SOIL ORGANISMS 95 (2) · August 2023 pp. 143–153

A new species of Entomobrya (Collembola, Entomobryidae) from southwestern France exhibiting conspicuous sexual dimorphism

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Received 25 April 2023 | Accepted 1 June 2023
Published online at www.soil-organisms.de 1 August 2023 | Printed version 15 August 2023
DOI 10.25674/so95iss2id316

Abstract

Entomobrya fourcesensis spec. nov. was found in an agricultural grassland habitat in the Occitanie region in SW France and described here based on adult females and males.
Male habitus and colour pattern closely resemble those of *E. schoetti* (Stach, 1922), whereas female habitus and colour pattern closely resemble those of *E. lanuginosa* (Nicolet, 1842). Clear differences in chaetotaxy prove otherwise, and point to sexual dimorphism within the *E. fourcesensis* population. The closely related species are compared with the new species using the most important characters for taxonomic identification of *Entomobrya* species. Measurements of adult specimens, detailed drawings of colouration patterns, chaetotaxy and most important morphological characteristics are presented.

Keywords Taxonomy | springtails | biodiversity | chaetotaxy | meadow

1. Introduction

Collembola are extremely important ecosystem components. They are very abundant in soil and leaf litter and have a positive effect on soil health playing a key role in soil aeration and decomposition.They exert control over stability and functioning of ecosystems (Hopkins 1997).

During infield studies on surface- and plant-dwelling arthropod fauna of a grassland habitat in SW France, large numbers of Collembola were sampled using a variety of sample types. Among a wide diversity of species, an unknown species of *Entomobrya* was encountered displaying three levels of pigmentation. A highly pigmented, a medium pigmented and a pale form could be distinguished from each other. Juveniles as well as both sexes were encountered during examination. They were present during different sample rounds in different seasons.

In the genus *Entomobrya*, species determination is mainly characterized by pigmentation and chaetotaxy.

Based on colour and pigmentation patterns by Gisin (1960), pale specimens were initially determined as *E. lanuginosa* (Nicolet, 1842) and pigmented specimens as *E. schoetti* (Stach, 1922). Pigmentation and colour patterns have been shown to be very variable in Entomobryidae, particularly in the genus *Entomobrya* (Bellinger et al. 1996-2023, Jordana 2012). Large variation in colouration of individuals is not only present within a single species but also within a single population as shown by Jordana & Baquero (1999).

In addition, a more careful microscopic study of morphological characteristics and macrochaetotaxy after Jordana & Baquero (2005) has been performed and the results presented in this paper.

2. Materials and Methods

Multiple infield studies on surface- and plant-dwelling arthropod fauna of a grassland habitat in SW France were performed on an agricultural meadow parcel of

ISSN 1864-6417 (print) · **ISSN** 2509-9523 (online)

about four ha (43.99730, 0.20652). This is located close to Landes de Gascogne Regional Natural Park, which is a protected area of pine forest, wetland and oceanic coastline.

Sampling happened from spring till late autumn and the new *Entomobrya* species was seen throughout different seasons.

The selection of the optimal sampling method depends strongly on the purpose of individual studies and the ecology and behavior of the arthropod groups targeted. A combination of different suitable methods is highly recommended in many cases as stated by Zou et al. (2012).

Following sampling methods were used: pitfall trap sampling, soil core sampling, suction sampling, sweep net sampling, weed sampling and stratified activity based sampling of soil micro-arthropods after Bakker et al. (2017).

All specimens were preserved in a 70% ethanol solution and transported to the Test Facility (Eurofins / Mitox, Amsterdam, the Netherlands), for species identification.

Determination based on colour and pigmentation was done under a Leica MZ6 binocular microscope.

For a closer look, 42 adults (23 ♂,19 ♀) were mounted in Hoyer medium. To get a clear detailed image of certain morphological characteristics, some specimens were cleared with Nesbitt solution. The slides were studied using an Olympus BX40CY phase contrast microscope. Measurements were taken using a measuring eyepiece (HWF10M) and calibration was done with an Objective Micrometer slide. Drawings were made using an Olympus drawing attachment U-DA to the Olympus BX40CY phase contrast microscope. Pictures were made with the Leica MC 170 HD camera attached to the DIC Leica DM 2500 LED.

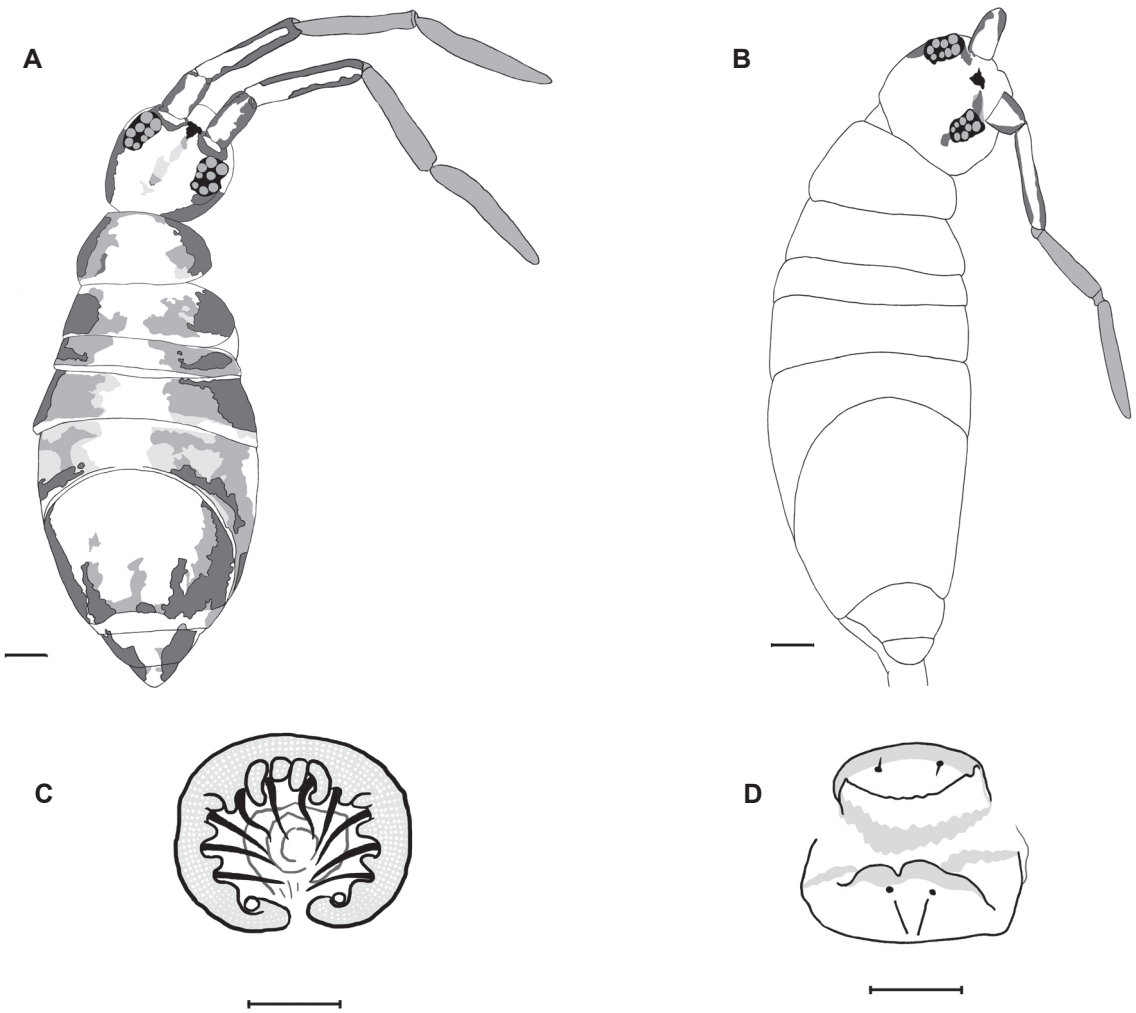

Figure 1. *Entomobrya fourcesensis* spec. nov.; (**A**) Colour pattern male (scale bar = 100µm); (**B**) Colour pattern female (scale bar = 100µm); (**C**) male genital plate (scale bar = 20µm); (**D**) female genital plate (scale bar = 20 µm).

From similar infield studies performed in more northern regions in The Netherlands and Germany, multiple specimens from *E. schoetti* were collected. Seven adults were mounted, measured and studied in the same way.

For comparison, a table with a set of characters used for identification of species of *Entomobrya* s.l. and values was created for *E. fourcesensis* spec. nov., *E. schoetti* and *E. lanuginosa* after Jordana & Baquero (2005).

Type material will be deposited at the Muséum national d'Histoire naturelle (MNHN) in Paris (France) and at Naturalis Biodiversity Center (NBC) in Leiden (The Netherlands).

3. Results

Family Entomobryidae

***Entomobrya fourcesensis* spec. nov., Delhem & Grove**

Type locality. France, Occitanie Region, Gers Department, Fourcès, Coordinates 43.995381, 0.216783, altitude 80 m asl.

Type material. Holotype, ♂ (non reproductive) specimen, cleared in Nesbitt's clearing fluid, mounted on slide using Hoyer's medium labeled as Ref. Col. no.: M00004, collected by Eurofins Mitox, deposited at MNHN in Paris.

Paratypes, 12 ♂ (four non reprod.) mounted on slide using Hoyer's medium labeled as Ref. Col. no.: P0002 and 13 ♀ mounted on slide using Hoyer's medium labeled as Ref. Col. no.: P0026, together with 25 paratypes in a tube in ethanol (70 %) labeled as ref.: 10697a deposited at MNHN in Paris.

Paratypes, six ♂ (five non reprod.) mounted on slide using Hoyer's medium labeled as Ref. Col. no.: P0001 and two ♀ mounted on slide using Hoyer's medium labelled as Ref. Col. no.: P0004, together with 25 paratypes in a tube in ethanol (70 %) labeled as ref.: 10697b deposited at NBC in Leiden.

Diagnosis

Sexual dimorphic Entomobrya species, male and female with ground colour pale yellow to white. Males with variable coloured patches from light grey to dark black. Females without body pigmentation. Chaetotaxic formula: 3-1-0-3-2(3) / 2-4 / 2-2 / 1-2-1 / 0-(1-5)-3-2-2.

Description

Maximum body length excluding antenna up to 1.9 mm. Ground colour pale yellow to white in alcohol. Male and Female specimens having a different colour pattern. Habitus and colour pattern from adult males with coloured patches from light grey to dark black (Fig. 1A). The male genital plate papillate (Fig. 1C). Body from adult females without pigmentation (Fig. 1B). The female genital plate as in Fig. 1D.

Eye patches dark. Antennae gradually darker from Ant.I to Ant. IV.

A detailed overview of the morphometrical data and the comparison with *E. schoetti* (Stach, 1922) and *E. lanuginosa* (Nicolet, 1842) is given in Tab. 1.

Head: Ommatidia 8+8, G and H smaller than E and F. Interocular setae as p, q, r, s, t. (Fig. 3A). Total length of the antenna up to 789 µm (mean n=35). Ant/Head ratio is 2.92 (mean n=35). Relative length of Ant 1 / 2.0 / 2.0 / 2.5. Ant IV with a bilobed apical bulb (Fig 2A). Clypeus with four labral smooth papillae (Fig. 2B).

Body: Abd. IV/III ratio is 3.42. Claw with four internal teeth with first pair at 52 % distance from the clawbase,

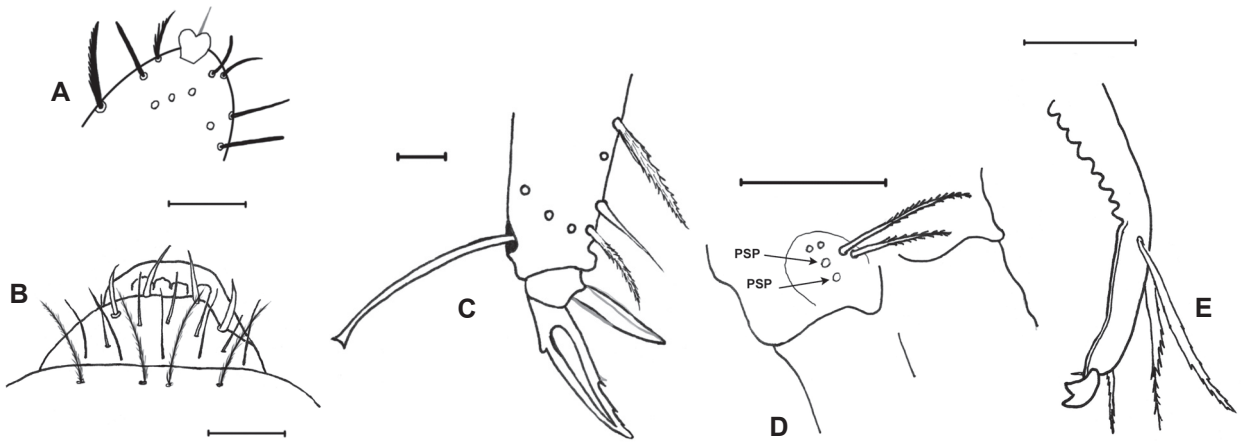

Figure 2. *Entomobrya fourcesensis* spec. nov.: (**A**) Ant IV with bilobed apical bulb (scale bar = 10µm); (**B**) Labrum with labral papillae (scale bar = 10µm); (**C**) Claw III (scale bar = 10µm); (**D**) Manubrial plate (scale bar = 50µm); (**E**) Mucro (scale bar = 100 µm).

first unpaired tooth at 74% and second unpaired tooth minute. Dorsal tooth in more basal position than the lateral ones. Empodium rather narrow and pointed, external edge smooth as in Fig. 2C. Mean furcal length = 582 ± 50 µm. Manubrial plate with four chaetae and two pseudopores (Fig. 2D). Mucro with basal spine, two teeth of equal size (Fig. 2E).

Chaetotaxy: Simplified formula: 3-1-0-3-2(3) / 2-4 / 2-2 / 1-2-1 / 0-(1-5)-3-2-2.

Head chaetotaxy. H1 area with three (An_2, An_3, An_{3a1}), H2 area with one (A_5), H4 area with three (S_1, S_3, S_4) and H5 area with two(three) (Ps_2, (Ps_3), Ps_5) macrochaetae present. H3 area with S'_0 absent (Fig. 3A).

Thorax chaetotaxy. T1 area with two (m_1, m_{2i}) and T2 area with four (a_5, m_4, m_{4i}, m_5) macrochaetae present (Fig. 3B, Fig. 6).

Abdomen chaetotaxy. Abd II: A1 area with two (a_2,a_3), A2 area with two (m_{3ep}, m_{3ea}) macrochaetae present (Fig. 3C, Fig. 7). Abd III: A3 area with one (a_1), A4 area with two (a_2, a_3) and A5 area with one (m_3) macrochaetae present (Fig. 3C, Fig 8).

Abd IV: A6 area without macrochaetae, A7 area with one (E_1) up to five variable, A8 area with three(A_{4a}, B_4, C_{2a}), A9 area with two(A_5, B_5) and A10 area with two(A_6, B_6) macrochaetae present (Fig. 3D).

Biology: *Entomobrya fourcesensis* spec. nov. was encountered in a meadow habitat with little agricultural practices. Vegetation structure indicates a humid, nutrient rich alkaline grassland with high coverage and moderately high plant species diversity. There was a homogeneous vegetation and soil constitution, without structures potentially causing irregular microclimates.

Dactilis glomerata (Cocks foot), *Convolvulus arvensis* (field bindweed), *Potentilla reptans* (Creeping cinquefoil) and *Geranium columbinum* (Long Stalked Crane's Bill) were dominant plant species in the meadow.

Using a large variety of sampling types, *E. fourcesensis* spec. nov. was mainly present in Pitfall trap samples and suction samples indicating it to be a soil dwelling epiedaphic species found in grass and low vegetation.

Table 1. Mean measurements (in µm) and standard deviations from *E. fourcesensis* spec. nov., *E.schoetti* (Stach, 1922) and *E. lanuginosa* (Nicolet, 1842).* data acquired from Jordana & Baquero (2005)

	***E. fourcesensis* spec. nov.**	***E. schoetti***	***E. schoetti ****	***E. lanuginosa ****
Head	270 ± 32	324 ± 21	424 ± 61	260
Th. II	163 ± 21	185 ± 21	257 ± 35	185
Th. III	129 ± 22	140 ± 28	169 ± 34	175
Abd. I	94 ± 19	105 ± 13	120 ± 37	80
Abd. II	133 ± 28	148 ± 31	176 ± 46	170
Abd. III	114 ± 25	132 ± 26	165 ± 33	125
Abd. IV	390 ± 60	430 ± 27	522 ± 58	450
Abd. V	107 ± 18	122 ± 17	111 ± 23	135
Abd. VI	68 ± 17	70 ± 17	76 ± 17	62
Body (+head)	1446 ± 194	1666 ± 106	2018 ± 249	1642
Body (Th/Abd)	1167 ± 177	1313 ± 76	1594 ± 209	1382
Manubrium.	252 ± 19	271 ± 20	344 ± 40	310
Dens	329 ± 34	360 ± 27	453 ± 48	385
Furca	582 ± 50	636 ± 42	798 ± 79	695
Man./Dens	0.77 ± 0.06	0.75 ± 0.04	0.8 ± 0.1	0,81
Ant. I	105 ± 17	110 ± 13	153 ± 31	125
Ant. II	208 ± 38	223 ± 27	268 ± 32	225
Ant. III	215 ± 45	219 ± 33	248 ± 26	210
Ant. IV	261 ± 35	287 ± 35	312 ± 32	275
Antenna	789	839	982 ± 97	835
Ant/Body	0,55	0,50	0.5 ± 0.05	0,51
Ant/Head	2,92	2,59	2,32	3,21
Abd. IV / III	3,42	3,26	3,16	3,60
	n=35	n=7	n=22	n=? (not mentioned in literature)

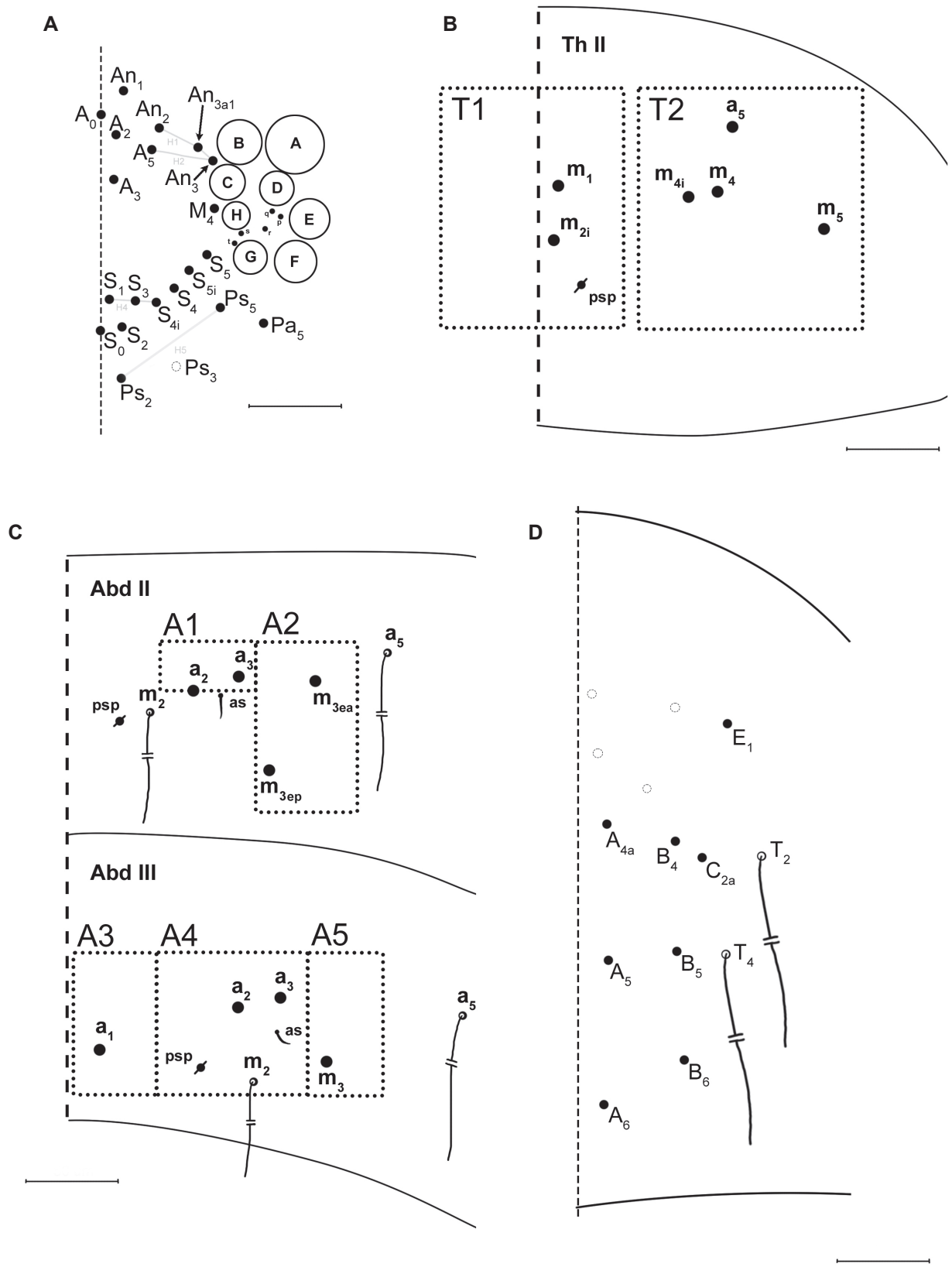

Figure 3. *Entomobrya fourcesensis* spec. nov. macrochaetotaxy. (**A**) Head (scale bar = 50µm); (**B**) Thorax II (scale bar = 50µm); (**C**) Abd II-III (scale bar = 50µm); (**D**) Abd IV (scale bar = 50µm).

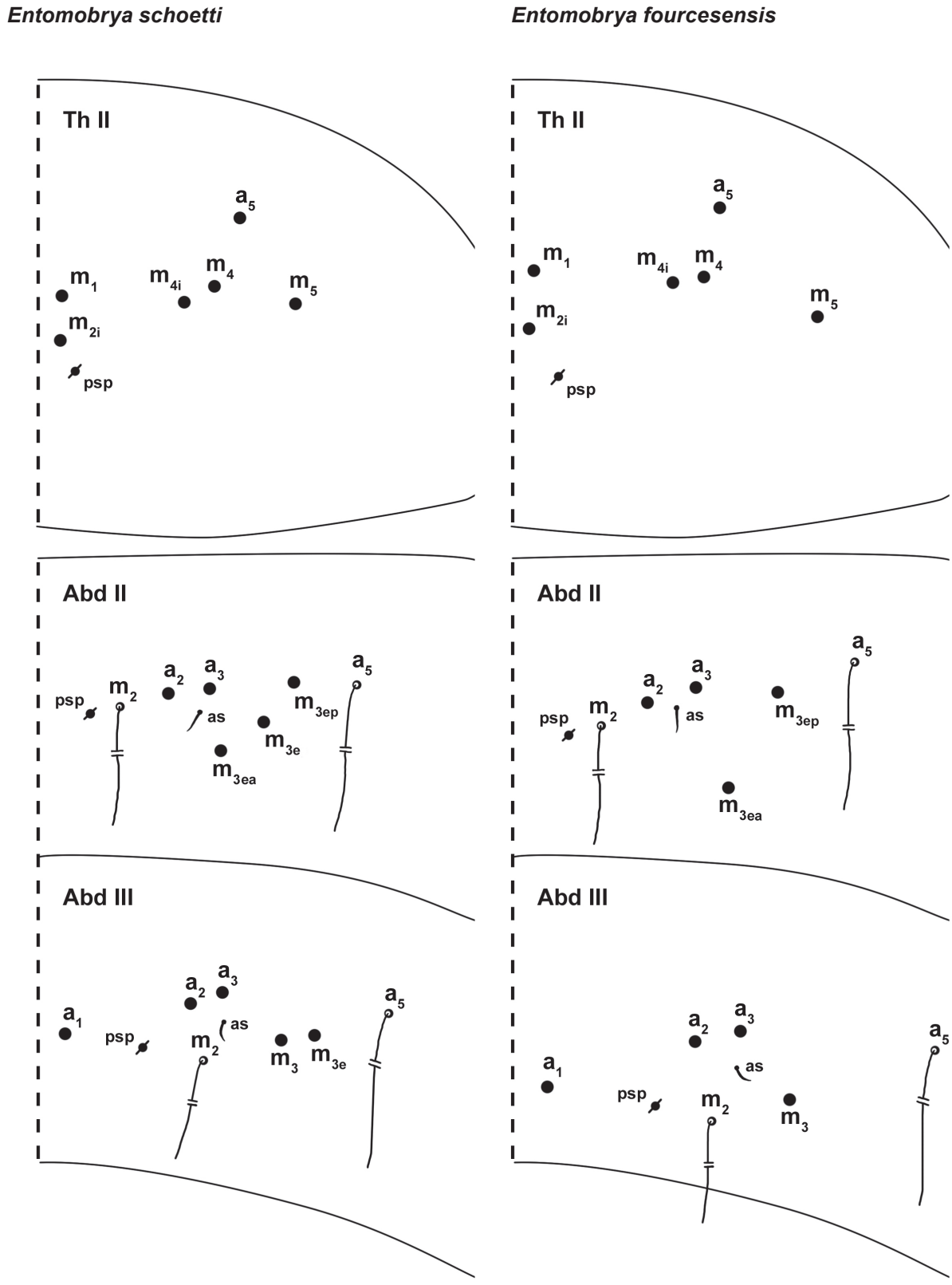

Figure 4. A comparison in macrochaetotaxy of ThII, AbdII and Abd III between *E. schoetti* and *E. fourcesensis* spec. nov.

Figure 5. *Entomobrya fourcesensis* spec. nov.: male genital plate lacking reproductive structures (scale bar = 20µm).

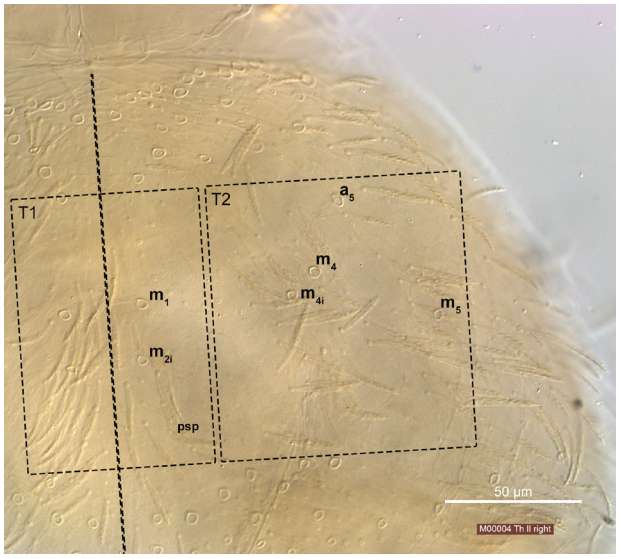

Figure 6. *Entomobrya fourcesensis* spec. nov. Macrochaetotaxy of Thorax II.

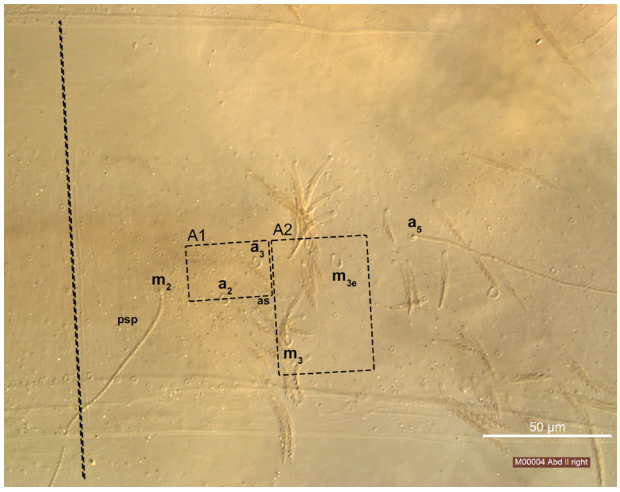

Figure 7. *Entomobrya fourcesensis* spec. nov. Macrochaetotaxy of Abdomen II.

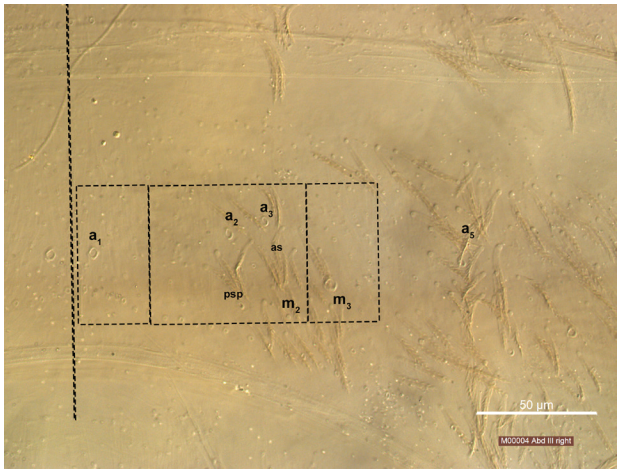

Figure 8. *Entomobrya fourcesensis* spec. nov. Macrochaetotaxy of Abdomen III.

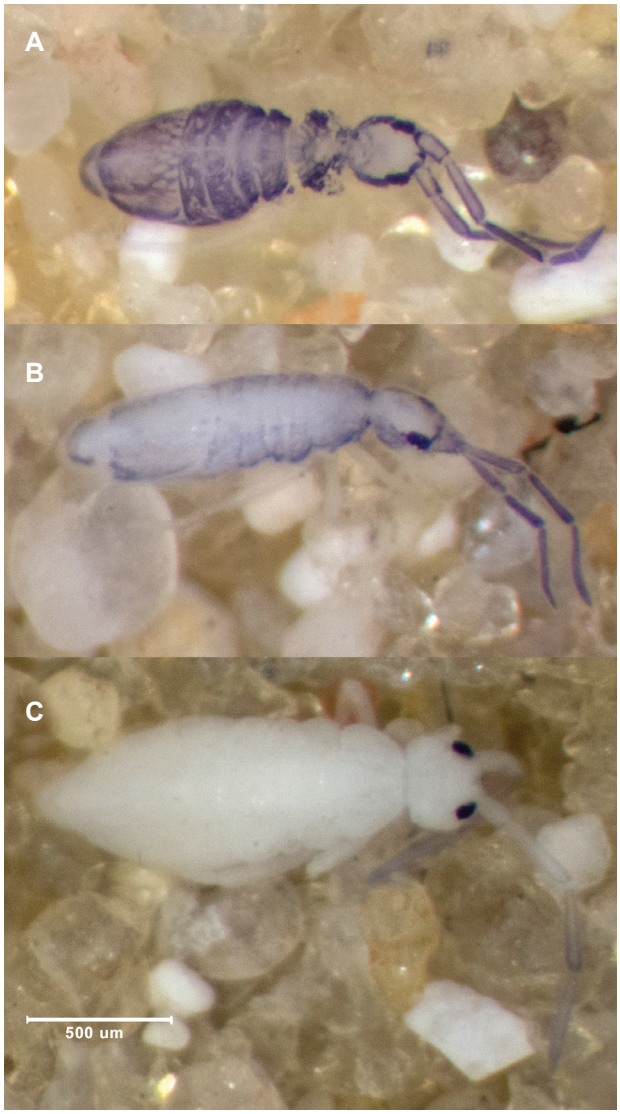

Figure 9. *Entomobrya fourcesensis* spec. nov. in three levels of pigmentation.

Table 2. Comparative Set of characters of *E. fourcesensis spec.nov., E. schoetti* (Stach, 1922) and *E. lanuginosa* (Nicolet, 1842). * data acquired from Jordana & Baquero (2005)

Character	Location	Description	value	*E. fourcesensis* n. sp.	*E. schoetti*	*E. schoetti* *	*E. lanuginosa* *
Ch.1	H1 (head)	An2- An3	1-6	3	3	3	3
Ch.2	H2	A5- A7	1-3	1	2	1	2
Ch.3	H3	S'0	0-1	0	0	0	0
Ch.4	H4	S1- S3- S4	0-3	3	3	3	3
Ch.5	H5	Ps2- Ps3- Ps5	0-3	2 (3)	3	2	3
Ch.6	labral papillae	1: simple and smooth 2: wrinkled or projections 3: chaeta like projection	1-3	2	2	2	1
Ch.7	ocelli G&H size	= E&F (1), < E&F (2)	1-2	2	2	2	2
Ch.8	apical antennal	no bulb (0), lobe simple(1),					
	retractible bulb	Bilobate (2), trilobate (3)	0-3	2	2	2	1
Ch.9	ratio Ant/ Head	>=3 (1),>=2<3 (2), <2 (3)	1-3	2	2	2	1
Ch.10	anterior dorsal mane ThII (Ms)	Ms type 1 (1), without Ms or Ms type 2 (2)	1-2	-	-	-	-
Ch.11	ThII, T1	chaetae m1-m2i2 or >4 (5)	0-5	2	2	2	3
Ch.12	ThII, T2	chaetae a5, m4-m5 or >8 (9)	0-9	4	4	4	3
Ch.13	smooth chaetae on tibiotarsi	0 or 1 on Tibiotars III (0) double file (1)	0-1	0	0	0	0
Ch.14	claw internal teeth	number teeth 1-4	1-4	4	4	4	4
	paired teeth	distance from clawbase %	-	51%	47%	40%	60%
	first unpaired teeth	distance from clawbase %	-	74%	71%	65%	72%
Ch.15	claw dorsal tooth	basal (1), internal teeth level (2)	1-3	1	1	1	1
Ch.16	claw internal edge	without ciliation (0), with ciliation (1)	0-1	0	0	0	0
Ch.17	external empodium	Smooth (0), serrate (1)	0-1	0	0	0	0
Ch.18	Abd II, A1	a2-a3	0-2	2	2	2	2
Ch.19	Abd II, A2	m3 series chaeta number	0-7	2	3	3	2
Ch.20	Abd III, A3	a1	0-1	1	1	1	1
Ch.21	Abd III, A4	above m2 chaeta number	0-3	2	2	2	2
Ch.22	Abd III, A5	m3-m4 series chaeta number	0-4	1	2	2	1

Character	Location	Description	value	*E. fourcesensis* n. sp.	*E. schoetti*	*E. schoetti* *	*E. lanuginosa* *
Ch.23	Abd IV, A6	a1-a5 (A1-D1) chaeta number, >8 (9)	0-9	0	0	0	0
Ch.24	A7 unpaired chaeta	ma0 (A03)	0-1	0	0	0	0
Ch.25	Abd IV, A7	ma1-ma4 (A2-E1) ch. Number, >9 (10)	0-10	1-5	5-6	4-6	2
Ch.26	A8 unpaired chaeta	m0 (A04)	0-1	0	0	0	0
Ch.27	Abd IV, A8	m1-m3 (A4a-C2a) ch. Number, >5 (6)	0-6	3	3	3	3
Ch.28	A9 unpaired chaeta	mp0 (A05)	0-1	0	0	0	0
Ch.29	Abd IV, A9	mp1-mp3 (A5-B5) ch. Number, >6 (7)	0-7	2	2	2	2
Ch.30	Abd IV, A10	p1a-p3 (A6-B6) ch. Number, >5 (6)	0-6	2	2	2	2
Ch.31	Abd IV, A11	T1 (ma4e) as trichobothrium	0-1	-	-	-	-
Ch.32	Abd IV, A12	T2 (m4) as trichobothrium	0-1	-	-	-	-
Ch.33	Abd IV, A13	T4 (mp4) as trichobothrium	0-1	-	-	-	-
Ch.34	Abd IV, A14	T6 (p4) as trichobothrium	0-1	-	-	-	-
Ch.35	ratio Abd IV / III	2< R< 4 (1), R> 4 (2)	1-2	1	1	1	1
Ch.X	Furca	total length (um)	-	582 um	636 um	797 um	715 um
Ch.36	manubrial plate	chaetae number; >10 (11)	0-11	4	4	4	4
Ch.37	manubrial plate	pseudopores 1-2	1-2	2	2	2	2
Ch.38	mucro	Sub-apical tooth. Without (0), normal (1), big (2)	0-2	1	1	1	1
Ch.39	mucro	basal spine absent (0), present (1)	0-1	1	1	1	1

Table 3. Sample locations and dates of mounted specimens of *E. fourcesensis* spec. nov and *E.schoetti* (Stach, 1922).

Localities	Coordinates	Dates	*Entomobrya fourcesensis* spec.nov. # mounted specimen	*Entomobrya schoetti* # mounted specimen
Fourcès field 1 (FR)	43.995381 0.216783	22.05.2012	1	0
		7.07.2012	42	0
Fourcès field 2 (FR)	43.991234 0.215001	3.09.2012	1	0
Fourcès field 3 (FR)	43.997575 0.204682	14.01.2017	6	0
Winden, Pfalz (DE)	49.092989 8.118021	6.06.2013	0	2
Tillburg (NL)	51.557391 5.041164	25.07.2013	0	1
		22.02.2014	0	1
Valencia (ES)	38.995681 -0.713497	9.03.2014	0	3
		1.07.2021	0	4

Etymology: The species was named after the type locality Fourcès. A small village in the Gers department in the Occitanie region in southwestern France.

4. Discussion

Considering the Chaetotaxy, two species have a similar chaetotaxy on the abdominal segments, but they are separated by the Thorax II and head chaetotaxy. These two species are *E. saxoniensis* after Jordana et al. (2011) and *E. multifasciata* (Tullberg, 1871), both with very different coloration.

The male colour appearance and close-leaning morphological characteristics of the new species show its affinity with *E. schoetti* (Stach, 1922). But a distinct difference in macrochaetotaxy is made clear in Fig. 4 and Tab. 2. On Abd II we clearly see the lacking of m_{3e} and on Abd III m_{3e} is not found on *E. fourcesensis* spec. nov..

By its chaetotaxy the three segments less variable within the genus *EFntomobrya* are Thorax II, Abdomen II and Abdomen III and its precisely on these segments that we see a very stable chaetotaxy.

Apart from the measured specimens, we collected *E. fourcesensis* on two more location during a total of five different sample dates within a time span of over 4 years (see Tab. 3). Within the whole sampled population, we found the same exact chaetotaxy.

Based on the original species description of *E. schoetti* from Stach (1922), two specimens were collected in Léva (Slovakia) and one in the environs of Krakow (Poland). During the re-description done by Jordana & Baquero (1999) large numbers were collected in Vergalijo, Navarra, Spain. The world distribution map from *E. schoetti* on http://collembola.org/ also suggests a bio-geographical distribution over the whole of Europe (Bellinger et al. 1996-2023).

In our own results of several infield studies on surface- and plant-dwelling, arthropod fauna throughout Europe we found *E. schoetti* at three different location during 5 different sampling dates (see Tab. 3)

Together with the data collected on https://www.gbif.org/species/4539582 which points out an occurrence in Germany, The Netherlands and France, we can assume that the distribution areas are intermingled with these of *E. fourcesensis* spec. nov.

On the other hand, as shown in Table 3, during nine different samplings we found just one or the other of the two resembling species. In another word, we do not have any record of their coexistence.

The large protected pine forest from Landes de Gascogne Regional Natural Park nearby might have influenced the species composition of the sampling location.

There is also a clear biological difference with *E. schoetti* (Stach, 1922) as the original species description mentions three pigmented specimens all being female and sexual dimorphism was not observed in the re-description done by Jordana & Baquero (1999).

As Figure 9 shows, *E. fourcesensis* spec. nov. was encountered in three levels of pigmentation. A dark, a medium and a pale form could be distinguished from each other. As mentioned in the description above, we found a clear sexual dimorphism. Both pigmented forms were determined as male specimens, the pale form as female.

Between the two pigmented forms we observed a difference in morphology of the male genital plate. In the dark form, we found the male genital plate lacking the associated reproductive structures as drawn in Fig. 5. Whereas in the medium form, the male genital plate with reproductive structures is present as seen in Figure 1C.

This phenomenon of specimens with typical male pigmentation lacking the reproductive structures was already observed by Mari Mutt J. A. (1981) in the redescription of *Willowsia jacobsoni,* saying: 'These specimens seem to be abnormal sterile males since their genital plate is unlike that of the female.'

5. Acknowledgments

We would like to thank Eurofins Mitox, our colleagues in Amsterdam and especially Frank Bakker for the facilities and opportunity to develop our knowledge and for the time and material necessary. Thanks to Farid and Micha for critical review of the manuscript.

With our findings we contacted Frans Janssens. He advised to contact Rafael Jordana, who gave very detailed feedback and confirmed we were most likely working on an undescribed species.

So a special thanks to Rafael Jordana and Frans Janssens for their specialist knowledge and clear advice and help when requested.

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