



# Breakaway from a globular body shape: molecular phylogeny reveals the evolutionary history of the enigmatic springtail *Mackenziella psocoides*

Clément Schneider<sup>1</sup>, Cyrille A. D’Haese<sup>2</sup>

<sup>1</sup> Abteilung Bodenzoologie, Senckenberg Gesellschaft für Naturforschung, Görlitz, Germany

<sup>2</sup> MECADEV, UMR 7179 CNRS MNHN, Muséum national d’Histoire naturelle, Paris, France

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Corresponding authors: Clément Schneider ([clement.schneider@senckenberg.de](mailto:clement.schneider@senckenberg.de)), Cyrille A. D’Haese ([cyrille.dhaese@mnhn.fr](mailto:cyrille.dhaese@mnhn.fr))

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## Abstract

*Mackenziella psocoides* Hammer, 1953 (Collembola: Mackenziellidae) is a widespread but uncommon springtail. Its unusual body shape (ovoid, with partial coalescence of abdominal segments) has puzzled the specialists for a long time, until the discovery of males allowed to relate the species to a family of globular springtails, the Sminthurididae. Yet, the precise phylogenetic position of *M. psocoides*, and hence of the Mackenziellidae, remained ambiguous. In this work, we report a new locality for *M. psocoides* in Germany. We provide the first DNA sequences (nuclear ribosomal DNA operon) for the species, as well as the first images using scanning electron microscopy. We investigate its phylogenetic position based on the molecular data and specify details on its morphology. Our results show that *M. psocoides* is nested inside of Sminthurididae, as the sister group of *Sphaeridia* Linnaniemi, 1912. Consequently, Mackenziellidae **syn. nov.** is here synonymized with Sminthurididae. We include *Mackenziella* and *Sphaeridia* in the Sphaeridiinae **subfam. nov.**, a replacement name for Sphaeridiinae Richard, 1968 that is a junior homonym of Sphaeridiinae Latreille, 1802 (Coleoptera: Hydrophilidae). Corresponding to its phylogenetic position within Sminthurididae, the evolutionary origin of *M. psocoides* is younger than previously thought (79 mya +/- 35 my). The lineage accumulated an unusual amount of body modifications involving, among others, the loss of the globular body shape. This rapid rate of evolution is, to our knowledge, unique in springtails. It shows that globular body shape is not an evolutionary dead-end, and the secondary acquisition of a linear body shape and recovery of longitudinal flexibility is still possible.

## Key words

Body shape evolution, Oxford Nanopore, body segmentation, SEM, Sminthurididae, Mackenziellidae, Symphyleona, new synonym

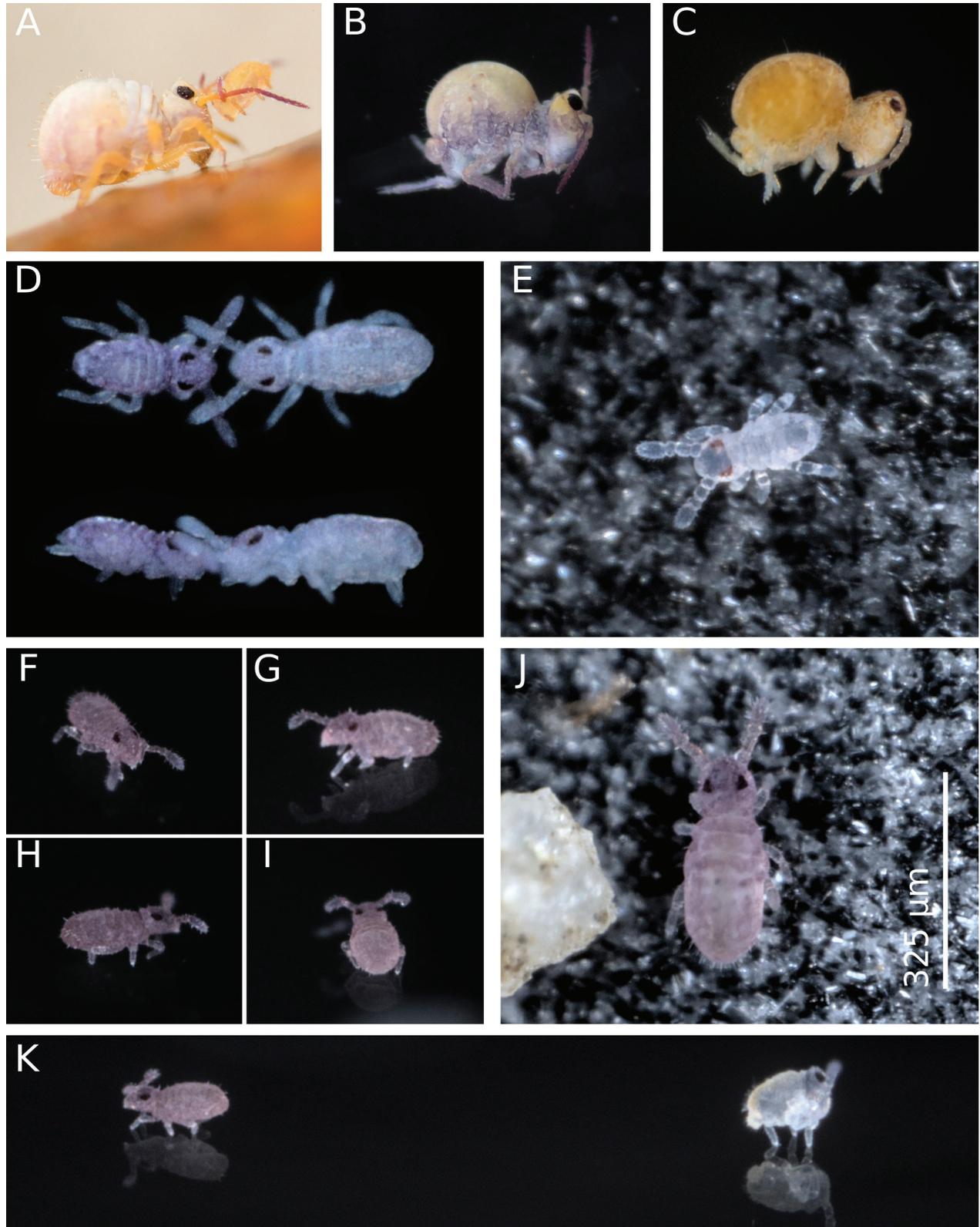
## 1. Introduction

Collembola (springtails) form one of the four classes of Hexapoda and are also the most ancient undebated Hexapoda found in the fossil record (Rhynie cherts, ear-

ly Devonian) (Hirst and Maulik 1926; Massoud 1967a; Greenslade and Whalley 1986). They are primitively of elongated shape, with three thoracic segments and six ab-

dominal segments, a morphology that is conserved in the order Poduromorpha. The orders Entomobryomorpha, Symphypleona and Neelipleona are characterized by the partial reduction of the prothorax (D'Haese 2003) (vol-

ume reduction and regression of the pronotum). Symphypleona and Neelipleona underwent a drastic evolutionary trajectory leading to the acquisition of a globular body shape (Fig. 1A–C) through the fusion of at least the third



**Figure 1.** Sampled species of Sminthuridae **A** *Sminthurides aquaticus*, **B** *Stenacidia violacea*, **C** *Sphaeridia pumilis*. *Mackenziella psocoides* **D** Male (left) and female (right) during courtship (fixed in ethanol), **E** juvenile, **F**, **G**, **H**, **I** female on water surface, various angles, **J** female (one of the largest specimens obtained), **K** *Mackenziella psocoides* (left) and a co-occurring young *Sphaeridia pumilis* (right).

thoracic to the fourth abdominal segments, with the second thoracic and fifth abdominal segments being further co-opted in this process in some lineages (Betsch 1980; Schneider 2017). In Neelipleona, the abdomen is reduced, smaller than the thorax, while in Symphypleona the abdominal region is larger than the thorax (Massoud 1976). In both orders, the head acquired a hypognathous posture.

*Mackenziella psocoides* Hammer, 1953 is the unique species of the family Mackenziellidae Yosii, 1961. It is also one of the smallest and strangest Collembola, being a tiny, ovoid animal with a prognathous head, and retaining a marked segmentation until the second abdominal segment (Fig. 1D–K), a prothorax devoid of dorsal chaetae and a small furca. It has puzzled the specialists for a long time and was in turn related to the families that are now classified in Poduromorpha (Hammer 1953; Hüther 1964), Neelipleona (Pactl 1956; Moen and Ellis 1984) and Symphypleona (Yosii 1961; Christiansen and Bellinger 1981). Salmon (1956) placed it in Poduridae (Poduromorpha) but estimated that it could be closer to Actaletidae (Entomobryomorpha). Massoud (1967b), followed by Christiansen and Bellinger (1981), guessing that the known specimens were juveniles. This history of early studies and opinions on *M. psocoides* has been reviewed in detail by Fjellberg (1989).

Fjellberg (1989) made a significant contribution to the systematics of the species by providing a detailed description based on many specimens, confirming that the species was described from adult females and describing the first males. He showed that males are equipped with the male antennal clasping organ (MACO), a modification of the antennae, allowing the male to grab the female antennae during courtship (Figs 1D, 4B, E, F). This organ is a synapomorphy of the Sminthurididae (Symphypleona). Fjellberg (1989) suggested that *M. psocoides* was the sister-group of Sminthurididae, with the following synapomorphies: presence of the MACO and absence of the female anal appendages (present in all the other families of Symphypleona). Fjellberg (1989) interpreted the elongate body shape and prognathous head as secondary modifications (apomorphies) of *M. psocoides*. Fjellberg's (1989) views were accepted by Sánchez-García and Engel (2016a) who reused the name Sminthuridida (a synonym of Sminthurididae) as a suborder grouping Sminthurididae and Mackenziellidae. Sminthuridida is sister to the Appendiciphora (all the other Symphypleona).

Despite the complete morphological redescription provided by Fjellberg (1989), the precise systematic position of *M. psocoides* has remained disputable. The absence of female anal appendages can currently only be seen as a plesiomorphy in Symphypleona (Bretfeld 1999), and hence their absence does not provide any clade support. The only clear synapomorphy of *M. psocoides* and the Sminthurididae is the MACO. Indeed, *M. psocoides* possesses the simple version of the MACO, almost identical to the one found in *Sphaeridia* Linnaniemi, 1912 (Massoud and Betsch 1972, Fjellberg 1989). From a strict parsimony point of view, this is counterweighted by the secondary acquisition of an elongated body shape and of the regression of the furcal segment (always well devel-

oped in Symphypleona). An alternative scenario from the Sminthuridida sensu Sánchez-García and Engel (2016a) hypothesis would be the sister relationship of Symphypleona and Mackenziellidae, involving a common ancestor characterized by an elongated body shape and a MACO, and the subsequent acquisition of globular shape in Symphypleona and the loss of the MACO in the Appendiciphora.

Could a globular springtail have made a U-turn on its overall body shape evolution and regain the elongated body shape? Answering this question requires to resolve the precise phylogenetic placement of *M. psocoides*. However, *M. psocoides* is a rare species that has never been sequenced so far (da Silva Medeiros et al. 2022). The absence of molecular data for *M. psocoides* was mentioned and regretted in recent phylogenetic works dealing with higher taxa of Collembola (Sun et al. 2020; Bellini et al. 2022) as well as in the recent review on Sminthurididae systematics by da Silva Medeiros et al. (2022).

We discovered a new locality for *M. psocoides*, in Saxony (Germany), that yielded over a hundred of specimens, allowing us to fill this gap and further study the species. In this work, we conducted a morphological investigation with light microscopy for comparison with the population described by Fjellberg (1989), examined the ultrastructure of the tegument and of the external organs using scanning electron microscopy (SEM), and revealed the phylogenetic position of *M. psocoides* within Collembola using the complete nuclear ribosomal DNA operon, with improved sampling in the Sminthurididae. The ecological, evolutionary, and systematic implications of our findings are discussed.

## 2. Methods

**Sampling.** The first sample of mosses growing on a concrete slab (Fig. 2A–C) was hand-collected on 12<sup>th</sup> February 2023 stored in a bag for 5 days at room temperature, then put on a Berlese (sample A). After 48 hours, *Mackenziella* specimens were noticed in the collection tube (99% ethanol). On 18<sup>th</sup> February 2023, the locality was visited again, the same habitat was sampled again twice: sample B and C, a few meters apart from each other, and each roughly twice the size of sample A. Additional samples were taken in the area, targeting similar mosses but growing directly on soil.

**Macrophotography.** Specimens from the second sampling were collected alive in a tube containing a moistened chunk of the original habitat. Macrophotographs of living individuals were taken using a Fujifilm X-T3, either with a Laowa Ultra-Macro 2.5-5X objective at f5.6 and 5× magnification, or mounted on a Leica S8AP0 stereomicroscope, at full magnification.

**Light microscopy.** Fifteen specimens (11 females, 4 males) were cleared in lactic acid and mounted on mi-

**Table 1.** List of species included in the phylogenetic analysis, with Genbank accession number. \*Accession to genome assembly (or biosample when assembly is yet unavailable), the extracted 18S and 28S rDNA sequences can be directly retrieved from the Zenodo data archive (<https://doi.org/10.5281/zenodo.8171774>).

Order	Family	Species	Genbank accession number	Data provider
Entomobryomorpha	Entomobryidae	<i>Lepidocyrtus violaceus</i>	OR149202	Schneider et al. in prep.
Entomobryomorpha	Entomobryidae	<i>Sinella curviseta</i>	GCA_004115045*	Zhang et al. (2019)
Entomobryomorpha	Isotomidae	<i>Desoria tigrina</i>	GCA_906901685*	Schneider et al. (2021)
Entomobryomorpha	Isotomidae	<i>Entomobrya marginata</i>	OR149203	Schneider et al. in prep.
Entomobryomorpha	Isotomidae	<i>Folsomia candida</i>	GCA_002217175*	Faddeeva-Vakhrusheva et al. (2017)
Entomobryomorpha	Isotomidae	<i>Folsomides angularis</i>	OR149205	Schneider et al. in prep.
Entomobryomorpha	Orchesellidae	<i>Orchesella cincta</i>	GCA_001718145*	Faddeeva-Vakhrusheva et al. (2016)
Neelipleona	Neelidae	<i>Megalothorax cf. minimus</i>	OR149198	Schneider et al. in prep.
Neelipleona	Neelidae	<i>Neelides folsomi</i>	SAMN25040855*	Collins et al. (2023) [preprint]
Neelipleona	Neelidae	<i>Neelus murinus</i>	SAMN25040856*	Collins et al. (2023) [preprint]
Poduromorpha	Poduridae	<i>Podura aquatica</i>	OR149201	Schneider et al. in prep.
Poduromorpha	Tullbergiidae	<i>Paratullbergia callipygos</i>	SAMN25040870*	Collins et al. (2023) [preprint]
Symphyleona	Katiannidae	<i>Sminthurinus elegans</i>	OR149196	This work
Symphyleona	Sminthuridae	<i>Sminthurus viridis</i>	OR149204	Schneider et al. in prep.
Symphyleona	Sminthurididae	<i>Mackenziella psocoides</i>	OR149199	This work
Symphyleona	Sminthurididae	<i>Sminthurides aquaticus</i>	GCA_906901655*	Schneider et al. (2021)
Symphyleona	Sminthurididae	<i>Sphaeridia pumilis</i>	OR149200	Schneider et al. in prep.
Symphyleona	Sminthurididae	<i>Stenacidia violacea</i>	OR149197	This work

crosscope slides in Marc-André II medium. Observations were done with a compound microscope with phase contrast, up to 100× magnification.

**Scanning Electron Microscopy (SEM).** Five specimens (2 females, 3 males) were transferred in 100% ethanol, critical point dried with a Leica EM CPD300 and platinum coated to a thickness of 7.13 nm with a Leica ACE600. Observations were carried out with a Hitachi SU3500 scanning electron microscope using 15 kV accelerating voltage and backscattered electron (BSE) for image magnifications ranging from 450× to 30,000×.

**DNA sequencing.** Genomic DNA (gDNA) was individually extracted from four specimens and an additional gDNA extract was made from a pool of five specimens, all using a modified protocol for the Qiagen MagAttract HMW extraction kit (Schneider et al. 2021). We also newly sequenced individuals of *Stenacidia violacea* (Reuter, 1881) and *Sminthurinus elegans* (Fitch, 1862) to improve the sampling of Sminthuridae and Katiannidae (Symphyleona). The ~6.4kb long nuclear rDNA operon was amplified with a single PCR, using primers newly designed (as part of a parallel work that will be separately presented) and the long range and high fidelity Q5® polymerase HotStart master mix (NEB). Forward primer: 5'-CTCAAAGATTAAGCCATGCATGTC-3', reverse: 5'-RAGTCTCAACGGATCGCAGC-3'. Amplification was done following NEB standard recommendations for the Q5 and using an annealing temperature of 65°C (computed using NEB Tm Calculator).

Two specimens, plus the pool, were amplified successfully. The amplicons were purified using the Qiagen MagAttract HMW kit purification steps and resuspending the purified DNA in water. The amount of purified

DNA was measured with a Quantus fluorometer (Promega) using the dsDNA assay kit. Libraries were prepared using the Nanopore Rapid Barcoding Kit 96 (SQK-RBK110-96). Amplicons were normalized to 50 ng prior to the tagmentation step and then pooled. Library preparation followed the standard protocol (protocol version RBK\_9126\_v110\_revD\_24Mar2021). The pooled library was sequenced on a Nanopore MinION using a Flongle flow cell (R9.4.1), and MinKNOW configured to run Fast basecalling. For each sequenced library, 1500 of the longest reads were selected and mapped to a reference sequence (*Folsomia candida*) using Geneious. A majority consensus was called after visual inspection and trimmed to the primer binding sites (excluded). The consensus was further polished by mapping 3000 of the longest reads on it.

**Phylogenetic reconstruction.** We used 18 collembolan species covering the four orders (Fig. 8, Genbank accession numbers provided in Table 1), for which the full-length nuclear rDNA was available. Fifteen sequences were obtained from third party projects. Sequences of *Desoria tigrina* Nicolet, 1842, *Folsomia candida* (Willem, 1902), *Orchesella cincta* (Linnaeus, 1758), *Sinella curviseta* (Brook, 1882) and *Sminthurides aquaticus* (Bourlet, 1842) were retrieved from publicly available genomes (Faddeeva-Vakhrusheva et al. 2016, 2017, Zhang et al. 2019, Schneider et al. 2021). The other nuclear rDNA sequences were obtained from, yet unpublished, PacBio based genome assemblies (Schneider et al. in prep.) and Illumina sequencing based genomes assemblies (Collins et al. 2023 preprint). Whole genomes will be released in a separate work. The sequences were aligned using MUSCLE (v3.8.31). Since one of the available species was missing the ITS1, 5.8S rDNA and

ITS2 (not assembled), we removed those regions from the alignment. The phylogeny was inferred under Maximum Likelihood criterion using IQ-TREE (v2.1.3; Minh et al. 2020), with automatic model test and selection (-m MFP), node support was assessed using nonparametric bootstrap using 100 replicates (-b 100) and SH-aLRT (-alrt 1000).

**Time calibration.** The same molecular data set was used to estimate the time of divergence of *Mackenziella*. The fossil record of Collembola is very scarce. Calibration was carried out using the most ancient known representative of a given group to provide an estimate of the age. The root age of Collembola is based on *Rhyniella praecursor* (at least 420 Mya); the age of Poduromorpha, Isotomidae, Entomobryidae and Sminthuridae are based on *Protodontella minicornis* Christiansen & Nascimbene 2006, *Proisotoma communis* Sánchez-García & Engel 2016b, *Entomobrya pilosa* Koch & Berendt 1854 and *Pseudosminthurides stoechus* Sánchez-García & Engel 2016a respectively. These were chosen from the exhaustive commented list of Collembola fossils by Sánchez-García and Engel (2016a,b) for an exhaustive and commented list of Collembola fossils, and the ages of amber inclusions were updated according to Seyfullah (2018). Divergence times were inferred using BEAST2 v.2.7.3 (Bouckaert et al. 2019), substitution models of all partitions were calculated using bModelTest package, calibrations were carried out with a relaxed lognormal molecular clock with an offset to the given group equal to the oldest estimated age of the fossil belonging to that group. Two independent runs of 150 10<sup>6</sup> generations were done. The first 22 % of the sampled trees were discarded as burn-in on checking likelihood trace plot, the tree files were then combined with LogCombiner and finally the resulting trees were summarized in a Maximum Clade Credibility with TreeAnnotator.

**Ancestral character states reconstruction.** The character states of the last common ancestor of *M. psocoides* and its closest found relative are inferred through direct optimization using an unweighted parsimony criterion (for discrete characters). Continuous ancestral character states (female maximal body size) are estimated with the function ace from the R package PHYTOOLS, using the Maximum Clade Credibility tree as input, and setting the method to ‘REML’ (Restricted Maximum Likelihood) and model to ‘BM’ (Brownian Motion). Maximal body sizes were directly observed or collected from literature (Fjellberg 1998a; Bretfeld 1999; Potapov 2001; Dunger and Schlitt 2011; Jordana 2012; Schneider 2017). The matrix of discrete morphological characters for Symphypleona is provided in Table S1, the body size of all sampled species is provided in Table S2. The complete analysis folder of the ancestral body size estimation is deposited on Zenodo (<https://doi.org/10.5281/zenodo.8171774>).

**Notes on species nomenclature.** The taxonomic status of worldwide populations of *M. psocoides* is arguably ambiguous, as only the specimens from Canary Islands

examined by Fjellberg (1989) are described with complete chaetotaxy details. The South African populations were referred as *M. cf. psocoides* (Liu et al. 2012). In this work we refer to *M. psocoides* as a single species (see Discussion). We decided to not abbreviate the genera names *Sphaeridia*, *Sminthurides* and *Stenacidia* in species names, to avoid confusion.

**Morphological nomenclature.** Nomenclature of the eye follows Guthrie (1906), nomenclature of the labial palp follows Fjellberg (1998b), nomenclature of male antennal article II and III after Massoud and Betsch (1972) and Fjellberg (1989). Abbreviations used: **Abd.** – Abdominal segment, **Ant.** – antennal article, **MACO** – male antennal clasp organ, **Th.** – Thoracic segment.

## 3. Results

### 3.1. Ecology

*Mackenziella psocoides* was found in the shallow matress of mosses (dominant *Brachythecium albicans* and intermixed *Ceratodon purpureus*) growing on a path of concrete slabs in a peri-urban context (Fig. 2A–C). The path is enclosed between a road and a concrete sidewalk, with only a thin border of ground covered with herbs. The mosses were sampled with its substrate, being a 1–2 cm deep layer of organic matter and soil particles mixed with coarse sand and small gravels. At the time of collection, the sample was humid, but unfrozen (sampling occurred 24 hours after the last frost event). The second collection occurred after one week of cold temperatures (two nights near 0°C, and up to 10°C), without precipitation. The day of collection itself was rainy and the new samples were well-moistened.

*Mackenziella psocoides* was very abundant in sample A (> 100 specimens), and in low abundances in sample B (4 specimens) and C (~ 30 specimens) (B and C each being roughly twice the size as sample A in terms of moss sampled). Even in sample A, *M. psocoides* was evidently the smallest contribution to the overall collembolan biomass.

*Hypogastrura vernalis* (Carl, 1901) was dominant in terms of relative abundance and biomass in all three samples. *Hemisotoma thermophila* (Axelson 1900) was rather common in all samples. *Folsomides angularis* (Axelson, 1905) was common in sample B but absent from A and C. Sminthuridae sp. (likely *Sminthurides* sp., only juveniles found, whitish, each with the tibiotarsal III organ) was rather common in sample A, and almost absent from sample B and C. *Sminthurinus cf. elegans* was found in low numbers in all samples. *Sphaeridia pumilis* (Krausbauer, 1898) was found only in sample C, in roughly the same numbers as *M. psocoides*. *Lepidocyrtus lanuginosus* (Gmelin 1790), *Orchesella cincta* (Linnaeus, 1758) and *Agrenia* sp. were found in moderate abundance (but high biomass) in sample A and C, but not B.



**Figure 2.** Habitat of the population of *M. psocoides* studied in this work, **A** context, **B**, **C** close-up.

### 3.2. Morphological examination

#### *Mackenziella psocoides* Hammer, 1953

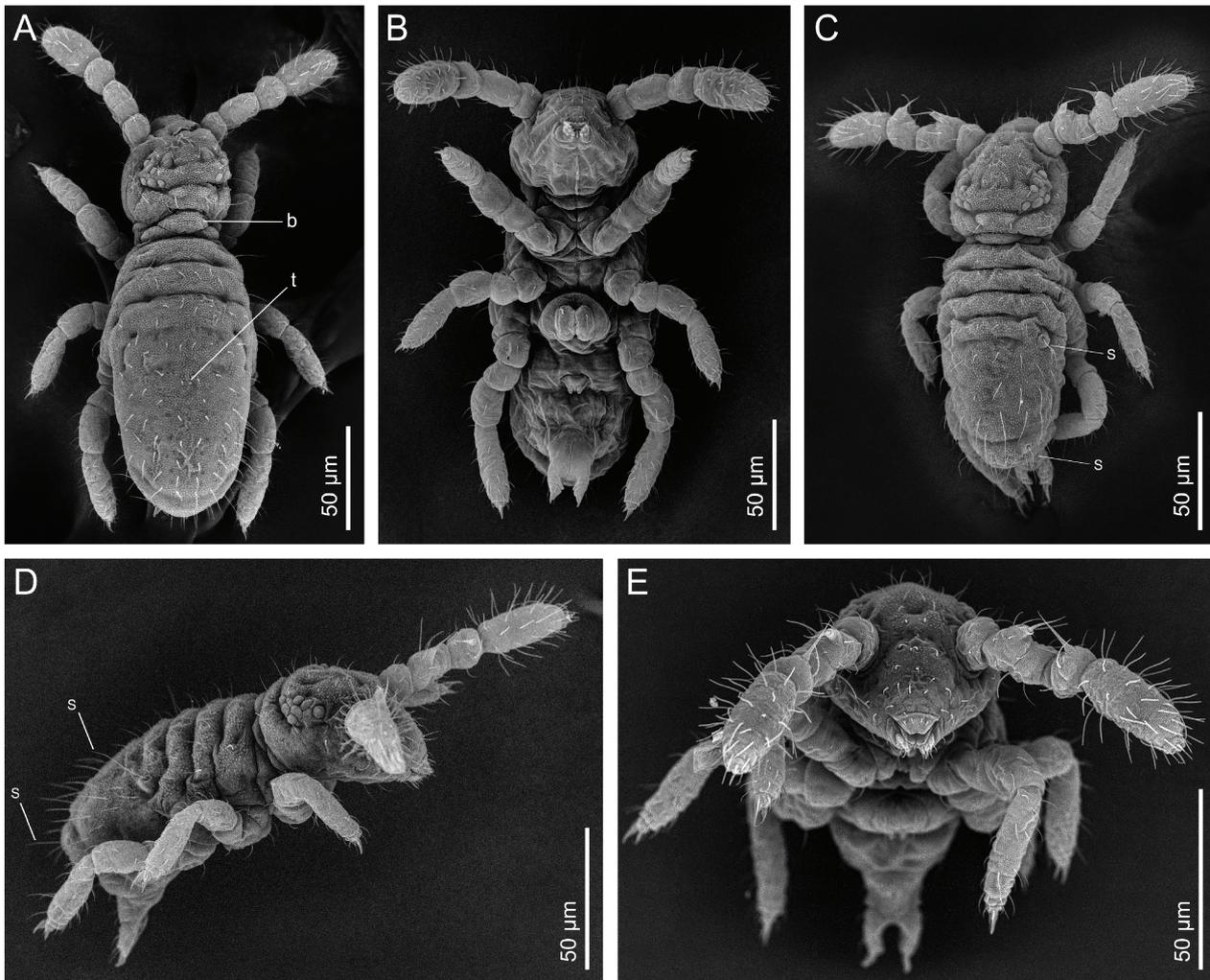
**Material examined for morphology.** Eight females and three males on eleven slides; Germany, Saxony, Tauchritz near Görlitz; 51.0689°N, 14.9340°E, alt. 210 m; 12 Feb. 2023; C. Schneider leg.; mosses and shallow substrate on a concrete slab; extracted with Berlese funnel; deposited in the Apterygota collection of the Senckenberg Museum für Naturkunde Görlitz; slides number AA00001 to AA00011. Three females and a male on four slides; same data as above; deposited in the Apterygota collection of the Muséum National d'Histoire Naturelle, Paris; slides number EA060065, EA060066, EA062721 and EA062722, SEM plate number EA030050.

**Additional description.** Our specimens are very similar to the descriptions of Fjellberg (1989, 1998b, 2007), which we do not intend to fully repeat here. Nonetheless, we report a few additional observations and precisions.

**Habitus as in Figs 1D–K, 3A–E.** Male with a higher ratio length head/trunk than female (Fig. 3A–D). Clypeal area reduced (Figs 3E, 5A). Dorsally on head, with a deep

transverse groove posterior to the eyes (Fig. 3A, C, D). With a dorsal bulge *b* at the head–Th. I insertion (Fig. 3A, D). With four dorsal bulges corresponding to Th. II–Abd. III, well-marked in either living, ethanol preserved and dried specimens (Figs 1D–J, 3A, C, D), but almost erased in lactic-acid induced swollen specimens. Abd. I and II bulges well-marked in male, but faint in female (Fig. 3A, C, D). Abd. IV delimited from Abd. V by a faint groove (Fig. 3A, C, D). Dorsally, Abd. III + IV region twice as long as Abd. I + II. Ventrally, retinaculum (Abd. III appendage) at mid-distance between the ventral tube (Abd. I) and the furca (Abd. IV appendage). Furca short, barely reaching the posterior side of the ventral tube when folded.

**Integument.** Integumentary secondary granules resulting from simple and individual outgrown primary granules (increased in size and elevated above the ordinary primary grain) (Fig. 6E). Presence limited to: postantennal area dorsally to the eyes (Fig. 5A, B), head–Th. I dorsal bulge, dorsal and lateral part of Th. II–Abd. V (Figs 3A, C, D, 6A). Absent from clypeal area and mouth part (Fig. 5A) and from all appendages. Dorso-median line of Th. II–Abd. III not marked, but terminal tubercle *t* present as a roundish prominence without secondary granules (Figs 3A, 6E). *Linea ventralis* straight, without additional in-



**Figure 3.** *Mackenziella psocoides* habitus SEM microphotographs. **A** dorsal view, female, **B** ventral view, female, **C** dorsal view, male, **D** lateral view, male, **E** frontal view, male. Abbreviations: b – head-Th. I bulge, t – integumental tubercle, s – sensillae in a depression on a papilla. Scale bars: 50 µm.

tegumentary channels (Fig. 4A), associated with three pairs of tubercles (Fig. 4A). Males without vesicles on Th. III.

**Antennae.** Chaetae smooth, without ornamentation (Fig. 4B–F). Most of the long chaetae of Ant. IV with a rounded apex (Fig. 4C–E). Male also with two long s-chaetae with rounded apex on Ant. III and three on Ant. II (Fig. 4B, E, F); ms on Ant. III as a microchaetae (Fig. 4B, F) (otherwise as described in Fjellberg 1989).

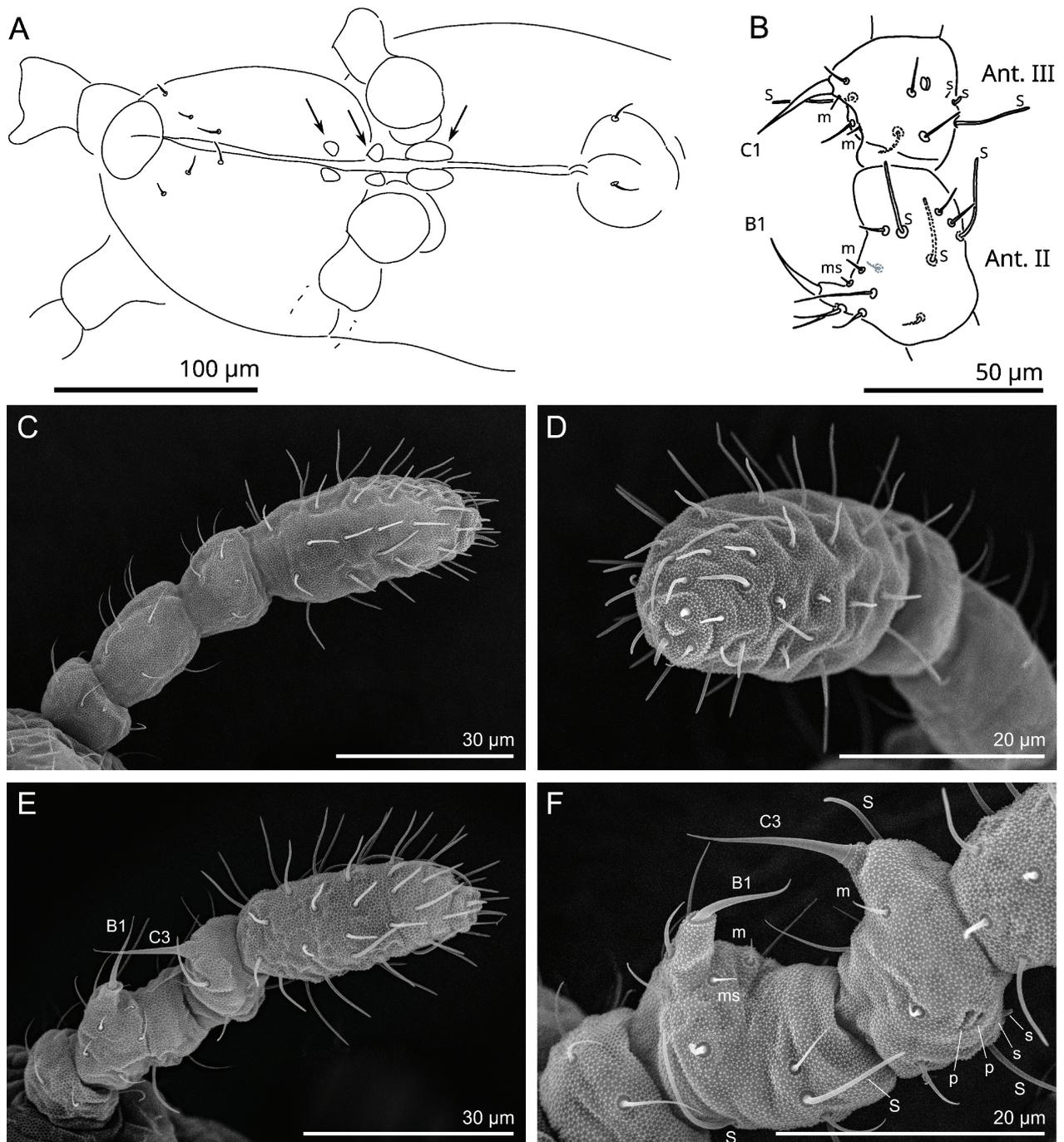
**Mouth.** Mouth as in Fig. 5C, without oral fold nor maxillary outer lobe. Labrum and labium as described in Fjellberg (1989, 1998b).

**Legs.** Tibiotarsus I apical row with chaeta *ja* flattened with an external groove, and appressed to the tegument (not erected), on ventral side (Fig. 6B). Unguis with a dorsal smooth lamella splitted in two basally, the two anterior and posterior halves each joining with a small pseudonichya, the dorsal, basal side of the claw forming a depression covered with primary grain (Fig. 6C). Unguiculus I to III respectively with: apical filament, short

apical filament, no apical filament (Fig. 6B–D); unguiculus tri-lamellate, each lamella with a smooth ridge (Fig. 6B–D).

**Furca.** Posterior part of dens with up to four chaetae ornamented with spicules (discovered with SEM, apparently smooth in some specimens) (Fig. 7B–D). Mucro either separate or fused to the dens (as reported in Fjellberg 1989 for juveniles), but fused form may be found in apparently mature specimens. Mucro posteriorly without lamellae, anteriorly, with a smooth lamella, either with an inner groove (separate mucro form) or not (fused mucro form). This smooth lamella extending to the apex of the mucro and shaping its rounded tip.

**Other ventral organs.** Sternite of Abd. IV with a pair of small chaetae (Fig. 7A) not mentioned in Fjellberg (1989). This pair of chaetae sometimes missing or incomplete (only on one side). Probably homologous to the ventral-anterior field of chaetae found on Abd. IV of most Symphypleona. Ventral tube of the male simple (Fig. 5D). Retinaculum simple, with 3+3 teeth and no basal tubercle (Fig. 7E).



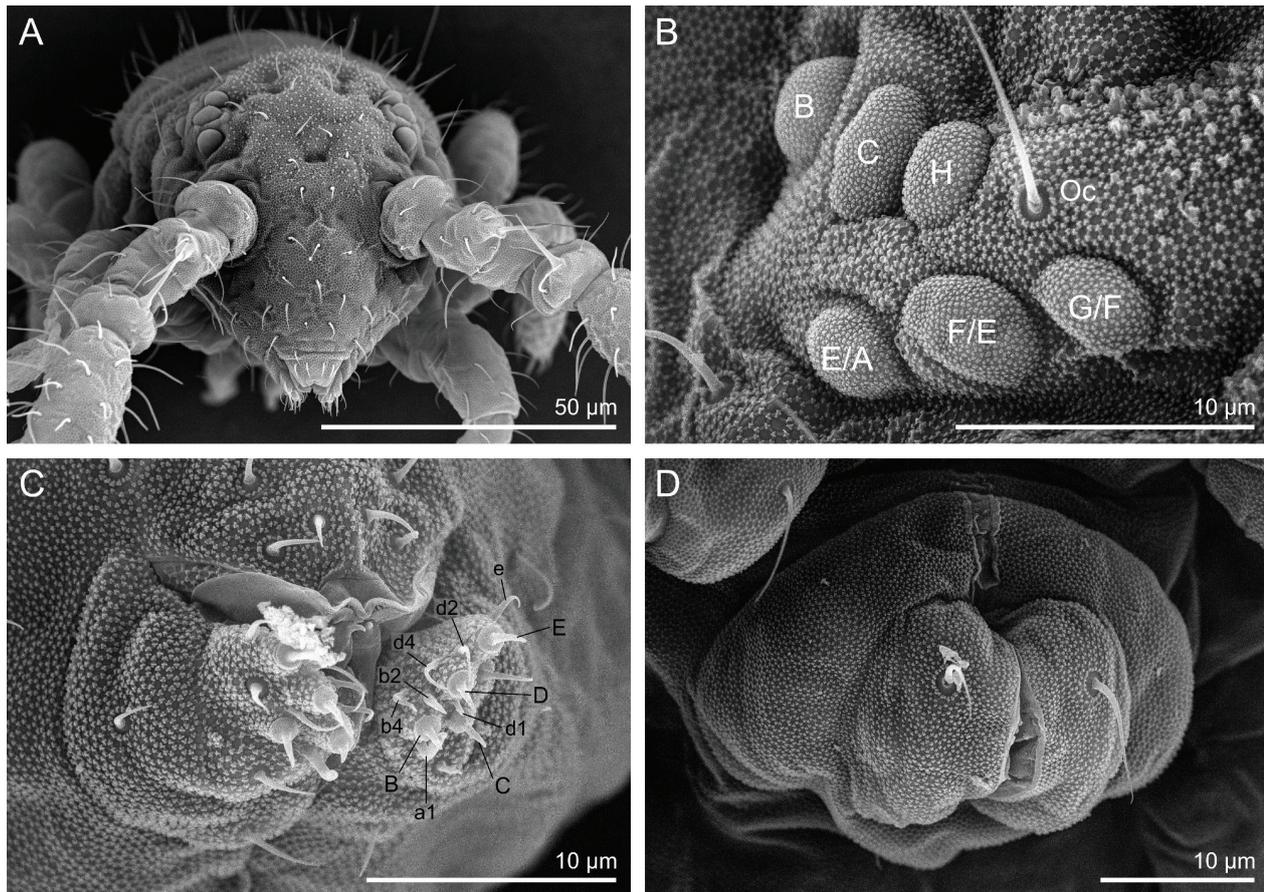
**Figure 4.** *Mackenziella psocoides*. **A** *Linea ventralis*, arrows indicate the ventral tubercles, **B** male ant. II and III (clamping organ), posterior side. Antennae SEM microphotographs. **C** right antenna dorsal view, female, **D** tip of antennal segment IV, female, **E** left antenna fronto-ventral view, male, **F** right antenna dorsal view, male. Abbreviations: B1 – chaeta b1, C3 – chaeta c3, m – microelement setiform, ms – microelement spine-like, p – Ant. III organ deep lateral pit, s – Ant. III organ sensillum (one very small, one larger), S – Ant. III large s-chaetae with rounded apex.

### 3.3. Sequencing and Phylogeny

The three sequenced libraries for *M. psocoides* resulted in three identical sequences. Thus, a single sequence was used to represent the species in the phylogenetic tree. The recovered tree (Fig. 8) was compatible with the monophyly of all orders. *Mackenziella psocoides* was found to be the sister group of *Sphaeridia pumilis*, this node being the sister of another clade formed by *Sminthurides aquaticus* and *Senacidia violacea*, all supported with

high bootstrap support ( $\geq 99\%$ ) in the ML tree and with maximum posterior probability (1) in the Bayesian analyses. In the rest of the text, we refer to Sminthuridae as a monophyletic group, including *M. psocoides*. Within Symphypleona, the relations were as such: (Katiannidae, (Sminthuridae, Sminthuridae)) with a moderate support to the basal node (bootstrap = 84.4%).

The mean crown age for *Mackenziella* + *Sphaeridia* was estimated at ~79 Ma (42–113 Ma). The crown age of *Sminthurides* + *Senacidia* was estimated at 68.79



**Figure 5.** *Mackenziella psocoides* SEM microphotographs. **A** head frontal view, male, **B** eyes, A, B, C, E, F, G, H – ocelli, Oc – ocular chaeta, **C** mouth, A, B, C, D, E – labial palp apical papillae, a1, b2, b4, d1, d2, d4, e – labial palp guard chaetae, e – labial palp guard chaetae. **D** ventral tube.

Ma (31–106), the crown age of Sminthurididae at ~126 Ma (113–158). The origin of the four Collembola orders seems to be rooted in the Paleozoic (or possibly Mesozoic considering the lower part of the range), with mean crown age of ~261 (169–363) Ma, ~285 (194–398) Ma, ~159 (100–309) Ma and ~336 (211–427) Ma for Entomobryomorpha, Symphyleona, Poduromorpha and Neelipleona respectively. These results have to be taken with caution, considering the restricted taxon sampling used in our analyses and, more importantly, the known collembolan fossil record being very scarce. The ML and Bayesian trees are independently shown in Fig. S1 and Fig. S2. The complete analysis folder is deposited on Zenodo (<https://doi.org/10.5281/zenodo.8171774>).

### 3.4. Ancestral character states reconstruction

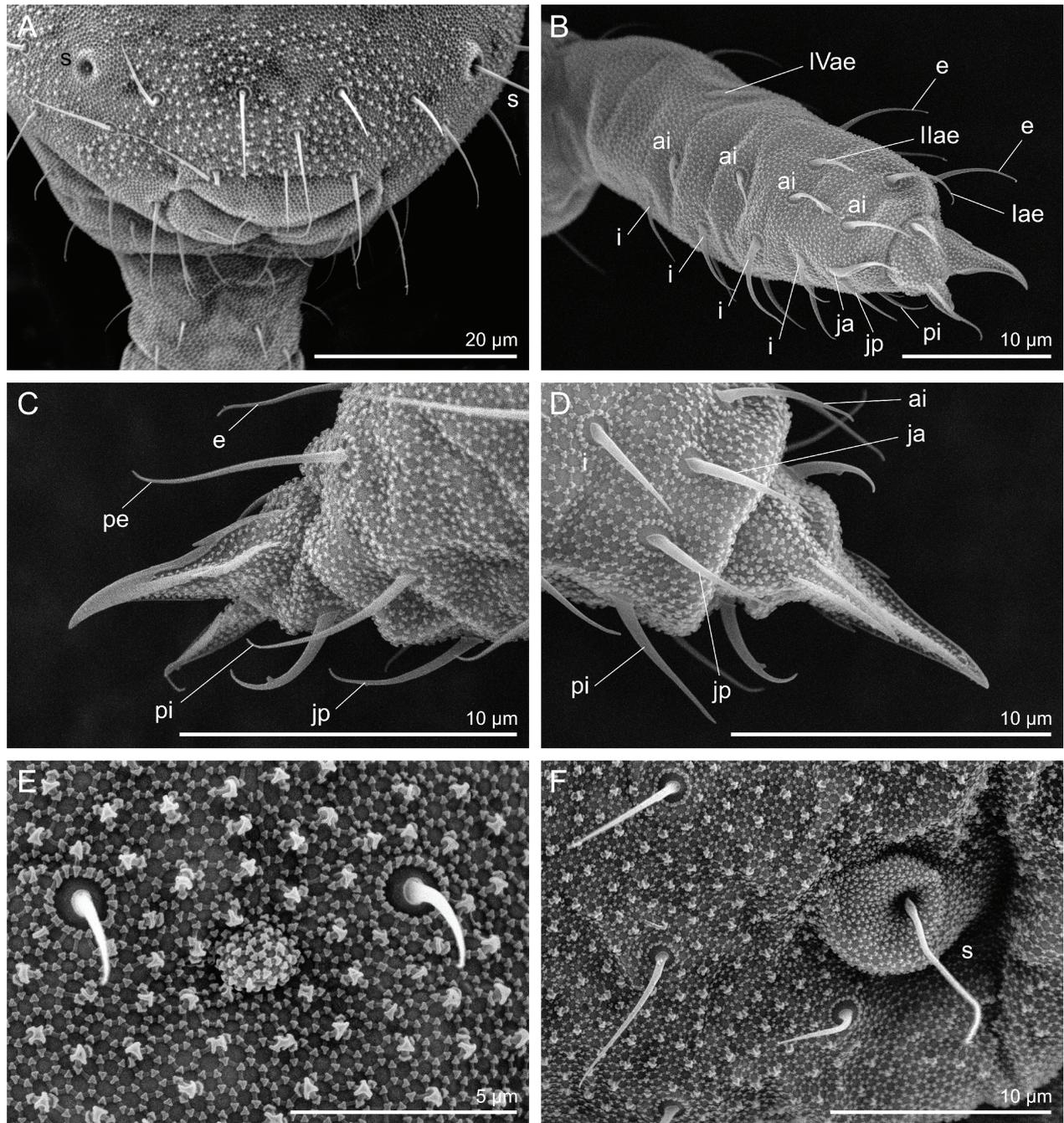
No evident morphological synapomorphies of *Sphaeridia* + *Mackenziella* could be found. Direct optimization of character states at the (*Sphaeridia*, *Mackenziella*) node results in: globular body shape, orthognathous head, simple MACO, mouth parts present, slender antennae, large clypeal area, complete mouth parts, eyes with eight ocelli, prothorax without prominent bulge, absence of vesicles on metathorax (male), long furca at least reaching

the prothorax segment when folded under the body, long mucro with a pair of posterior lamellae, retinaculum with presence of chaetae (adult) and basal tubercles, five pairs of abdominal trichobothria, absence of Tibiotarsus III organ. Those ancestral character states are all unchanged in the genus *Sphaeridia*, and also apply to the Sminthurididae ancestor (but state of MACO arguably ambiguous). The ancestral body size estimation indicates a reduction of the size in branch leading to *Sphaeridia* and *Mackenziella* (with an ancestor estimated around 650 µm, against 910 µm for the ancestor of Sminthurididae). The tree annotated with all estimated ancestral body sizes is provided in Fig. S3.

## 4. Discussion

### 4.1. Species distribution

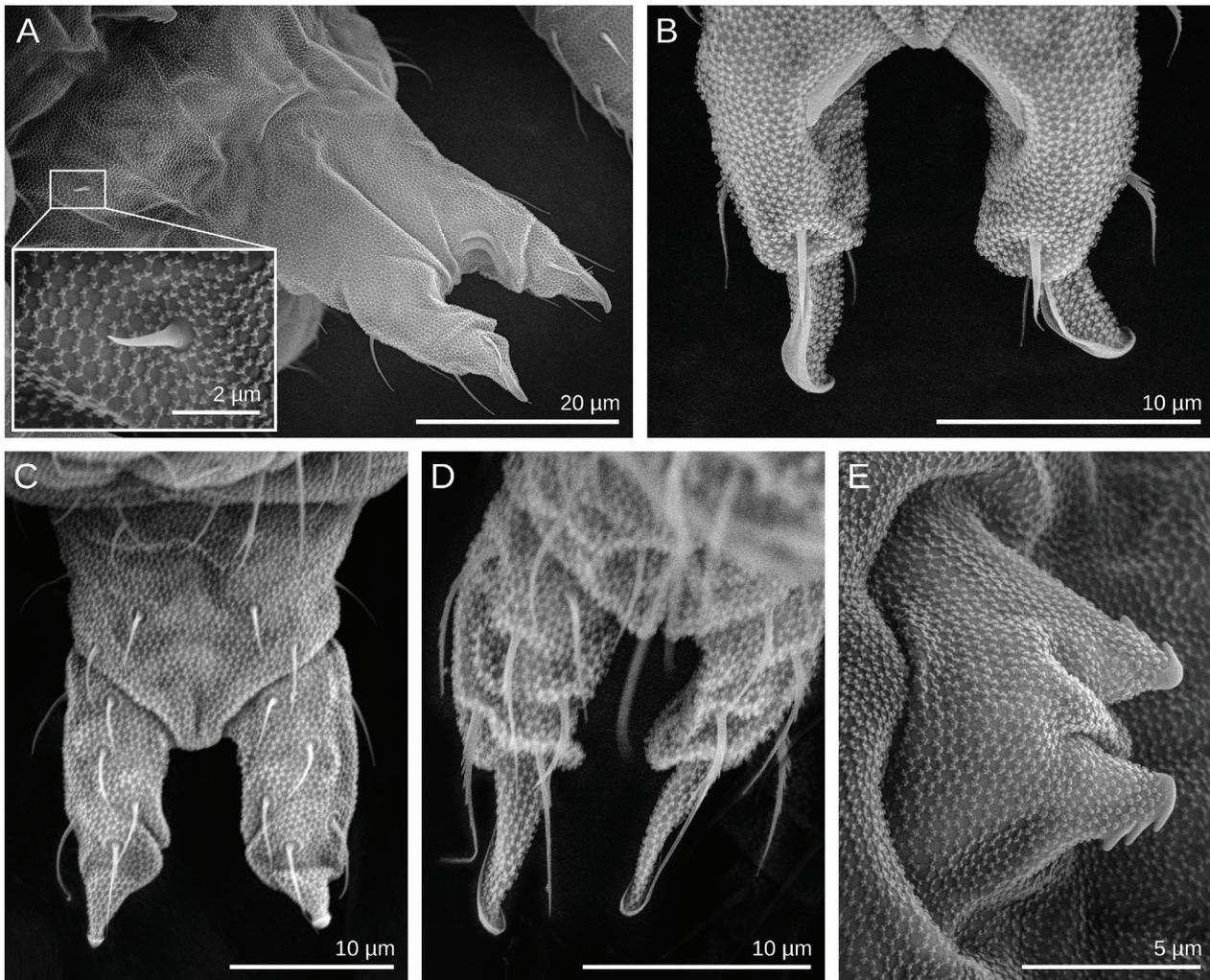
*Mackenziella psocoides* is reported from North America (Hammer 1953), Scandinavia (Fjellberg 1988, 2007), Western and Central Europe (Hüther 1961, Pomorski 2000, Berg 2018, this paper), North Africa (Fjellberg 1989) and South Africa (Liu et al. 2012). Known localities are indicated in Fig. 9 and Table S3. Despite this



**Figure 6.** *Mackenziella psocoides* SEM microphotographs. **A** Abdominal segment V and VI (anal valves), female, **B** tibiotarsus I ventral view, **C** tibiotarsus II dorso-lateral view, **D** tibiotarsus III ventral view, **E** Abd. III middorsal tubercle with first pair of axial chaetae, **F** Abd. II sensillum in a depression on a papilla, male. Abbreviations: s – sensillae, ae, ai, e, i, ja, jp, pe, pi – tibiotarsus chaetae, I, II, IV – tibiotarsus chaetae row.

wide distribution range, the species is uncommonly found. Of the two dozen reported findings, only Fjellberg (1989) found it in very large abundance (about 150 specimen), in the Canary Islands (Tenerife, La Palma and La Gomera). Another population of *Mackenziella* is regularly found in the region of Cape Town, South Africa (Liu et al. 2012 and C. Janion-Sheepers pers. comm.), where it is assumed to be invasive (Liu et al. 2012). Our specimens could not be distinguished from the description by Fjellberg (1989) based on specimens from the Canary Islands. Given that Fjellberg (1988, 2007) also recognized the same species in Scandinavia, we assume

that the European and North African populations belong to the same morphological species. Hammer (1953) original description based on two specimens from Canada, indicated 4+4 teeth on the retinaculum (instead of 3+3 as in our specimens). Fjellberg (1989) examined the type specimens but could not distinguish the actual state of their retinaculum. Novel collection in the Nearctic and in the Southern Hemisphere will be necessary to confirm the morphological homogeneity of *M. psocoides* populations worldwide. Since some collembolan species are known to hide a large molecular diversity behind homogeneous morphology (e.g., Schneider and D'Haese 2013,



**Figure 7.** *Mackenziella psocoides* furca SEM microphotographs. **A** furca anterior side, female; detail: chaetae on lateral side on base of furca, **B** furca anterior side, female, **C** furca posterior side, female, **D** furca posterior side, male, **E** retinaculum.

von Saltzwedel et al. 2017), further genetic investigations are also desirable.

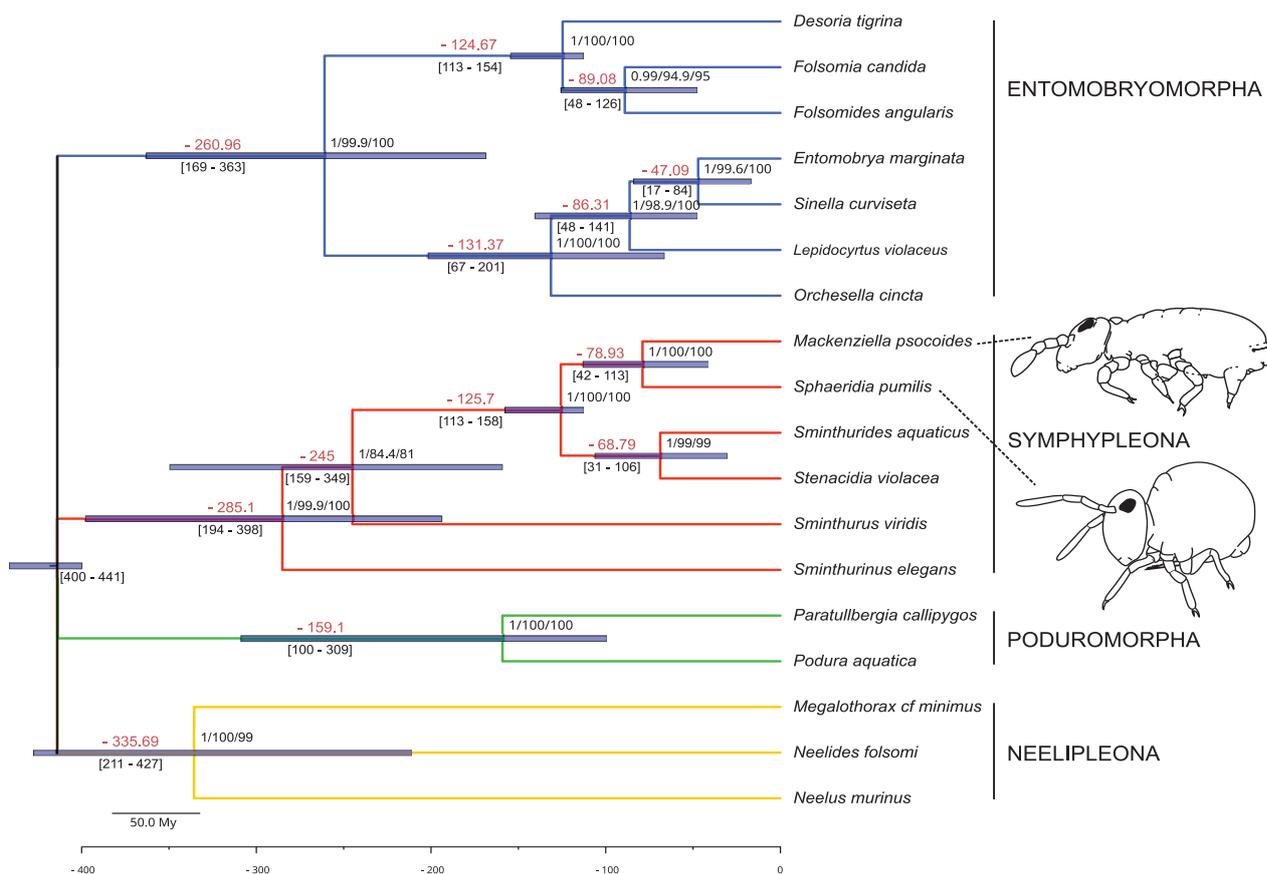
## 4.2. Species ecology

*Mackenziella psocoides* is related to poor habitats exposed to drought: moss and vegetation on sand and rocks, sandy meadow (Berg 2018). In the Canary Islands, it was found on wind swept open ridges in the forested zone with sparse moss cover on the ground (A. Fjellberg, pers. comm.). It was found in proximity to a coal mining site near Spremberg in Germany (D. Russel, pers. comm.); we sampled this locality in November 2022 but could not find it there. The population sampled by us for this paper was apparently restricted to the concrete slabs path; similar mosses sampled around, but growing directly on the ground, did not yield it. In summer, the shallow substrate on the slabs is likely to dry out, without any options for the individuals to escape in the depth. Fjellberg (1989, 2007) suggested *M. psocoides* to be adapted to dry habitat through drought resistant eggs. Berg (2018) reached the same conclusions by reviewing all the find-

ings of *M. psocoides* so far, indicating a preference for nutrient-poor, exposed habitats with shallow vegetations. This trait could also have allowed *M. psocoides* to reach a cosmopolitan distribution through airborne dispersal of the eggs (Fjellberg 1989). Our sampling shows the ability of *M. psocoides* to reach and colonize a small island of suitable habitat.

*M. psocoides* seems also to have a winter affinity. It was active shortly after the defrosting of its habitat. The defrosting may have triggered a rapid bloom of the population from diapause eggs, which was then already in decline one week later. However, fine observations would be needed to ascertain this. It is unclear if the individuals themselves can withstand drought or frost through mechanisms of anhydrobiosis or cryoprotective dehydration known in several springtail species (Holmsstrup 2018 and references therein).

Among the springtails found in the same habitat, we find notable the presence of *F. angularis* and *Sphaeridia pumilis*, both widespread and common species. *Folsomides angularis* is an indicator of dry habitats, well known for coping with drought through anhydrobiosis (Belgnaoui and Barra 1989). In Europe, it is common-



**Figure 8.** Maximum clade credibility tree based on the trimmed alignment of the combined 18S rDNA and 28S rDNA. Node support values: posterior probability/nonparametric bootstrap (100 replicates)/aLRT (1000 replicates); blue bars indicate 95% HPD intervals of the age estimates, age estimate in red above the branches. Nodes with less than 75% bootstrap or 0.7 posterior probability support are collapsed. Habitus of *Sphaeridia pumilis* and *M. psocoides* represented next to the corresponding labels (*M. psocoides* drawing modified after Fjellberg 1989).

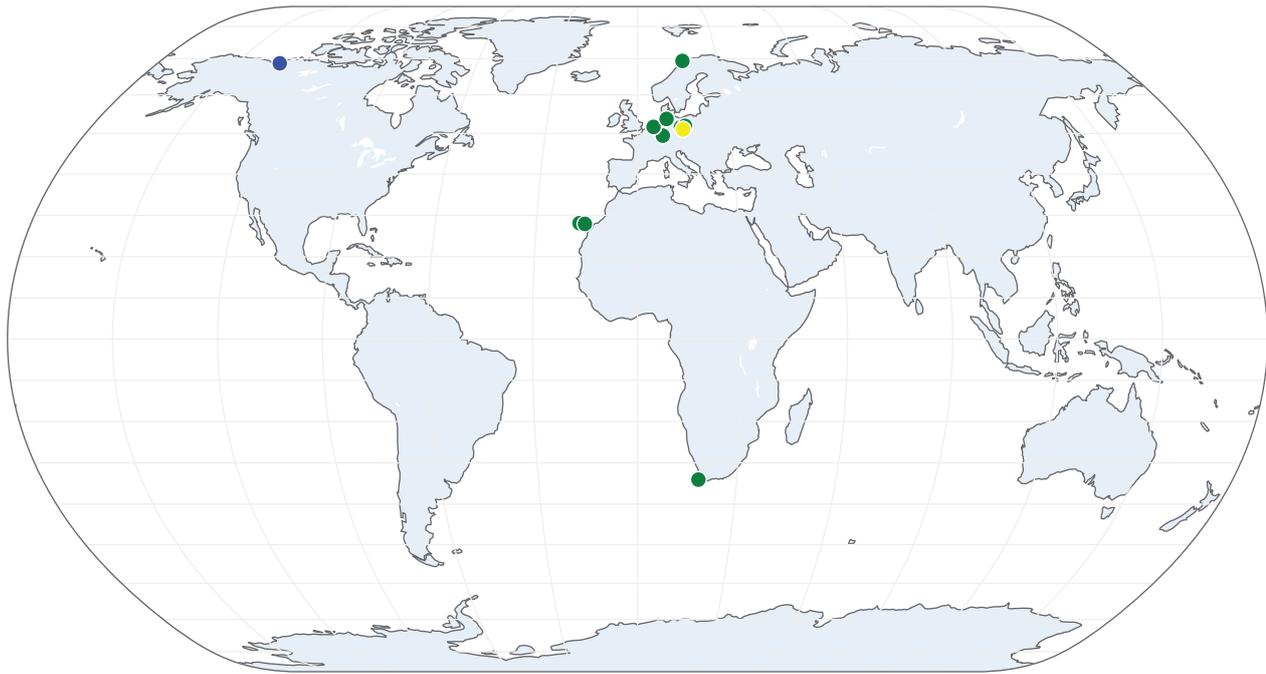
ly found in low-growing vegetation on exposed rocks (Fjellberg 2007), and it can settle in extreme environments such as the Namib desert gravel plains (Collins et al. 2019). *Sphaeridia* are the closest relatives of *M. psocoides*. *Sphaeridia pumilis* and *M. psocoides* were found together in one of the samples. This proximity was also observed by Fjellberg (1989) in the Canary Islands, who extracted a few individuals of *M. psocoides* from *Euphorbia balsamifera* litter and sand samples (collected dry, wetted before Berlese extractions), among several hundreds of *Sphaeridia pumilis* and *Folsomides* species (dominant *F. terrus* Fjellberg, 1992) (Fjellberg, comm. pers.). While we did not find any further mention of specific drought resistance for *Sphaeridia pumilis*, drought resistant eggs have been reported in *Sphaeridia* (Greenslade 1981). This trait could be ancestral to *Mackenziella* + *Sphaeridia* lineage. However, *Sphaeridia* does not only contain xerophilous species: the species from the tropical cloud forest of Ecuador (Bretfeld and Trinklein 2000) are likely never exposed to drought. The reduction of the mouthparts in *Mackenziella* (compared to the well-developed chewing-type mandibula in *Sphaeridia*) and its rarity suggest some specialization in food resources and micro-environmental conditions, while the co-occurring *Sphaeridia pumilis* may be more generalist.

We found *M. psocoides*, *F. angularis* and *Sphaeridia pumilis* in the same habitat, but with low overlap in the three distinct samples, hinting at a possible space or time exclusion of the species at fine scale. Further sampling is required to understand the dynamic of the springtail community in exposed habitats.

### 4.3. Phylogeny

Our phylogenetic inference based on the nuclear rDNA, not only confirms Fjellberg (1989) views that *Mackenziella* is related to Sminthurididae but reveals that *M. psocoides* is a member of Sminthurididae, actually a close relative to *Sphaeridia*. The precise phylogenetic positioning of *Mackenziella* allows to draw its singular evolutionary history, as a member of Sminthurididae that reverted to an elongated body shape. The present phylogeny also indicates the paraphyly of Appendiciphora, with two possible evolutionary scenarios:

- (1) the independent acquisition of the female anal appendages: once in Katiannidae (and presumably Arrhopalitidae not sampled here), and once in Sminthuridae (and presumably Bourletiellidae and Dicyrtomidae not sampled here)



**Figure 9.** Reported findings of *M. psocoides*. Marker: blue – type locality, yellow – this work new locality, green – previous records.

(2) loss of those appendages in Sminthurididae.

The bootstrap support remained weak and we do not aim to further address this question here.

#### 4.4. Comparative morphology

**Eye.** In most Sminthurididae, the eye is composed of eight ocelli (labeled A to H after Guthrie 1906). The ocelli C and D are usually reduced, e.g., in *Sminthurides aquaticus* and *Sphaeridia pumilis* (Fig. 10A, B). In species reported with reduced eyes such as *Sminthurides sexoculatus* Betsch and Massoud, 1970, C and D are missing. Palacios-Vargas et al. (2018) mislabelled the ocelli in the eye of *Denisiella betschi* Palacios-Vargas et al., 2018 and *Denisiella rhizophorae* Palacios-Vargas et al., 2018, and the small ocelli C and D have been overlooked (reduced, but still visible on their figs 12A and 25, in a similar configuration than in *Sminthurides* and *Sphaeridia*).

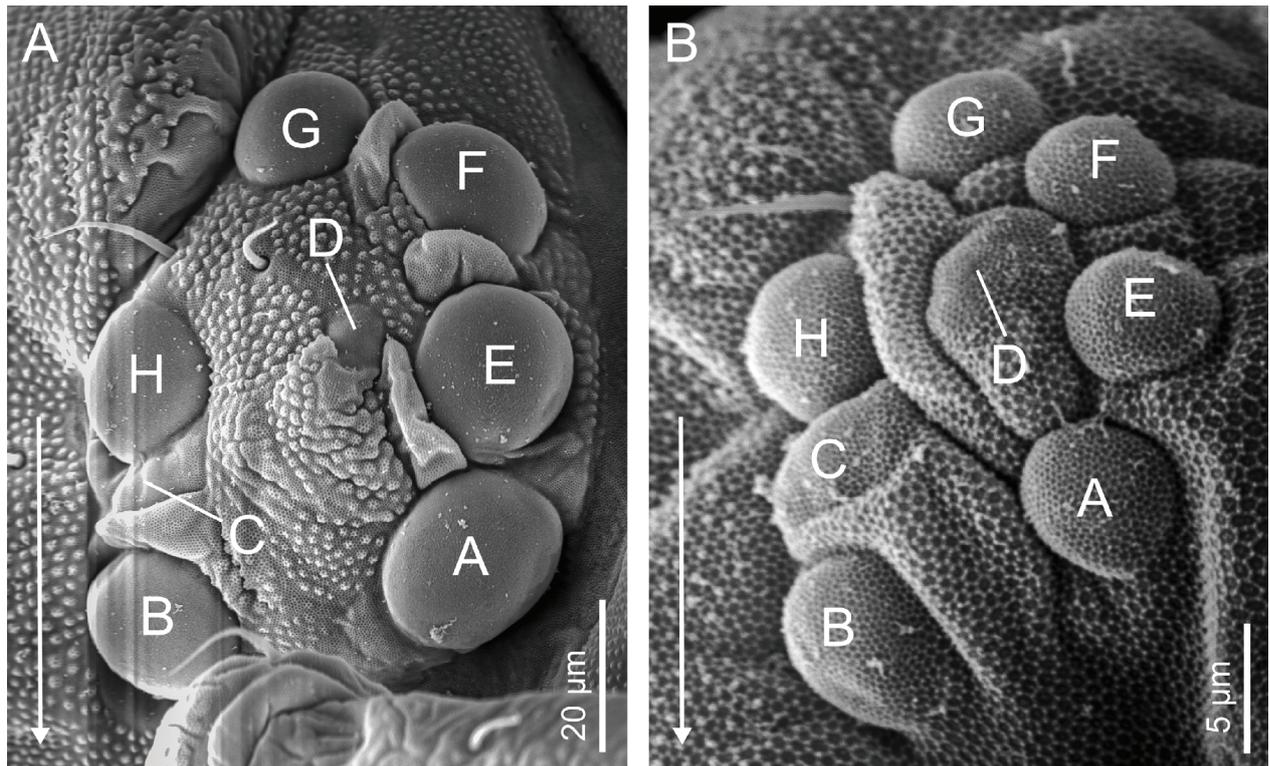
The reduction of the eye in *M. psocoides* can be described as follows: enlargement of C, loss of D, loss of either A or G (Fig. 5B). In *Mackenziella* a single chaeta is found between H and G, compared to two chaetae in the other genera of Sminthurididae (Figs. 5B, 10A) (state unknown in *Sminthuridia* Massoud and Betsch, 1972).

**Male antennal clasp organ (MACO).** The four represented genera of Sminthurididae in our dataset form two clades: one including species with larger body size and complex MACO (*Sminthurides* + *Stenacidia*) and one with smaller species with simple male antennal clasp organ (*Sphaeridia* + *Mackenziella*). Following a parsimony criterion, one would assume the simple MACO to be the ancestral trait of Sminthurididae. Indeed, all Sminthurididae possess the modified chaetae of the sim-

ple clasp organ (Massoud and Betsch 1972, refer also to fig. 1 in da Silva Medeiros 2022). Those are, on Ant. II: a large chaeta (*b1*) on a tubercle, at least one additional smaller chaetal element (two elements only in *Sminthuridia* Massoud and Betsch, 1972) being small spines in the simple organ version. On Ant III, the simplest form seems limited to the large chaeta *c3*, in *Sminthuridia*. In *Sphaeridia* and *Sminthuridia*, the modified macrochaetae, possibly homologous to *b2* and *b3* (Ant. II), were initially not named in the antennal nomenclature of the Sminthurididae (Betsch and Massoud 1970, Massoud and Betsch 1972). Massoud and Betsch (1972) noted the variability of their presence in *Sphaeridia* (from 0 to 2), based on the original species description of the time, and represented a MACO devoid of additional chaetal element as the simplest form found in *Sphaeridia* (their fig. 9). da Silva Medeiros et al. (2022) followed this view, but associated the microchaetae of *Sminthuridia* to *b2* and *b3*. At least several species of *Sphaeridia* possess two microchaetae in the same region.

The presence of *b2* and *b3* is possibly a plesiomorphy in Sminthurididae, with subsequent loss in *M. psocoides* and some species of *Sphaeridia*. However, a detailed re-analysis of *Sphaeridia* and *Sminthuridia* morphology and their internal phylogeny are necessary to gain a fine understanding of the homologies and evolution of the MACO. On the other hand, the complex MACO involves a large number of shared sub-organs following a similar organization. Those organs are on Ant. II: at least a trichobothria (*Tra1*) and from one to three additional modified chaetal elements (*b4*, *b5*, *b6*) generally mounted on a tubercle. On Ant. III from one to two additional modified chaetal elements (*c1* and *c2*), and additional unnamed small spines and processes (variable among the genera).

Suprageneric subdivisions of the Sminthurididae based on the MACO were once suggested by Richard



**Figure 10.** Left eye SEM microphotographs in frontal view. **A** *Sminthurides aquaticus*, **B** *Sphaeridia pumilis*. Arrow points in the anterior direction.

(1968) (in its restricted extent at the time and under the name Sminthuridinae): the Sminthuridini Börner, 1906 grouped the genera with a complex MACO and the Sphaeridiini Richard, 1968 accommodated *Sphaeridia*, the only genus with simple MACO at the time (also known now is *Sminthuridia*). However, those subdivisions were rejected by Betsch 1980 and not followed by any subsequent authors.

**Claws.** We note that the structure of the claws of *M. psocoides* is not perfectly matching the general description provided by Betsch (1980) for Symphypleona, stating “the external surface of the claw is convex and always without tegumentary grain” (translated from French). In *M. psocoides*, this is true in the apical part of the claw. On the basal part, the external smooth surface covers only the anterior and posterior edges of the claw, where it joins the weakly developed pseudonychia (Fig. 6C). We observed a similar condition in *Sphaeridia pumilis*, and *Sminthurides aquaticus* (for the last species, it is ascertained in claw II and III, but unclear in the fine and elongated claw I). The trait is difficult to assess with light microscopy. Claw II of *Denisiella rhizophorae*, also fine and long, is almost fully smooth (Palacios-vargas et al. 2018, their Fig. 12F). Further analyses are needed to understand the evolution of the claws in Symphypleona.

**Tibiotarsus I.** The modification of the ventro-apical chaeta *ja* we reported in *M. psocoides* is also apparent in *Sphaeridia pumilis*. After verification, we recognized that this chaeta was generally modified in Symphypleona (seen in representatives of Sminthurididae, Katiannidae,

Sminthuridae, Arrhopalitidae, Dycirtomidae and Bourletiellidae), a fact that is overlooked in the major syntheses on this order (Richard 1968, Betsch 1980, Bretfeld 1999).

**Ventral abdominal chaetotaxy.** The reduction or total loss of the ventral, anterior group of chaetae on Abd. IV may be the result of a common neotenic evolution of *Mackenziella* and *Sphaeridia*.

#### 4.5. Systematic conclusions

From a morphological point of view, the grouping of *Mackenziella* and *Sphaeridia* lacks clear synapomorphies. The simple MACO does not have specific innovations that would be missing in the lineages with the complex MACO and may be interpreted as the plesiomorphic state within Sminthurididae. However, the nuclear rDNA operon brings a strong support to the clade.

We expect the Sminthuridini (Börner, 1906) to be a natural group, including the 10 genera of Sminthurididae with a complex MACO (da Silva Medeiros et al. 2022). The Sphaeridiini Richard, 1968 may de facto be used to regroup *Sphaeridia* and *Mackenziella*. However, Frans Janssens drew our attention to the homonymy of Sphaeridiini Richard, 1968 with Sphaeridiini Latreille, 1802 (Coleoptera: Hydrophilidae). We propose the replacement name Sphaeridiaini (type-genus: *Sphaeridia* Linnaniemi, 1912) formed on the entire generic name as the stem, as per recommendation 29A of the Zoological Code of Nomenclature. To adjust to the current taxonom-

ic levels in use in Collembola higher taxa (Bellini et al. 2022), we suggest to raise those taxa to the subfamily level (Sminthuridinae and Sphaeridiinae). Mackenziellidae is a junior synonym of Sminthurididae.

We consider the position of *Sminthuridia* to remain unclear. *Sminthuridia* possesses the tibiotarsal III organ, an apomorphic character also present in most of the (above defined) Sminthuridinae, but absent in *Sphaeridia* and *Mackenziella*. Betsch (1980) used the presence-absence of the tibiotarsal III organ for the first split of its determination key of the Sminthurididae, and also represented this split in his “phyletic scheme” of the Symphypleona, while stating that the character was not informative for the family evolution. The presence of the tibiotarsal III organ but absence of a complex MACO suggests a sister relationship between *Sminthuridia* and the Sminthuridinae.

#### 4.6. *Mackenziella psocoides* evolution

Our phylogenetic reconstruction (Fig. 8) indicates that the common ancestor of *Sphaeridia* and *Mackenziella* was a roundish Sminthurididae, bearing traits similar to modern *Sphaeridia* species. The ancestral body size estimation indicates that the ancestor of *Sphaeridia* and *Mackenziella* underwent some size reduction. Of course, our sampling is limited and do not allow for a precise inference of ancestral body size. *Sphaeridia pumilis* (0.5 mm) is among the largest species of the genus (minimal 0.3 mm observed in *Sphaeridia furcata* Dunger & Bretfeld, 1989). Most species from the other genera of Sminthurididae have a body size  $\geq 0.5$  mm (0.7 mm for *Stenacidia violacea* and 1 mm for *Sminthurides aquaticus*), smaller species being exceptional (e.g., 0.35 mm for *Sminthurides monnnoti* Massoud & Betsch, 1966). Since our sampled Sminthurididae species are in the upper size range of their respective genus, increased sampling would likely result in inferior or equal body size estimation for the ancestor of *Sphaeridia* and *Mackenziella*.

The transformation process from a *Sphaeridia*-like ancestor toward *M. psocoides* can be described as follows:

- (1) Shortening and bulkening of the antennae
- (2) Reduction of the clypeal area
- (3) Reduction of the maxilla and loss of the oral fold, the maxillary outer lobe and of the mandibula.
- (4) Straightening of the head from an orthognathic to a more prognathic position.
- (5) Reduction of the eye.
- (6) Stretching of the trunk from a globular shape toward an elongated shape (including increased distance between the retinaculum and the ventral tube). The trunk deflation emphasizes the dorsal bulges aligned with the chaetal pattern, well-marked until Abd. II. The bulges are more or less marked in other Sminthurididae (Fig. 1A–C).
- (7) Formation of a granulated bulge (b) that could be interpreted as the reformation of a pronotum (or analogous structure). Alternatively, it could originate from the head occiput, pushed back dorsally during the head returns to horizontal position.
- (8) Reduction of the furca from the long one reaching the first thoracic segment, toward a short one barely the ventral tube (when folded under the body).
- (9) Modification of the long mucro with a pair of posterior lamellae, typical for Symphypleona and Neelipleona (Bretfeld 1986, 1999, Schneider 2017), into a short mucro with an anterior hardened ridge.
- (10) Loss of chaetae and basal tubercles on the retinaculum.
- (11) Loss of three of the five pairs of abdominal trichobothria (one of those remaining pairs being further reduced to small s-chaetae in the female). As suggested by Fjellberg (1989), the strongly reduced chaetotaxy of Th. II tergite is inherited from its globular ancestor.

The shortening of the antennae and the reduction of the mass of the abdomen may be the result of a neotenic process, since those traits are also observed in juveniles of Symphypleona (Fig. 1K). Assuming a “*Sphaeridia*-like” ancestor, we can wonder what conditions allowed *M. psocoides* lineage to undergo this specific evolutionary trajectory. Fjellberg (1989) suggested that *M. psocoides* was adapted to reach food in the inter-leaf spaces of the mosses. In general, it is clear that *M. psocoides* gained a much slender profile compared to *Sphaeridia*, from body elongation and size reduction. The straightening of the head and the reduction of anterior trichobothria may indeed allow *M. psocoides* to reach as far as possible into reducing spaces. The persistence of one anterior trichobothria in the male complicates this straightforward scenario: perhaps the male focuses more on finding females than feeding (the male is also even smaller than the female).

## 5. Conclusion

By solving the phylogenetic placement of *M. psocoides*, we demonstrated that the species evolved from one of the most advanced globular body shapes observed in Symphypleona toward an elongated morphology. While adapting to a specialized lifestyle in drought exposed habitat, a globular *Sphaeridia*-like ancestor made an evolutionary U-turn to reacquire an elongated body and straighten up its head. Neotenic processes probably took part in *M. psocoides* evolution. Indicators of its success are the wide distribution of *M. psocoides* on earth, and its ability to occupy a niche in habitats dominated by ancestrally elongated species, such as the drought resistant species *F. angularis*. We assign *Mackenziella psocoides* to family Sminthurididae and classify it in the newly established subfamily Sphaeridiinae, together with *Sphaeridia*.

## 6. Data availability

The ten novel DNA sequences are deposited on GenBank ([www.ncbi.nlm.nih.gov/Genbank](http://www.ncbi.nlm.nih.gov/Genbank)), accession numbers for the whole dataset are provided in Table 1. Retrieved nuclear rDNA sequences, alignment and parameter files for the phylogenetic analysis, datation and ancestral character states estimation (IQTREE2, BEAST and PHYTOOLS) are deposited in Zenodo (<https://doi.org/10.5281/zenodo.8171774>).

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## Supplementary Material 1

### Tables S1

**Authors:** Schneider C, D'Haese CA (2023)

**Data type:** .csv

**Explanation note:** Matrix of morphological character for the species of Symphypleona represented in this study, used to describe the evolution of *Mackenziella psocoides* and its last common ancestor with *Sphaeridia pumilis*.

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**Link:** <https://doi.org/10.3897/asp.81.e104522.suppl1>

## Supplementary Material 2

### Table S2

**Authors:** Schneider C, D'Haese CA (2023)

**Data type:** .csv

**Explanation note:** Species body size data used for the ancestral character estimation.

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**Link:** <https://doi.org/10.3897/asp.81.e104522.suppl2>

## Supplementary Material 3

### Table S3

**Authors:** Schneider C, D'Haese CA (2023)

**Data type:** .csv

**Explanation note:** Known records of *Mackenziella psocoides*, compiled from literature and from Edaphobase.

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**Link:** <https://doi.org/10.3897/asp.81.e104522.suppl3>

## Supplementary Material 4

### Figure S1

**Authors:** Schneider C, D'Haese CA (2023)

**Data type:** .pdf

**Explanation note:** Maximum Likelihood tree computed with IQTREE2. Node labels as: nonparametric bootstrap/aLRT.

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**Link:** <https://doi.org/10.3897/asp.81.e104522.suppl4>

## Supplementary Material 5

### Figure S2

**Authors:** Schneider C, D'Haese CA (2023)

**Data type:** .pdf

**Explanation note:** Maximum clade credibility tree and age estimates computed with BEAST2, age estimates in right position to the nodes, values in bracket and blue bars indicate 95% HPD intervals of the age estimates.

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**Link:** <https://doi.org/10.3897/asp.81.e104522.suppl5>

## Supplementary Material 6

### Figure S3

**Authors:** Schneider C, D'Haese CA (2023)

**Data type:** .pdf

**Explanation note:** Ancestral body size estimated with the ACE function of Phytools. Full output available in the Zenodo repository (<https://doi.org/10.5281/zenodo.8171774>).

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**Link:** <https://doi.org/10.3897/asp.81.e104522.suppl6>