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Reproductive Mode of *Corbicula tobae* (Martens, 1900): Brooding and Larval Morphology in Lake Toba (Indonesia)

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Abstract: Currently, the reproductive mode of the Indonesian endemic species *Corbicula tobae* from Lake Toba is yet to be investigated. In this study, we describe, for the first time, the reproductive mode of this endemic species based on a series of topotype specimens, combining anatomical and histological data. According to our data, *C. tobae* is a dioecious species, incubating D-shaped larvae in the inner demibranchs and having monoflagellate sperm. The head of the *C. tobae* spermatozoon differs from those of other Indonesian species of *Corbicula* by having the smallest length. A histological investigation of *C. tobae* gills revealed that the inner demibranch has larger interlamellar spaces compared with the outer demibranches. Our study highlights several specific reproductive traits may have evolved in narrowly endemic *Corbicula* clams within their native ranges.

Keywords: *Corbicula*; brooding; sperm morphology; reproduction; larval morphology; Indonesia

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1. Introduction

The reproduction features of species in the genus *Corbicula* Megerle von Mühlfeld, 1811 were intensively investigated during recent decades [1–6]. This is mainly due to their negative ecological and economic impacts, and the ability to develop unique reproductive systems [7,8]. The genus *Corbicula* consists of estuarine or freshwater clams with a wide spectrum of reproductive modes, ranging from development via free-swimming veliger larvae to the incubation of juveniles in the gills [2–4,9]. *Corbicula* clams can reproduce sexually with both sexes or through androgenesis, which is an unusual reproductive mode, when the offspring carries nuclear chromosomes from the male parent only [3,10]. It was shown that androgenetic *Corbicula* species are hermaphroditic and produce a biflagellate unreduced sperm, while sexual *Corbicula* have a reduced monoflagellate sperm [11–13].

Hermaphroditic lineages of *Corbicula* have a worldwide distribution, and only a few *Corbicula* species reproduce sexually [13]. Among these, *Corbicula sandai* Reinchardt, 1878, is endemic to Lake Biwa in Japan [14]; *C. japonica* Prime, 1864, is restricted to brackish waters of East Asia [15,16]; and a few freshwater sexual *Corbicula* lineages are known to occur in Indonesia [3,4]. Brooding in *Corbicula* is restricted to freshwater taxa [2,9]. There are a few nonbrooding freshwater taxa: *C. sandai* from Lake Biwa [14] and probably *Corbicula elatior* Martens, 1905 [17]. The larvae of these species transform into benthic juveniles after leaving the egg capsules [14]. In contrast, development via free-swimming larvae is a typical attribute of the brackish water *Corbicula* clams.

According to the body of literature, 17 species of the genus *Corbicula* have been recorded in Indonesia [18,19]. Among them, the validity of nine species was confirmed by means of a molecular genetic approach using the COI gene [3,8].

Currently, the type of brooding of five *Corbicula* species from the Indonesian islands Sumatra and Sulawesi has been studied, namely *Corbicula possoensis* Sarasin & Sarasin, 1898; *C. linduensis* Bollinger, 1914; *C. matannensis* Sarasin & Sarasin, 1898; *C. loehensis* Kruimel, 1913; and *C. moltkiana* Prime, 1878. The Indonesian *Corbicula* are mostly dioecious and

ovoviviparous with incubation of the larvae in the maternal gills [2–4]. However, the reproductive mode of several endemic species from Indonesia such as *C. tobae* has not been investigated. The present study aims to describe the first data on the reproductive mode of *C. tobae* from Lake Toba in Sumatra. The status of this taxon as a valid intra-lacustrine endemic species was recently confirmed on the basis of the DNA sequences of topotypes [8].

2. Materials and Methods

This work was based on an ethanol-preserved sample of *Corbicula* species (N = 34) from Lake Toba deposited in the collection of the Russian Museum of Biodiversity Hotspots (RMBH hereafter) of the N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (Arkhangelsk, Russia).

Specimens of *Corbicula tobae* from Lake Toba were dissected and the gills were investigated for incubating juveniles using a stereomicroscope (Leica M165C, Leica Microsystems, GmbH, Wetzlar, Germany). The length (SL) of larvae was measured with a research microscope (Axio Lab.A1, Carl Zeiss, Oberkochen, Germany). Photographs of the gills with larvae were obtained using the stereomicroscope with a digital camera (FLEXACAM C1, Leica Microsystems, Wetzlar, Switzerland).

A histological examination was performed on the gonads of 10 randomly selected mature specimens. After fixation, the tissues were dehydrated through a graded alcohol series and embedded in paraffin according to the approach of Korniushin and Glaubrecht [2]. Histological sections with a thickness of 6 µm were made using a rotary microtome (HM 325; Thermo Scientific, Waltham, MA, USA). The sections were stained with Harris hematoxylin and eosin (H&E) double stain for examination under a light microscope (Axio Lab.A1; Carl Zeiss, Oberkochen, Germany). Sperm morphology was studied on the basis of histological sections using a light microscope (Axio Lab.A1; Carl Zeiss).

3. Results

3.1. Larval Morphology

Based on our data, *Corbicula tobae* is a brooding species (Figure 1). Larvae of *C. tobae* were found only in the inner demibranches, located in the water tubes formed by interlamellar septae (Figure 1B–D). Incubated larvae of *C. tobae* are D-shaped, having an average length (SD) of 265.5 ± 1.9 µm (min − max 239 − 290 µm, N = 49) (Figure 1E).

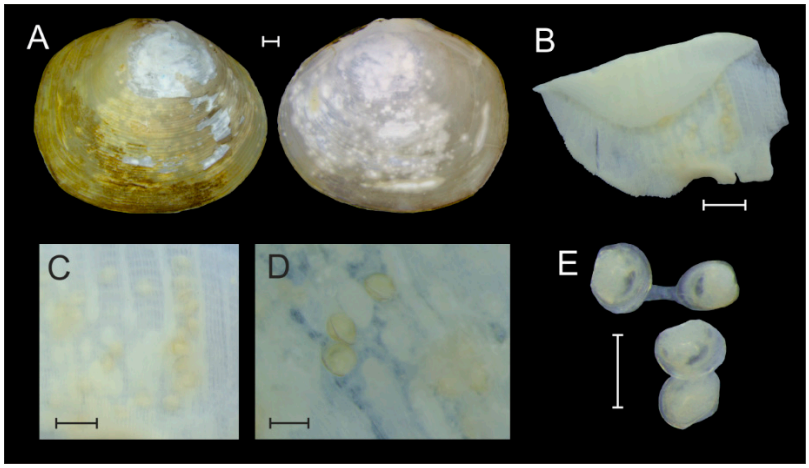

Figure 1. Morphology and anatomy of *Corbicula tobae* from Lake Toba, North Sumatra, Indonesia (lot Nos. RMBH MCorb-17 and RMBH MCorb-93). (**A**) Valve from the outer and inner sides; (**B**) gills of an incubating specimen; (**C**,**D**) detail of the same gill; (**E**) larvae. Scale bars (**A**,**B**) = 1 mm; (**C**) = 0.5 mm; (**D**,**E**) = 0.25 mm.

3.2. Histology

A histological investigation of *C. tobae* gills revealed that the inner demibranch has larger interlamellar spaces compared with the outer demibranches (Figure 2). According to our histological data, *C. tobae* is a dioecious species. Examination of the gonads in our specimens revealed the presence of either female (eggs) or male (spermatozoa) gametes in a single individual (Figure 3). In the female gonads, many mature eggs were recorded (Figure 3A). Aggregations of spermatozoa were observed in the gonads of males (Figure 3B). The state of the gonads of males and females indicates that the mollusks were at the spawning stage (Figure 3). Of the studied specimens, six were male and four were female.

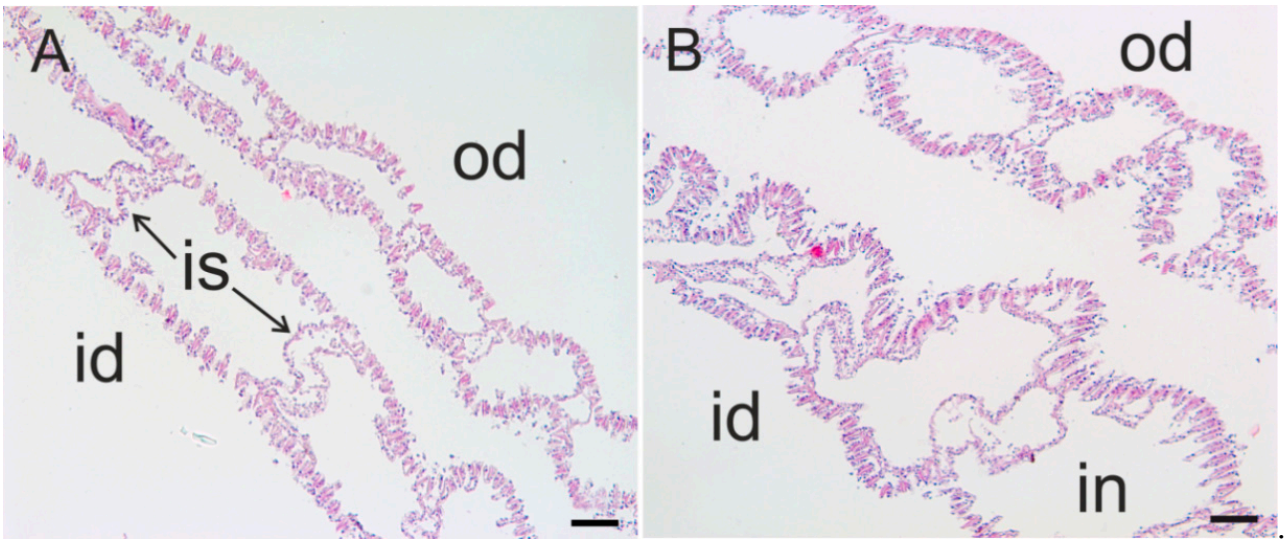

Figure 2. Cross-sections of gills in a brooding specimen: (**A**) left gills; (**B**) right gills. Abbreviations: in, interlamellar space; id, inner demibranch; is, interlamellar septa; od, outer demibranch. Scale bars (**A**,**B**) = 0.1 mm.

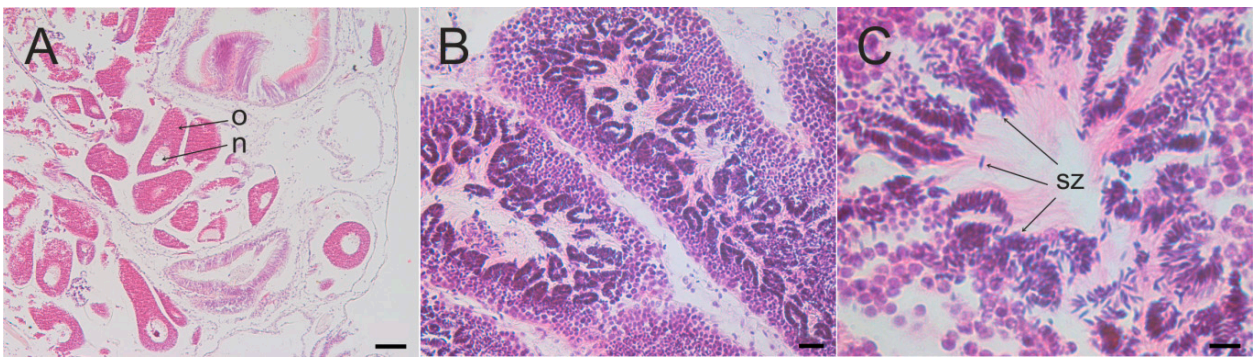

Figure 3. Gonad histology of *Corbicula tobae* from Lake Toba, North Sumatra, Indonesia (lot Nos. RMBH MCorb-17 and RMBH Corb-93): (**A**) section of the female gonad; (**B**) section of the male gonad; (**C**) aggregations of spermatozoa. Abbreviations: o, oocyte, n, nucleus; sz, spermatozoa. Scale bars = 100 µm (**A**), 20 µm (**B**), and 10 µm (**C**).

The analysis of *C. tobae* sperm revealed the presence of relatively small monoflagellate spermatozoa (Figure 3C, Table 1). The average head length (SD) of the spermatozoa was 3.8 µm (min − max 3.4 − 4.4 µm) (N = 50).

Table 1. Reproductive features of brooding *Corbicula* species from Indonesian islands.

Species	Maximum Shell Length, mm	Spermatozoon Type	Head Length of Spermatozoon, µm	Location of Brood	Larval Size, mm	Type of Released Larvae (Juveniles)	Type of Brooding	References
C. moltkiana Prime, 1878	30	Monoflagellate	11–12	Inner demibranches	0.25–0.40	D-shaped	Synchronous	[2–4]
C. linduensis Bollinger, 1914	17	Monoflagellate	8–9	Inner demibranches	Up to 1.5	Umbonal	Sequential	[2–4]
C. matannensis Sarasin & Sarasin, 1898	32.5	Monoflagellate	11–12	Inner demibranches	0.30–0.42	D-shaped	Synchronous	[2–4]
C. loehensis Kruimel, 1913	18	Monoflagellate	9–10	Inner demibranches	~0.35	D-shaped	Synchronous	[2–4]
C. possoensis Sarasin & Sarasin, 1898	29.4	Monoflagellate	10–11	Both demibranches	0.25–0.30	D-shaped	Synchronous	[2–4]
C. tobae (Martens, 1900)	15.5	Monoflagellate	3.4–4.4	Inner demibranches	0.23–0.29	D-shaped	Synchronous	[8]; this study

4. Discussion

In this study, we report on the discovery of another sexual and dioecious freshwater *Corbicula* species from Indonesia in addition to the eight sexual Far Eastern clams already described. It was established that *C. tobae*'s brooding mode is represented by D-shaped larvae in the inner demibranches (Figure 1E). A few other species have the same reproductive mode [2], e.g., *C. matannensis* from Lakes Matano and Mahalona (Sulawesi), *C. loehensis* from Lake Masapi (Sulawesi), and *C. moltkiana* from Lakes Maninjau and Singkarak (Sumatra) (Table 1).

In general, *C. tobae, C. matannensis, C. moltkiana,* and *C. loehensis* have an approximately comparable size of larvae (Table 1). Thus, *C. tobae* has a widely represented reproductive mode, which was mentioned by Korniushin and Glaubrecht [2] as a common trait for the brooding *Corbicula*. In contrast, *C. possoensis* from Lake Poso on Sulawesi is characterized by tetragenous brooding (i.e., the larvae develop in both demibranches). Prolonged incubation in the maternal gills was found in *C. linduensis* from the Lindu River system in Sulawesi [2]. In addition, *C. linduensis* is distinguished by the larger size of the brooding larvae (Table 1).

New data on the sperm morphology were produced for *C. tobae* (Figure 3, Table 1). According to our data, the head length of the *C. tobae* spermatozoon differs in having the smallest length compared with those of other Indonesian species of *Corbicula* (Table 1). An analysis of the published data shows that the head size of spermatozoa significantly varies among *Corbicula* species [2,20,21]. At the same time, a significant correlation of spermatozoon head size between biflagellate and monoflagellate spermatozoa was not found [2]. Hence, the head of biflagellate spermatozoa in several species of *Corbicula* is characterized by their larger size. For example, the spermatozoon head length of *Corbicula* aff. *fluminea* is 13.9 µm [20], that of *C. leana* is 16.9 µm [22], that of *C. fluminea* is 25 µm [21], and that of the monoflagellate *C. japonica* is 15 µm [23]. Conversely, the biflagellate spermatozoa of *C. australis* and the monoflagellate spermatozoa of Indonesian *Corbicula* species have a relatively small head size of 9.3 µm and 3.4–12.0 µm, respectively (Table 1). The size of the spermatozoa may correlate with the ploidy of the organism [21] and with the number of mitochondria [20]. Unfortunately, we do not have enough data to support or refute these hypotheses. Consequently, this issue requires additional research.

The mode of embryonic nutrition in brooding *Corbicula* clams is still disputable [2]. According to a number of authors, the eggs of *Corbicula* species are rich in nutrients that are essential for the developing embryos [9]. Furthermore, it was found that the interlamellar junctions of the inner demibranches in the Indonesian species *C. matannensis*, *C. loehensis*, *C. moltkiana*, and *C. possoensis* are characterized by thickening of the interlamellar septae and interfilamentary junctions and by the development of high cylindrical mucous cells in the epithelium [2]. The latter trait is considered as an adaptation for the incubation or release of juveniles and may probably serve as an alternative source of nutrition for embryos [2,9]. Similar changes have been identified in the interlamellar septa of *C. australis*

and *C. fluminea* [9]. We also found changes in the interlamellar spaces of *C. tobae*'s inner demibranches (Figure 2).

The preservation state of the available ethanol-fixed material did not permit detailed histological investigations of this species. However, the similar brooding type in the Indonesian *C. moltkiana*, *C. matannensis*, and *C. loehensis* indicates the probability of a structural change in the interlamellar epithelium and the presence of mucous cells in *C. tobae* (Figure 2). In contrast, the mucous cells of *C. linduensis* are not proliferating. According to Korniushin and Glaubrecht [2], matrotrophy could play a more important role in the larval development of the latter species. The authors also do not exclude the influence of brood cannibalism.

Currently, there are several hypotheses on the adaptive role of matrotrophy in different animal groups [24]. In our opinion, the reduced risk to vulnerable early life stages by retaining the offspring for longer or accelerating their maturation, thus enhancing survivorship by producing fewer but larger and fitter offspring, may play a more important role [25,26].

5. Conclusions

These data extend our knowledge of the diversity of reproduction in Indonesian endemic freshwater *Corbicula* clams. It was established that *C. tobae* is a sexual, dioecious, ovoviviparous, brooding species with D-shaped larvae in the inner demibranches. Furthermore, *C. tobae* produces monoflagellate spermatozoa having a small head size compared with those of other Indonesian species of *Corbicula*. We assume that mucous cells develop in the interlamellar epithelium of *C. tobae*, which may serve as an alternative source of nutrition for embryos. Future research should focus on studying the life cycles of Indonesian *Corbicula* species by means of population genetic, ecological, anatomical, and cytological methods.

Author Contributions: A.V.K., I.N.B. and Y.V.B. conceptualized the study. A.V.K. performed the histological studies. O.V.A. created the figures. A.V.K. and Y.V.B. wrote the article, with input from all authors. All authors have read and agreed to the published version of the manuscript.

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