The egg surface structure of Thalassophryne amazonica, a freshwater toadfish from the Amazon basin is described. Eggs of this species show a remarkable, highly unusual system of parallel ridges and intermittent grooves that originate at the equator of the egg and lead to the micropylar pit, at which they end in a spiralling pattern. A similar egg surface structure has so far been described only from a group of Asian anabantoid percomorphs, obviously not closely related to Thalassophryne. This egg surface pattern may enhance fertilization success by guiding sperm to the micropyle. We review museum records for T. amazonica, present an updated map of its occurrence in the Amazon basin, and provide information on its habitat.

Introduction

Toadfishes of the family Batrachoididae are a group of about 80 bottom dwelling, predominantly marine species of bony fishes. Their phylogenetic interrelationships are not entirely clear. Morphological characters appear to link them to the Lophiiformes (Patterson & Rosen, 1989), but authors of recent molecular analyses claim a closer affinity with Synbranchiformes (Miya et al., 2005).

Some toadfishes, like the oyster toadfish Opsanus tau, are well known for their ability to produce audible sounds with sonic muscles attached to their swimbladder when disturbed or during courtship (see Fish & Mowbray, 1970). Toadfishes also have highly developed parental care, in which the male guards the nest of eggs and the hatchlings for several weeks until they are free swimming (Arora, 1948; Dovel, 1960).

While almost all batrachoidid species inhabit marine or brackish waters, five live in freshwaters (Collette, 1966, 1973, 1995; Collette & Russo, 1981; Silfvergrip, 1990) and at least one, Thalassophryne amazonica Steindachner 1876, inhabits tributaries of the Amazon River that are hundreds or thousands of kilometers from the sea (Collette, 1966). Up until Collette’s (1966) study, this freshwater toadfish was only poorly known because the type specimens have been lost. Collette (1966) provided a redesription and illustration of T. amazonica based on specimens from the rio Itaya in Peru and rio Conambo and rio Corrientes in Ecuador, all tributaries of the upper Amazon.

Along with the five species of the genus Daector, the six species of Thalassophryne belong to the batrachoidid subfamily Thalassophryninae, which is characterized by one of the most highly developed venom apparatuses in the fish world (Collette, 1966). Venom glands are associated with the hollow spines of the dorsal fin and the hollow opercular spine. They can inflict extremely painful wounds to humans, which are accompanied by dizziness, fever, and even necrosis of tissue close to the area where the spine penetrated the skin (Haddad Junior et al., 2003).
There is no information available on the biology of *T. amazonica*, although this species has been exported now and then as an aquarium fish since the 1990s. We used the repeated spawning activities in captivity of a group of *T. amazonica* to study its egg morphology in detail and provide a description of its unusual egg surface structure.

**Material and Methods**

Six mature individuals of *T. amazonica* were purchased from a wholesaler in Germany and kept in the aquarium facilities of the Natural History Museum in London. They were kept in a tank with the dimensions 80 cm x 40 cm x 40 cm, with tap water, pH 8.5 and conductivity of 850 µS. The bottom consisted of a 5 cm high layer of fine sand. The fish spent all their time buried in the sand. They accepted only live food and were fed once a week with live river shrimps (*Palaemon* spp.). Egg deposition occurred a few days after a 50 per cent water change with deionised water (50-100 µS) and was most likely triggered by the decrease in conductivity. Eggs were found on the surface of the sandy substrate on which they sat with their adhesive foot. Eggs were removed from the tank and fixed in either formalin or glutaraldehyde and subsequently dehydrated in a graded series of ethanol: 15, 30, 50, 70, 80, 90, 95, and 100%. Eggs were then critical point dried with liquid CO2 in a critical point dryer and coated with gold and observed and photographed in a ZEISS DSM 940 scanning electron microscope.

We also made a brief survey of specimens of *Thalassophryne amazonica* that are deposited in collections known to have large number of Amazonian species, in order to provide information on the current distribution of this species. Museum acronyms are as follows: INPA (Instituto Nacional de Pesquisas da Amazônia, Manaus); MPEG (Museu Paraense Emílio Goeldi, Belém), and MZUSP (Museu de Zoologia da Universidade de São Paulo, São Paulo).

**Results**

**Egg structure**

On three occasions eggs were found in the tank with the six *Thalassophryne*: 22 eggs on 4 Dec 2008, 16 eggs on 1 Oct 2010, and 21 eggs on 26 Oct 2010. Spawned eggs of *Thalassophryne amazonica* are large and the yolk is amber coloured (Fig. 1). They measure up to 5.6 mm at the equator and up to 4.2 mm from pole to pole. They have a whitish translucent foot with which they rest on the sandy bottom. Under the stereomicroscope the animal egg pole opposite the foot shows a surface structure of folds or ridges that lead towards the micropyle.

Scanning electron microscopy reveals additional details (Fig. 2). The spiralling surface pattern on the 7 µm thick zona radiata starts at the equator of the egg and consists of obliquely running ridges and intermittent grooves that extend towards the animal egg pole. At the animal pole these ridges and grooves converge towards the micropylar region. Here they end in a counter-clockwise spiral. The deep micropylar pit sits at the top of a slightly elevated crater-like area at the animal egg pole. The 5 µm wide micropyle is located in the middle of the 20 µm wide micropylar pit. The foot of the egg consists of a homogenous matrix and a number of sand grains are attached to it.

**Distribution and habitat**

In addition to the localities listed in Collette (1966), *Thalassophryne amazonica* has since been collected from the following localities (see map in Fig. 3): INPA 37635, 1, ESEC Juami-Japurá, rio Juami, AM, at beach, 2° 8’5”S 68°19’W, 5 Aug 2005, F. Mendonça and crew. MPEG 12527, 7, Igarapé do Onça, Municipality of Coari, AM, 4°52’8”S 65°18’4”W, Nov 2006. MPEG 17776, 1, Igarapé do Onça, Municipality of Coari, AM, 4°52’9.7”S 65°18’2.8”W, Aug 2007. MZUSP 30324, 1, rio Tefé, Jurupari, AM, 3°22’S 64°44’18”W, 1 Aug 1979, M. Goulding. MZUSP 31402, 1, rio Tefé, Vista Escura AM, 3°22’S 64°44’18”W, 4 Aug 1979, M. Goulding.

Specimens from the Peruvian Amazon were collected by Hans-Georg Evers but were not preserved (see Fig. 4 for habitat): rio Itaya, along the road between Iquitos and Nauta, km 40, Puente Tocon, 4°4’5”S 73°26’2”W, 18 Sep 2005 and 03 May/2008; rio Momon 3°40’2”S 73°7’1”W.

For three localities we have also water parameters: in the rio Itaya near lqiuitos, the species was found in acidic water with a pH of 5.5 to 6, a very low conductivity of 14 to 89 µS and a temperature of 25.8 to 30.4°C. The bottom was mud and the water turbid. The locality in the rio Momon also had similar water parameters. The rio Juami, showed again similar parameters, pH 5.38, an extremely low conductivity of only 6.6 µS, a temperature of 27.1°C, and an O2 content of 6.92 mg/1. The bottom was a mixture of sand and mud typical of enclosed beaches with slow flowing waters. The only water parameter we have for Igarapé do Onça is pH 6.4 to 7.8, slightly higher than in the localities listed previously. The igarapé was 20 to 80 cm deep and 12 m wide with a sandy bottom.

**Discussion**

The little published information on the early life history of batrachoidids was recently summarized by Collette (2006). The most detailed information on reproduction and early development is available for *Opsanus tau* through the work of Dovel (1960). The relatively few eggs (ca. 100) are around 5 mm and are attached to the undersides of objects with the aid of an adhesive disc at the vegetal egg pole. The newly hatched larvae measure already 7.4 mm and sit on a very large yolk sac the bottom of which is still attached to the inside of the remnants of the egg shell via a large attachment organ at its tip. The larvae remain attached until the yolk sac is resorbed and leave the nesting site as small juveniles with fully formed fins at 18 mm standard length. Eggs and attached larvae are guarded and fanned by the male. The development of *Porichthys notatus* (Arora, 1948) resembles that of *Opsanus* in all important details and may indicate that this is the primitive condition for at least
Fig. 1. a, An adult individual of *Thalassophryne amazonica*. Note the two erected dorsal fin spines, the large pectoral fins, and the confluence of dorsal and caudal fins. b–d, spawned eggs of *T. amazonica*. Eggs are ellipsoid and relatively large with a large amount of yolk. They sit on top of the sand with the aid of their whitish adhesive foot. Note spiralling pattern clearly visible at 40-times magnification with light microscopy. Arrow in c points to micropyle.
the two batrachoidid subfamilies Batrachoidinae and Porichthyinae. No information has been published for any members of the third subfamily Thalassophryninae. From our observations of captive *Thalassophryne amazonica* it seems that the reproductive behaviour of this species differs in several ways from that of *Opsanus* and *Porichthys*. Eggs were not attached to any substrate but sat on top of the sand with the aid of their adhesive foot. There appeared to be no parental care, as the eggs were not fanned or guarded. We cannot, however, exclude the possibility that the eggs we discovered in the aquarium were not spawned and fertilised, but actually released by the female accidentally after the major water changes we performed.

The most remarkable feature of *T. amazonica* eggs is their surface pattern of parallel obliquely running ridges and grooves that end in a counter-clockwise spiral close to the micropyle. Ridged egg surfaces have been reported from few teleosts including, for example, the loricariid catfish *Sturisoma aureum* (Riehl & Patzner, 1991) the cyprinid *Puntius conchonius* (Amanze & Iyengar, 1990) and the characiform *Astyanax bimaculatus* (Rizzo et al., 2002). A spiralling pattern of ridges that run towards the micropylar area, however, is so
far only known from the anabantoid *Luciocephalus* (Riehl 
& Kokoscha, 1993; Britz et al., 1995) and its closest relatives  
*Sphaerichthys*, *Parasphaerichthys*, and *Ctenops* (Britz et al.,  
1995; Britz & Kottelat, 2002) and is now reported for *T.  
amazonica*. This spiralling system of ridges was hypothesized  
to increase success of fertilization and this may also apply to  
the spiralling pattern in *T. amazonica* eggs.

The adhesive foot of *Thalassophryne* eggs is also quite  
unusual among bony fishes with adhesive eggs, in that their  
adhesion is not the result of a system of filaments but of a  
homogenous substance. Adhesive egg filaments are quite  
widespread among teleosts and have been reported from  
gobies (Giulianini et al., 1994), blennies (Patzner, 1984),  
gobiesocids (Breining & Britz, 2000), chauhurid  
synbranchiforms (Kerle et al., 2000), different percoids (Mooi,  
1990; Mooi et al., 1990; Britz, 1997), cichlids (Stiassny & Mezey,  
1993) and are one of the defining synapomorphs of  
aitherinomorphs (Rosen & Parenti, 1981; Collette et al., 1983;  
White et al., 1983; Able, 1983). An adhesive foot consisting  
of a homogeneous substance is not very common and at  
least to our knowledge has so far been described only for  
*Indostomus paradoxus* (Britz, 2000), a member of the  
gasterosteiforms (Britz & Johnson, 2002). Clearly more  
information on the egg structure and other early life history  
characters of other batrachoidids and related taxa are needed  
to better evaluate the distribution and significance of the  
characters we report here for *T. amazonica*.

*Thalassophryne amazonica* is apparently a rare species  
known from relatively few specimens in collections, and it  
seems to be restricted to drainages from the middle and upper  
Amazon region. A preliminary survey of specimens from  
institutions that house large collections of Amazonian fish  
species (INPA, MPEG, and MZUSP), resulted in the record of  
5 lots all collected in tributaries of the Rio Amazonas above  
Manaus (Fig. 3).

It is clear from the water parameters reported to us that  
*Thalassophryne amazonica* prefers acidic waters with low  
conductivity and a higher temperature. The fish were found  
on a muddy or sandy bottom, in which they can supposedly  
bury themselves easily. A search for this type of habitat may  
help to locate this rare species also in additional streams of  
the upper Amazon.

In the original description of the species, Steindachner  
(1876:115) stated: “*Thalassophryne amazonica* scheint eine  
echte Süßwasser-Fischart zu sein, denn man kennt sie bisher  
nur von der Mündung des Rio negro und aus dem  
Amazonenstrom unterhalb Tabatinga (durch Natterer und  
Wesse1) sowie aus dem Xingu.” (“*T. amazonica* seems to be  
a true freshwater species, because it is only known from the  
mouth of the Rio Negro and from the Amazon river below  

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**Fig. 3.** Map of the northern portion of South America showing  
the distribution of *Thalassophryne amazonica* based on  
records we surveyed. Red dots indicate localities for which  
there is material deposited in collections; yellow dots indicate  
localities for which no specimens were deposited in  
collections. Black dots indicate type localities. The “?”  
indicates a possibly incorrect record for the species by  
Steindachner (see text for discussion).

**Fig. 4.** Habitat of *Thalassophryne amazonica* in two localities  
of the Peruvian Amazon where the species was collected.  
*a*. Río Itaya, along the road between Iquitos and Nauta, km 40,  
Puente Tocon (4°4’5”S 73°26’23”W);  
b. Río Momon (3°40’23”S  
73°17’14”W).
Tabatinga (by Natterer and Wessel), as well as from the Xingu”, our translation)

He thus reported it to be known from the mouth of the Rio Negro, from Tabatinga, and from the Rio Xingu. The first two localities are placed within the range of the species estimated from material deposited in the above mentioned collections, however we did not find specimens of Thalassophryne amazonica from the Rio Xingu. Unfortunately, the type specimens of this species could not be located (pers. comm. H. Wellendorf, 20 Jun 2012)

We checked Steindachner’s paper and found that Rio Xingu is mentioned four times, three of these in connection with the Thayer-expedition. The only time that there is no mention of the Thayer-expedition is in connection with T. amazonica. Natterer, a naturalist who travelled Brazil, and Wessel, a seller of natural history specimen, are mentioned on numerous occasions in Steindachner’s paper in connection with specimens from the mouth of the Rio Negro (Natterer) and Tabatinga (Wessel) respectively. It thus seems reasonable to assume that Steindachner had also at least two specimens available by these two individuals for his description of T. amazonica. There are no Xingu Thalassophryne specimens from the Thayer expedition in the collection on the Museum of Comparative Zoology (MCZ). However, there are two specimens of T. nattereri, a species Steindachner also described in the same paper, from the Thayer-expedition that were collected in Para. Based on our short review it appears most plausible to us that the Xingu locality mentioned by Steindachner was not based on specimens. We know of no T. amazonica specimen from the Xingu and all recent collections of this species are from the middle and upper Amazon exclusively (see Fig. 3 and Collette, 1966, 2003).

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Literature Cited


Salinópolis (Pará State) and Aracaju (Sergipe State), Brazil. Revista do Instituto de Medicina Tropical de São Paulo, 45: 221-223.


Silfvergrip, A. M. C. 1990. Additional specimens of the venomous toadfish Daector gerringi (Teleostei: Batrachoididae), hitherto known only from the holotype. Ichthyological Exploration of Freshwaters, 1: 253-256.
