CYST DEPOSITION BEHAVIOUR AND THE FUNCTIONAL MORPHOLOGY OF THE BROOD POUCH IN *STREPTOCEPHALUS TORVICORNIS* (BRANCHIOPODA: ANOSTRACA)

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A B S T R A C T

The cyst deposition behaviour of *Streptocephalus torvicornis* is described as the first documented case of active cyst deposition in Anostraca. The functional morphology of the brood pouch of *S. torvicornis* is described and illustrated using both Light Microscopy and Scanning Electron Microscopy. The brood pouch is an elongated tube-like structure with a subterminal crescent-shaped opening. During cyst deposition, the females insert this structure into the sediment to a depth of almost 10 mm, and deposit the resting cysts, analogous to an insect ovipositor. The opening mechanism of the genital pore is explained by contraction of a branched longitudinal muscle. The adaptive value of laying cysts into the soil and possible dispersal strategies are discussed.

Anostraca are inhabitants of ephemeral inland waters (e.g., Wiggins *et al*., 1980; Dumont and Negrea, 2002). They produce so-called "resting eggs"—actually cysts containing a gastrula embryo (Drinkwater and Clegg, 1991)—that can withstand long drought periods. In most cases, the resting cysts require periods of freezing or drought before they can hatch (Mossin, 1986; Brendonck, 1996). Soil samples of temporary waters often contain high numbers of anostracan cysts (Maffei *et al*., 2002). Cyst shell hardening and cyst maturation take place within the brood pouch, a ventral process of the last two thoracic segments, where the fertilized eggs mature into cysts and are kept until discharged. The brood pouch is also involved in insemination as the female genital pore is located terminally or subterminally on the brood pouch (Rogers, 2002). In many anostracan species, the brood pouch is more or less globular or longitudinally elongated. A conspicuous elongation of the brood pouch like in *Streptocephalus* is also found in other Streptocephalidae genera, in Branchinectidae and Thamnocephalidae, as well as in some small genera of other families within the Anostraca. The structures around the female genital pore in *Streptocephalus* seem complex and functionally not well explained (Wiman, 1981).

We have had the opportunity to observe a hitherto undescribed behavioural pattern in *Streptocephalus torvicornis* (Waga, 1842), which we think is connected to the specific morphology of the brood pouch.

MATERIALS AND METHODS

In September 1998, mature specimens of *S. torvicornis* were collected from ephemeral ponds in connection with the drainage system of a highway approximately 100 km south of Budapest, Hungary. A total of 200 specimens, including 50 adult females—mostly progeny of the originally collected specimens—was observed under laboratory conditions, in 50-L glass aquaria filled with de-ionised water, the bottoms covered with about 1 cm of soil from the original ponds. Swimming speed was recorded when the specimens (ten of each measured group) were swimming parallel to the front glass of the aquarium and measured in cm s^{-1}.

For morphological examination, ten adult females were fixed using 70% ethanol. For Scanning Electron Microscopy (SEM), three specimens were brought through a dehydration series of ethanol, transferred to acetone and critical point (CP) dried, using a BAL-Tech 030 CP-Dryer. Light Microscopy (LM) and SEM pictures were made at the Zoological Museum, University of Copenhagen, using a Leica MZ-95 light microscope and a JEOL JSM-840 or JEOL JSM-6335F scanning electron microscope with digital cameras.

RESULTS

Morphology

The twelfth and thirteenth thoracic segments of the female are produced ventrally. The brood pouch arises at the caudal margin of the thirteenth segment. It is an elongated tube-like structure, its end almost reaching the sixth abdominal segment (Fig. 1A).

The genital pore is located subterminally on the ventral side of the brood pouch. The opening is crescent-shaped and superficially divides the terminal part of the brood pouch into a ventral lip-like cover and a terminal tip-like portion (Fig. 1B, C). Light microscopy shows a longitudinal muscle on the dorsal side of the brood pouch, subdivided caudally into two branches (Fig. 1D). The ventral branch attaches medially to the dorsal wall of the genital opening, while the slightly thicker dorsal branch continues further into the tip-like portion and attaches dorsomedially, approximately 350 μm from the tip.

We interpret this muscle as the main component in opening the genital pore when the cysts are to be released. When contracting, the ventral branch pulls on the wall of the genital opening and widens its aperture, while the dorsal branch bends the tip-like portion dorsally (Fig. 1E, F). In conjunction, the described mechanism opens the genital aperture sufficiently for cyst release.

Behaviour

During our observations, the specimens swam continuously; males swam with an average speed of about 4.6 cm s^{-1}. Females with unfertilised eggs in their ovaries reached speeds in excess of 6.7 cm s^{-1}. Females with mature cysts in the brood pouch were less active and thus only reached speeds of 1.4 cm s^{-1}, sometimes hovering for longer periods.

Shortly before depositing the cysts, the females change their behaviour. They start to swim about one centimeter above the substratum touching it now and then with the dorsal side (Fig. 2A). After some minutes, the females turn around, now facing the substrate (Fig. 2B). The "thorax" is elevated and brought to almost right-angles with the abdomen (Fig. 2C). By actively swimming backwards towards the bottom, the brood pouch is inserted into the substratum (Fig. 2D). Maximum insertion depth is almost 10 mm, limited by the abdomen's position now touching the bottom. The females deposit the cysts, and a few seconds later the now empty brood pouch is pulled out of the substratum. The females swim off, once again taking up the normal inverted swimming position of anostracans. Insertion, cyst release, and extraction take a maximum of five seconds in total.

In some cases, if the substrate was of insufficient depth to insert the complete brood pouch, it was only inserted partly. The female swam three to four centimeters backwards leaving a groove in the surface. The brood pouch was not emptied in such cases, but it cannot be excluded that a few cysts were deposited. We observed up to ten such attempts within a period of approximately six minutes. None of the cyst-laying attempts, successful or not, took longer than five seconds. When no substrate was present, the females deposited the cysts into the slits between the aquarium glass and the sealant.

DISCUSSION

Usually, anostracans discharge their cysts into the water body (see Bludszuweit and Riehl, 1998). In some species, a preference for the periphery or the center of the inhabited ponds has been discussed (e.g., Maffei et al., 2002). A described exception is Artemiopsis stefanssoni Johansen, 1921. In this species, the genital pore is too small for cyst release. Daborn (1977) concludes that cysts of A. stefanssoni stay in the brood pouch until the decay of this structure after the female's death. In Eubranchipus (Siphonophanes) grubii (Dybowski, 1860), Gospodar and Winkelmann-Klöck (1982) reported a "clutch" of cysts attached to the leaf surface of a submersed plant. However, they could not confirm this observation under laboratory conditions, where the females always released their cysts into the water body.

The described behaviour of Streptocephalus torvicornis is the first documented case of directed cyst deposition in Anostraca. The brood pouch of S. torvicornis is actively used for cyst insertion, in analogy to the well-known insect ovipositor. Recently, D. Christopher Rogers reported observations of a very similar behaviour for Streptocephalus sealii Ryder, 1879, and Streptocephalus dorothae Mackin, 1942 (personal communication, Nov. 14, 2003).

The elongated shape of the brood pouch enables the females to inject the cysts into the soil to a depth of almost 10 mm. Wiman (1981) described the morphology of the brood pouch in other Streptocephalus species. He assumed that the female genital pore could be opened by muscular contraction to discharge fertilised
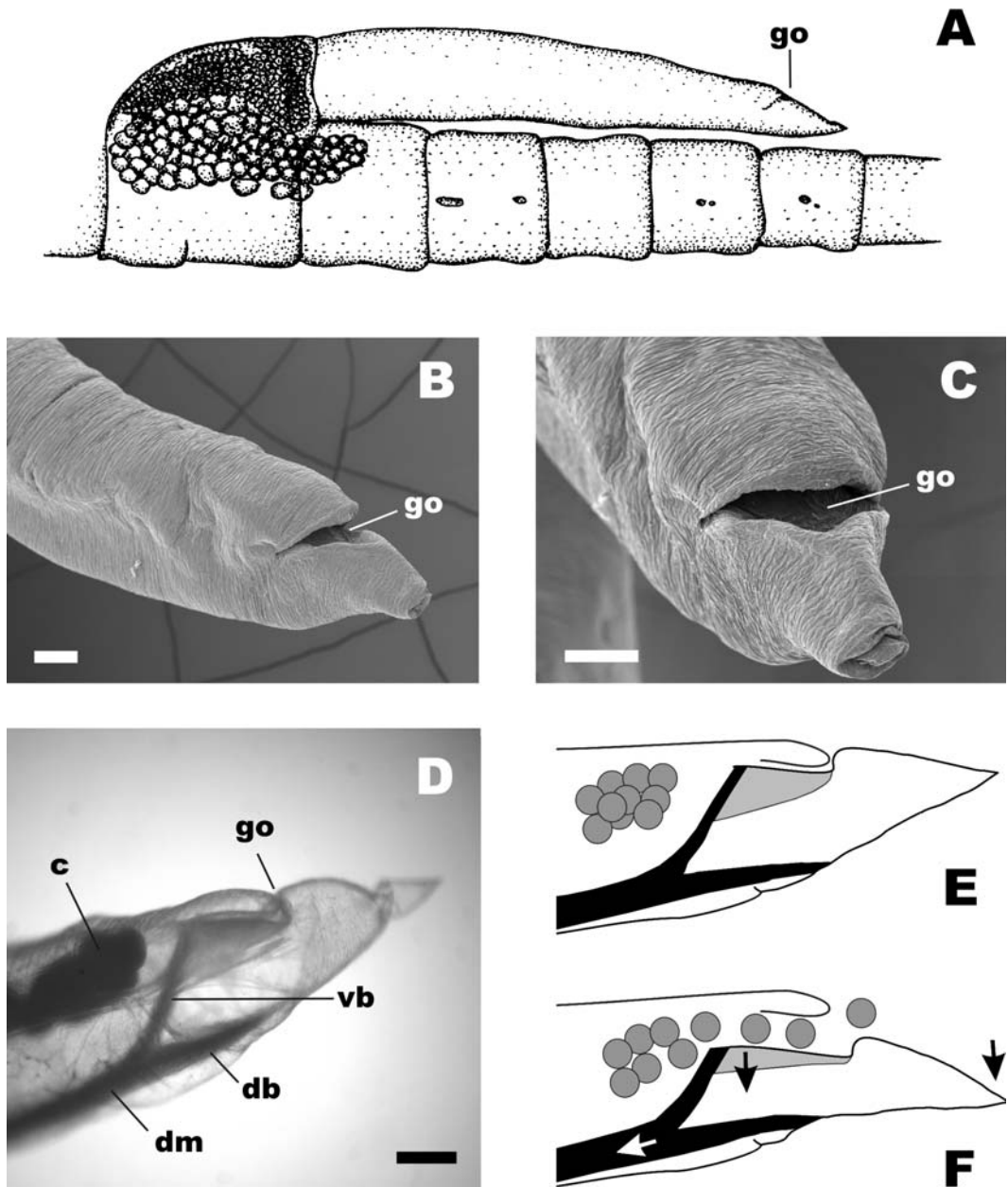
Fig. 1. Functional morphology of the brood pouch in *Streptocephalus torvicornis* (orientation: ventral side up). A, Lateral overview, based on light microscopy; B, lateral SEM view of brood pouch tip, genital pore slightly opened; C, caudal view on crescent-shaped genital pore; D, lateral LM view of brood pouch tip; E, F, schematic drawing of proposed cyst release mechanism, arrows indicate movement; E, genital pore closed; F, dorsal muscle contracts (white arrow), with its two branches simultaneously bending the brood-pouch tip and opening the pore (black arrows). Abbreviations: c = cysts, go = genital opening, dm = dorsal muscle, db = dorsal branch, vb = ventral branch. Scale bars are 100 μm.

cysts, but he apparently never observed the described mode of cyst-laying. During brood pouch insertion, the genital pore remains closed, possibly to avoid soil particles from entering.

When the brood pouch is fully inserted, the genital pore can be opened by the dorsal brood pouch muscle (Fig. 1D, E, F), thus allowing cyst deposition.
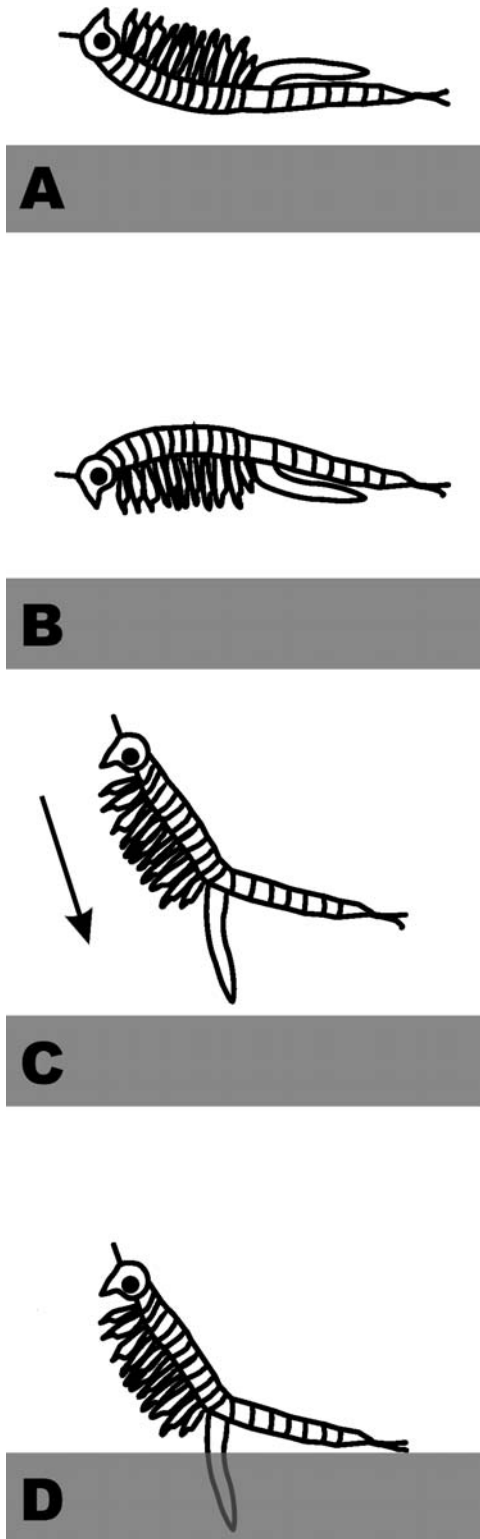
In two anostracan families, the brood pouch shape is a result of co-evolution of structures involved in the amplexus (Rogers, 2002). In all other families, there seem to be fewer constraints on the brood pouch morphology, except, for example, predation risk and number of cysts produced (Brendonck, 1995). The elongated brood pouch seems to be a pre-adaptation to the cyst deposition behaviour described above, but the presence of an elongated brood pouch does not impose such a behavioural pattern. Other anostracan species, e.g., *Branchinecta ferox* (Milne-Edwards, 1840), also have a conspicuously elongated brood pouch (see Petkovski, 1991), but discharge the cysts into the free water body.

The adaptive value of laying cysts into the soil remains uncertain. Possible explanations could be protection against predation of the cysts by notostracans, for example, and maybe also against radiation, which, however, is possibly provided by the cyst shell itself (Belk, 1970, for spinicaudatans). More importantly, wind-borne cyst dispersal is presumably reduced. This kind of nondirective dispersal may result in a relatively high loss of dispersal-prone cysts. Considering the fact that *S. torvicornis*, in comparison to other European anostracan species, is better adapted to deep longer-lived pools (Beladjal *et al.*, 2003), a nondirective dispersal strategy seems unfavourable in this case. Cyst dispersal via adult females ingested by water birds (Löffler, 1964; Procter, 1964) remains an infrequent but possible mode to encounter new suitable habitats, while a relatively stationary "safe" cyst bank stays present in the soil. A comparable, mixed (risk-spreading) dispersal strategy was described in the anostracan *Branchipodopsis wolfi* Daday, 1910, by Brendonck and Riddoch (1999).

Fig. 2. Phases of the cyst deposition behaviour in *Streptocephalus torvicornis*, schematic drawing. A, female

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approaching the sediment in normal inverted swimming position; B, female facing the substrate; C, dorsal body flexion, Female swimming backwards towards sediment; D, full insertion of brood pouch, cyst deposition.

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