Zootaxa 3881 (2): 195–200
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3881.2.8
http://zoobank.org/urn:lsid:zoobank.org:pub:A1C1EDFA-7154-416E-AB9B-0C552A6823AA

The breeding behaviour, advertisement call and tadpole of *Limnonectes dabanus* (Anura: Dicroglossidae)

JODI J. L. ROWLEY[1,4], DUONG THI THUY LE[2], HUY DUC HOANG[2] & RONALD ALTIG[3]

[1]*Australian Museum Research Institute, Australian Museum, 6 College St, Sydney, NSW, 2010, Australia.*
E-mail: jodi.rowley@austmus.gov.au
[2]*University of Science-Ho Chi Minh City, Faculty of Biology, 227 Nguyen Van Cu, District 5, Ho Chi Minh City, Vietnam*
[3]*Department of Biological Sciences, Mississippi State University, Mississippi State, MS 39762 U.S.A.*
E-mail: altig@biology.msstate.edu
[4]*Corresponding author. E-mail: jodi.rowley@austmus.gov.au*

Abstract

Fanged frogs (*Limnonectes*) are a group of dicroglossid frogs from Asia that often have reversed sexual dimorphism with larger males. *Limnonectes dabanus* is a poorly known species of fanged frog from forested habitats in southern Vietnam and eastern Cambodia. Adult males exhibit an extreme degree of megacephaly and possess bizarre head ornamentation. *L. dabanus* breeds in shallow, non-flowing or very slow-flowing pools, puddles, and drainage ditches. Eggs are laid as a widely spaced array, and the larvae have a morphology typical of pond-dwelling tadpoles. Although males of the species lack vocal sacs, they produce a low-pitched (0.4–0.6 kHz), single-note advertisement call that sounds like a drop of water falling into water. Given the spacing of calling males, presence of multiple females near breeding sites, and reversed sexual dimorphism, the mating system of *L. dabanus* may be an example of resource-defense polygyny, and the massive head of the male is likely used in male combat.

Key words: acoustics, amphibian, larvae, reproductive behaviour, Southeast Asia

Introduction

Frogs in the genus *Limnonectes,* also known as fanged frogs, are a group of dicroglossid frogs from Asia. The 61 currently known species of fanged frogs are distributed from southeastern China, southern Japan, and the Philippines south to the Sundas and Timor (Frost 2014). Many of the species in the genus have conserved morphology, and new species are continuously being revealed, particularly from within species complexes previously thought to be a single species (e.g., McLeod *et al*. 2011, 2012). The common name of the genus is derived from the presence of odontoid processes, or "fangs" on the lower jaw of both sexes. Many fanged frogs also have reversed sexual dimorphism, with larger males (unusual in frogs) that have longer and wider heads than females and hypertrophied jaw muscles (Inger 1966). Males often lack vocal sacs (Boulenger 1920; Inger 1954, 1966; Smith 1922).

Despite an awareness of the surprising number of breeding modes that have been reported for this genus—including viviparity (Iskandar & Tjan 1996), nest attendance (Brown and Iskandar 2000), and nidicolous development in nests (Rowley & Altig 2012)—details of breeding biology are lacking for most species. Additionally, previous studies have reported on male territoriality and combat in closely related congeners (Emerson 1992; Orlov 1997; Tsuji & Lue 1998).

Limnonectes dabanus (Smith 1922) is a charismatic and poorly known species known only from forested habitats on the Langbian Plateau, Lam Dong Province, Vietnam, and tributaries of the Mekong and Krong No rivers in Cambodia and southern Vietnam, below 900 m elevation (Frost 2014). Males have among the most extreme degree of megacephaly in the genus, with the head making up nearly half the body length. In addition, they are one of four species in which adult males have head ornamentation; a swollen 'cap', or caruncle on the top of

Accepted by M. Vences: 13 Oct. 2014; published: 4 Nov. 2014

their heads (Lambertz *et al*. 2014, Fig. 1A). Like other frogs in this group, the species also has prominent odontoids on their lower jaw. We provide information on breeding behaviour of the species, including advertisement call, eggs and larvae.

Material and methods

During fieldwork at Nui Ong Nature Reserve, Binh Thuan Province, Vietnam, we observed the breeding habitat and behaviour of *Limnonectes dabanus*, recorded the advertisement call and collected eggs, embryos and tadpoles. Advertisement call descriptions are based on the call of AMS R 173235 that was collected at the reserve (11.0175º N, 11.0966º E, 158 m elevation), and an un-vouchered adult male (107.7241ºN, 107.7524ºE, 720 m elevation). Advertisement calls were recorded with an Edirol R-09HR WAVE/MP3 Recorder (96 kHz sampling rate and 24-bit encoding) with a Røde NTG-2 condenser shotgun microphone. Calls were recorded at a distance of about 0.1–0.3 m, and ambient temperatures at the calling site were taken immediately after recordings with a Kestrel 3500 hand-held weather meter.

Calls were analysed with Raven Pro 1.3© software (http://www.birds.cornell.edu/raven). Audiospectrograms in the figures were calculated with fast-Fourier transform (FFT) of 512 points, 50% overlap and 172 Hz grid-spacing with Hanning windows. Temporal and spectral parameters of calls were measured with the definitions of Cocroft & Ryan (1995). We measured the call duration (ms), intercall interval (ms) and dominant frequency (kHz) for all calls recorded.

Eggs (AMS R 173227), embryos (AMS R 176218) and two tadpoles (AMS R 176542) of *Limnonectes dabanus* from a shallow, muddy pool on the forest floor in Nui Ong Nature Reserve were examined and photographed at 6–50 X. Staging followed the Gosner (1960) table, egg terminology is that of Altig and McDiarmid (2007), and tadpole terminology is that of Altig and McDiarmid (1999). Embryos and tadpoles were assigned to *L. dabanus* based upon mitochondrial 16S RNA sequence identity among tissue samples taken from an egg (AMS R 173227), embryo (AMS 176218), tadpole (AMS R 176542) and an adult female *L. dabanus* AMS R 173213 from the same location (531 bp; GenBank accession numbers KM516022–516024, KM875433).

Results

Breeding habitat. The breeding behaviour of *Limnonectes dabanus* was observed on several occasions. A calling adult male *L. dabanus* (AMS R 173212/UNS 0288; Fig. 1A) was observed with a female *L. dabanus* (AMS R 173213; Fig. 1B) in close proximity and another un-vouchered female nearby at 2230 h on 21 May 2009. These individuals were found near a rivulet in a seepy area beneath an overhanging rock in the forest, and eggs were scattered about the floor and ceiling of the overhang (Fig. 1B–C). The placement of at least 115 eggs is very difficult to understand. The eggs were arranged in a widely dispersed array on the rock ceiling (about 40 cm above the wet floor), on nearly vertical surfaces nearer the water, and on the floor of the area beneath the rock. There was only a small depression with standing water deeper than a few millimeters.

Two other male *Limnonectes dabanus* were observed calling at Nui Ong Nature Reserve. AMS R 173235 was calling from a 1 m wide, shallow (< 5 cm deep) drainage line in disturbed mixed evergreen and bamboo forest at 1945 h on 22 May 2009. The other, non-vouchered individual was at the base of a pandanus plant in a large, shallow (<30 cm deep) temporary pond in evergreen forest at 2120 h on 28 May 2009. The advertisement calls of both males recorded are described below. At all three instances where males were observed calling, there were no other males of the species heard or observed in the vicinity.

Two other species of *Limnonectes* were observed at Nui Ong Nature Reserve; *L. limborgi*, which has nidicolous larval development in terrestrial nests, and *L. poilani*, which were observed mainly along streams (tadpoles of *L. poilani* were collected from sandy pools within small streams; J. Rowley, pers. obs.). The only species that was observed sharing breeding sites with *L. dabanus* at Nui Ong Nature Reserve was *Rhacophorus annamensis* (eggs and tadpoles collected from the same pool as *L. dabanus* embryos and tadpoles).

Eggs. The eggs (AMS R 173227; Fig. 2A) in stage 14 with one jelly layer had a diameter of 5.7–6.1 mm and the embryos (AMS R 176218) with a darkly pigmented animal pole were 2.2–2.5 mm in diameter.

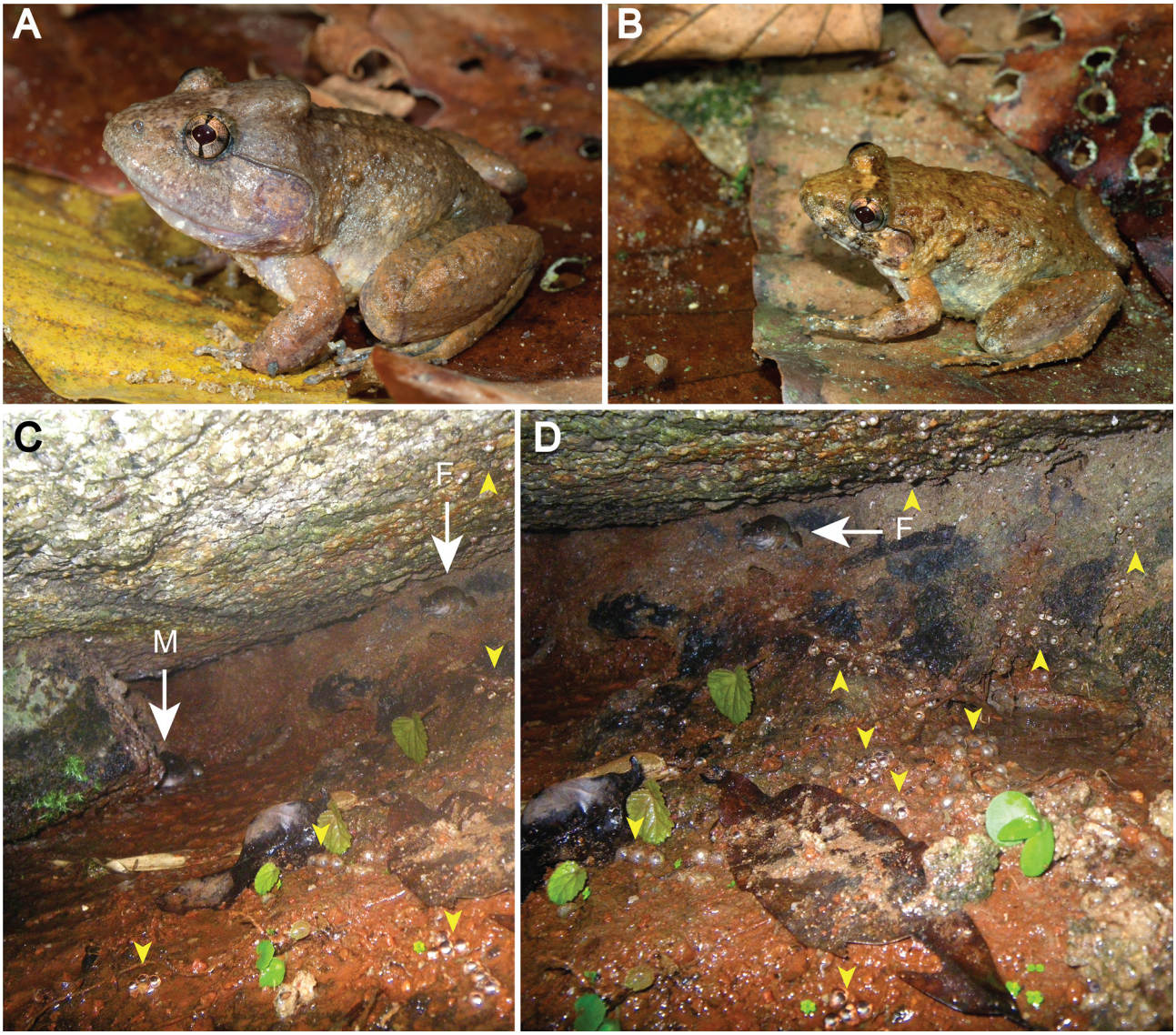

FIGURE 1. (A) Adult male *Limnonectes dabanus* (AMS R 173212/UNS0288) and (B) adult female *L. dabanus* (AMS R 173213). (C) Same adult male, M, and female, F, in seep area beneath an overhanging boulder with eggs on the ceiling (yellow up arrow) and floor (yellow down arrow). (D) Same adult female in same location, surrounded by eggs (yellow arrows).

Tadpole. Tadpoles in stages 29 and 31 (AMS R 176542) were collected from a muddy pool with leaf-litter on the forest floor. The stage 31 tadpole (tail tip damaged; Fig. 2B–D) is characterized as: 21.2 mm TL, 9.4 mm BL, Labial Tooth Row Formula 2(2)/3(1), with LTR P-3 about half the length of P-1-2, teeth rather coarsely spaced, upper jaw sheath medium wide with fine serrations, lower sheath V-shaped, oral disc emarginate with uniserial marginal papillae and a wide dorsal gap, eyes dorsal, vent dextral with a long tube, spiracle sinistral, and round nares large with a rimmed border. The coloration of the tadpole is typical for the genus: tail fins clear with random speckles and small, irregular blotches, body uniformly medium brown to slightly blotched. The depressed body and low tail fins are typical for the genus and probably represent adaptations for a benthic lifestyle in shallow water. The stage 29 tadpole (8.2 mm BL) was similar in all characteristics but was missing the tail (which was taken for molecular analysis).

Advertisement call. The calls of two adult male *Limnonectes dabanus* were recorded. Calls were between 141–197 ms in duration and consisted of a single note (Table 1, Fig. 3). Calls were highly amplitude modulated, with amplitude decreasing in the middle of the call and increasing toward the end and indistinctly pulsed. The dominant frequency (also the fundamental frequency) was 0.4–0.6 kHz, and harmonics were not evident. Calls were repeated relatively slowly, with intercall intervals of 3.8–14.0 s. To the human ear, the advertisement call of *L. dabanus* sounds similar to a large water drop falling into a pool of water.

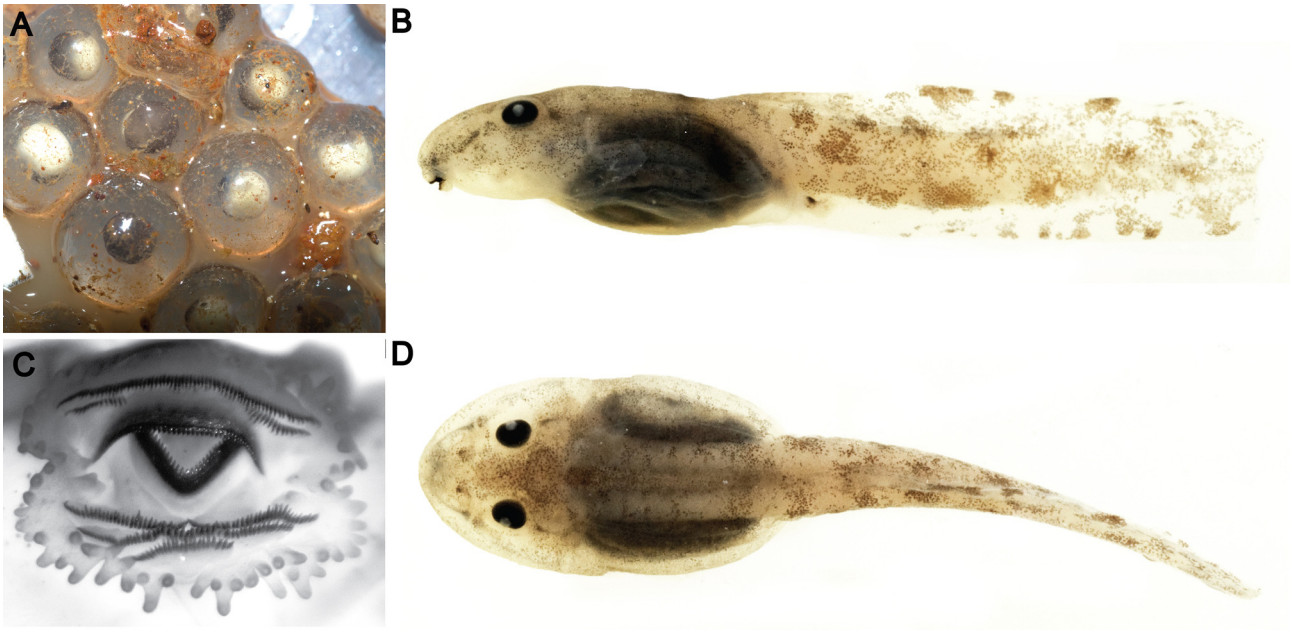

FIGURE 2. *Limnonectes dabanus* (A) eggs (AMS R 173227) grouped for photography, (B) tadpole in lateral view (AMS R 176542, stage 31, 21.2 mm TL, tail tip damaged). (C) Oral apparatus of same tadpole (stained with crystal violet to reveal translucent structures) and (D) dorsal view of same tadpole.

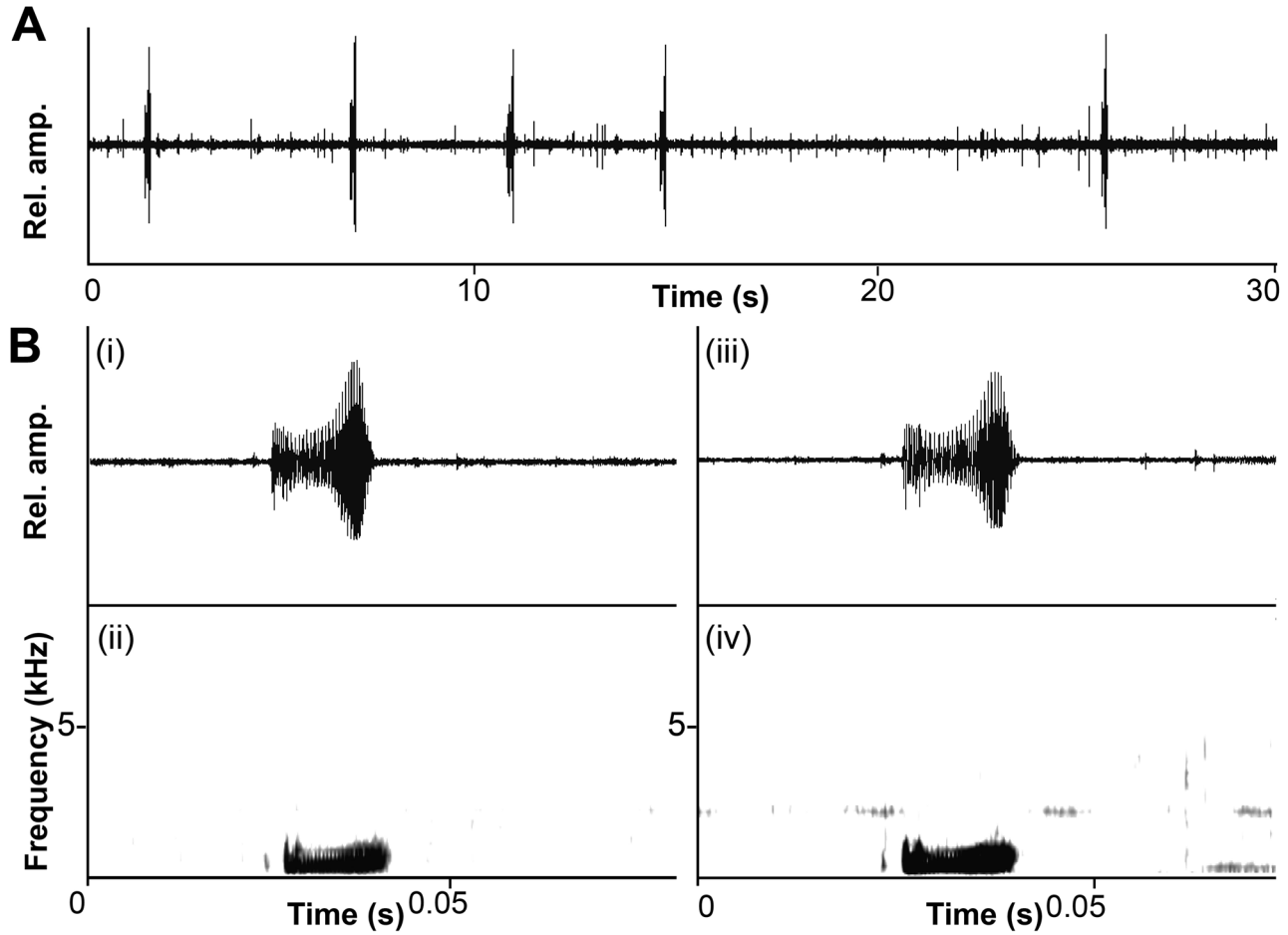

FIGURE 3. Advertisement call of *Limnonectes dabanus* AMS R 173235. (A) 30 s waveform of relative amplitude (Rel. amp.) over time for five calls, (B) (i, iii) waveform and (ii, iv) corresponding spectrogram of representative calls; all recorded at an ambient air temperature of 27.2º C.

TABLE 1. Measurements of advertisement call parameters for *Limnonectes dabanus.* Parameter values are given as means (and ranges).

	AMS R 173235	Non-vouchered
Dominant frequency kHz	0.56	0.38
Call duration (ms)	149 (141–156)	190 (182–197)
Intercall interval (s)	7.6 (3.8–14.0)	6.9 (5.5–8.5)
Temperature (ºC)	27.2	24.3

Discussion

Limnonectes dabanus breeds in shallow, non-flowing or slow-flowing pools, puddles, and drainage ditches. This breeding habitat is similar to the sympatric *L. kochangae* and some species in the *L.* cf. *kuhlii* complex (R. Altig & J. Rowley, pers. obs.). The advertisement calls of most *Limnonectes* species remain unknown, and some species have been reported to be voiceless (Emerson 1992). Although *L. dabanus* lacks vocal slits and a vocal sac (J. Rowley, pers. obs.), males produce an audible advertisement call. Because the call sounds like a water drop and has relatively low amplitude, it is possible that similar calls of related species have been overlooked.

At all three instances where males were observed calling, no other male *L. dabanus* were heard or observed in the vicinity. This, and the presence of two females near the first male (AMS R 173212/UNS 0288) suggests that males defend oviposition sites and possibly mate with multiple females. The mating system of *L. dabanus* may therefore be an example of resource-defense polygyny as has been suggested in congeners (Emlen & Oring 1977; Sullivan *et al*. 1995). The massive head of the species may be used in male combat, and male *Limnonectes* of other species have been reported to butt heads during combat (Orlov 1997; Tsuji & Matsui 2002). Indeed, it has been predicted that there is a positive correlation between the intensity of male-male combat and the degree of male-biased head dimorphism (Tsuji & Matsui 2002). If this is the case, male combat in *L. dabanus* may be among the most intense in the genus.

At this time we have no explanation for the placement of eggs on the rock ceiling. The structure of the site would not result in the chamber flooding more than a few centimeters. Based on known behaviours of other frogs, the ovipositional behaviour of ovipositing *Mixophyes* (Myobatrachidae; Anstis 2013; Hoskins 2008) present a possible option. After egg deposition, these females kick the eggs onto nearby moist, terrestrial surfaces well above the water surface. If this were the case, the eggs on the floor either fell off the ceiling or were merely scattered about by the female's actions.

Acknowledgments

Board and staff of Nui Ong Nature Reserve kindly facilitated surveys and issued permission to collect (B2008-18-34). C. Minshew and T. H. Phung assisted with fieldwork. This research was supported by funding from Ocean Park Conservation Foundation Hong Kong and ADM Capital Foundation to J. J L. Rowley and a Geddes Visiting Research Fellowship from the Australian Museum to R. Altig. D. McLeod greatly helped to improve an earlier version of this manuscript. For all this assistance we are most grateful.

References

Altig, R. & McDiarmid, R.W. (1999) Body plan: development and morphology. *In*: McDiarmid, R.W. & Altig, R. (Eds.), *Tadpoles: The Biology of Anuran Larvae*. University of Chicago Press, Chicago, pp. 24–51.

Altig, R. & McDiarmid, R.W. (2007) Diversity, morphology, and evolution of egg and clutch structure in amphibians. *Herpetological Monographs,* 21, 1–32. http://dx.doi.org/10.1655/06-005.1

Anstis, M. (2013) *Tadpoles and Frogs of Australia*. New Holland, Sydney, NSW, 832 pp.

Boulenger, G.A. (1920) A monograph of the South Asian, Papuan, Melanesian and Australian frogs of the genus *Rana*. *Records*

of the Indian Museum, 20, 1–223.
Brown, R.M. & Iskandar, D.T. (2000) Nest site selection, larval hatching, and advertisement calls of *Rana arathooni* from southwestern Sulawesi (Celebes) Island, Indonesia. *Journal of Herpetology*, 34, 404–413.
http://dx.doi.org/10.2307/1565364
Cocroft, R.B. & Ryan, M.J. (1995) Patterns of advertisement call evolution in toads and chorus frogs. *Animal Behaviour*, 49, 283–303.
http://dx.doi.org/10.1006/anbe.1995.0043
Emerson, S.B. (1992) Courtship and nest-building behavior of a Bornean frog, *Rana blythi*. *Copeia*, 1992, 1123–1127.
http://dx.doi.org/10.2307/1446654
Emlen, S.T. & Oring, L.W. (1977) Ecology, sexual selection, and the evolution of mating systems. *Science,* 197, 215–223.
http://dx.doi.org/10.1126/science.327542
Frost, D.R. (2014) Amphibian Species of the World: an Online Reference. Version 6.0 (1 August 2014). Available from: http://research.amnh.org/herpetology/amphibia/index.html. American Museum of Natural History, New York, USA. (accessed 21 October 2014)
Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 183–190.
Hoskins, C.J. (2008) Breeding behavior of the barred frog, *Mixophyes coggeri*. *Memoirs of the Queensland Museum*, 55, 1–6.
Inger, R.F. (1954) *Systematics and zoogeography of Philippine Amphibia*. Chicago Natural History Museum, Chicago, 370 pp.
http://dx.doi.org/10.5962/bhl.title.5571
Inger, R.F. (1966) The systematics and zoogeography of the Amphibia of Borneo. *Fieldiana, Zoology,* 52, 1–402.
Iskandar, D.T. & Tjan, K.N. (1985) The amphibians and reptiles of Sulawesi, with notes on the distribution and chromosomal number of frogs. *In*: Kitchener, D.J. & Suyanto, A. (Eds.), *Proceedings of the First International Conference on Eastern Indonesian-Australian Vertebrate Fauna*, Manado, Indonesia, pp. 39–45.
Lambertz, M., Hartmann, T., Walsh, S., Geissler, P. & McLeod, D.S. (2014) Anatomy, histology, and systematic implications of the head ornamentation in the males of four species of *Limnonectes* (Anura: Dicroglossidae). *Zoological Journal of the Linnean Society*, 172, 117–132.
http://dx.doi.org/10.1111/zoj.12171
McLeod, D.S., Horner, S.J., Husted, C., Barley, A.J. & Iskandar, D.T. (2011) “Same-same, but different”: an unusual new species of the *Limnonectes kuhlii* complex from West Sumatra (Anura: Dicroglossidae). *Zootaxa,* 2883, 52–64.
McLeod, D.S., Kelly J.K. & Barley, A.J. (2012) "Same-same but different": another new species of the *Limnonectes kuhlii* complex from Thailand (Anura: Dicroglossidae). *Russian Journal of Herpetology,* 19, 261–274.
Orlov, N. (1997) Breeding behavior and nest construction in a Vietnam frog related to *Rana blythii. Copeia*, 1997, 464–465.
http://dx.doi.org/10.2307/1447774
Rowley, J.J.L. & Altig, R. (2012) Nidicolous development in *Limnonectes limborgi* (Anura: Dicroglossidae). *Amphibia-Reptilia,* 33, 145–149.
http://dx.doi.org/10.1163/156853812X626179
Smith, M.A. (1922) The frogs allied to *Rana doriae*. *Journal of the Natural History Society of Siam*, 4, 215–229.
Sullivan, B.K., Ryan, M.J. & Verrell, P.A. (1995) Female choice and mating system structure. *Amphibian Biology,* 2, 469–517.
Tsuji, H. & Lue, K.-Y. (1998) Temporal aspects of the amplexus and oviposition behavior of the fanged frog *Rana kuhlii* from Taiwan. *Copeia,* 1998, 769–773.
http://dx.doi.org/10.2307/1447813
Tsuji, H. & Matsui, M. (2002) Male-male combat and head morphology in a fanged frog (*Rana kuhlii*) from Taiwan. *Journal of Herpetology,* 31, 520–526.
http://dx.doi.org/10.2307/1566203