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The general muscular architecture in *Tubiluchus troglodytes* (Priapulida)

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Abstract

We describe here the general muscular architecture of *Tubiluchus troglodytes* (Priapulida) based on phalloidin staining, confocal laser scanning and transmission electron microscopy. The body wall musculature is composed of a grid of circular and longitudinal muscle bundles, in the introvert, there is additional outer longitudinal musculature. *T. troglodytes* has pharynx protractors, whereas the introvert retractors could not be sufficiently resolved in this investigation. The muscular pharynx is followed by an organ with solid circular musculature, the polythyridium. The body wall musculature proceeds into the long tail. In the posterior region, anus and urogenital pores are surrounded by circular musculature.

Keywords: Priapulida, *Tubiluchus*, musculature, phalloidin, CLSM.

Introduction

Despite the fact that only 18 recent species from the taxon Priapulida are described, this small group is remarkably diverse concerning body size and reproductive biology. While species from the genera *Halicryptus*, *Priapulus*, *Priapulopsis* and *Acanthopriapulus* are large bodied (few centimeters up to about 40 cm), species from the genera *Tubiluchus*, *Meiopriapulus* and *Maccabeus* are only a few millimeters long. As far as investigated, the musculature does not vary much among priapulids belonging to different taxa. The body wall musculature is composed of outer circular and inner longitudinal musculature. In the introvert of *Priapulus caudatus* and *Halicryptus spinulosus*, an additional outer layer of longitudinal musculature is present (Adrianov & Malakhov 1996, Lemburg 1999). The introvert is everted by fluid pressure following the contraction of body wall muscles. The introvert is withdrawn with two sets of retractor muscles, a variable number (up to 25) of short and 8 long retractors (Matthisson et al. 1974, Storch et al. 1990, 1994, Storch 1991). At least in macroscopic species, further muscles acting as pharynx protractors may be present (Lemburg 1999).

The meiobenthic genus *Tubiluchus* is with seven species widely distributed from shallow to deeper waters. It is recognized particularly by a long thin tail in the posterior end. There are some fine

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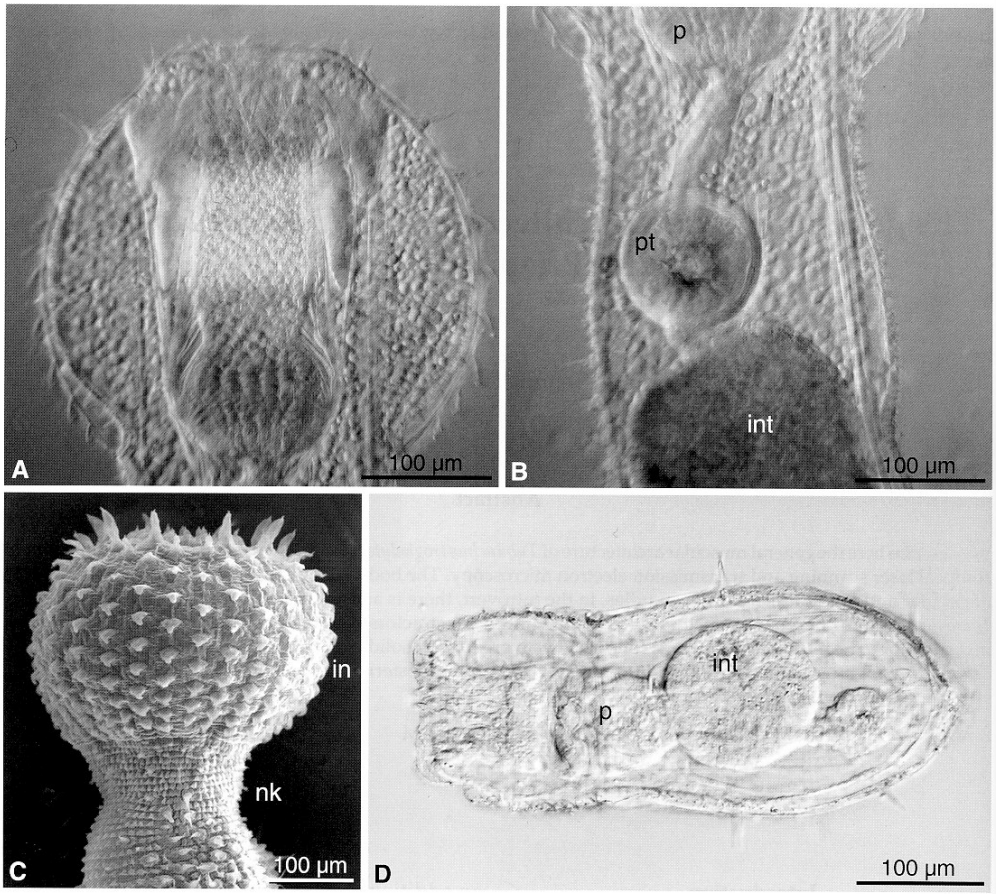
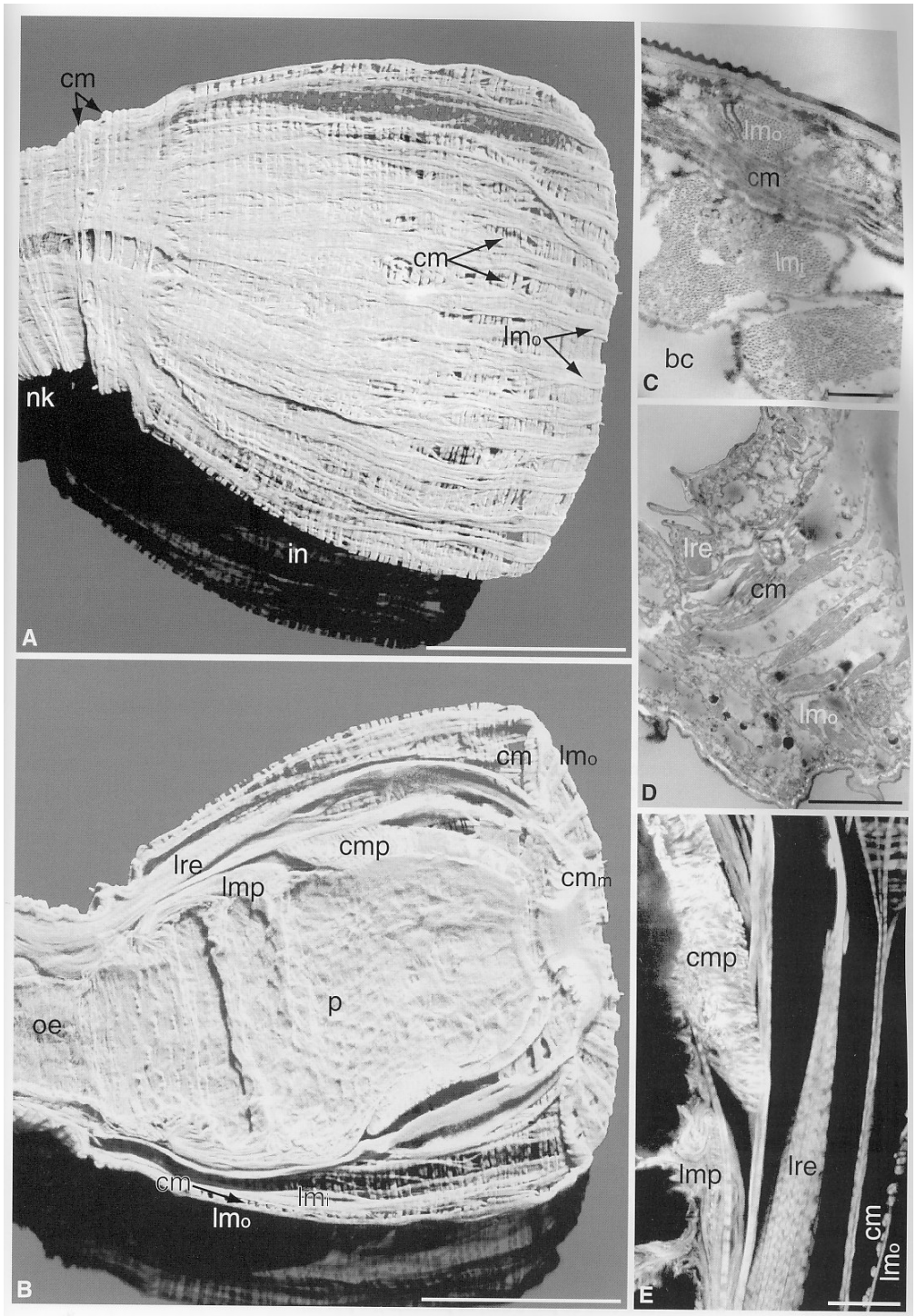


Fig. 1. General morphology of the adult (A-C) and larva (D) of *Tubiluchus troglodytes*. A,B,D with light microscopy, C with Scanning Electron Microscopy. **A.** Introvert with focus on the pharynx. **B.** Anterior trunk region showing transition from pharynx (p) to polythyridium (pt) and intestine (int). **C.** Everted introvert (in) and neck region (nk). **D.** Larva showing direct transition of pharynx to intestine, without a polythyridium.

Fig. 2. Musculature of the introvert of *Tubiluchus troglodytes*. A,B,E phalloidin labelling; A, B SFP-projections; E maximum-projection. C,D TEM. **A.** Dorsal view of the introvert (in) and neck (nk). In the introvert the integument consists of outer longitudinal muscle strands (lm_o) followed proximally by a layer of circular muscles (cm). **B.** View of the inner anatomy of the introvert (upper half of the full z-stack). The pharynx (p) is composed of an anterior circular muscle part (cmp) and a posterior longitudinal muscle part (lmp). In front of the pharynx is a concentration of circular muscles (cm_m) around the mouth opening. Next to the pharynx are the long retractor muscles (lre). Additionally to the outer longitudinal musculature (lm_o), an inner longitudinal muscle (lm_i) layer is distinguishable beneath the circular musculature (cm). oe = oesophagus. **C.** Introvert, median area, cross-section of the integument with the outer longitudinal musculature (lm_o) and the inner longitudinal muscles (lm_i), separated by circular musculature (cm), adjoining directly to the body cavity (bc). **D.** Cross-section of the anterior part of the introvert with the outer longitudinal musculature (lm_o), circular musculature (cm) and the long retractor muscles (lre). **E.** Detail of the long retractor (lre) beside the pharynx showing cross striation. Scale bars 100 µm (A,B); 20 µm (E), 5 µm (D), 1 µm (C).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structural investigations of the internal anatomy from the introvert (Calloway 1975), integument (Storch & Alberti 1985a), intestine (Storch & Alberti 1985b), nervous system (Rehkämper et al. 1989), protonephridia (Alberti & Storch 1986), the female reproductive system (Alberti & Storch 1988, 1989), spermatozoa (Alberti & Storch 1983, Storch et al. 1985, Storch & Higgins 1989; Ferraguti & Gabelli 2006) and the larva (Higgins & Storch 1989). The musculature is mentioned in several of these publications, but has not been a primary target of investigation. Using phalloidin staining and confocal laser scanning microscopy, we describe here the general architecture of the musculature from adults of *Tubiluchus troglodytes*, the most recently described species from Mediterranean submarine caves (Todaro & Shirley 2003; Todaro et al. 2006; Fig. 1).

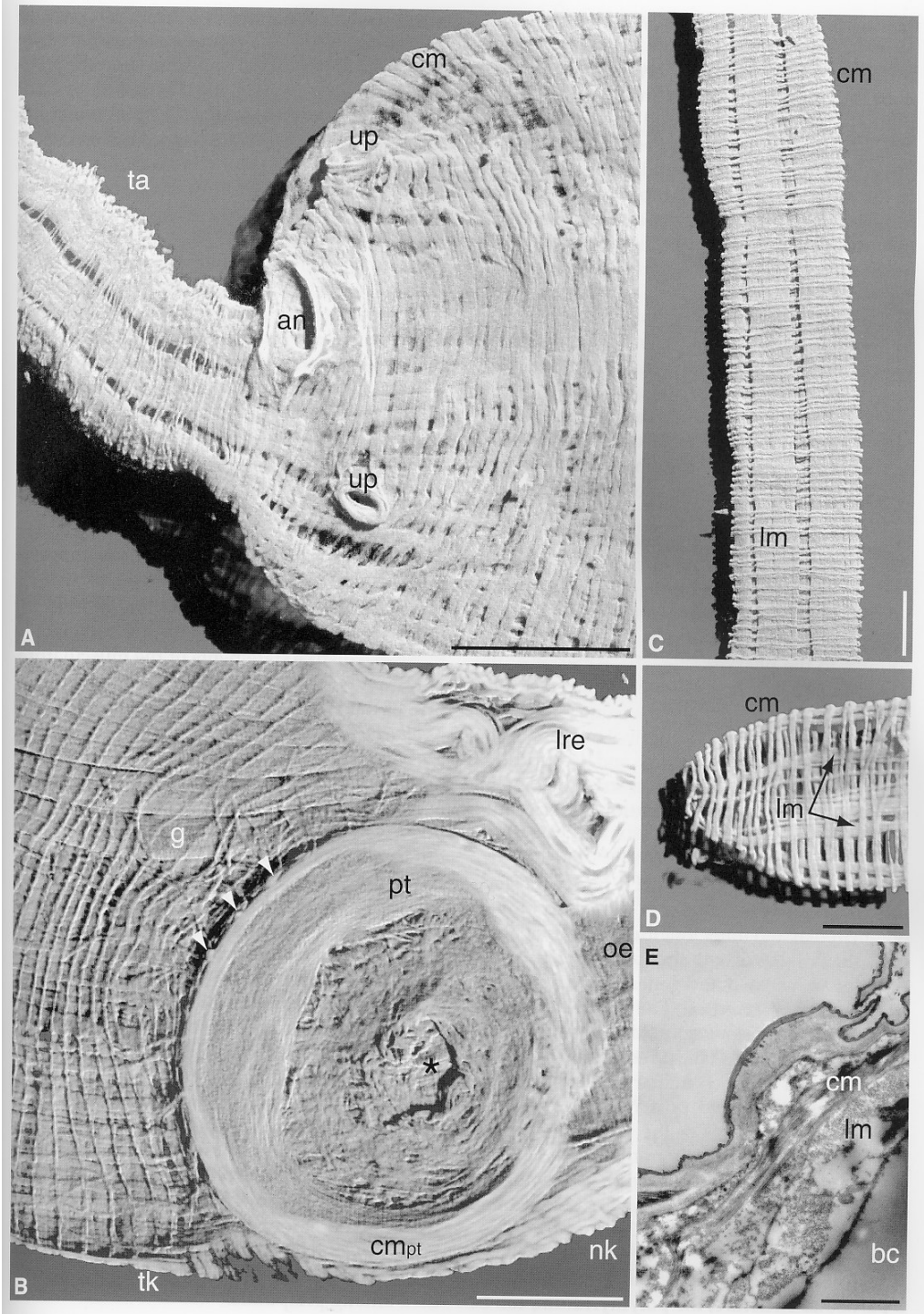
Material and methods

Priapulid-containing sandy samples were collected by scuba divers on 9th June 2005 from the type location, the marine cave 'Grotta Piccola del Ciolo' near Santa Maria di Leuca, Lecce, Italy and taken to the lab within 24 hours. In the laboratory a total of five specimens, one male and four females, were extracted alive by the narcotization-decantation technique using a 7 % $MgCl_2$ solution; two female specimens were observed *in vivo* under Nomarski optics (Leitz, Dialux 20), one male specimen was prepared in accordance to Todaro & Shirley (2003) and investigated with a Philips XL 30 SEM, one female was fixed in paraformaldehyde for CLSM investigation and one female was fixed in glutaraldehyde for TEM investigation.

For the CLSM investigation, the relaxed specimen was incubated in freshly made 4 % paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) at pH 7.4. Fixation was carried out at 0 °C overnight on ice. After fixation, specimens were washed several times with 0.1 M PBS and stored at 4 °C in 0.1 M PBS containing 0.05 % NaN_3 for up to several weeks. For staining, specimens were first preincubated for 24 h at 4 °C in a preincubation buffer containing 0.1 % Triton X 100, 0.25 % BSA and 0.05 % NaN_3 in PBS. The specimen was then incubated in TRITC-phalloidin (Sigma) (3 µl 3.8 µM solution in 50 µl preincubation buffer) for 12 h. Then the priapulid was rinsed in PBS several times and embedded in Citifluor on microscopical slides. Investigation took place with the confocal laser scanning microscope (CLSM) Leica TCS 2. Series of optical sections were projected in one maximum-projection (MP) image or visualized as a simulated fluorescence projection (SFP) for three-dimensional appearance.

For transmission electron microscopy the relaxed female specimen was fixed for 1 h at 4 °C in 2.5 % glutaraldehyde in 0.1 M sodium-cacodylate buffer, including a few crystals of ruthenium red. Postfixation was performed with 1 % OsO_4 in 0.1 M sodium-cacodylate buffer for 2 h at 4 °C. The fixed specimen was dehydrated in an acetone series and embedded in araldite. Ultrathin sections (about 70 nm) were made with an Ultracut E (Reichert), sections were automatically contrasted with uranyl acetate and lead citrate. Investigation of the sections was made with a Philips CM 100 transmission electron microscope operated at 60 and 80 kV.

Fig. 3. Musculature of the trunk and tail of *T. tubiluchus*. A-D phalloidin labelling, SFP-projections. E TEM. **A.** Ventral view of the posterior trunk and transition to the tail (ta). Anterior of the tailbase are three openings. Medioventral is the anus (an), anterolateral the two openings of the urogenital system (up). cm = circular musculature. **B.** Transition region of neck (nk) to trunk (tk) with the musculature of the polythyridium (pt). The upper integumental layer is extracted from the z-stack. The polythyridium consists in a solid layer of circular musles (cm_{pt}). The polythyridium is located intermediate between the oesophagus (oe) and gut (g), next to strands of the long retractor muscles (lre). The longitudinal muscles of the gut attach at the posterior opening of the polythyridium (arrow heads), the connective opening between polythyridium and oesophagus is situate at the ventral side (asterisk). **C.** Middle region of the tail, with several separated longitudinal muscles (lm), surrounded by a regular layer of circular muscles (cm). **D.** Tip of the tail with only delicate longitudinal muscles (lm), but distinct circular musculature (cm). **E.** Detail of the integument of the neck in cross-section, with a distal circular muscle layer and a proximate longitudinal muscle arrangement. The fluid of the body cavity (bc) appears inhomogeneously electron-dense. Scale bars 50 µm (A,B); 20 µm (C,D), 2 µm (E).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Results

Introvert. There are three layers of body wall muscles, from distal to proximal these are longitudinal, circular and longitudinal muscles (Fig. 2B,C). All muscles form bundles that do not attach directly to their neighbor bundles (Fig. 2A). Only in the anterior part of the introvert, about four bundles of the outer longitudinal musculature form larger bands that are clearly separated from other such bands (Fig. 2A). In the anteriormost region, longitudinal muscles appear to bend and proceed posteriorly between body wall and pharynx (Fig. 2B). They extend into the trunk region. From their position these muscles could represent the long retractor muscles (Fig. 2B,E). Another set of muscles attaches in the anterior region lateral of the mouth opening and runs to the posterior end of the pharynx. According to their position, these muscles are the pharynx protractors. The pharynx is a solid muscular bulb, extending along the entire introvert. The anterior part consists of circular musculature and is clearly separated from the posterior part where longitudinal muscles are dominating (Fig. 2B,E). In the posterior direction, an oesophagus follows to the pharynx (Fig. 2B). Anterior of the pharynx and clearly separated from it is a solid ring of circular musculature around the mouth opening (Fig. 2B). All longitudinal muscles (body wall muscles, long retractors and longitudinal pharynx musculature) are cross-striated (Fig. 2E). For the other muscles, the striation pattern could not be investigated.

Trunk. Starting from the neck region posteriorly, the body musculature is composed only of outer circular and inner longitudinal musculature (Fig. 3E). Bundles of both circular and longitudinal muscles are isolated and generally do not attach their neighbor bundles. They form a regular grid. In the posterior region, directly anterior of the transition to the tail, three openings can be distinguished by solid circular musculature. The slitlike anus is located posteriormost, and two slightly oval openings of the urogenital system are located anterolaterally of the anus. All three openings are surrounded by circular muscles which can act as a sphincter and extend a small distance into the animal (Fig. 3A).

Intestinal system. Posterior to the oesophagus, a particular structure, the polythyridium, can be recognized, which connects the oesophagus and the gut (Fig. 1B). The longitudinal muscles of the gut attach directly at the transition from the polythyridium to the gut (Fig. 3B). The polythyridium is mainly composed of solid circular musculature. Along the intestine, there is a spaced grid of small bundles of circular and longitudinal muscle fibres (Fig. 3B). The polythyridium is absent in larvae (Fig. 1D).

Tail. In the tail, the outer circular and inner longitudinal musculature of the trunk continue, only with a smaller number of bundles. The circular bundles are roughly arranged with regular spaces while the longitudinal musculature forms six bands composed of about 7-10 bundles (Fig. 3C). At the tip of the tail the longitudinal muscles fade out with only a few fibres reaching the tip, whereas the organization of the circular muscle do not change (Fig. 3D).

Discussion

The total number of two specimens (one for CLSM, one for TEM) is perhaps not sufficient to make exhaustive statements of the musculature, but it is possible to describe the general architecture of the musculature and compare it to other *Tubiluchus* species and to other priapulids.

Several aspects of the musculature of priapulids appear to be comparable in microscopic and macroscopic as well as their larvae. The body wall musculature in the trunk is a grid of outer circular and inner longitudinal musculature (Storch et al. 1989, 1994, Storch 1991). We can confirm this for *Tubiluchus troglodytes*, although the longitudinal musculature shows a pattern where some bundles can form larger bands. Whereas Storch et al. (1990, 1994) describe the body wall musculature in the introvert in two *Priapulus* species and *Halicryptus spinulosus* as similar to the trunk musculature, Adrianov & Malakhov (1996, Fig. 54) and Lemburg (1999) describe additional outer longitudinal muscles in *Halicryptus spinulosus* and *Priapulus caudatus*. For *Tubiluchus* species, there are hardly any data, but Rehkämper et al. (1989, Fig. 1) figure only outer circular and inner longitudinal musculature. As we found outer longitudinal musculature also in *T. troglodytes*, a tri-layered body wall musculature in the introvert appears to be commonly distributed among priapulids.

The retractors could not be documented convincingly in this investigation, but it appears that pharynx protractors are present in *T. troglodytes*. Such muscles are known from macroscopic species (Lemburg 1999), but have not been reported from *Tubiluchus* so far.

The polythyridium is a muscular organ posterior of the pharynx which is armed by cuticular plates and therefore probably has a "chewing" function (Kirsteuer 1976). It has been observed for the first time in *Tubiluchus corallicola* by Kirsteuer & van der Land (1970) and has subsequently been found in other *Tubiluchus*-species (*T. remanei*: van der Land 1982; *T. philippinensis*: Storch et al. 1985; *T. australensis*: van der Land 1985) as well as in *Meiopriapulus fijiensis* (Morse 1981, Storch et al. 1989). The polythyridium is probably lacking in the larva of *Tubiluchus* (Higgins & Storch 1989).

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