

Adhesive structures in the eggs of *Corydoras aeneus* (Gill, 1858; Callichthyidae)

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The surface structure of the eggs of the catfish *Corydoras aeneus* was examined and showed to be a unique pattern among teleosts. The surface was covered with small protuberances, which resemble attaching-filaments of teleost eggs. Eggs were, however, found to be very adhesive and since the species is known to inhabit turbid waters, this rare egg attachment mode could well be related to these environmental conditions.

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Modifications in the egg morphology of various teleosts often reflect the ecological challenges a species is faced with during its embryonic life stages. In this context, for instance, the thickness of the zona radiata interna, which has a protective function, is directly related to the exposure to mechanical strains (Riehl, 1996). Next to this, the zona radiata externa has been known to mediate egg adhesion in several teleosts and various modes of egg attachment are known (Laale, 1980; Patzner & Glechner, 1996; Riehl & Patzner, 1998; Rizzo *et al.*, 2002). Again, the presence and manner of egg attachment could reflect environmental constraints placed upon the eggs, or the species in general, during these developmental stages. Patzner & Glechner (1996) found fishes from different environments, which exhibited the same variety in attachment structures. Rizzo *et al.* (2002), on the contrary, related the absence or presence of egg adhesion in different species to their migratory behaviour and Morin & Able (1983) related the nature of adhesive structures found in the eggs of *Fundulus heteroclitus* (L.) to a variance in egg deposit sites. In addition Rizzo *et al.* (2002) point out that adhesive eggs are usually larger and laid in smaller numbers, as well as they relate egg adhesiveness to both the sedentary nature of species and possible parental care. In any case, the presence of adhesive eggs certainly indicates a behavioural strategy in which eggs are confined to a single locus until hatching,

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a strategy which could be related to environmental conditions, making it useful to take environmental factors into account when discussing egg morphology.

In the armoured catfish *Corydoras aeneus* (Gill), known to inhabit fast current freshwater systems in South America (Gosline, 1940; Nijssen, 1970; Kramer & Braun, 1983; Burgess & Quinn, 1992; Kohda *et al.*, 1995; Fuller, 2001), adaptations in egg morphology in order to cope with a similar turbulent environment can be expected. In particular, since Kohda *et al.* (1995) found adaptations in the species' insemination strategy, presumably to ensure a high insemination rate even in a turbulent habitat. This unique mode of insemination in *C. aeneus* consists of the female swallowing sperm and quickly transporting it through the intestine, emitting it at the anal opening into a previously emitted ventral egg pouch (Kohda *et al.*, 1995). For this study, eggs of *C. aeneus* were examined in order to obtain information on their morphology, this way possibly gathering some information on the environmental demands met by the species during its primary developmental stages. For this purpose, several commercially obtained *C. aeneus* specimens were induced to breed by imitation of rainy season conditions in the tank according to the protocol by Fuller (2001). The fertilized eggs were photographed with the use of a ColorView digital camera, mounted onto an Olympus SZX9 binocular microscope. Egg diameters were measured on these digital photographs using the AnalySIS toolpack (Münster, Germany). After this, eggs were fixed in 4% glutaraldehyde in a 0.2 M cacodylate buffer (pH 7.4). After washing, the eggs were dehydrated in a graded ethanol series and critical point dried under liquid CO₂. The eggs were further mounted and coated with gold using a Balzers SCD040 sputtercoater. For examination a Jeol SM840 scanning electron microscope (SEM) was used at 15 kV.

The eggs ($n = 21$) examined had a mean \pm s.e. diameter of 1.47 ± 0.20 mm and were always laid against either a leaf or the aquarium walls. A large yolk sac was present and, like in most teleosts (Riehl & Patzner, 1998), the eggs were spherical in shape (Fig. 1). The egg-surface was covered with 'little' projections,

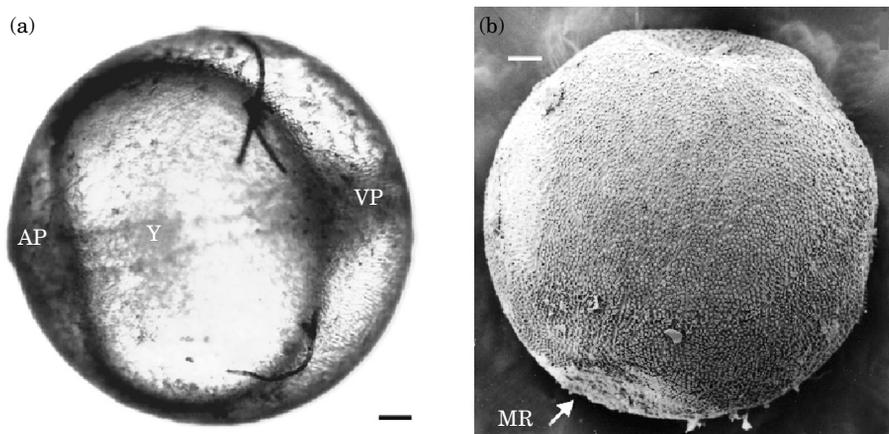


FIG. 1. Habitus of *Corydoras aeneus* eggs: (a) light microscopic and (b) scanning electron microscope view. AP, animal pole; MR, micropylar region; VP, vegetal pole; Y, yolk. Scale bar = 100 μ m.

with the exception of a small circular region surrounding the micropyle [Figs 1(b) and 2(e)]. These projections were *c.* 10 μm long, *c.* 5 μm wide and continuous with the outer layer of the zona radiata, *i.e.* the zona radiata externa [Fig. 2(a)], which was in total *c.* 13 μm thick. Both the size and position of these projections would suggest them to be villi-like protuberances according to the definition by Riehl & Patzner (1998). When comparing them to the villi described in *Leuciscus leuciscus* (L.), *Alburnoides bipunctatus* (Bloch) and *Rutilus rutilus* (L.) by Patzner & Glechner (1996), however, the shape of the villi seemed to differ from all these species. The protuberances found here were regularly hexagonally shaped [Fig. 2(c)], whilst the villi found in the former species were irregularly shaped. In addition, after spawning, these protuberances could lengthen considerably and act as adhesive structures in contact to both the substratum or other eggs [Fig. 2(b)]. After lengthening the projections resembled more attaching-filaments than villi-like protuberances. Attaching-filaments, however, are only fixed at the vegetal or animal pole or arranged in a disc or annular bulge (Riehl, 1996; Riehl & Patzner, 1998) excluding these projections from that category. In addition, this part of the zona radiata was perforated in *C. aeneus* with several small pores that lay in a clear hexagonal pattern in between the projections [Fig. 2(c)]. Similar hexagonal structures are suggestive of the honey-comb like pattern characteristic of fishes of the family Percidae (Riehl & Bless, 1995; Riehl & Patzner, 1998). This way, the arrangement and attachment mode found in the eggs of *C. aeneus* have not been described in other teleosts. Further, under the zona radiata externa lay the zona radiata interna, which was *c.* 7 μm thick and existed entirely out of pillar-shaped structures separated by pore-canals [Fig. 2(a)]. The thickness fits the teleost average, where a zona radiata of 5–15 μm is commonly found (Riehl, 1999), indicating that the eggs of *C. aeneus* do not have to cope with more than average mechanical stress. The deepest zone of the zona radiata was the zona radiata subinternus, which formed the boundary with the oocytosoma and was *c.* 0.2 μm thick. This part of the zona radiata was also highly perforated but pores were more randomly organized throughout this inner layer.

The micropylar apparatus in *C. aeneus* consisted of a flat, ellipse-shaped, micropylar pit which was *c.* 50 μm long and 20 μm wide [Fig. 2(d)]. In this pit lay the micropylar canal, which was *c.* 13 μm wide [Fig. 2(d), (f)]. Although the latter measurement was somewhat unreliable due to the unclear borders of the canal, this condition fits the type 2 micropylar configuration as described by Riehl & Götting (1974) and Riehl (1999). The micropyle itself lies at the end of the pit and is, after insemination, closed off by a micropylar plug to avoid polyspermy (Riehl, 1996).

The eggs in *C. aeneus* therefore exhibit a unique surface pattern with small villi-like protuberances which resemble attaching-filaments of teleost eggs. The presence of these structures can be related to the turbid habitat in which this species lives, but further studies on eggs of phylogenetically related and of non-related sympatric fishes will further have to clarify this hypothesis and demonstrate the true relation. The results of this study could possibly be used also in a taxonomic perspective, since morphological characters of teleost eggs have already been used this way (Kim & Park, 1996; Park & Kim, 2001) and phylogenetic relations within both *Corydoras* sp. and the Callichthyidae are

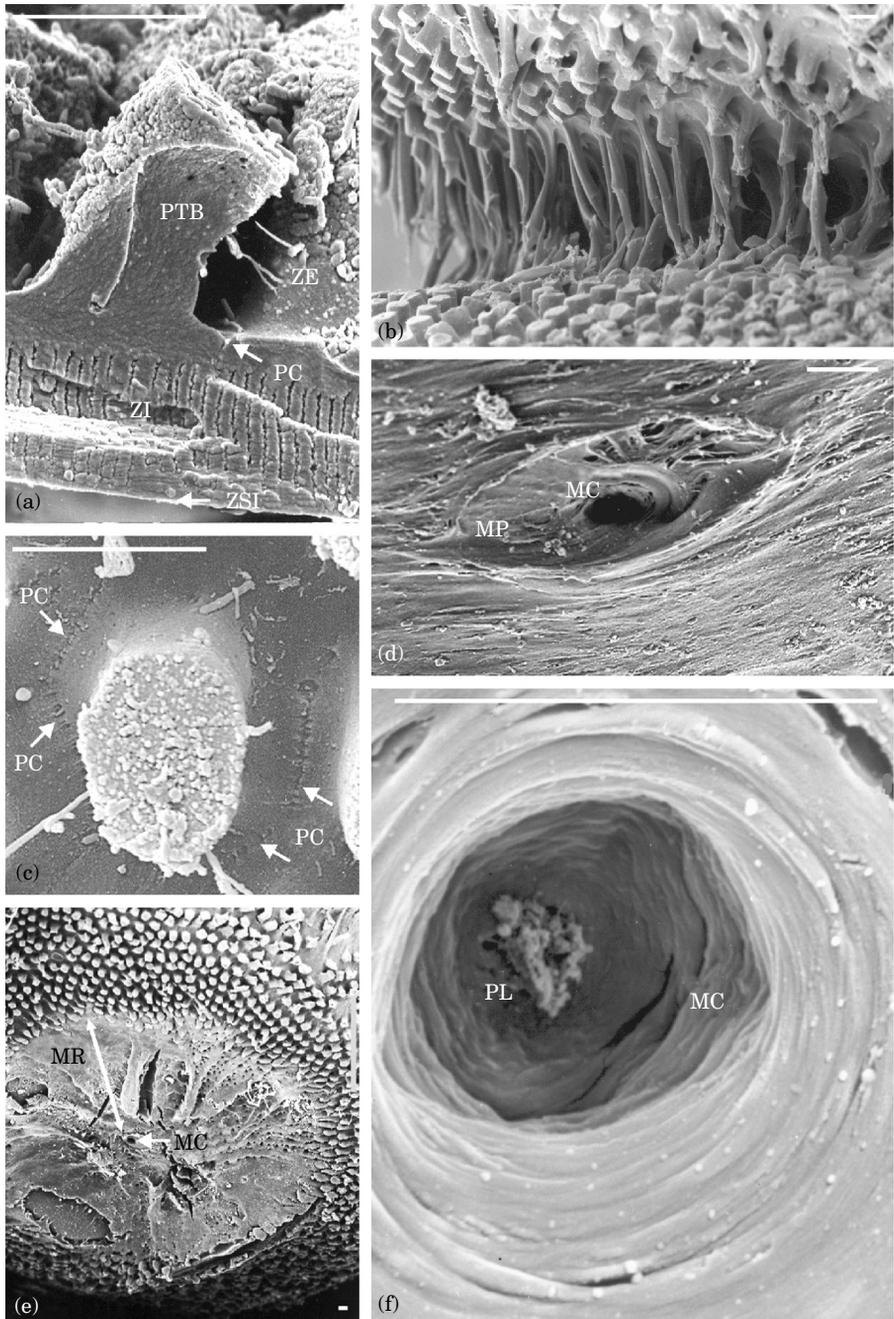


FIG. 2. Scanning electron microscope view of *Corydoras aeneus* egg morphology: (a) section of the zona radiata with protuberance, (b) attachment site between two separate eggs with lengthened and glued protuberances, (c) protuberance, (d) micropylar pit, (e) micropylar region and (f) micropylar canal. MC, micropylar canal; MP, micropylar pit; MR, micropylar region; PC, pore canal; PL, micropylar plug; PTB, protuberance; ZE, zona radiata externa; ZI, zona radiata interna; ZSI, zona radiata subinterna. Scale bar = 10 μ m.

still unclear (Britto & Castro, 2002). Patzner & Glechner (1996), however, question such an application since no association between egg morphology and taxonomy exists, at least not within the family Cyprinidae. Morin & Able (1983) seem to confirm this by reporting interspecific variation in *F. heteroclitus*, but, however, do state that egg morphology has proved to help identify major groups and species within the Cyprinodontiformes. Therefore, information on egg morphology of other callichthyids will have to reveal whether a similar pattern is also present in those species and to what extent, if any, the use of a similar character in the phylogenetic reconstruction of this family would be advisable.

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