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Research article

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Integrative description of two new species of the *Xenylla maritima* species complex (Collembola: Hypogastruridae) from Crete (Greece), with notes on the high intraspecific genetic divergence found in *X. maritima* Tullberg, 1869

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Abstract. A classical taxonomic analysis of materials from several European museums as well as our own collection along with the analysis of DNA barcoding data (*cox1*) allowed us to determine the taxonomic status of Cretan forms included in the recently revised *Xenylla maritima* complex. Two of them are recognized as distinct species: *Xenylla ellisi* sp. nov. and *Xenylla schulzi* sp. nov., and a new cryptic lineage was found within the morphological boundaries of the broadly distributed species *X. maritima*.

Keywords. DNA barcoding, Europe, overlooked diversity, *Xenylla ellisi* sp. nov., *Xenylla schulzi* sp. nov.

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Introduction

Crete is the largest Greek island and the fifth largest in the Mediterranean Sea. Its long period of isolation from the mainland alied to its complex geological and climatic history, as well as the long-term presence of humans, resulted in a great diversity and endemism of its local biota (Rackham & Moody 1996; Sfenthourakis & Triantis 2017). Crete is a biodiversity hotspot with a high proportion of threatened species (Spiliopoulou *et al.* 2021). All these factors make it an area of significant importance for taxonomy, biogeography and conservation biology.

Springtails (Collembola Lubbock, 1870) are a group of entognathic, wingless, mainly detrivorous arthropods that dwell primarily in moist habitats (Hopkin 1997). However, among them, there are many forms that have developed different life strategies, and some of them are well adapted to dry habitats. Undoubtedly, most species of the genus *Xenylla* Tullberg, 1869 can be included in this group. *Xenylla* is one of the largest genera in the family Hypogastruridae Börner, 1906 (139 species) distributed worldwide (Bellinger *et al.* 1996–2024) and exhibiting a high degree of local endemism (Babenko *et al.* 1994). Within the genus, there are several ecological groups: forest, steppe and littoral-compost species. They generally occur in mosses and lichens, under bark on tree trunks or within crevices in rocks (Babenko *et al.* 1994).

So far, about 170 species of Collembola have been recorded from Crete (Ellis 1976; Simon Benito 1985; Pomorski & Skarżyński 1997; Sterzyńska & Ehrnsberger 1997; Schulz & Lymberakis 2006; Schulz 2007, 2010; Smolis & Kaprus' 2009; Schulz & Burkhardt 2012; Skarżyński *et al.* 2017; Gwiazdowicz *et al.* 2022; Skarżyński & Gwiazdowicz 2022; Smolis *et al.* 2023). Among them there are five species/forms of the genus *Xenylla: X. maritima* Tullberg, 1869 (Ellis 1976; Schulz & Lymberakis 2006; Schulz 2007; Schulz & Burkhardt 2012; Gwiazdowicz *et al.* 2022), *X. brevisimilis brevisimilis* Stach, 1949 (Schulz 2007), *X. mediterranea* Gama, 1964 (Sterzyńska & Ehrnsberger 1997), *Xenylla* sp. 1 and *Xenylla* sp. 2 (*maritima* complex) (Gwiazdowicz *et al.* 2022). They all belong to the recently revised *maritima* complex (Skarżyński *et al.* 2018). In order to establish the taxonomic status of the Cretan forms included in this complex, a classical taxonomic analysis of materials from Naturalis Biodiversity Center, Leiden, Senckenberg Museum of Natural History, Görlitz and Museum and Institute of Zoology of the Polish Academy of Sciences, Warsaw and our own collection, along with the analysis of DNA barcoding data, was performed.

Material and methods

Morphological examination

Specimens stored in alcohol were cleared in Nesbitt's fluid (chloral hydrate, concentrated hydrochloric acid, distilled water), slide-mounted in a mixed medium of distilled water, arabic gum, glycerol and chloral hydrate. Observations were made using a Nikon Eclipse E600 phase contrast microscope. Figures were drawn with a camera lucida.

The following nomenclatural systems were used: for body chaetotaxy (Gama 1988; Thibaud *et al.* 2004), tibiotarsal chaetotaxy (Lawrence 1977; Deharveng 1983), chaetotaxy of anal valves (Hüther 1962), chaetotaxy of labium (Massoud 1967), labial palp (Fjellberg 1999) and maxilla (Fjellberg 1984, 1998).

Institutional abbreviations

DIBEC = Dep	partment of Invertebrate	Biology, I	Evolution and	Conservation,
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- University of Wrocław, Poland
- MHNF = Natural History Museum of Fribourg, Switzerland
- MIZ PAS = Museum and Institute of Zoology of the Polish Academy of Sciences, Warsaw, Poland
- NBCN = Naturalis Biodiversity Center, Leiden, Netherlands

SMNG = Senckenberg Museum of Natural History, Görlitz, Germany

Abbreviations for morphological terms

- Ant. I–IV = antennomeres I–IV
- Th. I–III = thoracic segments I–III
- Abd. I-VI = abdominal segments I-VI

Molecular analyses

Genomic DNA was extracted from 3-4 specimens for each species (all of them were collected from the same site, but from bark of different trees of the same species, Zelkova abelicea (Lam.) Boiss.: GREECE - Crete; Mt Kedros, Gerakari; 35.194829° N, 24.606713° E; 1255 m a.s.l.; 29 Sept. 2021; D.J. Gwiazdowicz leg.) using the Wizard®SV Genomic DNA Purification System (Promega, Madison, WI, USA). The cytochrome c-oxidase subunit 1, 5P fragment (cox1) was amplified with a universal primer pair, 5'-GGTCAACAAATCATAAAGATATTGG-3' (LCO1490) and 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (HCO2198) (Folmer et al. 1994). PCRs were prepared in a 25 µL reaction volume containing: 2.5 µL of DNA, 1.25 µL of both forward and reverse primers (10 µM), 2.5 µL of MgCl, (2.5 mM), 2.5 µL of deoxynucleotides (dNTPs, 10 mM), 5 µL of Green GoTaq Flexi Buffer (Promega), 0.125 µL of GoTaq® G2 Flexi DNA Polymerase (Promega), 5 µL and 9.875 µL of ddH₂O. Amplifications were run on a GeneAmp[®] PCR System 2700 (Applied Biosystems, Foster City, CA, USA) thermal cycler under the following conditions for each of the 35 cycles: a denaturation step at 95°C for 1 min, an annealing step at 50°C for 1 min and an elongation step at 60°C for 90 s. An additional initial denaturation step was set at 95°C for 5 min and a final extension step at 72°C for 7 min. For Xenylla ellisi sp. nov. an annealing temperature at 55°C was used to increase the specificity of the primers. PCR products were purified with the kit Wizard®SV Gel and PCR Clean-up System (Promega) and then sequenced on both strands using a DNA Analyzer ABI 3730 at Biofab (Rome, Italy) and BMR Genomics (Padua, Italy). Sequences were manually assembled and corrected in Benchling (2024) and AliView 1.28 (Larsson 2014).

The sequences produced for this study were analyzed with those from eight other species of *Xenylla* previously barcoded (GenBank accession numbers are presented in Table 1). Distance analyses were performed with MEGA7 (Kumar *et al.* 2016), using a Neighbor-Joining (Saitou & Nei 1987) algorithm with the Kimura-2 parameter model (K2P – Kimura 1980) to estimate genetic distances. The robustness of nodes was evaluated through bootstrap re-analysis of 1000 pseudoreplicates. The trees were replotted using R package ggtree (Xu *et al.* 2022).

Results

DNA barcoding

For the eight species of the genus *Xenylla (X. betulae* Fjellberg, 1985, *X. canadensis* Hammer, 1953, *X. humicola* (Fabricius, 1780), *X. maritima* L1, *X. mediterranea*, *X. nitida* Tullberg, 1871, *X. pomorskii* Skarżyński, Piwnik & Porco, 2018 and *X. szeptyckii* Skarżyński, Piwnik & Porco, 2018) included in this analysis, a clear barcode gap was found between a mean interspecifc distance of 23.7% (range 19.7–27.6%) and a mean intraspecifc distance of 1.2% (range 0–3%) (Fig. 1, Table 2). Similar intra- and interspecific distances were found for *Xenylla ellisi* sp. nov. (mean interspecific distance from the eight other species of *Xenylla* 22.4% (range 20–25.7%), intraspecific distances 0.7%) and *Xenylla schulzi* sp. nov. (mean interspecific distance from the eight other species of *Xenylla* 22.1% (range 19.7–26.8%), intraspecific distances 0.7%) (Table 2).

A highly divergent lineage was found within the morphological boundaries of the species of *X. maritima*. It exhibited a mean divergence with the other eight species of 23% (range 20.2–26.5%) (Table 2). More

species	GenBank accession number	publications with molecular data	country	
X. betulae Fjellberg, 1985	GU657274, GU657275	Porco et al. (2014)	Canada	
X. canadensis Hammer, 1953	GU657108, GU657184, GU657185, GU657186, GU657191	Porco et al. (2014)	Canada	
X. ellisi sp. nov.	PP844604, PP844605, PP844606, PP844607	this publication	Greece	
X. humicola (Fabricius O, 1780)	KF641975, KF642098, KF642176, KF642288, KF642591	Porco et al. (2014)	Canada	
X. maritima Tullberg, 1869 (L1)	HQ987412, HQ987413, HQ987414, HQ987415, HQ987428, HQ987429	Skarżyński et al. (2018)	Poland	
X. maritima Tullberg, 1869 (L2)	PP844611, PP844612, PP844613	this publication	Greece	
X. mediterranea Gama, 1964	HQ987416, HQ987417, HQ987418, HQ987419	Skarżyński et al. (2018)	Italy	
X. nitida Tullberg, 1871 (= X. brevisimilis Stach, 1949)	HQ987420, HQ987421, HQ987422, HQ987423, HQ987424	Skarżyński et al. (2018)	Poland	
X. pomorskii Skarżyński, Piwnik & Porco, 2018	KU565133, KU565134, KU565135, KU565136, KU565137	Skarżyński et al. (2018)	Poland	
X. schulzi sp. nov.	PP844608, PP844609, PP844610	this publication	Greece	
X. szeptyckii Skarżyński, Piwnik & Porco, 2018	HQ987425, HQ987426, HQ987427, KU565132, KU565139, KU565141	Skarżyński et al. (2018)	Poland	

Table 1. Sequence data of species of species of Xenylla Tullberg, 1869 used in molecular analysis.



Fig. 1. Neighbor joining tree (K2P) based on the *cox1* barcode sequences of eight species of *Xenylla* Tullberg, 1869 along with the two new species, *X. ellisi* sp. nov. and *X. schulzi* sp. nov., and the new cryptic lineage *X. maritima* L2 (previously barcoded Polish population in Skarżyński *et al.* 2018 is named *X. maritima* L1). The upper and lower sides of the triangle represent the maximum and minimum of genetic distances within the species respectively. Nodes supported by \geq 99% bootstrap values are marked with an asterisk.

Table 2. Intraspecific and interspecific K2P distances in analysed species of *Xenylla* Tullberg, 1869, measured in %.

		intraspecific	pecific interspecific									
	species		1	2	3	4	5	6	7	8	9	10
1	X. betulae Fjellberg, 1985	1.5										
2	X. canadensis Hammer, 1953	0.8	22.5									
3	X. ellisi sp. nov.	0.7	25.7	20.0								
4	X. humicola (Fabricius, 1780)	0.0	25.3	24.9	22.2							
5	<i>X. maritima</i> L1	2.0	27.6	26.1	23.5	21.9						
6	X. maritima L2	1.7	26.5	25.2	21.1	21.3	20.2					
7	X. mediterranea Gama, 1964	0.0	23.6	23.3	23.9	26.3	22.9	23.1				
8	X. nitida Tullberg, 1871	1.9	22.0	24.9	22.4	24.7	24.7	24.0	26.7			
9	X. pomorskii Skarżyński, Piwnik & Porco, 2018	3.0	23.3	23.5	21.4	25.9	24.2	25.0	24.4	21.3		
10	X. schulzi sp. nov.	0.7	26.1	25.6	22.2	21.2	17.8	20.2	23.3	20.7	23.9	
11	X. szeptyckii Skarżyński, Piwnik & Porco, 2018	0.4	24.5	26.8	21.6	20.5	20.3	21.1	25.9	23.9	24.7	19.7

Table 3. Morphological characteristic of species of the *Xenylla maritima* complex (species from Canada are not included).

	X. asiatica Martynova, 1975	<i>X. ellisi</i> sp. nov.	X. maritima Tullberg, 1869	X. mediterranea Gama, 1964	X. nitida Tullberg, 1871	<i>X. pomorskii</i> Skarżyński, Piwnik & Porco, 2018	X. schulzi sp. nov.	<i>X. szeptyckii</i> Skarżyński, Piwnik & Porco, 2018
size (mm)	1.3	0.5-1.1	1.1-1.6	0.7-1	0.7-1.3	0.6-1.1	0.6-1.6	0.7-1.3
number of sublobal hairs on outer lobe of maxilla	3	3	3	1	2	2	2	2
number of setae on leg III subcoxae 1	3	3	3	2	2	3	3	3
setae m ₅ on abd. IV	present	present	absent	present	present	present	absent	absent
no of teeth in retinaculum	3	3	3	2	3	3	3	3
number of setae in front of retinaculum on abd. III	1	1-2	0	1–2	1–2	1–2	0	1–2
ratio: mucrodens/claw III	2.3	1-1.4	1.9–2.4	1–1.6	1-1.3	2–2.3	1.2-1.7	1.6–2
apex of mucro	narrow, weakly upturned	narrow, weakly upturned	narrow, weakly upturned	narrow, weakly upturned	moderately broad and spoon-like	broad, strongly spoon-like	narrow, weakly upturned	narrow, weakly upturned

specifically, the Crete population (*X. maritima* L2) is 20.2% divergent from the lineage analysed from Poland (*X. maritima* L1; Table 2). The mean intralineage divergence of *X. maritima* L2 was 1.7%.

Taxonomy

Phylum Arthropoda Latreille, 1829 Class Collembola Lubbock, 1870 Order Poduromorpha Börner, 1913 Family Hypogastruridae Börner, 1906 Genus *Xenylla* Tullberg, 1869

Xenylla maritima Tullberg, 1869

Xenylla maritima Tullberg, 1869: 11.

Material examined (slide mounted unless otherwise stated)

GREECE – Crete • 12 $\Im \Im$, 12 $\Im \Im$, 17 juvs; Mt Kedros, Gerakari; 35.194829° N, 24.606713° E; 1255 m a.s.l.; 11 Oct. 2018; D.J. Gwiazdowicz leg.; bark of Zelkova abelicea (Lam.) Boiss. trees; DIBEC • 1 \bigcirc ; same data as for preceding; 29 Sept. 2021; D.J. Gwiazdowicz leg.; DIBEC • 18 \bigcirc \bigcirc , 25 33, 7 juvs; Dikti Mountains, Katharo; 35.148004° N, 25.567558° E; 1160 m a.s.l.; 9 Oct. 2018; D.J. Gwiazdowicz leg.; bark of *Z. abelicea* trees; DIBEC • 4 ♀♀, 4 ♂♂, 1 juv.; Dikti Mountains, Viannou; 35.064291° N, 25.469778° E; 1320 m a.s.l.; 9 Oct. 2018; bark of Z. abelicea trees; D.J. Gwiazdowicz leg.; DIBEC • 3 ♂♂, 27 juvs; Thripti Mountains, Thripti; 35.080588° N, 25.887408° E; 1150 m a.s.l.; 14 May 2019; D. Ghosn leg.; branches of dwarfed Z. abelicea individuals; DIBEC • 1 juv.; Levka Ori, Omalos; 35.31901° N, 23.91871° E; 1160 m a.s.l.; 21 May 2019; bark of Z. abelicea trees; D. Ghosn leg.; DIBEC • 3 ♀♀, 1 juv.; Levka Ori, Niato; 35.287527° N, 24.145503° E ; 1215 m a.s.l.; 21 May 2019; branches of dwarfed Z. abelicea individuals; D. Ghosn leg.; DIBEC • 2 \Im (on slides, formerly in alcohol); Levka Ori, Anopolis; 1991–1992; P. Lymberakis leg.; H.-J. Schulz det.; sample 34366; SMNG • 3 QQ (on slides, formerly in alcohol); Katharo Plateau, Kritsa; 23 May 2011; H.-J. Schulz leg.; sample 47304; SMNG • 1 ♀, 5 ♂♂ (on slides, formerly in alcohol); Agia Galini; sample 972.202; 30 Oct. 1972; Ellis leg.; collected manually, under large pebbles on the beach; NBCN • 1 \bigcirc ; Tsagarak; 15 Oct. 1972; A.C. and W.N. Ellis leg.; litter and topsoil (small hard lumps of dry loam) under an isolated *Ouercus coccifera* L. in phrygana (i.e., a very common low vegetation type, comparable to the French garigue, consisting mainly of small shrubs and geophytes); sample 972.234; ZMA.Col.P.36226 NBCN • 1 \bigcirc ; same data as for preceding; ZMA.Col.P.36227; NBCN • 1 juv.; same data as for preceding; ZMA. Col.P.36231; NBCN.

Remarks

The examined specimens of *X. maritima* mentioned by Schulz & Lymberakis (2006), Schulz & Burkhardt (2012) and Gwiazdowicz *et al.* (2022) fit the description of this species by Skarżyński *et al.* (2018). The specimens from the collection of Ellis (1976) that we had at our disposal, despite its poor state of preservation, can also be assigned to this species.

DNA barcoding revealed the existence of two genetic lineages of *X. maritma*: L1 from Poland and L2 from Crete. Since the analyzed material came from only one site on Crete (Kedros Mountain, Gerakari), it is not possible to further assess the extent of the genetic diversity of *X. maritima* on this island. More extensive research is needed to determine the barcode lineage status of *X. maritima* populations worldwide, including in Crete.

Xenylla ellisi sp. nov. urn:lsid:zoobank.org:act:1EB086E2-EC45-4464-AC9E-5ADC668B2DE6 Figs 1–2, 3A–B, Tables 1–3

Diagnosis

Body length 0.5–1.1 mm. Interior of body more or less pink. Chaetotaxy: b f h1 h2 q a4. Abd. IV with dorsal setae m_5 . Abd. III with 1–2 setae in front of retinaculum. Outer lobe of maxilla with 3 sublobal hairs. Subcoxae 1 of legs III with 3 setae. Retinaculum with 3+3 teeth. Mucrodens 1–1.4 × as long as inner edge of claws III, with 2 posterior setae, without or with only slightly marked ventral bend, with very low mucronal lamella, not reaching apex, which is narrow and weakly upturned. Anal spines small, situated on low basal papillae.

Etymology

Dedicated to Dr Willem N. Ellis, who initiated the research on the Collembola of Crete.

Type material

Holotype

GREECE – Crete • \bigcirc (on slide); Mt Kedros, Gerakari; 35.194829° N, 24.606713° E; 1255 m a.s.l.; 29 Sept. 2021; D.J. Gwiazdowicz leg.; bark of *Z. abelicea* trees; DIBEC.

Paratypes

GREECE – Crete • 5 $\bigcirc \bigcirc$, \circlearrowright (on slides), 20 spp. (in alcohol); same data as for holotype; DIBEC • 1 \circlearrowright (on slide); same data as for holotype; MHNF • 7 $\bigcirc \bigcirc$, 5 $\circlearrowright \circlearrowright$ (on slides); same data as for holotype; 11 Oct. 2018; DIBEC.

Other material examined (slide mounted unless otherwise stated)

GREECE – **Crete** • 1 \bigcirc ,1 \bigcirc ; Dikti Mountains, Viannou; 35.064291° N, 25.469778° E; 1320 m a.s.l.; 9 Oct. 2018; D.J. Gwiazdowicz leg.; bark of *Z. abelicea* trees; DIBEC • 18 \bigcirc \bigcirc , 14 \bigcirc \bigcirc , 9 juvs; Thripti Mountains, Thripti; 35.080588° N, 25.887408° E; 1150 m a.s.l.; 14 May 2019; D. Ghosn leg.; branches of dwarfed *Z. abelicea* individuals; DIBEC • 1 \bigcirc , 1 juv.; Levka Ori, Omalos; 35.31901° N, 23.91871° E; 1160 m a.s.l.; 21 May 2019; D. Ghosn leg.; bark of *Z. abelicea* trees; DIBEC • 1 \bigcirc , 2 \bigcirc \bigcirc ; same data as for preceding; 29 Sept. 2021; D.J. Gwiazdowicz leg.; DIBEC • 2 \bigcirc \bigcirc , 3 \bigcirc \bigcirc , 3 juvs; Levka Ori, Niato; 35.287527° N, 24.145503° E; 1215 m a.s.l.; 21 May 2019; D. Ghosn leg.; branches of dwarfed *Z. abelicea* individuals; DIBEC • 4 \bigcirc \bigcirc , 1 juv.; Levka Ori, Impros; 35.270546° N, 24.15315° E; 1175 m a.s.l.; 21 May 2019; D. Ghosn leg.; bark of *Z. abelicea* trees; DIBEC • 5 \bigcirc \bigcirc , 2 \bigcirc \bigcirc (on slides, formerly in alcohol); Katharo Plateau, Kritsa; 23 May 2011; H.-J. Schulz leg.; sample 47304; SMNG.

Description

Body length (without antennae) 0.5–1.1 mm. Colour (in alcohol): dorsal side blue gray to black, with pale spots all over body, ventral side paler. Interior of body more or less pink. Integument smooth or weakly verrucose with small primary hexagons. Posterior edge of sternum of Abd. II and anterior edge of sternum of Abd. III of reproductive males with 3–6 integumentary swellings (Fig. 2E).

Chaetotaxy: b f h1 h2 q a4. Dorsal chaetotaxy (Fig. 2A–C): setae short smooth or slightly serrated. Body sensilla (s) $2-5 \times as$ long as ordinary setae, fine and smooth. Head with seta c_1 absent (b), seta L_1 longer than seta L_3 (f). Th. II and III with seta a_2 displaced posteriorly compared with seta a_1 (h1), seta p_2 displaced anteriorly compared with seta p_1 (h2), with setae m_3 and la_1 present. Abd. I–III with seta p_5 present. Abd. IV without setae a_3 and setae m_5 present. Setae a_2 on Abd. V absent (q). Ventral chaetotaxy (Fig. 2D–E): head with setae p_1 and m_3 . Th. II and III with 1+1 setae. Abd. II with setae a_5 absent and



Fig. 2. *Xenylla ellisi* sp. nov. **A–D.** Paratype, $\stackrel{\bigcirc}{\rightarrow}$ (DIBEC). **A**. Dorsal chaetotaxy of head. **B**. Dorsal chaetotaxy of Th. II. **C**. Dorsal chaetotaxy of Abd. III–VI. **D**. Ventral chaetotaxy of head. **E**. $\stackrel{\bigcirc}{\rightarrow}$ (DIBEC), ventral chaetotaxy of Abd. II–IV.

setae p_1 and p_2 present, Abd. III with 1–2 setae in front of retinaculum, Abd. IV without seta m_1 (a4). Two anterior anal valves with 2 setae hr each.

Ant. IV with simple apical vesicle (av), subapical organite (so), microsensillum (ms) and 4 (3 dorsoexternal and 1 dorsointernal) cylindrical sensilla (A and B slightly thicker than C and D) as in Skarżyński *et al.* 2018: fig. 6. Ant. III-organ with two long (outer) and two short (inner) sensilla. Microsensillum on ant. III present. Ant. I with 7 setae.

Ocelli 5+5 (Fig. 2A). Labrum with apical papillae. Labral setae 5, 5, 4, prelabrals 4. Maxillary head as in Skarżyński *et al.* 2018: fig. 11. Labium with seta F ca $2.5 \times$ as long as seta E. Labial palp as in Skarżyński *et al.* 2018: fig. 12. Outer lobe of maxilla with 3 sublobal hairs.

Tibiotarsi I, II and III with 19, 19 and 18 setae, respectively, with setae A_2 and A_7 capitate (ratio capitate setae/claw III = ca 1.5). Femora I, II and III with 12, 11 and 10 setae, respectively, trochantera with 5, 5 and 4 setae, respectively, coxae I, II and III with 3, 7 and 7 setae, subcoxae 2 of legs I, II and III with 0, 2 and 2 setae, subcoxae 1 of legs I, II and III with 1, 2 and 3 setae, respectively. Claws with small inner tooth (Fig. 3A).

Ventral tube with 4+4 setae. Retinaculum with 3+3 teeth (Fig. 2E).

Mucrodens $1-1.4 \times$ as long as inner edge of claws III, with 2 posterior setae, without or with only slightly marked ventral bend, with very low mucronal lamella, not reaching apex, apex narrow and weakly upturned (Figs 2E, 3B).

Anal spines small, situated on low basal papillae (Fig. 2C).

Remarks

X. ellisi sp. nov. is similar to *X. mediterranea*, from which it differs in characters summarised in Table 3 and in the identification key. Moreover, it is worth emphasizing that the new species differs from all other members of the *X. maritima* complex in the morphology of reproductive males. The occurrence of integumentary swellings on the sterna of Abd. II and III may be considered as a manifestation of epitoky. This phenomenon has not been reported so far in representatives of the genus *Xenylla*.

Examination of the material mentioned above allowed us to conclude that this new species was reported from Crete by Schulz & Burkhardt (2012) as *X. maritima* and by Gwiazdowicz *et al.* (2022) as *Xenylla* sp. 2 *maritima* complex.

Xenylla schulzi sp. nov. urn:lsid:zoobank.org:act:C958C825-5CC9-4C55-A7EB-802B3193992E Figs 1, 3C–D, Tables 1–3

Diagnosis

Body length 0.6–1.6 mm. Interior of body white. Chaetotaxy: b f h1 h2 q a4. Abd. IV without setae m_s . Abd. III without setae in front of retinaculum. Outer lobe of maxilla with 2 sublobal hairs. Subcoxae 1 of legs III with 3 setae. Retinaculum with 3+3 teeth. Mucrodens $1.2-1.7 \times as$ long as inner edge of claws III, with 2 posterior setae, with marked ventral bend, with low mucronal lamella, not reaching apex, apex narrow and weakly upturned. Anal spines small, basal papillae almost absent.

Etymology

Dedicated to Dr Hans-Jürgen Schulz, who made a significant contribution to the knowledge of the fauna of Crete.

Type material

Holotype

GREECE – Crete • \bigcirc (on slide); Mt Kedros, Gerakari; 35.194829° N, 24.606713° E; 1255 m a.s.l.; 29 Sept. 2021; D.J. Gwiazdowicz leg.; bark of *Z. abelicea* trees; DIBEC.

Paratypes

GREECE – Crete • 15 $\bigcirc \bigcirc$, 11 $\bigcirc \bigcirc$, 1 juv. (on slides), 6 spp. (in alcohol); same data as for holotype; DIBEC • 1 \bigcirc (on slide); same data as for holotype; MHNF.

Other material examined (slide mounted unless otherwise stated)

GREECE – **Crete** • 4 $\Im \$, 5 $\Im \$, 3 juvs; Dikti Mountains, Viannou; 35.064291° N, 25.469778° E; 1320 m a.s.l.; 9 Oct. 2018; bark of *Z. abelicea* trees; D.J. Gwiazdowicz leg.; DIBEC • 7 $\Im \$, 12 $\Im \$, 1 juv.; Psiloritis Mountains, Rouvas; 35.164333° N, 24.922794° E; 1320 m a.s.l.; 10 Oct. 2018; D.J. Gwiazdowicz leg.; bark of *Z. abelicea* trees; DIBEC • 3 $\Im \$, 1 juv.; Thripti Mountains, Thripti; 35.080588° N, 25.887408° E; 1150 m a.s.l.; 14 May 2019; D. Ghosn leg.; branches of dwarfed *Z. abelicea* individuals; DIBEC • 16 $\Im \$, 4 juvs; Levka Ori, Omalos; 35.31901° N, 23.91871° E; 1160 m a.s.l.; 21 May 2019; D. Ghosn leg.; bark of *Z. abelicea* trees; DIBEC • 1 \Im ; same data as for preceding; 29 Sept. 2021; D.J. Gwiazdowicz leg.; DIBEC • 11 $\Im \$, 8 $\Im \$, 5 juvs; Levka Ori, Niato; 35.287527° N, 24.145503° E; 1215 m a.s.l.; 21 May 2019; D. Ghosn leg.; branches of dwarfed *Z. abelicea* individuals; DIBEC • 16 $\Im \$, 1 juv.; Levka Ori, Impros; 35.270546° N, 24.15315° E; 1175 m a.s.l.; 21 May 2019; D. Ghosn leg.; bark of *Z. abelicea* trees; DIBEC • 3 $\Im \$, 6 $\Im \$; Gingolos; May–June 2004; H.-J. Schulz leg.; sample 34626; SMNG • 1 $\Im \$, 2 $\Im \$, 2 juvs (on slides, formerly in alcohol); Katharo Plateau, Kritsa; 23 May 2011; H.-J. Schulz leg.; sample 47304; SMNG.



Fig. 3. A–B. *Xenylla ellisi* sp. nov., paratype, \bigcirc (DIBEC). A. Claw II. B. Mucrodens, lateral view. C–D. *Xenylla schulzi* sp. nov., paratype, \bigcirc (DIBEC). C. Mucrodens, lateral view. D. Mucrodens, dorsal view.

Description

Body length (without antennae) 0.6–1.6 mm. Colour (in alcohol): dorsal side blue gray to black, with pale spots over entire body, ventral side paler. Interior of the body white. Integument smooth or weakly verrucose with small primary hexagons.

Chaetotaxy as in X. *ellisi* sp. nov. (Fig. 2), but Abd. IV without dorsal setae m_5 and Abd. III without setae in front of retinaculum.

Ant. IV with simple apical vesicle (av), subapical organite (so), microsensillum (ms) and 4 (3 dorsoexternal and 1 dorsointernal) cylindrical sensilla (A and B thicker than C and D) as in Skarżyński *et al.* (2018): fig. 6. Ant. III-organ with two long (outer) and two short (inner) sensilla. Microsensillum on ant. III present. Ant. I with 7 setae.

Ocelli 5+5. Labrum with apical papillae. Labral setae 5, 5, 4, prelabrals 4. Maxillary head as in Skarżyński *et al.* 2018: fig. 11. Labium with seta F ca $2.5 \times$ as long as seta E. Labial palp as in Skarżyński *et al.* 2018: fig. 12. Outer lobe of maxilla with 2 sublobal hairs.

Tibiotarsi I, II and III with 19, 19 and 18 setae, respectively, with setae A_2 and A_7 capitate (ratio capitate setae/inner edge of claw III = ca 1.5). Femora I, II and III with 12, 11 and 10 setae, respectively, trochantera with 5, 5 and 4 setae, respectively, coxae I, II and III with 3, 7 and 7 setae, subcoxae 2 of legs I, II and III with 0, 2 and 2 setae, subcoxae 1 of legs I, II and III with 1, 2 and 3 setae, respectively. Claws with small inner tooth as in *X. ellisi* sp. nov. (Fig. 4).

Ventral tube with 4+4 setae. Retinaculum with 3+3 teeth.

Mucrodens $1.2-1.7 \times$ as long as inner edge of claws III, with 2 posterior setae, with marked ventral bend, with low mucronal lamella, not reaching apex, apex narrow and weakly upturned (Fig. 3C–D).

Anal spines small, situated on very low basal papillae.

Remarks

X. schulzi sp. nov. is most similar to *X. maritima* and *X. szeptyckii*, from which it differs in features presented in Table 3 and the key. Examination of the above-mentioned material revealed that this new species was reported from Crete by Schulz (2007) as *X. brevisimilis brevisimilis*, by Schulz & Burkhardt (2012) as *X. maritima* and by Gwiazdowicz *et al.* (2022) as *Xenylla* sp. 1 *maritima* complex.

Xenylla mediterranea Gama, 1964

Xenylla brevisimilis mediterranea Gama, 1964: 73.

Material examined

GREECE – Crete • 5 \bigcirc \bigcirc , 1 \bigcirc (on slides); wrack material; 3 Aug.1991; R. and S. Titgemeyer leg.; MIZ PAS.

Remarks

This species was recorded by Sterzyńska & Ehrnsberger (1997) from sea wrack accumulations in Crete. Examination of the specimens mentioned in this publication showed that they belong to *X. mediterranea*. Consequently, the occurrence of this species in Crete was confirmed.

Identification key to species of the Xenylla maritima complex

The key is based on Skarżyński et al. (2018).

1. _	Three sublobal hairs on outer lobe of maxilla2One or two sublobal hairs on outer lobe of maxilla4
2.	Setae in front of retinaculum on Abd. III and setae m ₅ on Abd. IV absent
_	Setae in front of retinaculum on Abd. III and setae m ₅ on Abd. IV present
3. -	Ratio: mucrodens/claw III length 2.3 X. asiatica Martynova, 1975 Ratio: mucrodens/claw III length 1–1.4 X. ellisi sp. nov.
4.	One sublobal hair on outer lobe of maxilla, retinaculum with 2+2 teeth
_	Two sublobal hairs on outer lobe of maxilla, retinaculum with 3+3 teeth
5. _	Setae m ₅ on Abd. IV absent, apex of mucro narrow, weakly upturned
6.	One or two setae in front of retinaculum on Abd. III
_	<i>X. szeptyckii</i> Skarżyński, Piwnik & Porco, 2018 Setae in front of retinaculum on Abd. III absent <i>X. schulzi</i> sp. nov.
7.	Ratio of mucrodens/claw III length 1–1.3, mucro spoon-like, two setae on leg III subcoxae 1 <i>X. nitida</i> Tullberg, 1871
_	Ratio of mucrodens/claw III length 2–2.3, mucro strongly spoon-like, three setae on leg III subcoxae 1

Discussion

By comparison within the genus of *Xenylla*, the two new species described here, *X. ellisi* sp. nov. and *X. schulzi* sp. nov., were found to be well defined morphologically and genetically. In particular, within the genus, their intra and interspecific genetic divergences were comparable to the ones measured among the eight other previously described species. Thus, DNA barcodes, by allowing the delineation of these two new species as discrete species-level genetic entities, supports their validity.

In addition, a new cryptic lineage was found within the morphological boundaries of the broadly distributed species *X. maritima*. This new lineage exhibited comparable genetic divergences with the other linage of *X. maritima* from Poland and with other species from the genus as well. These species-levels divergences suggest that this lineage could be a new species still to be described although we failed, for now, to found any diagnostic characters to sort *X. maritima* L1 and L2 apart. As in other cosmopolitan species, further sampling on a broader scale could allow us to uncover more of these cryptic lineages (Porco *et al.* 2012a, 2012b).

Previous taxonomic research on the *X. maritima* complex has already increased its number of species from three to eight (Skarżyński *et al.* 2018). However, taking into account the great diversification of this group, as indicated by Babenko *et al.* (1994), the potential for further increases in species number seems very likely. As the general distribution of species included in the complex is limited to the Palaearctic (except for *X. maritima*), and especially its warmer southern regions, further studies should be focused on these *Xenylla* diversity hotspots.

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References

Babenko A.B., Chernova N.M., Potapov M.B. & Stebaeva S.K. 1994. Collembola of Russia and Adjacent Countries. Family Hypogastruridae. Nauka, Moscow.

Bellinger P.F., Christiansen K.A. & Janssens F. 1996–2024. *Checklist of the Collembola of the World*. Available from http://www.collembola.org [accessed 3 Jun. 2024].

Benchling [Biology Software]. (2024). Available from https://benchling.com [accessed 8 Nov. 2024].

Deharveng L. 1983. Morphologie évolutive des Collemboles Neanurinae en particulier de la lignée Neanurienne. *Travaux du Laboratoire d'Ecobiologie des Arthropodes edaphiques, Toulouse* 4: 1–63.

Ellis W.N. 1976. Autumn fauna of Collembola from Central Crete. *Tijdschrift voor Entomologie* 119: 221–326.

Fjellberg A. 1984. Maxillary structures in Hypogastruridae (Collembola). *Annales de la Société royale zoologique de Belgique* 114: 89–99.

Fjellberg A. 1998. *The Collembola of Fennoscandia and Denmark, Part 1: Poduromorpha*. Fauna EntomologicaScandinavicavol.35.Brill,Leiden/Boston/Köln.https://doi.org/10.1163/9789004273634

Fjellberg A. 1999. The labial palp in Collembola. Zoologischer Anzeiger 237: 309–330.

Folmer O., Black M., Hoeh W., Lutz R. & Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3 (5): 294–299.

Gama M.M. da. 1988. Filogenia des espécies de *Xenylla* à escala mundial (Insecta, Collembola). *Evolución biológica, Coimbra* 2: 139–147.

Gwiazdowicz D.J., Skarżyński D., Fazan L., Fragnière Y., Ghosn D., Kozlowski G., Kuźmiński R., Remoundou E. & Zawieja B. 2022. Microarthropods living on the endemic tree *Zelkova abelicea* (Ulmaceae) with particular attention to Collembola diversity. *Forests* 13 (2): e195. https://doi.org/10.3390/f13020195

Hopkin S.P. 1997. *Biology of the Springtails (Insecta: Collembola)*. Oxford University Press, London. https://doi.org/10.1093/oso/9780198540847.001.0001

Hüther W. 1962. Beitrag zur Gattung *Willemia* Börner (Collembola). *Beiträge zur Entomologie* 12: 511–526.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. *Journal of Molecular Evolution* 16 (2): 111–120. https://doi.org/10.1007/BF01731581

Kumar S., Stecher G. & Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33 (7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Larsson A. 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics* 30 (22): 3276–3278. https://doi.org/10.1093/bioinformatics/btu531

Lawrence P.N. 1977. Studies on the tibiotarsal chaetotaxy of Collembola. *Systematic Entomology* 2: 313–317. https://doi.org/10.1111/j.1365-3113.1977.tb00380.x

Massoud Z. 1967. Monographie des Neanuridae, Collemboles Poduromorphes à pièces buccales modifiées. *In*: Delamare Debouteville C. & Rapoport E.H. (eds) *Biologie de l'Amérique Australe 3*: 7–399. CNRS, Paris.

Pomorski R.J. & Skarżyński D. 1997. A redescription of *Metaphorura denisi* Simon Benito, 1985 (Collembola, Onychiuridae, Tullbergiinae). *Genus* 8 (3–4): 489–496.

Porco D., Bedos A., Greenslade P., Janion C., Skarżyński D., Stevens M.I., Jansen van Vuuren B. & Deharveng L. 2012a. Challenging species delimitation in Collembola: cryptic diversity among common springtails unveiled by DNA barcoding. *Invertebrate Systematics* 26 (6): 470–477. https://doi.org/10.1071/is12026

Porco D., Potapov M., Bedos A., Busmachiu G., Weiner W.M., Hamra-Kroua S. & Deharveng L. 2012b. Cryptic diversity in the ubiquist species *Parisotoma notabilis* (Collembola, Isotomidae): a long-used chimeric species? *PLoS ONE* 7 (9): e46056. https://doi.org/10.1371/journal.pone.0046056

Porco D., Skarżyński D., Decaens T., Hebert P.D.N. & Deharveng L. 2014. Barcoding the Collembola of Churchill: a molecular taxonomic reassessment of species diversity in a sub-Arctic area. *Molecular Ecology Resources* 14: 249–261. https://doi.org/10.1111/1755-0998.12172

Rackham O. & Moody J. 1996. *The Making of the Cretan Landscape*. Manchester University Press, Manchester/New York.

Saitou N. & Nei M. 1987. The neighbor-joining method – a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4 (4): 406–425.

Schulz H.-J. 2007. Second contribution to the knowledge of the springtail fauna of the White Mountains (Lefká Óri) in West Crete (Insecta: Collembola). *Mitteilungen des internationalen entomologischen Vereins* 32: 175–183.

Schulz H.-J. 2010. A contribution to the Collembola fauna of Crete (Insecta: Collembola). *Mitteilungen des internationalen entomologischen Vereins* 35: 101–110.

Schulz H.-J. & Burkhardt U. 2012. Interessante Collembolennachweise aus Ostkreta (Hexapoda, Collembola). *Linzer biologische Beiträge* 44 (2): 1669–1676.

Schulz H.-J. & Lymberakis P. 2006. First contribution to the knowledge of the Collembola fauna of the White Mountains (Lefká Óri) in West Crete (Insecta, Collembola). *Senckenbergiana Biologica* 86: 229–234.

Sfenthourakis S. & Triantis K.A. 2017. The Aegean archipelago: a natural laboratory of evolution, ecology and civilisations. *Journal of Biological Research, Thessaloniki* 24: e4. https://doi.org/10.1186/s40709-017-0061-3

Simon Benito J.C. 1985. Colémbolos de suelos de sabinar en la provincia de Guadalajara. *Eos* 61: 293–318.

Skarżyński D. & Gwiazdowicz D.J. 2022. A redescription of *Hypogastrura gisini* Strenzke, 1954 (Collembola: Hypogastruridae) and description of a new related species from Crete (Greece). *Zootaxa* 5155 (1): 52–60. https://doi.org/10.11646/zootaxa.5155.1.2

Skarżyński D., Kahrarian M., Piwnik A. & Zawisza M. 2017. Description of *Hypogastrura ellisi* sp. n. (Collembola, Hypogastruridae), with notes on *H. tethyca* Ellis and the *trybomi* group. *ZooKeys* 719: 45–57. https://doi.org/10.3897/zookeys.719.14806

Skarżyński D., Piwnik A. & Porco D. 2018. Integrating morphology and DNA barcodes for species delimitation within the species complex *Xenylla maritima* (Collembola: Hypogastruridae). *Arthropod Systematics & Phylogeny* 76 (1): 31–43. https://doi.org/10.3897/asp.76.e31935

Smolis A. & Kaprus' I.J. 2009. Redescription of *Endonura cretensis* (Ellis, 1976) (Collembola: Neanuridae: Neanurinae) with a new record from Israel. *Annales Zoologici* 59: 1–6. https://doi.org/10.3161/000345409X432538

Smolis A., Skarżyński D. & Gwiazdowicz D.J. 2023. New species of Neanuridae (Collembola) living on the endemic tree *Zelkova abelicea* in Crete. *Zootaxa* 5278 (2): 333–350. https://doi.org/10.11646/zootaxa.5278.2.6

Spiliopoulou K., Dimitrakopoulos P.G., Brooks T.M., Kelaidi G., Paragamian K., Kati V., Oikonomou A., Vavylis D., Trigas P., Lymberakis P., Darwall W., Stoumboudi M.T. & Triantis K.A. 2021. The Natura 2000 network and the ranges of threatened species in Greece. *Biodiversity Conservation* 30: 945–961. https://doi.org/10.1007/s10531-021-02125-7

Sterzyńska M. & Ehrnsberger R. 1997. Marine algae wrack Collembola of European coasts. *Abhandlungen und Berichte des Naturkundemuseums Görlitz* 69 (2): 165–178.

Thibaud J.-M., Schulz H.-J. & Gama M.M. da. 2004. Synopses on Palearctic Collembola. Hypogastruridae. Vol. 4. *Abhandlungen und Berichte des Naturkundemuseums Görlitz* 75 (2): 1–287.

Xu S., Li L., Luo X., Chen M., Tang W., Zhan L., Dai Z., Lam T.T., Guan Y. & Yu G. 2022. Ggtree: A serialized data object for visualization of a phylogenetic tree and annotation data. *iMeta*: e56. https://doi.org/10.1002/imt2.56

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