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Bifidoachaetus, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach

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Abstract. Gastrotricha is a cosmopolitan phylum of aquatic and semi-terrestrial invertebrates that comprises ~820 described species. To date, freshwater gastrotrichs have not been the subject of faunistic or taxonomic research in the polar regions. In this paper, we present the first species-level description of a freshwater gastrotrich from the Arctic (Svalbard Archipelago). Evidence from morphology, morphometry and molecular analyses reveals that the species represents a new genus in Chaetonotidae: Bifidoachaetus arcticus, gen. et sp. nov. Taking into consideration many morphological similarities to Chaetonotus (Primochaetus) veronicae Kânneby, 2013 we propose to include C. (P.) veronicae in the newly established genus under the new combination Bifidoachaetus veronicae (Kânneby, 2013), comb. nov. In the phylogenetic analysis based on nuclear 18S rRNA, 28S rRNA and mitochondrial cytochrome c oxidase subunit I sequence data, B. arcticus, gen. et sp. nov. is nested within the family Chaetonotidae, as the sister group to the genus Lepidochaetus Kisielewski, 1991. In this paper we also present new taxonomic characters useful for gastrotrich taxonomy: the pharynx-to-intestine length ratio (I) and the spine bifurcation ratio (B).

Introduction

Gastrotricha is a group of microscopic acoelomate metazoans with a total body length of 50–3500 \(\mu\)m (Kisielewski 1997; Balsamo \textit{et al.} 2014; Kieneke and Schmidt-Rhaesa 2015). The phylum is divided into two orders: Chaetonotida Remane, 1925 [Rao & Clausen, 1970] and Macrodasyida Remane, 1925 [Rao & Clausen, 1970]. Gastrotrichs can be found in virtually all aquatic environments, including semi-aquatic ecosystems (peatbogs, alder woods, riparian forests, etc.) in both natural and artificial habitats (Kolicka \textit{et al.} 2013; Kolicka 2014). This includes microreservoirs that form between the leaves of Bromeliaceae species (Kisielewski 1991; Kolicka 2016), water in caves (e.g. Todaro \textit{et al.} 2006), and even extreme ecosystems such as hot springs (Guerne 1888) and hydrothermal vent zones (Kieneke and Zekely 2007). Despite their abundance in various habitats, gastrotrichs have often been neglected in biodiversity surveys and ecological studies, or mentioned only as a group without identification to the species level (e.g. Kotwicki \textit{et al.} 2005; Jankowska \textit{et al.} 2014). The ~820 described species probably represent only a small fraction of their real taxonomic diversity (Balsamo \textit{et al.} 2015; Todaro 2015).

Although marine gastrotrichs from the Arctic have been reported in several papers (Pfannkuche and Thiel 1987; Węsławski \textit{et al.} 1997; Włodarska-Kowalcuk \textit{et al.} 1999; Todaro \textit{et al.} 2005, 2009; Urban-Malinga \textit{et al.} 2005; Balsamo \textit{et al.} 2010; Grzelak and Kotwicki 2012; Górska \textit{et al.} 2014), none from the Svalbard Archipelago were identified to species level. Also, the sparse freshwater gastrotrich observations from the Svalbard area are limited only to the level of phylum or genus (Scourfield 1897; De Smet \textit{et al.} 1887, 1888; De Smet 1993; Coulson \textit{et al.} 2014). Furthermore, the observed genus Chaetonotus Ehrenberg, 1830, which is the most species-rich group within Chaetonotidae Gosse, 1864 (\textit{sensu} Leasi & Todaro, 2008), is currently regarded as a polyphyletic taxon encompassing species from various evolutionary lineages (Balsamo \textit{et al.} 2008, 2014; Kânneby \textit{et al.} 2012, 2013).
Because the genus is virtually cosmopolitan and probably unnatural, reporting its presence without indicating the species is largely uninformative.

The taxonomy of Gastrotricha still relies primarily on morphological characters (Balsamo et al. 2008, 2014; Kånneby et al. 2012, 2013); however, molecular data suggest that such an approach may be a misleading indicator of phylogenetic relationships (e.g. Kånneby et al. 2012, 2013; Balsamo et al. 2014). Comprehensive phylogenetic analyses based on morphological characters also indicate that relying on generally accepted features such as the presence and shape of scales and spines can be misleading and not necessarily directly correlated with phylogenetic relationships (Hochberg and Litvaitis 2000; Balsamo et al. 2008; Kånneby et al. 2012, 2013). Even though knowledge regarding gastrotrichs continues to progress, there is still a lack of a unified set of key characters that should be taken into account when describing a new species. This problem arises from the necessity of working with live specimens, hence there is a short time before the animal deteriorates, and important diagnostic characters become increasingly more difficult to study. Another drawback is that none of the standard fixation methods have been completely successful. Even well-fixed material does not lead to high-quality permanent mounts (archival type specimens), so more appropriate documentation, especially for freshwater chaetonotids, is often recommended such as microphotographs, illustrations and detailed measurements (Kisielewski 1981).

The estimated number of marine gastrotrich species suggests that only ~20% of species diversity is currently known for these animals (Appeltans et al. 2012). Similar estimates are also available for the number of freshwater taxa in Europe (Balsamo et al. 2015), and they suggest that most freshwater gastrotrich species are so far undescribed. This is due to the low number of specialists working on this phylum, as reflected in part in the fact that new species are described on a regular basis. In contrast to other groups of small invertebrates, such as Tardigrada, which are relatively well-researched in the Arctic (for a reviews see Zawierucha et al. 2013, 2015), the diversity of gastrotrich species in the area of Spitsbergen as well as all the rest of Svalbard is still unknown.

In this study, we describe a new freshwater gastrotrich genus and species from the Arctic (the Svalbard Archipelago) based on morphology, morphometrics and genetic data. In light of our results, Chaetonotus (Primochaetus) veronicae Kånneby, 2013 is also included in the newly established genus. We also give a unified terminology for morphological characters in Gastrotricha. As a consequence of detailed morphological analysis, we introduce two new characters: the pharynx-to-intestine length ratio (I) and the spine bifurcation ratio (B).

Materials and methods

Study area

The study was conducted in Spitsbergen, the largest island of the Svalbard Archipelago. Svalbard is a high Arctic archipelago located between 74°–81°N and 10°–35°E. It comprises five main islands (Spitsbergen, Nordaustlandet, Edgeøya, Barentsøya and Bjørnøya) with a total surface area of ~63 000 km², ~60% of which is permanently covered by snow and ice (Hagen et al. 2003). Geographically the islands are remote: they are located 700 km off the coast of northern Norway, which is the nearest mainland, and 600 km east of Greenland (Coulson 2007, 2013; Pilskog et al. 2014). The mean annual temperature in Spitsbergen is −6.7°C. The mean temperatures during the warmest month of the year, August, peak at +5.9°C, while in February, which is usually the coldest month, the average temperature reaches −14.6°C. The range of extreme temperatures listed for this island extends from −42.2°C to 17.0°C (Coulson 2013). The summer is short and lasts only four months, i.e. June through September, with a mean air temperature above 0°C. The ground may be snow-covered and the soil frozen for nine months during a year (Coulson et al. 1995; Norwegian Meteorological Institute 2014).

Sampling and documentation

The samples were collected on 7 and 8 August 2013 from temporary, small and shallow water reservoirs originating from melting snow or ice and surface runoff. These reservoirs were located in Longyearbyen, the capital city of the Svalbard Archipelago, at a distance of ~300–3000 m from the fjord coast (Fig. 1). Specimens of the new species were collected from the reservoir located ~500 m from the sea. Bottom sediments had organic and organic-clay characters and fine structure.

The top layer of bottom sediment together with ambient water from five reservoirs were collected by hand from shallow water zones into 100-cm³ plastic containers. Approximately 10–20 cm³ of the sediment and 80–90 cm³ of ambient water were placed into each container. The collected samples were subsequently placed in an isothermal box and transported to the laboratory within 24 h. In the laboratory, samples were oxygenated and placed in a thermal chamber at a temperature of 10°C to create conditions similar to those at the sampling site. Within 10 days of collection, a total of 0.3 cm³ of sediment from each sample was analysed. Specimens of Gastrotricha were extracted with a micropipette under an Olympus SZ51 stereomicroscope (Tokyo, Shinjuku, Japan). All specimens were observed, photographed and documented alive under an Olympus BX41 compound microscope (Tokyo, Shinjuku, Japan) equipped with phase contrast and an Array Artcam 300 digital camera (Tokyo, Shinjuku, Japan). Morphometric characters were measured in cellSens Entry 1.11 software (Olympus). All measurements are given in micrometres (μm) and all formulas are given as a percentage (%).

Morphological analyses

Measurements are provided for all sampled specimens of the new species and are given as ranges (Tables S1–S3). Because of the low taxonomic utility of fixed gastrotrichs, each specimen was photographed. The species description follows Hummon et al. (1992), where the position of certain morphological characters is given in units (U) proportional to total body length measured from the anterior end to the distal end of the furca. The present study uses a modified version of Kisielewski’s (1991) convention to identify Gastrotricha by means of particular morphological measurements and indices.
(formulas and ratios). All morphological terms and measurable characters used in this study are listed in Figs S1–S3. This study also proposes a new index: the pharynx-to-intestine length ratio (I), which determines the age of a given specimen. The pharynx-to-intestine length ratio is the quotient of pharynx length and intestine length: 

\[ I = \frac{\text{pharynx length}}{\text{intestine length}} \times 100\% \] (Fig. 2A). Juvenile and subadult specimens have a significantly higher I-ratio than adults. Furthermore, due to the presence of bifid spines in the taxon of interest, the spine bifurcation ratio (B) was introduced. The ratio is the quotient of the length of the bifid section of the spine and the length of the entire spine, expressed as a percentage:

\[ B = \frac{\text{length of the bifid section of the spine}}{\text{length of the entire spine}} \times 100\% \] (Fig. 2B).

Data accessibility

Microphotographs of the holotype and six paratypes (five adults and one subadult) were deposited in the Natural History Collections at Adam Mickiewicz University in Poznan under accession number NHC–GBA–5–1–20/h (holotype) and NHC–GBA–5–21–170/p (paratypes) in the Morphbank under accession numbers 859852 (holotype); 859869, 859870, 859871, 859873, 859874, 859875 (paratypes).

Molecular data

Total genomic DNA was extracted from single specimens of the new species (six adults and one subadult; seven samples in total) using the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) as described by Dabert et al. (2008). A 670-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified with a 1 : 1 mixture of two forward primers, bcdF08 and bcdF09 (developed in this study), and reverse primer bcdR04 (Dabert et al. 2010). A complete sequence of 18S rRNA was amplified in two overlapping fragments using 18Sfw/rev960 and fw390/rev18S primer pairs (Dabert et al. 2010), respectively. A 2365-bp fragment of 28S rRNA was amplified using the primer pair U178Forward/2450R (Telford et al. 2003). Details of the primers used in this study are given in Table 1. Polymerase chain reactions were carried out in 10-μL reaction volumes containing 5 μL of the Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany), 0.5 μM of each primer and 4 μL of the DNA template using a thermocycling profile with one cycle of 5 min at 95°C followed by 35 steps each of 30 s at 95°C, 90 s at 50°C, 1 min at 72°C and with a final step of 5 min at 72°C for all reactions.
After amplification, the PCR products were diluted with 10 μL of MQ water; 5 μL of the diluted PCR reaction was analysed by electrophoresis on 1% agarose gel. Samples containing visible bands were purified with exonuclease I and Fast alkaline phosphatase (Fermentas, Waltham, MA) and sequenced using a BigDye Terminator v3.1 kit and an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Waltham, MA), following the manufacturer’s instructions. Individual sequence reads were

---

**Table 1. Polymerase chain reaction primers used in this study**

<table>
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<tr>
<th>Primer name</th>
<th>Sequence (5’–3’)</th>
<th>Product</th>
<th>Use</th>
<th>Source</th>
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<td>CGATGRTTTTTTTTCHACWAACCAYAARGATATCGG</td>
<td>COI</td>
<td>A</td>
<td>This study</td>
</tr>
<tr>
<td>bcdF09</td>
<td>CGATGRTTTTTTTTCHACWAACCAYAARGACATTGG</td>
<td>COI</td>
<td>A, S</td>
<td>This study</td>
</tr>
<tr>
<td>bcdR04</td>
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<td>COI</td>
<td>A, S</td>
<td>Dabert et al. 2010</td>
</tr>
<tr>
<td>18Sfw</td>
<td>CTTGTCTCAAAGATTAAAGCATTGCA</td>
<td>18S rRNA</td>
<td>A, S</td>
<td>Dabert et al. 2010</td>
</tr>
<tr>
<td>rev480</td>
<td>GTTATTTTTCGTCACTACCT</td>
<td>18S rRNA</td>
<td>S</td>
<td>Dabert et al. 2010</td>
</tr>
<tr>
<td>rev960</td>
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<td>Dabert et al. 2010</td>
</tr>
<tr>
<td>fw1230</td>
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<td>Dabert et al. 2010</td>
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<tr>
<td>rev1460</td>
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<td>S</td>
<td>Dabert et al. 2010</td>
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<td>A, S</td>
<td>Dabert et al. 2010</td>
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<tr>
<td>U178Forward</td>
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<td>A, S</td>
<td>Dabert et al. 2010</td>
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<tr>
<td>1634LReverse</td>
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<td>S</td>
<td>Telford et al. 2003</td>
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<tr>
<td>1200F</td>
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<td>S</td>
<td>Telford et al. 2003</td>
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<tr>
<td>2450R</td>
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<td>A, S</td>
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<tr>
<td>300F</td>
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<td>S</td>
<td>Telford et al. 2003</td>
</tr>
<tr>
<td>1200R</td>
<td>GCATAGCCACATCGTTTGCC</td>
<td>18S rRNA</td>
<td>S</td>
<td>Telford et al. 2003</td>
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<td>UJR2176</td>
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<td>1600F</td>
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<td>18S rRNA</td>
<td>S</td>
<td>Telford et al. 2003</td>
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aligned and manually assembled into contigs in ChromasPro v. 1.32 (Technelysium, Brisbane, Australia) and GeneDoc v. 2.7.000 (Nicholas and Nicholas 1997). Genetic distance among the COI sequences was estimated using the Kimura 2-parameter model as implemented in MEGA 6 (Tamura et al. 2013).

Phylogenetic analyses

The nucleotide blast search of COI, 18S and 28S rRNA sequences of Bifidochaetus arcticus, gen. et sp. nov. suggested Heterolepidoderma macrops Kisielewski, 1981, Ichthydium (Furcicillichthys) skandicum Kånneby, Todaro et Jondelius, 2009 and Lepidochaetus zelinkai (Grünspan, 1908) as the most similar taxa. Therefore, in our phylogenetic analyses we used representatives of the main clades reconstructed in the published molecular phylogeny of Chaetonotidae (Kånneby et al. 2013), including species that displayed some potential morphological affinities with the new species, as well as representatives of all species that clustered with H. macrops, I. (F.) skandicum and L. zelinkai. As the outgroup we used sequences of Aspidiphorphus polystictos Balsamo et Todaro, 1987, which has been reconstructed as the sister group to all other species of Chaetonotidae by Kånneby et al. (2013). In total, 47 terminals were included in the analysis (Table 2).

Both rRNA markers were initially aligned by ClustalX 1.81 (Thompson et al. 1997) with default parameters; if necessary, the obtained alignment was subsequently adjusted manually. No hypervariable alignment positions in 28S rRNA were excluded from the dataset. The DNA substitution models for 18S and 28S rRNAs were tested with jModelTest 0.11 (Posada 2002) and MrModeltest (Nylander 2004) for maximum likelihood (ML) and Bayesian inference (BI), respectively. The GTR+I+G model was appropriate for both markers. For COI mtDNA sequences, the two-rate codon-based model was applied (Goldman and Yang 1994) with the invertebrate mtDNA genetic code. Tree inference was performed for the individual genes and for a concatenated dataset of the three genes using ML and BI with Markov Chain Monte Carlo. For the concatenated dataset, partitioned ML and BI analyses were performed with the appropriate substitution model for each partition. Maximum likelihood trees were searched using Garli v. 2.0 (Zwickl 2006) with 30 search replications. Bayesian inference analyses of four independent runs were performed on a parallel version of MrBayes 3.2 (Ronquist et al. 2012) using the online Poznan Supercomputing Network Center at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences, Poznan, Poland. Each run of the BI analyses was performed in 3–10 × 10⁸ generations, and the trees were sampled every 1000th generation. The final 50% majority rule consensus tree was generated after discarding the 25% burn-in fraction of initial trees after assessing the chain convergence in Tracer v. 1. (Rambaut and Drummond 2007) judged by the average standard deviation of split frequencies dropping below 0.01. Support for the tree branches was calculated by the non-parametric bootstrap method (BS; Felsenstein 1985) with 100 replicates for ML analyses. Tree editing was performed using the programs FigTree 1.4.2 (Rambaut 2014) and TreeView 1.66 (Page 1996) and the tree editing tool of MEGA 6 (Tamura et al. 2013).

Results

In the recorded material, 308 specimens belonging to eight species were found, including 42 specimens of the new species. The remaining species found in the material belong to the subgenera C. (Chaetonotus) Ehrenberg, 1830 and C. (Hystricochaetonotus) Schwank, 1990. These species are also new to science and will be described in future publications.

Taxonomic account

Phylum GASTROTRICHA Mečnikow, 1865

Order CHAETONOTIDA Remane, 1925 [Rao & Clausen, 1970]

Suborder PAUCITUBULATINA d’Hondt, 1971

Family CHAETONOTIDAE Gosse, 1864 (sensu Leasi & Todaro, 2008)

Subfamily CHAETONOTINAE Gosse, 1864 (sensu Kisielewski, 1991)

Genus Bifidochaetus Kolicka et Kisielewski, gen. nov.

Type species: Bifidochaetus arcticus Kolicka et Kisielewski, gen. et sp. nov.

Terra typica: Spitsbergen, Svalbard Archipelago, Norway.

Locus typicus: Small water reservoir in Longyearbyen (78°13′14.28″N, 15°38′37.14″E).

http://zoobank.org/urn:lsid:zoobank.org:act:793CDB50-09EF-48B6-9A
DA-41850438A9DB

Diagnosis

Total body length ranging from 107 to 154 μm. Dorsal, dorsolateral, lateral, ventrolateral and ventral sides of the body covered with thin, one-lobed scales without keels and notches. Scales located close to one another, without or with overlapping edges. Scales present on the inner part of the furca. Most body spines emerge vertically from the scales, strongly curved and divide into two spines of equal length. The pharyngeal section and a large part of the intestinal section of the ventral interciliary field are naked; scales present only in the posteriormost part of the field. The ventral interciliary field terminal scales large and with a keel. Head clearly five-lobed with a distinct cephalion, epiplera and hypopleria marked in the body outline. Two pairs of cephalic ciliary tufts. Posterior tufts with straighter and clearly thicker cilia than in the anterior tufts. Mouth ring located subterminally. Mouth ring internal reinforcements weakly separated, long and rod-like. Long cuticular bristles inside the mouth ring. Pharynx with weak anterior enlargement, then gradually widened towards the posterior end. Furcal base clearly marked, with external base of furcal appendages bearing hummocks with spined scales. Furca measures from 16.1 to 20.6 μm. Adhesive tubes thick, rigid and not tapered.
towards the blunt slightly rounded distal ends; measure from 9.0 to 12.0 μm.

Taxonomic affinities

Bifidochaetus, gen. nov. shows a set of morphological features justifying its placement in the family Chaetonotidae: head separated from the trunk by a constricted neck; cephalic plates and ciliary tufts as well as dorsal sensory bristles present; furca with a pair of distal adhesive tubes present in the posterior end; body surface covered with cuticular scales and spines; ventral interciliary field with scales and a larger pair of ventral interciliary field terminal scales; ventral locomotory cilia in two separate longitudinal bands. Moreover, molecular data also provide strong evidence for placement Bifidochaetus, gen. nov. within Chaetonotidae (see Figs 15, S4–S6).

The new genus presents a set of unique characters that separate it from all existing members of Chaetonotidae including: (1) thin rounded scales with distally bifurcating hair-like spines and (2) a bottle-shaped body with the

Table 2. DNA sequences used in phylogenetic analysis

<table>
<thead>
<tr>
<th>Species</th>
<th>18S</th>
<th>28S</th>
<th>COI</th>
</tr>
</thead>
<tbody>
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<td>Arenotus strixinoi</td>
<td>JQ798537</td>
<td>JQ798608</td>
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<td>Aspidiophorus polyxenous</td>
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<tr>
<td>Aspidiophorus tetracaudus</td>
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<td>JN185576</td>
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<td>JQ798723</td>
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<tr>
<td>Chaetonotus sp.</td>
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<td>JQ798692</td>
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<td>Chaetonotus (Priemochaeetus) acanthocephalus</td>
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hummock at the external base of the furcal appendages. Despite these differences, the new taxon shares some similarities with the various subgenera of Chaetonotus. For example, the new taxon has rounded scales that lack a keel or posterior notch and also has spines that emerge from the centre or posterior scale region: similar features are present in C. (Marinochaetus) Kisielewski, 1997 and C. (Primochaetus) Kisielewski, 1997. The new taxon is also characterised by a spine type with strong diametrically opposite lateral branches similar to strong diametrically opposite lateral denticles on spines found in C. (Schizochaetonotus) Schwank, 1990. Lastly, the new taxon shares with C. (Tristratachaetus) Kolicka, Kisielewski, Nesteruk & Zawierucha, 2013 the presence of closely arranged scales and spines that emerge vertically from the centre of the scale and curve strongly beyond the short basal segment. Moreover, the new taxon has spines with a vertically emerging basal section that is reminiscent of the peduncle in Aspidiophorus Voigt, 1903.

**Etymology**

From the Latin ‘bifidus’ (divided into two parts) and Greek ‘khaite’ (long hair, referring to the unique double spine).  

*Bifidochaetus arcticus* Kolicka et Kisielewski, gen. et sp. nov.  
(Figs 3–15; Tables S1–S3)  
http://zoobank.org/urn:lsid:zoobank.org:act:5725037D-AF42-4E52-A8B5-7DEB4219F085

**Material examined**

One sample, 42 specimens (36 adults, five subadults, one juvenile), all photographed.

**Diagnosis**

Adult total body length ranging from 137 to 154 μm. Juvenile and subadult total body length ranging from 107 to 139 μm. Scales subtriangular with strongly rounded, convex edges, located close to one another but without overlapping edges (Figs 5, 11A, C). Scale size differentiated within the body and all scales well distinct. Scales distributed in 36–45 longitudinal alternating rows with 30–42 scales in the central row. Entire scale surface lies on the cuticle. Three pairs of parafurcal scales with bifid spines are present on the lateral sides of the furcal appendages. Scales present on the inner part of the furca. The ventral interciliary field terminal scales separated, very large, oval with narrowed anterior part and with keel (Figs 4B, 14D). Head clearly five-lobed with a distinct cephalion, epipleuria and hypopleuria well marked in the body outline (Fig. 7). Two pairs of cephalic ciliary tufts. Posterior tufts with straighter and clearly thicker cilia than in the anterior tuft (Fig. 3A). Pharynx length in adult specimens ranging from 35.8 to 43.1 μm, in juvenile and subadult specimens ranging from 34.2 to 43.6 μm. Pharynx with weak anterior enlargement, then gradually widened towards the posterior end (Figs 4A, 11A). Intestine straight with anterior section marked as a narrow bend. Three pairs of dorsal sensory bristles. Furcal base clearly marked, with external base of furcal appendages bearing hummocks with spined scales (Fig. 14C). Pear-shaped furcal indentation (Figs 3, 4, 7–9).

**Description**

This new species has a slender, bottle-shaped body with a clearly marked neck constriction and furcal base (Figs 3, 4, 7). The head is five-lobed and parabolic in shape. Cephalion and pleuria are separated. However, the presence of strongly curved spines around the cephalion, epipleuria and hypopleuria gives the impression that the plates are weakly marked and almost invisible in the body outline (Figs 3, 4, 7–9). The cephalion (U1–U4) is narrow and adheres to the head along the entire length. The epipleuria (U5–U8) and hypopleuria (U9–U13) are similar in size and separated by shallow notches. The epipleuria are more convex and more rounded than the hypopleuria (Fig. 6). The epipleuria and hypopleuria are mostly located on the lateral side of the head and only small parts of them are visible from the dorsal side (Fig. 12A, B). The hypostomium (U4–U5) is small, narrow and oval, and its lateral edges are stronger than anterior and posterior ones (Fig. 4B). Two pairs of cephalic ciliary tufts emerge from between the cephalic plates; the anterior tuft has five cilia and the posterior one has five to six cilia. The area they emerge from is clearly visible between the pleuria on the dorsolateral side (Fig. 3B). The anterior tuft consists of four short cilia of similar length and a fifth, much longer, posteriormost cilium. The posterior tuft has three to four cilia of similar length located in a row, followed by a much longer cilium and a cilium that is shorter than the preceding one but longer than the initial cilia. All cilia in the posterior tufts are straighter and clearly thicker than the cilia in the anterior tufts (0.81–1.37 μm vs 0.4–0.9 μm) (Figs 3, 4). The mouth ring is located subterminally on U2–U3 and has long, weakly marked cuticular reinforcements as well as long cuticular mouth bristles (Figs 4B, 12B). The pharynx (from U3 to U30) has a weak anterior dilatation, beyond which it dilates gradually towards the posterior end (Fig. 11A; Table S1). The pharynx is heavily musculated, with muscles visible as horizontal bands (Figs 4A, 11A). It connects through the junction to the straight intestine, which extends from U30 to U86. The pharyngeal-intestinal junction is clearly marked, long and broad. The intestine has a distinct separate anterior section (from U30 to U31) that forms a narrow band located dorsally and partly laterally at the pharyngeal-intestinal junction (Figs 4A, 11B).

The head is wider than the neck. The neck tapers gradually from the head (from U13) to the beginning of the trunk (~U30) (Figs 3, 4, 7–9). The initial part of the trunk (from U30 to U35) is of the same width as the neck. The trunk dilates (initially rapidly, then gradually) from about U36 to about U61, where it is at its maximum width. Next it tapers (initially rapidly, then gradually) up to the furcal base (U87) (Fig. 7). From about U36 to about U86 the trunk is symmetrical, with a transverse axis of symmetry located at about U61, i.e. the shape of the trunk from the beginning of its wider part to its widest part is the same as from its widest part to the furcal base. The furcal base is clearly marked and narrow. On each furcal appendage, two clearly lateral indentations and one lateral hummock are present (Figs 3, 4, 7–9). The first indentation is located at U87 and is followed by a lateral hummock in the form of a thick fold of cuticle, and by a second indentation (U90). The furcal indentation (intrafurcal space) is pear-shaped. The furcal branches point slightly outwards at their distal ends and the
distance between their tips is greater than the width of the furcal base (Figs 3, 4, 7–9). The adhesive tubes are thick (1.9–2.6 μm), rigid, slightly curved towards the centre of the indentation and have the same width along their entire length. The ends of the adhesive tubes are blunt with slightly rounded edges (Figs 3, 4, 7–9).

The entire body is covered with thin, one-lobed scales that adhere with their entire surface to the cuticle (Figs 3, 4, 7, 8). The scales are distributed in 36–45 longitudinal alternating rows with 30–42 scales in the central row (see Fig. S3). The scales are located close to one another. Their edges meet and are juxtaposed but never overlap (Figs 5, 12A, C). The longitudinal rows of scales begin on the head directly behind the cephalion, epipleuria and hypopleuria (Fig. 12A). The central longitudinal row of scales extends straight down the dorsal side, parallel to the longitudinal axis of the body. Scales in each longitudinal row located laterally to the central longitudinal row (on dorsal, dorsolateral, lateral and ventrolateral body surfaces) are slightly posterior to the scales in the central row (Figs 3, 12A, C). Each subsequent row counting from the central one has scales placed lower than the preceding longitudinal row. Such a distribution forms a system of scales that converge in the direction of the central longitudinal row. The scales that converge in the direction of the central longitudinal row on the head and the neck form rounded arcs, while on the trunk they form straight lines that are slanted at ~40° relative to the central row. The ventrolateral and ventral scales of the three subsequent longitudinal rows located closest to the ventral ciliary bands have tops of scales rotated by ~30° towards the bands (Figs 4B, 14B). The spines that emerge from these bands are adequately rotated. The scales of the dorsal, dorsolateral, lateral, ventrolateral and ventral sides of the body, except for those of the dorsal posterior trunk region, have no keel and no indentation of the posterior edge. They are subtriangular, with strongly rounded, convex edges (Fig. 5). The scales of the dorsal, dorsolateral, lateral, ventrolateral and ventral surfaces of the head, neck and trunk gradually decrease in size in the direction of the ventral ciliary bands (see Table S2). The scales in the ventral longitudinal row located closest to the ciliary band are distinctly smaller than the other scales (Figs 4B, 14B; Table S2). The dorsal, dorsolateral, lateral, ventrolateral and ventral spines emerge vertically from the exact centre of the scales (Fig. 5). After a short (0.6–3.0 μm), straight base segment that emerges vertically from the scale, the spine strongly curves (at an angle of ~90°) and bifid, branches off at the curvature into two separate, thick, strongly curved spines of equal length (5.0–10.8 μm) that taper towards their hair-like ends (Figs 5,
The longest spines are on the head (Figs 12B, 13A, B) and gradually shorten on the neck to become still shorter on the trunk (Figs 12D, 13D; Table S3). In addition to this the longest spines are dorsal and gradually shorten in the direction of ventral ciliary bands (Figs 12B, D, 13A, B, D, 14B; Table S3). The spines of the head and neck are more curved at the point of bifurcation than the trunk ones (Fig. 12B, D; Table S3).

An area with single-spined scales is present on the dorsal posterior trunk region (Figs 3A, 14A). The anteriormost scales in this area are located in the central longitudinal row and the two adjacent to it (U82–U84); they are shaped like elongated triangles with strongly curved, convex edges, do not have a keel and are greater in size than the surrounding scales (3.7–5.6 μm × 3.1–5.1 μm) (scales 1) (see Figs S1, 6, 14A). Their spines emerge from the centre of the scale, are long (12.2–17.8 μm), singular and hair-like. The scales located in two, or three in some specimens, subsequent transverse rows on the dorsal posterior trunk region (U85–U86) are smaller (1.6–4.0 μm × 1.3–3.7 μm), but similar to the previous scales (scales 2). Their spines emerge at the posterior edge of the scale, are short (1.7–4.1 μm), simple and straight, and have blunt ends. A pair of large (4.2–5.4 μm × 3.2–5.1 μm), hexagonal scales with rounded edges, a very weak keel and a short (2.3–4.5 μm), simple and straight spine with a blunt end is present on each dorsolateral side of the furcal base at U86 (scales 3). Two pairs of triangular scales with strongly rounded, convex edges are present on the furcal base. The anterior pair is located dorsolaterally at U85 (2.9–4.9 μm × 2.5–4.2 μm) (scales 4). Long (14.5–19.6 μm), single, curved spines that taper towards the end emerge from the centre of these scales. The posterior pair is located laterally at U86 (2.4–3.9 μm × 1.9–3.5 μm) (scales 5). Their spines constitute the posteriormost pair of the lateral spines, reaching up to about three-quarters of the length of the adhesive tubes (U98–U99) and are stouter and stronger than the other spines of the body (18.3–22.1 μm). A pair of large scales (4.4–7.0 μm × 3.8–5.5 μm) is located at the dorsal base of each furcal appendage at U87–U88 (scales 6). These two scales are oval, taper towards the posterior end and have a weak keel and a simple, straight spine (12.9–5.3 μm) with blunt ends. Four keel-less oval scales that have spines with blunt ends emerging from the posterior side of the scales are present in the centre of the dorsal side of the furcal appendages (1.4–3.9 μm × 1.1–3.0 μm) (scales 7). The spines of the central scales reach up to the furcal indentation (1.6–4.4 μm). Three pairs of parafurcal scales (2.4–3.9 μm × 2.4–4.0 μm; of the same type as on the trunk) with bifid spines (7.4–9.7 μm) are present laterally on the furcal appendages at U88 (scales 8) (Figs 3A, 14A and Fig. S1). The intrafurcal scales are almost circular and present on the inner side of the furcal appendages at U90 (1.1–2.0 μm × 1.2–2.6 μm) (scales 9). The edges of these scales are curved, thus making the entire scale adhere to the narrow surface of the inner furcal indentation (Figs 14C, 14D; S1). The inner scales of the furca are distributed in two rows with six
Fig. 5. *Bifidochaetus arcticus*, gen. et sp. nov. (A) Scales with bifid spines; (B) arrangements of scales.

Fig. 6. *Bifidochaetus arcticus*, gen. et sp. nov. Arrangements of scales on dorsal posterior trunk region.
scales in the row closer to the dorsal side and four scales in the row closer to the ventral side. A long (5.9–18.4 μm), strongly curved, thin spine emerges from all these scales that is visible inside the furcal indentation (Figs 3, 4, 7–9, 14C). Spines that emerge from the central scales of the inner part of the furca are less curved than the spines of the lateral pairs of scales (Figs 3, 4).

Longitudinal ventral ciliary bands begin at U2 and run up to U86 (Fig. 4B). The ciliary bands on the head lie directly next to the epipleuria and hypopleuria, enter the inside of the notch between them, and surround the lateral edges of the hypostomium. The ciliary bands are wider on the head and neck than on the trunk. The ventral interciliary field has no cuticular structures (Fig. 14B) except for the dorsal posterior trunk region (from U81 to U87) (Fig. 14D). Here they are oval, have a short, weakly marked keel and are distributed in three to four longitudinal rows with five scales in each row (Figs 4B, 8C, 9C, 14B, D). The longitudinal row of ventral field scales closest to the ventral ciliary bands has smaller scales than in the central rows (Fig. 14B; Table S2). The terminal scales of the ventral interciliary field are very large (9.3–15.0 μm × 4.6–7.9 μm), oval and have narrowed anterior parts as well as a weak keel (Figs 4B, 8, 9C, 14D).

The species has three pairs of dorsal sensory bristles (Fig. 3A). The first, anterior pair is located on the dorsal side of the head at U3, near the epipleuria. The bristles emerge from small, spherical papillae. The second pair of sensory bristles is located on the dorsal side of the neck and emerges from small, spherical papillae present between the third and fourth longitudinal rows of scales, counting from the central longitudinal row (U25). The third, posterior pair of sensory bristles is located dorsally at U81 before the area with the single-spined scales. These bristles emerge from scales shaped like an inverted heart with two keels and very weakly notched posterior edges.

The X-organ of this species (observed in six specimens) is located at U84–U86 and surrounds the terminal part of the intestine. It is bilobed, built from two extensions enveloped in a thin coat, and connected by a thinner band located behind the intestine at the ventral side. The extensions have a granular, grain-like structure. The thin coat and the cellular bridge connecting the extensions have a smooth and homogeneous structure (Fig. 10D). The sperm packets of this species take the form of long, coiled strings and are located at U65–U70 on the sides of the intestine (Fig. 10C).

Sequence diversity and phylogenetic relationships
Two different COI haplotypes were found among the six adult and one subadult specimens of B. arcticus, gen. et sp. nov. that were analysed; one of the haplotypes was shared by six individuals, and the second haplotype was found in one specimen with a developing egg. Although the mean genetic distance between the two haplotypes was relatively low and amounted to 0.13% (SD 0.08), one of the three nucleotide substitutions resulted in substitution of isoleucine by valine. No intraspecific sequence variation was found in the 18S and 28S rRNA sequences.

The total dataset for phylogenetic analysis comprised 4918 nucleotide positions (nps), including 657 nps for COI, 1741 nps for 18S rRNA and 2520 nps for 28S rRNA. Bayesian inference and ML phylogenetic analyses of the combined dataset provide strong support (PP = 1, BS = 84) for placement of B. arcticus, gen. et sp. nov. in the Chaetonotidae clade containing representatives of five genera: Chaetonotus Ehrenberg, 1830, Aspidiophorus Voigt, 1903, Lepidodermella Blake, 1933, Ichthydium Ehrenberg, 1830 and Lepidochaetus Käselewski, 1991 (Fig. 15). The individual gene trees either strongly support this clade in 28S rRNA analysis (PP = 0.99, BS = 71 for ML) or do not conflict with it (18S rRNA, COI) (Figs S4–S6). In the BI tree for the combined dataset, B. arcticus, gen. et sp. nov. is resolved with strong support (PP = 1) as a sister group to the genus Lepidochaetus. This relationship is consistent, but varies in support across the datasets (COI, 28S rRNA and COI+18S+28S) and analysis methods (BI, ML), with one exception: in the ML and BI trees for 18S rRNA, B. arcticus, gen. et sp. nov. is resolved as basal to the clade grouping Aspidiophorus tetrachaetus, Chaetonotus (Primochaetus) acanthocephalus, C. (P.) acanthodes, C. (P.) heideri and Lepidodermella, but this relationship is unsupported supported (PP = 0.77, BS = 22).

Differential diagnosis
Among all the species known to date, Bifidochaetus arcticus, gen. et. sp. nov. is most similar to Chaetonotus (Primochaetus) veronicae Kanneby, 2013, reassigned to the genus...
Bifidochaetus, gen. nov. in the present study (see below). These two species share the following characters: thin scales with no posterior notch that adhere to the cuticle with their entire surface, long bifid spines that curve strongly beyond a straight basal segment and taper towards their hair-like ends, spines present inside the inner furcal indentation (intrafurcal spines), the last pair of lateral trunk spines stronger than other spines of the body, hummocks on the lateral sides of furcal appendages, thick and rigid adhesive tubes, as well as muscular pharynx with marked anterior and no distinct posterior dilatation. However, B. arcticus, gen. et sp. nov. differs from B. veronicae, comb. nov. in terms of: body size (B. veronicae, comb. nov. is smaller and adults measure 123–135 µm); shape, size and arrangement of pleurae (in B. veronicae, comb. nov. they are small and weakly marked on head outline); thickness of cephalic cilia (B. veronicae, comb. nov. has cilia in both pairs of cephalic tufts of similar thickness); pharynx length (in B. veronicae, comb. nov. pharynx in adults measures 34–35 µm); number of scales (in B. veronicae, comb. nov. scales are distributed in 30–33 longitudinal alternating rows with 27–30 scales in central row); scale shape and type (B. veronicae, comb. nov. has round to suboval scales with overlapping edges, of which anterior parts are fused to the body surface); distinct scales in the furcal base (in B. veronicae, comb. nov. scales on the furcal base are more reduced than other scales of the dorsal body surface); presence

Fig. 8. Bifidochaetus arcticus, gen. et sp. nov. Holotype (phase contrast microphotographs): (A) dorsal body view; (B) internal body view; (C) ventral body view.
of scales with spines at the lateral side of furcal appendages (in *B. veronicae*, comb. nov. lateral scales are not present); location of intrafurcal scales with long, single spines (in *B. veronicae*, comb. nov. intrafurcal scales are located at ventral body side); the difference in scale size on the body (in *B. veronicae*, comb. nov. scales are a similar size all over the body, although slightly smaller anteriorly); the position where the spines emerge from scales (in *B. veronicae*, comb. nov. spines arise from the posterior part of each scale); length of basal (not bifid) part of spines (in *B. veronicae*, comb. nov. the basal part of spines is longer and is ~1/3 of the total spine length); thickness of bifid part of spines (in *B. veronicae*, comb. nov. the bifid part of spines is significantly thinner); degree of ventrolateral spine curvature (*B. veronicae*, comb. nov. has ventrolateral spines only slightly curved); number of pairs of dorsal sensory bristles (*B. veronicae*, comb. nov. has only one pair of dorsal sensory bristles, on the trunk); shape, type and size of ventral interciliary field terminal scale (*B. veronicae*, comb. nov. has keeled suboval scales strongly overlapping, which measure 6–8 µm × 4–5 µm); shape of furca (in *B. veronicae*, comb. nov. the furca is straight).

**Remarks**

In *B. arcticus*, gen. et sp. nov., three pairs of dorsal sensory bristles were observed. The feature is rarely listed among the freshwater members of the family Chaetonotidae and, so far, has been observed only in *Chaetonotus* (*Chaetonotus*) *brevispinosus* Zelinka, 1889 and *C. (C.) sanctipauli* Kisielewski, 1991. They are much more common in marine and brackish species, e.g. *Aspidiophorus lamellophorus* Balsamo, Hummon, Todaro et Tongiorgi, 1997 (see Kolicka et al. 2015). A larger number of dorsal sensory bristles (up to six pairs) is typically characteristic of marine genera, such as *Heteroxenotrichula* Wilke, 1954 and *Xenotrichula* Remane, 1927 (from Xenotrichulidae Remane, 1927).

The presence of intrafurcal scales similar to those observed in *B. arcticus*, gen. et sp. nov. is a very common character in representatives of the genus *Lepidodermella* Blake, 1933 and is also observed in some species from *Chaetonotus* Ehrenberg, 1830 – e.g. *Chaetonotus* (*Primochaetus*) *acanthocephalus* Valkanov, 1937, *C. (P.) brachyurus* Balsamo, 1980, *C. (Chaetonotus) heterospinosus* Balsamo, 1978, *C. (C.) pseudopolyspinosus*...
Kisielewski, 1991 and C. (C.) ventrochaetus Kisielewski, 1991. However, other previously described species do not have intrafurcal scales with long, single, curved spines located internally in the furcal indentation. Chaetonotus representatives that have intrafurcal scales always have them located dorsally or ventrally, never internally. Among the observed specimens, one had a large developing egg. Furthermore, the X-organ was observed in six specimens and a maturing set of sperm cells was observed in two specimens. It should be noted that body size (length) is not an accurate indicator of age in Gastrotricha. In B. arcticus, gen. et sp. nov., the specimen with the developed egg and the specimen with the marked X-organ (whose presence may indicate a post-parthenogenetic life phase) both had body sizes similar to that of one of the subadult specimens. Clear differences between the adult and subadult or juvenile specimens were observed for head size (the head was disproportionately large in relation to the rest of the body in young specimens) (e.g. Remane 1936; Mock 1979; Balsamo and Todaro 1987; Hochberg 1998; Balsamo et al. 2014). Usually in juveniles the head is wider than the trunk and in adults the trunk is wider than the head (Balsamo et al. 2014). However, this rule does not apply to all species, e.g. B. arcticus, gen. et sp. nov. has a bottle-

Fig. 10. Bifidochaetus arcticus, gen. et sp. nov. (phase contrast and brightfield microphotographs). (A) Specimen with mature egg; (B) mature egg; (C) a developing package of sperm; (D) X-organ.
shaped body and its head is always narrower than the trunk, even in very young individuals. Therefore, we propose as a more adequate indicator the pharynx-to-intestine ratio (I), whose value in juvenile specimens is greater or equal to 55% (≥55% in juveniles or subadults and <55% in adults) and is not strongly associated with a species-specific body shape (Table S1).

**Locality**
Small water reservoirs in Longyearbyen (78°13’14.28”N, 15°38’37.14”E), Spitsbergen, Svalbard Archipelago, Norway.

**Etymology**
From the name of the geographic region and to commemorate the first freshwater species described from the area.

*Bifidochaetus veronicae*, comb. nov. (Kånneby, 2013)

**Diagnosis**
Adult total body length ranging from 123 to 135 μm. Scales of similar size although slightly smaller anteriorly. Scales distributed in 30–33 longitudinal alternating rows with 27–30 scales in central row. Anterior part of scales fused with body surface, some scales reduced. Scales round to suboval with overlapping edges. Parafurcal scales on lateral sides of furcal appendages absent. Scales present on inner part of furca. Ventral interciliary field terminal scales large, oval with keel and with overlapping edges. Head five-lobed with a small cephalion, epipleuria and hypopleuria weakly marked in body outline. Two pairs of cephalic ciliary tufts; their cilia of similar thickness. Pharynx length of adult specimens ranging from 34 to 35 μm. Pharynx with weak anterior enlargement, then gradually widening towards the posterior end. Intestine straight without differentiated anterior section. One pair of dorsal sensory bristles, only on trunk. Furcal base clearly marked, with external base of furcal appendages bearing hummocks. Furca straight.

**Remarks**
The reason for transferring this species to the genus *Bifidochaetus*, gen. nov. is the presence of bifid spines as opposed to the single spines of the genus *Chaetonotus*. In the original description and drawing (see Kånneby 2013: fig. 23), as well as in selected holotype microphotographs (see Kånneby 2013: fig. 22), only simple non-bifid spines were present and an incorrect impression is given with respect to whether this species has bifid spines arising from dorsal, dorsolateral, lateral,
ventrolateral and ventral scales (see Kånneby 2015). The following features justify the inclusion of this species in the newly established genus: the presence of bifid spines and a bottle-shaped body with a hummock at the external base of the furcal appendages; thin, one-lobed scales; thick, rigid adhesive tubes; mouth ring with weakly marked cuticular reinforcements and long cuticular mouth bristles; heavily muscularised pharynx with weak anterior dilatation.

**Discussion**

Our phylogenetic analyses of DNA sequences indicate a close relationship between *Bifidochaetus*, gen. nov. and *Lepidochaetus* Kisielewski, 1991 (Figs 15, S4–S6). Morphological features suggesting this relationship are as follows: most of body covered in scales with rounded edges, without posterior notches or keel (except for scales with posterior sensory bristles and a single pair of scales at the posterior part of the trunk in *Bifidochaetus*, gen. nov.); presence of longer spines of different type on the furcal base than the other spines on the dorsal, dorsolateral, lateral and ventral sides of the posterior part of trunk; short cuticular reinforcements of the mouth ring with long internal cuticular hairs; ventral locomotor cilia bands wider in the head region; and thick, rigid adhesive tubes. However, the two genera differ significantly in other essential characters, such as: body habitus (*Lepidochaetus* has a large, stocky body with a

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**Fig. 12.** *Bifidochaetus arcticus*, gen. et sp. nov. (brightfield microphotographs). Dorsal view of scales: (A) on the head and neck; (C) on the trunk. Dorsal view of spines: (B) on the head and neck; (D) on the trunk.
weak neck constriction and not a clearly marked furcal base); scale type (Lepidochaetus exhibits extraverted scales, i.e. with double anterior edges, connected to the body surface by their anterior part only); scale type and size variation (all scales in Lepidochaetus are of similar size and shape); type of spines (Lepidochaetus possesses dentate posterior spines), as well as the type of furcal base and shape of adhesive tubes (Lepidochaetus has an indistinct furcal base, the furcal indentations are narrow and the adhesive tubes are straight). Foregoing similarities and differences in morphology between these two genera and the clear separation of the clades in our phylogenetic trees indicate the high evolutionary distinctiveness of Bifidochaetus–Lepidochaetus.

The results of our molecular phylogenetic analyses are mostly consistent with the previous molecular phylogeny of Chaetonotidae (Kånneby et al. 2013), except for relationships among species of the most distal clade of Chaetonotidae (see Kånneby et al. 2013: fig. 4). In the earlier reconstruction (Kånneby et al. 2013), the Lepidochaetus clade was sister group to a clade grouping species of Chaetonotus (Primochaetus), Aspidophorus, Lepidodermella and Ichthydium. In our tree, the genus Lepidochaetus forms a clade with Bifidochaetus arcticus, gen. et sp. nov.; this monophyletic group is the sister clade to the Ichthydium–Aspidophorus tetrachaetus–Lepidodermella group. Although Bifidochaetus, gen. nov. shares several morphological characters with other groups within

Fig. 13. Bifidochaetus arcticus, gen. et sp. nov. (brightfield microphotographs). (A) Internal view of head and neck. Lateral view: (B) of head and neck; (C) of scales on trunk; (D) of spines on trunk.
Chaetonotidae, especially with certain subgenera of Chaetonotus (see ‘Taxonomic affinities’ for the genus), these characters may reflect homoplasy in the perspective of the phylogenetic relationships at genus level inferred from molecular data (see Kånneby et al. 2013). Further sampling for molecular studies including detailed morphological and morphometric analyses would be of great value in solving the taxonomy of Chaetonotidae, where most current genera are indeed polyphyletic. Further research could result in significant changes to the systematics of this group.

From a morphological point of view, a hypothesised evolutionary scenario could see the bifid spines evolve through the formation of a thin lamella partially and subsequently entirely linking the bifid spine sections, thus forming a pedunculate scale similar to those present in Aspidiophorus Voight, 1903. A similar evolutionary scenario could also be the case for ancestors of Chaetonotus (Schizochaetonotus) Schwank, 1990. On the other hand, the opposite is also possible, i.e. a reduction of the pedunculate secondary scales in Aspidiophorus to a bifid or dentate spine as in Bifidochaetus, gen. nov. and C. (Schizochaetonotus), respectively.

In many representatives of chaetonotid genera, characters considered to be distinguishing at the genus level are ambiguous and can appear in varying proportions in particular species (e.g. scales, spines or lamellae). Many species display characters that are present in several different genera or characteristics considered to be representative of another genus. Species with non-specific features for their genus are highly common within Chaetonotidae. According to Kånneby et al. (2013), while differences in cuticular coverage constitute a very accurate indicator of specific level, they have no diagnostic validity for genera. In case of present discrepancies between morphological and molecular data, future research will have to analyse both detailed morphological and molecular data and carefully examine their consistency. Fiers and Kotwicki (2013) demonstrate that analysing only selected, easily discernible morphological characters can be misleading. The two authors used a morphological analysis to prove that a taxon of cosmopolitan Harpacticoida (Crustacea, Copepoda), considered to be one species, constitutes in fact four species with significant differences in external morphology and geographical distribution. In Gastrotricha, Kånneby et al. (2012) drew attention to the morphological differences between two separate clades that were currently considered species, Lepidodermella squamata (Dujardin, 1841), obtained based on molecular analyses. These authors, however, find that morphological differences between the clades could be explained by developmental flexibility during cuticle formation, like that observed by Amato and Weiss...
(1982) in research on clonal individuals of this species, but also speculate that cryptic speciation may be taking place (Kånneby et al. 2012). Also, earlier studies (e.g. Fregni et al. 1998) suggest that the species L. squamata should be divided into distinct species based on differences in shape and number of dorsal, dorsolateral, lateral, ventrolateral and ventral scales; type, shape and number of ventral interciliary field terminal scales and the presence or absence of parafurcal spines. Likewise, Leasi and Todaro (2009), using confocal laser scanning microscopy, found significant differences in the construction of the muscular system in two geographically separate groups currently known as species Xenotrichula intermedia Remane, 1934. Their observation indicates that morphological differences of species regarded as cryptic are possible. Within Gastrotricha it is highly plausible that numerous taxa currently considered to be cryptic species will prove to be a complex of species that are similar yet differentiable in terms of appearance after thorough morphological analysis.

During gastrotrich postembryonic development, the number and size of cuticular structures as well as the degree of their separation from the cuticle can change (Amato and Weiss 1982). Moreover, in juvenile and subadult specimens the spines can be thinner and do not possess marked lateral denticles even if this structure exists in adult individuals (Kisielewski 1997). In our study, we proposed a new parameter: the pharynx-to-intestine length ratio (I), which enables an objective determination of the degree of development of an individual. This index can help identify whether the individual is a juvenile, subadult or adult even without other means to estimate age (such as a developing egg or visible X-organ). Gauging age is important to avoid errors in species identification and description of morphological characters. Also, to improve the quality of morphological differentiation, we suggest the use of spine bifurcation ratio (B) parameters. The point at which the single spine divides into two separate spines is important in distinguishing the two species now present within L. squamata, gen. nov., and may be useful if additional species are described in the genus.

It should be emphasised that some characters may have appeared independently during the evolution of Gastrotricha, such as the presence of pedunculated scales in Aspidiophorus Voigt, 1903, some representatives of Polymerurus Remane, 1927, Lepidoderma Blache, 1933, as well as the marine Xenotrichula Remane, 1927 and Draculiceteria Hammon, 1974.

The taxon discovered in our research is characterised by a unique composition of morphological features. The combination of these characters did not allow us to assign the taxon to any of the currently known species or genera. Molecular examination of the taxon also indicated its distinctness from the other included Chaetonotidae. Therefore, in this paper we included
a description of a previously described species for which we believe a separate genus should be defined.

Conclusion
In this paper we propose the new genus Bifidochaetus, gen. nov., within the family Chaetonotidae (Gastrotricha) based on morphological characters and its placement in a molecular phylogenetic analysis. Bifidochaetus arcticus, gen. et sp. nov. is the first species of freshwater gastrotrich determined to species level from the Arctic. Moreover, the redescription of Chaetonotus (Primochaetus) veronicae Känneby, 2013 (Känneby 2015) brings characters into light that place it in our newly proposed genus, hence it is reclassified as B. veronicae, comb. nov. (Känneby 2013). Detailed morphological comparisons of these taxa indicate they are two separate species. Moreover, we propose the use of two new parameters: the pharynx-to-intestine length ratio (I) and the spine bifurcation ratio (B).

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