

The Anabantoid Genera *Ctenops*, *Luciocephalus*, *Parasphaerichthys*, and *Sphaerichthys* (Teleostei: Perciformes) as a Monophyletic Group: Evidence from Egg Surface Structure and Reproductive Behaviour

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Abstract The surface structures of eggs of the anabantoid species *Ctenops nobilis*, *Luciocephalus pulcher*, *Parasphaerichthys ocellatus* and *Sphaerichthys osphromenoides* are described for the first time after observations with scanning electron microscope. Eggs of these species share a distinctive surface pattern which is not found in any other anabantoid species or even in any other teleost. It consists of a system of almost equidistant ridges. They originate at the vegetal pole and run parallel towards the animal pole where they end in a counter-clockwise spiral near the micropyle. This peculiar pattern presents strong evidence for the monophyly of a group which consists of the genera *Ctenops*, *Luciocephalus*, *Parasphaerichthys* and *Sphaerichthys*. In addition, the derived pear shape of the eggs of *Luciocephalus* and *Sphaerichthys* indicates that they are more closely related to each other than either is any to *Ctenops*. Due to the lack of information about the egg shape of spawned eggs of *Parasphaerichthys* the precise phylogenetic placement of that genus within the monophyletic four-taxon-group remains open.

The monophyletic origin of that group is further corroborated by the shared mouthbreeding behaviour in *Ctenops*, *Luciocephalus* and *Sphaerichthys*. To date the reproductive behaviour of *Parasphaerichthys* is unknown.

The egg surface structure of *Luciocephalus* sp., a new anabantoid species has been described in a recent SEM-study (Riehl and Kokoscha, 1993). The zona radiata of its eggs possesses a system of parallel ridges that spiral towards the micropyle. No other teleost was known whose eggs exhibit such a peculiar surface pattern. Comparative data based on SEM-studies of other anabantoid eggs were not available at that time apart from the short descriptions of the eggs of *Anabas testudineus* (Moitra et al., 1987) and *Sandelia capensis* (Cambray, 1990). It is the purpose of this paper to compare the distinct egg surface pattern found in *Luciocephalus* sp. with that of other labyrinth fishes and to evaluate the phylogenetic significance.

Material and Methods

Specimens of the species *Luciocephalus pulcher* (Gray, 1830), *Ctenops nobilis* M'Clelland, 1844 and

Sphaerichthys osphromenoides Canestrini, 1860 were bred in captivity. The newly spawned fertilized eggs were fixed either in 4% glutaraldehyde in cacodylate buffer or in 2.5% glutaraldehyde in veronal acetate buffer. The samples were washed with the same buffers, dehydrated in a graded series of ethyl alcohol, critical point dried with liquid CO₂ in a Polaron E 3000 series II critical point drying apparatus and mounted on aluminium stubs. All mounts were coated with 20 nm gold-palladium in a Balzers SCD 030 sputtering device. They were observed and photographed using a Cambridge Stereoscan 250 Mk2.

Unfertilized eggs of the poorly known species *Parasphaerichthys ocellatus* Prashad and Mukerji, 1929 were obtained from a gravid female. This specimen was obtained by Tyson Roberts on the market in Mandalay, Burma, in April 1993 and fixed in formalin. The female was carefully dissected and the eggs removed from the body cavity. The adhering maternal tissue was removed with a pair of fine

tweezers after ultrasonic treatment. Afterwards the eggs were submitted to the same procedure as described above.

All fixed eggs and formalin fixed adult specimens are part of the private collection of one of the authors (coll. Britz, Tübingen) and will be stored in a museum collection after work is completed.

Results

Surface structure of the eggs

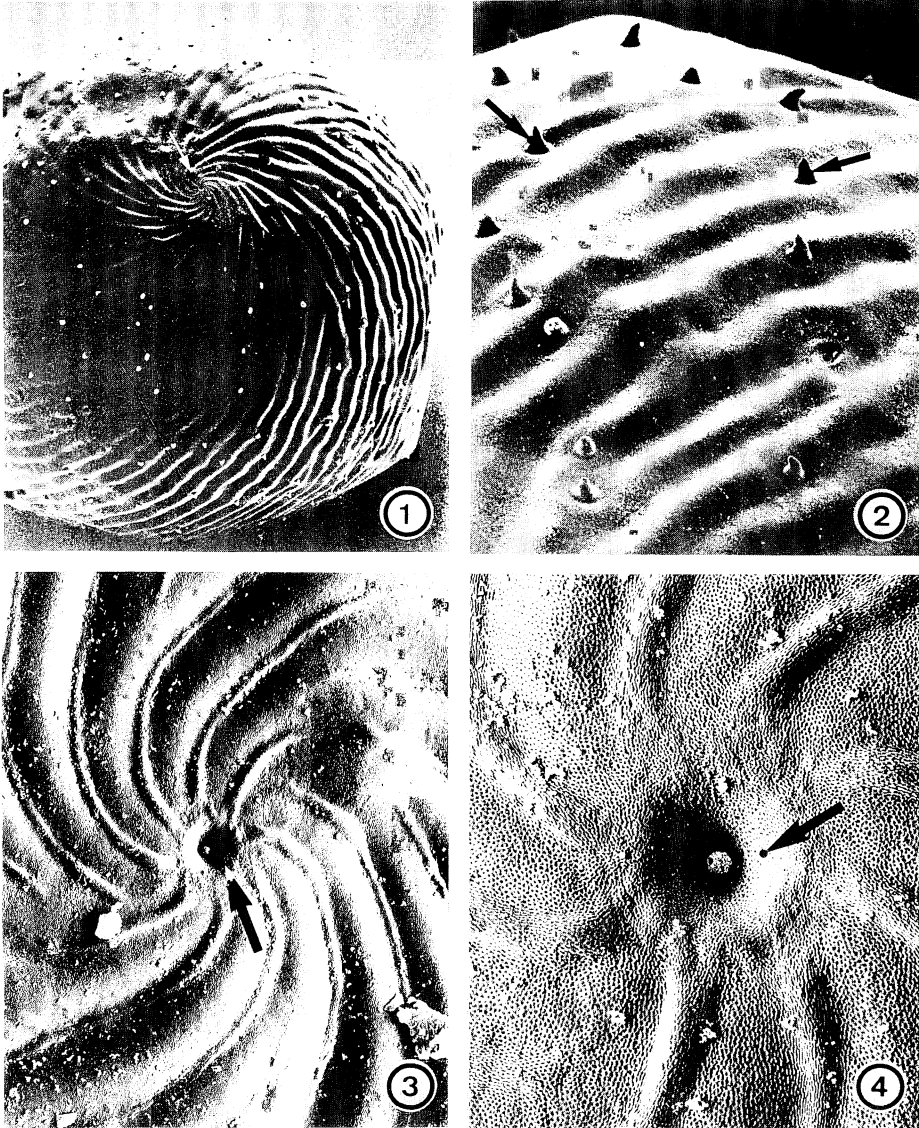
Ctenops nobilis (Figs. 1–4).—The almost spherical eggs of *C. nobilis* measure from 1.9 to 2.1 mm in diameter ($n=5$). Eggs of one-year old F1 specimens (not fully grown) are somewhat smaller, ranging from 1.5 to 1.7 mm ($n=5$). In the SEM the eggs show a zona radiata with a special surface structure of parallel ridges. Originating from an irregular pattern at the vegetal pole (i.e. the pole opposite the micropyle) the ridges run parallel towards the micropyle. Together with the increasing diameter of the egg from each pole to the equator also the number of ridges increases, being highest in the equatorial region of the egg. Most of the ridges stop at various distances from the micropyle. Only some of them actually reach the micropylar region and end at the edge of a micropylar pit in a counter-clockwise directed spiral (Fig. 3). The pit has a width of 10–13 μm . The micropyle canal is about 2.5 μm in diameter and lies inside the pit (Fig. 4). On the ridges and within the intermediate furrows of the zona radiata a pattern of protuberances can be recognized (Fig. 2). These conical projections, not observed in any other species, measure about 20 μm in basal diameter and also in length. In addition circular grooves of a similar diameter occur on the surface but are much less frequent. They probably represent deformed projections. At higher magnifications (see Figs. 4, 8, 12, 16) the pore canal openings are visible on the whole egg surface. They are reported for a wide range of teleost eggs (see for example Stehr and Hawkes, 1983 and the review of Riehl, 1991) and were also found in all investigated anabantoids. All surface structures including the projections are already present in ovarian eggs of *Ctenops*.

Sphaerichthys osphromenoides (Figs. 5–8).—The eggs of *S. osphromenoides* are pear-shaped (see Fig. 5). The distance from the micropyle to the

opposite pole varies from 2 to 2.2 mm ($n=3$). The diameter of the transversal axis ranges from 1.9 to 2.0 mm ($n=3$). The zona radiata shows a similar surface structure of ridges as described for *C. nobilis*. They originate at the vegetal pole (Fig. 6), run parallel towards the micropyle and end in a counter-clockwise spiralling pattern at the micropylar pit (Fig. 7) similar to that in *Ctenops*. Again the number of ridges is highest at the widest part of the egg (see Fig. 5). Their number decreases towards each pole as in *C. nobilis*. The micropylar pit has a width of 17 μm . The micropyle canal lying inside of that pit measures 2.5 μm in diameter. There are no projections on the zona radiata.

Parasphaerichthys ocellatus (Figs. 9–12).—Only unfertilized eggs from the ovary of *P. ocellatus* were available for the study. It is known from other teleosts that the egg diameter increases and the outer shape changes when the eggs are laid due to the formation of the perivitelline space (Laale, 1980). These changes were also observed in the eggs of anabantoids investigated in this study, e.g. *Luciocephalus pulcher* and *S. osphromenoides*. Thus, no observations are available concerning the actual shape or size of spawned eggs of *P. ocellatus*. The biggest eggs taken from the ovary were supposed to be the ripest ones. They have a diameter of about 1.2 mm. Their egg surface exhibits a very similar pattern of ridges (Fig. 9) as those described for the eggs of the preceding species. Here too the ridges originate from an irregular pattern at the vegetal pole (Fig. 10) and only some of them actually reach the animal pole, where they spiral in a counter-clockwise direction to the micropyle (Figs. 9, 11). The micropylar pit appears to be more shallow than in *Ctenops* and *Sphaerichthys* but possibly becomes deeper when the eggs are spawned. The diameter of the pit is 6.7 μm . The micropyle canal has a width of 1.9 μm (Fig. 12). No projections are present on the zona radiata. Likely this holds for spawned eggs of *P. ocellatus* too, because the projections on the eggs of *Ctenops* can already be recognized in ovarian eggs.

Luciocephalus pulcher (Figs. 13–16).—The eggs of *L. pulcher* are similar to those of the undescribed *Luciocephalus* sp. investigated by Riehl and Kokoscha (1993). They are almost pear-shaped. The tapering vegetal pole provides space for the head of the developing embryo (R. Britz, pers. obs.). The distance from pole to pole varies from 3.1 to 3.2 mm, that of the transversal axis from 2.9 to 3.2 mm ($n=5$). A similar pattern on the zona radiata is present



Figs. 1-4. *Ctenops nobilis*.

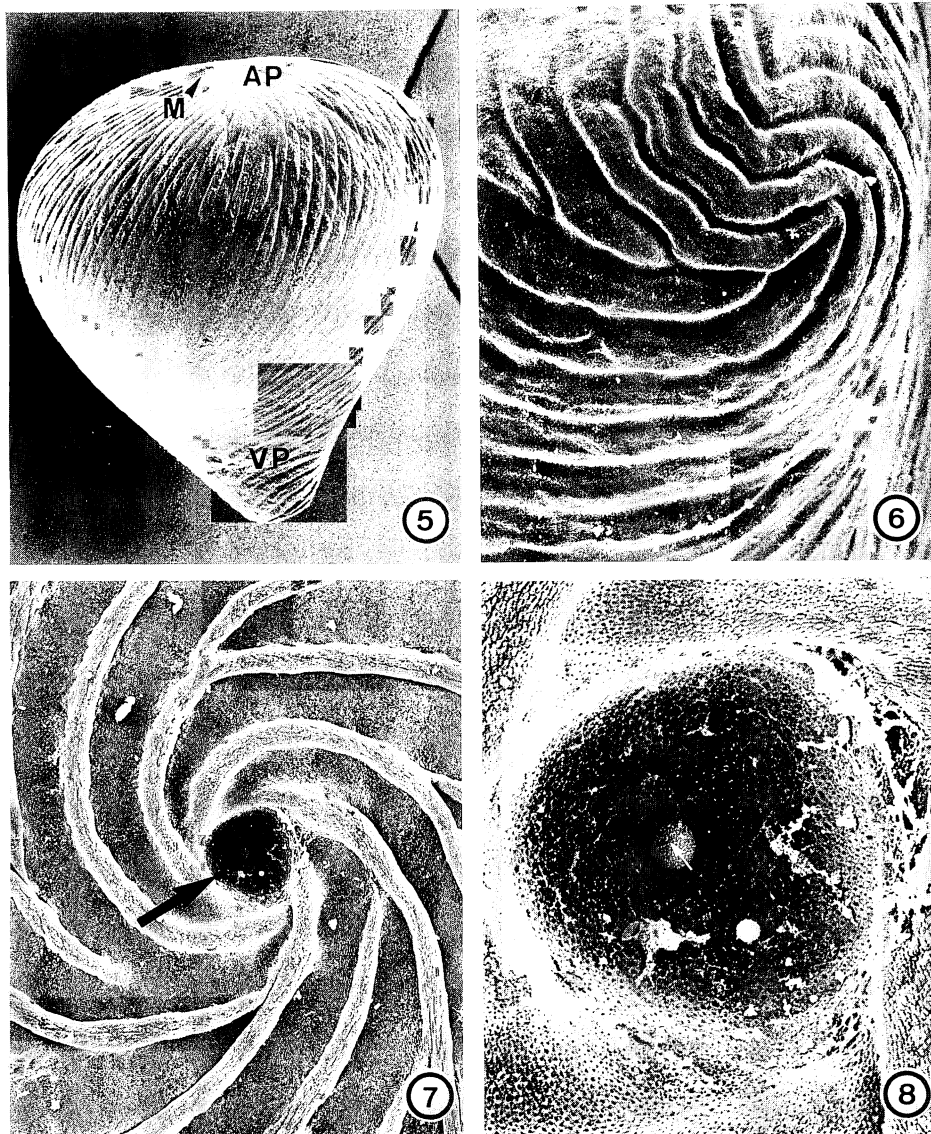
Fig. 1. View on the animal pole of the egg at low magnification. The white arrow points to the micropyle. Note the parallel ridges and the conical projections 60.5 \times .

Fig. 2. Part of the zona radiata at higher magnification to demonstrate the conical projections (black arrows) 213 \times .

Figs. 3, 4. Micropylar region at higher magnifications. Note the spiralling ridges which end at the micropylar pit (arrows point to the micropyle). Fig. 3: 620 \times , Fig. 4: 1860 \times .

as described for *Ctenops*, *Sphaerichthys* and *Parasphaerichthys*. A system of ridges originates at the vegetal pole of the egg (Fig. 14). They run parallel towards the animal pole and stop in a counter-clockwise directed spiral at the micropylar pit (Figs. 15, 16). The pit varies in width from 17.5 to

22.8 μm . The diameter of the micropyle canal which lies inside of the pit ranges from 1.5 to 1.7 μm . No projections are present on the zona radiata. The micropyle of the eggs of all four species belong to Type I according to the classification of Riehl and Götting (1974) and Riehl (1991).



Figs. 5–8. *Sphaerichthys osphromenoides*.

Fig. 5. The pear-shaped egg of *S. osphromenoides*. The arrowhead points to the micropyle 47.3 \times . AP—animal pole, VP—vegetal pole.

Fig. 6. The tapering vegetal pole at higher magnification 230 \times .

Fig. 7. The micropylar region showing the spiralling ridges ending at the micropylar pit (arrow) 835 \times .

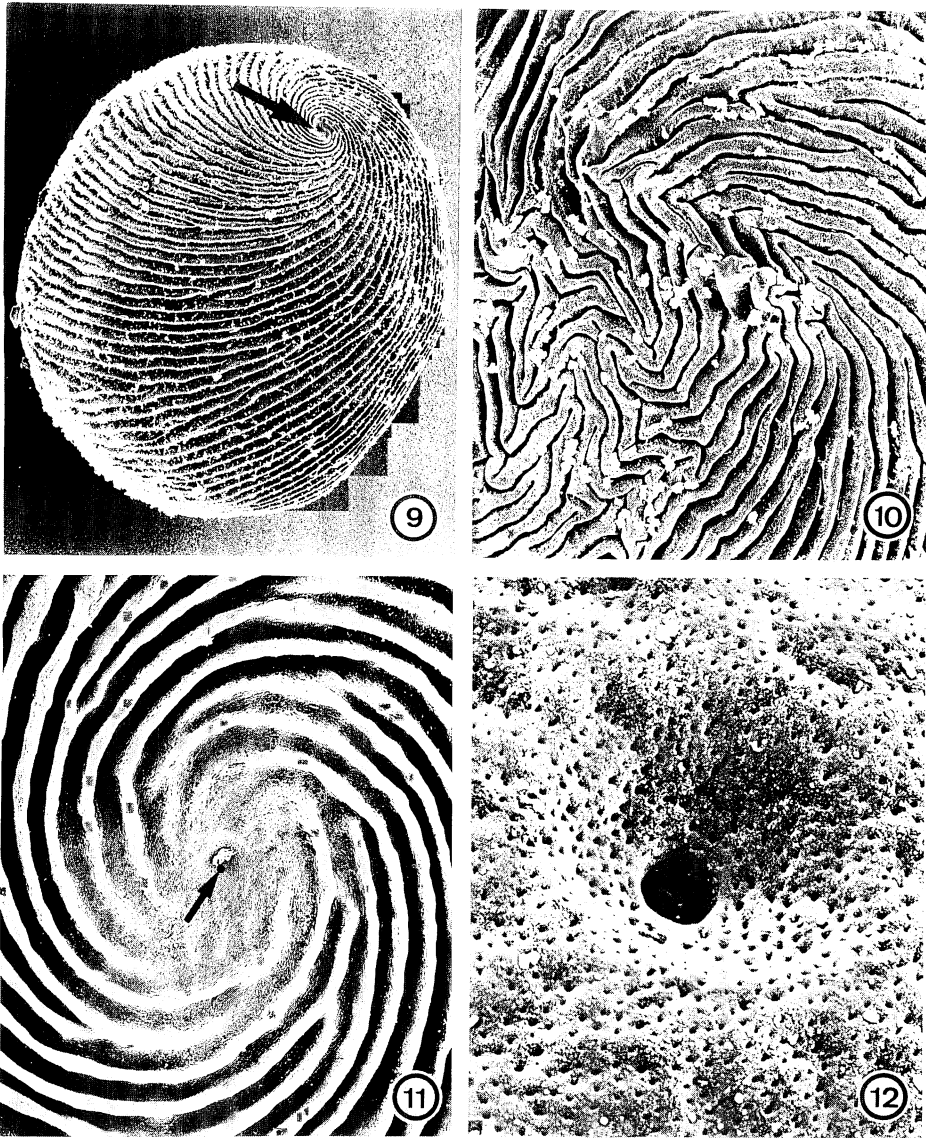
Fig. 8. The micropyle at higher magnification 3700 \times . The white arrowhead marks the micropyle canal.

Reproductive behaviour

The rare *C. nobilis* was recently discovered to be a mouthbrooding species (see Armitage, 1987; Bilke, 1992; Britz, 1992, 1993). It was demonstrated that the male broods the eggs (Britz, 1992) and not the female as had been assumed before.

Both *S. osphromenoides* and the closely related *S. acrostoma* are reported to be mouthbrooders, too (see Richter, 1972; Godfrey, 1976; Linke, 1990). In *S. acrostoma* the male mouthbroods, whereas in *S. osphromenoides* it is the female.

To date the reproductive behaviour of *P. ocellatus* is not known.



Figs. 9–12. *Parasphaerichthys ocellatus*.

Fig. 9. View on the spiralling ridges on the zona radiata with the micropyle marked by an arrow 140 \times .

Fig. 10. Irregular ridges at the vegetal pole 147 \times .

Fig. 11. The micropylar region with the spiral pattern around the micropyle (arrow) 844 \times .

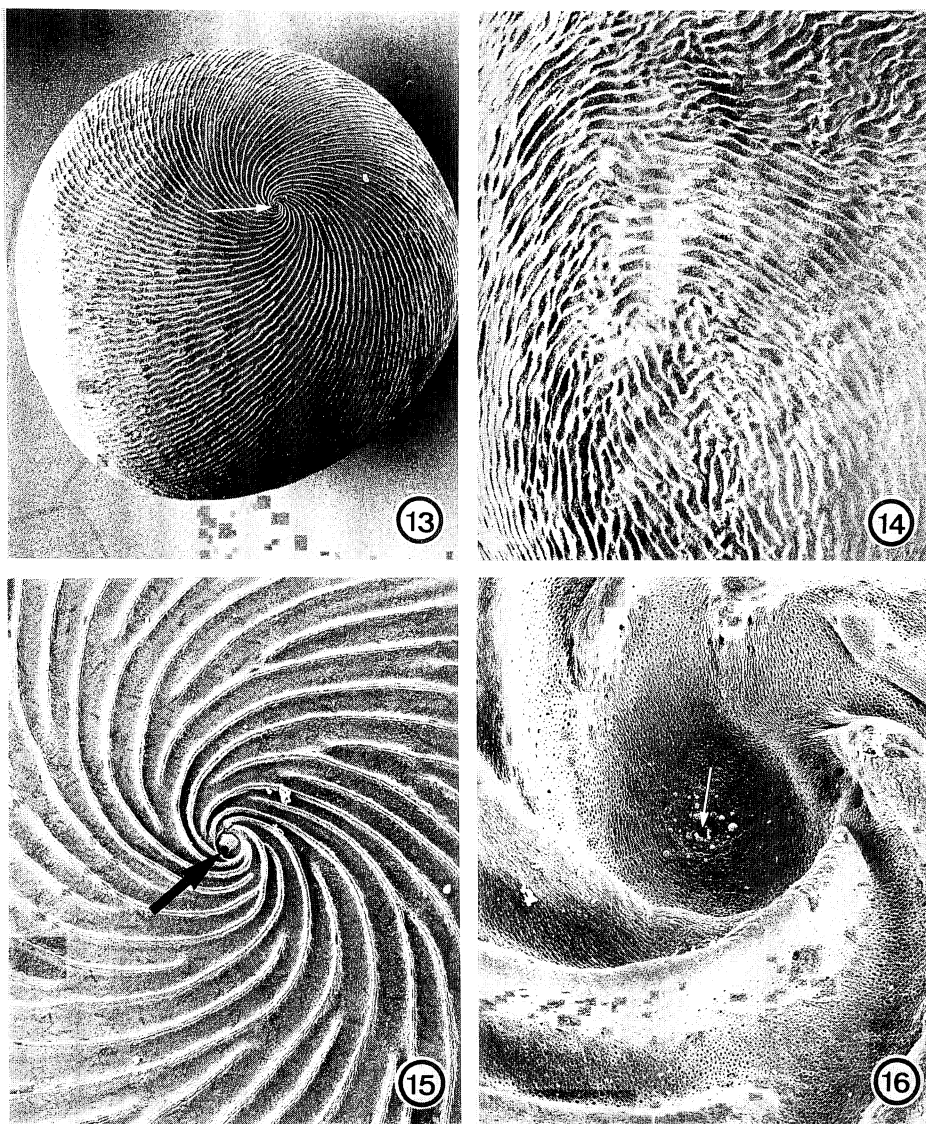
Fig. 12. The micropyle at higher magnification (5300 \times) to show the micropyle canal.

Volz (1904), Marsack (1951), Alfred (1966) and Korthaus (1978) consistently noticed during field observations that *L. pulcher* also exhibits mouth-brooding. In addition, Korthaus (1978) observed that the male carries the eggs and Kokoscha (1988) and Britz (1994a) confirmed this when they succeeded in breeding *L. pulcher* in captivity. The same reproductive behaviour is reported for the un-

described *L. sp.* (Riehl and Kokoscha, 1993).

Discussion

It has been shown that the genera *Ctenops*, *Luciocephalus*, *Parasphaerichthys* and *Sphaerichthys* share strikingly similar egg surface patterns. A broad



Figs. 13–16. *Luciocephalus pulcher*.

Fig. 13. View on the animal pole of the egg 27.8 \times . The white arrow marks the micropyle.

Fig. 14. Tapering vegetal pole showing the irregular pattern of ridges 60 \times .

Fig. 15. Animal pole at higher magnification 232 \times . The arrow points to the micropyle.

Fig. 16. Micropylar pit with micropyle canal (white arrow) 1920 \times .

survey of egg surface structures in anabantoids, including representatives of the genera *Ctenopoma*, *Anabas*, *Osphronemus*, *Belontia*, *Colisa*, *Trichogaster*, *Macropodus*, *Trichopsis*, *Malpulutta*, *Pseudosphromenus*, *Parosphromenus* and *Betta* (Britz, in prep.) reveals that none of these species has anything approaching this kind of pattern. It was also not reported for the anabantid *Sandelia* (see Cambray,

1990). The only anabantoid genus whose eggs could not be studied is *Helostoma*. However, this genus seems to be only distantly related to the genera under consideration (Lauder and Liem, 1983). Thus, the distinct egg surface structure is interpreted as a synapomorphy of the genera *Ctenops*, *Luciocephalus*, *Parasphaerichthys* and *Sphaerichthys* and as evidence for the monophyly of that assemblage (Fig.17).

Phylogenetic Relationships of *Luciocephalus*

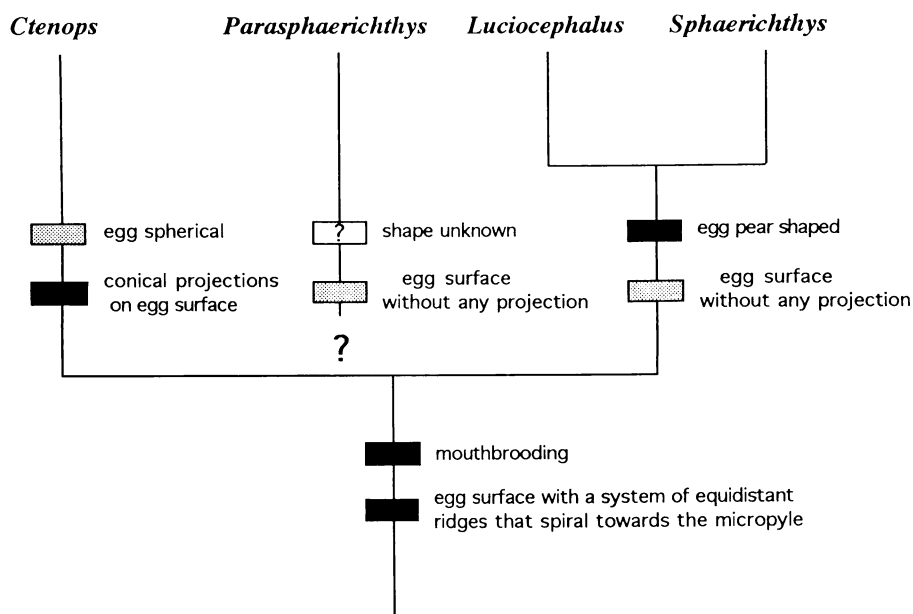


Fig. 17. Phylogenetic tree summarizing the results of this study. Black squares symbolize apomorphic, grey squares plesiomorphic character states. Apart from the egg surface structure all other characters shown on the tree are unknown in *Parasphaerichthys* and its precise position within this monophyletic assemblage remains unclear at present. Theoretically it could be the sister group of 1) all three genera, 2) *Ctenops* only, 3) *Luciocephalus* + *Sphaerichthys*, 4) *Luciocephalus* only and 5) *Sphaerichthys* only.

Apart from *C. nobilis*, a spherical or ovoid egg-shape was observed in all anabantoid species with the exception of the genera *Luciocephalus* and *Sphaerichthys*. Judging from the present hypothesis about anabantoid intrarelations (see Lauder and Liem, 1983 and its modification by Britz, 1994b), a spherical egg shape represents the plesiomorphic character state for the Anabantoidei. This is further supported by the fact that in taxa supposed to be close relatives of the Anabantoidei like Badidae, Nandidae or Channidae, the egg shape is also spherical (for *Badis* and the nandid *Polycentrus* see Barlow et al., 1968, for *Nandus* see Parameshwaran et al., 1971, for *Channa* see Willey, 1910). The shared derived pear-shape of the eggs of *Luciocephalus* and *Sphaerichthys* may indicate that these two genera are more closely related to one another than is either of them to *Ctenops* (Fig. 17). The actual shape of spawned eggs of *P. ocellatus* remains unknown. Therefore, its phylogenetic position within the monophyletic four taxon group *Ctenops* + *Parasphaerichthys* + *Luciocephalus* + *Sphaerichthys* cannot be given more precisely. The conical projections on the zona radiata of *Ctenops* are an autapomorphy of that taxon (Fig. 17).

Riehl and Kokoscha (1993) proposed a sperm guidance function for the spiral surface pattern in *Luciocephalus* sp. This explanation could hold also for the eggs of *C. nobilis*, *L. pulcher*, *P. ocellatus* and *S. osphromenoides*. However further observations are required to confirm this hypothesis.

The reproductive behaviour adds to the evidence for a close relationship of the genera *Ctenops*, *Luciocephalus* and *Sphaerichthys*. Due to the lack of relevant data from *P. ocellatus* this species cannot be considered at present. As long as there is no contradictory evidence, the shared mouthbrooding behaviour of *Ctenops*, *Luciocephalus* and *Sphaerichthys* can be hypothesized as another synapomorphic character of this group (Fig. 17). Apart from these genera, mouthbrooding occurs within the anabantoids only in some members of the genus *Betta* and is therefore interpreted as a parallel development within that genus. Consequently, at the present state of knowledge mouthbrooding evolved only twice in anabantoids, unless it is convincingly shown that it evolved more than once in the genus *Betta*. This rejects Vierke's (1991) thoughts about a multiple evolution of mouthbrooding in anabantoids. A detailed comparative analysis of the reproductive behaviour of

the genera *Ctenops*, *Luciocephalus* and *Sphaerichthys* (Britz and Kokoscha, in prep.) provides additional support for the phylogenetic relationships hypothesized in this study.

Liem (1963) divided the Anabantoidei into four families: Anabantidae, Helostomatidae, Osphronemidae and Belontiidae. Lauder and Liem (1983) added the family Luciocephalidae with the single genus *Luciocephalus* to the Anabantoidei. There it forms the sister group of the remaining labyrinth fishes. The genera *Ctenops*, *Sphaerichthys* and *Parasphaerichthys* are members of the family Belontiidae according to Liem (1963, 1965). He classified them in different subfamilies: *Sphaerichthys* and *Parasphaerichthys* belong to his Trichogasterinae, whereas *Ctenops* was included in his Macropodinae and should be most closely related to the genus *Trichopsis* (Liem, 1965).

However, Liem's (1963) osteological definitions of the belontiid subfamilies were not based on shared derived characters and the hypothesized monophyly first has to be evaluated. Furthermore, in a recent investigation on the anatomy and osteological development of anabantoids it has been demonstrated that the characters for the phylogenetic placement of *Luciocephalus* given by Lauder and Liem (1983) are not corroborated (Britz, 1994b). Instead of representing the sister group of all remaining Anabantoidei, *Luciocephalus* is hypothesized to form a monophyletic group together with the Belontiidae, including *Osphronemus* (Britz, 1994b). Yet, the phylogenetic position of *Luciocephalus* within the three taxon group Belontiidae + *Osphronemus* + *Luciocephalus* remained unresolved in that study. The present investigations on the egg surface structure are relating to totally different aspects but are, nevertheless, in full agreement with the hypothesis of Britz (1994b). They even help to determine the phylogenetic position of *Luciocephalus* more precisely.

The results of this study may have importance for the classification of the anabantoid families. There is now evidence from two areas of investigation (Britz, 1994b and this paper) that *Luciocephalus* is not the sister group of all remaining anabantoids but is more closely related to a group within Liem's (1963) family Belontiidae. Needless to say that our hypothesis should be tested with other characters, but at the moment there is no evidence that contradicts the monophyletic origin of the genera *Ctenops*, *Parasphaerichthys*, *Sphaerichthys* and *Luciocephalus*. As a

consequence of this hypothesized phylogenetic position, the familial rank of *Luciocephalus* may become obsolete.

Acknowledgments

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卵の表面構造と繁殖行動にもとづくキノボリウオ亜目の *Ctenops*, *Luciocephalus*, *Parasphaerichthys* および *Sphaerichthys* の単系統性

Ralf Britz • Michael Kokoscha • Rüdiger Riehl

キノボリウオ亜目魚類の *Ctenops nobilis*, *Luciocephalus pulcher*, *Parasphaerichthys ocellatus* および *Sphaerichthys osphromenoides* の卵を電子顕微鏡を用いて観察し、その表面構造を初めて記載した。これらの魚類の卵は他のキノボリウオ亜目魚類および真骨魚類に見られない特有の表面構造を共有している。表面構造はほぼ等距離にある隆起から形成されている。隆起は植物極から始まり、動物極に向かって並行して走り、動物極の卵門付近で反時計方向の螺旋を形成する。この独特の構造は *Ctenops*, *Luciocephalus*, *Parasphaerichthys* および *Sphaerichthys* を含むグループの単系統性を強く示す。さらに、*Luciocephalus* と *Sphaerichthys* の派生的な洋ナシ形の卵は、これら2属の相互の類縁関係がそれぞれの属と *Ctenops* の類縁関係よりも近いことを示している。*Parasphaerichthys* の産出卵の形が不明なため本属の正確な系統的位置は不明である。上述の4属で構成されるグループの単系統性は *Ctenops*, *Luciocephalus* および *Sphaerichthys* が口内保育をすることで支持される。*Parasphaerichthys* の生殖行動は現在のところ不明である。