

Chorion morphology of the eggs of *Lethocerus delpontei*,
Kirkaldyia deyrolli, and *Horvathinia pelocoroides*
(Heteroptera: Belostomatidae)

Морфология хориона яиц *Lethocerus delpontei*, *Kirkaldyia deyrolli*
и *Horvathinia pelocoroides* (Heteroptera: Belostomatidae)

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КЛЮЧЕВЫЕ СЛОВА. отцовская забота о потомстве, пластронное дыхание, Nepomorpha, Lethocerinae, Horvathininae.

ABSTRACT. The chorion morphology of *Lethocerus delpontei*, *Kirkaldyia deyrolli*, and *Horvathinia pelocoroides* is here described. The surface of all species' eggs is covered with a plastron. The chorion consists of three layers, a superficial one functioning as plastron, and intermediate one, with multiple pores running through it, and a deep respiratory layer with wide spaces presumably filled with air. On the micropylar zone, the respiratory layer is interrupted. Over the hypopyle, filiform outgrowths are covering the surface and the glue does not reach the surface of the egg, leaving an empty space between them. The morphology of the chorion of *H. pelocoroides* is practically identical to that of Lethocerinae, suggesting an emergent brooder strategy in this enigmatic genus.

РЕЗЮМЕ. Описана морфология хориона *Lethocerus delpontei*, *Kirkaldyia deyrolli* и *Horvathinia pelocoroides*. Поверхность яиц всех видов покрыта пластроном. Хорион состоит из трех слоев. Внешний слой функционирует как пластрон, промежуточный слой пронизан многочисленными порами, а внутренний респираторный слой обладает обширными пустотами, предположительно заполненными воздухом. В области микропиле респираторный слой отсутствует. Поверхность над гидропиле покрыта нитевидными выростами, за счет которых клей не достигает поверхности яйца, оставляя свободное пространство между ними. Морфология хориона *H. pelocoroides* практически иден-

тична таковой Lethocerinae. Это позволяет предположить, что для загадочного рода *Horvathinia* также характерна отцовская забота о потомстве вне воды.

Introduction

Species of the subfamily Lethocerinae lay their eggs outside water, on substrates such as moss [DeCarlo, 1962, for *L. mazzai*] and on other vegetation. The male takes care of them by providing water to prevent desiccation and protects them from predators [Ichikawa, 1988; Smith & Larsen, 1993; Ohba, 2002]. This egg-laying mode was named “emergent brooder” by Smith [1997]. This author hypothesized that this type of breeding was plesiomorphic with respect to the type seen in Belostomatinae, where females lay their eggs on the back of the male (thus “back brooder”). The egg-laying behaviour of *Horvathinia* (Horvathininae), which belongs to the third of the three subfamilies of Belostomatidae, is not known.

Material and Methods

Egg masses of *Lethocerus delpontei* were collected in Salta Province, Argentina; and those of *Kirkaldyia deyrolli* were found in Hyogo Prefecture, Japan. Eggs of *Horvathinia pelocoroides* were dissected from specimens collected in Torres City, southern Brazil.

Eggs were preserved in 70% ethanol until observation. Portions of the egg were cut with a scalpel blade, and rinsed in alcohol with an ultrasonic cleaner for 15 minutes. The selected portions were allowed to dry, and then observed with a Keyence VE8800 scanning electron microscope without metallic coating.

Results

The egg of *L. delpontei* is 3–4 mm long, 2–2.5 mm wide (Fig. 1), whereas the egg of *K. deyrolli* is somewhat bigger, 4–5 mm long, 2.5–3 mm wide. Both are ovoid, with the anterior third wider. The anterior half is pale yellow in *K. deyrolli* and 10–12 dark longitudinal stripes are visible on the dorsal side, which are faint on the ventral (micropylar) side of the egg. In *L. delpontei* only irregular dark patches with a longitudinal trend were found (Fig. 1). The pseudopericulum is approximately 0.7 mm in diameter and circular. It remains attached to the egg as in the remaining Belostomatidae [Cobben, 1968]. The pseudopericulum is clearly separated from the rest of the egg by a pale line, which coincides with the eclosion (weakness) line in *L. delpontei*, but not in *K. deyrolli*. An ellipsoidal micropylar zone is observed, sub-apically on the pseudopericulum, which is recognized by being much darker than the rest of the zone. However in the middle it is paler. The basal half is of dark chestnut colour in both species. In the postero-ventral zone of the egg, the chorionic hypople is approximately 0.8 mm wide, covered with a fine pubescence in a hexagonal pattern. This position matches that in other Lethocerinae, but also the one in Nepidae [Cobben, 1968].

The whole chorion is covered by a hexagonal pattern delimited by ridges in the three studied species. The hexagons are 18 to 45 μm wide, with an average of 31 μm wide (Fig. 2). Inside each hexagon, a meshwork is visible (Fig. 4). According to Hinton [1981] the entire surface is responsible for the gas exchange, and functionally a plastron. The chorion can be divided in three different zones: Plastron layer, intermediate, and deep respiratory layer (Fig. 6).

The plastron layer is made of finger-shaped outgrowths, 800 nm to 1 μm in diameter, which leave spaces of similar size between them (Figs 3–4). This layer is approximately 2.5 μm deep. The intermediate layer underneath it (10–12 μm) is relatively massive with thin channels (600–700 nm diameter) across the layer, from the bottom of the spaces between the outgrowths of the plastron, to the first respiratory layer (Figs 3, 6).

However, irregularly distributed thick channels (almost 1 μm diameter) are visible, but no specialized structure is present on the external surface, which presumably out ruling them being aeropyles (Fig. 6). Two deep respiratory meshwork layers are visible against the inner side of the chorion. The outermost is about 1.5 to 4 μm wide, with wide spaces between the trabecules. The deepest layer is wider (3–7 μm) and the meshwork is much denser (Fig. 6).

There are 8 to 10 micropyles, arranged in an ellipsoid pattern (Fig. 7) in both studied Lethocerinae species. This is coincident with the observation of Hinton [1981] on an undetermined *Lethocerus* species from Africa, but different from the semicircle described by Cobben in *L. indicus*. On the micropylar zone, the hexagonal pattern is visible but these are much smaller and the plastron is not developed (Fig. 7). Correspondingly, there is no respiratory layer inside the chorion. The chorion is massive and about 20–23 μm thick (Fig. 9).

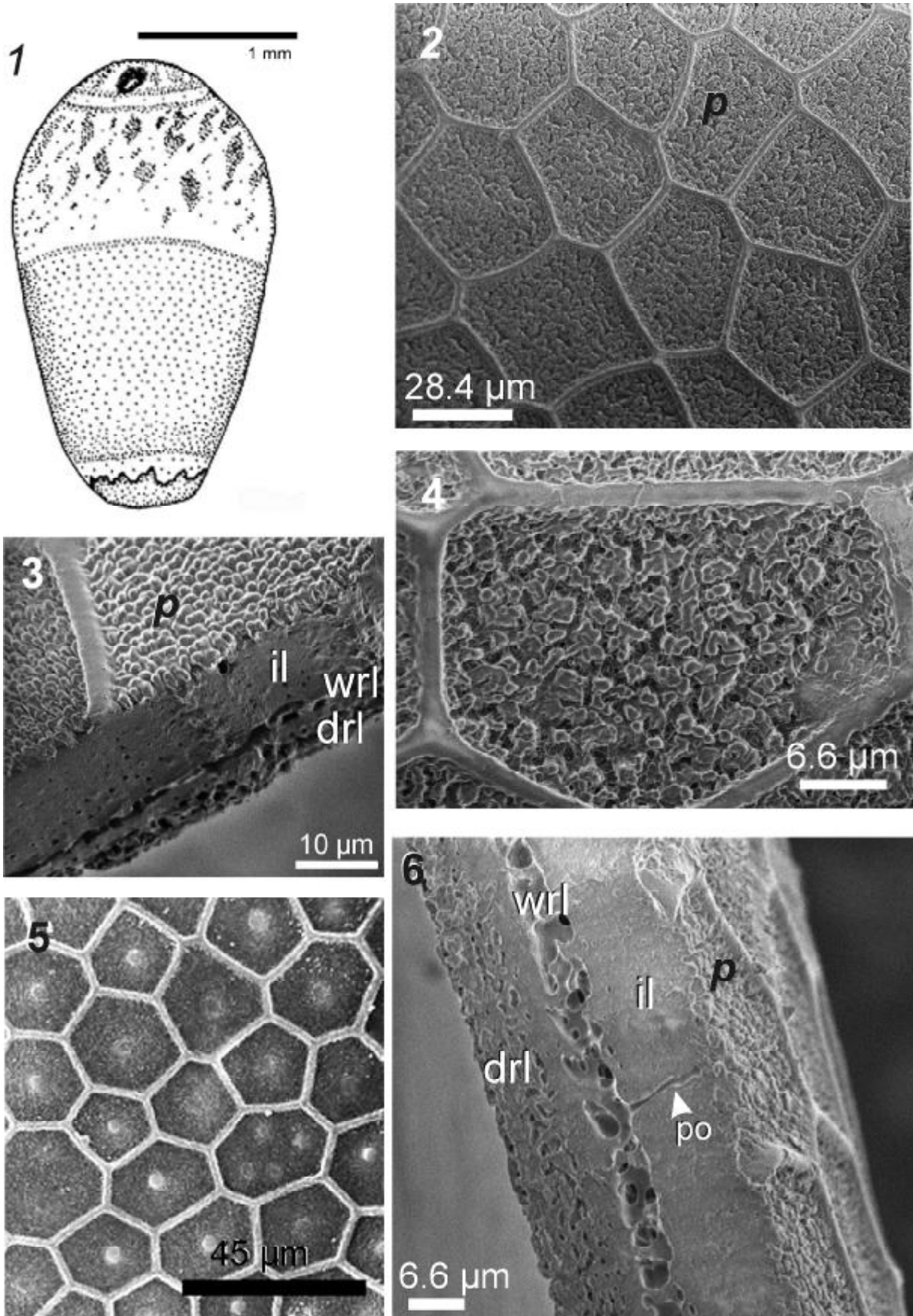
The basal half of the egg, which is dark chestnut seen with the naked eye, seems to have a relatively different organization on the surface. Inside the hexagons, even though coarsely rugose, there are almost no digitiform outgrowths. The pores communicating with the underlying respiratory layer are visible (Fig. 10).

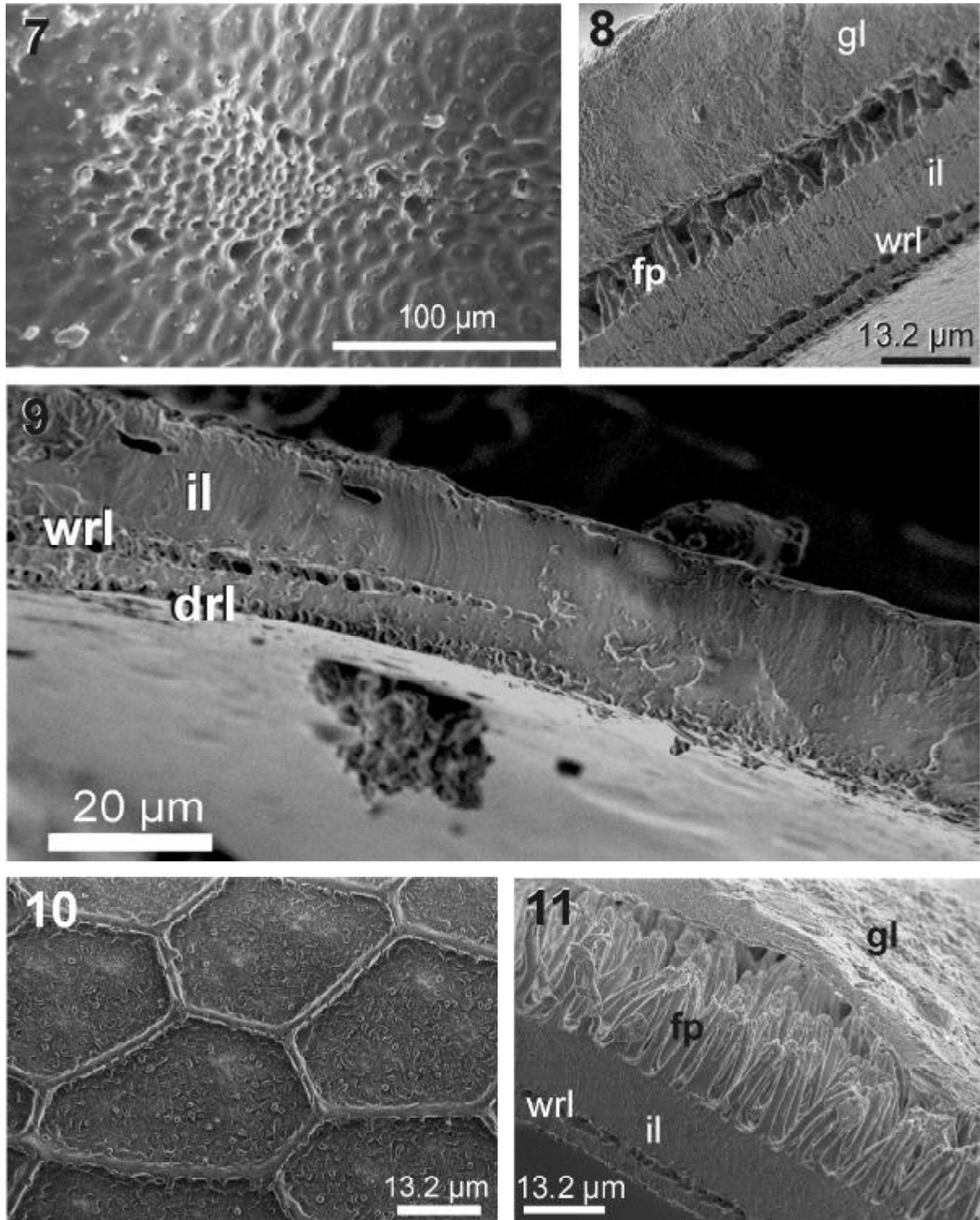
The chorionic hypople surface shows the hexagonal pattern, but it is covered with filiform outgrowths, which are approximately 10 to 12 μm long and clump together in the preparation. The deep structure of the chorion is almost the same as in the rest of the egg (Fig. 8), no specialized structure is visible as in Nepidae [Hinton, 1961], and the respiratory layers are continuous all around the egg (except in the micropylar zone as noted before). Between the filiform outgrowths, thin channels open through the intermediate layer to the respiratory layer. In deposited eggs, the hypople and most of the posterior forth of the egg is covered with a secretion, which presumably acts as glue. The filiform outgrowths under the glue are not collapsed, and the glue does not reach the surface of the egg. An empty layer about 6 to 8 μm wide is formed by these outgrowths between the glue and the chorion of the egg (Figs 8, 11). The glue is hydrophilic and presumably water may be retained in the space between the glue and the chorion.

The chorion of *Horvathinia pelocoroides* is remarkably similar to that of Lethocerinae. The whole surface is covered with a hexagonal pattern. These hexagons are approximately the same size (22–45 μm) and also covered with a plastron meshwork (Fig. 5).

Figs 1–6. 1 — external aspect of egg of *Lethocerus delpontei*; 2 — mid chorion external aspect of *Kirkaldyia deyrolli*, showing hexagonal plastron pattern; 3 — *K. deyrolli* chorion cut, showing the internal layers; 4 — *K. deyrolli* detail of one hexagon of plastron; 5 — chorion external aspect of *Horvathinia pelocoroides*, showing hexagonal plastron pattern; 6 — idem figure 3, left: inside, right: outside the egg; (p — plastron; po — irregular thick pore; il — intermediate layer; wrl — wide spaces respiratory layer; drl — dense spaces respiratory layer).

Рис. 1–6. 1 — внешний вид яйца *Lethocerus delpontei*; 2 — внешний вид хориона *Kirkaldyia deyrolli* в средней части; показана гексагональная структура пластрона; 3 — срез хориона *K. deyrolli*; показаны внутренние слои; 4 — *K. deyrolli*, один из элементов гексагональной структуры; 5 — внешний вид хориона *Horvathinia pelocoroides*, показана гексагональная структура пластрона; 6 — то же, что на рис. 3, внутренний слой слева, наружный — справа; (p — пластрон; po — нерегулярно расположенная толстая пора; il — промежуточный слой; wrl — респираторный слой с обширными полостями; drl — респираторный слой с плотно расположенными полостями).





Figs 7–11. 7 — micropylar zone of *L. delpontei*; 8 — hydropylar zone of *K. deyrolli*; 9 — micropylar zone of *K. deyrolli*, showing fainting respiratory layer to the right, closer to the micropylar zone; 10 — posterior chorion external aspect of *K. deyrolli*; 11 — hydropylar zone of *K. deyrolli*; (gl — glue; fp — filiform outgrowths; il — intermediate layer; wrl — wide spaces respiratory layer; drl — dense spaces respiratory layer).

Рис. 7–11. 7 — микропилярная зона *L. delpontei*; 8 — гидропилярная зона *K. deyrolli*; 9 — микропилярная зона *K. deyrolli*; показано исчезновение респираторного слоя справа, ближе к микропилярной зоне; 10 — внешний вид хориона *K. deyrolli* в задней части; 11 — гидропилярная зона *K. deyrolli*; (gl — клей; fp — нитевидные выросты; il — промежуточный слой; wrl — респираторный слой с обширными полостями; drl — респираторный слой с плотно расположенными полостями).

Discussion

The hypothesis of Smith [1997] suggests an “ancillary evolution” in which, due to the increase in size of the adults in order to have access to a vertebrate diet, the eggs should also increase in size. As a consequence, eggs would have physiological problems to develop without the assistance of an adult. In a primitive state, the eggs would have been laid outside the water to have a higher oxygen supply, but thus forcing the adults to keep them from drying up because of the extensive plastron surface (both Lethocerinae [Hinton, 1981] and Horvathininae described in this study for the first time). And in a derived state in which, the eggs would be carried on the back of the parent, whom in turn would have to aerate periodically (Belostomatinae). Our data support the position of the Horvathininae in this schema.

The importance of such an extended plastron becomes evident when one considers the size of the eggs. Following Smith [1997], the evolutionary scenario for the breeding behaviour in this family is the following. Body size increases in the family, and because the number of larval instars is fixed, the egg also increases in size. The oxygen demand of the egg increases with the greater volume, but the surface does not increase at the same ratio. Hence the gas exchange possibilities under water are not sufficient for the embryo. The Lethocerinae solved this problem by breeding exposed to the air, with an egg completely covered with plastron. But such an extensive plastron would make the egg sensitive to desiccation, which would be the reason why the male must provide the eggs with water very often [Ichikawa, 1988; Smith & Larsen, 1993; Ohba, 2002].

Considering that the sister group of Belostomatidae, the Nepidae [Hinton, 1981; Cobben, 1968; Wichard et al., 1995; Lopez Ruf & Perez Goodwyn, 2002] has a plastron developed exclusively over specialized respiratory horns and none over the chorion surface, the plastron function of the chorion may be interpreted as an evolutionary novelty in the Nepomorpha. Nevertheless, the chorion of certain Corixidae possess a similar network, but Hinton [1981] suggested that it could work as a plastron. Corixidae is most probably the sister group of the clade Nepidae + Belostomatidae [Mahner 1993; Hebsgaard et al., 2004], and thus the plastron developed on the chorion may be interpreted as a plesiomorphy.

Accepting Lethocerinae as the most primitive group of Belostomatidae [Lauck & Menke, 1961; Mahner, 1993]; the development of a chorion all over the egg may be interpreted as a plesiomorphy for Belostomatidae. The derived Belostomatinae subfamily genera such as *Belostoma*, *Diplonychus* and *Limnogeton* [Cobben, 1968; Hinton, 1981; Baker, 1987], which are all back-brooders, possess a plastron-function surface only over one eighth of surface of the egg, with several aeropyles also present. The morphology of those Belostomatinae can be easily derived from an

egg completely covered by plastron. These eggs are bred under water, and the egg-carrying male keeps them in contact with the water surface. The aeration and care of the male is indispensable for the development of the embryo [Smith, 1997].

Following this hypothesis, the chorion morphology would clearly indicate that *Horvathinia* is an emergent brooder, and not a back brooder. Lauck & Menke [1961] considered Lethocerinae as the most primitive group, and Horvathininae as a second group derived from the stem. Mahner [1993] made a phylogenetic analysis, and considered Lethocerinae as the most primitive group, and Horvathininae as sister group of all Belostomatinae, with the fused endosoma and ventral diverticulum as synapomorphy. The plastron over the egg and the breeding mode would be shared characters with the Lethocerinae, however this would be a shared plesiomorphy.

Until now, an embryonic cuticle was detected in Lethocerinae [Ohba, 2002] but not in Belostomatinae. The functional importance of this cuticle may be for enhanced protection against desiccation because of the emergent brooding behaviour; thus also unnecessary for back brooders Belostomatinae. This membrane may also play a role in synchronizing the hatching of eggs in a cluster [Ohba, 2002]. The presence in *Horvathinia* could not be confirmed.

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