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TITLE: The phylogenetic position of Neogosseidae (Gastrotricha: Chaetonotida) and the origin of planktonic Gastrotricha

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ABSTRACT

1
2 Planktonic forms of Gastrotricha have been known since the 1850s, despite the fact
3 that they are rather uncommon and difficult to collect. They are characterized by a
4 round sack-shaped body, an absence of furcal adhesive tubes, and a different
5 distribution of the locomotory ciliation compared to epibenthic and periphytic
6 gastrotrichs. Today, planktonic gastrotrichs are classified into the three taxa –
7 Dasydytidae, Neogosseidae, and *Undula* – but their origin and whether they share a
8 recent common ancestor remains largely unknown. A long held view is that
9 planktonic taxa derive from benthic ancestors related to *Chaetonotus (Zonochaeta)*,
10 but the hypothesis has never been properly tested. Here, in order to elucidate the
11 phylogeny and origin of planktonic Gastrotricha, we provide the first molecular data
12 on the very rare genera *Kijanebalola* and *Neogosseia*, both members of the family
13 Neogosseidae. We use Bayesian and maximum likelihood phylogenetics to analyze
14 sequences of 18S rDNA, 28S rDNA and COI mtDNA spanning 71 taxa in total. We
15 find high support for a common origin of planktonic gastrotrichs, with monophyly of
16 both Dasydytidae and Neogosseidae. Planktonic forms have evolved from epibenthic
17 or periphytic ancestors, and the closest extant clade comprises members of
18 *Chaetonotus (Zonochaeta)* + *Chaetonotus heteracanthus* Remane, 1927. These results
19 further imply that the motile spines and underlying muscle patterns that control them
20 in species of Dasydytidae are adaptations to the planktonic environment that evolved
21 independently of those in other species of Gastrotricha.
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40 **KEYWORDS:** Meiofauna; Freshwater; Planktonic; Phylogeny; Paucitubulatina
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INTRODUCTION

1 Gastrotricha is a small phylum of aquatic acoelomate animals with approximately 850
2 species (see Balsamo et al. 2009; 2014; Hummon and Todaro 2010; Kieneke and
3 Schmidt-Rhaesa 2014; Todaro et al. 2014; and references therein). The group is a
4 common component of the meiofauna and is hypothesized to act as an important link
5 between the microbial loop and larger invertebrate predators (Balsamo and Todaro
6 2002). The phylum is divided into the two orders Chaetonotida and Macro-dasyida.
7 Chaetonotida, present in both freshwater and marine habitats, are generally tenpin-
8 shaped with adhesive tubes confined to the posterior end, and with the cuticle
9 generally sculptured into various arrangements of scales and spines. Macro-dasyida,
10 with few exceptions are entirely marine and vermiform, with adhesive tubes not
11 confined to the posterior end, and with a smooth or sculptured cuticle.

22 Freshwater gastrotrichs within Chaetonotida are very small, ranging from 60
23 μm to 770 μm in total body length. Most species are epibenthic, periphytic or
24 interstitial, but some also have a planktonic life-style. The first records of planktonic
25 gastrotrichs were those of *Dasydytes goniathrix* Gosse, 1851 and *Dasydytes*
26 *antenniger* Gosse, 1851 (now *Neogosseia antennigera*). In the years leading up to the
27 20th century several findings of new planktonic gastrotrichs were published, e.g.
28 *Chitonodytes longisetosus* (Metschnikoff, 1865), *Haltidytes saltitans* (Stokes, 1887),
29 *Stylochaeta fusiformis* (Spencer, 1890) and *Setopus bisetosus* (Thompson, 1891).
30 Zelinka (1889) separated *N. antennigera* (Gosse, 1851) from the rest of the planktonic
31 gastrotrichs based on the cephalic tentacles. Daday (1905) erected the families
32 Dasydytidae and Gosseidae (now Neogosseidae) and gave diagnostic characters for
33 each group. The very rare *Kijanebalola* was described from an Ugandan lake by
34 Beauchamp (1932), and regarded as a close relative to *Neogosseia*. A leap in the
35 knowledge of planktonic forms was made during the 1980s by Kisielewski (1991),
36 who found several new and very interesting semi-planktonic gastrotrichs from an
37 evolutionary point of view, among them *Undula*, *Ornamentula* and *Kijanebalola*.
38 Common features of the planktonic species are the absence of the furcal adhesive
39 tubes and a rearrangement of the locomotory ciliation. In planktonic taxa the ciliation
40 includes discrete tufts along the trunk region and at least a band of long strong
41 propelling cilia, which more or less completely encircles the head. Today planktonic
42 gastrotrichs are classified into three taxa: (i) the monotypic *Undula*, forming the
43 subfamily Undulinae, which is provisionally considered a sister group of the
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1 subfamily Chaetonotinae (Kisielewski 1991); (ii) Dasydytidae, which is characterized
2 by long and movable spines (Kisielewski 1991; Kieneke and Ostmann 2012); and (iii)
3 Neogosseidae, characterized by long posterior spines, either distributed in an unpaired
4 median group (*Kijanebalola*) or in a paired lateral group (*Neogossea*), and a pair of
5 club-shaped cephalic tentacles (Todaro et al. 2013).
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9 The origin and evolution of planktonic gastrotrichs remain largely unknown
10 (Hochberg and Litvaitis 2000; Kieneke et al. 2008a). However, the long-held
11 hypothesis that at least some planktonic forms may have been derived from benthic
12 ancestors (e.g. related to *Chaetonotus* (*Zonochaeta*) Remane, 1927a; see Kisielewski
13 1991) has gained support in a recent phylogenetic study based on molecular data
14 (Kånneby et al. 2013). In the latter study the authors showed that the planktonic
15 Dasydytidae is nested within a non-monophyletic Chaetonotidae. However, the non-
16 monophyly of Chaetonotidae had been known for quite some time prior to that study,
17 based on morphological data (e.g. Hochberg and Litvaitis 2000; Kieneke et al.
18 2008a). It should be emphasized that the systematics of the entire order Chaetonotida,
19 and especially within the largest group Chaetonotidae, is unstable. This is mainly due
20 to the classification's heavy reliance on cuticular structures and ornamentation,
21 characters that are extremely variable and thereby inconsistent on higher levels of
22 classification (e.g. genus, family etc.) (see Kånneby et al. 2013). A phylogenetic
23 approach based on molecular data may prove to be helpful in the process of re-
24 systematization of chaetonotidan taxa, similar to what is happening in the
25 systematization of the Macrotrichida (Todaro et al. 2012; 2014) However, several
26 groups of chaetonotidans have not yet been sampled for molecular data; this includes
27 Dichaeturidae, Neogosseidae and Proichthyidiidae, mainly because of lack of material.
28 It should be emphasized that members of these groups are very uncommon, with
29 some species only reported from their respective, often remote, type localities; as a
30 consequence, information on these animals is generally very poor and their origin and
31 phylogenetic alliances remain obscure.
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51 In this study, in order to shed some light on the hypothesized relationship
52 between Dasydytidae and Neogosseidae suggested by previous authors, we have
53 obtained specimens and sequences of 18S rDNA, 28S rDNA and COI mtDNA for the
54 uncommon planktonic genera *Kijanebalola* and *Neogossea*, comprising Neogosseidae
55 (Table 1). We also hope, if possible, to shed light on the origin of planktonic
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1 Gastrotricha. Although efforts were made to find *Undula paraënsis* Kisielowski,
2 1991, at its type localities in Brazil, these attempts were fruitless.
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5 MATERIALS AND METHODS 6

7 *Collection and documentation* 8

9 *Kijanebalola devestiva* Todaro et al. 2013 (Fig. 1A) and *Neogosseia acanthocolla*
10 Kisielowski, 1991 (Fig. 1B) were collected in February 2013 from a freshwater pond
11 in the iSimangaliso wetland park (Kwazulu-Natal, South Africa); for details on
12 specimen sampling, recording and storage prior to DNA processing refer to Todaro et
13 al. (2013). Individuals of *Neogosseia antennigera* (Fig. 1C, D) were collected with a
14 plankton net with a mesh size of 25 µm at Bryan Country Club Lake, Bryan, Texas,
15 USA (30°38'32.18"N; 96°21'44.05"W) on two occasions, May 23, 2012 and June 26,
16 2012. Collected samples contained *Ceratophyllum* spp. and *Lemna* spp. It is also
17 noteworthy that the water of the lake as well as ambient sediment contain arsenic
18 levels that exceed the human health criterion and are not optimal for aquatic life
19 (Clark et al. 1998). Subsamples were treated with a 1% MgCl₂-solution and
20 subsequently studied under an Olympus LMS225R dissecting microscope.
21 Gastrotrichs were picked out with a micropipette, mounted on a slide and documented
22 alive with an Olympus BH-2 microscope equipped with DIC optics and a Canon EOS
23 Rebel T1i digital camera or a Nikon Eclipse 80i DIC microscope equipped with a
24 Nikon Digital Sight DS-Fi1 digital camera. For molecular studies, specimens were
25 recovered from the slide and put in 95-100% EtOH and stored at -18 to -20°C until
26 further treatment. The specimens of Neogosseidae used in the phylogeny have been
27 photographed and the photos have been deposited as collection 855412 in Morphbank
28 (<http://www.morphbank.net/855412>) (Table 1). Also refer to collection 799280 in
29 Morphbank (<http://www.morphbank.net/799280>) for photographs of the other
30 specimens used in our analysis (Table 1).
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51 *Phylogenetic analyses* 52

53 DNA extraction, amplification and sequencing of 18S rDNA, 28S rDNA and COI
54 mtDNA follow the protocol presented in Kånneby et al. (2012). The new sequences
55 were analyzed together with most of the sequences obtained by Kånneby et al. (2013).
56 However, to keep the dataset more manageable, only a single sequence per
57 morphological species per gene was included, with the exception of Neogosseidae
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1 where in some instances two sequences from the same morphological species were
2 used (Table 1).

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4 The nuclear genes were aligned with Muscle (Edgar 2004) implemented in
5 Geneious v. 7.1.8 (Kearse et al. 2012) using the default settings. COI was aligned in
6 the same way as the nuclear genes using the translation align option, ensuring that
7 gaps in the alignment correspond to the translated amino acid sequences. The
8 concatenated dataset (18S rDNA, 28S rDNA and COI mtDNA) was analyzed with
9 MrBayes v.3.2.3 (Ronquist *et al.* 2012) on the CIPRES Science Gateway v. 3.3
10 (Miller et al. 2010) under the mixed model setting with gamma distributed rate
11 variation across sites and an estimated proportion of invariable sites. The gamma
12 shape parameter, the substitution rates for the GTR-model, the proportion of
13 invariable sites and the character state frequencies were all unlinked. The dataset was
14 partitioned according to each nuclear gene and by codon position for COI. To ensure
15 sufficient mixing and a reliable sample from the posterior distribution the dataset was
16 analyzed with 8 MCMC chains for each run and a heating parameter of 0.1. The
17 analysis started from a random starting tree and was run for 40 million generations.
18 Convergence of the two runs was ascertained by checking the log likelihood graphs,
19 the average standard deviation of split frequencies and the potential scale reduction
20 factor (PSRF+) (Gelman & Rubin 1992). After a burn-in of 5 million generations,
21 chains were sampled every 1000th generation. *Musellifer delamarei* (Renaud-
22 Mornant, 1968) within Muselliferidae, a group hypothesized to be the sister group of
23 all other Paucitubulatina (e.g. Hochberg and Litvaitis 2000; Todaro et al. 2006; Leasi
24 and Todaro 2008; K anneby et al. 2014), was used as outgroup. The alignments of the
25 concatenated dataset and the resulting consensus trees from the Bayesian and the
26 maximum likelihood analyses has been deposited in TreeBASE at
27 <http://purl.org/phylo/treebase/phyloids/study/TB2:S17533>
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RESULTS

The final alignment of the concatenated dataset yielded 7109 positions, with 1830, 4612 and 667 positions for 18S rDNA, 28S rDNA and COI mtDNA, respectively.

1 In general the concatenated phylogenies based on both the Bayesian and the
2 maximum likelihood alignments follow the results obtained by K anneby et al. (2013).
3 Given the included data, none of the currently recognized subgenera appear
4 monophyletic except for *Chaetonotus (Zonochaeta)*. *Lepidochaetus*, considered a
5 subgenus within *Chaetonotus* by Balsamo et al. (2009), is also monophyletic. The
6 hypothesized reinvasion of certain Chaetonotidae (*Halichaetonotus* and marine
7 *Heterolepidoderma* in a sister group relation to the freshwater taxon *Chaetonotus*
8 *schultzei* Metschnikoff, 1865) from a limnic environment to a marine environment by
9 K anneby et al. (2013) also gains high support in this study (pp=0.98) (Fig. 2).

16 For individual gene trees there are no major conflicts when taking into account
17 bootstrap support values above 70. Deeper nodes within the Chaetonotidae +
18 Dasydytidae + Neogosseidae clade generally have very low bootstrap support (results
19 not shown).

23 With regard to the main aims of the current study, the Bayesian analysis gives
24 high support for monophyly of the planktonic gastrotrichs
25 (Dasydytidae+Neogosseidae) in our study (pp=1). Within this clade both Dasydytidae
26 (pp=0.99) and Neogosseidae (pp=1) are monophyletic (Fig. 2). For Neogosseidae,
27 both *Kijanebalola (K. devestiva, n=2)* and *Neogosseia (N. acanthocolla, n=1 + N.*
28 *antennigera, n=2)* are monophyletic with high bootstrap support (pp=1). *N.*
29 *acanthocolla* and *N. antennigera* are sister taxa. Within Dasydytidae two major clades
30 can be discerned: *Haltidytes squamosus + Ornamentula para ensis* and *Dasydytes +*
31 *Stylochaeta* (Fig. 2). Within the latter clade *Stylochaeta fusiformis* and *Stylochaeta*
32 *scirtetica* are in a sister group relation to *Dasydytes (Prodasydytes) elongatus*
33 Kisielewski, 1991 and *Dasydytes (P.) papaveroi* Kisielewski, 1991 (pp=1). The
34 subgenus *Dasydytes (Prodasydytes)* is non-monophyletic, because of the sister group
35 relationship of *Dasydytes (P.) carvalhoae* Kisielewski, 1991 to *D. (P.) elongatus + D.*
36 *(P.) papaveroi* and *Stylochaeta* (pp=1).

49 The sister group of the planktonic gastrotrichs (Dasydytidae + Neogosseidae)
50 appears as *Chaetonotus (Zonochaeta) spp. + Chaetonotus heteracanthus* Remane,
51 1927 (pp=0.99). *Polymerurus* is the sister group of the latter clade and the planktonic
52 gastrotrichs (Fig. 2).

56 The maximum likelihood analysis of the concatenated dataset supports
57 monophyly of planktonic Gastrotricha (bs=90). However, Dasydytidae appear non-
58 monophyletic, although with very low support (bs=55), since *Stylochaeta+Dasydytes*

1 is in a sister group relationship to Neogosseidae. *Haltidytes squamosus* Kisielewski,
2 1991 and *Ornamentula paraënsis* Kisielewski, 1991 form the sister group of
3 *Stylochaeta*+*Dasydytes*. Further, the support for *Chaetonotus* (*Zonochaeta*) as the
4 sister clade of planktonic gastrotrichs is also very low (bs=45). The deeper nodes of
5 the maximum likelihood phylogeny generally have very low support.
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10 DISCUSSION

11 Inside the primarily benthic phylum Gastrotricha, the occurrence of planktonic taxa is
12 in many respects of particular interest. For instance, within an evolutionary
13 framework these animals may prove to be excellent for studies dealing with the origin
14 and evolution of adaptations to the planktonic environment. Knowledge of the co-
15 location of pelagic dwellers along the gastrotrich evolutionary tree would benefit
16 studies dealing with, for example, ancestral character patterns and evolution of traits
17 such as movable spines and muscle systems arrangement (Kieneke and Ostmann
18 2012).
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27 Unfortunately, the origin and phylogenetic alliances of planktonic Gastrotricha
28 has not been studied to any great extent. Kisielewski (1991) considered Dasydytidae
29 and Neogosseidae as two separate lineages, and discussed their possible origin from a
30 benthic ancestor within Chaetonotidae. This hypothesized ancestor would have
31 developed a sack-shaped body, by reduction of the caudal lobes and adhesive tubes.
32 The ventral ciliation would also have been modified into tufts and bands encircling
33 the head. However, whether the two groups arose from a common ancestor or not was
34 left open.
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42 Based on morphological observations, both Remane (1927a) and Kisielewski
43 (1991) agree on a close relationship between *Chaetonotus* (*Zonochaeta*) and
44 Dasydytidae (and possibly Neogosseidae). In fact, members of *Chaetonotus*
45 (*Zonochaeta*) and Dasydytidae both possess movable spines of similar structure and
46 function, e.g. strongly cuticularised, scaleless spines with a notched apex, used for
47 locomotion and/or defence (Schwank 1990; Kisielewski 1991; Kieneke and Ostmann
48 2012). In his 1991 authoritative paper on Brazilian freshwater Gastrotricha,
49 Kisielewski described the subgenus *Dasydytes* (*Prodasydytes*), whose members he
50 considered to have retained the plesiomorphic character states among the planktonic
51 Gastrotricha. Comparing morphology it is evident that *Dasydytes* (*P.*) *carvalhoae*,
52 *Dasydytes* (*P.*) *elongatus* and *Dasydytes* (*P.*) *papaveroi* exhibit several similarities
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1 with members of the putative Dasydytidae sister taxon, *Chaetonotus (Zonochaeta)*.
2 These traits are: (i) long and specialized spines with denticles and bifurcated tips; (ii)
3 similar shape of scales; and (iii) long parafurcal spines. In addition, dasydytids
4 possess long ventrolateral and/or dorsolateral motile spines. To be more specific,
5 dasydytids, by virtue of a peculiar complex system made up of serially arranged
6 oblique muscles and segmental longitudinal muscles, can quickly raise their spines,
7 which causes the animal to leap through the water, e.g. in response to a predator or
8 other threat (Kieneke et al. 2008a; Kieneke and Ostmann 2012). On the other hand,
9 species of *Chaetonotus (Zonochaeta)* are further characterized by a peculiar
10 transverse row of spines, commonly known as the girdle, across the trunk. These
11 spines can also be raised by muscle action. Consequently, the combination of
12 movable spines and the associated musculature can be seen as potentially homologous
13 characters shared by the dasydytids and species of *Chaetonotus (Zonochaeta)* (e.g.
14 Kisielewski 1991).

15
16 The first cladistic analysis of Gastrotricha, based on 81 morphological
17 characters, showed a sister group relationship between Dasydytidae and Neogosseidae
18 in close alliance to Proichthyridae and Dichaeturidae (Hochberg and Litvaitis 2000).
19 A subsequent, more inclusive analysis based on 135 morphological characters found a
20 monophyletic Neogosseidae nested within Dasydytidae, although with low statistical
21 support at nodes (Kieneke et al. 2008b). In the latter work, planktonic Gastrotricha
22 appeared as a subset of Chaetonotidae, with low statistical support at nodes, although
23 not in a close relationship with *Chaetonotus (Zonochaeta)*. In the recent phylogenetic
24 analysis, based on three molecular markers, K anneby et al. (2013) found a sister
25 group relation between Dasydytidae (*Dasydytes*, *Haltidytes*, *Ornamentula* and
26 *Stylochaeta*) and a clade containing *Chaetonotus (Zonochaeta)*, and thereby
27 somewhat confirming the early hypothesis by Remane (1927a) and Kisielewski
28 (1991).

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30 The current study supports the general phylogenetic scenario obtained by
31 K anneby et al. (2013) and provides further evidence, on a molecular basis, for a
32 common origin of Dasydytidae and Neogosseidae. The major findings of our study
33 are: (i) Dasydytidae and Neogosseidae both appear as monophyletic groups; (ii)
34 Dasydytidae and Neogosseidae are in a sister group relation; and (iii) the planktonic
35 lineage evolved from a benthic or periphytic ancestor within Chaetonotidae. Strong
36 nodal support and congruence with previous phylogenies (e.g. K anneby et al. (2013)

1 for the general scenario and Hochberg and Litvaitis (2000) for the sister group
2 relationship between Dasydytidae and Neogosseidae) make our findings very likely.

3 Neogosseidae is monophyletic and the clade has high support in our analyses.
4 Neogosseidae can be separated from Dasydytidae morphologically by the presence of
5 a pair of club-shaped cephalic tentacles in neogosseids. Within Neogosseidae,
6 *Kijanebalola* and *Neogossea* (Fig. 1) are sister groups in our phylogeny, and can be
7 separated morphologically from each other based on distinct autapomorphic traits of
8 the posterior end, which appears truncate and provided with a pair of tufts in
9 *Neogossea*, and rounded with a median group of spines in *Kijanebalola* (Todaro et al.
10 2013).

11 Dasydytidae also appears monophyletic in our analysis (Fig. 2).
12 Morphologically, the presence of groups of motile spines along the trunk, that aid
13 ciliary swimming can be considered an autapomorphy for the family (e.g. Kieneke
14 and Ostmann 2012). Likewise, the peculiar system of somatic oblique and segmented
15 lateral muscles associated with the cuticular movable spines, may at least
16 provisionally be considered an additional autapomorphy of Dasydytidae (Kieneke and
17 Ostmann 2012). Within Dasydytidae two major clades can be distinguished (Fig. 2).
18 The first contains two species, *Haltidytes squamosus* and *Ornamentula paraënsis*.
19 The second contains five species, *D. (P.) elongatus*, *D. (P.) carvalhoae*, *D. (P.)*
20 *papaveroi*, *Stylochaeta fusformis* and *Stylochaeta scirtetica*. The grouping of
21 *Haltidytes* + *Ornamentula* in one clade and *Dasydytes* in another provides support to
22 the in-group evolutionary scenario envisioned by Kisielewski (1991) on the basis of
23 the structure and organization of the cuticular apparatus (scales and spines), but is in
24 contrast to the phylogenetic hypothesis put forward by Kieneke and Ostmann (2012;
25 see position of *Ornamentula* and *Stylochaeta* in Fig. 10) based mainly on characters
26 of the muscular system. All the sampled species in our phylogeny belong to the
27 subgenus *Dasydytes* (*Prodasydytes*), which is, according to the results, non-
28 monophyletic due to the early divergence of *D. (P.) carvalhoae* along the *Dasydytes* +
29 *Stylochaeta* branch. By virtue of the highly uniform anatomy of species of *Dasydytes*,
30 and the strong difference compared to species of *Stylochaeta*, we consider this result
31 quite unlikely and perhaps an artifact due to contamination.

32 Our phylogeny supports the existence of a monophyletic planktonic clade, as
33 shown by Hochberg and Litvaitis (2000) and Kieneke et al. (2008b). However, in
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1 contrast to the latter study, Neogosseidae appear to be the sister taxon of Dasydytidae
2 and not nested within it.

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4 In the present study the planktonic clade appears as an offshoot of the
5 Chaetonotidae as repeatedly indicated in the literature, for example, by Kisielewski
6 (1991). Due to lack of material we did not include specimens of Dichaeturidae nor
7 Proichthydidae, and because of this, the hypothesis by Hochberg & Litvaitis (2000),
8 where the planktonic taxa are most closely related to these groups, cannot be properly
9 tested. It should be emphasized that Dichaeturidae and Proichthydidae are very rare,
10 that the original descriptions are poor, and that both these conditions could make the
11 very existence of these taxa as independent evolutionary lines open to doubt. For
12 instance, the recent and most accurate description of a third species of Dichaeturidae
13 (Suzuki et al. 2013), reports characteristics that could well fit for a taxon belonging to
14 the current Chaetonotidae. However, a taxonomic revision of Dichaeturidae and
15 Proichthydidae lies beyond the scope of the present paper; yet in agreement with
16 Kisielewski (1991), we consider our finding very likely. According to our results the
17 evolution of the genuine planktonic life-style in Chaetonotida occurred only once
18 (Dasydytidae+Neogosseidae) and probably from a benthic/periphytic ancestor.
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31 From a morphological point of view, a lineage originally affiliated with the
32 Chaetonotidae and evolving to the split between Dasydytidae and Neogosseidae,
33 would have undergone several morphological adaptations to cope with the change
34 from a benthic or periphytic life-style to a permanent planktonic life-style. Both
35 Dasydytidae and Neogosseidae lack a furca and adhesive tubes, and have a rounded
36 posterior end. There are also reductions and rearrangements of the locomotory
37 ciliature: while benthic chaetonotids commonly have ventral longitudinal bands of
38 cilia, planktonic taxa possess transverse bands or tufts of cilia along the trunk and
39 around the head, which may increase speed and maneuverability when swimming.
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47 These dramatic changes would also have involved the ancestors that the
48 Dasydytidae + Neogosseidae clade share with the sister group revealed by the
49 Bayesian analysis in our study, e.g. *Chaetonotus (Zonochaeta)* spp. + *Chaetonotus*
50 *heteracanthus*. The maximum likelihood analysis does not show high support for this
51 sister group relation, suggesting *Polymerurus* as a possible alternative sister group.
52 However, we note that the most relevant autapomorphic trait of *Polymerurus* is the
53 extraordinarily long furca, which makes it hard to unite taxa lacking a furca
54 (planktonic gastrotrichs) with taxa possessing a particularly long furca. On the other
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1 hand the presence of strong scaleless spines could testify in favor for a close
2 relationship between Dasydytidae + Neogosseidae and *Chaetonotus (Zonochaeta)*
3 spp. + *C. heteracanthus*. In this scenario we acknowledge the possible independent
4 origin of the musculature, associated with the movable spines, in dasydytids and
5 *Chaetonotus (Zonochaeta)*, respectively. Future morpho-functional studies on the
6 musculature of species of *Chaetonotus (Zonochaeta)* could support or disprove this
7 hypothesis.
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12 To summarize, the phylogenetic hypothesis generated by this study gives
13 strong support for a common origin of Dasydytidae and Neogosseidae, and also a
14 hypothesized common origin of planktonic Gastrotricha. It also appears very plausible
15 that the planktonic clade has evolved from a benthic or periphytic ancestor, by
16 morphological adaptations to a life in the water column. Morphology and molecular
17 data prefers *Chaetonotus (Zonochaeta)* spp. (and *C. heteracanthus*) as the sister group
18 of planktonic Gastrotricha. The close relationship of these groups to *Polymerurus* is
19 somewhat confusing and should be evaluated in future studies.
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FIGURE LEGENDS

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4 Figure 1. Species of Neogosseidae included in this study. A. *Kijanebalola devestiva*,
5 habitus of adult specimen. Note the rounded posterior end with single median group
6 of spines. B. *Neogossea acanthocolla*, habitus of adult specimen. Note the truncate
7 posterior end with a pair of tufts. C. *Neogossea antennigera*, anterior two thirds of
8 body of adult specimen. D. *N. antennigera*, posterior end of body of adult specimen.
9 Note the truncate posterior end with a pair of tufts. Scale bars: A and B, 50 μm ; C and
10 D, 25 μm .
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18 Figure 2. Majority rule consensus tree based on the Bayesian analysis of the
19 concatenated dataset. Numbers at nodes represent posterior probability. Asterisks
20 indicate a bootstrap support value above 75, obtained from the maximum likelihood
21 analysis of the concatenated dataset. The star indicate the clade where a permanent
22 planktonic life-style has evolved. The clades containing members of Muselliferidae
23 (outgroup) and Xenotrichulidae have been collapsed for esthetic reasons (see Table 1
24 for full set of taxa).
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Table 1. Taxa used in the phylogenetic analyses in this study with Genbank accession numbers and reference. The Morphbank Id number is given in parentheses for most specimens and photographs can be accessed at <http://www.morphbank.net/799280> or at <http://www.morphbank.net/855412>. Na, Not available.

Taxon (Morphbank ID)	Genbank Accession #			Reference
	18S	28S	COI	
Family Chaetonotidae				
<i>Arenotus strixinoi</i> Kisielewski, 1987 (791776)	JQ798537	JQ798608	JQ798677	Kånneby et al. 2013
<i>Aspidiophorus ophiodermus</i> Balsamo, 1983 (791777–791778)	JN185463	JN185510	NA	Kånneby et al. 2012
<i>Aspidiophorus paramediterraneus</i> Hummon, 1974 (791779)	JQ798538	JQ798609	JQ798678	Kånneby et al. 2013
<i>Aspidiophorus polystictos</i> Balsamo & Todaro, 1987	JQ798598	JQ798665	JQ798727	Kånneby et al. 2013
<i>Aspidiophorus tentaculatus</i> Wilke, 1954 (791780)	JQ798553	JQ798625	JQ798690	Kånneby et al. 2013
<i>Aspidiophorus tetrachaetus</i> Kisielewski, 1986 (791781)	JN185505	JN185540	JN185576	Kånneby et al. 2012
<i>Aspidiophorus</i> sp. 3	JQ798559	JQ798629	NA	Kånneby et al. 2013
<i>Chaetonotus acanthocephalus</i> Valkanov, 1937 (791825–791826)	JQ798569	NA	JQ798701	Kånneby et al. 2013
<i>Chaetonotus acanthodes</i> Stokes, 1887 (791828– 791830)	JQ798552	JQ798624	NA	Kånneby et al. 2013
<i>Chaetonotus aemilianus</i> Balsamo, 1978 (791819)	JQ798556	JQ798626	JQ798693	Kånneby et al. 2013
<i>Chaetonotus armatus</i> Kisielewski, 1981 (791832–791835)	JQ798594	NA	JQ798723	Kånneby et al. 2013
<i>Chaetonotus (Zonochaeta) bisacer</i> Greuter, 1917 (791848– 791849)	JQ798565	JQ798635	NA	Kånneby et al. 2013
<i>Chaetonotus</i>	JQ798549	JQ798621	JQ798687	Kånneby et al.

1	<i>daphnes</i> Balsamo & Todaro, 1995 (791782–791784)				2013
2					
3	<i>Chaetonotus cf. dispar</i> Wilke, 1954 (791844–791846)	JQ798561	JQ798631	JQ798696	Kånneby et al. 2013
4					
5	<i>Chaetonotus heideri</i> Brehm, 1917 (791836–791837)	JQ798547	JQ798619	JQ798685	Kånneby et al. 2013
6					
7	<i>Chaetonotus heteracanthus</i> Remane, 1927 (791840–791842)	JQ798543	JQ798615	JQ798681	Kånneby et al. 2013
8					
9	<i>Chaetonotus cf. hystrix</i> Metschnikoff, 1865 (791821)	JQ798603	JQ798670	JQ798732	Kånneby et al. 2013
10					
11	<i>Chaetonotus cf. laroides</i> Marcolongo, 1910 (791788–791789)	JQ798602	JQ798669	JQ798731	Kånneby et al. 2013
12					
13	<i>Chaetonotus mariae</i> Todaro, 1992 (791824)	JQ798558	JQ798628	NA	Kånneby et al. 2013
14					
15	<i>Chaetonotus cf. maximus</i> Ehrenberg, 1838 (791793)	JQ798574	JQ798646	JQ798706	Kånneby et al. 2013
16					
17	<i>Chaetonotus microchaetus</i> Preobrajenskaja, 1926 (791801)	JQ798583	JQ798651	JQ798714	Kånneby et al. 2013
18					
19	<i>Chaetonotus neptuni</i> Wilke, 1954	JQ798595	JQ798662	JQ798724	Kånneby et al. 2013
20					
21	<i>Chaetonotus cf. novenarius</i> Greuter, 1917 (791822–791823)	JQ798566	JQ798636	JQ798699	Kånneby et al. 2013
22					
23	<i>Chaetonotus cf. oculifer</i> Kisielewski, 1981 (791802)	JQ798576	JQ798647	JQ798708	Kånneby et al. 2013
24					
25	<i>Chaetonotus polyspinosus</i> Greuter, 1917 (791804–791805)	JQ798563	JQ798633	JQ798698	Kånneby et al. 2013
26					
27	<i>Chaetonotus schultzei</i> Metschnikoff, 1865	JQ798596	JQ798663	JQ798725	Kånneby et al. 2013
28					
29	<i>Chaetonotus similis</i> Zelinka, 1889 (791808–791809)	JQ798578	JQ798648	JQ798710	Kånneby et al. 2013
30					
31	<i>Chaetonotus cf. sphagnophilus</i> Kisielewski, 1981	JQ798604	JQ798671	JQ798733	Kånneby et al. 2013
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1	<i>Chaetonotus uncinus</i> Voigt, 1902 (791847)	JQ798540	JQ798611	NA	Kånneby et al. 2013
2					
3	<i>Chaetonotus (Zonochaeta)</i> sp. 1 (791852–791853)	JQ798593	JQ798661	NA	Kånneby et al. 2013
4					
5	<i>Chaetonotus (Zonochaeta)</i> sp. 2 (791854–791856)	JQ798587	JQ798655	JQ798718	Kånneby et al. 2013
6					
7	<i>Chaetonotus</i> sp. 1 (791817–791818)	JQ798601	JQ798668	JQ798730	Kånneby et al. 2013
8					
9	<i>Halichaetonotus aculifer</i> (Gerlach, 1953)	JQ798550	JQ798622	JQ798688	Kånneby et al. 2013
10					
11	<i>Halichaetonotus euromarinus</i> Hummon & Todaro, 2010 (791857–791859)	JQ798551	JQ798623	NA	Kånneby et al. 2013
12					
13	<i>Halichaetonotus paradoxus</i> (Remane, 1927)	JQ798599	JQ798666	JQ798728	Kånneby et al. 2013
14					
15	<i>Halichaetonotus</i> sp. 2	JQ798600	JQ798667	JQ798729	Kånneby et al. 2013
16					
17	<i>Halichaetonotus</i> sp. 4	JQ798560	JQ798630	JQ798695	Kånneby et al. 2013
18					
19	<i>Heterolepidoderma acidophilum</i> Kånneby et al., 2012 (791871–791872)	JN185462	JN185509	JN185543	Kånneby et al. 2012
20					
21	<i>Heterolepidoderma loricatum</i> Schrom, 1972	JQ798541	JQ798612	NA	Kånneby et al. 2013
22					
23	<i>Heterolepidoderma macrops</i> Kisielewski, 1981 (791860–791863)	JN185469	JN185515	JN185548	Kånneby et al. 2012
24					
25	<i>Heterolepidoderma ocellatum</i> (Metschnikoff, 1865) (791867–791868)	JN185476	JN185520	JN185555	Kånneby et al. 2012
26					
27	<i>Heterolepidoderma</i> sp. 2 (791875–791876)	JN185485	JQ798644	JN185563	Kånneby et al. 2013
28					
29	<i>Heterolepidoderma</i> sp. 3 (791877–791879)	JQ798572	JQ798641	JQ798704	Kånneby et al. 2013
30					
31	<i>Ichthydium skandicum</i> Kånneby et al., 2009 (791880–791881)	JQ798573	JQ798645	JQ798705	Kånneby et al. 2013
32					
33	<i>Ichthydium squamigerum</i> Balsamo & Fregni, 1995 (791882–791883)	JQ798607	JQ798674	JQ798736	Kånneby et al. 2013
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1	<i>Lepidochaetus</i>	JN185495	JQ798658	JN185568	Kånneby et al.
2	<i>brasiliense</i>				2012; 2013
3	Kisielewski, 1991				
4	(791884–791887)				
5	<i>Lepidochaetus</i>	JN185486	JN185527	JN185564	Kånneby et al.
6	<i>zelinkai</i> (Grünspan,				2012; 2013
7	1908) (791897–				
8	791899)				
9	<i>Lepidodermella</i>	JN185468	JN185514	JN185547	Kånneby et al.
10	<i>intermedia</i>				2012
11	Kånneby et al.,				
12	2012 (791904–				
13	791906)				
14	<i>Lepidodermella</i>	JN185474	NA	JN185553	Kånneby et al.
15	<i>minor minor</i>				2012
16	(Remane, 1936)				
17	(791907–791908)				
18	<i>Lepidodermella</i>	JN185479	JN185523	JN185558	Kånneby et al.
19	<i>squamata</i>				2012
20	(Dujardin, 1841)				
21	(791915–791917)				
22	<i>Polymerurus</i>	JN185502	JN185537	JN185573	Kånneby et al.
23	<i>nodicaudus</i> (Voigt,				2012
24	1901)				
25	<i>Polymerurus</i>	JN185493	JN185533	JN185567	Kånneby et al.
26	<i>rhomboides</i>				2012
27	(Stokes, 1887)				
28	(791931–791933)				
29	Family				
30	Dasydytidae				
31	<i>Dasydytes</i>	JQ798570	JQ798639	JQ798702	Kånneby et al.
32	<i>carvalhoae</i>				2013
33	Kisielewski, 1991				
34	(791934–791937)				
35	<i>Dasydytes</i>	JQ798568	JQ798638	JQ798700	Kånneby et al.
36	<i>elongatus</i>				2013
37	Kisielewski, 1991				
38	(791938–791941)				
39	<i>Dasydytes</i>	JQ798571	JQ798640	JQ798703	Kånneby et al.
40	<i>papaveroi</i>				2013
41	Kisielewski, 1991				
42	(791948–791950)				
43	<i>Haltidytes</i>	JQ798567	JQ798637	NA	Kånneby et al.
44	<i>squamosus</i>				2013
45	Kisielewski, 1991				
46	(791951–791954)				
47	<i>Ornamentula</i>	JQ798562	JQ798632	JQ798697	Kånneby et al.
48	<i>paraënsis</i>				2013
49	Kisielewski, 1991				
50	(791955–791956)				
51	<i>Stylochaeta</i>	JN185471	JN185517	JN185550	Kånneby et al.
52	<i>fusiformis</i>				2012
53	(Spencer, 1890)				
54	(791957–791959)				
55	<i>Stylochaeta</i>	JN185492	JN185532	NA	Kånneby et al.
56	<i>scirtetica</i> Brunson,				2012
57	1950 (791960–				
58	791962)				
59	Family				

	Muselliferidae				
1	<i>Musellifer</i>	AM231775	NA	NA	Todaro et al. 2006
2	<i>delamarei</i>				
3	(Renaud-Mornant,				
4	1968)				
5	<i>Musellifer</i>	KF578503	NA	NA	Kånneby et al.
6	<i>reichardti</i>				2014
7	Kånneby et al.,				
8	2014				
9	Family				
10	Neogosseidae				
11	<i>Kijanebalola</i>	KR822112	KR822117	KR822120	This study
12	<i>devestiva</i> Todaro et				
13	al. 2013 (855405–				
14	855406)				
15	<i>Kijanebalola</i>	KR822113	KR822118	NA	This study
16	<i>devestiva</i> Todaro et				
17	al. 2013 (855405–				
18	855406)				
19	<i>Neogosseia</i>	KR822114	KR822119	KR822121	This study
20	<i>acanthocolla</i>				
21	Kisielewski, 1991				
22	(855408)				
23	<i>Neogosseia</i>	KR822110	KR822115	NA	This study
24	<i>antennigera</i>				
25	(Gosse, 1851)				
26	(855197-855198)				
27	<i>Neogosseia</i>	KR822111	KR822116	NA	This study
28	<i>antennigera</i>				
29	(Gosse, 1851)				
30	(855197-855198)				
31	Family				
32	Xenotrichulidae				
33	<i>Draculiciteria</i>	JN185470	JN185516	JN185549	Kånneby et al.
34	<i>tesselata</i> (Renaud-				2012
35	Mornant, 1968)				
36	(791964–791967)				
37	<i>Heteroxenotrichula</i>	JQ798542	JQ798613	NA	Kånneby et al.
38	<i>squamosa</i> Wilke,				2013
39	1954 (791968)				
40	<i>Xenotrichula</i>	JF357664	JF357712	JF432047	Todaro et al. 2011
41	<i>intermedia</i>				
42	Remane, 1934				
43	<i>Xenotrichula</i>	JN185464	JN185511	NA	Kånneby et al.
44	<i>punctata</i> Wilke,				2012
45	1954				
46	<i>Xenotrichula velox</i>	JN185488	JQ798652	NA	Kånneby et al.
47	Remane, 1927				2012
48	(791969–791971)				

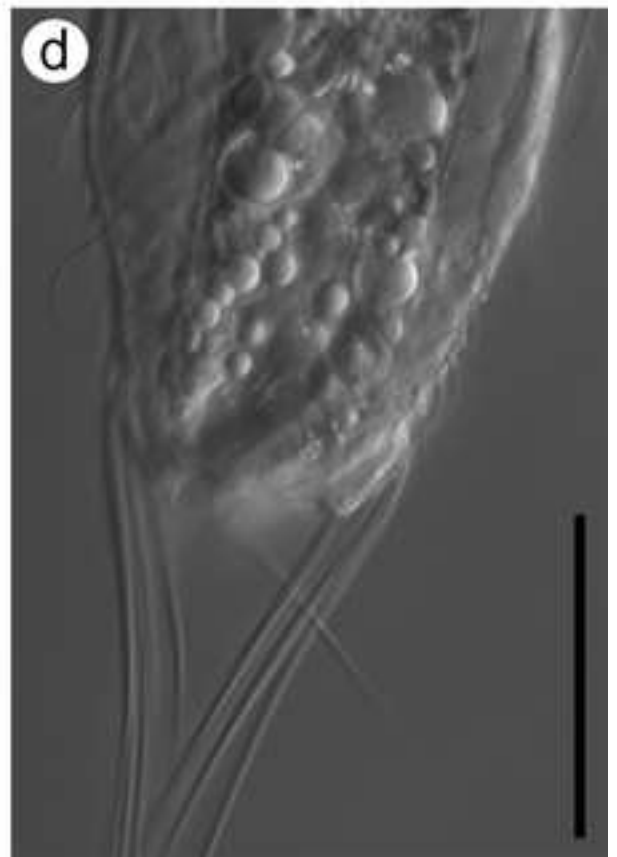
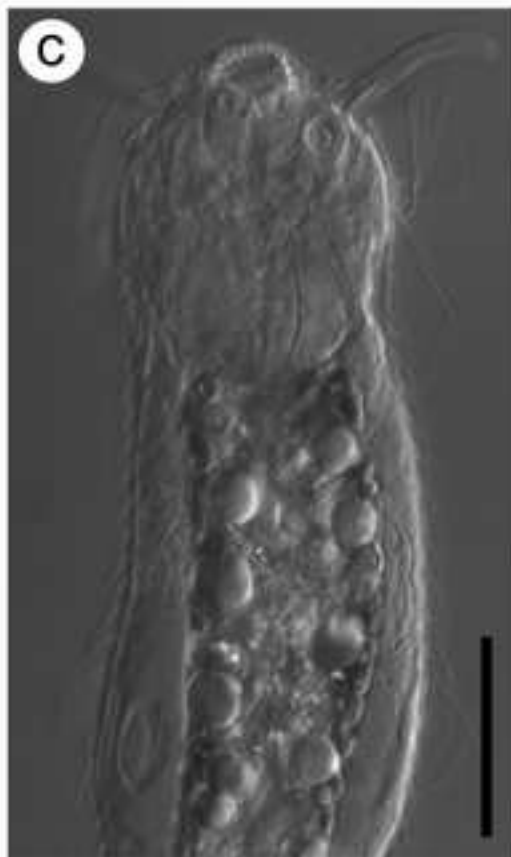
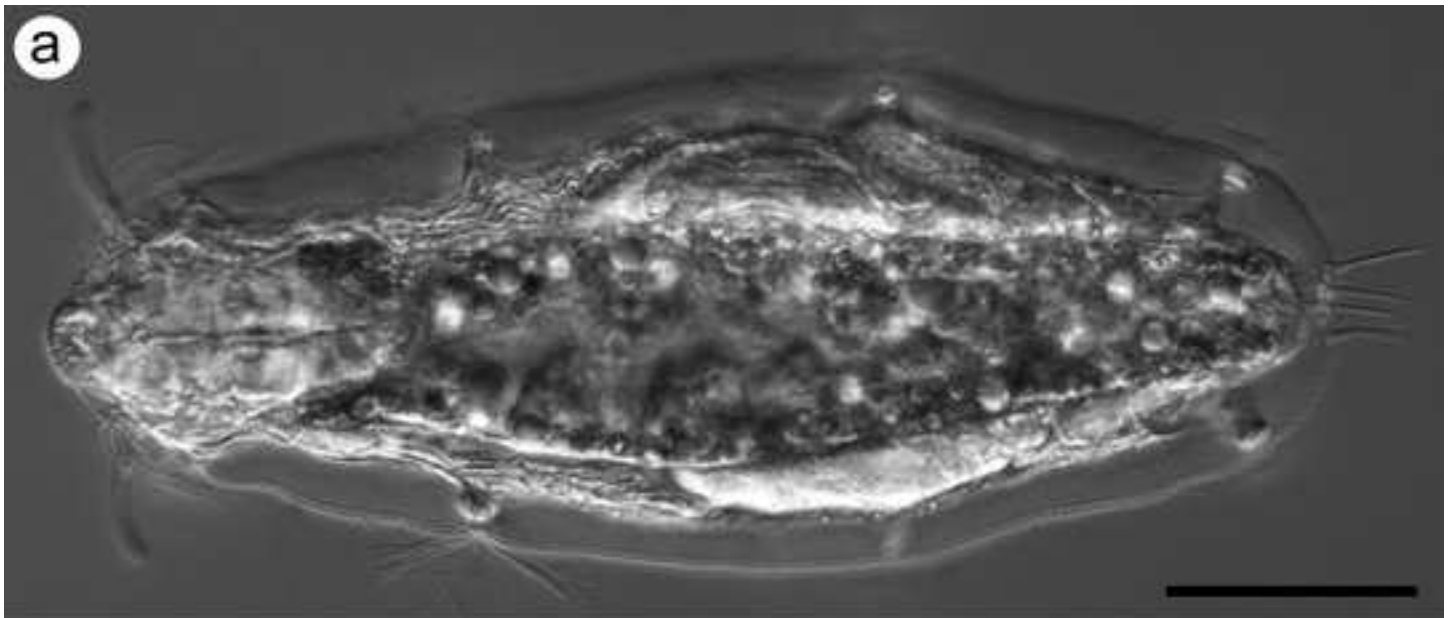


Figure 2 Phylogeny

