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New ultrastructural (SEM) data of *Tubiluchus arcticus* (Priapulida)

Running title ultrastructure of *Tubiluchus*

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Abstract We present new data on *Tubiluchus arcticus,* the northernmost species of the genus. The ultrastructure of the scalids and the buccal papillae is documented. The pharyngeal teeth of *T. arcticus* are unique among priapulids in being long and slender, with multiple fringes of bristles and an apical comb (pecten) of bristles. These descriptions extend the knowledge about *T. arcticus* and make it a very peculiar species within the genus. The new specimen is larger than the ones previously described and may be the only adult specimen so far.

Introduction

Priapulids of the microscopically small genus *Tubiluchus* are usually reported to occur in warm regions, from the tropics to the temperate zone (Mediterranean) (e. g. Todaro & Shirley 2003, Schmidt-Rhaesa et al. 2013, 2017). *Tubiluchus arcticus,* described by Adrianov et al. in 1989 is an exception, as it was found in the White Sea, close to the Arctic Circle. The original description is based on two females and an immature specimen (Adrianov et al. 1989). Few SEM (scanning electron microscopy) images and additional TEM (transmission electron microscopy) images of this species were included in the monography on

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Priapulida by Adrianov & Malakhov (1996), but it is unclear whether this is based on additional material or on the material from the original description. We had the opportunity to investigate two specimens from the White Sea and, although only a single of these specimens revealed good results, we can add some important new observations concerning this species.

Tubiluchus specimens consist of a trunk and an eversible anterior introvert with the apical mouth opening. Some authors distinguish a neck region between introvert and trunk (e. g. Van der Land 1970, 1985, Todaro & Shirley 2003). Characteristic for *Tubiluchus* is a muscular tail that can be distinctly longer than the trunk. Trunk and tail are covered by cuticular elevations called tumuli. Two types of sensory structures, the tubuli and the flosculi, are scattered along the body. Tubuli are lacking in the neck region while flosculi are usually abundant there. The introvert is covered with scalids, which form 25 longitudinal rows and differ morphologically along these rows. In the anterior region of scalids, a ring of eight larger scalids is present and further scalid-like structures are present in the anteriormost region of the introvert in some species. Different terms are applied to these structures (e. g. buccal papillae, buccal scalids). Posterior to the mouth opening is the pharynx, which is lined with cuticular pharyngeal teeth. In several publications, the pharynx is completely everted, which makes all teeth accessible for observation. However, such an extreme eversion is never observed in live animals and may be the product of pressure under the coverslip (Kirsteuer & Van der Land 1970) or other treatment during observation. The teeth have a comb-like shape and are called pectinate teeth (Van der Land 1970). Van der Land (1970) distinguishes three parts of the teeth: a cellular basis, a cuticular manubrium as "stem" and an apical pecten composed of a comb-like row of spines.

Species of *Tubiluchus* differ most strongly in the genital region of males, which usually includes similar cuticular structures, but in different arrangement patterns (Schmidt-Rhaesa et al. 2013). Originally, it was assumed that different species are otherwise indistinguishable (Van der Land 1985), but it turned out that there are also differences in other characters, most specifically in the structure of scalids, buccal papillae and pharyngeal teeth (e. g. Todaro & Shirley 2003, Schmidt-Rhaesa et al. 2017).

Material and Methods

The two investigated specimens were both collected in Kandalaksha Bay in the White Sea, close to the White Sea Biological Station. They belong to the collection of the White Sea Biological Station and have the accession numbers WS11097 (specimen not described in detail) and WS029857 (specimen described below in detail). The latter specimen was collected on June 18, 1996 by scuba diving at 18 m depth (66°33'22.15"N 33°6'28.51"E) by A. B. Tzetlin and extracted from a sample of sand. It was fixed in glutaraldehyde and postfixed with OsO_4 buffered with cacodylate. Both specimens were photographed with a Keyence VHX-7000

digital light microscope. For SEM, both specimens were dehydrated in a series of increasing ethanol concentrations, critical point dried in a Leica EM CPD300, sputter coated with platinum in a Polaron SC7640 Sputter Coater and investigated with a LEO SEM 1524.

Results

The large investigated specimen (WS029857) is a female. It has a trunk length of 1.3 mm, the extended introvert is 550 µm long and the tail is at least 4 mm long (Fig. 1 A). Measurements after preparation for SEM are slightly shorter (trunk: 960 µm, introvert 435 µm;

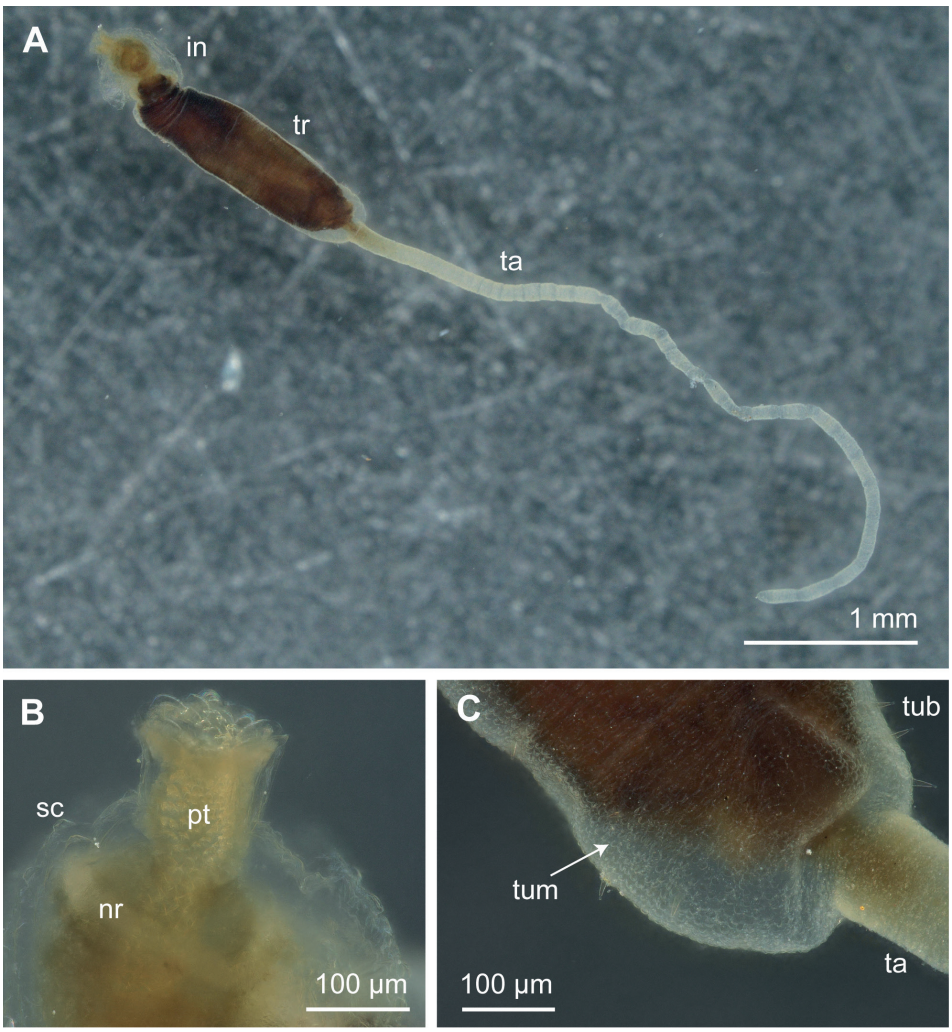

Fig. 1 *Tubiluchus arcticus*, light microscopical images. A: Entire specimen with body subdivision into introvert (in), trunk (tr) and tail (ta). B: Magnification of the anterior introvert, the pharyngeal teeth (pt) and the nerve ring (nr) shine through. The introvert is covered by scalids (sc). C: Posterior part of the trunk and attachment of the tail (ta). The trunk is covered by tumuli (tum) and scattered tubuli (tub).

Fig. 2 A), probably due to shrinkage by dehydration. The trunk is covered by tumuli, which are roundish cuticular elevations (Fig. 1 C, 2 B). The surface of the tumuli is smooth, and radial ridges are lacking. Tubuli are present in irregular distribution on almost the entire trunk (Fig. 1 C, 2 B – D). They are lacking in the anteriormost region, which may therefore be called neck region. Tubuli possess a small accessory tubulus at the base (Fig. 2 C) (see discussion for terminology). Within the apical opening of the tubuli, a substructure of septae is recognizable (Fig. 2 D). Flosculi are scattered over the entire trunk and neck region. They possess 12 – 14 short petal-like apical extensions (Fig. 2 E – G).

On the introvert, symmetry patterns of scalids could not be well observed due to foldings in the introvert surface. We distinguish between buccal papillae, primary scalids

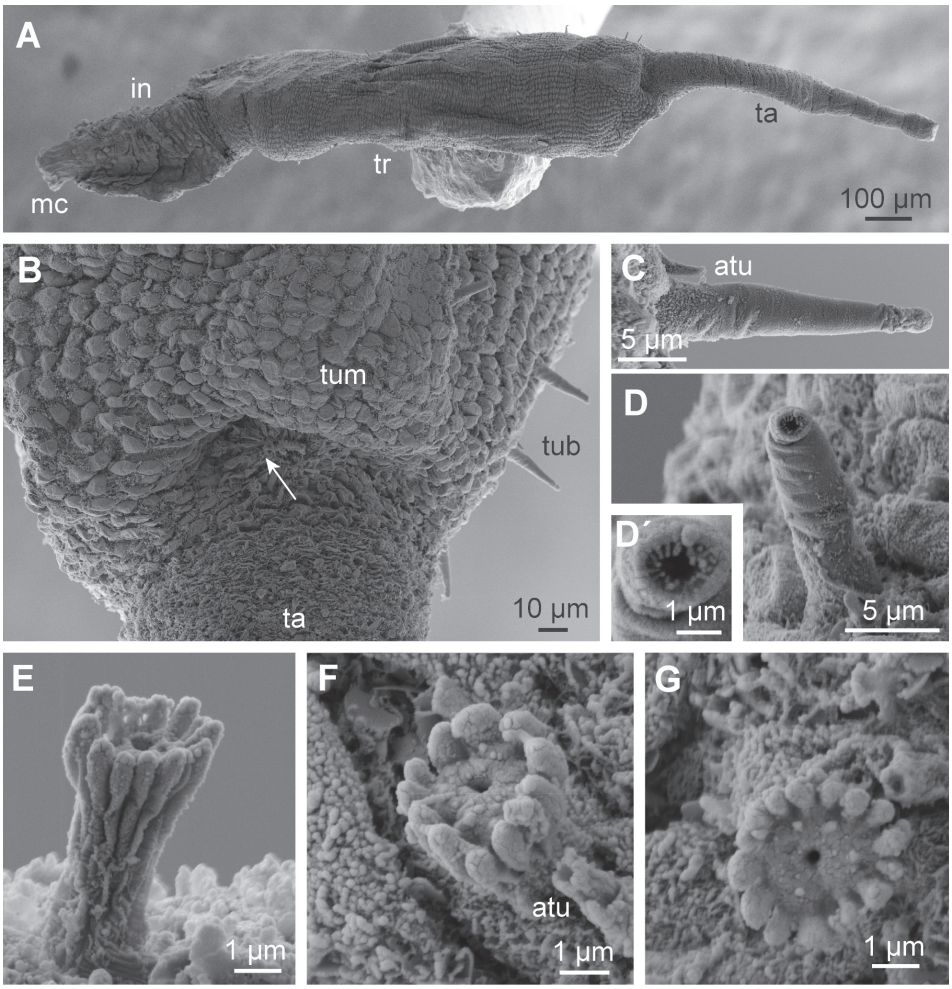

Fig. 2 *Tubiluchus arcticus*, SEM images. A: Entire specimen with body subdivision into introvert (in), trunk (tr) and tail (ta). The anterior part of the introvert is the mouth cone (mc). B: Posterior region of the trunk showing covering of tumuli (tum), scattered tubuli (tub) and the anal/genital opening (arrow). C, D: Tubuli, in C with accessory tubulus (atu). D´ is a magnification of D showing a pattern of septae within the apical opening. E – G: Three different flosculi, in F with accesscory tubulus (atu).

and (regular) scalids, see discussion for an explanation of these terms. There are three rings of buccal papillae. The first ring of 5 buccal papillae is on the edge of the everted mouth cone (Fig. 3 A, B, 5 A). Each papilla originates directly anterior of the pharyngeal

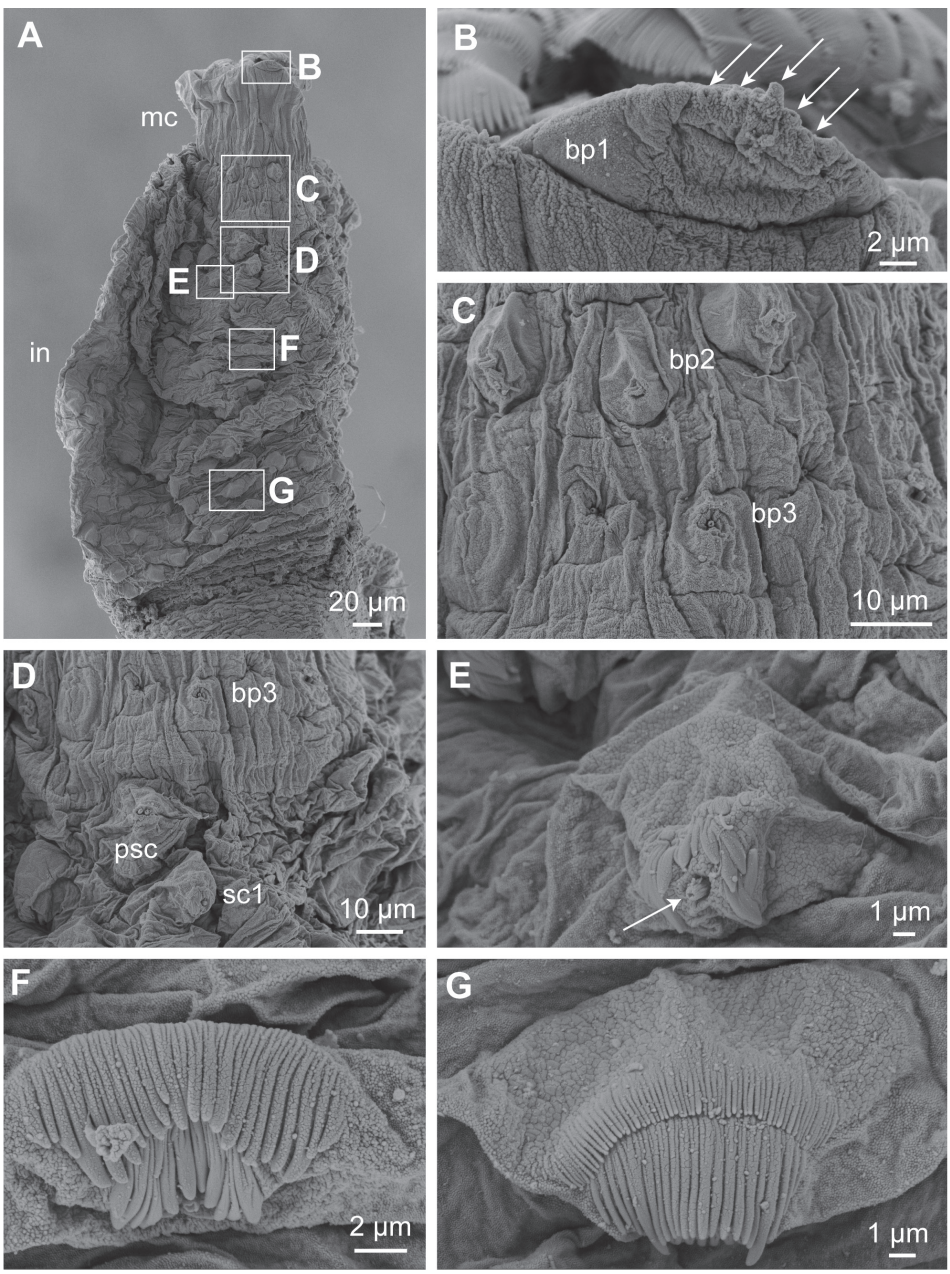

Fig. 3 *Tubiluchus arcticus*, SEM images of the introvert. A: Introvert (in) with mouth cone (mc), indicating the position of the structures figured in B – G. B: Buccal papilla 1 on the edge of the mouth cone. Arrows indicate 4 very short and one slightly longer tubulus. C: Second and third ring of buccal papillae (bp2 and bp3). D: Primary scalid (psc) and scalid of the second ring (sc2). E: Magnification of a scalid of the second ring, arrow shows the apical tubulus. F, G: Posterior scalids with two fringes of bristles.

teeth, on the inner side of the mouth cone and projects over its anterior border, often turning backwards on the outer side of the mouth cone (Fig. 3B, 5A). The papillae are broad (20µm) and have a number of tubuli on their tip. In the papilla that could be observed best, there are 5 tubuli, four very short ones and a central one slightly protruding (Fig. 3B). The second ring of buccal papillae is at the base of the mouth cone, about 75µm posterior of the first ring of buccal papillae. It contains more than 5 papillae, but the exact number could not be counted; this also accounts for the other buccal papillae and scalids. The second-ring buccal papillae are slightly conical and have 1–3 tubuli on their apex (Fig. 3C, 4A, B). Buccal papillae of the third ring appear as small depressions, in which one or two central tubuli are present (Fig. 3B, 4C, D). This ring follows about 10µm behind the second ring of buccal papillae.

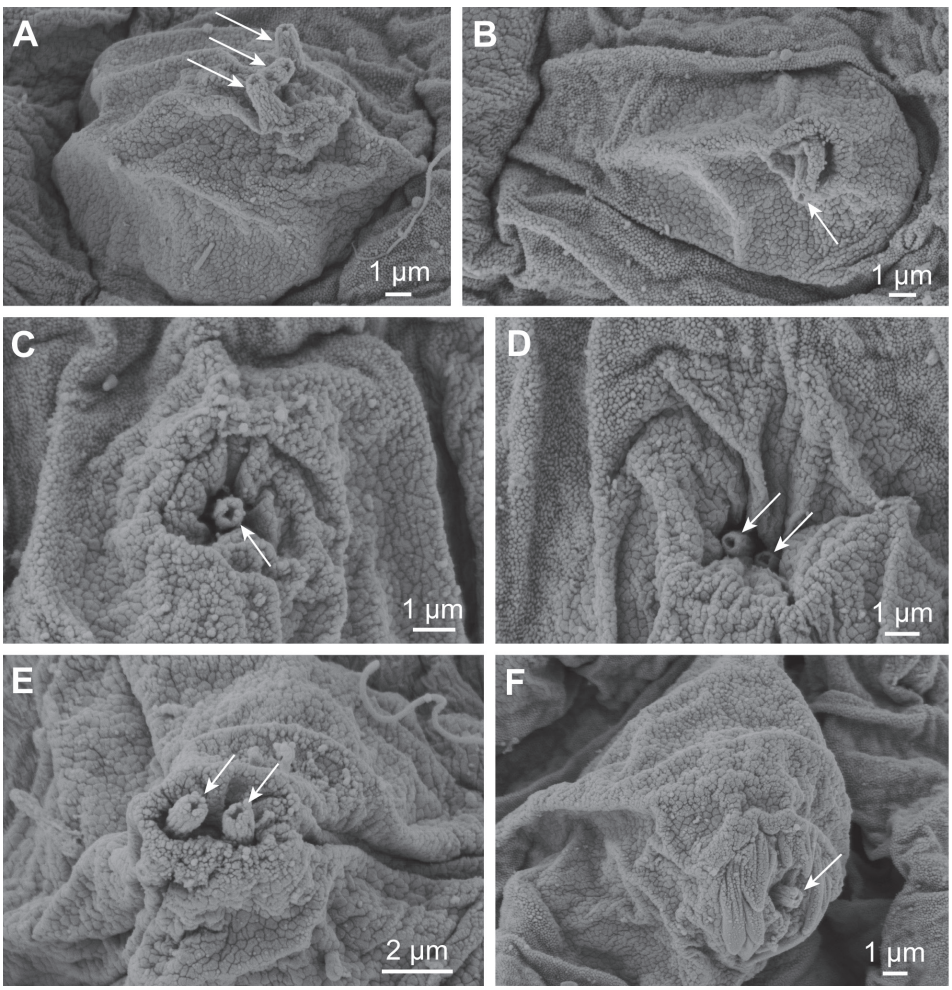

Fig. 4 *Tubiluchus arcticus*, SEM images of buccal papillae and scalids. A, B: Buccal papillae of the second ring with one (B) or three (A) tubuli. C, D: Buccal papillae of the third ring with one (C) or two (D) tubuli. E: Primary scalid with two tubuli. F: Scalid of the second ring with one tubulus and leave-like bristles. Tubuli are marked by arrows.

Further posterior, approximately 20 µm of the last ring of buccal papillae, is the first ring of scalids (Fig. 3 D). These primary scalids (see discussion for explanation of terms), from which only one could be observed in detail (Fig. 3 D, 4 E), are slightly larger than the following ones and have two apical tubuli (Fig. 3 D, 4 E). The primary scalids are in close proximity to the following (second) ring of scalids, the first "regular" scalids (Fig. 3 D). These scalids have a roughly structured surface (like all previously described scalids and buccal papillae, see Fig. 4 A – F). Close to the apex are several petal-like structures that surround one central tubulus (Fig. 3 E, 4 F). Further scalids have two or three cuticular fringes (Fig. 3 F, G), in the anterior scalids the bristles of the distal fringe are flattened

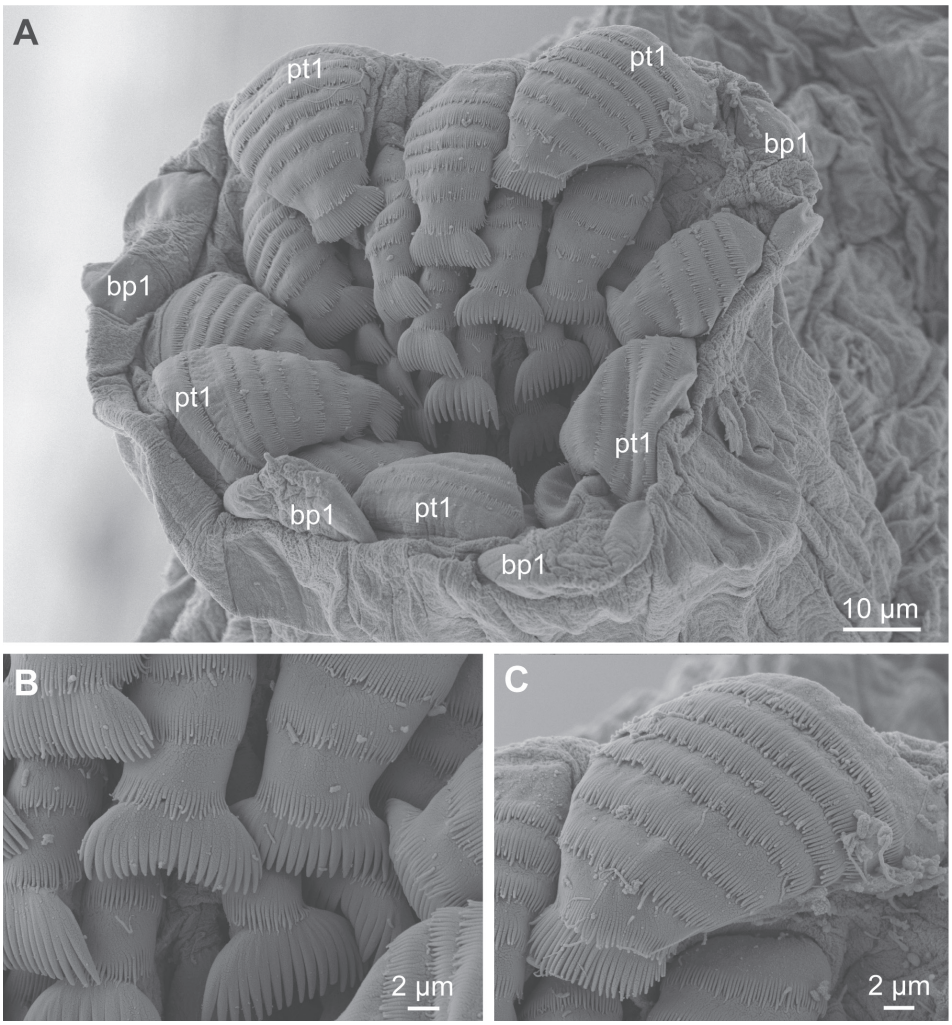

Fig. 5 *Tubiluchus arcticus*, SEM images of pharyngeal teeth. A: Overview on the entire mouth opening showing first ring of buccal papillae (bp1) and pharyngeal teeth. The five teeth of the first ring are marked with pt1, the other teeth could not be assigned to rings with certainty. B: Magnification of the posterior, slender teeth. C: Magnification of one first-ring tooth.

and resemble the petal-like structures of the second ring of scalids (Fig. 3F). In the other scalids, the bristles of both fringes are more slender.

The pharyngeal teeth are slightly everted and allow observation of the first rings of teeth (Fig. 5A). The teeth are likely arranged in rings, but they are densely positioned (and probably slightly distorted), so that their exact arrangement cannot be revealed. Only in the first ring, it can be assumed that 5 teeth belong to one ring (Fig. 5A). All observed teeth are elongate and contain up to eight transverse rows of fine bristles (Fig. 5A-C). The distal structure appears as a fringe in the anterior teeth (Fig. 5A, C) and as a row of spines of equal size in posterior teeth (Fig. 5A, B). The first-ring teeth are the largest and broadest. Each first-ring tooth is broadest in about the middle of its length and get narrower towards the distal end. The distal fringe is slightly broader than the distal end of the tooth and it´s bristles are only slightly longer and thicker than the bristles in the transverse fringes along the tooth (Fig. 5C). The posterior teeth become more slender and their distal "comb" is distinctly broader than the distal part of the tooth (Fig. 5B). The spines in the comb are approximately 20 in number, and distinctly more solid than the bristles in the transverse fringes (Fig. 5B).

The small specimen (WS11097) has a trunk length of about 485µm and 445µm tail length (total length about 930µm; measured from light microscopic images). As the introvert is completely withdrawn in WS11097 and the specimen is very poor in condition, the detailed description is not included here. Few scattered trunk tubuli and flosculi were observed and correspond in structure to the larger specimen.

Discussion

Tubiluchus arcticus is a very special member of the genus *Tubiluchus* due to its unusual distribution. It occurs close to the Arctic Circle (Adrianov et al. 1989), whereas the majority of other *Tubiluchus* specimens were found in distinctly warmer oceans. The exact number of specimens used for the original description of the species is not known, but there are at least two females and a juvenile (Adrianov et al. 1989), males were not reported so far.

The original description of *T. arcticus* includes a number of scanning electron microscopical (SEM) images of flosculi and tubuli, but only drawn images of scalids and teeth (Adrianov et al. 1989). Our description confirms some of the characters, contradicts others, and expands the description of this species.

The specimen in our investigation is a female, because all regions on the trunk look similar and no specialized genital region was detected. Such a region is known from other species (see overview in Schmidt-Rhaesa et al. 2013) and represents the main differential character between species of the genus. The specimen investigated by us is larger than the specimens investigated by Adrianov et al. (1989). They report a length of trunk and introvert of 1.08mm, whereas our specimen is 1.85mm long. The tail in Adrianov et

al.´s specimen is quite short (0.9 mm), while in our specimen it is considerably longer (4 mm), assuming our specimen represents an older life stage. Moreover, measurements are influenced by the state of contraction and the condition of the tail.

The tumuli covering the trunk and the tail have radial ridges in some species (*T. corallicola:* Kirsteuer & Van der Land 1970; *T. troglodytes:* Todaro & Shirley 2003). In other species including *T. arcticus* such ridges are lacking (*T. soyoae* and *T. pardosi:* Schmidt-Rhaesa et al. 2017). The observed distribution of flosculi and tubuli in our specimens corresponds to the description by Adrianov et al. (1989), and only the number of "petals" of the flosculi is higher in our investigation (12 – 14) compared to Adrianov et al. (1989) (7 – 11).

The term "tubulus" is used in different ways in priapulids. First, it is used for elongate, tapering structures on the trunk. Usually, these tubuli have a small tube-shaped and tapering structure at their base, which was originally termed "accessory seta". As it has an apical opening, the term "accessory tubulus" seems more appropriate than "seta". Furthermore, "tubulus" is used to describe the putative receptive part of different kinds of receptors, as this is often a short or longer tube. Such tubuli occur on scalids, but also on trunk papillae, tail vesicles or teeth (e. g. in *Priapulopsis bicaudatus,* see Schmidt-Rhaesa & Raeker 2023).

The original scalid description by Adrianov et al. (1989) does not correspond completely to our observations. Adrianov et al. (1989) mention that the first scalids have three apical tubuli and that the following ones have two transverse rows of bristles, with some of them lacking a tubulus. However, we observed that the number of apical tubuli appears to be variable and the presence of exactly three tubuli occurs only in the second-ring buccal papillae. Rows of bristles (here: fringes) occur on scalids of the second ring and posterior.

It is not easy to compare cuticular structures on the introvert among priapulids, but it appears likely that, at least in the macroscopic priapulids and in *Tubiluchus,* there is one ring of scalids, which are always 8 in number and which are usually larger than the remaining scalids. These are the primary scalids and they appear well suited as a landmark on the introvert. In macroscopic priapulids, it has been shown that primary scalids and the subsequent scalids are arranged in a certain pattern that creates the 25 longitudinal rows of scalids (Adrianov & Malakhov 2001, Schmidt-Rhaesa & Raeker 2023). The 8 primary scalids and this particular pattern of scalids also are present in *Tubiluchus,* as demonstrated in several species (*T. corallicola:* Calloway 1975; *T. vanuatensis:* Adrianov & Malakhov 1996, 2001; *T. troglodytes:* Todaro & Shirley 2003). In most, if not all priapulids, there are additional scalid-like structures anterior of the primary scalids. These structures can only be clearly observed when the introvert is fully everted, and their number and structure are difficult to compare among priapulids. In *Tubiluchus,* Van der Land (1970) has named these structures "buccal papillae", a term that we also use for all structures between the primary scalids and the pharyngeal teeth. Adrianov & Malakhov (1996) use the term "buccal scalids", which refers to the fact that their basic structure is similar to scalids.

The greatest dissent between the original description and our investigation is in the structure of teeth. As pharyngeal teeth were not documented by SEM images by Adrianov et al. (1989), we assume that they were not easy to observe. Nevertheless, the authors describe the teeth as having a large central spine and up to 5 smaller lateral spines. Such a tooth is very particular, as it is more or less a cuspidate tooth, which occurs in macroscopic priapulids and in larvae of *Tubiluchus* (Kirsteuer 1976), but not in adults of *Tubiluchus* (Van der Land 1970). Our observation of the pharyngeal teeth differs fundamentally from this description and shows a unique form of pectinate teeth that has not been described for any priapulid before. Pectinate teeth, as present in other *Tubiluchus* species (e. g. Schmidt-Rhaesa et al. 2017), have a comb-like series of equally shaped spines, to which *T. arcticus* corresponds. The shape of the tooth "body" is unique by being much longer than in any other species and also covered by repeated fringes of bristles. Fringes additional to the apical comb are present in other species as well, for example in *T. pardosi* (Schmidt-Rhaesa et al. 2017), but there is only one such fringe and teeth are not as elongate as in *T. arcticus*.

Despite these differences to the original description, we still assume that the specimen investigated by us represents *T. arcticus*. Some characters may be difficult to investigate and especially the investigation of pharyngeal teeth depends on the state of eversion of the introvert. Given the smaller size and the possible presence of cuspidate teeth in the original description of *T. arcticus,* it may be the case that Adrianov et al. (1989) studied non-adult, postlarval stages of this species. The postlarval development of *Tubiluchus* is not known and therefore it remains possible that cuspidate teeth are present not only in the larval stage, but also in early postlarval stages.

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