THE RAFFLES BULLETIN OF ZOOLOGY 2006 **54**(2): 465-467
Date of Publication: 31 Aug.2006

THE TADPOLE OF *RANA GLANDULOSA* BOULENGER (ANURA:RANIDAE)

Robert F. Inger
The Field Museum, Department of Zoology, 1400 S. Lake Shore Drive, Chicago, Illinois 60605-2496, U.S.A.
Email: ringer@fieldmuseum.org (Corresponding author)

Robert B. Stuebing
Grand Perfect Sdn. Bhd., Bintulu, Sarawak, Malaysia

Bryan L. Stuart
The Field Museum, Department of Zoology, 1400 S. Lake Shore Drive, Chicago, Illinois 60605-2496, U.S.A.
The University of Illinois at Chicago, Department of Biological Sciences, 845 W. Taylor, Chicago, Illinois 60607, U.S.A.
Email: bstuart@fieldmuseum.org

***ABSTRACT*. –** The identity of the tadpole of *Rana glandulosa* Boulenger, a lowland species of frog widely distributed in Malaysia and Sumatra, Indonesia, has been uncertain. In this paper we show by means of molecular identification that tadpoles collected from Sarawak belong to *Rana glandulosa* Boulenger. These tadpoles have slender bodies, glands in three conspicuous pairs of clusters and in one median ventral cluster, no glands in the tail, five labial tooth rows on the anterior lip, and three or four rows on the posterior lip.

***KEY WORDS.* –** Sarawak, amphibian, *Rana glandulosa*, tadpole, molecular identification.

INTRODUCTION

Tadpoles have been attributed to the widely distributed, lowland species, *Rana glandulosa* Boulenger, only once. Berry (1972) collected tadpoles from a small stream at 1280 m elev. in Pahang, Peninsular Malaysia, and assigned them to *R. glandulosa* on the basis of the webbing and shape of the head in several reared to metamorphosis. These tadpoles had a very distinctive coloration—body and tail reddish brown with dark brown spots (Berry, 1972: fig. 2, 8-9). Inger (1985), Manthey and Grossmann (1997), and Leong and Chou (1999) repeated Berry's identification, but had no new tadpoles. Leong and Lim (2003) found tadpoles agreeing with those described by Berry in coloration, general form, and labial tooth formula at 1300 m elev. in Perak. However, Leong and Lim (2003) assigned their larvae to the new species they described as *Rana banjarana*, noting the similarity of metamorphosing individuals to the adults of *R. banjarana* and the known lowland restriction of *R. glandulosa*. Consequently, the tadpole of *R. glandulosa* has remained unknown.

We have recently obtained a small sample of tadpoles from a seasonally flooded freshwater swamp forest in Sarawak (Bukit Sarang, Bintulu Division) and saved tissue for molecular identification from one tadpole. Adult *Rana glandulosa* and *R. baramica* were extremely abundant and males were actively calling at the time of our work (1-12 Nov.2004). Our identification of *R. baramica* was based on the clarification of that species by Leong et al. (2003). Because these larvae had clusters of glands generally similar to those of larval *R. laterimaculata* (Leong & Lim, in press), we assumed our tadpoles might be those of either the very similar *R. baramica* or *R. glandulosa*. We sequenced mitochondrial DNA from adults of both those species (from the same locality as the tadpole) and compared those sequences with that from a tail clip of one of the tadpoles at issue. As will be shown below, the mitochondrial DNA sequence from the tadpole is essentially identical to that from the adult *R. glandulosa*, but differs substantially from that of the adult *R. baramica.* We, therefore, assign these tadpoles to *R. glandulosa*.

MATERIALS AND METHODS

Tadpoles were collected by dip net and adults by hand and preserved in 10% buffered formalin. Adults were later transferred to 70% ethanol. Tissue samples were taken by preserving a tail clip from a tadpole and pieces of liver from adults in 95% ethanol before specimens were fixed in formalin. Specimens were deposited in the Field Museum of

Natural History (FMNH). The following measurements were made on tadpoles by means of an ocular micrometer: total length (TL), headbody length (HBL), maximum headbody width (HBW), maximum headbody depth (HBD), tail length (TlL) measured from the midline of the juncture of tail and body, and maximum tail depth (TlD).

Total genomic DNA was extracted from ethanol-preserved tissue of one tadpole (FMNH 266571), one adult *R. glandulosa* (FMNH 266573), and one adult *R. baramica* (FMNH 266574) using PureGene Animal Tissue DNA Isolation Protocol (Gentra Systems, Inc.). A 595-597 bp piece of mitochondrial DNA that encodes part of the 16S ribosomal RNA (16S) gene was amplified by the polymerase chain reaction (PCR; 94°C 45s, 60°C 30s, 72°C 1 min) for 35 cycles using the primers L-16SRanaIII (5'-GAGTTATTCAAATTAGGCACAGC-3') and H-16SRanaIII (5'-CATGGGGTCTTCTCGTCTTAT-3'). PCR products were electrophoresed in a 1% low melt agarose TALE gel stained with ethidium bromide and visualized under ultraviolet light. The bands containing DNA were excised and agarose was digested from bands using GELase (Epicentre Technologies). PCR products were sequenced in both directions by direct double strand cycle sequencing using Big Dye version 3 chemistry (Perkin Elmer) and the amplifying primers. Cycle sequencing products were precipitated with ethanol, 3M sodium acetate, and 125 mM EDTA, and sequenced with a 3730 DNA Analyzer (ABI). Sequences were edited and aligned with Sequencher version 4.1 (Genecodes), and deposited in GenBank (accession numbers AY994202-04).

RESULTS

***Molecular results.* –** The tadpole (FMNH 266571) differed from the adult *R. glandulosa* (FMNH 266573) at only two of 597 positions (uncorrected pairwise distance 0.34%), but from the adult *R. baramica* (FMNH 266574) at 48 of 595 positions (uncorrected pairwise distance 8.07%). Additionally, there is a 2 bp insertion-deletion in the sequences of the tadpole and the adult *R. glandulosa* compared with the sequence of the adult *R. baramica*. Consequently, we identify the tadpole as *R. glandulosa*.

Rana glandulosa Boulenger, 1882

(Fig. 1)

Rana glandulosa Boulenger, 1882: 73.

***Material Examined.* –** Two lots of larvae (FMNH 266571-72) from Bukit Sarang, Bintulu Division, Sarawak, Malaysia (2°39.21'N 113°03.09'E). FMNH 266573 adult male *Rana glandulosa* from Bukit Sarang (see above).

***Comparative material.* –** FMNH 266574 adult male *Rana baramica* from Bukit Sarang (see above).

***Larval diagnosis.* –** Body slender, elongate; tail tapering gradually from mid-length to narrow, rounded tip. Three pairs of glandular clusters, interobital, dorsolateral, and ventrolateral pairs and a large median cluster behind the oral disk. No glands on tail. Color uniformly dark brown without markings.

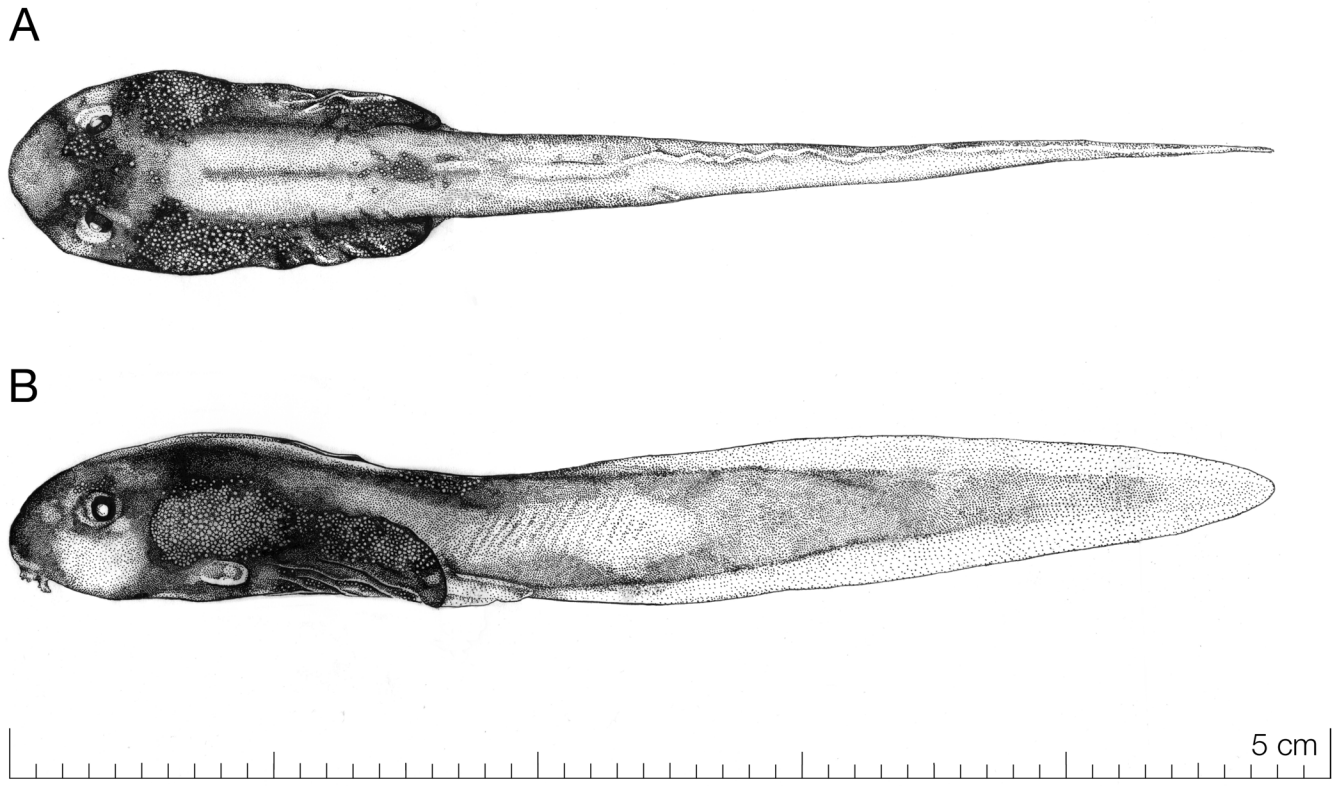

Fig. 1. Dorsal (A) and lateral (B) views of the tadpole of *Rana glandulosa* (FMNH 266571) in preservative.

Larval description. – Stages 27-37. Headbody slender, flattened above and below; maximum body width just anterior to level of spiracle. Nares dorsal, without raised rim. Eyes dorsolateral, not visible from below; interorbital greater than internarial. Spiracular tube adherent to body wall, opening closer to eye than to end of body, opening below midline of side.

Tail slender, margins barely convex, tapering gradually in distal fourth to rounded tip. Origin of dorsal fin behind end of body, distance from origin to end of body about equal to 2/3 eye-naris distance; dorsal fin as deep as caudal muscle only in distal fourth of tail. Ventral fin slightly shallower than dorsal, origin at end of body.

Oral disk ventral, subterminal, width slightly less than half width of body. Margin of anterior lip with papillae in lateral quarter. Papillae in two or three staggered rows across the margin of the posterior lip, thinning to a single row in center of margin. Papillae homogenous in length. Labial teeth usually 5(2-5)/3(1) or 4(2-4)/3(1). Median break in P1 very narrow. All posterior rows subequal in length. Jaw sheaths black in marginal halves; upper sheath broadly U-shaped without median convexity; lower sheath V-shaped; margins smooth.

Glandules arranged in clusters on headbody in three pairs and one median group. An interorbital pair of elongately oval groups, each group close to the border of eye, distance between groups slightly greater than width of a group. A dorsolateral pair, each cluster beginning behind the eye and extending almost to end of body, cluster widest near anterior end, distance between a cluster and eye about 2/3 eye-naris distance. A ventrolateral pair of clusters, each beginning just behind level of the spiracle and extending to end of body, maximum width in posterior half, maximum width less than that of dorsolateral cluster. A large median cluster behind the oral disk, anterior border strongly concave, longitudinal axis greatest at lateral margins. In some individuals scattered glandules between anterior ends of dorsolateral clusters. No glandules on the tail.

Color in preservative dark brown, slightly lighter on tail. Both fins colored as caudal muscle.

Total lengths (mm) 39.7-43.3 (stage 27), 50.3 (stage 36), 52.5 (stage 37) (Gosner stages). HBL/Total length 0.34-0.38 (median 0.360, n=5); TlL/Total length 0.62-0.68 (median0.662, n=5); HBW/HBL 0.44-0.49 (median 0.472, n=5); HBD/HBL 0.34-0.40 (median 0.371, n=4); TlD/TlL 0.20-0.24 (median 0.225, n=4). Total lengths 39.7-43.3 (stage 27), 50.3-52.5 (stages 36, 37).

Ecological notes. – The tadpoles were collected at Bukit Sarang, Bintulu Division, Sarawak, on 7 Nov.2004 in a pool (100 x 200 x 20cm) in a flooded freshwater swamp forest. At the time of collection, a large portion (ca >25%) of the surface consisted of pools of various sizes, from < 1m to 5 m in diameter. Species of *Channa, Clarias,* and *Betta* were common in the pools on the floor of that forest, though we did not record any fishes in the pool from which these tadpoles were taken. Although adult frogs of 11 species were abundant and most were actively calling at the time of sampling, we succeeded in finding only five lots of tadpoles in the more than 50 pools we searched. It is tempting to ascribe the dearth of tadpoles to the density of predaceous fishes, although we have no direct evidence. Three of the five lots of tadpoles consisted of forms with clusters of glandules in the skin: the two lots of *R. glandulosa* and one lot of *Rana chalconota* (sensu latus). Fish avoid eating larval *R. chalconota* (Liem, 1961), which have glands similar to those on the present tadpoles. We assume that the glands on the larval *R. glandulosa* also make them distasteful to fish.

ACKNOWLEDGEMENTS

We are grateful to Datuk Cheong Ek Choon for permission to work in Sarawak, to Freddy Yulus and Patrick Francis for their skillful assistance in the field, for the help of Nyegang Megom, Supiandi, Aman, and Eddi, and for the hospitality of Mr. Su Ah Kong at Bukit Sarang. We are also grateful to T. M. Leong and K. K. P. Lim for giving us access to their manuscript (in press) and to S. Drasner for drawing the tadpole. The field work was supported by the Marshall Field III Fund, The Field Museum, and by Grand Perfect Sdn. Bhd. Sequencing was done in The Field Museum's Pritzker Laboratory for Molecular Systematics and Evolution operated with support from the Pritzker Foundation.

LITERATURE CITED

Berry, P. Y., 1972. Undescribed and little known tadpoles from West Malaysia. *Herpetologica*, **28**(4): 338-346.

Boulenger, G. A., 1882. *Catalogue of the Batrachia Salientia S. Ecaudata in the Collection of the British Museum*. Second Edition. Taylor and Francis, London. 495 pp.

Inger, R. F., 1985. Tadpoles of the forested regions of Borneo. *Fieldiana Zoology New Series*, **26**: 1-89.

Leong, T. M. & L. M. Chou, 1999. Larval diversity and development in the Singapore Anura (Amphibia). *Raffles Bulletin of Zoology*, **47**(1): 81-137.

Leong, T. M. & B. L. Lim, 2003. A new species of *Rana* (Amphibia: Anura: Ranidae) from the highlands of the Malay Peninsula, with diagnostic larval descriptions. *Raffles Bulletin of Zoology*, **51**(1): 115-122.

Leong, T. M. & K. K. P. Lim, in press. Larval description of *Rana laterimaculata*. *Zoological Science*.

Leong, T. M., M. Matsui, H. S. Yong & A. A. Hamid, 2003. Revalidation of *Rana laterimaculata* Barbour et Noble, 1916 from synonymy of *Rana baramica* Boettger, 1901. *Current Herpetology*, **22**(1): 17-27.

Liem, K. F., 1961. On the taxonomic status and the granular patches of the Javanese frog *Rana chalconota* Schlegel. *Herpetologica*, **17**(1): 69-71.

Manthey, U. & W. Grossmann, 1997. *Amphibien & Reptilien Südostasiens*. Natur und Tier, Münster. 512 pp.