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# The Evolution of Collembola Higher Taxa (Arthropoda, Hexapoda) Based on Mitogenome Data [†]

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**Abstract:** Mitogenomes represent useful tools for investigating the phylogeny of many metazoan clades. Regarding Collembola, the use of mitogenomics has already shown promising results, but few published works include sufficient taxon sampling to study its evolution and systematics on a broader scale. Here, we present a phylogenetic study based on the mitogenomes of 124 species from 24 subfamilies, 16 families, and four orders—one of the most comprehensive datasets used in a molecular study of Collembola evolution to date—and compare our results with the trees from recently published papers and traditional systematic hypotheses. Our main analysis supported the validity of the four orders and the clustering of Poduromorpha with Entomobryomorpha (the traditional Arthropleona). Our data also supported the split of Symphypleona *s. str.* into the Appendiciphora and Sminthuridida suborders, and the division of the Neelipleona into two subfamilies: Neelinae and Neelidinae subfam. nov. On the other hand, the traditional Symphypleona *s. lat.*, Isotomoidea, and all the Isotomidae subfamilies were refuted by our analyses, indicating a need for a systematic revision of the latter family. Though our results are endorsed by many traditional and recent systematic findings, we highlight a need for additional mitogenomic data for some key taxa and the inclusion of nuclear markers to resolve some residual problematic relationships.

**Keywords:** mitogenomic phylogeny; Entomobryomorpha; Poduromorpha; Neelipleona; Symphypleona

## 1. Introduction

Springtails (Collembola) are tiny arthropods, with the majority of the species smaller than 5 mm [1]. Because of this limited size, they are unknown to most people. Even so, springtails are among the most widely distributed and abundant extant terrestrial animals [2]. Also, Collembola represents the richest lineage among the early diverging ("apterygotan") hexapod lineages, gathering more species than Protura, Diplura, Archaeognatha, and Zygentoma combined. Compared to other non-Holometabola hexapods, this clade is only smaller in number of species than the Hemiptera and Orthoptera insect orders [3]. Even with more than 9400 described species, many others are yet to be described [2,4]. The evolution and systematics of the Collembola main lineages have been debated in several revisions for more than a century. Many traditional propositions to organize the supraspecific taxa and infer their evolution have been discussed by different authors. Such hypotheses were based on the external morphology of adult specimens, first instar and post-embryonic development patterns, and even internal structures. However, they mostly resulted in discrepant propositions, showing little consensus compared to each other [5–18].

The largest internal groups of springtails, nowadays considered as orders, were also continuously reorganized. They were first suggested as only two main lineages: the Arthropleona and Symphypleona *sensu* Börner, 1901 [5,6], going up to five orders, including the ambiguous Metaxypleona [9,16]. More recently, different studies agreed that there are four main well-delimited lineages: Neelipleona, Symphypleona, Entomobryomorpha, and Poduromorpha [19,20], but even so, some species and genera show so many autapomorphies that their placements question the validity and boundaries of such orders [8,16,21].

With the advent of molecular tools and modern bioinformatics to investigate biological evolution, many traditional and more recent views on the systematics of the Collembola were put to the test. Taxonomic evolution, affinities, boundaries, and validity were investigated at different scales, confirming or refuting previous hypotheses. Starting from a smaller taxonomical scope, intra- and interspecific levels of genetic variation were investigated to provide data on species delimitation, population structuring, and the existence of cryptic species [4,22–33]. Based on similar methodologies, species–groups affinities and validity were also analyzed [34–37]. The placement and relationships of genera within subfamilies, as well as their monophyletic status, were also evaluated using molecular-based approaches [38–41]. Likewise, the limits, affinities, and main diagnostic features of subfamilies, families, and superfamilies were the main subject of many recent studies, especially among the Entomobryomorpha [42–47]. Finally, on a broader scale, the relationships of the current four Collembola orders were also recently investigated, but so far they show very little consensus regarding their affinities [27,28,35,46,48–57].

Mitogenomes are useful tools for studying the phylogeny of many metazoan lineages. They can provide a large set of useful comparative data for arthropod systematics, especially when confronted with single locus markers [55,58–60]. Regarding Collembola, the use of mitogenomics already showed promising results, but most of the published papers were limited to a small sample size and/or focused on a specific higher taxon [41,52,53,55,57,60–64]. The main exception is the recent study of Cucini et al. [56], which provided a wider view of Collembola phylogenetics based on a large dataset of mitogenome sequences, using first and second codon positions in the analyses. In this work, many important observations on the use of mitogenomes for phylogenetic investigation and Collembola gene orders were provided, but the authors opted to discuss their systematic findings in light of recent molecular studies, avoiding in-depth comparisons of their results with traditional hypotheses or looking for morphological evidence to support their results. Additionally, some incongruence between traditional systematics and the obtained results was observed, like the finding of polyphyletic Tomoceridae, Neelidae, and Entomobryomorpha.

In the current study, we aimed to provide a large-scale investigation of the main clades within Collembola based on mitogenomes, using a broad set of species, genera, subfamilies, and families of its four orders. In contrast to Cucini et al. [56], we used amino acid sequences in our analyses, similarly to Sun et al. [53]. Such efforts resulted in one of the most representative phylogenies for the entire class so far, facilitating our ability to investigate the affinities of higher taxa and, at the same time, confirming previously published hypotheses concerning smaller clades. Additionally, we provided a comprehensive review of the main previous systematic hypotheses based on morphology and molecular data and how they compare with our results.

## 2. Materials and Methods

### 2.1. Species and Mitogenomic Data Acquisition

We surveyed the NCBI database in May 2022 and retrieved mitogenomic data for 123 collembolan, two dipluran, and two proturan species for analyses. Our dataset gathers data published in 38 studies, with 12 sequences never used in mitogenomic phylogenetic studies before (*Ascocyrtus cinctus* Schäffer, *Desoria tigrina* (Tullberg), *Lepidosira calolepsis* (Börner), *Plutomurus gul* (Yosii), *Pogonognathelus flavensis* (Tullberg), *Pogonognathelus longicornis* (Müller), *Pseudobourletiella spinata* (MacGillivray), *Seira boneti* Denis, *Sminthurides aquaticus* (Bourlet), *Tomocerus maximus* (Liu, Hou and Li), *Tomocerus nigrus* Sun, Liang and Huang,

and *Tomocerus vulgaris* (Tullberg)). Only one species newly sequenced was included in our dataset: *Seira boneti*. The paper with its mitogenome announcement and description is under preparation.

A few mitogenomes were previously discarded from our dataset due to the absence of some coding genes or incorrect phylogenomic placement (possible identification errors). Only mitochondrial protein coding genes were used for the analyses. In total, the ingroup included 124 taxa of Collembola: three species representing the Neelipleona, nine Symphypleona, 15 Poduromorpha, and 97 Entomobryomorpha, totaling 24 sampled subfamilies and 16 families. Four outgroups, two species of Protura and two of Diplura, were chosen based on molecular studies focused on basal hexapods, which supported a closer relationship between these three groups [49,50,65–67].

The detailed classification information and voucher numbers of the 128 species analyzed in this study are listed in Table S1.

*2.2. Phylogenetic Analyses*

Firstly, we organized matrices of nucleotide data (with and without the third codon) and ran a priori analyses. Nucleotide data were investigated with different alignment and trimming tools/parameters. At the beginning of each run, IQTree performs a composition chi-square test to verify the homogeneity of the character composition in the alignment. This test showed that 87 sequences failed to achieve the optimal value of chi2, suggesting possible inconsistencies with our nucleotide matrices. The resulting ML trees of nucleotide data, with or without the third codon, failed to support Collembola (with the outgroup clustered together with Actaletidae using the partitioned dataset), or to support more than two orders at the same time (with Neelipleona and Entomobryomorpha as polyphyletic taxa in the same topology using the unpartitioned dataset). Because of this, we dismissed nucleotide data from our final analyses.

TransDecoder v5.5.0 [68] was used to translate the nucleotide sequences of the 13 protein-coding genes (PCGs) of each species into amino acids. MAGUS strategy [69], employing MAFFT [70], was used to align each PCG independently. BMGE v1.12 [71] performed the trimming, with the defaults. PhyKIT v1.9.0 [72] was used to concatenate the genes and generate the matrix and partition scheme. Maximum Likelihood (ML) analyses were performed with IQTREE v2.0.7 [73], 1000 ultrafast bootstrap [74], and SH-aLRT replicates. The "edge-linked-proportional partition model with separate substitution models and separate rates across sites" method was used for two ML partitioned analyses: one tree was constructed using ModelFinder [75] to select the best-fitting substitution model for each gene partition (Table S2), and another tree was made with the mixture model (EX-EHO). The resultant tree of the mixture model was used as a guide for the unpartitioned method with the posterior mean site frequency (PMSF) model option "-m mtART + C60 + FO + R" [76]. Additionally, the traditional unpartitioned method was tested with the best model (mtZOA + F + R10) selected by ModelFinder. The Bayesian Inference (BI) tree was reconstructed using Phylobayes-MPI v1.8 [77], unpartitioned dataset, default model CAT+GTR with discrete gamma (four categories). Two chains were run until the likelihood had satisfactorily converged (maxdiff < 0.3). The consensus tree was generated using a burn-in of 1000 trees and subsampling every 10 trees. Maximum Parsimony (MP) analysis was performed using MPBoot v1.1.0 [78], with 1000 bootstrap replicates. All resulting phylogenies were visualized and first edited in FigTree v1.3.1 [79]. Concerning ML and MP analyses, we considered clades with 95–100 of bootstrap as resolved (with high node support), 90–94.9 as acceptable, and lower than 90 as unresolved. For BI analysis, any clade with less than a 0.95 posterior probability was considered as unresolved.

A priori, we considered the different types of analyses, partition schemes, and models as equally capable of solving the phylogeny. To choose the main topology, we defined the following criteria: (1) all four orders should be recovered as monophyletic groups; (2) superfamilies and families with strong morphological evidence supporting their validities should also be recovered, as the Neanuroidea, Entomobryoidea, Sminthuroidea,

Sminthurididae, and Neelidae; (3) since most sampled taxa belong to Entomobryoidea, the higher Entomobryidae (the group with elongate fourth abdominal segment) should also be recovered, following the findings of [43–45,60]; and (4) an overall higher node support for the main relationships, above the subfamily level, should be recovered (only bootstrap for ML analyses [74]). Due to the high number of terminals, we manually reconstructed all trees highlighting the sampled springtails higher taxa (main subfamilies, families, superfamilies, suborders, and orders, and a few genera). To better illustrate these branches, we drew potential representatives of each lineage loosely based on the photograph repository of [1] using CorelDraw 2021, except for *Spinactaletes* Soto-Adames (Actaletidae), which was adapted from [80], and the depictions of the orders, adapted from [81].

*2.3. Tree Topology Tests*

Using the RELL approximation method with 10,000 replicates [74], we performed tree topology tests on the constraining monophyly of some of the suprageneric taxa of Collembola. The following six hypotheses were proposed: (1) best tree without any constraints (Figure 1); (2) Monophyly of Orchesellidae (including Orchesellinae and Heteromurinae as independent taxa) and Paronellidae; (3) Monophyly of Hypogastruridae; (4) (Sminthuridae + Sminthurididae) + Bourletiellidae; (5) Sminthurididae + Sminthuroidea; (6) (Bourletiellidae + Dicyrtomidae) + (Sminthuridae + Sminthurididae). Kishino-Hasegawa (KH), Shimodaira-Hasegawa (SH), weighted KH (WKH) and weighted SH (WSH), expected likelihood weight (ELW), and approximately unbiased (AU) tests were performed in IQTREE.
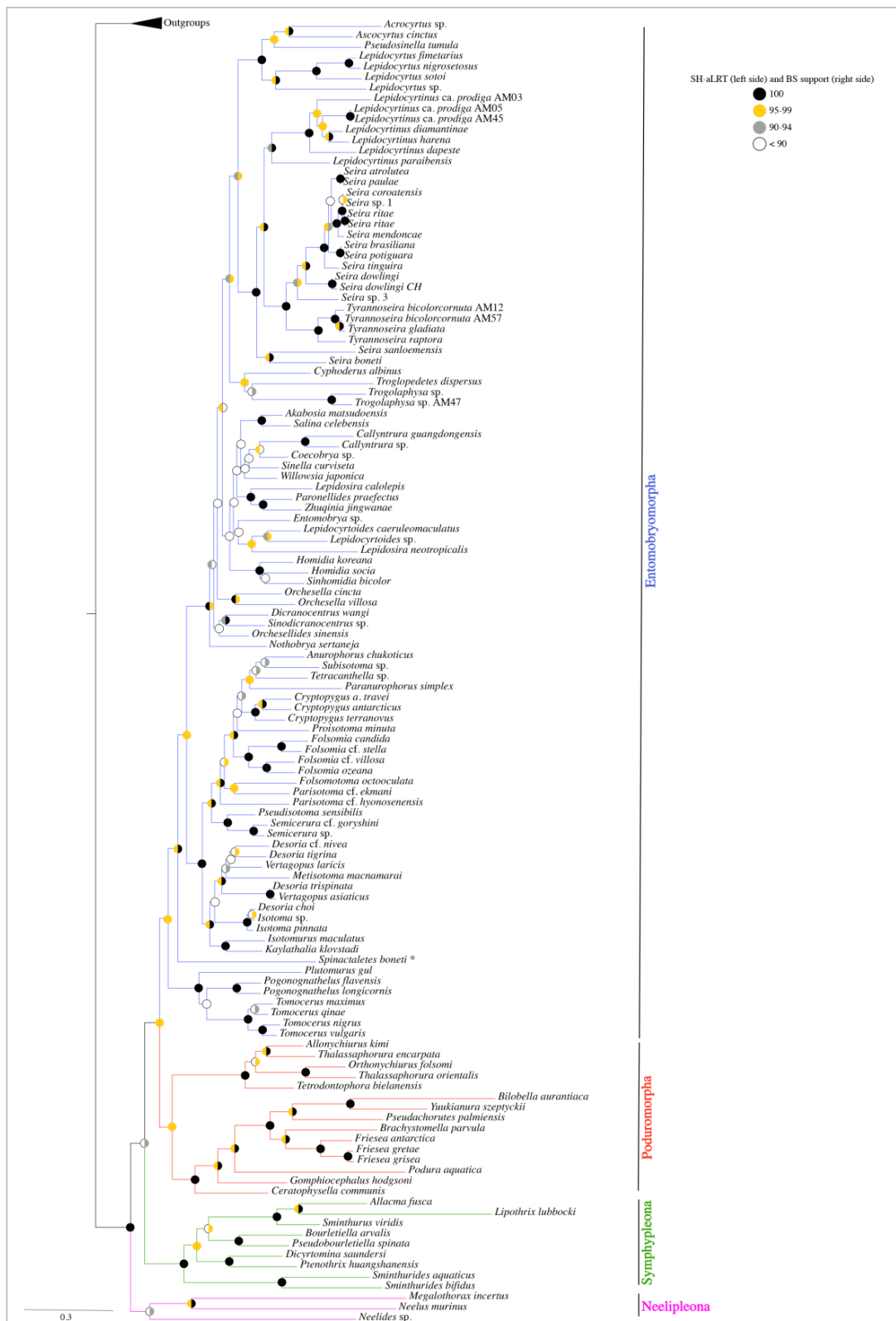
**Figure 1.** Phylogeny of Collembola inferred from Maximum Likelihood unpartitioned dataset substitution model mtZOA + F + R10 (ML_1). Node circles represent SH-aLRT support (left side) and bootstrap values (right side), respectively; colors represent the following scores: black = 100; yellow = 95–99.9; grey = 90–94.9; white < 90. Branches of each Collembola order are represented in different colors.

## 3. Results

### 3.1. Matrices and Trees

The data matrix used for the phylogenetic analyses included 128 taxa and 3003 amino acid sites representing the 13 mitochondrial protein-coding genes. Six trees were generated based on different models or phylogenetic inference methods. Among our analyses, the ML tree, unpartitioned, under the model mtZOA + F + R10 (best model suggested by Model Finder), better represented the currently accepted classifications of the higher taxa of Collembola in topology and overall node support, as discussed ahead. This phylogeny was chosen as our main tree and is represented in Figures 1 and S6 (the latter with detailed node support values). The other five trees are depicted in the Supplementary Materials as Figures S1–S5. A synthesis of the different analyses, parameters, tree codes, and related figures is presented in Table 1.

**Table 1.** Different types of analyses, partition schemes (for ML analyses), models, and respective tree codes used in this study and their respective resulting figures.

| Type of Analysis | Partition Scheme | Models | Tree Code | Figure |
|---|---|---|---|---|
| Maximum Likelihood | Unpartitioned | mtZOA + F + R10 | ML_1 * | Figures 1 and S6 |
| Maximum Likelihood | Partitioned | EX-EHO | ML_2 | Figure S1 |
| Maximum Likelihood | Unpartitioned | mtART + C60 + FO + R | ML_3 | Figure S2 |
| Maximum Likelihood | Partitioned | Table S2 | ML_4 | Figure S3 |
| Bayesian Inference | - | CAT + GTR | BI | Figure S4 |
| Maximum Parsimony | - | - | MP | Figure S5 |

Legends: '*' = main phylogeny of this study; '-' = not applicable.

### 3.2. Phylogeny of the Orders

The ordinal relationships of all obtained trees are summarized in Figure 2. Our main phylogeny recovered the four Collembola orders as monophyletic taxa, with the following topology: Neelipleona + (Symphypleona + (Entomobryomorpha + Poduromorpha)) (Figures 1 and 2A). Two other ML trees also reached this same topology concerning the orders (ML_2 and ML_4), while ML_3 resolved (Neelipleona + Symphypleona) + (Entomobryomorpha + Poduromorpha) (Figures 2A,B and S1–S3). Our BI and MP trees did not recover the monophyly of all orders, with Neelipleona as an ingroup of Poduromorpha in the former, and Actaletidae outside of Entomobryomorpha in the latter (Figures 2C,D, S4 and S5).

Entomobryomorpha + Poduromorpha, the traditional Arthropleona grouping, was strongly supported by our main phylogeny. The bootstrap support for Symphypleona as its sister group was lower (91), slightly below of a 95 optimal threshold, while the SH-aLRT support for this relationship was only 58.1. On the other hand, the Neelipleona appeared as the most basal order of Collembola, with absolute node support (Figures 1, 2A and S6). Similar results were obtained for ML_4 (Figure S3), while ML_2 reached the same order topology but with higher support for the Arthropleona + Symphypleona clade (Figure S1). The ML_3 tree also recovered the Arthropleona with bootstrap support = 95 and SH-aLRT = 89.7, while the clade Symphypleona + Neelipleona was recovered with very low support levels (Figure S2). The Bayesian Inference tree found a very low posterior probability value for Neelipleona as an ingroup of Poduromorpha (Figure S4), while the Entomobryomorpha lacking Actaletidae was recovered with a bootstrap value of only 59 in the MP analysis (Figure S5).

Our main tree supported all orders with bootstrap values above 95, except for Neelipleona, with 94 (Figures 1 and S6). The Maximum Likelihood analyses using other models achieved a higher node support for Neelipleona (Figures S1–S3), while BI and MP reached a posterior probability value of 0.63, and parsimony bootstrap of 80, respectively (Figures S4 and S5).
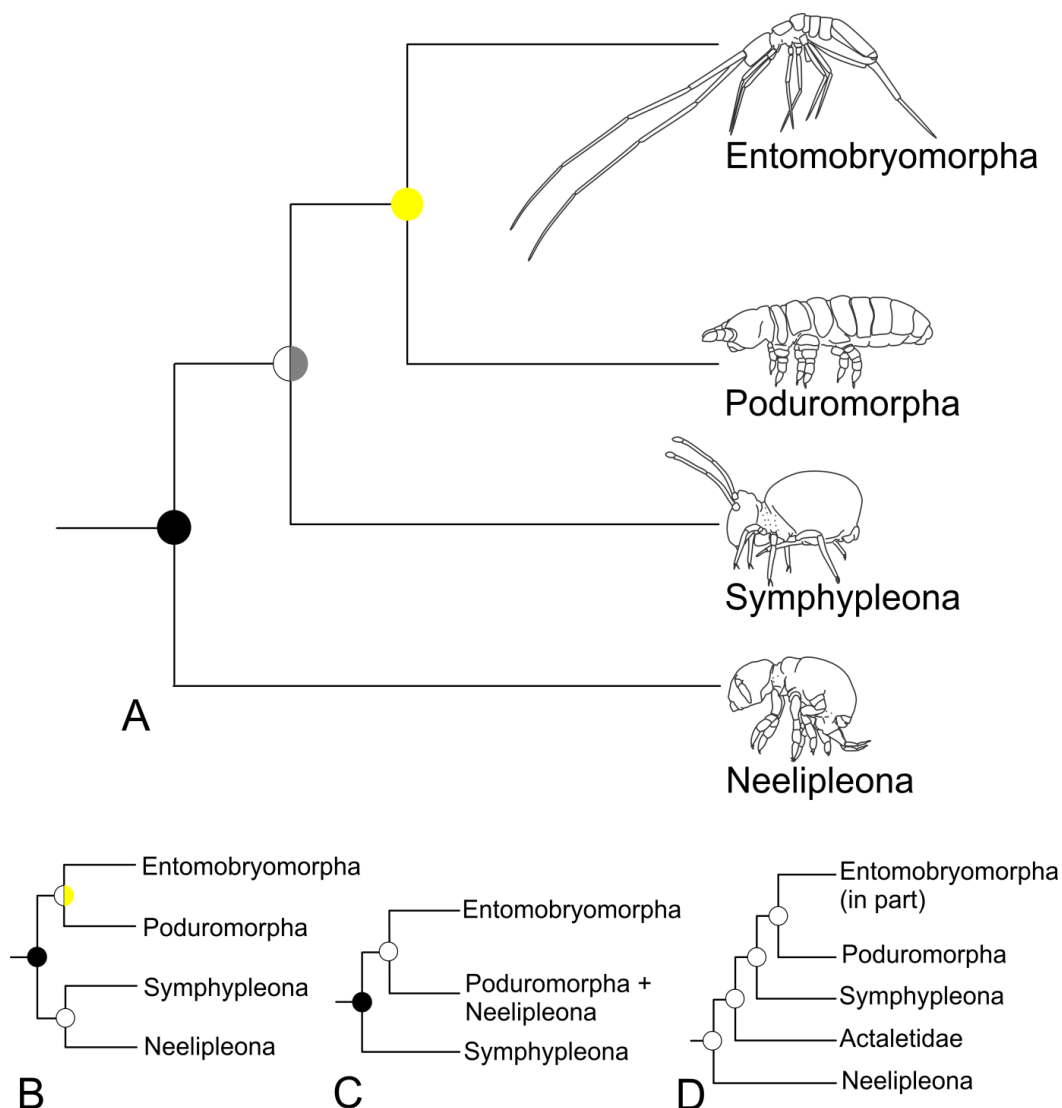
**Figure 2.** Summarized phylogenetic relationships of the Collembola orders obtained in this study: (**A**) topology of ML_1, ML_2 and ML_4 trees (nodes depict only ML_1 support levels); (**B**) topology of ML_3 tree; (**C**) topology of BI tree; (**D**) topology of MP tree. Node circles represent SH-aLRT support (left side) and bootstrap values (right side), respectively, for ML trees, posterior probability for BI tree, and maximum parsimony bootstrap for MP tree. Colors represent the following scores: black = 100 (1 for BI); yellow = 95–99.9 (0.95–0.99 for BI); grey = 90–94.9 (0.9–0.94 for BI); white < 90 (<0.9 for BI).

*3.3. Entomobryomorpha*

The phylogenies of the Entomobryomorpha higher taxa are summarized in Figure 3. Our main tree supported the topology Tomoceridae + (Actaletidae + (Isotomidae + Entomobryoidea)), as well as our BI analysis (Figures 1, 3A and S4). Similar trees were obtained by other ML models, but with different internal organizations for the Entomobryoidea (Figures 3B,D and S1–S3), while the MP tree recovered the Entomobryomorpha without Actaletidae (Figures 3E and S5). All trees supported Tomoceridae as the most basal branch of Entomobryomorpha, and did not support the Isotomoidea *sensu* Soto-Adames et al. [18], clustering Isotomidae and Actaletidae, and all subfamilies of Isotomidae, with Pachyotominae (*Paranurophorus* Denis), Proisotominae (*Proisotoma* Börner and *Subsitoma* Stach), Anurophorinae (*Anurophorus* Nicolet, *Cryptopygus* Willem, *Folsomia* Willem, and *Tetracanthella* Schött) mixed and as ingroups of Isotominae (the other isotomid genera) (Figures 1 and S1–S5).
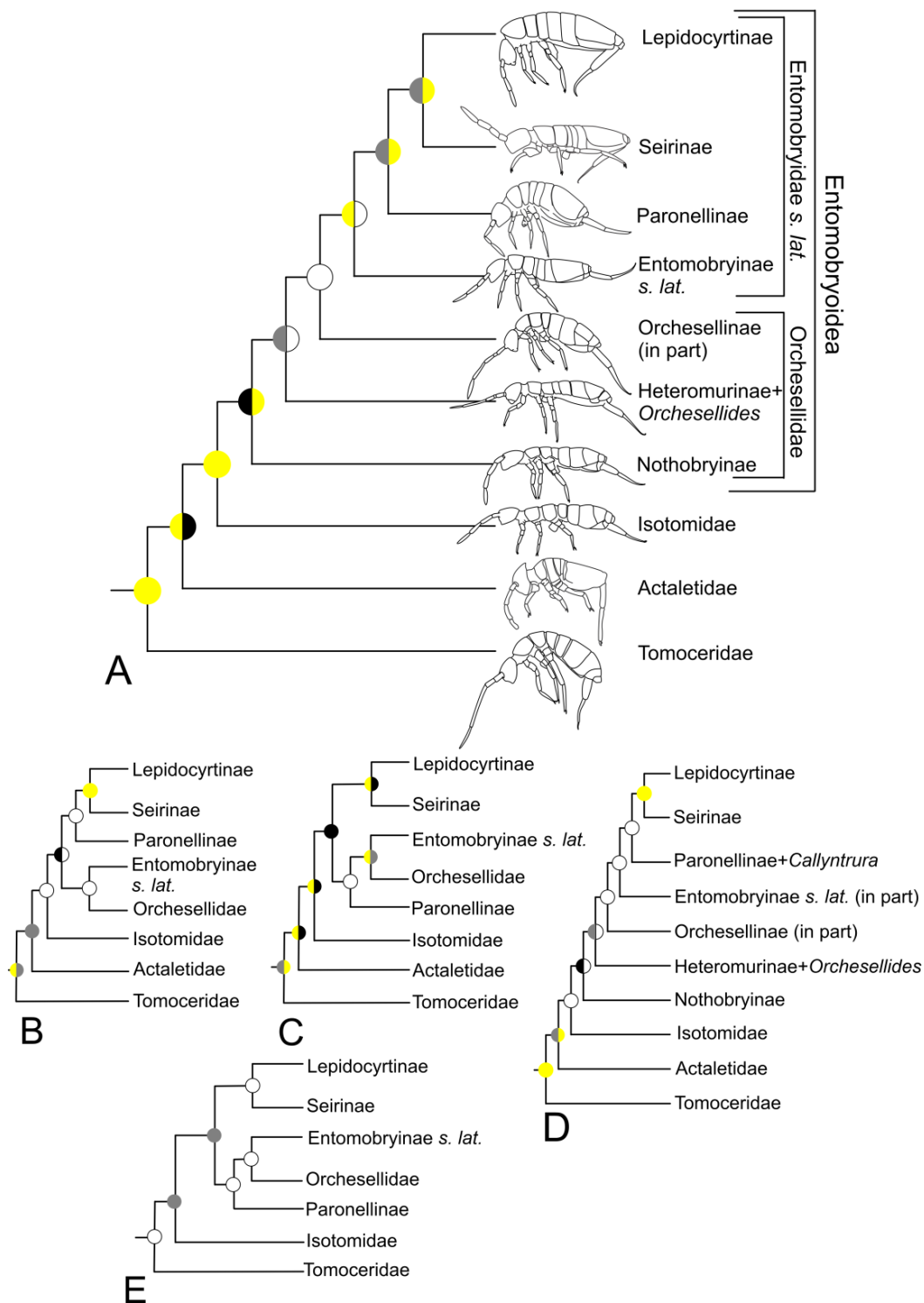
**Figure 3.** Summarized phylogenetic relationships of the higher Entomobryomorpha: (**A**) topology of ML_1 and BI trees (nodes depict only ML_1 support levels); (**B**) topology of ML_2 tree; (**C**) topology of ML_3 tree; (**D**) topology of ML_4 tree; (**E**) topology of MP tree (Actaletidae omitted since it was recovered outside of the main Entomobryomorpha branch). Node circles represent SH-aLRT support (left side) and bootstrap values (right side), respectively, for ML trees, and maximum parsimony bootstrap for MP tree. Colors represent the following scores: black = 100; yellow = 95–99.9; grey = 90–94.9; white < 90.

The ML_1 and BI trees found Entomobryidae *s. lat.* topology as Entomobryinae *s. lat.* + (Paronellinae + (Lepidocyrtinae + Seirinae)), with Entomobryinae *s. lat.* gathering the Salininae (*Akabosia* Kinoshita, *Callyntrura* Börner, and *Salina* MacGillivray) and the Entomobryinae *sensu* Zhang et al. [44]. The same trees did not support the Orchesellidae and Orchesellinae, while Heteromurinae appeared as an ingroup of part of Orchesellinae, and Nothobryinae was recovered as the most basal branch of Entomobryoidea (Figures 1, 3A, and S4). Similar results were found by ML_4, but with *Callyntrura* as an ingroup of Paronellinae (Figures 3D and S3). The ML_2, ML_3 and MP trees recovered the Orchesellidae, but failed to support the Orchesellinae as well (Figures 3B,C,E, S1, S2 and S5). Tree topology tests aimed at investigating the Entomobryoidea inner relationships also rejected, with full confidence, both Orchesellidae and the traditional Paronellidae in the same topology. Such tests also refuted independent Orchesellinae and Heteromurinae (Table S3). The main phylogeny support levels were high or acceptable for the majority of the main Entomobryoidea branches, with the most important exceptions of the Entomobryinae *s. lat.* cluster, with a bootstrap support of 85 and an SH-aLRT of 67.3, and Entomobryidae *s. lat.* + *Orchesella* Templeton, with a bootstrap support of 79 and an SH-aLRT of 80.1 (Figures 1 and S6). The Bayesian Inference analysis, which found the most similar topology to the main tree, recovered Entomobryidae *s. lat.* with very high node support, but the clade Paronellinae + (Lepidocyrtinae + Seirinae) was supported by a posterior probability value of only 0.65 (Figure S4).

### 3.4. Poduromorpha

The internal relationships of the Poduromorpha higher clades of all trees are summarized in Figure 4. All ML analyses supported the following internal topology for the order: Onychiuridae + (*Ceratophysella* Börner + (*Gomphiocephalus* Carpenter + (Poduridae + Neanuroidea))) (Figures 1, 4A and S1–S3). A close topology was obtained by MP analysis, but the internal organization of the Onychiuridae and Neanuroidea subfamilies was different from ML results (Figures 4A,C and S5). Bayesian Inference recovered the Neelidae as an ingroup of Poduromorpha, and as the sister group of the Onychiuridae (Figures 4B and S4). All analyses dismissed the Hypogastruridae, represented by *Ceratophysella* and *Gomphiocephalus*, and included the Brachystomellidae as an ingroup of the Neanuridae, represented by the Frieseinae, Pseudachorutinae and Neanurinae subfamilies (Figures 1, 4, and S1–S5). Hypogastruridae was also rejected by most of the tree topology tests (Table S3).

All internal nodes of Poduromorpha had high support in our main analysis, with the exception of the SH-aLRT value supporting the Onychiurinae (represented by *Allonychiurus* Yoshii, *Orthonychiurus* Stach, and *Thalassaphorura* Bagnall), a result similar to the ML_2 tree (Figures 1, S1 and S6). Low node support values were found by BI clustering the Neelidae, Neelidae + Onychiuridae, and Poduromorpha + Neelipleona clades (Figure S4). The Maximum Parsimony analysis had mixed bootstrap values, with some internal nodes of Neanuroidea and Onychiuridae with low support (Figure S5).

### 3.5. Symphypleona and Neelipleona

The relationships of the Symphypleona families and Neelipleona genera of all the obtained trees are summarized in Figures 5 and 6, respectively. Concerning the Symphypleona, in all analyses the sampled families were recovered as monophyletic independent groups (Figures 1, 5 and S1–S5). All ML models reached the same results for the order, with the Sminthurididae (suborder Sminthuridida *sensu* Sánchez-García and Engel [82]) as the sister group of the suborder Appendiciphora *sensu* Bretfeld [17]. Within the latter group, Dicyrtomidae was found to be the sister group of the Sminthuroidea (Sminthuridae + Bourletiellidae) (Figures 1, 5A and S1–S3). Alternative hypotheses which disregarded the suborders of Symphypleona were recovered by BI: Dicyrtomidae + (Sminthurididae + (Sminthuridae + Bourletiellidae)); and MP: (Dicyrtomidae + Bourletiellidae) + (Sminthurididae + Sminthuridae) (Figures 5B,C, S4 and S5). The main tree showed high node support

values for all the internal branches of Symphypleona, with the exception of an SH-aLRT value of 66.2 for the Sminthuroidea clade (Figures 1 and S6). Other ML analyses also achieved lower SH-aLRT support for this node, as well as for other internal groups of Appendiciphora (Figures S1–S3). Tree topology tests better supported two competing hypotheses: (1) Appendiciphora + Sminthuridida (the unconstrained ML_1 tree); and (6) (Bourletiellidae + Dicyrtomidae) + (Sminthuridae + Sminthurididae) (found by MP tree) (Table S3).
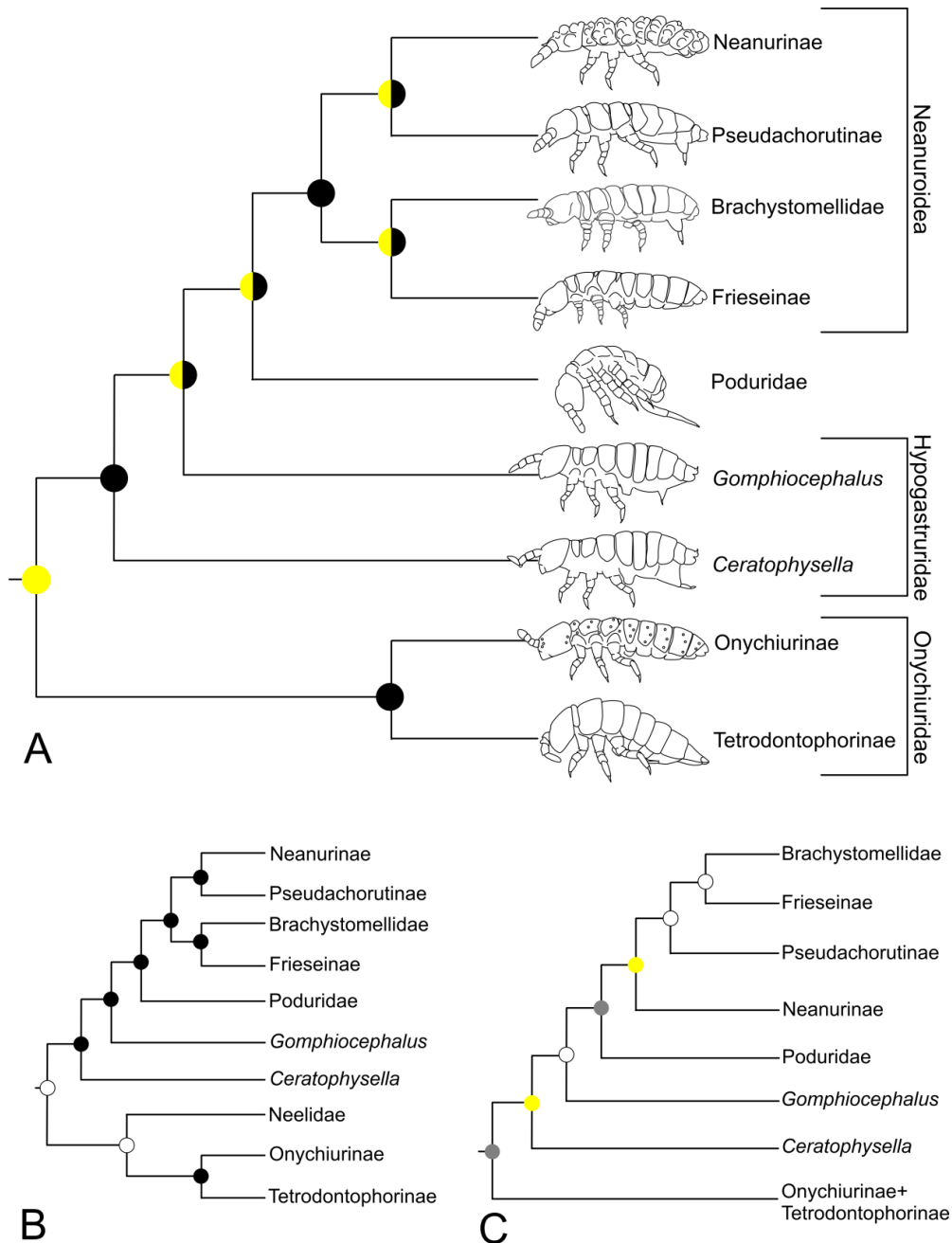


**Figure 4.** Summarized phylogenetic relationships of the higher Poduromorpha: (**A**) topology of ML_1, ML_2, ML_3 and ML_4 trees (nodes depict only ML_1 support levels); (**B**) topology of BI tree; (**C**) topology of MP tree. Node circles represent SH-aLRT support (left side) and bootstrap values (right side), respectively, for ML tree, posterior probability for BI tree, and maximum parsimony bootstrap for MP tree. Colors represent the following scores: black = 100 (1 for BI); yellow = 95–99.9 (0.95–0.99 for BI); grey = 90–94.9 (0.9–0.94 for BI); white < 90 (<0.9 for BI).

**Figure 5.** Summarized phylogenetic relationships of the higher Symphypleona: (**A**) topology of ML_1, ML_2, ML_3 and ML_4 trees (nodes depict only ML_1 support levels); (**B**) topology of BI tree; (**C**) topology of MP tree. Node circles represent SH-aLRT support (left side) and bootstrap values (right side), respectively, for ML tree, posterior probability for BI tree, and maximum parsimony bootstrap for MP tree. Colors represent the following scores: black = 100 (1 for BI); yellow = 95–99.9 (0.95–0.99 for BI); grey = 90–94.9 (0.9–0.94 for BI); white < 90 (<0.9 for BI).
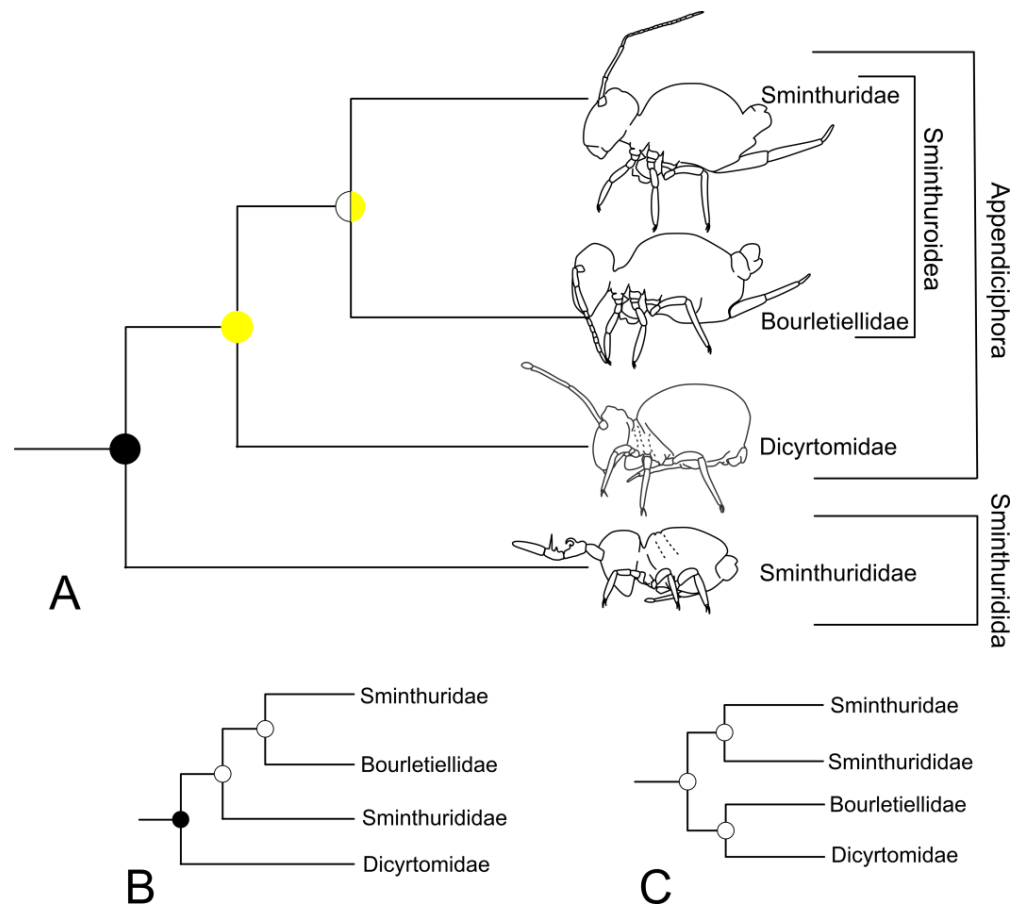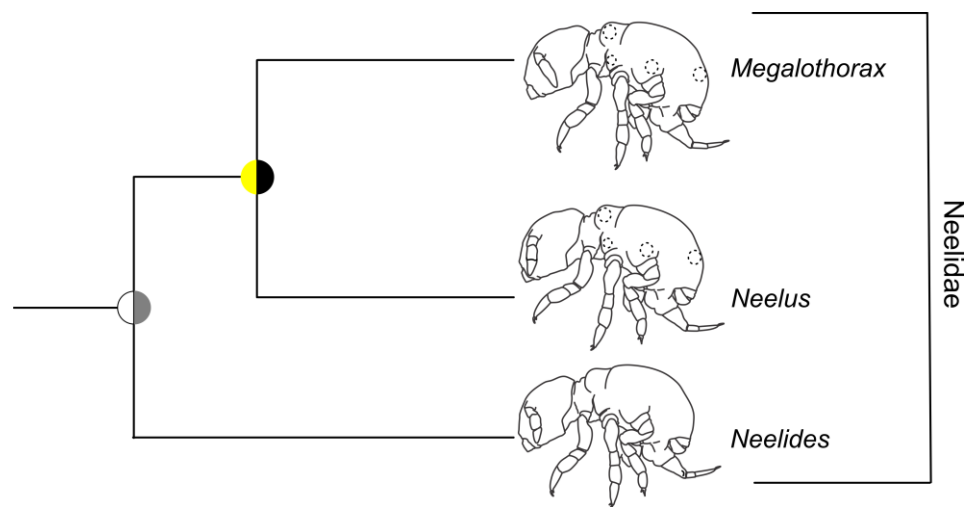


**Figure 6.** Summarized phylogenetic relationships of the Neelipleona genera obtained by the ML_1, ML_2, ML_3, ML_4, BI, and MP trees. Node circles represent SH-aLRT support (left side) and bootstrap values (right side), respectively, for ML_1 tree. Colors represent the following scores: black = 100; yellow = 95–99.9; grey = 90–94.9; white < 90.

Regarding the Neelipleona (Neelidae), all trees recovered the same topology for the sampled genera: *Neelides* Caroli + (*Neelus* Folsom + *Megalothorax* Willem) (Figures 1, 6, and S1–S5). The support values were mostly high for both nodes within the order in all ML analyses, except for an SH-aLRT support of 58.6 in the basal branch of ML_1 tree (Figures 1 and S6). Bayesian Inference found a low value of posterior probability for the node containing the Neelipleona within Poduromorpha (Figure S4), while in the MP analysis, the bootstrap support for the order was 80 (Figure S5).

Finally, Figure 7 translates the full view of the Collembola higher taxa relationships obtained in our main ML phylogeny, ML_1 (Figure 1).
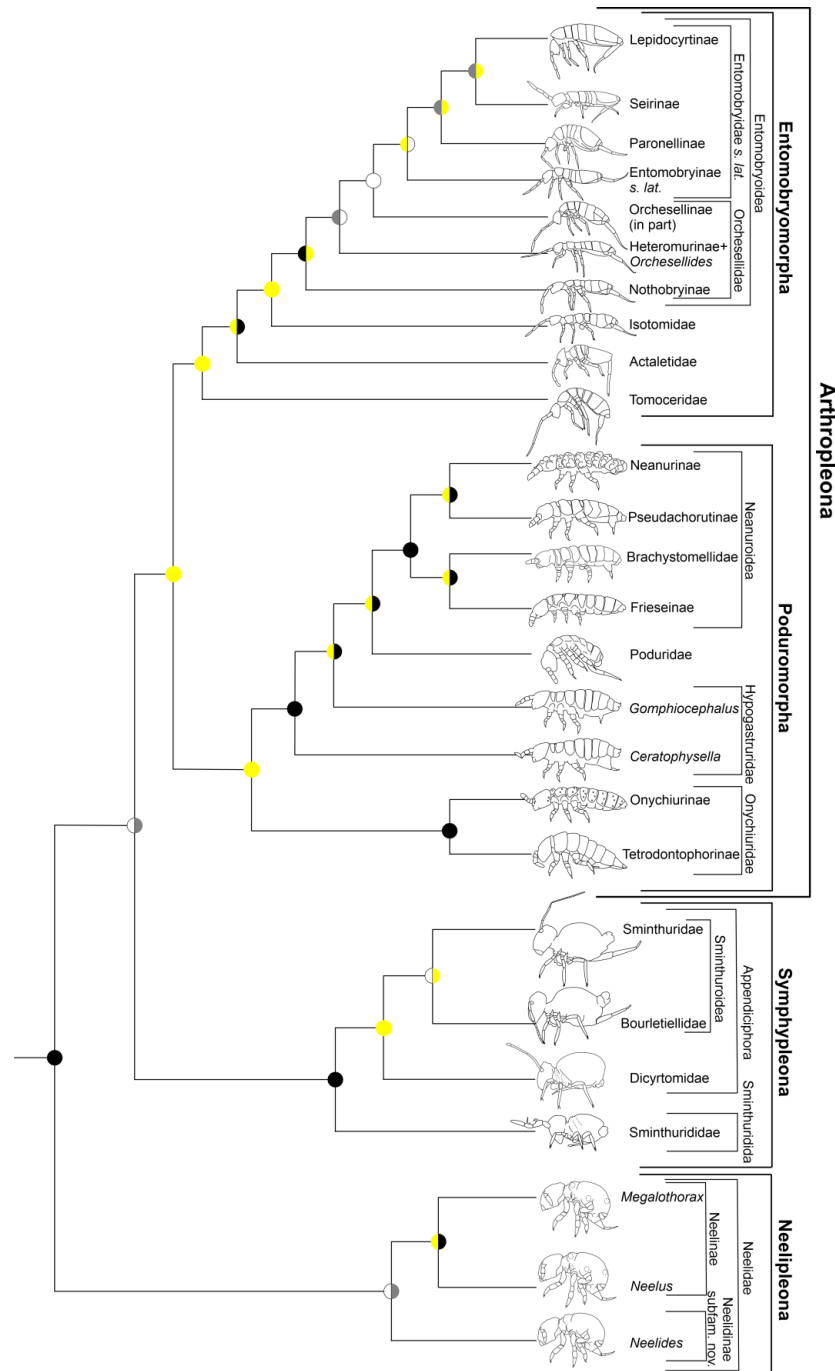


**Figure 7.** Summarized phylogenetic relationships of the sampled higher Collembola based on ML_1 tree. Node circles represent SH-aLRT support (left side) and bootstrap values (right side), respectively. Colors represent the following scores: black = 100; yellow = 95–99.9; grey = 90–94.9; white < 90.

## 4. Discussion

### 4.1. Phylogeny of the Orders

　　The validity and affinities of the current four Collembola orders have been subjects of many systematic revisions and phylogenetic studies. Although some previous published trees, as well as our BI and MP analyses, did not recover the monophyly of all four orders, the internal and at least part of the external morphology advocate that they are distinct monophyletic lineages [10,12,13,15,17,18,83,84]. Even the puzzling *Podura* Linnæus, *Mackenziella* Hammer, and Actaletidae, which were previously allocated in the distinct order Metaxypleona by Salmon [9], have morphological traits which assign them to the Poduromorpha, Symphypleona, and Entomobryomorpha, respectively [18,21,84,85]. Molecular phylogenetic studies based on mitogenomes or other markers also support the current systematic placing of *Podura* and Actaletidae, while the placement of *Mackenziella* has yet to be evaluated with genetic data [27,28,35,46,48–53,55,56,63,86].

　　Assuming the four orders are monophyletic taxa, a more important question arises: what are their relationships? A traditional view provided by Börner proposed the higher clade Arthropleona to gather the Poduromorpha and Entomobryomorpha, and Symphypleona *s. lat.* to gather the current Symphypleona *s. str.* and Neelipleona [5,6]. Such division was mainly based on body shape and the presence or absence of clear abdominal segments. Concerning the Arthropleona, more recently published trees supported this clade to some extent [27,35,48–51,53], while others refuted it [28,46,54–57,87]. Even our results did not reach a consensus for the relationships of these orders, with all ML trees supporting the Arthropleona, while the BI and MP trees rejected this clade (Figure 2). At first, it may be tempting to consider the Arthropleona as a monophyletic taxon compared to the very dissonant trunk morphologies of Symphypleona *s. str.* and Neelipleona. However, the body tagmosis of Poduromorpha and Entomobryomorpha is likely a plesiomorphy and potentially has no phylogenetic signal to group them, as a segmented ancestral state for the Collembola is expected when observing the relationships of basal hexapods and the segmented Protura and Diplura bauplans [65–67]. In this scenario, for now, there is no clear, striking morphological trait that could be assigned as a synapomorphy of Arthropleona, whereas the loss of the pronotum in Entomobryomorpha is even shared with Neelipleona and Symphypleona *s. str.* (except for Mackenziellidae), and the first instar tibiotarsal chaetotaxy may point to a closer relationship between the Entomobryoidea and Symphypleona [1,54,88]. So, the morphological support for this higher clade is, for now, unfulfilling. If the Arthropleona clade is valid, the synapomorphies of the group are likely to be more discrete and can be related to the chaetotaxy or even to the internal morphology, as studied by Cassagnau [12].

　　Contrastingly, the clade Symphypleona *s. lat.* has less support based on more recent molecular-based phylogenetic studies, and it was recovered only by Xiong et al. [51] and Schnider et al. [28], while it was refuted by most of the published trees, including those phylogenies relying on mitogenomes [27,35,46,50,54–57]. The findings of Sun et al. [53] were conflicting regarding this clade, with the phylogeny based on mitogenomes partitioned dataset of nucleotide sequences dismissing the Symphypleona *s. lat.*, whereas the tree inferred from amino acid sequences supporting it, but with a bootstrap value of 73.9. In our analyses, only the ML_3 tree recovered Symphypleona *s. str.* + Neelipleona, but with very low node support (Figures 2 and S2). Bretfeld [17,83] suggested three apomorphies for the Symphypleona *s. lat.*: thoracic and first abdominal segments fused to some extent, resulting in a globular body; the presence of neosminthuroid chaetae on the parafurcal area (furca basis), which were posteriorly lost in different internal lineages of Symphypleona *s. str.* and Neelipleona, and the presence of a gutterlike mucro. Other morphological traits, however, may refute a closer relationship between the two orders. The spherical trunk seen in Neelipleona is likely not homologous to the one of Symphypleona *s. str.*, as, in the former, the large abdomen is mostly constituted by thorax II and III, with a reduction of abdominal segments in size and chaetotaxy (at least in most genera), while the Symphypleona *s. str.* have a large abdomen mostly formed by abdominal segments

I–IV, holding a more complex chaetotaxy compared to the thorax [28,35,83,89–91]. Other features of the trunk chaetotaxy are also not clearly comparable between both orders, like the presence of papillate long bothriotricha on the large and small abdomens of Symphypleona *s. str.*, which already emerge in the first instar. In Neelipleona, if these are present, they are short discrete non-papillate bothriotricha, clearly not homologous compared to the latter [28,35,90,91]. The complex chaetotaxy of the small abdomen seen in the Symphypleona *s. str.*, even during the first instar, does not match the strongly reduced chaetotaxy of the Neelipleona [28,83,91–93]. The tibiotarsal chaetotaxy of Neelidae is also strongly reduced compared to the Symphypleona first instar, and it may be more in line with the Poduromorpha [28,35,91,93–96]. Finally, the reduced antennae, long coxae, tenaculum shape, and midgut morphology of the Neelipleona advocate they may not be related to the Symphypleona *s str.* as well [1,83,91].

Our main tree topology, Neelipleona + (Symphypleona + (Entomobryomorpha + Poduromorpha)) (Figures 1 and 2), was also recovered by Gao et al. [50], and was similar to Sun et al. [53] tree based on mitogenomic partitioned nucleotide sequences. The disparity of tree topologies regarding Collembola orders in recent studies is high and does not point to any clear consensus [56]. We believe the inclusion of large sets of nuclear markers would improve the results obtained by the use of mitogenomes or few isolated mitochondrial markers in this case. Nuclear DNA can be more resilient to mutation compared to mitochondrial genes, making the latter markers more fitting to investigate a more recent evolution [97]. In this case, deeper relationships within the Collembola phylogeny could be better investigated with the inclusion of high amounts of nuclear DNA, which hold the potential to unveil more solid data to evaluate the validity and affinities of the current four orders.

*4.2. Entomobryomorpha*

The Entomobryoidea higher clades have been investigated in many recent phylogenetic studies, which provided further grounds for their evolutionary affinities and insights into the phylogenetical signal of morphological traits on which traditional systematics relied on [38–45,56,60–63,98,99]. For instance, body scales have emerged more than once within the clade and have little or no phylogenetic signal within the Entomobryinae *s. lat* [43]. Similarly, the straight smooth dens has also emerged more than once within the superfamily, and the traditional Paronellidae *sensu* Soto-Adames et al. [18] is likely a polyphyletic group [39,43,44], as supported by our ML, BI, and MP analyses and tree topology tests. On the other hand, trunk chaetotaxy patterns show more reliable attributes to group the higher Entomobryoidea, especially concerning the sensilla [14,43–45].

The results of our ML_1 and BI trees (Figures 1, 3A, and S4) are similar to recently published studies. Our data support the Seirinae as the sister group of Lepidocyrtinae, similarly to Godeiro et al. [41,60,63] trees based on mitogenomes, but differently from the BI tree of Cucini et al. [56] based on the first and second codon positions. Other ML and MP trees also retrieved the same topology, reinforcing the validity of this clade (Figures 3B–E, S1–S3 and S5). Morphology also better supports this relationship rather than the alternative hypothesis of Lepidocyrtinae + (Entomobryinae *s. lat.* + Seirinae) [14,44,100]. Within Seirinae, our data indicate that at least some oriental species of *Seira* Lubbock (*Seira sanloemensis* Godeiro and Cipola and *S. boneti* Denis) may represent an independent clade of *Lepidocyrtinus* Börner + (*Seira* + *Tyrannoseira* Bellini and Zeppelini), similar to the results obtained by Godeiro et al. [61]. Within the Lepidocyrtinae, we reached a very similar internal topology compared to Godeiro et al. [60], which was also based in mitogenomes. We found the Paronellinae closer to the Lepidocyrtinae, which was expected (see [44]), but as the sister group of Seirinae + Lepidocyrtinae, differently from other studies, such as those from Zhang et al. [39,43,44]. Nevertheless, our results better solved the position of the Paronellinae within Entomobryoidea when compared to other mitogenomic phylogenies [53,56,60], matching at some level morphology and the obtained topology based on a larger dataset of Paronellinae, Seirinae, and Lepidocyrtinae.

If our results are confirmed in future studies, this would mean the resemblance of the Paronellinae to the Lepidocyrtinae, like the reduction of dorsal macroquetotaxy and the same sensillar pattern [14,44], are possibly due to the plesiomorphies of this clade, and the more complex dorsal macrochaetotaxy of Seirinae was achieved posteriorly within the lineage.

The Entomobryinae *s. lat.* was recovered by our ML_1 (Figure 1) and BI (Figure S4) trees with high node support and gather genera with a wide range of dorsal chaetotaxy and furca morphologies. This clade clusters unscaled and scaled genera, with highly variable dorsal main chaetotaxy, ranging from a polymacrochaetotic coverage (more common) to a reduced dorsal macrochaetotaxy [39,43,44]. Within this branch, the dens varies from crenulate to smooth, while the mucro morphology is somewhat similar to the Paronellinae in lineages like the Salininae *sensu* Zhang et al. [44], *Zhuqinia* Zhang, Ma and Greenslade, and *Paronellides* Schött, or more on par with the Entomobryidae *s. str* [43,44]. The main morphological feature grouping these very distinct taxa is the trunk sensillar pattern of 2, 2|1, 2, 2 from the mesothorax to the third abdominal segment. However, even this potential synapomorphy is secondarily modified in some internal branches, like the Salininae [44]. As in many other recent papers based on mitogenomes or other markers, our data could not clearly resolve some internal relationships within the subfamily, as the lower node support of our ML_1 and BI trees pointed out.

Our analyses could not solve the internal relationships of the Orchesellidae, with the ML_1, ML_4, and BI trees supporting a polyphyletic condition of the family, with some branches having low node support (Figures 1, 3A,D, S3, S4 and S6). Our tree topology tests rejected the family as well (Table S3). The trees which recovered the Orchesellidae, ML_2, ML_3, and MP, could not separate the Heteromurinae from the Orchesellinae (Figures 3B,C,E, S1, S2 and S5), a result also endorsed by our tree topology tests (Table S3). A similar problem was found in the BI tree of Godeiro et al. [60], which was also based on mitogenomes. Apparently, the lack of nuclear genes in the analyses obscures the relationships of the Orchesellidae, which were better solved in studies based on a few mitochondrial and nuclear markers combined, like in Zhang et al. [38,39,42,43] and Nunes et al. [40]. This may represent a similar issue to the one discussed in the previous topic, in which the absence of more mutation-resilient markers prevents a clearer understanding of deeper nodes within the phylogeny. Even so, it is worth noting that there is no clear synapomorphy to circumscribe the Orchesellidae, and the reduced abdomen and the presence of a postantennal organ in some genera are likely plesiomorphic features of the Entomobryoidea. On the other hand, morphology, to some extent, advocates that Orchesellinae and Heteromurinae are distinct lineages [14,60,98], a result not supported by our findings. We believe our limited dataset for the Orchesellidae and the mitogenomes alone are insufficient to clearly unveil the affinities and validities of the basal Entomobryoidea suprageneric taxa.

All our ML, BI, and MP trees did not support the current systematics of the Isotomoidea in two main lines: firstly, the internal division of the Isotomidae subfamilies as presented in Bellinger et al. [1] or as proposed by Potapov [101]; secondly, the positioning of Actaletidae next to Isotomidae, following Soto-Adames et al. [18]. The essential revision provided by Potapov [101] suggested three subfamilies for the Isotomidae: Pachyotominae, Anurophorinae, and Isotominae, disregarding Proisotominae due to its similarities with Anurophorinae. The author also highlighted the absence of "absolutely strict differences" between Anurophorinae and Isotominae and the absence of phylogenetic grounds to support his classification. Contrarily, the Bellinger et al. [1] database kept Proisotominae as a valid subfamily, separating it from Anurophorinae based on the number of body sensilla. The morphology within Isotomidae is remarkably variable, and the boundaries among the different subfamilies, genera, and subgenera may be difficult to determine in some cases [18,101]. Our data point to Pachyotominae, Anurophorinae, and Proisotominae as being internal groups of Isotominae. Moreover, *Proisotoma* and *Subsitoma*, the representative Proisotominae taxa in our analyses, were recovered apart from each other,

within a clade gathering all sampled Anurophorinae (*Anurophorus*, *Cryptopygus*, *Folsomia*, and *Tetracanthella*) together with *Paranurophorus*, the sole Pachyotominae we sampled (Figures 1 and S1–S5). Although such results support Potapov's [101] view of merging Anurophorinae with Proisotominae, they did not sustain Pachyotominae as a full subfamily. Additionally, the clade mixing Pachyotominae, Anurophorinae, and Proisotominae genera is not independent as it emerges inside a higher group with *Pseudisotoma* Handschin, *Semicerura* Maynard, *Parisotoma* Bagnall, and *Folsomotoma* Bagnall (all Isotominae genera) in its base (Figures 1 and S1–S5). Similar results were also obtained by some other studies dealing with Isotomidae taxa, with at least the Anurophorinae as an ingroup of a paraphyletic Isotominae [46,55,56,63]. Our data are not definitive by any means, as some of the internal nodes of Isotomidae had low support in our analyses (Figures 1 and S1–S6). However, the high number of isotomid taxa used in this study is unmatched compared to the previously cited papers, providing some level of robustness to our findings. Such results point to a need for an urgent systematic/phylogenetic revision of the Isotomidae, including a review of the features used to delimit its subfamilies.

In contrast to the uncertainties of the internal systematics of the Isotomidae, all of our phylogenies supported it as a valid independent family, refuting the hypothesis of Yosii [8] and the findings of D'Haese [48], Schneider et al. [27,28], and Schneider and D'Haese [35]; and as the sister group of the Entomobryoidea (Figure 3), as hypothesized by Szeptycki [14] and was recovered in many recent published trees [46,47,51,53,55–57,63]. Our analyses also place Actaletidae apart from Isotomoidea, differently from Cassagnau [12], Massoud [13], and Soto-Adames et al. [18]. Our ML and BI trees supported Actaletidae as the sister group of Isotomidae + Entomobryoidea, suggesting the similarities between Actaletidae and Isotomidae, like the presence and shape of the postantennal organ, the absence of a trochanteral organ, body scales and head bothriotricha, plus other chaetotaxic features [18,80], may actually be due to the resemblance of both families with the ancestral of the branch Actaletidae + (Isotomidae + Entomobryoidea). In order to endorse this hypothesis, basal Entomobryoidea, like part of Nothobryinae, also share a similar postantennal organ and absence of scales with the Actaletidae and Isotomidae, while having a reduced number of metatrochanteral spines [102,103]. Previously, only the recent study of Godeiro et al. [63] (based on mitogenomes) used the Actaletidae in molecular phylogeny, reaching a topology different from our results: Actaletidae + (Tomoceridae + (Isotomidae + Entomobryoidea)). The Entomobryomorpha dataset of the previous study was limited compared to ours, and the overall high node support retrieved in our ML and BI analyses combined to the morphological evidence of a potentially closer relationship of Isotomidae and Actaletidae, suggest the clade Tomoceridae + (Actaletidae + (Isotomidae + Entomobryoidea)) is more plausible.

Our ML, MP, and BI trees found the Tomoceroidea to be the most basal branch of Entomobryomorpha (Figure 3). Such results are on par with the findings of Sun et al. [53] tree based on partitioned nucleotide sequences and Yu et al. [47], but contrast with Sun et al. [53] tree inferred from amino acid sequences, Xiong et al. [51], and Yu et al. [46]. Our dataset is limited regarding the Tomoceroidea, with only three genera of Tomocerinae (Tomoceridae) (Table S1). With the absence of Oncopoduridae and Lepidophorellinae representatives, we cannot clearly compare our results with the previously cited papers. Nevertheless, none of our trees clustered the Tomoceridae with Poduromorpha, and found the sampled Tomocerinae as an ingroup of Entomobryomorpha with overall high node support (with the exception of MP analysis, Figure S5), as endorsed by the morphology [18].

*4.3. Poduromorpha*

The main results obtained by the ML analyses (Figure 4) are remarkably similar to other published phylogenies based on mitogenomes [52,53,55–57]. These studies, as well as our data, support the Onychiuroidea apart from the clade gathering the Hypogastruridae, Poduridae, and Neanuroidea lineages. Sun et al. [53] ML tree inferred from amino acid sequences and Cucini et al. [56] BI tree excluding the third codon position recovered the Onychiuroidea as a valid superfamily, gathering Onychiuridae and Tullbergiidae. On the

other hand, Sun et al. [53] ML tree based on partitioned nucleotide sequences and Leo et al. [55] BI tree based only on the first and second codon positions did not support the clade Onychiuridae + Tullbergiidae, while Carapelli et al. [52], Ma et al. [57], and our study only sampled the Onychiuridae. Most of these studies also refuted the Hypogastruridae in the same way we found in our ML, BI, and MP analyses and the majority of our tree topology tests, putting apart *Ceratophysella* and *Gomphiocephalus*, with the exception of Sun et al. [53] ML tree based on partitioned nucleotide sequences and Carapelli et al. [52], which only sampled *Gomphiocephalus*. Additionally, in most phylogenies, including ours, Poduridae was recovered as the sister group of the Neanuroidea, with the exception of Ma et al. [57], which did not sample *Podura*. Finally, Cucini et al. [56] and our ML and BI trees achieved the same internal topology for the Neanuroidea, with Neanurinae as the sister group of Pseudachorutinae, and Brachystomellidae as an ingroup of Neanuridae and the sister group of Frieseinae.

On the other hand, other phylogenetic studies based on fewer genes recovered mixed results. We found D'Haese's [48] tree achieved a quite similar topology compared to our ML phylogenies, but a straightforward comparison would be unwise since D'Haese's dataset was remarkably better represented than ours. Nevertheless, this study found the Onychiuroidea + *Triacanthella* Schäffer apart from the clade gathering most of the polyphyletic Hypogastruridae plus Poduridae and Neanuroidea. An unexpected basal position for *Triacanthella* was obtained by Luan et al. [49] and Xiong et al. [51]. These data combined support that the actual genus placement should be revised in future systematics studies of the Poduromorpha. Luan et al. [49] also found the topology Hypogastruridae in part + (Poduridae + Neanuroidea); however, their dataset for the Poduromorpha was limited to five species. Greenslade et al. [86] found the Onychiuroidea in part as the sister group of the branch clustering the paraphyletic Hypogastruidae, Poduridae, and Neanuroidea; however, in this study, the sister group of the latter was *Gomphiocephalus*, and the Poduridae appeared mixed with Hypogastruridae lineages. Contrarily, Schneider et al. [27,28] trees found Poduridae as an ingroup of Neanuroidea, and Hypogastruridae as a monophyletic family; Xiong et al. [51] recovered the topology Neanuroidea + (Tullbergiidae + (Poduridae + Hypogastruridae in part)), and Yu et al. [46] found Hypogastruridae in part + ((*Triacanthella* + Odontellidae) + (Poduridae + Neanuroidea)).

At least part of our findings is also supported by morphology. D'Haese's [87] detailed morphology-based phylogeny also pointed out for a non-monophyletic Hypogastruridae, and the Poduridae closer to the Neanuroidea. The developed chewing mouthparts seen among the Hypogastruridae are undoubtedly a plesiomorphy shared by most lineages of the other orders of springtails and supposedly do not have any phylogenetical signal within the Poduromorpha. Conversely, the modified mouthparts of the Neanuroidea, with the reduction or complete loss of mandibles, notable modifications on the maxillae capitulum, and variable elongations of the oral cone, are clearly synapomorphies of derived taxa. Still, it is not clear if the Odontellidae belongs to the Neanuroidea, as some morphological traits have supported [87,104], or if its peculiar maxillae lacking the cardo point to an independent path of mouthparts modification within the Onychiuroidea, or even of an independent basal Poduromorpha branch, as supported by some molecular studies [27,28,35,48,51,86]. Our findings also endorse that Brachystomellidae should be considered a subfamily of Neanuridae, following Massoud's [104] view. A similar placement for Brachystomellidae was also obtained by D'Haese [48] and Cucini et al. [56]. The limited dataset of Neanuroidea in our analyses, with the absence of representatives of Caputanurininae, Morulininae, and Uchidanurinae, prevents us from providing further comments on the systematics of the superfamily. We are aware that Dr. D'Haese's team is currently working on a large phylogenetic study of the Neanuroidea, which will likely be more conclusive about the validity and internal relationships of its subfamilies.

*4.4. Symphypleona and Neelipleona*

Recent advances in the systematics of Collembola using molecular markers provided different views for the internal relationships of the Symphypleona *s. str.* families and the order's validity. For example, D'Haese [48] and Luan et al. [49] found the Symphypleona *s. str.* to be the paraphyletic basal stock of all springtails, a result not endorsed by morphology or traditional systematics of Collembola [6,10,15,17,20,83,105]. On the other hand, more recent studies recovered the Symphypleona *s. str.* as a valid order, but with different internal topologies. Using five sampled species, Xiong et al. [51] found the ML/BI tree: Sminthurididae + (Bourletiellidae + Sminthuridae); Schneider et al. [27,28] and Schneider and D'Haese [35], with nine sample taxa, found the MP/ML tree: (Katiannidae + Arrhopalitidae) + (Dicyrtomidae in part + (*Dicyrtoma* Bourlet + (Bourletiellidae + Sminthuridae))); using six taxa, Yu et al. [46] found the BI/ML tree (Bourletiellidae + (Katiannidae + Sminthuridae)) + (Sminthurididae in part + (*Sminthurides* Börner + Dicyrtomidae)); while Sun et al. [54], based on four species, found the ML tree: Arrhopalitidae + (Sminthurididae + (Sminthuridae + Bourletiellidae)).

Concerning the use of mitogenomes to investigate Symphypleona *s. str.* phylogeny, the results were also divergent. Leo et al. [55] and Ma et al. [57], with four and three species, respectively, and based on first and second codon positions, reached BI and ML trees with the same topology: Dicyrtomidae + (Sminthuridae + Bourletiellidae). Using seven sampled species and based on protein-coding genes, Nardi et al. [64] reached the BI tree Sminthurididae + (Sminthuridae + (Bourletiellidae + Dicyrtomidae)). Sun et al. [53], with six species and based on partitioned nucleotide sequences, found the ML tree Sminthuridae in part + (Dicyrtomidae + ((Katiannidae + Sminthurididae) + (*Sminthurus* Latreille + Bourletiellidae))), while using amino acid sequences recovered (Dicyrtomidae + (Sminthurididae + Katiannidae)) + (Bourletiellidae + Sminthuridae)). The more recent study of Cucini et al. [56], based on nine species and on first and second codon positions, reached the BI tree Bourletiellidae/Dicyrtomidae + (Sminthuridae + (Katiannidae + Sminthurididae)).

All the above discrepant results are due to different types and analysis parameters (similar to our conflicting ML, BI, and MP trees), mismatched sampled taxa, and/or molecular markers. It is also worth noting that, so far, no representative molecular phylogeny of the Symphypleona *s. str.* has been published, with datasets reaching at most nine species from few genera, as in Schneider et al. [27,28], Cucini et al. [56], and in our study. In this scenario, all conclusions about the internal evolution of the Symphypleona *s. str.* based on molecular data should be taken as preliminary. Nevertheless, our ML trees (Figure 5) support previous hypotheses of the internal relationships of the order. The suborders Sminthuridida and Appendiciphora Bretfeld [17] *sensu* Sánchez-García and Engel [82] were recovered with high node support in all ML trees, while our tree topology tests recovered mixed results, both supporting Appendiciphora and Sminthuridida, or refuting them as independent taxa (Table S3). The morphology strongly supports the two suborders, as the Sminthurididae and Mackenziellidae (Sminthuridida) share the apomorphic antennal clasper of the males and the plesiomorphic short and spherical ventral tube sacs and absence of the subanal appendages; at the same time, the Appendiciphora, gathering all the other families of Symphypleona *s. str.*, share the derived long ventral tube sacs and the subanal appendage of the females [10,17,21,83]. The absence of any Katiannoidea taxa (Katiannidae, Spinothecidae, Arrhopalitidae, and Collophoridae) in the analyses prevented us from testing Börner's [6,105] original view of a closer relationship between Sminthurididae and Katiannoidea, which was endorsed by the results of Sun et al. [53] and Cucini et al. [56]. Additionally, *Mackenziella psocodes* Hammer, the sole species of Mackenziellidae, has never been included in any molecular phylogeny so far. Such an omission prevents a clearer view of its relationships with other Collembola lineages, as its morphology is unique, and its systematic position within different orders was disputed by several authors [8,16,21,106,107].

Our ML trees also supported the Sminthuroidea *sensu* Bretfeld [108], a superfamily that gathers the Sminthuridae and Bourletiellidae. Such lineages are remarkably similar,

with some taxa presenting overlapping morphologies between them [109]. Contrarily, our ML and BI trees did not support Sminthurinae and Sphyrothecinae, as *Lipothrix* Börner, a representative of the latter, was found inside the former (Figure 1 and Figures S1–S4). At this time, with only three species and genera of Sminthuridae sampled, such results are insufficient to propose the fusion or regrouping of these subfamilies. Additionally, our analyses did not sample any Songhaicinae taxon, the third subfamily of Sminthuridae [110].

We could not compare our results concerning the Neelipleona with other studies investigating molecular phylogenies of the group. In most of them, the dataset was restricted to a single species [46,50,51,53], or the presence of a species without an assigned genus obscured the comparison between the topologies [55,56]. The detailed trees of Schneider et al. [27,28] and Schneider and D'Haese [35] only sampled two genera, *Neelus* and *Megalothorax*, and focused mainly on the latter internal lineages. The same sampled genera limitation was observed in the phylogeny of Ma et al. [57]. Although our data are also limited by sample size, the use of the three main genera of Neelipleona in the analyses provided us with an important insight on the internal evolution of the order. The sensory fields are unique structures without morphological parallel in other Collembola taxa, likely representing an autapomorphy of the Neelipleona [89]. Some authors consider *Neelides* as the sole genus of Neelidae lacking sensory fields [83,93], while others provided evidence that they are present but are rudimentary and discrete [89,91]. In all other genera of the family, including *Neelus* and *Megalothorax*, such structures are very well delimited and hold specialized inner chaetae [83,91,93]. In our analysis, *Neelides* was recovered as the most basal branch of the order, with *Megalothorax + Neelus* as its sister group (Figure 6). Based on this finding, we believe the sensory fields present in *Neelides* (if homologous to the ones seen in other genera [91]) represent a more basal state of this character in Neelipleona internal evolution, while the well-delimited sensory fields are a derived state, a synapomorphic trait of the higher taxa. This hypothesis, supported by our data, allows us to suggest the subdivision of Neelidae into two subfamilies: Neelinae Handlirsch, 1929 [83], including the sampled *Neelus* and *Megalothorax*, the largest genera of the order [1], plus *Zelandothorax* Delamare Deboutteville & Massoud, *Spinaethorax* Papáč and Palacios-Vargas and *Acanthoneelidus* Bretfeld & Griegel; and Neelidinae subfam. nov. Bellini, Godeiro and Zhang, represented by *Neelides* species. This proposition is included in Figure 7.

## 5. Conclusions

Our results advocate that mitogenomes are highly suitable for the study of Collembola phylogeny, supporting many previously traditional and more recent hypotheses regarding the relationships between its main internal lineages. For instance, our main tree supported the validity of the four current orders, the superorder Arthropleona, the Entomobryoidea as the sister group of Isotomidae, the Neanuroidea (putting Brachystomellidae inside Neanuridae), the split of Symphypleona *s. str.* into the Appendiciphora and Sminthuridida suborders, the Sminthuroidea clustering Sminthuridae and Bourletiellidae, and *Neelides* as a basal branch of Neelipleona, allowing us to divide the Neelidae into two subfamilies: Neelinae and Neelidinae subfam. nov. Contrarily, our main tree refuted the superorder Symphypleona *s. lat.*, Paronellidae *sensu* Soto-Adames et al. [18], all subfamilies of Isotomidae, Hypogastruridae, and Poduridae as a basal lineage of Poduromorpha. Though this study presents one of the most taxonomically and genetically comprehensive springtail phylogenies to date, it also highlights a great need for the inclusion of additional representative taxa and nuclear markers to better resolve the evolutionary relationships among some persistently problematic springtail lineages.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15010007/s1. Figure S1: Phylogeny of Collembola inferred from Maximum Likelihood, partitioned dataset, mixture model (ML_2). Node labels show SH-aLRT support and bootstrap values, respectively; Figure S2: Phylogeny of Collembola inferred from Maximum Likelihood, unpartitioned dataset, model mtART + C60 + FO + R (ML_3). Node labels show SH-aLRT support and bootstrap values, respectively; Figure S3: Phylogeny of Collembola

inferred from Maximum Likelihood, partitioned dataset detailed in Table S2 (ML_4). Node labels show SH-aLRT support and bootstrap values, respectively; Figure S4: Phylogeny of Collembola inferred from Bayesian Inference (BI), default model CAT+GTR with four categories. Node labels show posterior probability support; Figure S5: Phylogeny of Collembola inferred from Maximum Parsimony (MP), '*' marks Actaletidae outside the Entomobryomorpha. Node labels show bootstrap support; Figure S6: Phylogeny of Collembola inferred from Maximum Likelihood, unpartitioned dataset, substitution model mtZOA + F + R10 (ML_1). Node labels show raw SH-aLRT support and bootstrap values, respectively. Branches of each Collembola order are represented in different colors. Table S1: Taxonomic information, GenBank accession numbers, country and information source of all analyzed species; Table S2: Partitioning scheme and substitution models selected by Model Finder used for partitioned maximum likelihood analyses. Table S3: Tree topology tests of non monophyletic groups of Collembola higher taxa.

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