J. Mar. Biol. Ass. U.K. (2001), **81**, 939–942
Printed in the United Kingdom

Larval brooding and development of the micromorph rhynchonellid *Tethyrhynchia mediterranea* (Brachiopoda: Recent)

Carsten Lüter

Museum für Naturkunde der Humboldt-Universität zu Berlin, Institut für Systematische Zoologie,
Invalidenstrasse 43, D-10115 Berlin, Germany. E-mail: carsten.lueter@rz.hu-berlin.de

Two developmental stages of the micromorph rhynchonellid *Tethyrhynchia mediterranea* (Brachiopoda: Tethyrhychiidae) are described using scanning electron microscopy (SEM). They were found in niches of the mantle cavity of adult females, as *T. mediterranea* broods its offspring between the protecting valves of the shell. The developmental stages of *T. mediterranea* are very small (~120 μm), but relative to adult body size of up to 1.2 mm in length they are larger than any other lecithotrophic brachiopod larva. Dispersal ability and phylogeography of *T. mediterranea* in the Mediterranean Sea is discussed.

INTRODUCTION

Only seven years ago, Logan described the micromorph rhynchonellid *Tethyrhynchia mediterranea* from Mediterranean submarine caves along the coast between Marseille and La Ciotat and from the Gulf of Tunis, where it inhabits the dark zones of caves together with the megathyridid species *Argyrotheca cistellula* (Searles-Wood) and *Megathiris detruncata* (Gmelin) (see Logan & Zibrowius, 1994). The subsequent discovery of *T. mediterranea* in the Adriatic Sea (Simon & Willems, 1999) implies a widespread distribution of this species in the Mediterranean, possibly overlooked in the past because of its cryptic habitat and small size. Logan & Zibrowius gave a detailed description of the shell morphology in their original account, but only two figures showed complete animals, the transparent shells of which allowed a glimpse of what was postulated to be gonads (Logan & Zibrowius, 1994: plate 1, figures 8 & 9). Because of the small body size and the trocholophe lophophore, which is common as a juvenile character of larger-sized articulate brachiopods, the authors discussed the possibility that they were describing an unknown juvenile of an already described brachiopod species, but decided that their specimens were mature and that *T. mediterranea* was a new micromorph, possibly neotenous species. If this is so, one would expect the new species to brood its larvae, because brooding is a common trait in all micromorph brachiopods described so far (Lacaze-Duthiers, 1861; Grobe & Lüter, 1999 and references therein; C.L., personal observation). Here I report the discovery of brooded larvae in the mantle cavities of *T. mediterranea* from the Adriatic Sea, thus corroborating the conclusions of Logan & Zibrowius (1994). *Tethyrhynchia mediterranea* is indeed a valid species, reaching maturity at only about 1 mm body length, and the hypothesis that it represents a juvenile of an already known species can definitely be excluded.

MATERIALS AND METHODS

Specimens of *Tethyrhynchia mediterranea* Logan, 1994 were collected in submarine limestone caves (−23 m depth) at Unije Island, Croatia, Adriatic Sea in July 1998 by Gregory Willems using SCUBA. Specimens were fixed in 4% formalin and stored in 80% ethanol.

Larger individuals were opened and checked for larvae under a dissecting microscope.

Developmental stages were separated from the adults with a glass pipetter, stained with eosin for 24 h and dehydrated in an alcohol series. For SEM inspection, larvae were critical point dried in a BAL-TEC CPD 030 and subsequently sputtered with gold in a BAL-TEC SCD 050.

An SEM examination was performed using a LEO 1450 VP scanning electron microscope. Micrographs of larvae were made as digital images and photographic panels were arranged using Adobe Photoshop® 5.0 and Adobe Illustrator® 7.0.

RESULTS

When dissected, adult females of *Tethyrhynchia mediterranea* revealed different stages of developing larvae in the mantle cavity as well as series of ripening eggs in the coelom. Thus, *T. mediterranea* could be confirmed to brood its larvae in the mantle cavity, as was expected from investigations of other micromorph brachiopods (see above). In contrast to the rhynchonellid *Notosaria nigricans* (Sowerby), brooded larvae of *T. mediterranea* were not associated with lophophoral tentacles, but were situated in niches of the inner mantle on either side of the parental feeding organ. A maximum of eight brooded larvae per adult female could be observed. Although absolute size of the lecithotrophic larvae is small (~120 μm in length), the three-lobed stages of *T. mediterranea*, which are competent to metamorphose, are huge relative to the adult female's minute body size (<1.2 mm in length). Compared to other brachiopods with lecithotrophic larvae, the ratio of larval length to length of the adult shell is by far the largest in *T. mediterranea* compared to other brachiopods with lecithotrophic larvae (see Table 1).

The apical lobe is strongly ciliated with long cilia forming a girdle used to propel the larva forward (Figure 1A–C). Apart from ciliation, the apical lobe carries an undulating row of cells protruding from the hind part of the apical lobe (Figure 1B). The ultrastructure of this undulating row of cells is unknown, but it resembles the so-called vesicular bodies of other lecithotrophic brachiopod larvae and may have a comparable excretory function.

In early three-lobed stages, cilia can also be observed on the mantle and pedicle lobes (Figure 1A). Later, these cilia vanish and, prior to settlement, mantle and pedicle lobes of the competent larvae are free of cilia (Figure 1B,C). Setal sacs in the mantle lobe produce four bundles of long larval setae, which provide buoyancy and may also be important for larval defence (Figure 1B,C). The larval setae are similar to larval setae of other articulate brachiopods in lacking an enamel layer covering the setal surface so that the single setal canals are visible in the SEM

Table 1. *Comparison of larval length/adult shell length ratio in different brachiopod species with lecithotrophic larvae. Measurements are from single individuals taken as representatives for the species, and do not represent statistically validated mean values.*

Species	Length of larva (μm)	Length of adult shell (mm)	Length ratio
Novocrania anomala	200	10	1:50
Thecidellina sp.	250	4	1:16
Notosaria nigricans	280	15	1:54
Terebratulina retusa	150	15	1:100
Calloria inconspicua	250	20	1:80
Tethyrhynchia mediterranea	120	1.2	1:10

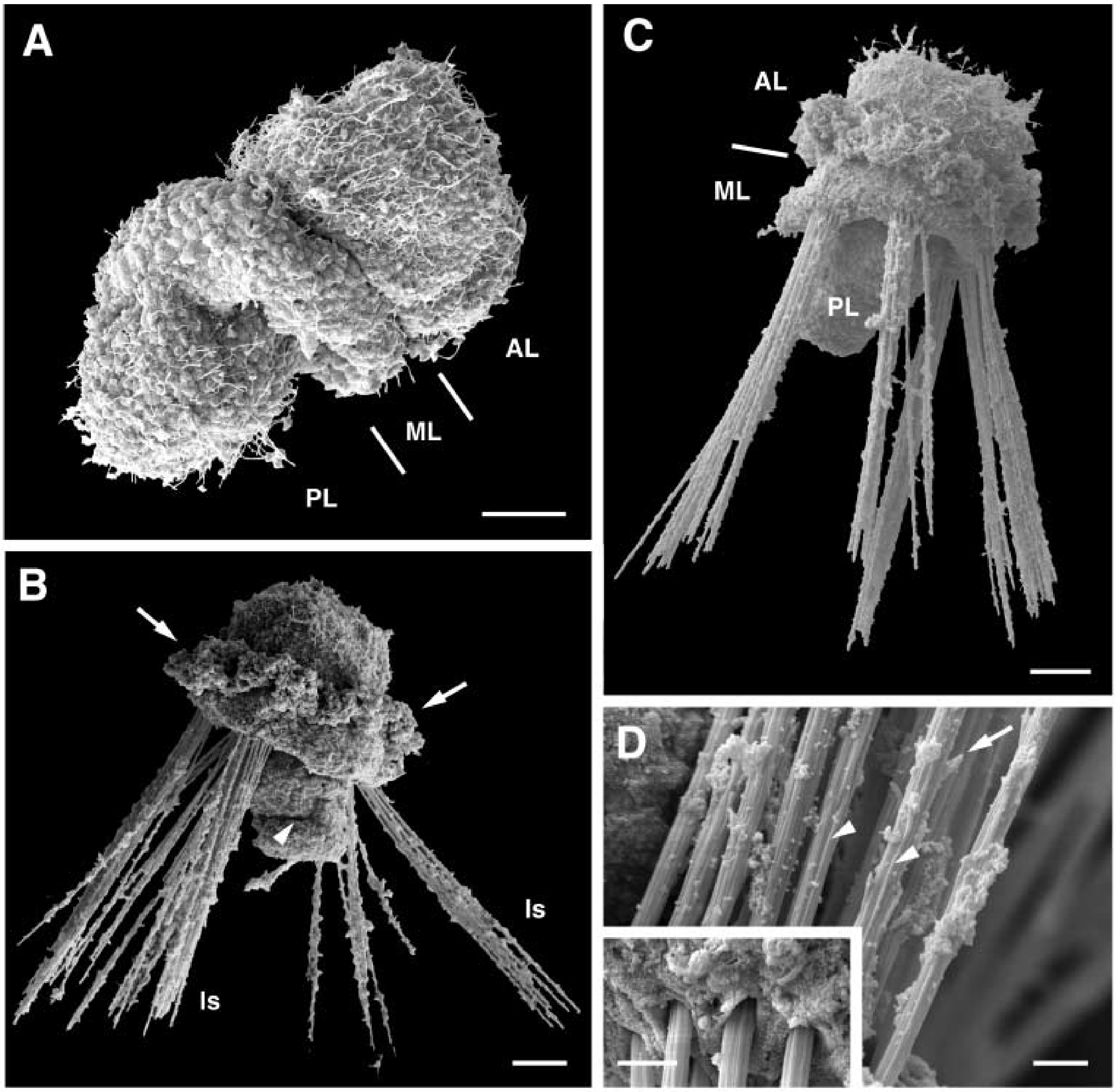

Figure 1. *Tethyrhynchia mediterranea*, developmental stages. (A) Early three-lobed stage from the mantle cavity of an adult female. Growth of the mantle lobe (ML) has just started. Notice ciliation on all three lobes; (B) contracted late three-lobed stage, side view, with fully developed larval setae (ls) arranged in four bundles, and a contracted mantle lobe. Arrows point to the undulating row of large cells, which may be equivalent to vesicular bodies (see text). Notice the groove where longitudinal muscles are attached to inner side of the pedicle (arrowhead); (C) late three-lobed stage, stretched, dorsolateral view; (D) details of larval setae showing peripheral setal canals (arrowheads) ending in spines (arrow). The inset shows surface details like microvilli on apices of seta-bearing cells. AL, apical lobe; PL, pedicle lobe. Scale bars: A–C, 20 μm; D, 2 μm.

(Figure 1D). Peripheral setal canals end in spines, which are irregularly distributed on each seta (Figure 1D). Where setae protrude from the mantle lobe, microvilli densely covering the epidermal cells can be observed (Figure 1D, inset). A sensory complex consisting of larval setae and collar receptors as described for larvae of the terebratellid *Calloria inconspicua* (Sowerby) (cf. Lüter, 2000) could not be found, but may be invisible in the SEM.

The pedicle lobe when contracted during fixation shows the inner attachment site of longitudinal muscles (Figure 1B), which are necessary for mantle reversal during metamorphosis. The tip of the pedicle lobe is slightly retracted, thereby forming a concave pit (not shown). In other lecithotrophic brachiopod larvae the epidermal cells in this area are glandular and play an important role for cementation of the larva on a hard substrate during metamorphosis and settlement (Chuang, 1996; James, 1997). Although a histological examination of *T. mediterranea* larvae is still pending, it is highly likely that the epidermal cells at the tip of the pedicle lobe of this species are glandular and responsible for glue production during cementation of the metamorphosing larva.

DISCUSSION

Tethyrhynchia mediterranea, the smallest rhynchonellid known so far and, similarly, the only representative of this superfamily in the Mediterranean (Logan & Zibrowius, 1994; Simon & Willems, 1999) has been confirmed to brood its larvae in special niches of the mantle cavity on either side of the lophophoral organ. Larval brooding is not uncommon among articulate brachiopods (see James et al., 1992; Williams et al., 1997) and is widely distributed among micromorph forms, which depend on high parental investment in their offspring to ensure reproductive success (Swedmark, 1964, 1967; Strathmann & Strathmann, 1982; Strathmann et al., 1984; Levin & Bridges, 1995). The small number of larvae present at the same time in the mantle cavity of adult females (=fecundity) is low and lies in the range of what is known from other micromorph brachiopod species (Grobe & Lüter, 1999; C.L., personal observation). The overall similarity of the three-lobed stages of *T. mediterranea* to the same larval stages of other articulate brachiopods is striking. Not only are the larval setae identical in construction (from SEM observations) (see Lüter, 2000), but also the development of ciliation of the larval body is the same (Lüter, 1998a,b). Apart from the size difference, larvae of *T. mediterranea* mostly resemble those of the only other known rhynchonellid larvae of the New Zealand species *Notosaria nigricans* (Percival, 1960; Hoverd, 1985; Lüter, 1998b). The undulating row of large cells on the apical lobe, which may be a functional equivalent to the vesicular bodies known from terebratellid brachiopod larvae (Stricker & Reed, 1985; Lüter, 1998b), are similar in both species and may represent an autapomorphic character of Rhynchonellida.

The collecting localities (see Logan & Zibrowius, 1994; Simon & Willems, 1999) suggest that *T. mediterranea* is distributed in submarine caves throughout the Mediterranean Sea. Taking into account that: (i) these minute rhynchonellids were only found in the dark, inner sections of the caves; and (ii) that larval release from parental females is supposed to take place immediately prior to settlement and simultaneous metamorphosis, it is unlikely that the reported distribution is a product of a recent colonization. In contrast, a low dispersal ability of *T. mediterranea* is highly likely and, therefore, the present biogeographical pattern may represent relict populations of a wider distribution in the Mesozoic Tethys.

Thanks are due to Eric Simon (Institut Royal des Sciences Naturelles de Belgique, Brussels) and Gregory Willems (Rhisnes, Belgium) for providing the specimens of *Tethyrhynchia mediterranea* for examination. I am grateful to B.L. Cohen (University of Glasgow, Scotland) for comments on an earlier draft of this paper. This study was partly supported by a grant from Deutsche Forschungsgemeinschaft (Ba 1520/1–3).

REFERENCES

Chuang, S.H., 1996. The embryonic, larval and early postlarval development of the terebratellid brachiopod *Calloria inconspicua* (Sowerby). *Journal of the Royal Society of New Zealand*, **26**, 119–137.

Grobe, P. & Lüter, C., 1999. Reproductive cycles and larval morphology of three Recent species of *Argyrotheca* (Terebratellacea: Brachiopoda) from Mediterranean submarine caves. *Marine Biology*, **134**, 595–600.

Hoverd, W.A., 1985. Histological and ultrastructural observations of the lophophore and larvae of the brachiopod, *Notosaria nigricans* (Sowerby, 1846). *Journal of Natural History*, **19**, 831–850.

James, M.A., 1997. Brachiopoda: internal anatomy, embryology, and development. In *Microscopic anatomy of invertebrates*, vol. 13: Lophophorata, Entoprocta, and Cycliophora (ed. F.W. Harrison and R.M. Woollacott), pp. 297–407. New York: Wiley-Liss.

James, M.A., Ansell, A.D., Collins, M.J., Curry, G.B., Peck, L.S. & Rhodes, M.C., 1992. Biology of living brachiopods. *Advances in Marine Biology*, **28**, 175–387.

Lacaze-Duthiers, H., 1861. Histoire naturelle des Brachiopodes vivants de la Méditerranée. *Annales des Sciences Naturelles, Série 4*, **15**, 260–330.

Levin, L.A. & Bridges, T.S., 1995. Pattern and diversity in reproduction and development. In *Ecology of marine invertebrate larvae* (ed. L. McEdward), pp. 1–48. Boca Raton: CRC Press.

Logan, A. & Zibrowius, H., 1994. A new genus and species of rhynchonellid (Brachiopoda, Recent) from submarine caves in the Mediterranean Sea. *Pubblicazioni della Stazione Zoologica di Napoli: Marine Ecology*, **15**, 77–88.

Lüter, C., 1998a. Note: embryonic and larval development of *Calloria inconspicua* (Brachiopoda, Terebratellidae). *Journal of the Royal Society of New Zealand*, **28**, 165–167.

Lüter, C., 1998b. *Zur Ultrastruktur, Ontogenese und Phylogenie der Brachiopoda*. Göttingen: Cuvillier Verlag.

Lüter, C., 2000. Ultrastructure of larval and adult setae of Brachiopoda. *Zoologischer Anzeiger*, **239**, 75–90.

Percival, E., 1960. A contribution to the life-history of the brachiopod *Tegulorhynchia nigricans*. *Quarterly Journal of Microscopical Science*, **101**, 439–457.

Simon, E. & Willems, G., 1999. *Gwynia capsula* (Jeffreys, 1859) and other Recent brachiopods from submarine caves in Croatia. *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Biologie*, **69**, 15–21.

Strathmann, R.R. & Strathmann, M.F., 1982. The relationship between adult size and brooding in marine invertebrates. *American Naturalist*, **119**, 91–101.

Strathmann, R.R., Strathmann, M.F. & Emson, R.H., 1984. Does limited brood capacity link adult size, brooding and simultaneous hermaphroditism? A test with the starfish *Asterina phylactica*. *American Naturalist*, **123**, 796–818.

Stricker, S.A. & Reed, C.G., 1985. The ontogeny of shell secretion in *Terebratalia transversa* (Brachiopoda, Articulata) I. *Journal of Morphology*, **183**, 233–250.

Swedmark, B., 1964. The interstitial fauna of marine sand. *Biological Reviews*, **39**, 1–42.

Swedmark, B., 1967. *Gwynia capsula* (Jeffreys), an articulate brachiopod with brood protection. *Nature, London*, **13**, 1151–1152.

Williams, A., James, M.A., Emig, C., MacKay, S. & Rhodes, M., 1997. Brachiopod anatomy. In *Treatise on invertebrate Paleontology, part H: Brachiopoda (revised)*, vol. 1 (ed. R.L. Kaesler), pp. 7–188. Boulder and Lawrence: The Geological Society of America and University of Kansas Press.

Submitted 31 July 2001. Accepted 10 October 2001.