INTRODUCTION

The phylum Rotifera comprises about 2,000 described species classified into the four major clades of Acanthocephala, Seisonacea, Bdelloidea, and Monogononta (Brusca, Moore, & Shuster, 2016; Segers, 2002a). The latter three taxa constitute Rotifera as recognized traditionally, with all species being microscopic (ca. 200–500 µm in length), aquatic, and characterized by the presence of a rotatory organ consisting of ciliary bands (Segers, 2002b, 2004). Although free-living rotifers were among the first organisms noticed...
by the early microscopists (Rousselet, 1902), the group as a whole generally remains poorly characterized systematically. For example, initial descriptions of many species are unreliable and ambiguous, and many species have not been re-discovered since their initial description (e.g., see Hollowday, 2002). Numerous new species continue to be discovered, in part as cryptic species within new species complexes (e.g., as for Brachionus manjavacas Fontaneto, Giordani, Melone, & Serra, 2007; Brachionus ibericus Cíos-Pérez, Gómez, & Serra, 2001), but it remains that most species are badly characterized both morphologically and especially molecularly. Comprehensive phylogenetic studies of the group are also generally lacking with a few exceptions including an analysis based on trophi morphology (Sørensen, 2002) and one relying on combined morphological and molecular data (Sørensen & Giribet, 2006). As such, although the major clades within Rotifera are well defined, their relationships to one another remain uncertain (Fontaneto & De Smet, 2015).

A good example of this situation is presented by Synchaetidae, a clade of monogonont rotifers supported by derived features including a plank-shaped fulcrum, reduced unc, a prominent V-shaped hypopharynx muscle, and stiff sensory setae or palps on the apical field (De Smet & Segers, 2008; Fontaneto & De Smet, 2015). There are about 56 valid morphospecies (after Segers, 2007) that are accommodated in the three genera Synchaeta Ehrenberg, 1832, Polyarthra Ehrenberg, 1834, and Ploesoma Herrick, 1885. (A fourth potential genus Pseudoploesoma Myers, 1938 was reassigned to Notommataeae by De Smet and Segers (2008).) However, despite being relatively species poor, Synchaetidae is comparatively highly successful among rotifers. Many species have cosmopolitan distributions and are individually adapted to a wide range of freshwater, brackish, and/or marine habitats (see Hollowday, 2002; Koste, 1978), where they often constitute the dominant member of the rotifer community (Garcia et al., 2002; McNaught et al., 1999; Stemberger & Gilbert, 1985; Thorp & Casper, 2002).

Despite their ecological importance, Synchaetidae as a group is poorly known systematically. As for rotifers as a whole, many initial species descriptions are of questionable utility, which again might explain why several species have only ever been found once. Confounding this general situation is the difficulty in examining, identifying, and delineating species of Synchaetidae given their sensitivity toward preservation as well as the high interspecific similarity and intraspecific variability that is typical within this group (e.g., Koste, 1978; Ruttner-Kolisko, 1972). Finally, apart from a few species targeted for phyleogeographic studies (e.g., Synchaeta pectinata Ehrenberg, 1832 in Kimpel, Gockel, Gerlach, and Bininda-Emonds (2015) and Obertegger, Fontaneto, and Flaim (2012)), molecular data for most species are few, if not missing entirely. As such, the phylogenetic position of Synchaetidae within Monogononta is only roughly known (see Sørensen, 2002; Sørensen & Giribet, 2006) and the internal relationships within the group, including the monophyly of the three genera, if not of the entire family itself, to our knowledge have never been examined in a robust phylogenetic framework. At best, a rough clustering within Synchaeta could be argued for based on the different virgate mastax types present in the genus (i.e., Synchaeta tremula-type versus S. pectinata-type; see Koste, 1978), but even then for only a handful of species.

Therefore, as a first step toward elucidating the phylogenetic relationships of Synchaetidae, we provide the first phylogenetic analyses for this group based on single and combined data sets of both morphological and molecular information. In so doing, we have generated new morphological and molecular data from extensive re-examinations of 19 species (14 from Synchaeta, three from Polyarthra and two from Ploesoma). Our re-examinations have also revealed several characters within the group that we hold to have been misinterpreted in the past and for which we supply new hypotheses of homology. Finally, we apply our phylogeny to elucidate potential key morphological character transformations that might be responsible for making Synchaetidae such an ecologically successful rotifer taxon.

2 | MATERIAL AND METHODS

2.1 | Included species

Our phylogenetic analysis includes all species of Synchaetidae considered valid in the rotifer checklists of Segers (2007) and/or Jersabek et al. (2018) as well as all new species described thereafter. Species of doubtful identity (species inquirenda; according to Hollowday, 2002 and Segers, 2007) were excluded as were Synchaeta jollyae Shiel & Koste, 1993, Synchaeta rufina Kutikova & Vassiljeva, 1982, and Synchaeta monopus Plate, 1889, which we have argued elsewhere are likely misidentifications of other, valid Synchaeta species (Wilke, Ahlrichs, & Bininda-Emonds, 2018a, 2018b, 2019a). Overall, our data set comprises 34 species of Synchaeta, 11 of Polyarthra, and eight of Ploesoma, plus two species of Notommata as a suitable outgroup (see Sørensen & Giribet, 2006; Appendix 2).

2.2 | Data generation and processing

2.2.1 | Morphological data

Morphological data were obtained from re-examinations of fresh material wherever possible and otherwise supplemented with literature information. In the latter case, morphological descriptions were obtained mainly from the review papers of Rousselet (1902), Voigt (1956–1957), Koste (1978), Hollowday (2002), and Leutbecher (2004); from detailed re-examinations of single species (e.g., Friedrich & De Smet, 2000; Labuice & Strake, 2017; Rougier & Pourriot, 2006; Rougier, Pourriot, & Lam-Hoai, 2000); and from the original descriptions of individual species. Additional information for some species was also derived from our interpretations of their photographs and drawings in the Rotifer World Catalog (Jersabek & Leitner, 2013).

Altogether, we re-examined 14 species of Synchaeta, three of Polyarthra, and two of Ploesoma that were sampled between June 2013 and August 2017 in northwest Germany from freshwater (Oldenburg in Lower Saxony and Tecklenburger Land in
North Rhine-Westphalia) as well as brackish and marine habitats (Wilhelmshaven in Lower Saxony; Appendix 2). For these re-examinations, the behavior of living, undisturbed specimens was initially observed using a LEICA MZ 12.5 high-performance stereomicroscope. Thereafter, the specimens were sedated with carbonated water and examined in detail using a LEICA DMLB with differential interference contrast. Digital images were taken with a Canon EOS 5D Mark II camera. The habitus and the trophi of individuals of each species were also investigated using a scanning electron microscope (SEM). For habitus examinations, specimens were sedated with carbonated water and examined in detail using a LEICA DMLB with differential interference contrast. Digital images were taken with a Canon EOS 5D Mark II camera. The habitus and the trophi of individuals of each species were also investigated using a scanning electron microscope (SEM). For habitus examinations, specimens were sedated with carbonated water and subsequently fixed with picric acid-formaldehyde of either 240 mOsmol for freshwater (following the protocol of Melone & Ricci, 1995), 600 mOsmol for brackish, or 1,200 mOsmol for marine specimens. Preparations of the trophi were accomplished by dissolving the surrounding tissue of the specimens using a dissolving agent (0.1 g DDT [AppliChem, Darmstadt, Germany] in a 5-m1 stock solution of 5.2 g SDS + 0.24 g NH2HCO3 in 100 ml aqua dest) following the protocol of Kleinow, Klusemann, and Wratil (1990). For this step, single specimens were transferred into a droplet of DTT for about 15 min before the isolated trophi were carefully and repeatedly washed with distilled water. Both habitus and trophi samples were dehydrated using an ascending, graded ethanol series followed by critical-point drying before being attached onto a SEM stub. Finally, both sample types were coated with a 15-nm thick layer of gold-palladium and examined on a Hitachi S-3200N SEM.

2.2.2 | Morphological character matrix and character descriptions

A nexus-formatted character matrix (Maddison, Swofford, & Maddison, 1997) of the morphological data was compiled using the Nexus Data Editor (NDE, v0.5.0; Page, 2001; Appendix 3). The matrix contains 53 species and 78 equally weighted, parsimony informative characters, of which 56 were binary and 22 were multistate. Following the approach of Strong and Lipscomb (1999), character states were coded as "inapplicable" ("-"") for those species where the focal structure was absent in contrast to "missing" ("?") in cases of uncertain or missing information. This distinction, although important in terms of accuracy, is largely semantic because most phylogenetic algorithms do not distinguish between inapplicable and missing character states and treat both as missing data.

The list of characters, including their explanations and further comments, is given in the appendix (Appendix 1). To minimize potential uncertainty, the form of each character state is referenced as to how it appears in a particular exemplar species.

2.2.3 | Molecular genetic data

DNA sequence data were obtained either from GenBank or newly generated by us from specimens collected within this study (Appendix 2). For the latter, isolated individuals were starved for approximately 30 min in filtered pond water (5–8 µm filter) to avoid any potential contamination caused by ingested food before being transferred singly into 0.2-µl Eppendorf tubes. DNA extraction was accomplished by covering the isolated specimens with 50 µm InstaGene™ Matrix (Bio-Rad) following the protocol of Montero-Pau, Gómez, and Muñoz (2008). Two genes were targeted for sequencing: a partial fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI) and almost the entire nuclear 18S rRNA sequence (18S) where less than 80 bps were missing at each of the 5’ and 3’ ends.

Cytochrome c oxidase subunit I was amplified in PCR reagent mix vessels of Illustra™ PuReTaq™ Ready-To-Go™ PCR Beads (GE Healthcare) in a final volume of 20 µl comprising 2.5 µl template DNA and 0.2 µl of each of the standard Folmer, Black, Hoeh, Lutz, and Vrijenhoek (1994) primers LCO1490 (5’-GGT CAA CAA ATC ATA AAG ATA TTG G-3’) and HCO2189 (5’-TAA ACT TCA GGG TGA CCA AAA AAT CA-3’). The amplification process consisted of one cycle of initial denaturation of 5 min at 94°C, followed by 40 elongation cycles of 30 s at 94°C, 1 min at 48°C, and 1 min at 72°C before concluding with a final, single elongation cycle of 5 min at 72°C. Amplification of 18S followed a similar procedure, but used the primers 18A1mod (5’-CTG GAT CCT GCT GCC AGT CAT ATG C-3’) and 1,800 mod (5’-GAT CCT TCC GCA GGT TCA CCT ACG-3’) (Raupach, Mayer, Malyutina, & Wägele, 2009) instead and with the PCR parameters comprising one cycle of 5 min at 94°C; followed by 40 cycles of 30 s at 94°C, 40 s at 52°C, and 3 min at 72°C; and one final cycle of 5 min at 72°C.

All amplicons were sent to GATC Biotech for purification and Sanger sequencing in both directions. The same Folmer primers were used for COI sequencing, whereas sequencing of 18S rRNA was performed in three sections via primer walking using the four internal primers from Kimpel et al. (2015): 18SV4r (5’-GCA CCA GAC TTC GGC TCC TCC AAT-3’), 18SV4f (5’-ATT GGA GGG CAA GTC TGG TGC-3’), 18SV7r (5’-GTT TCA GCT TTG CAA CCA TA-3’), and 18SV7f (5’-TAT GGT TGC AAA GCT GAA/AC3’) in addition to the two external primers above from Raupach et al. (2009). Excluding the primer sequences and any major indels, the target sequences had lengths of either 661 (COI) or ca. 1,800 bps (18S). All sequences used in this study are listed in Appendix 2, including GenBank accession numbers for the newly generated sequences.

2.3 | Data analysis

2.3.1 | Morphological data analysis

The morphological data matrix was analyzed under a parsimony framework using PAUP* v4.0a163 (Swofford, 2002) using both a conventional heuristic search (random addition sequence of 100 replicates) and a parsimony ratchet (Nixon, 1999) consisting of 500 replicates of 200 iterations each, followed by a brute-force search of all equally most parsimonious trees that were found as directed by the
Perl script perlRat v2.0 (Bininda-Emonds, 2019a). Both analyses used TBR branch swapping and leveraged the strengths of a more comprehensive search strategy (the conventional analysis) with one designed more to find the shortest length, but not necessarily all trees of this length (the ratchet). Both analyses recovered trees of the same length (209 steps), with the conventional search yielding more (2,267 vs. 655) and which we summarized using a fully resolved 50% majority-rule consensus tree. Support values for the nodes on the latter were determined using a non-parametric bootstrap analysis (Felsenstein, 1985) of 1,000 replicates, with each replicate using the same heuristic search parameters as the conventional search above.

2.3.2 | Molecular genetic data analysis

For the molecular genetic data sets, all GenBank sequences for a given species were represented by the single sequence that was closest to their consensus sequence using the Perl script seqCleaner.pl v1.2 (Bininda-Emonds, 2019b). All novel sequences that we generated were retained so that a given species could be represented by several specimens, including one from GenBank (Appendix 4). Phylogenetic analyses of the COI (aligned length 689 bps), 18S (aligned length 1,780 bps), and COI + 18S (aligned length 2,469 bps) data sets were performed in a maximum-likelihood framework using RAxML v8.2.12 (Stamatakis, 2014) using a rapid, non-parametric bootstrap analysis (n = 1,000 replicates; Felsenstein, 1985; Stamatakis, Hoover, & Rougemont, 2008) followed by a thorough search to generate the maximum-likelihood topology. All analyses used a GTR + gamma model (with gamma being modeled according to the CAT approximation; Stamatakis, 2006), with separate parameters being estimated for each of the 18S and COI partitions when the two data sets were combined. All aligned data sets and the associated trees have been uploaded to TreeBASE (http://purl.org/phylo/treebase/phylows/combined. All aligned data sets and the associated trees have been study/ TB2:S25626; Piel et al., 2009; Piel, Donoghue, & Sanderson, 2018).

2.3.3 | Combination of molecular and morphological data

For the combined analysis including the morphological data, each of the 18S and COI data sets was pruned to only a single specimen per species, again using seqCleaner.pl. Doing so did not change the alignment lengths for either gene. Where possible, we gave preference to those sequences that we generated ourselves over GenBank sequences and then those where the 18S and COI sequences came from the same individual to minimize problems associated with uncertain species identifications (Appendix 5). The maximum-likelihood analysis followed the same procedure for the combined 18S + COI analyses, with the morphological partition being analyzed under a multistate model in combination with the ascertainment bias correction method of Lewis (2001). The resulting tree was then used to reconstruct the evolutionary history of selected morphological characters in Mesquite v3.6 (Maddison & Maddison, 2018) in an attempt to elucidate possible adaptations underlying the success of Synchaetidae as a group.

3 | RESULTS AND DISCUSSION

3.1 | Hypotheses of homology between and clarifications of several morphological characters

Our re-examination of Synchaetidae revealed several characters that were possibly misinterpreted in the literature as well as specific structures that we hypothesize can be homologized across the genera. In all cases, these characters should be re-examined in detail to rigorously test the hypotheses we put forth.

3.1.1 | “Streams of pigment granules” and “frontal eyespots” in Synchaeta

In the head region, streams of pigment granules leading to the frontal margin of the apical field as well as “frontal eyespots” (Appendix 1, characters 22 and 23) have been variously reported in several (mainly brackish and marine) Synchaeta species. For instance, they are simultaneously present in Synchaeta bicornis Smith, 1904, with streams of pigment granules being connected to “frontal eyespots”; individually present in Synchaeta verrucosa Nikpov, 1961 or Synchaeta prominula Kutikova & Vassilieva, 1982; entirely absent in S. pectinata (Figure 1a); and variably present in Synchaeta oblonga Ehrenberg, 1832 (Hollowday, 2002). We argue, however, that these apparently independent characters actually represent different expressions of the same one.

The streams of pigment granules appear to arise from the glandular retrocerebral organ (RCO) that is located dorsally to the brain and comprises an unpaired retrocerebral sac and two lateral subcerebral glands (Fontaneto & De Smet, 2015). Each subcerebral gland exhibits an efferent duct that runs, together with one branch of the bifurcating duct of the retrocerebral sac, toward the frontal margin of the apical field, where they open into frontal orifices (Fontaneto & De Smet, 2015; Figure 2b, go). Because the efferent ducts are usually transparent, they would appear as “streams of pigment granules leading to the frontal margin” (Figure 1d, arrow; Hollowday, 2002, e.g., p. 120) when they are filled with the red-colored granules that are occasionally found in the RCO or in the retrocerebral sac in particular (Fontaneto & De Smet, 2015; Koste, 1978). Based on our re-examinations, we hypothesize that the “frontal eyespots” (see, e.g., Hollowday, 2002, p. 107) are simply aggregations of these same pigment granules in the frontal orifices (e.g., as in Synchaeta triophthalma Lauterborn, 1894; Figures 1e and 2b, go). In this context, it is noteworthy that frontal "eyespots" are often connected to the streams of pigment granules in several species. The differential expression of this character among species of Synchaeta or even within some species (pers. obs.; see also Koste, 1978 and Hollowday, 2002) might be tied in part to nutrition, which is known to influence
the intensity of the color of eye pigmentation (pers. obs.; also Birky, 1964).

In our morphological phylogenetic analyses, we have coded these features as two separate characters. Methodologically, this is unproblematic because this is equivalent to coding a true multistate character, which we hypothesize to be the case here, using additive binary coding.

### 3.1.2 | Sensory receptors on the apical field in Synchaeta, Polyarthra, and Ploesoma

The apical field among members of Synchaetidae is equipped with numerous apical, dorsal, and dorsolateral sensory receptors (Figure 1a–c, ar, dr, dlr) that differ from each other in their specific morphology (Appendix 1, characters 48–55). Again, our re-examinations suggest the homology of the specific sets of receptors among the genera based on their identical positions. For instance, the apical receptors are always situated in the center of the apical field between the mouth and the dorsal ciliary fields in all genera although they are manifested as two bundles of short sensory cilia in Synchaeta (Figures 1a and 2b, ar), a continuous row of cilia in Polyarthra (Figures 1b and 2a, ar), or paired palps with long cilia in Ploesoma (Figure 1c, ar). Similarly, the dorsal sensory receptors are always located on the dorsal apical field below the narrow gap between the dorsal and dorsolateral ciliary bands being in the form of styles (= compound cilia) in Synchaeta (Figures 1a and 2b, dr), small ciliated papillae in Polyarthra (Figure 1b, dr), or ciliated palps in Ploesoma (Figure 1c, dr). Finally, the dorsolateral sensory receptors are always situated near the gap between the dorsolateral and lateral ciliary fields despite variously appearing as styles in Synchaeta (Figures 1a and 2b, dlr), ciliated palps in Polyarthra (homology with those of Synchaeta was already proposed by Remane, 1929; Figures 1b and 2a, dlr) and trinomial unciliated palps in Ploesoma (Figure 1c, dlr).

### 3.1.3 | Unci and rami in Synchaeta

The apical parts of the trophi that bend dorsally in species of Synchaeta (Figure 2e–f, ara) have always been considered to be the unci (e.g., in Obertegger, Braioni, Arrighetti, & Flaim, 2006); however, we hypothesize that they belong to the rami because of their specific location and morphology. The presence of many strong, stout teeth on the inner margins of the structure (e.g., S. tremula (Müller, 1786); Figure 2e–f, ara) corresponds to the typical morphology of dorsally recurving rami in virgate trophi (Fontaneto & De Smet, 2015; Koste, 1978) where the inner parts can be edentulous or possess a series of tooth-like projections (Hollowday, 2002; Koste, 1978; Sørensen, 2002). The rami of the remaining Synchaetidae are representative here, with several Polyarthra species (Figure 2c–d,
ara) and, for example, *Ploesoma africanum* Wulfert, 1965, likewise possessing teeth along their inner sides (Hollowday, 2002) and toothless inner margins being known in some rotatorivorous species of *Ploesoma* (pers. obs.; Hollowday, 2002). The latter condition is typical of *Synchaeta* as well (Figure 2e–f, ara) and speaks toward a homology with the rami of both *Polyarthra* and *Ploesoma*. Generally, the teeth of the unci in virgate trophi, if present, are weak, with only one tooth usually being of a strong and rod-shaped build (Fontaneto & De Smet, 2015; Sørensen, 2002). Therefore, we conjecture that the rod-shaped tooth dorsal to the apical ramus in *Synchaeta* (the “frontal hook”; see, e.g., Hollowday, 2002, p. 91; Figure 2f, un) more likely represents the remnant of the actual uncus in this genus. If correct, this would make *Synchaeta* consistent with the remaining members of Synchaetidae in exhibiting up to two unci teeth only (see Hollowday, 2002) as well as having reduced unci (see Appendix 1, character 77; De Smet & Segers, 2008).

### 3.2 Phylogeny of Synchaetidae

The three primary trees (morphology, COI, and 18S; Figures 3–5) are largely incongruent, with more than half of all clades being different in each of the possible pairwise comparisons (i.e., normalized $d_s$ values are >0.5; Table 1). However, when accounting for nodal bootstrap support, the weighted, normalized $d_s$ values all sink to around 0.3, indicating that much of the conflict involves more poorly supported clades. Unanimous agreement among the primary trees is limited to the monophyly of each synchaetid genus, the sister species pair of *S. tremula* and *Synchaeta tremuloida* Pourriot, 1965, and that *Synchaeta hutchingsi* Brownell, 1988 and *S. triophthalma* are part of a clade including *Synchaeta neapolitana* Rousselet, 1902 (for which only morphological data were available) as the possible sister species of *S. hutchingsi*. These clades, together with those comprising the individual species in the COI and 18S trees, also tended to exhibit the highest support values in their respective trees.

These latter results are also found in the combined analysis of all three primary data sets (Figure 7), together with some additional, but better supported resolution. For instance, as for the morphological analyses, a sister group relationship between *Polyarthra* and *Ploesoma* is well-supported (bootstrap = 92.4%). However, the relationships within each genus remain unclear because the general lack of sequence data means that most internal resolution derives from the morphological data with its weaker signal (usually <50%). In addition, the different analyses often disagree about the relationships of those species that they have in common (e.g., *Ploesoma hudsoni* Imhof, 1891) in Figures 3–6 compared to Figure 7). In addition, the morphological and molecular trees disagree strongly about the placement of *Polyarthra major* Burckhardt, 1900, which might obtain from our uncertain identification of our *P.* (“cf.”) *major* specimens. Finally, although the GenBank COI sequence for *Polyarthra vulgaris* Carlin, 1943 is most closely related to those we obtained for this species, the respective sequences are still very distinct (Figure 4), hinting at either another potential misidentification or a case of cryptic speciation.

A similar situation is also true for *Synchaeta*, although a case can be made to support several groups within the genus despite them not always being universally or robustly supported and notwithstanding differences arising from species sampling. For instance, most analyses place *Synchaeta grandis* Zacharias, 1893, *S. pectinata*, *Synchaeta stydata* Wierzejski, 1893, and *Synchaeta longipes* Gosse, 1887 (morphology only) basal to the remaining species of *Synchaeta*, albeit in different constellations and usually not as a monophyletic group. The monophyly of the remaining *Synchaeta* species enjoys

**Figure 2** Scanning electron microscope images of the frontal habitus of (a) *Polyarthra dolichoptera* and (b) *Synchaeta triophthalma*, and of the trophi (c, e: ventral and d, f: frontal) of (c, d) *Polyarthra dolichoptera* and (e, f) *Synchaeta tremula*. ar, apical receptors; ara, apical ramus; dr, dorsal receptors; dlr, dorsolateral receptors; fu, fulcrum; go, gland opening of the retrocerebral organ; hyp, hypopharynx; ma, manubrium; ra, ramus; un, uncus. Scale bars = 20 µm.
high support in the combined analysis (91.0%; Figure 7) and indeed unusually high support for such a large, intrageneric clade with these data sets. Additional groups include a clade comprising Synchaeta vorax Rousselet, 1902 and Synchaeta fennica Rousselet, 1909 and then as the sister group to all other Synchaeta species barring those in the S. pectinata “group” (Figures 3 and 7), a Synchaeta kitina Rousselet, 1902/S. hutchingsi/S. triopthalthma group (Figures 3, 5–7), a Synchaeta baltica Ehrenberg, 1834/S. bicorns group (Figures 3 and 7), and a Synchaeta grimpei Remane, 1929/Synchaeta lakowitziana Lucks, 1930 group (Figures 3, 4, 6 and 7).

Robust phylogenetic analyses of rotifers are rare and tend to be based on morphological (e.g., Clément, 1980; Sørensen, 2002) or molecular data (e.g., Derry, Hebert, & Prepas, 2003) alone, with
the latter often relying on a single gene only (e.g., Gómez, Serra, Carvalho, & Lunt, 2002). The exception in this regard is the total evidence analysis of Sørensen and Giribet (2006). However, only three species of Synchaetidae were included in the latter study, none of which belonged to Synchæta. As such, our total evidence phylogeny for Synchaetidae (sensu Kluge, 1989), which is based on three different data partitions (morphology, mtDNA, and nDNA), remains the best working hypothesis available for this group and also highlights the continuing role that morphological data can play in phylogenetic analyses, particularly those of rotifers. Despite the increasing ease with which sequence data can be generated, rotifers as a group continue to have only sparse molecular coverage. Indeed, our study was the first to generate sequence data for several morphospecies including *S. triophthalma*, *Synchæta gyrina* Hood, 1887, and *S. hutchingsi*. Moreover, because molecular information is still missing for many species of Synchaetidae, arguably the most comprehensive phylogenetic signal in our analysis derives from the morphological partition. Even so, missing data here remain problematic because we
could not re-examine several morphospecies and a few have never been re-discovered since their initial description (e.g., *Synchaeta rousseleti* Zelinka, 1927 and *S. prominula*; see Hollowday, 2002). For the less well-known species, often decades can lie between documented occurrences (e.g., ca. 60 years for *S. tremuloida*; Pourriot, 1965, Wilke, Ahlrichs, & Bininda-Emonds, 2017) and the known morphological information for them tends to derive entirely from their initial descriptions with their focus on diagnostic autapomorphies, which, although useful for species delineation and identification, are of little avail for phylogenetic reconstructions, where synapomorphies are required for establishing relationships.

For the future, we recommend that re-examinations of established species or descriptions of new ones be as comprehensive as possible to obtain both morphological (habitus and trophi) and molecular data, with the former being obtained from live, unpreserved specimens (see Wilke, Ahlrichs, & Bininda-Emonds, 2018a, 2019a) and the latter in association with a representative voucher specimen from the same population given that species within Synchaetidae are exceedingly difficult to identify correctly (e.g., Koste, 1978; Ruttner-Kolisko, 1972). In addition, the use of more genes would also appear to be necessary given the low support values associated with 18S and COI, both individually and in combination. COI (and arguably the hypervariable regions of 18S) is commonly used for species taxonomy and barcoding (Fontaneto, Flot, & Tang, 2015) and is typically used to resolve relationships on the species and even population levels (Avise et al., 1987; Tautz, Arctander, Minelli, Thomas, & Vogler, 2003); however, it rapidly loses signal above these levels (Ballard & Rand, 2005). The same is largely true for the hypervariable regions of 18S rRNA. By contrast, the non-hypervariable regions of 18S generally evolve much slower than COI (Wiens & Penkrot, 2002) and should therefore complement it. Within rotifers, however, these regions show an uncharacteristically low degree of variation within each of the major clades (Bininda-Emonds, in prep) such that they might often be too slow for reconstructing the phylogenetic relationships within them.

**3.3 Evolution of and key character transformations within Synchaetidae**

Synchaetidae is a successful planktonic rotifer taxa with a high morphological and ecological diversity within and among its genera (e.g., see Fontaneto & De Smet, 2015). From an inferred most recent common ancestor that was pelagic, found in freshwater, and
had a food spectrum including rotifers (= rotatorivory; Figure 7, gp2), several key character transformations appear to have enabled the success of Synchaetidae in pelagic environments and so become one of the most widely distributed and abundant rotifer taxa (Table 2). These transformations include additional modifications to the virgate trophi, particularly to elements that were already presumably more expanded and delicate in the most recent common ancestor as compared to its closest relatives (e.g., rami), as well as adaptations to facilitate hatching and feeding independent of either benthic or periphytic habitats.

### 3.3.1 Colonizing pelagic habitats

In contrast to the benthic and/or periphytic Notommata outgroup (Figure 7, gp17), Synchaetidae are truly planktonic and can occur in pelagic environments that are several kilometers away from shore (Hollowday, 2002; Koste, 1978). Our analyses indicate that successful colonization of the water column and a life independent from benthic and periphytic habitats evolved somewhere along the lineage leading to Synchaetidae. However, because all plausible close relatives of Synchaetidae except Microcodon are benthic and/or periphytic (see Sørensen, 2002 and Sørensen & Giribet, 2006), the transition to a pelagic lifestyle is likely to be a derived feature of Synchaetidae and might have triggered an adaptive radiation within this taxon (Figure 7, gp2). Indeed, the majority of Synchaetidae are exclusively planktonic with only a few exceptions including some periphytic Ploesoma species (e.g., *Ploesoma triacanthum*; see Hollowday, 2002) and some non-predatory, freshwater members of *Synchaeta* that frequently attach themselves to objects in the water (e.g. *S. tremula*; Appendix 1, characters 2–4).

A planktonic lifestyle is generally associated with specific adaptations (after Allen, 1968) such as the reduction in the foot (up to being entirely absent in the exclusively planktonic *Polyarthra*; Figure 7, gp15; Appendix 1, character 35), the development of movable appendages such as fins (e.g., in *Polyarthra*; Figure 7, gp15; Appendix 1, characters 24–34) and/or strategies for hatching eggs in environments that are usually free of any substrate or plants to which to attach them. In the latter case, pelagic species either carry their eggs on their body or produce eggs that are able to float in the water column via an internal oil droplet, an outer gelatinous layer, or through numerous external spines (Allen, 1968; Appendix 1, characters 6–8). Although information on subitan egg morphology and hatching behavior in Synchaetidae is sparse, it is known that both these general strategies occur within the group, with our analyses indicating that the production of floating subitan eggs was already present in the synchaetid common ancestor (Figure 7, gp2). However, whether or not this feature is derived for Synchaetidae could not be determined because of the lack of information regarding it for species of Notommata and most other potential outgroup taxa. This condition is retained in *Ploesoma* and the basalmost *Synchaeta* lineages (i.e., the *S. pectinata* “group”), with *S. pectinata* reducing the time of egg floating by having hatching occur directly after the egg is laid or even within the mother (= oovivipary; Koste, 1978). By contrast, the derived condition whereby eggs are carried on the body apparently evolved independently in *Polyarthra* (eggs attached to the cloacal region; Figure 7, gp15) and in brackish and marine *Synchaeta* species (eggs attached to the tips of the toes; see below; Figure 7, gp4).

The pelagic lifestyle of Synchaetidae is also reflected in the evolution of their sensory system (Figure 7, gp2; Appendix 1, characters 48–61), where the sensory receptors are distributed around almost the entire body to receive input from as much of the surrounding water column as possible. Thus, several conspicuous and large receptors including sensory styles or palps are present on the apical field (also Fontaneto & De Smet, 2015), a dorsal antenna is located caudal to the cerebral eye, and sensory antennae are situated ventrolaterally or laterally on the caudal part of the trunk. Possibly aiding stimulus reception from the environment is a typical swimming motion of rotating along the longitudinal axis. By contrast, the sensory receptors of the Notommata outgroup are restricted to the

### Table 1

Unweighted and weighted partition metrics \((d_s\text{)}\) for all pairs of trees

| Tree 1          | Tree 2          | Shared taxa | Normalized unweighted \(d_s\) | Normalized weighted \(d_s\) |
|-----------------|-----------------|-------------|-------------------------------|-----------------------------|
| All data        | 18S             | 20          | 0.500                         | 0.229                       |
| All data        | COI             | 24          | 0.591                         | 0.208                       |
| All data        | Morphological   | 55          | 0.453                         | 0.164                       |
| All data        | Combined molecular | 24        | 0.318                         | 0.114                       |
| Combined molecular | 18S         | 20          | 0.389                         | 0.111                       |
| Combined molecular | COI          | 24          | 0.591                         | 0.161                       |
| Combined molecular | Morphological | 24          | 0.545                         | 0.232                       |
| 18S             | COI             | 20          | 0.667                         | 0.290                       |
| 18S             | Morphological   | 20          | 0.500                         | 0.261                       |
| COI             | Morphological   | 24          | 0.727                         | 0.312                       |

**Note:** Values are normalized to account for tree size and fall between 0 (no or 0% difference) and 1 (maximum or 100% difference). Weighted values account for nodal bootstrap support values.

**Abbreviations:** 18S, 18S rRNA sequence; COI, cytochrome c oxidase subunit I gene.
and advantageous for the crawling behavior typical of their benthic and periphytic lifestyle (Figure 7, gp17).

3.3.2 Colonizing brackish and marine environments

The ancestral colonization of the water column in Synchaetidae undoubtedly happened in freshwater environments (Figure 7, gp1; Appendix 1, character 1) and the majority of the extant species, including all of Ploesoma and Polyarthra, occur exclusively in such habitats. Synchaeta, however, is noteworthy among rotifers because a large proportion of its species (approximately half) inhabit brackish and marine environments (Hollowday, 2002; Koste, 1978). Our reconstructions show that the invasion into saline habitats within Synchaeta was a unique evolutionary event and one that, based on the extant species sample, coincides with carrying subitan eggs on the toes (Figure 7, gp4). Whereas the ancestral state of floating subitan eggs is probably suitable for delimited waterbodies like...
Figure 7. Maximum-likelihood tree (lnL = −12,284.960615) of the combined molecular (18S, COI) and morphological data for 53 species of Synchaetidae and two notommatid outgroups. Both molecular data sets were pruned to only a single sequence for each species that was closest to their consensus sequence (Appendix 5). Bootstrap values (n = 1,000) are listed above each node, whereas the ground patterns of selected ancestral nodes are listed below the relevant nodes as “gpX” (see Table 2). The scale bar represents the amount of morphological and molecular change along each branch, where molecular change is measured in the average number of substitutions per site per unit time.
| Node | Character |
|------|-----------|
| gp1  | (#1) Habitat: fresh to slightly brackish water  
(#5) Nutrition: feeding on other rotifers either additionally or exclusively (= rotatorivore)  
(#6) Subitan egg—carried: no  
(#10) Epidermis—flexibility: flexible  
(#47) Esophagus—morphology: long and tubular, narrow along its entire length (= no proventriculus-like structure)  
(#65) Rami—teeth: rami edentulous or with serrated/corrugated margin  
(#73) Manubrium—extension of the clava: very small  
(#75) Manubrium—location of the cauda: lateral within the trophi |
| gp2  | (#2) Microhabitat: exclusively pelagic  
(#8) Subitan egg—adapted to floatation: yes (possibly already present in gp1, but information for the Notommata outgroup is lacking)  
(#57) Dorsal antenna—location: posterior to the cerebral eye  
(#59) Lateral antennae—location in relation to transversal axis: ventrolateral  
(#62) V-shaped hypopharynx muscle—presence: present  
(#63) Trophi—overall symmetry: symmetrical  
(#64) Rami—overall structure and morphology: delicate, forming lateral wing-shaped extensions  
(#70) Fulcrum—expansion of the distal end in dorsal view: none, fulcrum of equal width  
(#77) Uncus—number of teeth: one or two distinct teeth |
| gp3  | (#48) Apical receptors—morphology: two bundles of short sensory cilia  
(#51) Dorsal sensory receptors on the apical field—morphology: styles  
(#52) Dorso-lateral sensory receptors on the apical field—morphology: styles  
(#73) Manubrium—extension of the clava: very extensive  
(#75) Manubrium—location of the cauda: dorso-lateral within the trophi |
| gp4  | (#1) Habitat: brackish to marine or inland saline  
(#6) Subitan egg—carried: yes  
(#8) Subitan egg—adapted to floatation: no  
(#65) Rami—teeth: development of teeth in form of states 1, 3, or 4 (all equally parsimonious) |
| gp5  | (#5) Nutrition: herbivorous or feeding on small ciliates or bacteria  
(#47) Esophagus—morphology: slender proximally, widened distally to form a proventriculus-like structure |
| gp6  | (#65) Rami—teeth: dorsal teeth distinct, ventral teeth comb-like |
| gp7  | (#1) Habitat: fresh to slightly brackish water |
| gp8  | (#65) Rami—teeth: all teeth distinctly incised |
| gp9  | (#1) Habitat: fresh to slightly brackish water |
| gp10 | (#1) Habitat: fresh to slightly brackish water |
| gp11 | (#5) Nutrition: feeding on other rotifers either additionally or exclusively (= rotatorivore)  
(#47) Esophagus—morphology: long and tubular, broad along its entire length (= loss of proventriculus-like structure) |
| gp12 | (#65) Rami—teeth: dorsal teeth comb-like, ventral teeth distinct |
| gp13 | (#65) Rami—teeth: one strong tooth at the tip of the ramus, remainder are irregular and blunt |
| gp14 | (#1) Habitat: fresh to slightly brackish water |
| gp15 | (#5) Nutrition: herbivorous or feeding on small ciliates or bacteria  
(#6) Subitan egg—carried: yes  
(#8) Subitan egg—adapted to floatation: no  
(#10) Epidermis—flexibility: partially flexible  
(#24) Dorso- and ventrolateral fins—presence: present  
(#35) Foot—presence: absent  
(#48) Apical receptors—morphology: continuous row of long cilia  
(#51) Dorsal sensory receptors on the apical field—morphology: ciliated papillae  
(#52) Dorso-lateral sensory receptors on the apical field—morphology: ciliated palps  
(#65) Rami—teeth: one strong tooth distinctly removed from the ramus tip, remaining plate smooth or slightly serrated  
(#73) Manubrium—extension of the clava: medium |
| gp16 | (#10) Epidermis—flexibility: stiff  
(#48) Apical receptors—morphology: paired palps with long cilia  
(#51) Dorsal sensory receptors on the apical field—morphology: ciliated palps  
(#52) Dorso-lateral sensory receptors on the apical field—morphology: trinomial unciliated palps  
(#64) Rami—overall structure and morphology: of medium robustness |

(Continues)
freshwater lakes, there is a greater risk of the eggs drifting away in more turbulent open waters such that carrying them attached to the toes becomes a beneficial adaptation to improve recruitment in brackish and marine environments (Figure 7, gp4; Appendix 1, characters 1, 6–7).

The reconstructions show several freshwater species (S. kitina, the S. tremula/S. tremuloida group, S. oblonga, and the S. lakowitiziana group) occurring amidst the brackish/marine taxa. The most parsimonious explanation is one of multiple secondary re-invasions into freshwater habitats (Figure 7, gp7, gp9, gp10, and gp14), which is further supported by all these species (as far as data on hatching behavior are present) retaining the habit of carrying subitan eggs that is otherwise typical for brackish and marine species (Figure 7, gp4), even if the duration of carrying the eggs is slightly (e.g., S. tremula) to significantly (e.g., S. oblonga) reduced compared to the non-freshwater species.

### 3.3.3 Feeding strategy and feeding habit in pelagic environments

The common ancestor of Synchaetidae exhibited virgate trophi, which are symplesiomorphic for this taxon and shared with the Notommata outgroup, if not virtually all plausible alternative sister groups of Synchaetidae. Generally, virgate trophi are adapted to swallowing food whole by pumping without any crushing or piercing of it (Fontaneto & De Smet, 2015). Within the ground pattern of Synchaetidae, however, the colonization of the pelagial meant that the pumping action of the virgate trophi needed to be optimized for capturing food directly out of the water column. Whereas Ploesoma retained the inferred ancestral state of stout lateral clava of the manubria found in the Notommata outgroup (pers. obs.; Figure 7, gp1), Polyarthra and Synchaeta convergently greatly enlarged the lateral clava (Figure 7, gp3 and gp15; Appendix 1, character 73) to presumably enhance the pumping action of the mastax. Likewise, whereas the rami are stout in the benthic/periphytic Notommata outgroup, larger, more delicate lamellae are found across Synchaetidae (Figure 7, gp2 and gp16; Appendix 1, character 64), again to apparently increase the efficiency of food capture.

A final, crucial adaptation to this end in all Synchaetidae is that the rami and manubria jointly form a trophi cavity, the volume which is controlled by the strong v-shaped hypopharyngeal muscle attached to the distal end of the antagonistic fulcrum (Figure 7, gp2; Appendix 1, character 62). Together with reduced unci, this latter feature is diagnostic for Synchaetidae (e.g., De Smet & Segers, 2008; Fontaneto & De Smet, 2015) and both are already present in its most recent common ancestor (Figure 7, gp2). The entire complex surrounding the trophi cavity, however, is most pronounced within Synchaeta, where the extensive rami and the enlarged clava lamella of the manubrium encompass the entire trophi cavity.

Although knowledge about food preferences in Synchaetidae is relatively sparse, our reconstruction indicates that the ancestral food spectrum and that in the Notommata outgroup at least partly comprised rotifers (Figure 7, gp1; Appendix 1, character 5). Rotatorivory is retained in and typical for all recent species of Ploesoma as well as in the basalmost lineages of Synchaeta (up to and including the S. fennica/S. vorax clade), whereas a non-rotatorivorous feeding habit evolved in the ground pattern of Polyarthra and is inferred to be present in all recent species (Figure 7, gp15).

Within Synchaeta, the shift to a non-rotatorivorous feeding habit (Figure 7, gp5) and a single secondary gain (at least in S. baltica; Figure 7, gp11) of rotatorivory appear to correlate with the morphology of the esophagus (Appendix 1, character 47) and with the unique development of a proventriculus-like structure (Figure 7, gp5) in particular. In contrast to the tensile, tubular esophagus found in rotatorivorous species that might be necessary for ingesting large (rotifer) prey whole, a proventriculus-like structure might act as a form of pre-digestive, food-storage organ for ingested food particles that is potentially advantageous for a food spectrum comprising algae and bacteria. Supporting this hypothesis is that S. baltica has reverted to a rotatorivorous diet and also lost its proventriculus-like structure. A future test of this hypothesis would be to ascertain the feeding habits of the remaining members of the S. baltica clade (Figure 7, gp11), where rotatorivory would be predicted given that at least Synchaeta johanseni Harring, 1921 and S. bicorns also lack the proventriculus-like structure.

Although the associations are not as clear cut, rotatorivory in Synchaeta might also be associated with a strongly convex apical field (Appendix 1, character 16) and large, caudally directed auricles (Appendix 1, characters 18 and 19). Both sets of features are found in the basal rotatorivorous species as well as in the S. baltica clade and could be advantageous for chasing and capturing (rotifer)
prey (Appendix 1, characters 16, 18 and 19). If this association is true, it provides another testable hypothesis that the feeding habit of the remaining members of the *S. baltica* clade is indeed rotatorivorous.

By contrast, the convergent shifts to a non-rotatorivorous feeding habit within each of *Polyarthra* and *Synchaeta* (Figure 7, gp15 and gp5, respectively) appear to benefit from the development of distinct rami teeth from the ancestral state of edentulous or serrated rami for Synchaetidae (Appendix 1, character 65). The association, however, is not perfect insofar as the species in the rotatorivorous *S. fennica*/*S. vorax* clade also possess a single strong tooth with those in the *S. baltica* clade, which were inferred above based on other characters to be secondarily rotatorivorous, all possessing (or having retained) either distinct or comb-like rami teeth.

Koste (1978) had previously distinguished between species of *Synchaeta* according to whether their rami possessed teeth (S. *tremula*-type) or not (S. *pectinata*-type). However, given that no robust phylogeny of the genus existed at this time, it was unclear how these types related to the evolutionary history of the group. Elsewhere, we added four further trophi types (see Wilke, Ahlrichs, & Bininda-Emonds, 2019b; Appendix 1, character 65), and herein, we can see an overall tendency toward an increased development of rami teeth within *Synchaeta* from the ancestral state of edentulous rami in the basalmost species (up to the *S. stylata*/*S. longipes* monophylum; Figure 7, gp1; = *S. pectinata*-type after Koste, 1978) to rami possessing a single strong tooth in the *S. fennica*/*S. vorax* clade (Figure 7, gp4) to the development of several rami teeth (Figure 7, gp5). The latter evolutionary event, in turn, yielded a diversity of forms, including the *S. tremula*-type of Koste (1978) with distinct teeth (Figure 7, gp8), from which rami with several, dorsal comb-like teeth (Figure 7, gp12, *S. baltica*/*S. johanseni* clade) or teeth that are entirely blunt except for a single strong and sharp tooth (Figure 7, gp13, *Synchaeta tamara* Smirnov, 1932/*Synchaeta pachypoda* Jaschnov, 1922/*S. lakowitziana* clade) are derived. An alternative path (Figure 7, gp6, *S. triophthalma*/*S. kitina* clade) led to the development where several rami teeth are ventrally comb-like. Unfortunately, further information regarding the trophi remains sparse among species of *Synchaeta*, meaning that further re-examinations are needed to help establish a more definitive evolutionary pattern and so to assess its phylogenetic and functional importance.

### 4 | CONCLUSION

Our phylogenetic tree for Synchaetidae represents the first systematic treatment of this group based on a comprehensive, integrative data set (morphological data, mtDNA and nDNA) combined with a robust method of analysis. The current tree provides insight into some possible key innovations within Synchaetidae (e.g., the adoption of a pelagic lifestyle with associated adaptations) and within *Synchaeta* in particular (e.g., changes to the ancestral virgate rami or colonization of non-freshwater habitats) that have contributed to the success of each taxon. Although several species are missing the associated morphological or ecological data underlying these presumed adaptations, the correlations we have identified predict what these missing data should be and so function to test the hypotheses we put forth once these data become known.

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APPENDIX 1
Character descriptions

In the following, descriptions of the individual characters in the morphological character matrix are provided together with one or more exemplar species (in parentheses) for the individual character states. All characters refer to the states in fully grown females only. The associated matrix is presented in Appendix 5.

Several sets of characters form complexes insofar as they all pertain to a single structure (e.g., the foot or the apical field). This often creates a problem of non-independence among the characters, especially when the first one refers to the absolute presence versus absence of the structure. However, we have knowingly chosen this option because the use of multistate characters is impractical when many characters are involved and also often too fine-grained, thereby potentially minimizing the amount of resolution. Although it makes no difference with respect to the phylogenetic analysis, we followed Strong and Lipscomb (1999) in coding inapplicable characters using a dash (i.e., “–”) so as to distinguish them from cases where the character state is truly unknown (coded using a “?”).

(1) Habitat: 0 = fresh to slightly brackish water (Synchaeta oblonga, S. kitina); 1 = brackish to marine or inland saline (Synchaeta vorax or S. cylindrica, respectively).

Rotifers predominantly occur in freshwater environments (ca. 85% of the species; Fontaneto & De Smet, 2015) up to a salinity of 1.5‰, a value that constitutes a boundary at which the freshwater rotifer biocoenosis changes significantly (Ruttner-Kolisko, 1971, as cited in Fontaneto & De Smet, 2015). Although species richness is held to decrease with increasing salinity (Ruttner-Kolisko, 1980), approximately half of all known species of Synchaeta occur in brackish and marine environments (Hollowday, 1887). For the present analysis, only definitive findings were included, isolated records of individuals in otherwise strongly atypical salinities for that species were not considered (e.g., reports of S. oblonga from saline and strongly brackish habitats or of S. tavina from freshwater habitats; Hollowday, 1887) because it cannot be excluded that the observations stem from misidentifications.

(2) Microhabitat: 0 = benthic/ periphytic (Ploesoma triacanthum, Notommata codonella); 1 = pelagic, but with frequent adherence to objects (Synchaeta tremula); 2 = exclusively pelagic (Synchaeta pectinata).

Less than one-third of all rotifer genera contain some of the 5%-8% of all rotifer species that are adapted to lifestyle that is at least partly planktonic (Ruttner-Kolisko, 1972). This character refers to the microhabitat of individuals of a species. A benthic/ periphytic lifestyle applies to the Notommata outgroup and some species of Ploesoma and includes species that predominantly live in contact with any substrate or occur among vegetation. A pelagic lifestyle includes species that are found in the water column and is further subdivided into those that are exclusively pelagic (all Polyarthra, some of Ploesoma, and most of Synchaeta) or ones that regularly frequent and adhere to objects (some of Synchaeta).

(3) Adherence to objects: 0 = no (S. pectinata, Polyarthra dolichoptera); 1 = yes (S. tremula, S. oblonga).

This character refers to the absolute ability to adhere to objects in the microhabitat by a mucus thread secreted from the pedal glands and does not concern the frequency and duration of such events.

(4) Adherence to objects—duration: 0 = regularly and for a longer time (S. tremula); 1 = sporadically, and then for a short time (S. oblonga).

Adherence to objects tends to be either frequent and long-lasting (e.g., S. tremula) or a short, sporadic event that predominantly occurs during disturbances (e.g., S. oblonga; Wilke et al., 2018b).

Species that never adhere to objects were coded as inapplicable for this character.

(5) Nutrition: 0 = herbivorous or feeding on small ciliates or bacteria (S. tremula); 1 = feeding on other rotifers either additionally or exclusively (= rotatorivorous; S. pectinata).

Most members of Synchaetidae (all Polyarthra and many Synchaeta species) are herbivorous or feed on small ciliates or bacteria. All species of Ploesoma and some of Synchaeta, however, additionally or exclusively feed on other rotifers. For the condition when rotifers comprise at least part of the diet, we have introduced the term “rotatorivorous.” The rotifer prey can be congeneric or even conspecific and is either ingested whole (e.g., Synchaeta grandis) or its internal organs are sucked out (e.g., in Synchaeta longipes, Ploesoma hudsoni) through the pumping function of the virgate trophi (Fontaneto & De Smet, 2015; Wallace, Snell, Ricci, & Nogrady, 2006). Notommata species are omnivorous and often prey on other rotifers as well (e.g., N. codonella; Fontaneto & Melone, 2003).

(6) Subitan egg—carried: 0 = no (S. pectinata); 1 = yes (Synchaeta triophthalma, S. oblonga).

Subitan eggs can be released into the water column, attached to the substrate or carried attached by mucus to the toes or cloacal region of the female (Fontaneto & De Smet, 2015; Wallace et al., 2006). This character refers to the absolute presence of eggs attached to the body and does not concern itself with the duration of this event.

(7) Subitan egg—duration of carrying eggs: 0 = sporadically, for a short time (S. oblonga); 1 = for a long time (S. triophthalma).

In carrying subitan eggs attached to the body, the females of some species carry them only sporadically as well as for a short time only, whereas others hold them until their hatching. Species that never carry eggs were coded as inapplicable for this character.

(8) Subitan egg—adapted to floatation: 0 = no (S. oblonga); 1 = yes (S. pectinata, P. hudsoni).

Several species produce subitan eggs that are modified so as to aid their floatation in the water column (Ruttner-Kolisko, 1972), either via an internal oil drop (e.g., S. grandis) or by being covered by either an external gelatinous layer (Ploesoma species or S. pectinata) or numerous long spines (e.g., Synchaeta stylata) (Allen, 1957), the latter of which also yield an additional
protective function (Nipkow, 1961). With this coding, it is recognized that state 1 can include possibly non-homologous mechanisms of floatation; however, our primary goal here was to examine whether there was any relationship between egg floatation per se and a pelagic microhabitat.

(9) Body length: 0 = <250 µm (S. kitina); 1 = more than 250 µm (S. oblonga).

Within the present analysis, only mature specimens were measured (i.e., those with a distinct ovary), with body length being taken from the anterior most part of the apical field to the tips of the toes, thereby disregarding any contribution from cilia. From literature data, only the maximal sizes of the respective species were considered to exclude measurements of immature specimens.

(10) Epidermis—flexibility: 0 = flexible (S. pectinata); 1 = partially flexible (P. dolichoptera); 2 = medium stiffness (P. hudsoni); 3 = stiff (P. triacanthum).

This character essentially refers to the morphology and consistency of the intracytoplasmic lamina (ICL) that is found in all rotifers underneath the external cell membrane of the epidermal cells. In the case of a very thin ICL, the epidermis is entirely flexible as in Synchaeta. However, it can also be thickened and stiffened to various degrees by cross-linked keratin-like proteins (Fontaneto & De Smet, 2015; Wallace et al., 2006), thereby forming a robust, stiffened body armor as in Ploesoma. This stiffening can also be restricted to specific body regions to serve as an insertion point for musculature as for the fins in Polyarthra (Allen, 1957).

(11) Stiffened epidermis—ventral sulcus along the trunk: 0 = absent (Ploesoma murrayi); 1 = present (P. triacanthum).

This character refers to the presence of a ventrally located, slit-shaped region of flexible ICL that, in turn, forms an invagination between the flanking, stiffened epidermis (Fontaneto & De Smet, 2015). In Ploesoma murrayi, a species that otherwise exhibits a stiffened epidermis, the sulcus is present only as a small ventral flexible aperture. Species without a stiffened epidermis were coded as inapplicable for this character.

(12) Stiffened epidermis—anterior shape of the head shield: 0 = lobed (P. truncatum); 1 = with spine(s) (P. triacanthum).

The shape of the anterior margin of the head shield that overlaps the head region of specimens with a stiffened epidermis can be either lobed or possesses spines. Species without a stiffened epidermis were coded as inapplicable for this character.

(13) Stiffened epidermis—posterior margin of the trunk region: 0 = rounded (P. triacanthum); 1 = elongated (P. africanum).

The posterior margin of specimens with a stiffened epidermis can be either rounded or elongated to a blunt or acute process. Species without a stiffened epidermis were coded as inapplicable for this character.

(14) Apical field—distal post-oral end of the corona forming a "chin": 0 = present (Notommata allantois); 1 = absent (S. pectinata for Synchaetidae).

The distal post-oral margin of the corona projects to form a distinct ventral "chin" in species of the outgroup Notommata (Pourriot, 1995); it is flat in species of Synchaetidae.

(15) Apical field—width in relation to the trunk: 0 = narrower than the trunk (P. triacanthum); 1 = as wide as or wider than the trunk (S. pectinata).

This character refers to the widest expansion of the apical field in relation to the widest expansion of the trunk region. Any lateral contribution by the cilia of the apical field was not considered.

(16) Apical field—elevation: 0 = flat to medium (P. dolichoptera, S. tremula, S. oblonga); 1 = strong (S. grandis).

This character refers to the convexity of the apical field.

(17) Auricles—presence: 0 = present (S. pectinata); 1 = absent (P. dolichoptera).

The auricles are two lateral, fleshy protuberances bearing the cilia of the lateral ciliary fields of the rotatory organ and are used for swimming.

(18) Auricles—size: 0 = small to medium (S. tremula); 1 = large (S. grandis).

This character refers to the size of the auricles, ignoring any contribution from the cilia. Species without auricles were coded as inapplicable for this character.

(19) Auricle—orientation: 0 = lateral to slightly caudal (S. tremula); 1 = strongly caudal (S. longipes); 2 = pointing forward (N. codonella).

This character refers to the orientation of the auricles, again ignoring any contribution from the cilia. Species without auricles were coded as inapplicable for this character.

(20) Head delimited from the trunk by a neck region: 0 = yes (S. oblonga); 1 = no (P. dolichoptera).

A neck region is often present within Monogononta (Fontaneto & De Smet, 2015) and consists of at least one pseudosegment that clearly distinguishes the head from the trunk region. Within Polyarthra (Hollowday, 1887) and Ploesoma, however, a distinct neck region is absent and the head passes directly over into the trunk region.

(21) Saccular appendages in the neck region—presence: 0 = absent (S. pectinata); 1 = present (Synchaeta bicornis).

The saccular integumental appendages in the dorsal or ventral neck region of some species of Synchaeta are thought to compensate for pressure changes in the body fluid by increasing their volume through increased internal pressure caused by contraction of the body (Remane, 1929).

(22) Pigment granules of the retrocerebral organ (RCO)—in streams: 0 = absent (S. pectinata); 1 = present (Synchaeta baltica).

Pigment granules can be present in the efferent ducts of the RCO, thereby appearing as streams leading to the frontal margin (see Results and Discussion). However, we have observed that the intensity of the pigmentation can vary within a species, possibly because they are influenced by nutrition like the granules of the cerebral eye (Birky, 1988). In addition, conservation (e.g., preservation in alcohol) might also influence the intensity. Therefore, we restricted coding pigment granules as being “absent” for only those species where we examined a high number of live individuals from several different populations. Literature information relying on only a few live specimens or exclusively on preserved material was coded as “?” for this character.
(23) Pigment granules of the RCO—in frontal aggregation (“frontal eye spots”): 0 = absent (S. pectinata); 1 = present (S. triophthalma). The present character is similar to the previous one, including the restrictions on coding it as being absent, but refers to whether pigment granules are aggregated in the orifices of the RCO in the frontal region, thereby potentially appearing as “frontal eye spots” (see Results and Discussion).

(24) Dorso- and ventrolateral fins—presence: 0 = absent (S. pectinata, P. hudsoni); 1 = present (P. dolichoptera).

A characteristic feature of Polyarthra is the presence of two dorso- and two ventrolateral bundles, each comprising three fins. The fins are integumental structures that are attached to strong muscle strands on each lateral side of body and used for locomotion (Allen, 1957).

(25) Dorso- and ventrolateral fins—overall length: 0 = <100 µm (Polyarthra indica); 1 = over 100 µm (Polyarthra euryptera).

Species without fins were coded as inapplicable for this character.

(26) Dorso- and ventrolateral fins—length in relation to body size: 0 = shorter than the body length (P. euryptera); 1 = longer than total body length (P. longiremis).

Species without fins were coded as inapplicable for this character.

(27) Dorso- and ventrolateral fins—symmetry between bundles: 0 = symmetrical, all fins are more or less identical (P. dolichoptera); 1 = asymmetrical, fins of different shape or length between bundles on one body side (Polyarthra luminosa).

An example of asymmetrical fins is found in P. luminosa, where the dorsal bundles contain differently shaped fins compared to the ventral ones. A left-right asymmetry was observed in Polyarthra minor, where the right dorsal bundle of fins is twice as long as remaining bundles. Species without fins were coded as inapplicable for this character.

(28) Dorso- and ventrolateral fins—symmetry within each dorsolateral bundle: 0 = fins are of the same shape (Polyarthra vulgaris); 1 = fins are of different shape (P. minor).

An example of asymmetry is found in P. minor, where one fin is dagger shaped and the remaining two are slender, but of equal width along their lengths. Species without fins were coded as inapplicable for this character.

(29) Dorso- and ventrolateral fins—shape: 0 = narrow, of even width (P. dolichoptera); 1 = broadened near base, decreasing in width distally (P. minor); 2 = lanceolate; broadest in the middle or caudal third (P. vulgaris); 3 = leaf-like, very broad (P. euryptera).

Species without fins were coded as inapplicable for this character.

(30) Dorso- and ventrolateral fins—margin shape: 0 = feathery (P. euryptera); 1 = serrated (P. dolichoptera).

The margins of the fins can be either widely incised, thereby resembling a feather, or serrated. Species without fins were coded as inapplicable for this character.

(31) Dorso- and ventrolateral fins—length of the central rib: 0 = not reaching the tip of the fin, a distinct gap is present (P. euryptera); 1 = (almost) reaching the tip of the fin (P. dolichoptera).

All Polyarthra species possess a midrib of variable length on each fin. Species without fins were coded as inapplicable for this character.

(32) Ventral fins—presence: 0 = absent (S. pectinata, Polyarthra remata, Ploesoma hudsoni); 1 = present (P. vulgaris).

This character refers to the absolute presence versus absence of two short ventral fins that are situated between the ventrolateral fins in several Polyarthra species (e.g., P. vulgaris). This character is specific to some species of Polyarthra and was coded as missing for species of Ploesoma and Synchaeta.

(33) Ventral fins—length in relation to the main fins: 0 = ca. 1/2 of the main fins (P. indica); 1 = ca. 1/3 to 1/4 of the main fins (P. vulgaris); 2 = ca. 1/4 of the main fins (P. dolichoptera).

The ventral fins vary in length among the species of Polyarthra, but are always shorter than the main fins. Species without ventral fins were coded as inapplicable for this character.

(34) Ventral fins—shape of margin: 0 = smooth (Polyarthra platensis); 1 = serrated (P. dolichoptera).

Species without ventral fins were coded as inapplicable for this character.

(35) Foot—presence: 0 = present (S. pectinata, P. hudsoni); 1 = absent (P. dolichoptera).

Although a foot is usually present in rotifers, it is either partly or entirely reduced in planktonic monogononts (Fontaneto & De Smet, 2015) because it is of little benefit when swimming in the free water column (Allen, 1957). In Polyarthra, a foot is briefly present embryonically, but is entirely reduced before hatching and is absent in adults (Sudzuki, 1955; as cited in Allen, 1957).

(36) Foot—shape: 0 = short, conical (S. oblonga, S. triophthalma); 1 = long, slender (S. longipes); 2 = long, broad (Synchaeta pachypoda, S. johanseni); 3 = tubular and flexible (P. hudsoni).

All species of Polyarthra, which lack feet as adults, were coded as inapplicable for this character.

(37) Foot—origin on the trunk: 0 = caudally (S. pectinata); 1 = ventrally (P. triacanthum).

The foot can originate from the caudal trunk region as in species of Synchaeta or Notomnata or can be displaced ventrally as in Ploesoma (Fontaneto & De Smet, 2015; Wallace et al., 2006). All species of Polyarthra, which lack feet as adults, were coded as inapplicable for this character.

(38) Foot—strongly annulated: 0 = no (S. pectinata); 1 = yes (P. hudsoni).

The foot in all species of Ploesoma appears annulated because of numerous rings along its entire length. All species of Polyarthra, which lack feet as adults, were coded as inapplicable for this character.

(39) Foot—dorsolateral spur: 0 = absent (S. pectinata); 1 = present (Synchaeta hutchingsi).

A dorsolateral spur is present on the foot of S. hutchingsi and Synchaeta neapolitana, species that are otherwise noteworthy for exhibiting a single toe only. However, similar to the spur of S. neapolitana (assumed by Rousselet, 1902; contra Lie-Pettersen, 1905), the “spur” in S. hutchingsi likely represents a second toe turned upwards because a pedal gland terminates in it (pers. obs.). All species of Polyarthra, which lack feet as adults, were coded as inapplicable for this character.
(40) Pedal glands—opening: 0 = in the tips of the toes (S. pectinata); 1 = in the cloacal region? (P. dolichoptera).

Mucus threads for the attachment of eggs or adherence to the substrate are commonly secreted by pedal glands that terminate in the tips of the toes (Koste, 1989; Wallace et al., 2006). In adult specimens of species of Polyarthra, where the foot and toes are absent, the pedal glands are held to be either lacking (Fontaneto & De Smet, 2015) or displaced to the cloacal region (Allen, 1957) where glands are located that are responsible for egg attachment. However, this hypothesis of primary homology to the pedal glands of the remaining rotifers requires additional support through embryological studies of one or more species of Polyarthra (Allen, 1957), including gene expression analyses.

(41) Pedal glands (in the foot)—symmetry: 0 = symmetrical (S. pectinata); 1 = asymmetrical (S. triophthalma).

Paired, symmetrical pedal glands are common for monogonont rotifers possessing a foot (Fontaneto & De Smet, 2015). However, some species within Synchaeta exhibit asymmetrical glands, with one gland being either partially or completely reduced (e.g., S. triophthalma). Species of Polyarthra were coded as inapplicable for this character because true pedal glands in the foot are absent.

(42) Pedal glands (in the foot)—length: 0 = of foot length (S. grandis); 1 = shorter than the foot (S. pectinata); 2 = longer than the foot, extending into the caudal trunk (P. triacanthum).

Species of Polyarthra were coded as inapplicable for this character because true pedal glands in the foot are absent.

(43) Pedal glands (in the foot)—shape: 0 = club-shaped (S. tremula); 1 = tubular (S. longipes); 2 = with distal reservoir (S. oblonga); 3 = tubular and attached proximally to the trunk (S. pachypoda).

This character refers to the morphology of pedal glands. “Tubular” glands are of more or less of equal width along their entire length, whereas “club-shaped” glands are voluminous proximally and decrease gradually in width moving distally. Glands “with distal reservoir” are similar to the latter except that the decrease in width is more abrupt and the distal end widens again to form a reservoir. Tubular glands that are attached proximally to the trunk via thin strands of unknown composition seem to be present exclusively in S. pachypoda. Species of Polyarthra were coded as inapplicable for this character because true pedal glands in the foot are absent.

(44) Toe(s)—presence: 0 = present (S. pectinata); 1 = absent (P. dolichoptera).

As for the foot, toes can be absent or vestigial in plankton rotifers (Fontaneto & De Smet, 2015). Species of both Ploesoma and Synchaeta possess toes (although they are often very small in the latter), whereas they are entirely absent in adult specimens of all species of Polyarthra, which all lack a foot.

(45) Toe(s)—size: 0 = large, longer than 15 µm (P. hudsoni); 1 = small, shorter than 15 µm (S. pectinata).

All species of Polyarthra, which lack feet and toes as adults, were coded as inapplicable for this character.

(46) Toe(s)—symmetry: 0 = symmetrical (S. pectinata); 1 = asymmetrical (S. triophthalma).

Although most rotifers possess paired, symmetrical toes (Wallace et al., 2006), one toe is either partially or completely reduced in some species or apparently modified into a spur as is presumed to be the case for S. Hutchingsi and S. neapolitana. All species of Polyarthra, which lack feet and toes as adults, were coded as inapplicable for this character.

(47) Esophagus—morphology: 0 = long and tubular, narrow along its entire length (S. pectinata); 1 = long and tubular, broad along its entire length (S. baltica); 2 = slender proximally, widened distally to form a proventriculus-like structure (S. oblonga); 3 = very short, stomach reaches the distal part of the mastax (P. vulgaris).

The esophagus is morphologically diverse within Synchaetidae, with four distinct morphological states based on its length and width as well as the possible presence of a proventriculus-like structure distally. Several species possess a long, tubular esophagus that is either narrow (e.g., S. pectinata) or broad (e.g., S. baltica) along its entire length, but always with numerous longitudinal striae. The broad esophagus of state 1 is remarkably tensile and can expand to form a “sack” along its entire length (e.g., S. vorax). Some species within Synchaeta exhibit an esophagus where the distal half widens abruptly to form a highly tensile, sack-like proventriculus-like structure (e.g., S. oblonga). By contrast, species of Polyarthra possess a very short esophagus only such that the stomach reaches the distal mastax region.

(48) Apical receptors—morphology: 0 = two bundles of short sensory cilia (S. tremula, S. pectinata); 1 = continuous row of long cilia (P. dolichoptera); 2 = paired palps with long cilia (P. hudsoni).

This character refers to the morphology of the apical receptors that are located in the center of the apical field between the mouth and the dorsal ciliary fields (see Discussion).

(49) Apical receptors—distance between the bundles of short sensory cilia: 0 = not completely separated from one other (S. vorax); 1 = slightly separated from one other (S. oblonga); 2 = distinctly separated from one other (S. pectinata).

For species where the apical receptors each consist of paired bundles of short sensory cilia, the distance between these two bundles can vary. By contrast, species of both Ploesoma and Polyarthra exhibit apical receptors lacking these paired bundles (see character 48) and were therefore coded as inapplicable for this character.

(50) Apical receptors—elevation of the paired bundles of short sensory cilia: 0 = slightly raised on the central elevation of the apical field (S. oblonga); 1 = strongly raised on the distinct elevation of the apical field, snout-like (S. grandis); 2 = raised on two small distant bulges (S. triophthalma); 3 = raised on two medium, distant “pimples” (S. hyperboreum); 4 = raised on strong, paired elevations (tentacles; S. pectinata); 5 = raised on a single tubular elevation (S. vorax).

Species of both Ploesoma and Polyarthra were coded as inapplicable for this character because they exhibit apical receptors lacking these paired bundles (see character 48).
(51) Dorsolateral sensory receptors on the apical field—morphology: 0 = styles (S. pectinata); 1 = ciliated papillae (P. dolichoptera); 2 = ciliated palps (P. hudsoni).

This character refers to the morphology of the dorsal sensory receptors that are located on the dorsoapical field approximately below the narrow gap between the dorsal and dorsolateral ciliary bands (see Discussion).

(52) Dorsolateral sensory receptors on the apical field—morphology: 0 = styles (S. pectinata); 1 = ciliated palps (P. dolichoptera); 2 = trinominal unciliated palps (P. hudsoni).

This character refers to the morphology of the dorsolateral sensory receptors of the apical field (see Discussion) that are situated near the gap between the dorsolateral and lateral ciliary fields.

(53) Dorsolateral sensory receptors on the apical field—elevation of the dorsolateral styles: 0 = not to slightly elevated (S. tremula); 1 = medium elevation (S. oblonga); 2 = distinctly elevated (S. vorax).

This character refers to the triangularly shaped elevation underneath the dorsolateral styles, the latter of which are specific to Synchaeta. The dorsolateral sensory receptors of all species of Ploesoma and Polyarthra do not consist of styles (see character 52), and all species were therefore coded as inapplicable for this character.

(54) Styles—length: 0 = medium to long (S. pectinata, S. vorax); 1 = weak to short (Synchaeta grimei).

All species without styles (i.e., those of Ploesoma and Polyarthra) were coded as inapplicable for this character.

(55) Dorsolateral sensory receptors on the apical field—length of the ciliated palps: 0 = short (Polyarthra leleki); 1 = medium to long (P. vulgaris).

This character refers to the length of the ciliated palps that are specific to species of Polyarthra (see character 52). All species of both Ploesoma and Synchaeta were therefore coded as inapplicable for this character.

(56) Dorsal antenna—opening: 0 = round (S. oblonga); 1 = slit-shaped (S. pectinata).

A dorsal antenna is found in all rotifers (Fontaneto & De Smet, 2015). Within Synchaetidae, it commonly protrudes through a round opening in the integument, but through a slit-shaped one in S. pectinata and S. grandis.

(57) Dorsal antenna—location: 0 = anterior to the cerebral eye (N. allantois); 1 = posterior to the cerebral eye (S. pectinata).

The dorsal antenna is always located on the dorsal side of the body, but at different points along the longitudinal axis of the body.

(58) Lateral antenna(e)—symmetry: 0 = symmetrical, paired (S. pectinata); 1 = asymmetrical, only one is present (S. triphthalma).

At least one lateral antenna is present in all monogonont rotifers, with the typical state being paired antennae that are situated laterally in the posterior half of the trunk (Fontaneto & De Smet, 2015). Paired, symmetrical lateral antennae are also common within Synchaetidae, although some species within Synchaeta possess only a single lateral antenna.

(59) Lateral antenna(e)—location in relation to the transversal axis: 0 = slightly dorsolateral (N. allantois); 1 = ventrolateral (S. pectinata); 2 = directly lateral (S. tremula).

A slight dorsolateral positioning of the lateral antenna(e) relative to the transversal axis of the body is found in species of the Notommatidae outgroup, whereas a direct lateral or slightly ventrolateral positioning is found in Synchaetidae.

(60) Lateral antenna(e)—location in relation to the longitudinal plane: 0 = posterior third or fourth of the trunk (S. pectinata) or above posterolateral corners of the body (P. remata); 1 = caudalmost trunk region (P. dolichoptera) or near the base of the foot in Synchaeta (S. tremula); 2 = on lateral lobes located caudally to the cloaca (S. grimei only).

This character refers to the location of the lateral antenna(e) in relation to the longitudinal axis of the body.

(61) Lateral antenna(e)—base: 0 = tubular or papillary base (S. oblonga, S. baltica, S. johanseni); 1 = no papilla (S. pectinata).

Each lateral antenna projects through a circular opening in the integument that itself can be protruded to form a tubular or papillary fold encasing the base of the antenna.

(62) V—shaped hypopharynx muscle—presence: 0 = absent (N. allantois); 1 = present (S. pectinata).

The large hypopharynx muscle interconnects the distal end of the fulcrum with the dorsal manubria in species with virgate trophi (Fontaneto & De Smet, 2015). A v-shaped hypopharynx muscle is diagnostic for Synchaetidae (De Smet & Segers, 2003).

(63) Trophi—overall symmetry: 0 = symmetrical (S. pectinata); 1 = asymmetrical (N. codonelae).

Synchaetidae have bilaterally symmetrical trophi, with, at most, only slight asymmetries in the form and/or number of ramus teeth in some species. The trophi in species of Notomma, however, are strongly asymmetrical with the left side being distinctly more robust (Pourriot, 1995).

(64) Rami—overall structure and morphology: 0 = robust, no extensions (N. allantois); 1 = of medium robustness, extensions present, but not as distinct as in species of Synchaeta and Polyarthra (P. hudsoni); 2 = delicate, forming lateral wing-shaped extensions (S. pectinata, P. dolichoptera).

Most rotifers possess rami that are thick and robust (Sørensen & Giribet, 2006), but virgate trophi show a tendency toward broader, dorsally recurved rami that form a dome-like structure (Fontaneto & De Smet, 2015), likely as an adaptation for the pumping function of this trophi type. Nevertheless, the rami of the virgate trophi of species of Notomma remain rather stout and robust, with modifications toward larger and more delicate lamellae being more expressed within Synchaetidae and especially in species of Synchaeta and Polyarthra. Species of Ploesoma possess rami that are intermediate in form between those of species of Notomma and those of species of Synchaeta and Polyarthra.

(65) Rami—teeth: 0 = rami edentulous or with serrated/corrugated margin (S. pectinata—type after Koste (1989)); 1 = one strong tooth at the tip of the ramus, remaining plate serrated
(S. vorax); 2 = one strong tooth at the tip of the ramus, remainder are irregular and blunt (Synchaeta verrucosa, S. pachypoda); 3 = all teeth distinctly incised (S. tremula-type after Koste (1989)) (S. tremula, S. oblonga); 4 = dorsal teeth distinct (see Figure 2f), ventral teeth comb-like (S. triophthalma); 5 = dorsal teeth comb-like, ventral teeth distinct (S. baltica); 6 = one strong tooth distinctly removed from the ramus tip, remaining plate smooth or slightly serrated (P. dolichoptera); 7 = one strong tooth distinctly removed from the ramus tip, remaining teeth distinct but smaller and of differing morphologies from one another (P. indica); 8 = two strong, interdigitating teeth that are distinctly removed from the ramus tip (P. vulgaris); 9 = three sharp teeth at the tip of the ramus, remaining plate smooth (P. africanum); A = asymmetrical, only one side of ramus with small teeth (N. codonella).

The present character identifies the specific morphology of the teeth along the inner margin of the rami. Evidence suggests that what has previously been recognized as the unci in species of Synchaeta are actually parts of the rami (see Results and Discussion).

(66) Rami—sulcus: 0 = absent (Synchaeta gyrina, S. pectinata); 1 = present (S. oblonga, S. triophthalma).

In some species, a deep indentation (sulcus) on the anterior inner side of the rami separates the ramus teeth into two distinct groups.

(67) Rami—posterior ridge (apophyses): 0 = rod-like, without bifurcating or knobbed distal end (S. pectinata, P. leleki, P. hudsoni); 1 = distally bifurcating (P. luminosa); 2 = with distal knob (Polyarthra major).

Within Synchaetidae, the lamellar rami are each reinforced posteriorly by a strong, short ridge that is more or less perpendicular to the proximal end of the fulcrum. Within Polyarthra, these structures are referred to as apophyses and are homologous to the posterior ridges in Synchaeta and Ploesoma (pers. obs.). This character refers to the distal end of these reinforcements, which are either rod-like, bifurcating, or knobbed.

(68) Fulcrum—length in relation to the rami: 0 = as long as or longer (S. triophthalma); 1 = shorter (P. vulgaris).

The fulcrum is strongly elongated in the trophi of the virgate type (Fontaneto & De Smet, 2015; Wallace et al., 2006) and is often either as long as or longer than the rami.

(69) Fulcrum—shape in lateral view: 0 = blade-like and with more or less parallel edges, slender (S. pectinata); 1 = machete-like to semicircular (S. oblonga, S. grimpei); 2 = abruptly broadens distally, axe-like (P. leleki); 3 = slightly broadens distally (P. euryptera); 4 = curved, gradually tapering distally to a pointed distal end (N. allantais).

This character identifies the different shapes of the fulcrum in lateral view.

(70) Fulcrum—expansion of the distal end in dorsal view: 0 = none, fulcrum of equal width along its entire length (S. pectinata); 1 = distal expansion present (N. allantais).

The distal end of the fulcrum is broadened in species of Notommata when viewed dorsally. By contrast, it is of equal width along its entire length in members of Synchaetidae.

(71) Fulcrum—dorsal part thickened along its entire length: 0 = absent (S. pectinata); 1 = present (S. grimpei).

In some species, the fulcrum is distinctly thickened dorsally and also possesses a thinner ventral lamella that is attached along its entire length. Together, these structures reveal a “T”-shaped fulcrum in cross-section.

(72) Fulcrum—shape of the distal margin in lateral view: 0 = oblique (S. oblonga); 1 = rounded (S. pectinata).

Within Synchaeta, an oblique distal end of the fulcrum is mainly found in those species where the fulcrum is machete-shaped or semicircular (see character 69) and the ventral side joins the straight dorsal side at an acute angle. However, a fulcrum with parallel edges, such as that found in S. pectinata, commonly possesses a rounded distal end.

(73) Manubrium—extension of the clava: 0 = very small (P. hudsoni); 1 = medium (P. dolichoptera); 2 = very extensive (S. pectinata).

Manubria of Ploima usually consist of an expanded clava and a distal elongated shaft called the cauda (Fontaneto & De Smet, 2015; Wallace et al., 2006). The present character refers to the extension of the clava, which itself is composed of three manubrial chambers. In Synchaetidae, these chambers are modified into thin lamellae, a feature that likely constitutes a modification of the otherwise more robust manubria of the remaining rotifers (Sørensen, 2002). Whereas Ploesoma exhibit robust manubria, the clava lamellae are larger and more delicate in Polyarthra and the most expanded in Synchaeta, where they function as the lateral walls of the trophi cavity.

(74) Manubrium—lateral triangular protrusion: 0 = absent (S. pectinata, P. dolichoptera); 1 = present (P. hudsoni).

The clava protrudes laterally in a triangular form in species of Ploesoma species, a character state that is absent in the remaining taxa examined in the present study.

(75) Manubrium—location of the cauda: 0 = lateral within the trophi (P. dolichoptera, P. hudsoni); 1 = dorsolateral within the trophi (S. pectinata).

The cauda, which reinforces the lamellar clava, is commonly located laterally within the trophus. In species of Synchaeta, however, the cauda is pushed dorsally because of the highly enlarged clava lamellae that are specific for this genus.

(76) Manubrium—thickness of the cauda excess: 0 = normal, robust (S. pectinata, S. longipes); 1 = very thin, slender (S. triophthalma).

In contrast to many other species of rotifer, the cauda excess is very thin and slender in some species of Synchaeta.

(77) Uncus—number of teeth: 0 = one or two distinct teeth (P. triacanthum, P. dolichoptera, “frontal hook” in Synchaeta); 1 = more than two distinct teeth (N. codonella).

The “frontal hook” described in various species of Synchaeta likely represents the uncus (see Results and Discussion).

(78) Uncus—uncus tooth with a spine: 0 = absent (S. pectinata); 1 = present (S. tremula).

A small ventral spine can be found on the uncus tooth in some species of Synchaeta.
APPENDIX 2

Summary of available data for each species

Morphological data were available from the literature for all species in the table. Species listed in bold were also collected locally (OL, Oldenburg; TL, Tecklenburger Land; WHV, Wilhelmshaven) and morphologically re-examined in this study. For the molecular data, GenBank accession numbers in bold or that are in italics represent sequence data that were generated in this study or in Wilke et al. (2017, 2018b), respectively. Morph: Number of specimens examined morphologically.

| Species                        | Sampling locality and date | Morph | 18S     | COI                      |
|-------------------------------|----------------------------|-------|---------|-------------------------|
| Synchaeta arcifera Xu, 1998   | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta atlantica Zelinka, 1907 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta bacillifera Smirnov, 1933 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta baltica Ehrenberg, 1834 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta bicornis Smith, 1904  | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta cecilia Rousselet, 1902 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta cylindrica Althaus, 1957 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta fennica Rousselet, 1909 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta glacialis Smirnov, 1932 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta grandis Zacharias, 1893 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta greversi Remane, 1929  | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta gyrina Hood, 1887     | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta hutchingsi Brownell, 1887 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta hyperborea Smirnov, 1932 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta johanseni Harring, 1921 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta kitina Rousselet, 1902  | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta lakowitziana Lucks, 1930 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta longipes Gosse, 1887   | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta neapolitana Rousselet, 1902 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta oblonga Ehrenberg, 1832 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta pachypoda Jaschnow, 1922 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta pectinata Ehrenberg, 1832 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta pectinata Ehrenberg, 1832 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta pectinata Ehrenberg, 1832 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta prominula Kutikova & Vassiljeva, 1982 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta rousseleti Zelinka, 1927  | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta squamadigitata De Smet, 2006 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta stylata Wierzejski, 1893 | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta tamara Smirnov, 1932   | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |
| Synchaeta tavina Hood, 1893      | WHV, Germany Jan. & Apr. 2016 ca. 35 | MK896813–MK896814 | MK905843–MK905848 |

(Continues)
| Species | Sampling locality and date | Morph | 18S | COI |
|---------|---------------------------|-------|-----|-----|
| **Synchaeta tremula** (Müller, 1786) | OL, Germany Mar. to May 2016 | ca. 90 | KY751511–KY751522 | KY751495–KY751510; EU499817–EU499906 |
| **Synchaeta tremuloida** Pourriot, 1965 | OL, Germany Nov. to Jan. 2015 & 2016 | ca. 70 | KY751523–KY751538 | KY751476–KY751494 |
| **Synchaeta triophthalma** Lauterborn, 1894 | WHV, Germany Apr. 2016 | ca. 25 | MK896828–MK896829 | MK905794–MK905803 |
| **Synchaeta verrucosa** Nipkow, 1961 | — | — | — | — |
| **Synchaeta vorax** Rousselet, 1902 | WHV, Germany Apr. 2016 | 17 | MK896832–MK896835 | MK905832–MK905842 |
| **Polyarthra** | | | | |
| **Polyarthra dolichoptera** Idelson, 1925 | OL, Germany Feb. 2016 | 19 | — | JN936494–JN936510; KC618866–KC619279; KJ460384–KJ460500 |
| **Polyarthra euryptera** Wierzejski, 1891 | OL, Germany Jun. 2017 | 3 | — | MK905769–MK905770 |
| **Polyarthra indica** Segers & Babu, 1999 | — | — | — | — |
| **Polyarthra ileli Koste & Tobias, 1989** | — | — | — | — |
| **Polyarthra longiremis** Carlin, 1943 | — | — | — | — |
| **Polyarthra luminosa** Kutikova, 1962 | — | — | — | — |
| **Polyarthra major** Burchhardt, 1900 | — | — | “cf” major: MK896806 | “cf” major: MK905771 |
| **Polyarthra minor** Voigt, 1904 | — | — | — | — |
| **Polyarthra platensis** José de Paggi & Paggi, 2011 | — | — | — | — |
| **Polyarthra remata** Skorikov, 1896 | — | — | DQ297716 | DQ297789: EU499859–EU499866 |
| **Polyarthra vulgaris** Carlin, 1943 | OL, Germany Jun. 2017 | 14 | MK896807–MK896808 | MK905772–MK905776; KJ460378–KJ460383; KY749517–KY749532 |
| **Ploesoma** | | | | |
| **Ploesoma africanum** Wulfert, 1965 | — | — | — | — |
| **Ploesoma asiaticum** Trinh Dang, Segers, & Sanoamuang, 2013 | — | — | — | — |
| **Ploesoma hudsoni** (Imhof, 1891) | OL, Germany Aug. 2017 | 7 | MK896809–MK896810; DQ297714 | MK905777; DQ297787 |
| **Ploesoma lenticulare** Herrick, 1885 | — | — | — | — |
| **Ploesoma murrayi** Wulfert, 1961 | — | — | — | — |
| **Ploesoma peipsiense** Mäemets & Kutikova, 1979 | — | — | — | — |
| **Ploesoma triacanthum** (Bergendal, 1892) | TL, Germany Apr. 2017 | 11 | MK896811–MK896812 | MK905778–MK905782 |
| **Ploesoma truncatum** (Levander, 1894) | — | — | DQ297715 | DQ297788 |
| **Notommata** | | | | |
| **Notommata allantois** Wulfert, 1935 | — | — | DQ297710 | DQ297784 |
| **Notommata codonella** Harring & Myers, 1924 | — | — | DQ297711 | DQ297785 |
### APPENDIX 3
Character matrix of 78 morphological characters for all 54 species of Synchaetidae plus two species of Notommata (outgroup)

| Species               | 1s | 10s | 20s | 30s | 40s | 50s | 60s | 70s |
|-----------------------|----|-----|-----|-----|-----|-----|-----|-----|
| Synchaeta arcifera   | 1??0???00 | ---| 1100?0? | 1?0----- | -0--00000 | 01?010?022 | 00?0-01021 | 010241?000 | 01201100 |
| Synchaeta atlantica  | 1???0?000 | ---| 1100000 | 0?30----- | -0--00000 | 02?010???? | 0001-????? | ?102307010 | 10201000 |
| Synchaeta bacillifera | 1???1110 | ---| 1101110 | 1?0----- | -0--020000 | 01?020???? | 0001-????? | ?102317000 | 0120101 |
| Synchaeta baltica    | 120-111010 | ---| 1110110 | 0110----- | -0--020000 | 0120101001 | 0020-01010 | 010250000 | 01201001 |
| Synchaeta bicomis    | 1???11?10 | ---| 1111010 | 1110----- | -0--00000 | 002070?011 | 0020-01???? | ?102317000 | 0120101 |
| Synchaeta cecilia    | 1???111?00 | ---| 1100000 | 0?30----- | -0--00000 | 1000112022 | 00?0-01021 | 110247000 | 012011???? |
| Synchaeta cylindrica | 1??0???00 | ---| 1100000 | 0?30----- | -0--00000 | 02010???? | 0000-01????? | ?1023?710 | 0020100? |
| Synchaeta fennica     | 1???011?00 | ---| 1100000 | 0?30----- | -0--00000 | 0010100005 | 0070-01010 | 110210?010 | 1020101 |
| Synchaeta grandis    | 120-010-110 | ---| 1110110 | 0000----- | 0-010000 | 0010100001 | 0011-11010 | 110200000 | 00201000 |
| Synchaeta grimpei    | 120-11010 | ---| 1100000 | 0110----- | -0--020000 | 0120102010 | 0001-01022 | 110200000 | 01201001 |
| Synchaeta gyrina     | 12110010 | ---| 1100000 | 0?30----- | -0--00000 | 0120102010 | 0000-01010 | 010230001 | 0020301 |
| Synchaeta hutchingsi | 120-011000 | ---| 1100000 | 0110----- | -0--00000 | 1000112022 | 0010-01121 | 110240000 | 01201100 |
| Synchaeta hyperborea | 1200????10 | ---| 1100000 | 0?30----- | -0--00000 | 0020102023 | 0001-01010 | 110230?010 | 10201001 |
| Synchaeta johanseni  | 12?????100 | ---| 1110110 | 0110----- | -0--020000 | 0120101005 | 0020-01010 | 010251??? | ??????? |
| Synchaeta kitina     | 0110010000 | ---| 1100000 | 0110----- | -0--00000 | 0000100023 | 0010-01021 | 010247000 | 01201001 |
| Synchaeta lako witziana | 0????1000 | ---| 1110000 | 0?30----- | -0--020000 | 0120102010 | 0011-01010 | ?10270010 | ??201301 |
| Synchaeta longipes   | 020-10-700 | ---| 1110100 | 0000----- | -0--01000 | 0010100001 | 0020-01010 | 110200000 | ?0201000 |
| Synchaeta neapolitana | 1???1???00 | ---| 1100000 | 0?30----- | -0--00000 | 1000112022 | 0010-01070 | 110247000 | 01201001 |
| Synchaeta oblonga    | 02110010 | ---| 1100000 | 0000----- | -0--01000 | 0120102010 | 0010-01010 | 010231000 | 00201001 |
| Synchaeta pachyproda | 0??????010 | ---| 1100000 | 0?30----- | -0--020?0 | 0130002???? | 0071-01070 | 110220?010 | ?10201000 |
| Synchaeta pachypondia | 0??????010 | ---| 1100000 | 0?30----- | -0--020?0 | 0130002???? | 0071-01070 | 110220?010 | ?10201000 |
| Synchaeta pectinata  | 020-10-110 | ---| 1110110 | 0000----- | -0--00000 | 0110100024 | 00?0-11010 | 110200000 | 01201000 |
| Synchaeta prominula  | 0??????010 | ---| 1100000 | 0?30----- | -0--00000 | 0700102010 | 0000-01021 | 11023??00 | ??2010?? |
| Synchaeta rousseletti | 1???1???00 | ---| 1100000 | 0?30----- | -0--00000 | 0000102010 | 0000-01021 | 11023??00 | ??2010?? |
| Synchaeta squamadigitata | 1??????00 | ---| 1110000 | 0?30----- | -0--00000 | 002010?02 | 0001-01070 | ?102300010 | 10201001 |
| Synchaeta stylata    | 020-10-110 | ---| 1110110 | 0000----- | -0--01000 | 0010100001 | 0020-01010 | 110200000 | 00201000 |
| Synchaeta tamara     | 12?????100 | ---| 1100000 | 0?30----- | -0--00000 | 110011010 | 0001-01???? | ?102?0010 | 1020101 |
| Synchaeta tavina     | 1211?????00 | ---| 1100000 | 0?30----- | -0--00000 | 0020102010 | 0000-01010 | 11023???10 | ?0201?? |
| Synchaeta tremula    | 0110010010 | ---| 1100000 | 0000----- | -0--00000 | 0000102010 | 0000-01021 | 110231000 | 01201001 |
| Synchaeta tremuloida | 0110010010 | ---| 1100000 | 0000----- | -0--00000 | 0000102010 | 0000-01021 | 110231000 | 01201001 |

(Continues)
| Species                     | 1s     | 10s    | 20s   | 30s   | 40s   | 50s   | 60s   | 70s   |
|----------------------------|--------|--------|-------|-------|-------|-------|-------|-------|
| Synchaeta triophthalma     | 120–011010 | 10110000000 | 1001112022 | 102410000 | 01201100 |
| Synchaeta verrucosa        | 0??????10 | 0?10000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Synchaeta vorax            | 120–011010 | 011100001 | 1121100001 | 11–010261 | 110200010 |
| Polyarthra dolichoptera    | 020–011001 | 10110000000 | 1001112022 | 102410000 | 01201100 |
| Polyarthra euryptera       | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Polyarthra indica          | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Polyarthra platensis       | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Polyarthra lelekhi         | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Polyarthra longiremis      | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Polyarthra luminosa        | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Polyarthra major           | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Polyarthra minor           | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Polyarthra remata          | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Polyarthra vulgaris        | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Ploesoma hudsoni           | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Ploesoma asiaticum         | 0???????12 | 0?10000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Ploesoma pelipiense        | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Ploesoma trisacanthum      | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Ploesoma truncatum         | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Ploesoma lenticulare       | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Ploesoma africanus         | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Ploesoma murrayi           | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Notommata codonella        | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
| Notommata allantois        | 020–011001 | 00110000000 | 0120102?? | 000101010 | 0?2200010 | 1020100 |
### APPENDIX 4

List of species that were included in the molecular analyses with their respective GenBank accession numbers for 18S rRNA and COI

All sequences for a given species that were obtained from GenBank were represented by that sequence that was closest to their overall consensus sequence. By contrast, all novel sequences that we generated in this study (accession number in bold face) or in Wilke et al. (2017, 2018b) (accession number in italics) were retained.

| Species | 18S | COI |
|---------|-----|-----|
| *Synchaeta baltica* Ehrenberg, 1834 |     |     |
| *S. baltica* Specimen 1 | MK896813 | MK905847 |
| *S. baltica* Specimen 2 | — | MK905843 |
| *S. baltica* Specimen 3 | — | MK905844 |
| *S. baltica* Specimen 4 | — | MK905848 |
| *S. baltica* Specimen 5 | MK896814 | MK905846 |
| *S. baltica* Specimen 6 | — | MK905845 |
| *Synchaeta cecilia* Rousselet, 1902 |     |     |
| *S. cecilia* Specimen 1 | — | MK905881 |
| *Synchaeta grandis* Zacharias, 1893 |     |     |
| *S. grandis* | — | JN936521 |
| *S. grandis* Specimen 1 | MK896815 | MK905828 |
| *S. grandis* Specimen 2 | MK896816 | MK905829 |
| *S. grandis* Specimen 3 | MK896817 | MK905830 |
| *S. grandis* Specimen 4 | — | MK905831 |
| *S. grandis* Specimen 5 | — | MK905827 |
| *Synchaeta grimpei* Remane, 1929 |     |     |
| *S. grimpei* Specimen 1 | — | MK905784 |
| *S. grimpei* Specimen 2 | MK896818 | MK905783 |
| *S. grimpei* Specimen 3 | MK896819 | MK905785 |
| *Synchaeta gyrina* Hood, 1887 |     |     |
| *S. gyrina* Specimen 1 | — | MK905806 |
| *S. gyrina* Specimen 2 | — | MK905807 |
| *S. gyrina* Specimen 3 | — | MK905805 |
| *S. gyrina* Specimen 4 | — | MK905808 |
| *S. gyrina* Specimen 5 | MK896820 | MK905810 |
| *S. gyrina* Specimen 6 | MK896821 | MK905811 |
| *S. gyrina* Specimen 7 | — | MK905809 |
| *S. gyrina* Specimen 8 | — | MK905804 |
| *Synchaeta hutchingsi* Brownell, 1988 |     |     |
| *S. hutchingsi* Specimen 1 | — | MK905787 |
| *S. hutchingsi* Specimen 2 | MK896836 | MK905786 |
| *S. hutchingsi* Specimen 3 | MK896837 | MK905792 |
| *S. hutchingsi* Specimen 4 | MK896838 | MK905793 |
| *S. hutchingsi* Specimen 5 | — | MK905788 |
| *S. hutchingsi* Specimen 6 | — | MK905789 |
| *S. hutchingsi* Specimen 7 | — | MK905790 |
| *S. hutchingsi* Specimen 8 | — | MK905791 |

(Continues)
### APPENDIX 4 (Continued)

| Species               | 18S     | COI      |
|-----------------------|---------|----------|
| **Synchaeta kitina**  |         |          |
| S. kitina             | —       | JN936601 |
| S. kitina specimen 1  | —       | MK905871 |
| S. kitina specimen 2  | —       | MK905879 |
| S. kitina specimen 3  | —       | MK905880 |
| S. kitina specimen 4  | —       | MK905872 |
| S. kitina specimen 5  | —       | MK905873 |
| S. kitina specimen 6  | —       | MK905874 |
| S. kitina specimen 7  | —       | MK905877 |
| S. kitina specimen 8  | —       | MK905878 |
| S. kitina specimen 9  | —       | MK905869 |
| S. kitina specimen 10 | —       | MK905870 |
| S. kitina specimen 11 | MK896830| MK905867 |
| S. kitina specimen 12 | MK896831| MK905868 |
| S. kitina specimen 13 | —       | MK905875 |
| S. kitina specimen 14 | —       | MK905876 |
| **Synchaeta lakowitziana** |   |          |
| S. lakowitziana       | —       | JN936538 |
| **Synchaeta oblonga** |         |          |
| S. oblonga            |         |          |
| S. oblonga specimen 1 | —       | MH481739 |
| S. oblonga specimen 2 | —       | MH481738 |
| S. oblonga specimen 3 | —       | MH481740 |
| S. oblonga specimen 4 | MH481723| MH481728 |
| S. oblonga specimen 5 | MH481724| MH481729 |
| S. oblonga specimen 6 | —       | MH481730 |
| S. oblonga specimen 7 | MH481725| MH481732 |
| S. oblonga specimen 8 | MH481726| MH481733 |
| S. oblonga specimen 9 | —       | MH481731 |
| S. oblonga specimen 10| MH481727| MH481734 |
| S. oblonga specimen 11| —       | MH481737 |
| S. oblonga specimen 12| —       | MH481735 |
| S. oblonga specimen 13| —       | MH481736 |
| **Synchaeta pectinata** |    |          |
| S. pectinata          | KF561106|         |
| S. pectinata specimen 1| MK896822| MK905851 |
| S. pectinata specimen 2| MK896823| —        |
| S. pectinata specimen 3| —       | MK905850 |
| S. pectinata specimen 4| —       | MK905857 |
| S. pectinata specimen 5| —       | MK905849 |
| S. pectinata specimen 6| —       | MK905852 |
| S. pectinata specimen 7| —       | MK905853 |
| S. pectinata specimen 8| —       | MK905854 |
| S. pectinata specimen 9| —       | MK905858 |
| S. pectinata specimen 10| —      | MK905856 |
### APPENDIX 4 (Continued)

| Species              | 18S     | COI     |
|----------------------|---------|---------|
| S. pectinata specimen 11 |         | MK905855|
| S. pectinata specimen 12 |         | MK905859|
| S. pectinata specimen 13 |         | MK905862|
| S. pectinata specimen 14 |         | MK905864|
| S. pectinata specimen 15 |         | MK905860|
| S. pectinata specimen 16 |         | MK905861|
| S. pectinata specimen 17 |         | MK905865|
| S. pectinata specimen 18 |         | MK905866|
| S. pectinata specimen 19 |         | MK905863|
| Synchaeta stylata Wierzejski, 1893 |     |         |
| S. stylata specimen 1 | MK896826 |         |
| S. stylata specimen 2 |         | MK905815|
| S. stylata specimen 3 |         | MK905819|
| S. stylata specimen 4 |         | MK905822|
| S. stylata specimen 5 | MK896827 | MK905818|
| S. stylata specimen 6 |         | MK905812|
| S. stylata specimen 7 |         | MK905821|
| S. stylata specimen 8 |         | MK905814|
| S. stylata specimen 9 |         | MK905823|
| S. stylata specimen 10 |         | MK905813|
| S. stylata specimen 11 |         | MK905820|
| S. stylata specimen 12 | MK896824 | MK905816|
| S. stylata specimen 13 | MK896825 | MK905817|
| S. stylata specimen 14 |         | MK905824|
| S. stylata specimen 15 |         | MK905825|
| S. stylata specimen 16 |         | MK905826|
| Synchaeta tremula (Müller, 1786) |     |         |
| S. tremula |         | EU499825|
| S. tremula specimen 1 | KY751514 | KY751495|
| S. tremula specimen 2 | KY751520 | KY751502|
| S. tremula specimen 3 | KY751521 | KY751503|
| S. tremula specimen 4 | KY751522 | KY751504|
| S. tremula specimen 5 | KY751515 |         |
| S. tremula specimen 6 |         | KY751496|
| S. tremula specimen 7 | KY751516 | KY751497|
| S. tremula specimen 8 | KY751517 | KY751498|
| S. tremula specimen 9 | KY751518 | KY751499|
| S. tremula specimen 10 | KY751519 | KY751500|
| S. tremula specimen 11 |         | KY751501|
| S. tremula specimen 12 |         | KY751505|
| S. tremula specimen 13 |         | KY751509|
| S. tremula specimen 14 | KY751511 | KY751510|
| S. tremula specimen 15 |         | KY751506|
| S. tremula specimen 16 | KY751512 | KY751507|
| S. tremula specimen 17 | KY751513 | KY751508|

(Continues)
### Synchaeta tremuloida Pourriot, 1965

| Species                  | 18S   | COI    |
|--------------------------|-------|--------|
| S. tremuloida specimen 1 | —     | KY751476 |
| S. tremuloida specimen 2 | KY751538 | KY751479 |
| S. tremuloida specimen 3 | KY751536 | KY751480 |
| S. tremuloida specimen 4 | KY751537 | KY751481 |
| S. tremuloida specimen 5 | —     | KY751482 |
| S. tremuloida specimen 6 | KY751535 | KY751477 |
| S. tremuloida specimen 7 | KY751529 | KY751489 |
| S. tremuloida specimen 8 | KY751530 | KY751490 |
| S. tremuloida specimen 9 | KY751531 | KY751491 |
| S. tremuloida specimen 10| KY751523 | KY751483 |
| S. tremuloida specimen 11| KY751524 | KY751484 |
| S. tremuloida specimen 12| KY751525 | KY751485 |
| S. tremuloida specimen 13| KY751526 | KY751486 |
| S. tremuloida specimen 14| KY751527 | KY751487 |
| S. tremuloida specimen 15| KY751528 | KY751488 |
| S. tremuloida specimen 16| KY751532 | KY751492 |
| S. tremuloida specimen 17| KY751533 | KY751493 |
| S. tremuloida specimen 18| KY751534 | KY751494 |

### Synchaeta triophthalma Lauterborn, 1894

| Species                  | 18S   | COI    |
|--------------------------|-------|--------|
| S. triophthalma specimen 1| MK896828 | MK905800 |
| S. triophthalma specimen 2| —     | MK905795 |
| S. triophthalma specimen 3| —     | MK905803 |
| S. triophthalma specimen 4| —     | MK905794 |
| S. triophthalma specimen 5| MK896829 | MK905801 |
| S. triophthalma specimen 6| —     | MK905798 |
| S. triophthalma specimen 7| —     | MK905799 |
| S. triophthalma specimen 8| —     | MK905802 |
| S. triophthalma specimen 9| —     | MK905797 |
| S. triophthalma specimen 10| —    | MK905796 |

### Synchaeta vorax Rousselet, 1902

| Species                  | 18S   | COI    |
|--------------------------|-------|--------|
| S. vorax specimen 1      | MK896835 | —     |
| S. vorax specimen 2      | MK896833 | MK905838 |
| S. vorax specimen 3      | —     | MK905841 |
| S. vorax specimen 4      | —     | MK905834 |
| S. vorax specimen 5      | MK896832 | MK905837 |
| S. vorax specimen 6      | MK896834 | MK905839 |
| S. vorax specimen 7      | —     | MK905832 |
| S. vorax specimen 8      | —     | MK905833 |
| S. vorax specimen 9      | —     | MK905842 |
| S. vorax specimen 10     | —     | MK905840 |
| S. vorax specimen 11     | —     | MK905835 |
| S. vorax specimen 12     | —     | MK905836 |

### Polyarthra dolichoptera Idelson, 1925

| Species                  | 18S   | COI    |
|--------------------------|-------|--------|
| P. dolichoptera          | —     | JN936506 |

(Continues)
### APPENDIX 5

**Molecular data included in the combined phylogenetic analysis with morphological data**

For each molecular data set, the sequence for a given species was represented by that sequence that was closest to the consensus sequence of all sequence from that species, with a preference given to sequences generated in this study or those of Wilke et al. (2017), Wilke et al. (2018).

| Species                                      | 18S   | COI   |
|----------------------------------------------|-------|-------|
| *Synchaeta arcifera* Xu, 1998                | —     | —     |
| *Synchaeta atlantica* Zelinka, 1907          | —     | —     |
| *Synchaeta bacillifera* Smirnov, 1933        | —     | —     |
| *Synchaeta baltica* Ehrenberg, 1834          | MK896813 | MK905843 |
| Species                           | 18S   | COI    |
|----------------------------------|-------|--------|
| Synchaeta bicornis Smith, 1904   |       |        |
| Synchaeta cecilia Rousselet, 1902|       |        |
| Synchaeta cylindrica Althaus, 1957|       |        |
| Synchaeta fennica Rousselet, 1909|       |        |
| Synchaeta glacialis Smirnov, 1932|       |        |
| Synchaeta grandis Zacharias, 1893| MK896817 | MK905831 |
| Synchaeta grimei Remane, 1929    | MK896818 | MK905784 |
| Synchaeta gyrina Hood, 1887      | MK896821 | MK905806 |
| Synchaeta hutchingsi Brownell, 1988| MK896836 | MK905787 |
| Synchaeta hyperborea Smirnov, 1932|       |        |
| Synchaeta johansenii Harring, 1921|       |        |
| Synchaeta kitina Rousselet, 1902 | MK896831 | MK905877 |
| Synchaeta lakowitziana Lucks, 1930|       | JN936538 |
| Synchaeta longipes Gosse, 1887   |       |        |
| Synchaeta neapolitana Rousselet, 1902|       |        |
| Synchaeta oblonga Ehrenberg, 1832|       |        |
| Synchaeta pachypoda Jaschnov, 1922|       |        |
| Synchaeta pachypoda Kutikova & Vassiljeva, 1982|       |        |
| Synchaeta pectinata Ehrenberg, 1832| MK896822 | MK905851 |
| Synchaeta prominula Kutikova & Vassiljeva, 1982|       |        |
| Synchaeta rousseleti Zelinka, 1927|       |        |
| Synchaeta squamadigitata De Smet, 2006|       |        |
| Synchaeta stylata Wierzejski, 1893| MK896827 | MK905824 |
| Synchaeta tamara Smirnov, 1932   |       |        |
| Synchaeta tavina Hood, 1925      |       |        |
| Synchaeta tremula (Müller, 1786) | KY751516 | KY751499 |
| Synchaeta tremuloida Pourriot, 1965| KY751528 | KY751490 |
| Synchaeta triophthalma Lauterborn, 1894| MK896828 | MK905795 |
| Synchaeta verrucosa Nipkow, 1961|       |        |
| Synchaeta vorax Rousselet, 1902  | MK896835 | MK905842 |
| Polyarthra dolichoptera Idelson, 1925|       | JN936506 |
| Polyarthra euryptera Wierzejski, 1891|       | MK905770 |
| Polyarthra indica Segers & Babu, 1999|       |        |
| Polyarthra leleki Koste & Tobias, 1989|       |        |
| Polyarthra longiremis Carlin, 1943|       |        |
| Polyarthra luminosa Kutikova, 1962|       |        |
| Polyarthra major Burckhardt, 1900|       |        |
| Polyarthra minor Voigt, 1904     |       |        |
| Polyarthra platensis José de Paggi & Paggi, 2011|       |        |
| Polyarthra remata Skorikov, 1896 | DQ297716 | DQ297789 |
| Polyarthra vulgaris Carlin, 1943 | MK896808 | MK905775 |
| Ploesoma africanum Wulfert, 1965 |       |        |
| Ploesoma asiaticum Trinh Dang et al., 2013|       |        |
| Ploesoma hudsoni (Imhof, 1891)    | MK896809 | MK905777 |
| Ploesoma lenticulare Herrick, 1885|       |        |

(Continues)
### APPENDIX 5  (Continued)

| Species                                      | 18S    | COI     |
|----------------------------------------------|--------|---------|
| *Ploesoma murrayi* Wulfert, 1961             | —      | —       |
| *Ploesoma peipsiense* Mäemets & Kutikova, 1979 | —      | —       |
| *Ploesoma triacanthum* (Bergendal, 1892)     | MK896811 | MK905782 |
| *Ploesoma truncatum* (Levander, 1894)        | DQ297715 | DQ297788 |
| *Notommata allantois* Wulfert, 1935          | DQ297710 | DQ297784 |
| *Notommata codonella* Harring & Myers, 1924  | DQ297711 | DQ297785 |