **18**: R374–R375.

Blackburn, D. C., D. Bickford, A. C. Diesmos, D. T. Iskandar & R. Brown (2010): An ancient origin for the enigmatic flat-headed frogs (Bombinatoridae: *Barbourula*) from the islands of southeast Asia. – PLoS One, **5**: e12090.

Blackburn, D. C., J.A. Gray & E. L. Stanley (2024): The only "lungless" frog has a glottis and lungs. – Current Biology, **34**: R492–R493.

Bosch, J., L. Afuang, M. Miñarro, C. Lansac, R. Márquez, R. Brown & I. De la Riva (2023): The calls of the poorly known Philippine Flat-headed frog, *Barbourula busuangensis* (Anura: Bombinatoridae). – Salamandra, **59**: 297–303.

Boulenger, G. A. (1886): Remarks in connection with the preceding note. – The Annals and Magazine of Natural History, **17**: 463–464.

Breder Jr. C. M. & D. E. Rosen (1966): Modes of reproduction in fishes. – Natural History Press, New York.

Brown, H. A. (1990): Morphological variation and age-class determination in overwintering tadpoles of the tailed frog, *Ascaphus truei*. – Journal of Zoology, **220**: 171–184.

Brown, W. C. & A. C. Alcala (1983): Modes of reproduction of Philippine anurans. – pp. 416–428 in: Rhodin, G. J. & K. Miyata (eds): Advances in Herpetology and Evolutionary Biology. – Museum of Comparative Zoology, Cambridge, Massachusetts.

Cadle, J. E. & R. Altig (1991): Two lotic tadpoles from the Andes of Southern Peru: *Hyla armata* and *Bufo veraguensis*, with notes on the call of *Hyla armata* (Amphibia: Anura: Hylidae and Bufonidae). – Studies on Neotropical Fauna and Environment, **26**: 45–53.

Clarke, B. T. (1987): A description of the skeletal morphology of *Barbourula* (Anura: Discoglossidae), with comments on its relationships. – Journal of Natural History, **21**: 879–891.

Conradie, W. & C. Conradie (2015): Correlation between development and increase of number of labial tooth rows in Ghost Frog tadpoles (Anura: Heleophrynidae). – Acta Herpetologica, **10**: 143–148.

Crump, M. L. (1974): Reproductive strategies in tropical anuran communities. –Miscellaneous Publication, University of Kansas Museum of Natural History, **61**: 1–68.

Crump, M. L. (ed.) (2024): Lost Frogs & Hot Snakes. Herpetologists' Tales from the Field. – Cornell University Press, Ithaca, New York.

De la Riva, I. (1995): A new reproductive mode for the genus *Adenomera* (Amphibia: Anura: Leptodactylidae): taxonomic implications for certain Bolivian and Paraguayan populations. – Studies on Neotropical Fauna and Environment, **30**: 15–29.

de Sá, F. P., C. Canedo, M. L. Lyra & C. F. B. Haddad (2015): A new species of *Hylodes* (Anura, Hylodidae) and its secretive underwater breeding behavior. – Herpetologica, **71**: 58–71.

Di Cerbo, A. R. & C. M. Biancardi (2010): Morphometric study on tadpoles of *Bombina variegata* (Linnaeus, 1758) (Anura; Bombinatoridae). – Acta Herpetologica, **5**: 223–231.

Dias, P. H., F. Vera Candioti, A. F. Sabbag, G. Colaço, H. R. da Silva, C. F. B. Haddad, A. M. P. Carvalho-e-Silva & T. Grant (2021): Life on the edge: tadpoles of Cycloramphidae (Amphibia; Anura), anatomy, systematics, functional morphology, and comments on the evolution of semiterrestrial tadpoles. – Journal of Zoological Systematics and Evolutionary Research, **59**: 1297–1321.

Doumbia, J., L. Sandberger-Loua, A. Schulze & M.-O. Rödel (2018): The tadpoles of all five species of the West African frog family *Odontobatrachidae* (Amphibia, Anura). – Alytes, **36**: 63–92.

Duellman, W. E. (1978): The biology of an equatorial herpetofauna in Amazonian Ecuador. – Miscellaneous Publications, Museum of Natural History, The University of Kansas, **65**: 1–352.

Duellman, W. E. (2005): Cusco Amazónico: the lives of amphibians and reptiles in an Amazonian rainforest. – Cornell University Press, Ithaca, New York.

Duellman, W. E. & M. L. Crump (1974): Speciation in frogs of the *Hyla parviceps* group in the upper Amazon Basin. – Occasional Papers of the Museum of Natural History, The University of Kansas, **23**: 1–40.

Duellman, W. E. & E. Lehr (2009): Terrestrial-breeding frogs (Strabomantidae) in Peru. – Natur und Tier Verlag, Münster.

Duellman, W. E. & L. Trueb (1986): Biology of Amphibians. – McGraw-Hill Book Co., New York.

Feng, A. S., P. M. Narins, C.-H. Xu, W.-Y. Lin, Z.-L. Yu, Q. Qiu, Z.-M. Xu & J.-X. Shen (2006): Ultrasonic communication in frogs. – Nature, **440**: 333–336.

Flores, G. M. A., A. B. A. Flores, R. J. I. B. Mabitasan, J. M. F. Lopus & L. J. Paguntalan (2024): Abundance and microhabitat association of *Barbourula busuangensis* (Anura: Bombinatoridae) in Busuanga Island, Philippines. – Phyllomedusa, **23**: 65–78.

Frost, D. R. (2024): Amphibian species of the World: An Online Reference. Version 6.1 (3). – American Museum of Natural History, New York, USA. Electronic database https://amphibiansoftheworld.amnh.org/index.php, accessed 16 October 2024.

Gan, L. L., S. T. Hertwig, I. Das & A. Haas (2016): The anatomy and structural connectivity of the abdominal sucker in the tadpoles of *Huia cavitympanum*, with comparisons to *Meristogenys jerboa* (Lissamphibia: Anura: Ranidae). – Journal of Zoological Systematics and Evolutionary Research, **54**: 46–59.

Gosner, K. L. (1960): A simplified table staging anuran embryo and larvae with notes on identification. – Herpetologica, **16**: 183–190.

Grafe, T. U., D. Preininger, M. Sztatecsny, R. Kasah, J. M. Dehling, S. Proksch & W. Hödl (2012): Multimodal communication in a noisy environment: a case study of the Bornean rock frog *Staurois parvus*. – PLoS One, **7**: e37965.

Haas, A. & S. J. Richards (1998): Correlations of cranial morphology, ecology, and evolution in Australian suctorial tadpoles of the genera *Litoria* and *Nyctimystes* (Amphibia: Anura: Hylidae: Pelodryadinae). – Journal of Morphology, **238**: 109–141.

Haas, A., J. Wolter, S. T. Hertwig & I. Das (2009): Larval morphologies of three species of stream toads, genus *Ansonia* (Amphibia: Bufonidae) from East Malaysia (Borneo), with a key to known Bornean *Ansonia* tadpoles. – Zootaxa, **2302**: 21–18.

Haddad, C. F. B. & A. A. Giaretta (1999): Visual and acoustic communication in the Brazilian torrent frog, *Hylodes asper* (Anura, Leptodactylidae). – Herpetologica, **55**: 324–333.

Haddad, C. F. B. & C. P. A. Prado (2005): Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. – BioScience, **55**: 207–217.

Hernandez, A. & G. Espallargas (2020): *Bombina maxima* (Boulenger, 1905): observations of the breeding habitat and reproductive behavior in north-western Yunnan province, China. – Bulletin de la Société Herpétologique de France, **173**: 57–60.

Hirschfeld, M., M. F. Barej, S. P. Loader & M.-O. Rödel (2012): Description of two *Werneria* tadpoles from Cameroon (Amphibia: Anura: Bufonidae). – Zootaxa, **3172**: 65–68.

Hora, S. L. (1930): Ecology, bionomics and evolution of the torrential fauna. – Philosophical Transactions of the Royal Society, London B, **218**: 172–281.

Hora, S. L. (1934): Further observations on the bionomics of the tadpoles of *Rana afghana* Günther. – Records of the Indian Museum, **36**: 321–325.

Infante, C. R., R. M. Brown, A. C. Diesmos, G. Gee & J. Dimalibot (2002): The search for the lost treasure of Palawan: the Philippine flat-headed frog. – Haring Ibon, 1st quarter, **2002**: 12–15.

Inger, R. F. (1954): Systematics and zoogeography of Philippine amphibia. – Fieldiana Zoology, **33**: 181–531.

IUCN (2022): The IUCN Red List of Threatened Species. Version 2022–2. – International Union for Conservation of Nature. http://www.iucnredlist.org

Kaplan, R. H. & E. G. King (1997): Egg size is a developmentally plastic trait: evidence from long term studies in the frog *Bombina orientalis*. – Herpetologica, **53**: 149–165.

Lamotte, M. & J. Lescure (1977): Tendances adaptatives l'affranchissement du milieu aquatique chez les amphibiens anoures. – Terre et Vie, **30**: 225–312.

Liedtke, H. C., J. J. Wiens & I. Gómez-Mestre (2022): The evolution of reproductive modes and life cycles in amphibians. – Nature Communications, **13**: 7039.

Lötters, S. (2001): Tadpole of *Atelopus mindoensis* Peters (Anura, Bufonidae) from northwestern Ecuador. – Copeia, **2001**: 276–278.

MacArthur, R. & E. O. Wilson (1967): The Theory of Island Biogeography. – Princeton University Press, Princeton, New Jersey.

Matsui, M., T. Shimada & A. Sudin (2010): A new species of *Meristogenys* (Amphibia, Anura, Ranidae) from Sabah, Borneo. – Zoological Science, **27**: 61–66.

Narvaes, P. & M. T. Rodrigues (2005): Visual communication, reproductive behavior, and home range of *Hylodes dactylocinus* (Anura, Leptodactylidae). – Phyllomedusa, **4**: 147–158.

Nokhbatolfoghahai, M., K. W. Conway, L. Atherton, P. B. Budha, M. J. Jowers & J. R. Downie (2020): Larval description and developmental staging of *Amolops* tadpoles from Nepal, including ultrastructure of the oral disc and sucker. – Salamandra, **56**: 317–328.

Nunes de Almeida, C. H. L., C. F. B. Haddad & L. F. Toledo (2021): A revised classification of the amphibian reproductive modes. – Salamandra, **57**: 413–427.

Pezzuti, T. L., I. R. Fernandes, F. S. F. Leite, C. E. De Sousa, P. C. A. Garcia & D. Rossa-Feres (2016): The tadpoles of the neotropical *Scinax catharinae* group (Anura, Hylidae): ecomorphology and descriptions of two new forms. – Zoologischer Anzeiger, **261**: 22–32.

Pezzuti, T. L., F. S. F. Leite, D. D. C. Rossa-Feres & P. C. A. Garcia (2021): The tadpoles of the Iron Quadrangle, Southeastern Brazil: a baseline for larval knowledge and anuran conservation in a diverse and threatened region. – South American Journal of Herpetology, **22**: 1–107.

Přikryl, T., P. Aerts, P. Havelková, A. Herrelv & Z. Roček (2009): Pelvic and thigh musculature in frogs (Anura) and origin of anuran jumping locomotion. – Journal of Anatomy, **214**: 100–139.

Rachmayuningtyas, B. A., D. P. Bickford, M. Kamsi, S. N. Kutty, R. Meier, U. Arifin, A. Rachmansah & D. T. Iskandar (2011): Conservation status of the only lungless frog *Barbourula kalimantanensis* Iskandar, 1978 (Amphibia: Anura: Bombinatoridae). – Journal of Threatened Taxa, **3**: 1981–1989.

Rafińska, A. (1991): Reproductive biology of the fire-bellied toads, *Bombina bombina* and *B. variegata* (Anura: Discoglossidae): egg size, clutch size and larval period length differences. – Biological Journal of the Linnean Society, **43**: 197–210.

Roček, Z., N. Baleeva, A. Vazeille, A. Bravin, E. van Dijk, C. Nemoz, T. Prikryl, E. M. Smirina, R. Boistel & L. Claessens (2016): Contribution to the head anatomy of the basal frog *Barbourula busuangensis* and the evolution of the Anura. – Russian Journal of Herpetology, **23**: 163–194.

Salthe, S. N. & W. E. Duellman (1973): Quantitative constraints associated with reproductive mode in anurans. – pp. 229–249 in: Vial, J. L. (ed.): Evolutionary biology of the anurans. – University of Missouri Press, Columbia.

Salthe, S. N. & J. S. Mecham (1974): Reproductive and courtship patterns. – pp. 309–521 in: Lofts, B. (ed.): Physiology of the amphibians, vol. II. – Academic Press, New York.

Schneider C. A., W. S. Rasband & K. W. Eliceiri (2012): NIH Image to ImageJ: 25 Years of Image Analysis. – Nature Methods, **9**: 671–675.

Stephenson, B. & P. Verrell (2003). Courtship and mating of the tailed frog (*Ascaphus truei*). – Journal of Zoology, **259**: 15–22.

Summers K., C. S. McKeon, H. E. Heying, J. Hall & W. Patrick (2006): Social and environmental influences on egg size evolution in frogs. – Journal of Zoology, **271**: 225–32.

Taylor, E. H. & G. K. Noble (1924): A new genus of discoglossid frogs from the Philippine Islands. – American Museum Novitates, **121**: 1–4.

Vera Candioti, F., D. Baldo, S. Grosjean, M. O. Pereyra & J. Nori (2023): Global shortfalls of knowledge on anuran tadpoles. – npj Biodiversity, **2**: 22.

Supplementary data

The following data are available online:

Supplementary Video 1. The first nest of *Barbourula busuangensis* tadpoles, found in November 2022 in San Rafael (New Busuanga, Palawan). A group of metamorphs were found, all together and pressed against each other on the interface between the water and the rock walls of the crevice.

Supplementary Video 2. The second nest of *Barbourula busuangensis* tadpoles, found in April 2023 in San Rafael (New Busuanga, Palawan). A group of tadpoles with the same developmental stage (approximately Gosner 36) were found behind an adult *B. busuangensis*, fully submerged in water and adhered to the rock wall.