



Adhesiveness and surface patterns of eggs in neotropical freshwater teleosts

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Eggs of 23 Characiformes and eight Siluriformes, belonging to nine families with diverse reproductive behaviour, were ultrastructurally analysed. The migratory species exhibited non-adhesive eggs, whereas, most of the sedentary species presented some degree of egg adhesiveness. Among the Characiformes, non-adhesive eggs showed zona radiata with pore-canals or a fibrillar net at the surface; weakly adhesive eggs presented only zona radiata with pore-canals while adhesive eggs exhibited zona radiata with apparatus like globules, filaments, villi or honeycomb-like pores depending on the systematic group. The 'jelly' coat is strongly related to the Siluriformes eggs apparently without relationship with adhesiveness. A micropylar disc was present in adhesive eggs of a few species of both Characiformes and Siluriformes. Some patterns were characteristic of the animal pole, others of the vegetal pole, and others were common to both poles. The radial ridges converging to the micropyle in *Astyanax bimaculatus lacustris* appear to be related to fertilization. In general, egg surface structures in the Characiformes varied according to the genus, whereas all Siluriformes showed a similar egg surface pattern, regardless of the group analysed. Multivariate analysis allowed the identification of eight clusters among the Characiformes and three among the Siluriformes showing relationships between reproductive style and egg characteristics. It is suggested that egg surface and adhesiveness may be related to reproductive patterns and to phylogenesis.

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Key words: adhesiveness; egg surface; cluster analysis; Characiformes; Siluriformes; freshwater teleosts.

INTRODUCTION

The neotropical freshwater fish fauna, mostly concentrated in two large orders, Characiformes and Siluriformes, is the most diversified and richest in the world (Lowe-McConnell, 1987), encompassing species with reproductive strategies adapted to diverse aquatic habitats. Various aspects of the life cycle of this fauna are still unknown, for which reason knowledge of its evolutionary history and of the phylogenetic relationships among its groups is limited (Vari & Malabarba, 1998). Previous comparative analyses have shown that some oogenesis patterns are common to fishes of the same family in neotropical freshwater fishes (Bazzoli & Rizzo, 1990; Bazzoli, 1992; Bazzoli & Godinho, 1994, 1995). The egg surface

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TABLE I. Adhesiveness and egg surface patterns in 31 species of neotropical freshwater teleosts

Order/family/species	Reproductive strategy	Adhesiveness	Surface arrangement	
			Animal pole	Vegetal pole
Characiformes				
Characidae				
<i>Acestrorhynchus britskii</i> ¹	Sedentary ⁵	Adhesive	Knobs	Filaments
<i>Acestrorhynchus lacustris</i> ¹	Sedentary ³	Adhesive	Knobs	Filaments
<i>Asiyanax bimaculatus lacustris</i> ²	Sedentary ⁴	Weakly adhesive	Zona radiata ridges	Zona radiata pore-canal
<i>Bryconops</i> cf. (= <i>Creatochanes</i>) <i>affinis</i> ¹	Sedentary ⁵	Adhesive	Globules	Globules
<i>Brycon lundii</i> ²	Migratory ⁴	Non-adhesive	Zona radiata pore-canal	Zona radiata pore-canal
<i>Brycon orbignyana</i> ²	Migratory ³	Non-adhesive ³	Zona radiata pore-canal	Zona radiata pore-canal
<i>Colossoma macropomum</i> ²	Migratory ³	Non-adhesive ³	Zona radiata pore-canal	Zona radiata pore-canal
<i>Piaractus mesopotamicus</i> ²	Migratory ³	Non-adhesive ³	Zona radiata pore-canal	Zona radiata pore-canal
<i>Salminus brasiliensis</i> ²	Migratory ⁴	Non-adhesive	Zona radiata pore-canal	Zona radiata pore-canal
<i>Salminus maxillosus</i> ²	Migratory ⁵	Non-adhesive ³	Fibrillar net	Fibrillar net
<i>Serrasalminus spilopleura</i> ¹	Migratory ⁵	Non-adhesive ³	Fibrillar net	Fibrillar net
<i>Triportheus guentheri</i> ²	Sedentary ³	Adhesive	Micropylar disc	Honeycomb-like pores
<i>Triportheus guentheri</i> ²	Sedentary ⁴	Weakly adhesive	Zona radiata pore-canal	Zona radiata pore-canal
Erythrinidae				
<i>Hoplias</i> cf. <i>lacerdae</i> ¹	Sedentary ³	Adhesive ³	Villi	Villi
Anostomidae				
<i>Leporinus elongatus</i> ²	Migratory ⁴	Non-adhesive	Fibrillar net	Fibrillar net
<i>Leporinus friderici</i> ²	Sedentary ³	Non-adhesive ³	Fibrillar net	Fibrillar net
<i>Leporinus piau</i> ²	Sedentary ⁴	Non-adhesive	Fibrillar net	Fibrillar net
<i>Leporinus reinhardtii</i> ²	Migratory ⁴	Non-adhesive	Fibrillar net	Fibrillar net
<i>Leporinus taeniatus</i> ²	Migratory ⁴	Non-adhesive	Zona radiata pore-canal	Zona radiata pore-canal
<i>Schizodon kneri</i> ²	Sedentary ⁴	Adhesive	Globules	Globules
Curimatidae				
<i>Curimatella lepidura</i> ²	Sedentary ⁴	Weakly adhesive	Zona radiata pore-canal	Zona radiata pore-canal

TABLE I. *Continued*

Order/family/species	Reproductive strategy	Adhesiveness	Surface arrangement	
			Animal pole	Vegetal pole
Characiformes <i>continued</i>				
Prochilodontidae				
<i>Prochilodus affinis</i> ²	Migratory ⁴	Non-adhesive	Fibrillar net	Fibrillar net
<i>Prochilodus lineatus (=scrofa)</i> ²	Migratory ³	Non-adhesive ³	Fibrillar net	Fibrillar net
<i>Prochilodus marginatus</i> ²	Migratory ⁴	Non-adhesive	Fibrillar net	Fibrillar net
Siluriformes				
Doradidae				
<i>Franciscodoras marmoratus</i> ²	Sedentary ⁴	Adhesive	Jelly coat	Jelly coat
Pimelodidae				
<i>Conorhynchus conirostris</i> ²	Migratory ⁴	Non-adhesive	Jelly coat	Jelly coat
<i>Lophiosilurus alexandri</i> ²	Sedentary ⁴	Adhesive	Jelly coat	Jelly coat
<i>Paulicea luetkeni</i> ²	Migratory ³	Non-adhesive ³	Jelly coat	Jelly coat
<i>Pimelodus maculatus</i> ²	Sedentary ⁴	Non-adhesive	Jelly coat	Jelly coat
<i>Pseudoplatystoma coruscans</i> ²	Migratory ⁴	Non-adhesive	Jelly coat	Jelly coat
<i>Rhamdia quelen</i> ²	Sedentary ⁴	Non-adhesive	Jelly coat	Jelly coat
Loricaridae				
<i>Rhinelepis aspera</i> ²	Sedentary ⁴	Adhesive	Micropylar disc	Jelly coat

¹Eggs collected through spontaneous or ²induced spawning; ³according to Lamas (1993); ⁴according to Sato (1999); ⁵unpubl. obs.

has so far been examined only in a few Characiformes and Siluriformes (Wirz-Hlavacek & Riehl, 1990; Rizzo & Bazzoli, 1993; Rizzo *et al.*, 1998; Riehl & Patzner, 1998).

The zona radiata covering the egg is a complex extracellular matrix with pore-canal filled by oocyte microvilli and follicular cell extensions. It is usually composed of two main layers with different morphological characteristics (Bazzoli & Rizzo, 1990; Bazzoli, 1992). The inner layer, consisting mainly of proteins with few carbohydrates, is similar to the zona pellucida of mammals and its functions during fertilization and embryonic development were conserved during evolution (Murata *et al.*, 1997; Scapigliati *et al.*, 1999). The outer layer presents a specific macromolecular composition, besides glycoproteins, it may show carboxylated and sulphated polysaccharides and rarely sialic acid (Bazzoli & Rizzo, 1990; Rizzo & Bazzoli, 1991; Bazzoli, 1992) that are responsible for the interactions between the egg and the aquatic environment.

After spawning, the zona radiata undergoes extensive molecular modifications that lead to the formation of the chorion (Yamagami *et al.*, 1992). The ultimate chorionic structure reflects adaptations to variable environmental conditions, pelagic eggs usually having a thin chorion and demersal eggs having a thicker and more complex chorionic membrane (Stehr & Hawkes, 1979). The appendages associated with the outer layer of the zona radiata suffer physical-chemical changes that allow them to attach to different substrata (Laale, 1980; Riehl & Patzner, 1998). Less elaborate outer layers are seen in non-adhesive eggs (Rizzo & Bazzoli, 1993; Rizzo *et al.*, 1998).

Neotropical freshwater teleosts bearing free or non-adhesive eggs are highly fecund, their eggs are small in size, with relatively large perivitelline space, quick embryonic development, and frequently the eggs are not subjected to parental care (Sato, 1999). On the other hand, adhesive eggs are spawned in smaller numbers, they are larger, with a smaller perivitelline space (Sato, 1999). Under natural conditions, usually the eggs of migratory species drift freely downstream, for oxygenation and dispersion while those of the sedentary species remain close to the area where they were laid, usually attached to different substrata (Lamas, 1993; Sato, 1999).

Thus, egg characteristics may be helpful to the understanding of the phylogenetic relationships between the different groups of teleosts (Britz *et al.*, 1995; Britz, 1997; Chen *et al.*, 1999; Sato, 1999; Breining & Britz, 2000). The objective of this study was to establish the relationship between adhesiveness and egg surface patterns in Characiformes and Siluriformes fishes and to provide basic knowledge which may help to elucidate the life history of South American freshwater fishes.

MATERIAL AND METHODS

Recently ovulated, unfertilized eggs from 23 Characiformes and eight Siluriformes species of the neotropical freshwater ichthyofauna were used. They were obtained either from natural reproduction in the wild or from induced or spontaneous spawning in hatcheries (Table I). At least 20 eggs of three to five females from each species were examined.

EGG ADHESIVENESS

The eggs were analysed for adhesiveness by placing them in a petri dish with fresh water immediately after spawning. Adhesiveness was classified into one of the following

categories after macroscopic evaluation of the egg: 1, adhesive, when they stuck firmly to each other and formed a coherent egg mass; 2, weakly adhesive, when they adhered to each other but then became free under slight agitation; 3, non-adhesive, when they were completely free. The data on egg adhesiveness of some species in [Table I](#) were obtained from [Lamas \(1993\)](#).

EGG FIXATION

The eggs were fixed in 2.5% glutaraldehyde or Karnovsky (2.5% glutaraldehyde and 2% paraformaldehyde) solution, both in 0.1 M phosphate buffer, pH 7.3, during 18 to 22 h at 4–8° C. Post-fixation was performed in 1% osmium tetroxide during 2 h at room temperature, followed by 1% tannic acid during 20 min and finally 1% osmium tetroxide for another 2 h.

SCANNING AND TRANSMISSION ELECTRON MICROSCOPY

Following fixation, the specimens were dehydrated, dried under CO₂, mounted on stubs, metallized with gold during 1–2 min at 15 mV, and examined with a Zeiss DSM-950 scanning electron microscope at 10 to 20 kV. The images were digitized and submitted to image processing programmes. The eggs were also subjected to routine procedures for transmission electron microscopy. In this case, after fixation they were embedded in epon-araldite, sectioned with a diamond knife, contrasted with uranyl acetate and lead citrate, and analysed with a Zeiss EM-10 microscope. Measurements of structures were made with scanning and transmission electron images.

STATISTICAL ANALYSIS

The data were submitted to multivariate cluster analysis (Statistic for Windows) in order to organize them into meaningful groupings. The following values were attributed to the egg adhesiveness characters: non-adhesive (1), weakly adhesive (2) and adhesive (3). The egg surface characters were classified as follows: zona radiata pore-canals (1), fibrillar net (2), zona radiata ridges (3), honeycomb-like pores (4), filaments (5), villi (6), globules (7), jelly coat (8) and micropylar disc (9). Among the various methods tested, weighted pair-group centroid-median (WPGMC) with Euclidean distance best joined the species.

RESULTS

EGG ADHESIVENESS

Various degrees of egg adhesiveness were found among the Characiformes and Siluriformes. The migratory species exhibited non-adhesive eggs whereas most sedentary species presented some degree of egg adhesiveness ([Table I](#)).

EGG SURFACE PATTERN

The following arrangements were identified in the egg surface.

Zona radiata pore-canals

This arrangement corresponded to the undecorated smooth zona radiata with visible pore-canals forming the less complex structural arrangement observed on the egg surface of the fish species examined. The diameter and density of the pores varied according to the egg region. At the vegetal pole, they had a similar diameter and were uniformly distributed [[Fig. 1\(a\)](#)], whereas at the animal pole they varied in diameter and their density increased towards the micropyle [[Fig. 1\(b\)](#)]. This pattern was present in species of three Characiformes families: Characidae [*Brycon lundii* Reinhardt, *Brycon orbignyanus* Valenciennes, *Colossoma macropomum* (Cuvier), *Piaractus mesopotamicus* (Holmberg) and

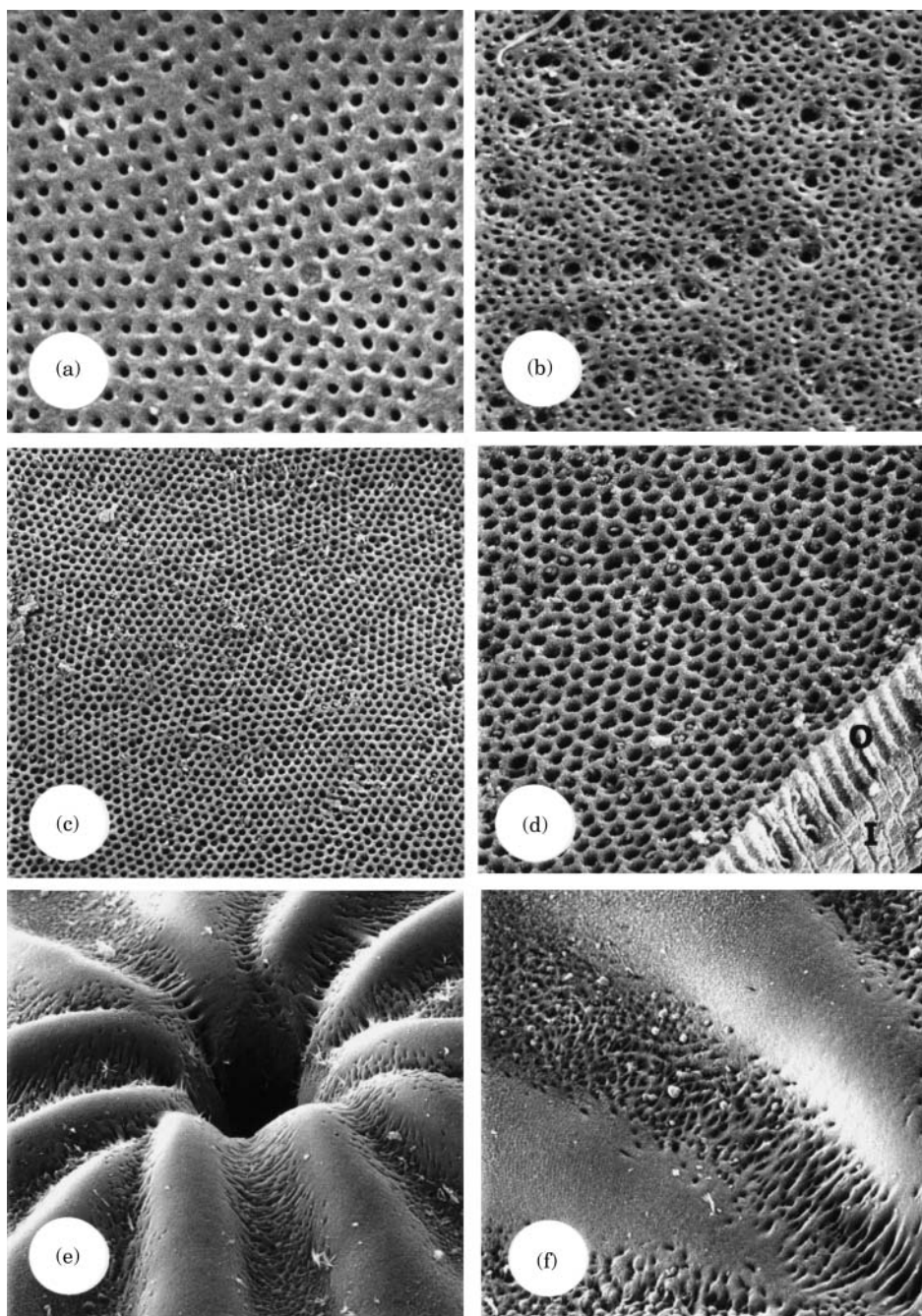


FIG. 1. Arrangements of the zona radiata in Characiformes eggs. (a) Uniform distribution of pore-canals at the vegetal pole of *Piaractus mesopotamicus* egg, $\times 7060$; (b) pore-canals of different diameters at the animal pole of *Triportheus guentheri* egg, $\times 3530$; (c) honeycomb-like pores at the vegetal pole of *Serrasalmus spilopleura* egg, $\times 1410$; (d) fractured zona radiata (O, outer layer; I, inner layer) of *Serrasalmus spilopleura* egg showing canals of the outer zona radiata opening on the egg surface, $\times 3590$; (e) zona radiata ridges radially arranged around the micropyle of *Astyanax bimaculatus lacustris* egg, $\times 1410$; (f) large pore-canals between ridges in *Astyanax bimaculatus lacustris* egg, $\times 2570$.

Triportheus guentheri (Garman)], Anostomidae (*Leporinus taeniatus* Lütken) and Curimatidae (*Curimatella lepidura* Eigenmann & Eigenmann).

Honeycomb-like pores

This special arrangement of the outer zona radiata was observed in *Serrasalmus spilopleura* Kner. Their pores were wider than those of the previous arrangement, polygonal in shape and organized in a honeycomb fashion [Fig. 1(c),(d)].

Zona radiata ridges

These were folds of the zona radiata formed by ridges and grooves radially arranged toward the micropyle [Fig. 1(e)]. The ridges, perforated by fine pores, extended towards the micropyle vestibule. The pores located on the grooves were larger than those on the ridges [Fig. 1(f)]. This arrangement was characteristic of *Astyanax bimaculatus lacustris* (Reinhardt) eggs.

Globules

These were peculiar electron-dense projections (length=10–20 μm , thickness=4–10 μm) attached to the outer zona radiata, forming a compact arrangement at the vegetal pole [Fig. 2(a),(b)]. These globules became progressively flattened and irregularly shaped towards the micropyle. Their size and organization varied according to the species and egg pole. In *Schizodon knerii* (Steindachner), they were irregularly distributed on the egg surface; in *Bryconops* cf. *affinis* (Günther), they were arranged in radial rows toward the micropyle, whereas at the vegetal pole they were organized in whirls.

Filaments

These were long and thin threads (length=5–8 μm , thickness=0.4–0.8 μm) forming a dense cover on the zona radiata at the vegetal pole of *Acestrorhynchus lacustris* (Reinhardt) and *Acestrorhynchus britskii* Menezes eggs. These electron-dense filaments were continuous with the zona radiata [Fig. 2(c),(d)].

Villi

These were thick and short projections (length=2–5 μm , thickness=1.0–1.5 μm) sparsely arranged among the zona radiata pores in eggs of *Hoplias* cf. *lacerdae* Ribeiro [Fig. 2(e)]; these structures became shorter as they approached the micropyle.

Knobs

These were small and round bulges (thickness=0.2–0.4 μm) located among the pores of the zona radiata; they occurred only in the animal pole of *Acestrorhynchus* spp. eggs [Fig. 2(f)].

Fibrillar net

This was composed of delicate fibrils that were present on the surface of the vegetal pole (Fig. 3(a),(b)). This network, forming a thin layer (thickness=0.4–0.8 μm), became progressively reduced at the animal pole, especially around the micropyle, where it was dispersed among the pores. The fibrillar net was seen in

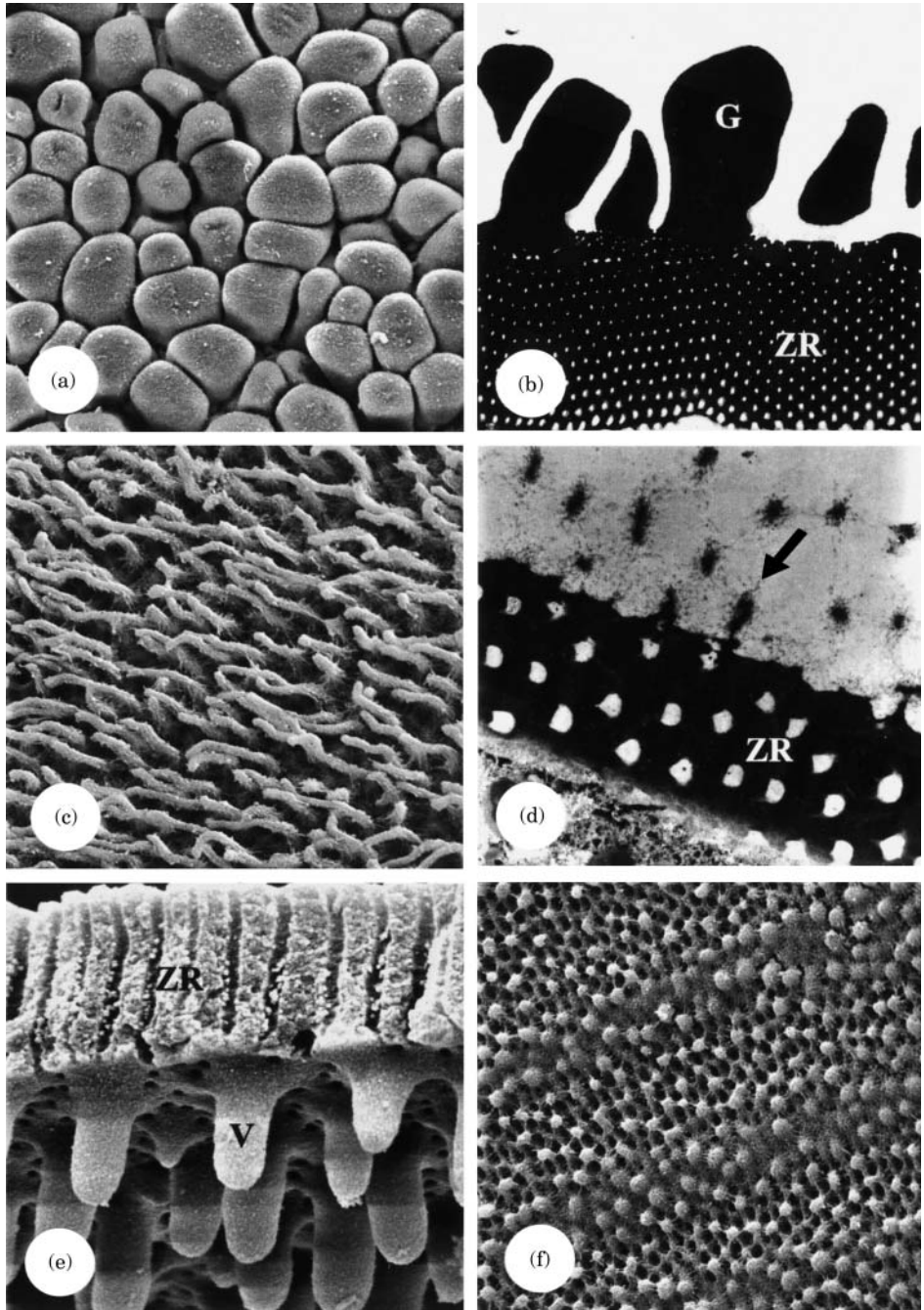


FIG. 2. Appendages of the zona radiata in Characiformes eggs. (a) Irregular arrangement of the globules at the vegetal pole of *Schizodon knerii* egg, $\times 1410$; (b) ultra-thin section of the surface of *Schizodon knerii* egg showing globules (G) continuous with the zona radiata (ZR), $\times 2340$; (c) filaments at the vegetal pole of *Acestrorhynchus britskii* egg, $\times 2820$; (d) ultra-thin section of the surface of *Acestrorhynchus lacustris* egg with filaments (\blackrightarrow) associated with the zona radiata (ZR), $\times 5640$; (e) fractured surface at vegetal pole of *Hoplias lacerdae* egg with villi (V) continuous with the zona radiata (ZR), $\times 7060$; (f) knobs at the animal pole of *Acestrorhynchus britskii* egg, $\times 7060$.

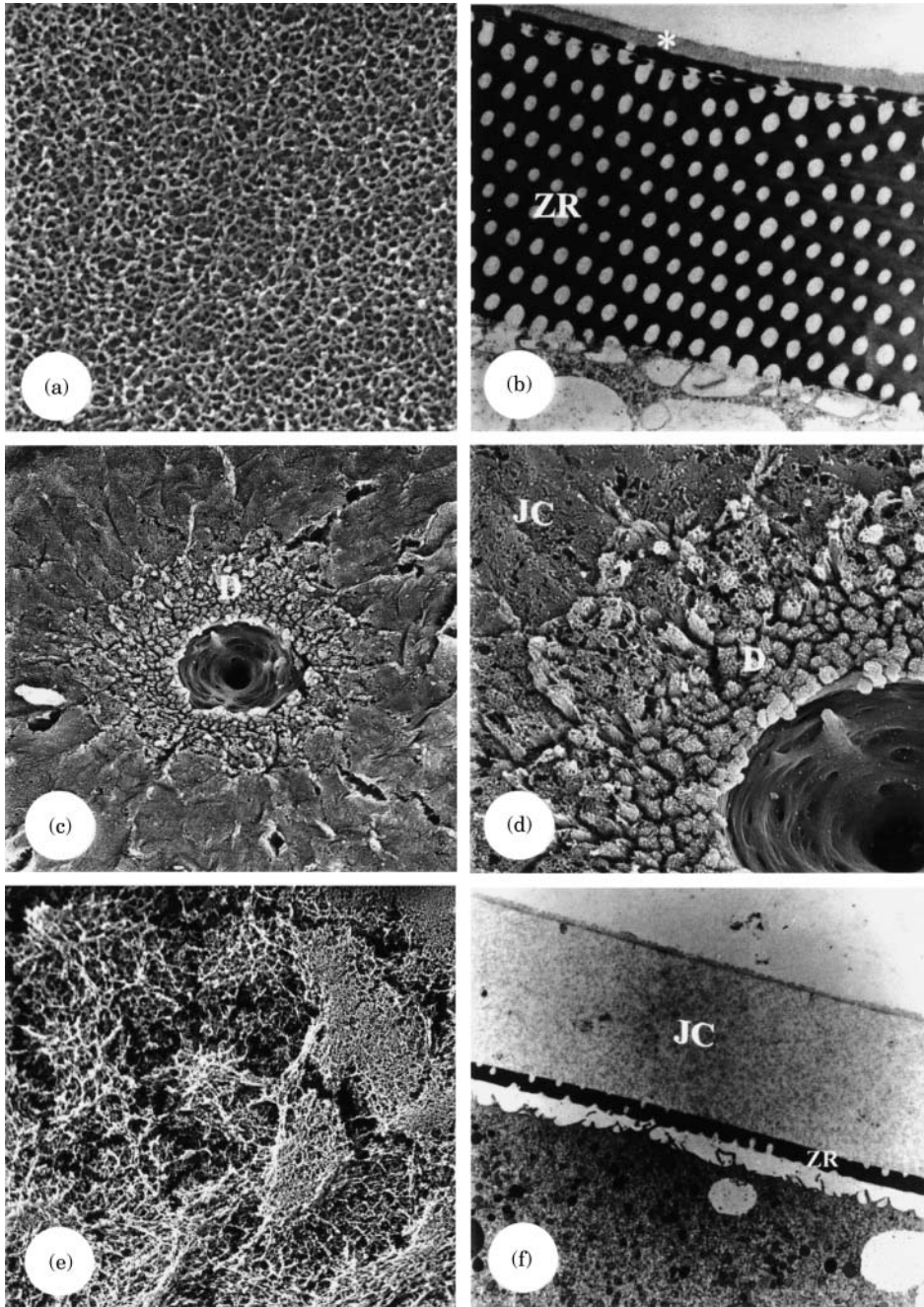


FIG. 3. Coats on the zona radiata in Characiformes and Siluriformes eggs. (a) Fibrillar net at the vegetal pole of *Prochilodus marggravii* egg, $\times 7060$; (b) ultra-thin section of *Prochilodus affinis* egg with fine layer of fibrils (*) covering the zona radiata (ZR), $\times 4420$; (c) micropylar region of *Rhinelepis aspera* egg with micropylar disc (D), $\times 740$; (d) micropylar disc continuous with the jelly coat of *Rhinelepis aspera* egg, $\times 2120$; (e) dense jelly coat on the surface of *Lophiosilurus alexandri* egg, $\times 2120$; (f) ultra-thin section of *Pseudoplatystoma coruscans* egg showing electron-lucent jelly coat (JC) on the zona radiata (ZR), $\times 2120$.

several groups of Characiformes: *Salminus* spp., most *Leporinus* spp., and *Prochilodus* spp. and was the simplest of structures deposited on the zona radiata.

Micropylar disc

This was circumscribed mucous strip (width=15–30 μm) of fine threads on the zona radiata surrounding the micropyle [Fig. 3(c),(d)]. This arrangement was present in *S. spilopleura* and *Rhinelepis aspera* Agassiz eggs.

Jelly coat

This was a mucous layer (thickness=5–30 μm) covering the zona radiata and constituted of entangled delicate fibrils with an electron-lucent aspect [Fig. 3(e),(f)]. All Siluriformes eggs examined exhibited this structure, which varied in thickness and distribution among the species. Thus, the eggs of *Franciscodoras marmoratus* (Reinhardt) and *Lophiosilurus alexandri* (Steindachner) had a thick jelly coat, whereas in the other Pimelodidae the jelly coat was thinner. It was distributed essentially at the animal pole in the eggs of *Paulicea luetkeni* (Steindachner), *Pimelodus maculatus* Lacépède and *Pseudoplatystoma coruscans* (Agassiz), irregularly arranged around the micropyle in *Rhamdia quelen* (Quoy & Gaimard), or on the entire egg surface in *R. aspera*. The jelly coat constituting a continuous layer on both egg poles was seen only in *F. marmoratus* and *L. alexandri*.

ADHESIVENESS AND SURFACE PATTERN

Within the Characiformes (Table I), the species with non-adhesive eggs showed either a smooth zona radiata with pore-canals, or a fibrillar net covering the zona radiata. The species bearing adhesive eggs presented distinct arrangements on the egg surface: honeycomb-like pores, globules, filaments, villi, and a micropylar disc depending on the systematic group. All species with weakly-adhesive eggs exhibited zona radiata with pore-canals.

The Siluriformes (Table I), on the other hand, invariably showed a thick jelly coat covering the zona radiata in adhesive eggs and a thin jelly coat in non-adhesive eggs. In *R. aspera*, the adhesive eggs showed, in addition to the jelly coat, a micropylar disc.

CLUSTER ANALYSIS

This analysis revealed eight clusters among the Characiformes and three among the Siluriformes (Fig. 4): I, Characiformes (Characidae) with weakly adhesive eggs and radial ridges; II, Characiformes (Characidae, Anostomidae and Prochilodontidae) with non-adhesive eggs and fibrillar net; III, Characiformes (Characidae and Curimatidae) with weakly adhesive eggs and smooth zona radiata with pore-canals; IV, Characiformes (Characidae) with non-adhesive eggs and smooth zona radiata with pore-canals; V, Siluriformes (Pimelodidae) with non-adhesive eggs and thin jelly coat; VI, Siluriformes (Pimelodidae and Doradidae) with adhesive eggs and thick jelly coat; VII, Siluriformes (Loricariidae) with adhesive eggs, thick jelly coat and micropylar disc; VIII, Characiformes (Characidae) with adhesive eggs and filaments; IX, Characiformes (Characidae) with adhesive eggs, honeycomb-like pores and

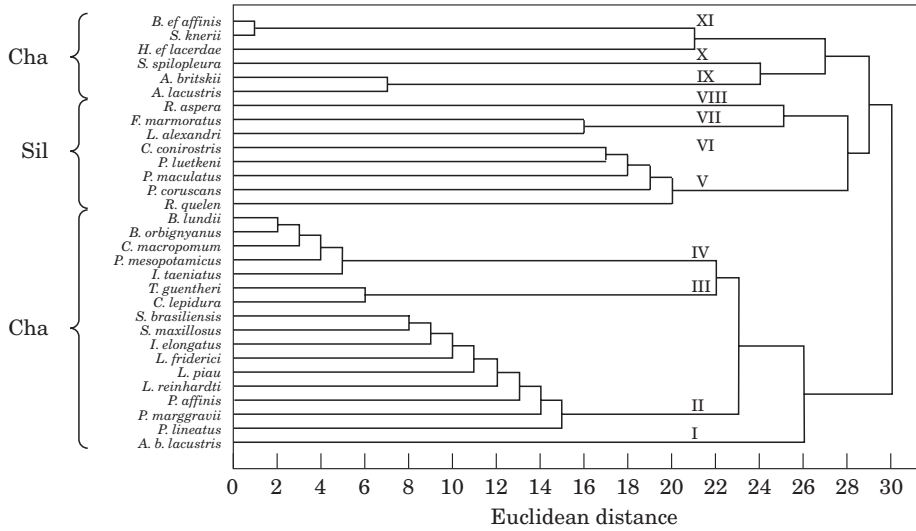


FIG. 4. Dendrogram of cluster analysis of 31 neotropical teleost species based on adhesiveness and egg surface patterns. The clusters I to XI were separated using WPGMC (weighted pair-group centroid–median) and Euclidean distance. Cha, Characiformes; Sil, Siluriformes.

micropylar disc; X, Characiformes (Erythrinidae) with adhesive eggs and villi; XI, Characiformes (Characidae and Anostomidae) with adhesive eggs and globules.

DISCUSSION

In spite of recent data (Rizzo & Bazzoli, 1993; Rizzo *et al.*, 1998; Riehl & Patzner, 1998), the relationship between egg surface and degree of adhesiveness in teleosts still remains poorly understood. The large diversity of reproductive modes (Balon, 1984) and the difficulty in acquiring newly spawned eggs or reliable series in nature are probably among the main constraints to understanding the relationship.

Various egg surface patterns emerged from the analyses. The non-adhesive eggs of migratory Characiformes showed either fibrillar net or zona radiata with pore-canals. Although sedentary, *Leporinus friderici* (Bloch) and *Leporinus piau* Fowler showed similar adhesiveness and egg surface pattern as seen in migrant Characiformes. The weakly adhesive eggs of the sedentary Characiformes *A. bimaculatus lacustris*, *T. guentheri* and *C. lepidura* showed zona radiata with pore-canals. According to Riehl & Patzner (1998), a smooth zona radiata constitutes a less complex egg attachment apparatus that would not assure strong adhesiveness. The zona radiata ridges around the micropyle in *A. bimaculatus lacustris* eggs were also observed in the cyprinid *Barbus conchoni* (Hamilton) and appear to act as a device to capture and conduct spermatozoa to facilitate fertilization (Amanze & Iyengar, 1990).

Regarding the adhesive eggs of the sedentary Characiformes, the analysis revealed variable patterns according to the systematic group. Thus, the globules of *S. knerii* appear to be similar to the protuberances associated with the zona

radiata reported in vitellogenic oocytes of *Schizodon nasutus* (Kner), a sedentary, multiple spawner (Benjamin, 1996). Besides, *A. lacustris* and *A. britskii* that are sedentary, multiple spawners, with no parental care (Bazzoli & Godinho, 1991; Lamas, 1993) showed adhesive eggs with filaments at the vegetal pole. Similar appendages occur in a large number of teleost families (Riehl & Patzner, 1998) but the long and flexible filaments found in adhesive eggs of *Oryzias latipes* (Temminck & Schlegel) (Hart *et al.*, 1984) and *Odonthestes bonariensis* (Cuvier & Valenciennes) (Guandalini *et al.*, 1994) are apparently different structures. The knobs at the animal pole of *Acestrorhynchus* spp. eggs could be filaments that become shorter as they approached the micropyle. They are located among the zona radiata pore-canals leaving them open and not plugged as reported in salmonids (Groot & Alderdice, 1985).

The piranha *S. spilopleura* is a sedentary, multiple spawner with adhesive eggs and parental care (Lamas & Godinho, 1996). Submerged plants and roots seem to be the main substratum for the eggs of piranhas maintained in aquaria and probably also of those in the wild (Leão, 1996). The honeycomb-like pores and micropylar disc seen in *S. spilopleura* (present work) and in the piranha *Pygocentrus* (= *Serrasalmus*) *nattereri* (Kner) (Wirz-Hlavacek & Riehl, 1990) were also observed in *Serrasalmus brandtii* Reinhardt (unpubl. obs.), suggesting that this pattern may be characteristic of the group. Honeycomb-like pores are comparable to the chorionic respiratory system of the insect egg (Wourms & Sheldon, 1976), suggesting that this arrangement may contribute to fish egg oxygenation. The carboxylated and sulphated mucosubstances found at the external layer of the zona radiata of various piranha species, including *S. spilopleura*, may indicate a relationship between these substances and egg adhesiveness (Rizzo & Bazzoli, 1991; Bazzoli, 1992). Accordingly, an irregular pattern of a honeycomb-like arrangement containing mucopolysaccharides, probably responsible for egg adherence to sand and rocks, is seen in the asprete *Romanichthys valsanicola* Dumitrescu, Banarescu & Stoica, and in other percids (Riehl & Patzner, 1998).

Hoplias cf. *lacerdae*, a sedentary Characiformes, is a multiple spawner, bearing adhesive eggs, with parental care (Lamas, 1993). The villi present at its egg surface are probably responsible for the egg's strong adhesiveness. This pattern is similar to that found in various cyprinid species in which the villi have variable length and distribution and exhibit a strong carbohydrate histochemical reaction (Riehl & Patzner, 1998).

The jelly coat is present in adhesive eggs of several Siluriformes: *Pimelodella lateristriga* (Müller & Troschell) (Ihering & Azevedo, 1936a), in some *Rhamdia* spp. (Godinho *et al.*, 1978; Espinach Ros *et al.*, 1984; Sato, 1999; present work), in *Silurus* spp., *Ictalurus* spp. and *Chrysichthys* spp. (Legendre *et al.*, 1996), *F. marmoratus*, *L. alexandri* and *R. aspera* (Sato, 1999, present work). The jelly coat also occurs in Siluriformes non-adhesive eggs of *Conorhynchus conirostris* (Valenciennes), *P. maculatus*, *Pseudopimelodus zungaro* (Humboldt), *P. coruscans* and *P. luetkeni* (Sato, 1999; present work) indicating that it apparently bears no relationship with egg adhesiveness. The jelly coat is also present in other teleost groups such as Perciformes, Cypriniformes, Cyprinodontiformes (Riehl & Patzner, 1998), in the characiform *A. bimaculatus lacustris* (Ihering & Azevedo, 1936b) and in cartilaginous fishes such as the sturgeons (Cherr & Clark, 1985).

In the present work, various arrangements of the jelly coat were observed among different eggs of a single batch (mainly in *R. aspera*) and among eggs of different species. Such differences may be ascribed to artefacts since the jelly coat may be extracted by egg friction or during histological preparations (Cherr & Clark, 1985; Groot & Alderdice, 1985).

The micropylar disc of *S. spilopleura* and *R. aspera* resembles the adhesive disc of *P. nattereri* (Wirz-Hlavacek & Riehl, 1990), *Clarias gariepinus* (Burchell) (Riehl & Appelbaum, 1991), *Cichlasoma nigrofasciatum* Günther (Busson-Mabillot, 1977) and *Polypterus* spp. (Bartsch & Britz, 1997). In *S. spilopleura* and *R. aspera* this apparatus does not contain filaments as reported in other species. Despite the absence of data that elucidate the mechanism by which eggs of these species adhere to substrata, it could be postulated that the micropylar disc may play a role in their adhesiveness. Since the micropyle in these fishes is located in the centre of the micropylar disc, synchronization of male and female spawning behaviour is of striking importance (Riehl & Appelbaum, 1991). In this case, the eggs should be fertilized before they attach to the substratum as in *P. nattereri* (Wirz-Hlavacek & Riehl, 1990).

In conclusion, the egg surface pattern is related to the degree of egg adhesiveness and is the same at the gender level in Characiformes; on the other hand, there is a strong correlation between jelly coat and Siluriformes eggs, apparently without a relationship between this structure and adhesiveness.

RELATIONSHIPS AMONG SPECIES

The Characiformes of clusters II (except *L. friderici* and *L. piau*) and IV and the Siluriformes of cluster V (except *P. maculatus* and *R. quelen*), all of which are migratory species with non-adhesive eggs, correspond to the nonguarding, egg-scattering pelagic spawners, a reproduction style that might be considered ancestral in the evolution of fishes (Balon, 1984). Eggs of these fishes exhibited three different surface patterns: thin fibrillar net (cluster II), smooth zona radiata with pore-canals (cluster IV) or thin jelly coat (cluster V). These less complex patterns may constitute less evolved surface arrangements of Characiformes and Siluriformes eggs.

The jelly coat on the egg surface of Siluriformes is one of the main features that allowed cluster differentiation between Characiformes and Siluriformes (Sato, 1999; present study). In the analysis, Characiformes with non-adhesive eggs were placed on one side and the Characiformes with adhesive eggs, on the opposite side, with the Siluriformes between them. The Characiformes with non-adhesive or weakly adhesive eggs formed a large group (clusters I to IV). The Siluriformes with non-adhesive eggs (cluster V) and with adhesive eggs (clusters VI and VII) were also placed together in a large group, due to the presence of the jelly coat.

Few works have been published on the phylogenetic relationships between the different groups of Characiformes (Fink & Fink, 1996), and no consensus exists as to the number of families to recognize (Nelson, 1994). According to Vari (1998), Prochilodontidae and the Curimatidae are sister-groups of a lineage that comprises Anostomidae and Chilodontidae. In the present study, egg adhesiveness and surface pattern were similar among the member of Anostomidae (except

L. taeniatus and *S. knerii*) and Prochilodontidae (cluster II), whereas the eggs of the curimatid *C. lepidura* had distinct characteristics that placed it together with *T. guentheri* (cluster III).

Characiformes with adhesive eggs (clusters VIII to XI) were placed separately from those with non-adhesive or weakly adhesive eggs. Each of these groups exhibited a characteristic egg surface pattern. Although the Characiformes are considered more primitive than the Siluriformes (Nelson, 1994), the adaptive irradiation to which they were subjected during the separation of the continents led to a greater speciation, resulting in a wide variation of reproductive behaviour, which then could explain the distancing between their clusters. According to Winemiller (1989) the phenotypic variations of the reproductive behaviour patterns of Characiformes represent evolutionary divergences from an ancestor with more limited traits. He considers that if life history traits represent adaptations, and hence are derived from natural selection, they are not merely phenotypic artefacts. Thus, different taxa sometimes exhibit subsequent convergence in characters, as occurred with *S. knerii* and *B. cf. affinis* that showed globules in the egg surface.

Characidae is a large, diversified group of fishes, on which few phylogenetic studies have been conducted and whose classification could be subject to changes (Nelson, 1994; Weitzman & Malabarba, 1998). Buckup (1998) proposed that *Acestrorhynchus* should be excluded from Characidae, as it is more closely related to other groups, such as the erythrinid *Hoplias*. According to Buckup (1998), *Bryconops*, included in the Tetragonopterinae, should also be subjected to a phylogenetic analysis. With regard to the egg surface pattern and degree of egg adhesiveness in *B. cf. affinis* (cluster XI), it differed from those of the Tetragonopterinae of cluster I. Similarly, the oogenesis of *B. cf. affinis* differs from that of other Tetragonopterinae (Bazzoli & Godinho, 1994).

Oligosarcus spp. is another group subject to discussion within Characidae since it appears to be more closely related to Tetragonopterinae than to *Acestrorhynchus* spp. (Buckup, 1998). In fact, eggs of *Oligosarcus argenteus* Günther and *Oligosarcus paranensis* Menezes & Géry showed ridges arranged radially to the micropyle (unpubl. obs.), a characteristic present in various Tetragonopterinae, such as *A. bimaculatus lacustris* (present paper), *Hemmigramus marginatus* Ellis and *Hyphessobrycon eques* (Steindachner) (unpubl. obs.). Therefore, the egg surface pattern of *Oligosarcus* spp. differs from that of *Acestrorhynchus* spp.

Apparently, Serrasalminae constitutes a monophyletic group (Machado-Alisson, 1983) which includes *Serrasalmus*, *Colossoma* and *Piaractus*, among others. Although they tend to be considered as a family, no phylogenetic evidence exists that could suggest that they are not related to Characidae (Buckup, 1998). The eggs of *S. spilopleura* (cluster IX) showed a surface arrangement that is typical of the genus, yet different from the eggs of *C. macropomum* and *P. mesopotamicus* (cluster IV).

As concerns the Siluriformes, most Pimelodidae in this study had non-adhesive eggs and a similar egg surface pattern. In the clusters, however, *L. alexandri* was closer to the doradid *F. marmoratus* and to the loricariid *R. aspera* than to the other pimelodids probably as a consequence of the heterogeneity of the Pimelodidae (Pinna, 1998).

Although the silurid *R. aspera*, from the Upper Paraná River basin, had been considered as a migratory fish with no parental care (Agostinho & Ferreira Júlio Jr, 1999; Suzuki *et al.*, 2000), the presence of a micropylar disc and thick jelly coat in its adhesive eggs approximated it to the sedentary fishes with parental care, as indicated also by Sato (1999) in individuals from the São Francisco River basin.

As concerns the strategy of egg guarders, evolution tends to progress from less to more protective styles (Balon, 1984). In this respect, the Serrasalminae and Erythrinidae (among the Characiformes) and *F. marmoratus*, *L. alexandri* and *R. aspera* (among the Siluriformes) exhibited more derived reproductive characteristics (Sato, 1999). It may thus be suggested that egg surface pattern (honeycomb-like pores, villi, thick jelly coat and micropylar disc) in these fishes can be considered as having evolved from more primitive egg surface patterns.

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