



Research article

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Two new species of the genus *Siro* Latreille, 1796 (Opiliones, Cyphophthalmi, Sironidae) in the European fauna

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Abstract. Two new opilionid species from suborder Cyphophthalmi, family Sironidae, *Siro franzi* Karaman & Raspotnig sp. nov. and *Siro ozimeci* Karaman sp. nov., from Austria and Croatia respectively, are described and illustrated. Both species show a close relation to two other relict sironid species from the southern and eastern parts of the Alps, *Siro valleorum* and *Siro crassus*. All four species are treated here as a monophyletic, alpine group of genus *Siro*, opposed to the remaining two European sironids, *S. rubens* and *S. carpaticus* (palaeoeuropean *Siro* group). The history of the alpine *Siro* group parallels the history of a part of the dynamic European archipelago in the Mediterranean Tethys area, which became a part of the Alpine orogeny. Diversification of the alpine *Siro* group is the result of the orogenic evolution of the Alps, linked to the Austroalpine and South Alpine tectonic units.

Keywords. Taxonomy, phylogeny, alpine *Siro* group, Alpine orogeny, relict.

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Introduction

The first established cyphophthalmid genus *Siro* Latreille, 1796 has a long and dynamic history. *Siro rubens* Latreille, 1804 was the first species described in the genus, and it was the only species for many years. A number of species and subspecies, assumed to belong to this single genus, have been described many years later.

Recently, all species of the genus *Siro* from the Balkan Peninsula and Asia Minor were transferred into the resurrected genus *Cyphophthalmus* Joseph, 1869 (Boyer *et al.* 2005; Karaman 2005). The first

two discovered sironid species from North America were described as separate genera which were later synonymized with the genus *Siro* (Ewing 1923; Newell 1943). According to the currently widely accepted composition of the genus, it is distributed across wider areas of two continents, Europe and North America. The origin of such a composed genus is considered to be linked to areas of West Laurasia during the Triassic, i.e., representatives of the genus *Siro* have shared more than 200 Ma years of history as it has been suggested in an evolutionary time-tree of Cyphophthalmi Simon, 1879 (Giribet *et al.* 2012).

In Europe, the genus *Siro* is disjunctly distributed (Fig. 1) with 4 extant species: *S. rubens* from an area in southeastern France, *S. valleorum* Chemini, 1990 and *S. crassus* Novak & Giribet, 2006 from two remote areas in the Eastern Alps, and *S. carpaticus* Rafalski, 1956 from an area in the Eastern Carpathian Mountains. The only two fossil species were described from Europe, *S. platypedibus* Dunlop & Giribet, 2004 from Bitterfeld amber and *S. balticus* Dunlop & Mitov, 2011 from Baltic amber. In North America the genus is represented by 10 species, nine across the west coast and one species in the Appalachian Mountains to the east (Giribet & Shear 2010; Giribet *et al.* 2017).

For the last twelve years, we have studied a number of specimens of two new species of *Siro* from Austria and Croatia which we have not described yet for several reasons. First of all, we considered the (low) number of specimens of the new species from Croatia to be insufficient for a valid description. However, after several unsuccessful attempts, we gave up further searchings for additional specimens. In the case of the Austrian species, the absence of a character we considered synapomorphic for the genus *Siro* – and the thereby arising unclear generic affiliation – required additional, genetic research.

Material and methods

Morphological study

Dissection of the specimens and slide mounting were done as described in Karaman (2009). The separated body parts were prepared by dehydration through graded alcohol and acetone series before

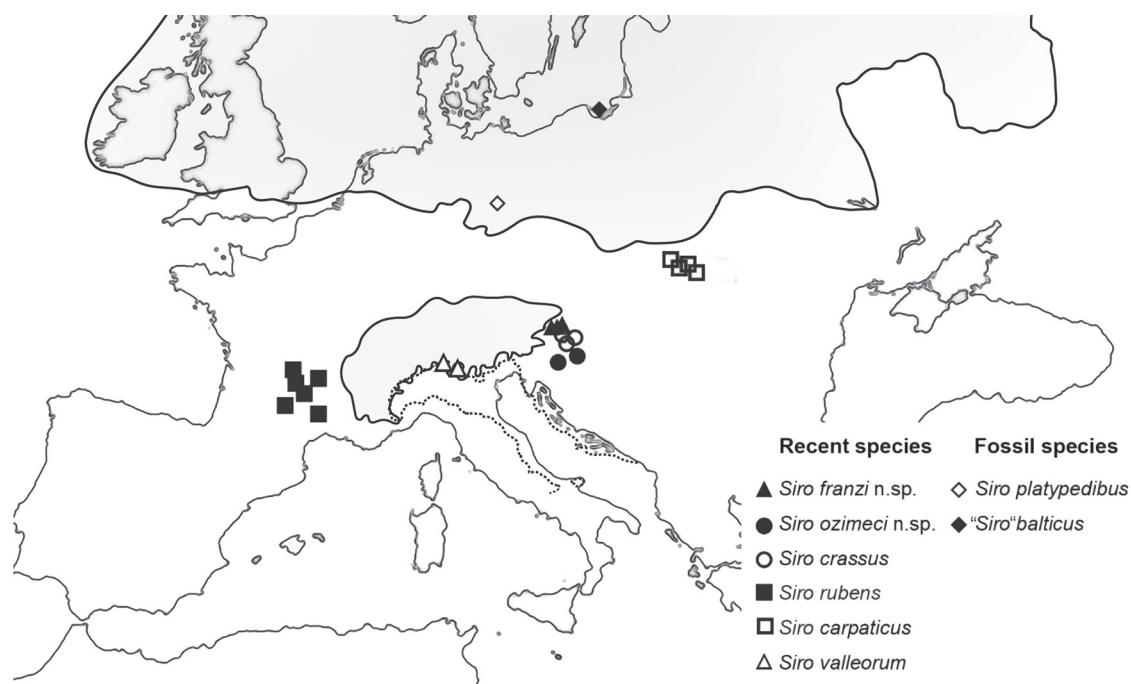


Fig. 1. Distribution of species of *Siro* Latreille, 1796 in Europe. Shaded areas: the maximum extent of glacial ice in north Europe and Alps during the Pleistocene; dashed line: extension of the northern part of the Adriatic Sea in the early Pliocene.

Table 1. Primer sequences for four gene fragments and corresponding references.

Gene	Primer	Sequence (5' → 3')	Reference
18S rRNA			
	1F	TACCTGGTTGATCCTGCCAGTAG	Giribet <i>et al.</i> 1996
	3F	GTTCGATTCCGGAGAGGGA	
	5R	CTTGGCAAATGCTTTCGC	
	9R	GATCCTTCCGCAGGTTACCTAC	
	18S a2.0	ATGGTTGCAAAGCTGAAAC	Whiting <i>et al.</i> 1997
	18S bi	GAGTCTCGTTCGTTATCGGA	
28S rRNA			
	28S a	GACCCGTCTTGAAACACGGA	Whiting <i>et al.</i> 1997
	28S b	TCGGAAGGAACCAGCTAC	
	28S rD1a	CCCSCGTAAAYTTAGGCATAT	Edgecombe & Giribet 2006
	28S D1F	GGGACTACCCCCTGAATTTAAGCAT	Park & Foighil 2000
16S rRNA			
	16S ar	CGCCTGTTTATCAAAAACAT	Xiong & Kocher 1991
	16S b	CTCCGGTTTGAAGTCAGATCA	Edgecombe <i>et al.</i> 2002
COI			
	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer <i>et al.</i> 1994
	HCOoutout	GTAAATATATGRTGDGCTC	Schwendinger & Giribet 2005
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> 1994

being cleaned in a sonicator. Dried samples mounted on stubs were prepared in a Baltec SCD005 sputter coater. Scanning electron microscopy (SEM) photographs were recorded with a JEOL-JSM-64601v SEM microscope under high vacuum. Microscopic photographs were done on a Zeiss Axio Imager A1. Drawings based on photographs were made in Adobe Illustrator CS2 on a Genius graphics tablet.

DNA-extraction, PCR and sequencing

DNA was extracted by means of the Chelex method following the protocol (CH1, 95°C for 20 min) described in Lienhard & Schäffer (2019). Genes or gene fragments of four different loci were analysed. Two nuclear ribosomal genes, namely the 18S rRNA (18S) and the 28S rRNA (28S), as well as the mitochondrial ribosomal 16S rRNA (16S), and the mitochondrial protein-encoding cytochrome c oxidase subunit 1 (COI) were investigated. Primer sequences and references are given in Table 1 (annealing conditions for PCR were performed as recommended by cited authors). DNA purification (with the enzyme cleaner ExoSAP-IT, Affymetrix; and the Sephadex G-50 resin, GE Healthcare) and sequencing steps (using the BigDye Sequence Terminator ver. 3.1 Cycle Sequencing Kit, Applied Biosystems) as well as Sanger sequencing steps were conducted as described by Koblmüller *et al.* (2004) on an automated capillary sequencer (ABI PRISM 3130xl). Sequencing was performed in both directions.

Table 2 (continued on next page). Investigated specimens with analysed loci and GenBank accession numbers and corresponding references.

Species	GenBank accession numbers				References
	18S rRNA	28S rRNA	16S rRNA	COI	
<i>Siro acaroides</i>	AY639490	DQ513128	AY639551	DQ825643	Boyer <i>et al.</i> 2005
	DQ513143	DQ513130	–	DQ513114	Giribet <i>et al.</i> 2006
<i>Siro boyerae</i>	DQ513139	DQ513125	–	DQ513112	Giribet <i>et al.</i> 2006
	KY352083	KY352111	–	KY352180	Giribet <i>et al.</i> 2017
<i>Siro calaveras</i>	KY352084	KY352113	KY352161	KY352181	Giribet <i>et al.</i> 2017
<i>Siro carpaticus</i>	KJ857536	KJ857539	KJ857545	KJ857542	Dreszer <i>et al.</i> 2015
<i>Siro clousi</i>	KJ857537	KJ857540	–	KJ857543	Dreszer <i>et al.</i> 2015
<i>Siro exilis</i>	AY639491	DQ825585	–	AY639579	Boyer <i>et al.</i> 2005
<i>Siro kamiakensis</i>	KY352087	KY352116	–	KY352182	Giribet <i>et al.</i> 2017
<i>Siro ligiae</i>	KY352086	KY352118	–	KY352184	Giribet <i>et al.</i> 2017
<i>Siro rubens</i>	AY428818	DQ825584	–	DQ513111	Giribet <i>et al.</i> 2004
<i>Siro shasta</i>	KY352090	KY352120	KY352162	KY352185	Giribet <i>et al.</i> 2017
	DQ513149	DQ513136	KJ857535	KJ857531	Giribet <i>et al.</i> 2006
<i>Siro valleorum</i>	AY639492	DQ513123	AY639552	AY639580	Boyer <i>et al.</i> 2005
<i>Cyphophthalmus duricorius</i>	KJ857509	KJ857512	KJ857515	KJ857527	Groh & Giribet 2015
<i>C. ere</i>	AY639462	DQ825593	AY639527	AY639557	Boyer <i>et al.</i> 2005
<i>C. gjorgjevici</i>	AY639464	DQ825587	AY639529	AY639559	Boyer <i>et al.</i> 2005
<i>C. gjorgjevici</i>	–	–	–	AY639560	Boyer <i>et al.</i> 2005
<i>C. martensi</i>	AY639471	DQ825589	AY639536	AY639563	Boyer <i>et al.</i> 2005
<i>C. minutus</i>	AY639473	DQ825591	AY639537	AY639565	Boyer <i>et al.</i> 2005
<i>C. rumijae</i>	AY639477	DQ825588	AY639539	AY639569	Boyer <i>et al.</i> 2005
<i>C. solentiensis</i>	KJ857519	KJ857523	KJ857533	KJ857529	Dreszer <i>et al.</i> 2015
	KJ857518	KJ857522	KJ857532	KJ857528	Dreszer <i>et al.</i> 2015
<i>C. teyrovskyi</i>	AY639482	DQ513118	AY639544	AY639571	Boyer <i>et al.</i> 2005
<i>C. zetae</i>	AY639485	AY639515	AY639546	AY639574	Boyer <i>et al.</i> 2005
<i>Paramiopsalis anadonae</i>	KY352076	JF934991	JF935024	JF786390	Giribet <i>et al.</i> 2017; Giribet <i>et al.</i> 2012
<i>P. ramblae</i>	KY352077	KY352104	KY352153	KY352173	Giribet <i>et al.</i> 2017
<i>P. ramulosus</i>	AY639489	DQ513121	AY639550	DQ825641	Boyer <i>et al.</i> 2005
	KY352070	KY352105	KY352154	KY352174	Giribet <i>et al.</i> 2017
<i>Paramiopsalis</i> sp.	EU638284	EU638287	EU638281	EU638288	Murienne & Giribet 2009
<i>Iberosiro rosae</i>	KY352066	KJ857524	KJ857534	KJ857530	Giribet <i>et al.</i> 2017
	KY352067	KY352097	KY352146	KY352166	Giribet <i>et al.</i> 2017

Table 2 (continued). Investigated specimens with analysed loci and GenBank accession numbers and corresponding references.

Species	GenBank accession numbers				References
	18S rRNA	28S rRNA	16S rRNA	COI	
<i>Parasiro coiffaiti</i>	EU638283	EU638286	KY352156	–	Murienne & Giribet 2009
<i>P. minor</i>	JF934958	JF934992	JF935025	JF786391	Giribet <i>et al.</i> 2012
<i>Suzukielus sauteri</i>	DQ513138	DQ513116	DQ518086	DQ513108	Giribet <i>et al.</i> 2006
	DQ825541	DQ825583	DQ825615	DQ825640	Boyer <i>et al.</i> 2007
<i>Siro crassus</i> (S2372)	ON023753	ON023735	–	–	own study
<i>Siro franzi</i> sp. nov. (P22KN)	ON023754	ON023736	ON074586	ON039634	own study
<i>Siro franzi</i> sp. nov. (P23KN)	ON023755	ON023737	ON074587	ON039635	own study
<i>Siro acaroides</i>	–	–	–	AY639578	Boyer <i>et al.</i> 2005
	–	–	–	DQ513113	Giribet & Shear 2010
	–	–	–	KY352177	
	–	–	–	KY352178	Giribet <i>et al.</i> 2017
<i>Siro rubens</i>	–	–	–	KY352179	
	–	–	–	AY428842	Giribet <i>et al.</i> 2004
<i>Siro ligiae</i>	–	–	–	KY352183	Giribet <i>et al.</i> 2017

Phylogenetic analyses

Included sequences and accession numbers are given in Table 2. Alignments were generated by means of the program MEGA7 (Kumar *et al.* 2016) and were aligned by eye. Genes were analysed individually, as mitochondrial (16S & COI) and nuclear (18S & 28S) datasets, and with all gene partitions combined. The concatenated datasets were trimmed using Gblocks ver. 0.91b (http://phylogeny.lirmm.fr/phylo.cgi/one_task.cgi?task_type=gblocks&tab_index=2, Talavera & Castresana 2007) applying default settings. After the Gblocks cut the remaining alignment contained 4936 bp in total (18S: ~1761 bp, 28S: ~2093 bp, COI: ~656 bp, 16S: ~426 bp). All built topologies show congruent results concerning the relationships within and among *Siro* spp. Because of the highest statistical support, the concatenated and the COI tree are shown in Figs 2 and 3, respectively. For these Bayesian analyses, Bayesian 50% majority-rule consensus trees were generated by means of MrBAYES ver. 3.2.6 (Ronquist *et al.* 2012) applying a MC3 simulation with 10 million generations (5 chains, partition by gene and/or by codon, 2 independent runs, 10% burn-in, GTR+I+G model). Results were analysed in TRACER ver. 1.7.1 (Rambaut *et al.* 2018) to check for convergence and to ensure the stationarity of all parameters. Newly generated sequences have been deposited in GenBank under accession numbers ON023753–ON023755, ON023735–ON023737, ON074586, ON074587, ON039634, and ON039635.

The acronyms used in the text are as follows:

GMV = Natural History Department of Varaždin City Museum, Croatia
 GRC = Guenther Raspotnig collection at the Institute of Biology, University of Graz, Austria

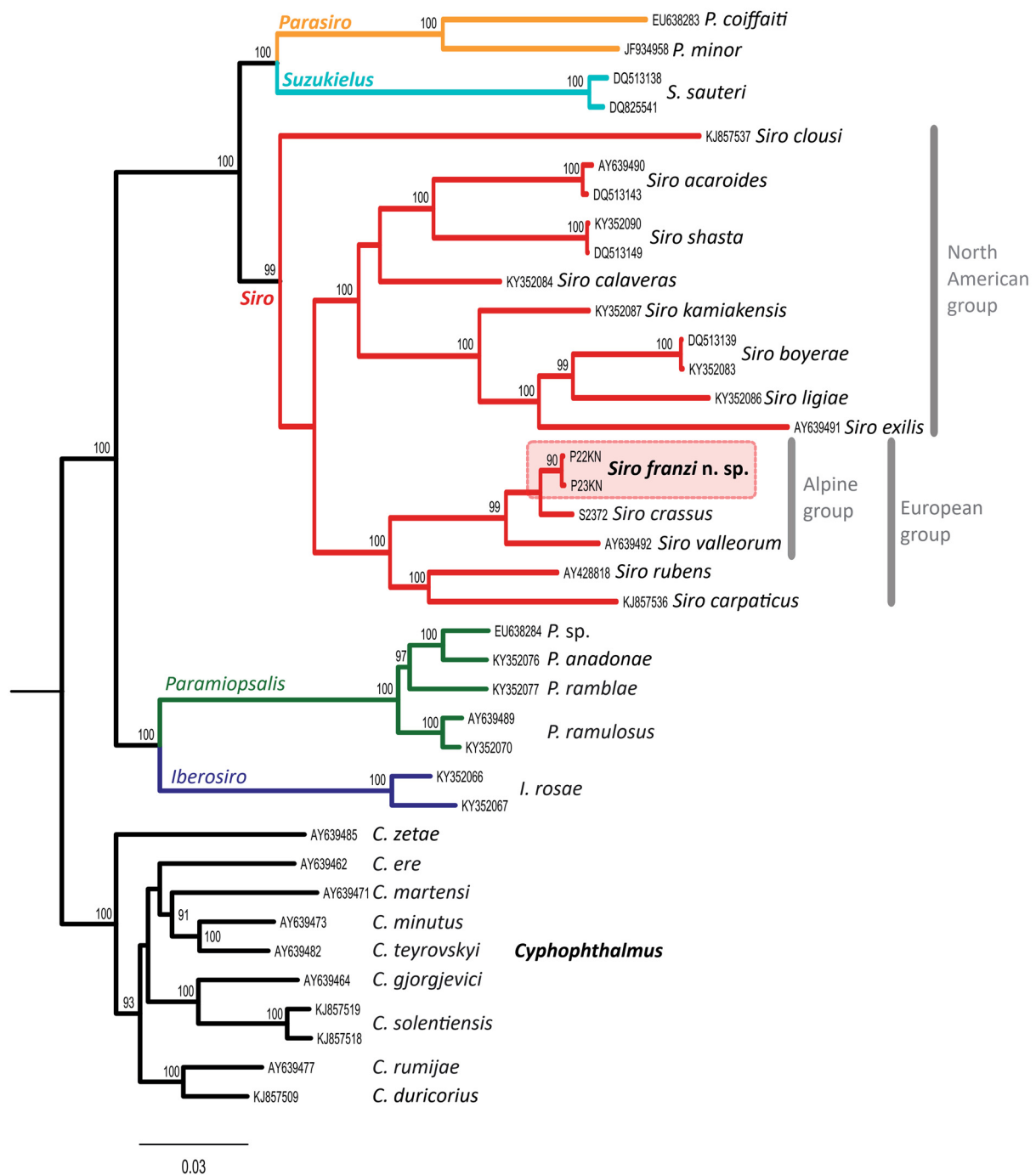


Fig. 2. Bayesian inference topology of the combined dataset (18S, 28S, 16S, COI; 4936 bp) of Sironidae Simon, 1879 comprised of 38 taxa. Posterior probabilities >90 are shown close to nodes. Coloured branches refer to different genera.

- IKC = Ivo Karaman collection at the Department of Biology and Ecology, University of Novi Sad, Serbia
 NHMW = Natural History Museum Vienna, Austria
 SEM = Scanning Electron Microscope

Results

Molecular data analyses

All built topologies confirm the monophyly of the genus *Siro* and the included European species with high statistical support (Figs 2–3). The new *Siro franzi* Karaman & Rasputnig sp. nov. is firmly nested within the European *Siro*, albeit missing some autapomorphic traits of the genus. It is further supported as representing an independent lineage close to the Eastern Alpine *Siro valleorum*, with both species being sister to the Eastern Alpine *Siro crassus*.

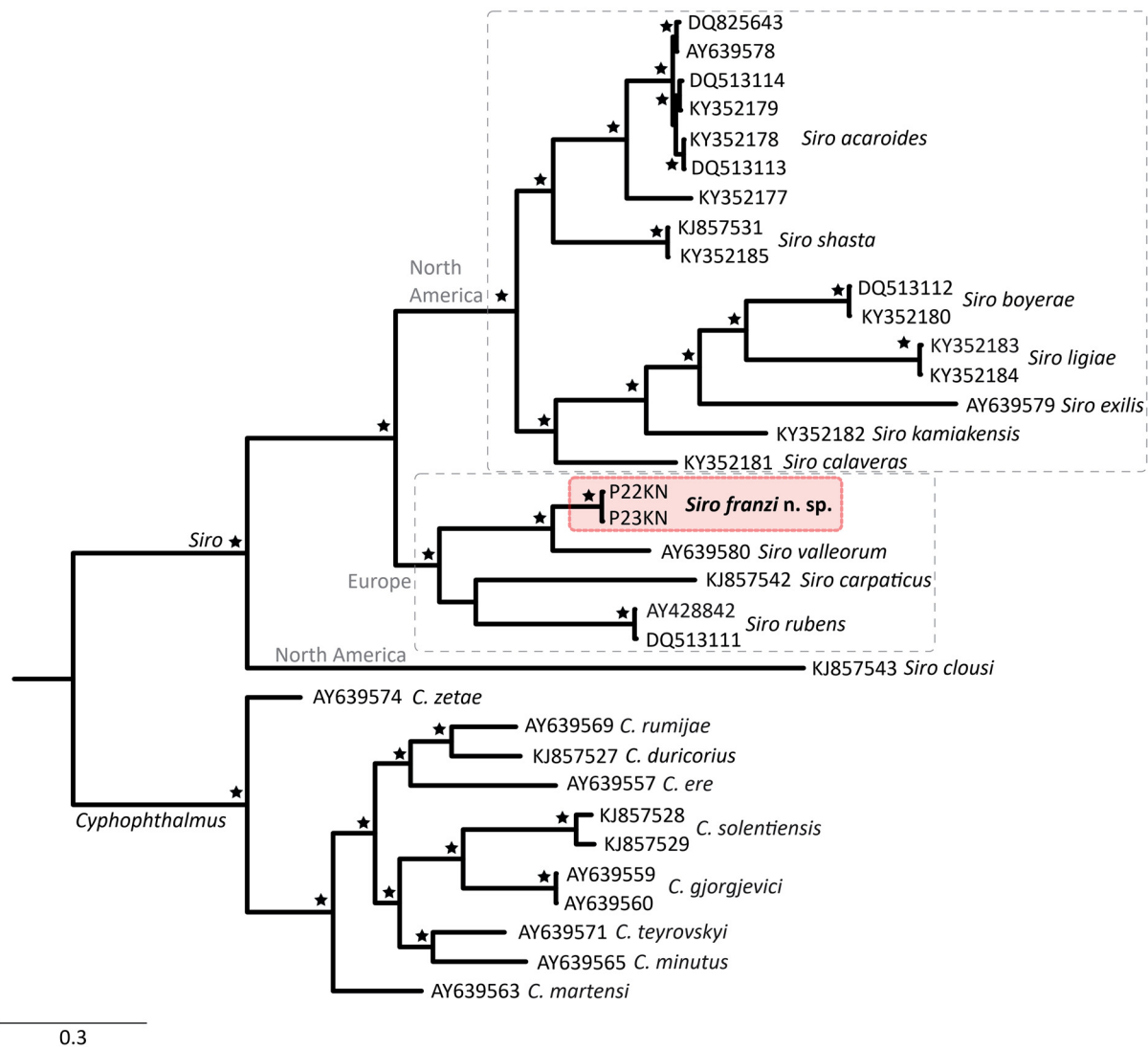


Fig. 3. Bayesian inference topology of the COI dataset (657 bp, 34 taxa) of *Siro* spp. Posterior probabilities >90 are indicated as stars and shown close to nodes.

Taxonomy

Class Arachnida Lamarck, 1801
Order Opiliones Sundevall, 1833
Suborder Cyphophthalmi Simon, 1879
Family Sironidae Simon, 1879

Genus *Siro* Latreille, 1796

Type species

Siro rubens Latreille, 1802.

Siro franzi Karaman & Raspotnig sp. nov.

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Figs 4–7

Diagnosis

Smallest European species of *Siro*, with a slender body and short legs. Females without both protruded anal region and elongate setae on it.

Etymology

The species is dedicated to Professor Herbert Franz, a famous coleopterist from Austria who first found this rare species.

Material examined

Holotype

AUSTRIA – **Styria** • ♂; Staritsch; 46°41'42.40" N, 15°11'45.46" E; 510 m a.s.l.; 8 Oct. 2016; K. Niklos leg.; NHMW 29919.

Paratypes

AUSTRIA – **Styria** • 1 ♂, 1 ♀; same collection data as for holotype; NHMW 29920 • 2 ♂♂; same collection data as for holotype; GRC KN39, KN40 • 1 ♀; same collection data as for holotype; 4 Jun. 2016; GRC KN24 • 3 ♂♂, 1 ♀; same collection data as for holotype; 22 Jun. 2016; GRC KN25, KN26, KN29, KN30 • 1 ♀; same collection data as for holotype; 26 Jun. 2016; NHMW 29921 • 1 ♂; same collection data as for holotype; GRC KN31 • 1 ♂; same collection data as for holotype; 29 Jun. 2016; NHMW 29922 • 1 ♂; same collection data as for holotype; GRC KN33 • 3 ♀♀; St Oswald ob Eibiswald; 46°40–42' N, 15°05–08' E; 1050 m a.s.l.; 9 Jul. 1960; H. Franz leg.; NHMW 28757 • 1 ♀; E Soboth, “Golobsattel”; 46°39–41' N, 15°05–07' E; 800–1100 m a.s.l.; 15 Jul. 1964; H. Franz leg.; NHMW 28758 • 1 ♀; Reinischkogel, St Oswald ob Eibiswald – surroundings or St Oswald in Freiland? [collection information uncertain]; 22 Jul. 1964; H. Franz leg.; NHMW 28759 • 1 ♂; Krumbachgraben, W St Oswald ob Eibiswald; 46°41–43' N, 15°03–06' E; 1150 m a.s.l.; 21 Aug. 1965; H. Franz leg.; NHMW 28760 • 1 ♂; Krumbach, ca 2 km W of St Oswald ob Eibiswald, Mauthnereck area, forest road to the left of road B69; 46°41' N, 15°06–07' E; 800–1000 m a.s.l.; 27 Jun. 2010; G. Raspotnig and I. Karaman leg.; IKC1538. – **Carinthia** • 1 ♂; southern slopes of the Koralpe, near St Vinzenz, near the border between Carinthia and Styria; 46°40–42' N, 15°00–01' E; 1070–1300 m a.s.l.; 14 Aug. 1965; H. Franz leg.; NHMW 28761 • 1 ♂; Soboth upper area of the Höllgraben, near forest road, approx. 20 m after the Styrian border; 46°39'(59") N, 15°02'(01") E; ca 1070 m a.s.l.; Jun. 1980; R. Schuster leg.; GRC-RS1.

Additional material

AUSTRIA – Styria • 1 ♂ (used for SEM); Staritsch; 46°41'42.40" N, 15°11'45.46" E, 510 m a.s.l.; 4 Jun. 2016; K. Niklos leg.; GRC KN21 • 1 ♂ (used for SEM); same collection data as for preceding; 8 Oct. 2016; GRC KN35 • 2 ♀♀ (used for SEM); same collection data as for preceding; 22 Jun. 2016;

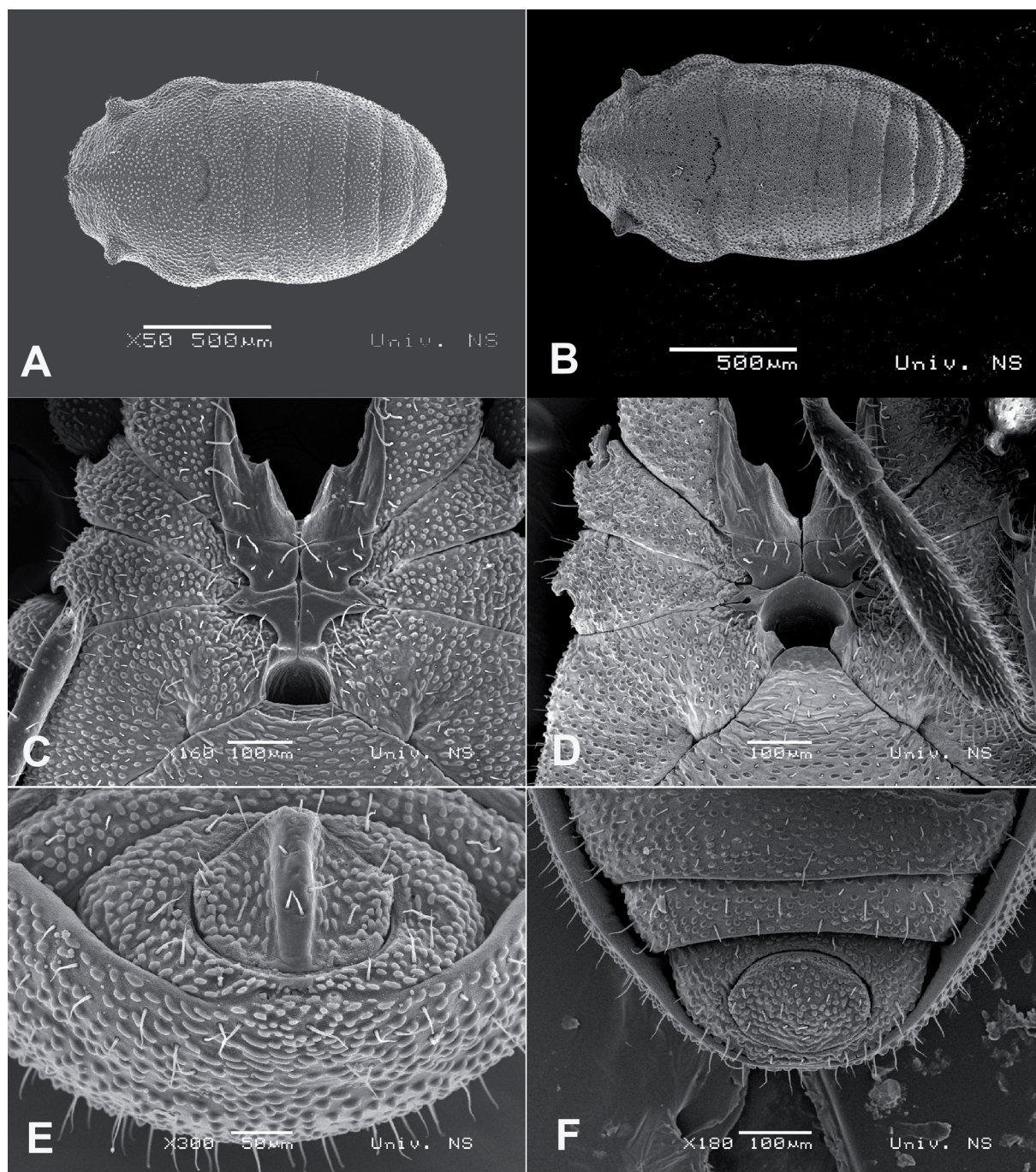


Fig. 4. *Siro franzi* Karaman & Raspotnig sp. nov. **A, C, E.** Paratype, ♂ (IKC1538). **B, D, F.** Paratype, ♀ (NHMW 28759). **A–B.** Dorsum. **C–D.** Ventral prosomal complex. **E.** Anal region, subventral view. **F.** Anal region, ventral view.

K. Niklos leg.; GRC KN27, KN28 • 2 ♂♂ (used for genetics); same collection data as for preceding; 4 Jun. 2016; K. Niklos leg.; GRC KN22, KN23

Description

Male

BODY. If not otherwise stated, measurements for the male holotype: body length 1.47 mm (males: 1.38–1.6 mm). Body uniformly light brown in color. Dorsum narrow, elongated (almost twice as long as wide) (Fig. 4A); anterior margin of prosoma widely convex; anterolateral margins short, slightly sinuate; posterior margin of opisthosoma bluntly rounded. Ozophores as long as wide at their bases, dorso-laterally oriented (type 2 – after Juberthie 1970). Anal plate ornamented with smooth, narrow and pronounced longitudinal medial ridge (Fig. 4E). All three pores of anal glands close to each other.

CHELICERAE. Short (Fig. 5A) (in paratype male 1.47 mm: basal article 0.73 mm long; second article 0.66 mm long), with pronounced ventral bulge and shallow dorsal depression on basal article. Basal article granulated on dorsal lateral and ventral side; medially sparsely granulated. Second article smooth.

PEDIPALPS. Of normal proportions (Fig. 5B) (in 1.47 mm long paratype 1.20 mm long without coxa and apotele); trochanter shorter than patella, half length of femur. Legs slightly elongated, all basitarsi and telotarsi without ornamentation. Telotarsus I (as on Fig. 5C) elongated (L/W ratio: 3.75), with distinct soleae. Claws I smooth and short. Telotarsus IV elongate (L/W ratio: 3) with adenostyle at base of

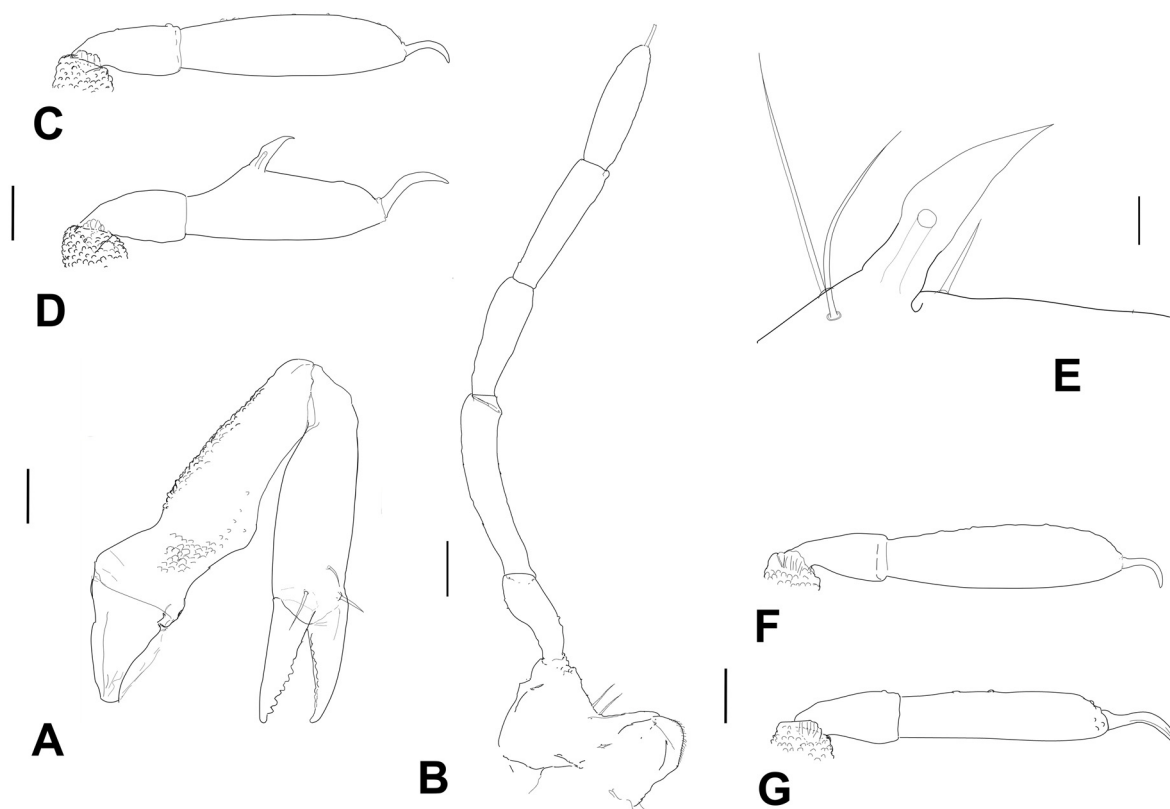


Fig. 5. *Siro franzi* Karaman & Rasputnig sp. nov. A–E. Paratype, ♂ (IKC1538). F–G. Paratype, ♀ (NHMW 28759). A. Chelicerae, medial view. B. Pedipalp, medial view. C. Basitarsus and telotarsus, leg I. D. Basitarsus and telotarsus, leg IV. E. Adenostyle. F. Basitarsus and telotarsus, leg I. G. Basitarsus and telotarsus, leg IV. Scale bars: A–D, F–G = 100 µm; E = 20 µm.

second third of its length (as on Fig. 5D); adenostyle of tubular lancet form (Fig. 5E). Measurements of legs (without coxae and claws): I 1.50 mm; II 1.45 mm; III 1.21 mm; IV 1.53 mm.

VENTRAL PROSOMAL COMPLEX (Fig. 4C). Coxal lobes II anteriorly wider for a third of its posterior width, less than three times as wide as long; coxal lobes III short, medially slightly protruded between coxal lobes II; conical processes situated laterally on frontal margin of gonostome.

SPERMATOPOSITOR (Fig. 6). Short and wide, with short terminal lobe. Distal part of terminal lobe slightly scale-like sclerotized. Setae terminales (5) short, about third of spermatopositor length; setae dorsales (6) long, two lateral pairs almost as long as spermatopositor. Three setae laterales on each side. Setae ventrales (3) standing on the terminal fourth of spermatopositor length. Movable fingers short, slightly sclerotized, curved outward, reach margin of median lobe; sensory papillae widely separated.

Female

Body length 1.5–1.72 mm, without protruding of anal region and without long lateral setae on it (Fig. 4F). Dorsum (Fig. 4B) narrow elongated, almost twice as long as wide; ventral prosomal complex (Fig. 4D) – coxal lobes II as in male, coxal lobes III short, meeting each other in middle, medially protruded between coxal lobes II. Ovipositor apical lobes (Fig. 7) more than three times as long as previous article. Receptacles of saccate form. Openings of receptacles situated on half of apical lobe length. Each apical lobe bearing one terminal, one ramified and 15 simple setae. Measurements of legs, without coxae and claws (female 1.5 mm body length): I 1.62 mm; II 1.42 mm; III 1.25 mm; IV 1.645 mm.

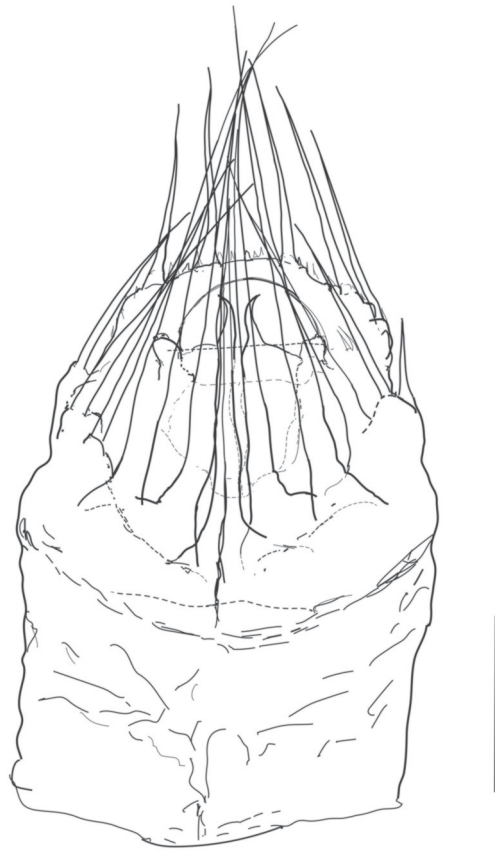


Fig. 6. *Siro franzi* Karaman & Rasputnig sp. nov., paratype, ♂ (IKC1538). Spermatopositor. Scale bar = 50 µm.

Remarks

Initial information on this new species was previously presented in Raspotnig *et al.* (2011). There, we speculatively treated *S. franzi* sp. nov. as a member of a new genus based on the absence of a protruded anal region in females of this species, which is a striking feature of all other European species of *Siro* (including *S. ozimeci* Karaman sp. nov., described below). We consider this character a synapomorphy of European *Siro*. This character is also not present in North American species of *Siro*, which are considered members of separate evolutionary lineages. Genetic analyses indicated the new species as sister to *Siro valleorum* and *S. crassus*, both of which are alpine species. The close relatedness of *S. franzi* and *S. valleorum* is also supported by some morphological characters. The structure of the spermatopositor and the coxosternal region (coxosternite II) are more similar among these two species compared to other species of the genus *Siro*. The absence of a protruded anal region in females of *S. franzi*, a character that is extremely pronounced in *S. valleorum*, is thus interpreted as a character loss. The structure of the anal plate, with a narrow and pronounced longitudinal carina followed by tightly spaced pores of anal glands (whose total spacing corresponds to the width of the carina), is a common feature of all alpine species of *Siro*, i.e., *S. valleorum*, *S. crassus*, *S. ozimeci* and *S. franzi*. In the remaining two European species of *Siro* (*S. rubens* and *S. carpaticus*) the longitudinal carina is low and wide, especially in the posterior part, accompanied by spaced pores.

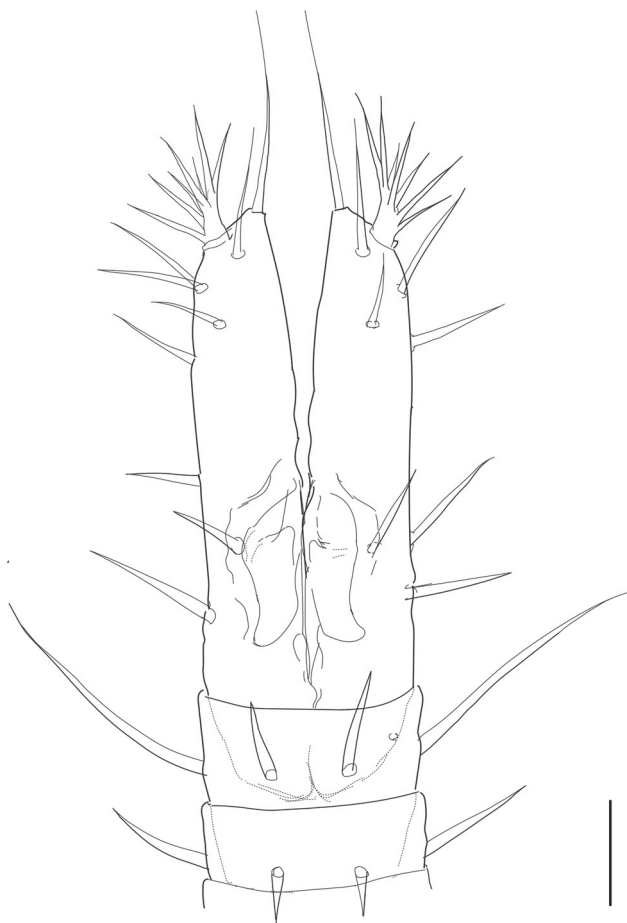


Fig. 7. *Siro franzi* Karaman & Raspotnig sp. nov., paratype, ♀ (NHMW 28759). Distal portion of ovipositor, ventral view. Scale bar = 50 µm.

***Siro ozimeci* Karaman sp. nov.**

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Figs 8–10

Diagnosis

Long-legged robust species of *Siro* with wide body. Female with pronounced anal protrusion of cubic form. Metatarsi of legs I–IV ornamented.

Etymology

The species is dedicated to its finder, our colleague and friend from Zagreb, Roman Ozimec.

Material examined

Holotype

CROATIA • ♀; Mt. Medvednica, Horvatove stube; 700 m a.s.l.; 13 Apr. 2008; R. Ozimec leg.; GMV 100066.

Paratype

CROATIA • 1 ♂; Mt. Žumberak, Sekulići, entrance of a small cave – Špilja kod Juraševe livade; 780 m a.s.l.; 6 Jan. 1998; T. Rubinić leg.; GMV 100067.

Description

Female (holotype)

BODY. Length 2.43 mm (L/W 1.56); body uniformly light brown in color. Dorsum wide, stocky (Fig. 8B); anterior margin widely convex; anterolateral margins short, sinuate; opisthosoma with deep transverse sulci; posterior margin of opisthosoma with pronounced anal protrusion of cubic form, three times as wide as long (Fig. 8D). Anal plate without longitudinal medial ridge. Ozophores as long as wide at their bases, dorso-laterally oriented (type 2).

CHELICERAE (Fig. 9A). Slightly elongated and robust. Basal article, 1.2 mm, with strongly pronounced ventral bulge; granulated on dorsal lateral and ventral side; medially sparsely granulated; second article 1.3 mm long, smooth, medially with a longitudinal row of sparse denticles.

PEDIPALPS (Fig. 9B). Elongated, 2.14 mm long (without coxa and apotele); trochanter sparsely granulated, significantly shorter than patella, half length of femur.

LEGS. Elongated, all basitarsi ornamented. Telotarsus I (Fig. 9C) elongated (L/W ratio: 4.5), with distinct soleae. Claws I smooth and slightly curved. Telotarsus IV elongate (L/W ratio: 4.44) (Fig. 9D). Claws IV strong. Measurements of legs (without coxae and claws): I 3 mm; II 2.83 mm; III 2.56 mm; IV 3.12 mm.

VENTRAL PROSOMAL COMPLEX (Fig. 8C). Coxal lobes II anterior width more than twice as wide as posterior width, 3.7 times as wide as long; coxal lobes III short, medially slightly protruded between coxal lobes II. Spiracles (Fig. 8E) circular in shape.

OVIPOSITOR APICAL LOBES (Fig. 10). More than three times as long as terminal article. Receptacles of saccate form, narrowed in basal third. Openings of receptacles situated in base of terminal half of apical lobe length. Each apical lobe bearing one terminal, one ramified and 23 simple setae.

Male (paratype)

The paratype male 2.32 mm long (L/W 1.56) (Fig. 9B). It was inadequately preserved, dried and mounted on glue board and for this reason some morphological details could not be researched. No further

material became available to study the spermatopositor and details of the anal plate. Coxal lobes II same as in holotype female. Gonostome semicircular, twice as wide as long. Conical processes of coxal lobes IV situated antero-laterally on anterior margin of gonostome. Spiracles circular in shape as in females.

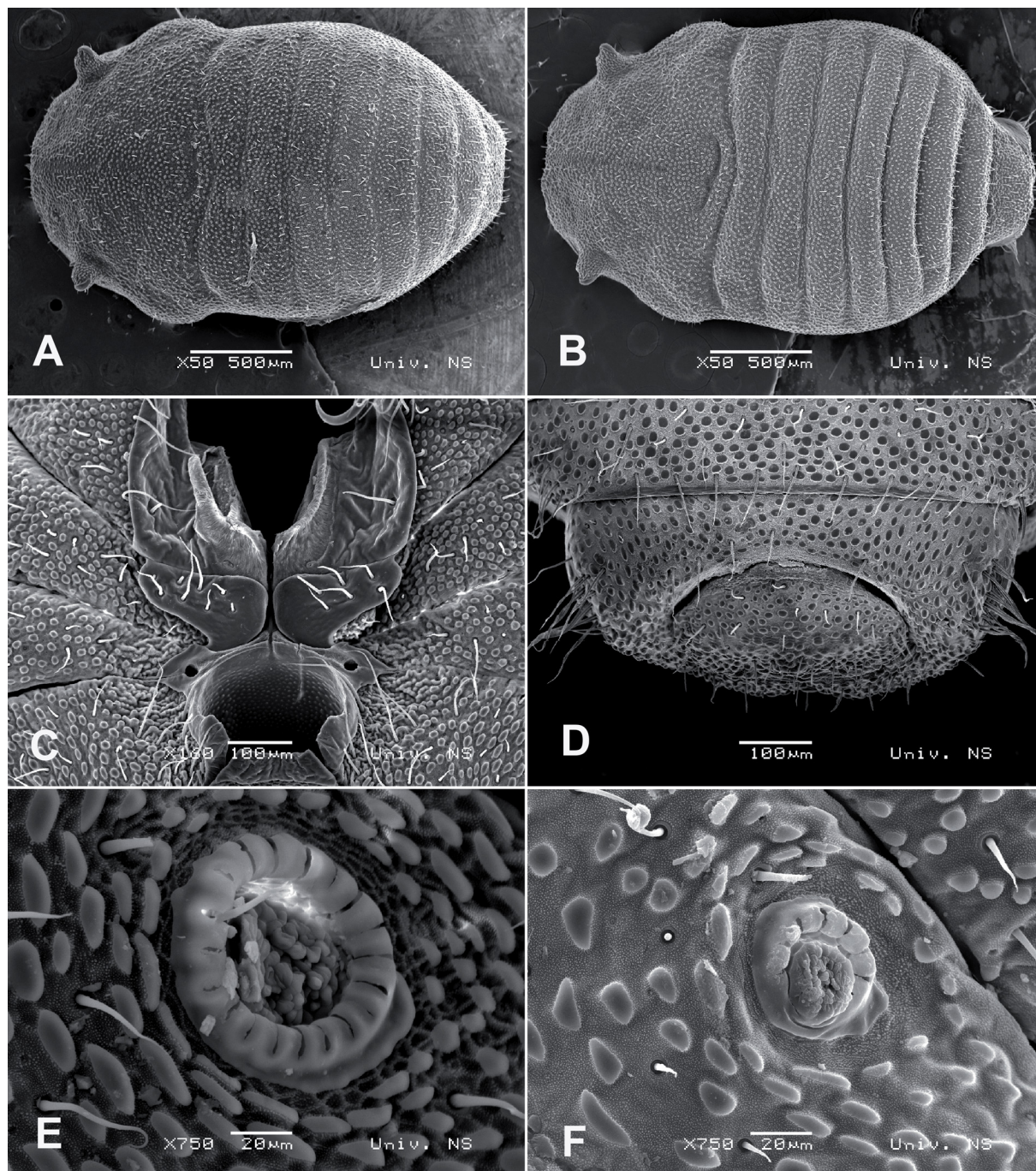


Fig. 8. *Siro ozimeci* Karaman sp. nov. **A.** Paratype, ♂ (GMV 100067). **B–E.** Holotype, ♀ (GMV 100066). **F.** *Siro franzi* Karaman & Rasputnig sp. nov. (IKC1538). **A–B.** Dorsum. **C.** Ventral prosomal complex. **D.** Anal region, ventral view. **E–F.** Spiracle.

Remarks

Siro ozimeci sp. nov. is considered to be closely related to *S. crassus*, from which it is distinguished by a more robust and wider body as well as a more pronounced anal protrusion in females. Minor differences are also expressed in the profile of the coxal lobes II and III. Coxal lobes III and the posterior part of coxal lobes II of *S. ozimeci* are narrower than in *S. crassus*. Both species are exceptional in the genus by the robustness of their bodies, elongated legs and fully ornamented basitarsi, and the extended coxal lobes II. The similar profile of coxal lobes II, spiracles, chelicerae structure, proportions of pedipalp articles and ovipositor setation also point to their close kinship, probably representing the two closest species in the genus. They are also spatially close. The southernmost known locality for *S. crassus* is only 40 km air distance from the northernmost known locality (type locality) of *S. ozimeci*.

In Novak & Giribet (2006: fig. 27), the leg I of a *S. crassus* female is erroneously indicated as IV. That is the reason for the striking difference between the illustrations of leg IV in these two closely related species.

Deep transverse opisthosomal sulci and, because of that, a wrinkled appearance of the *S. ozimeci* sp. nov. holotype female is most likely not a characteristic feature females in this species rather than an aberration.

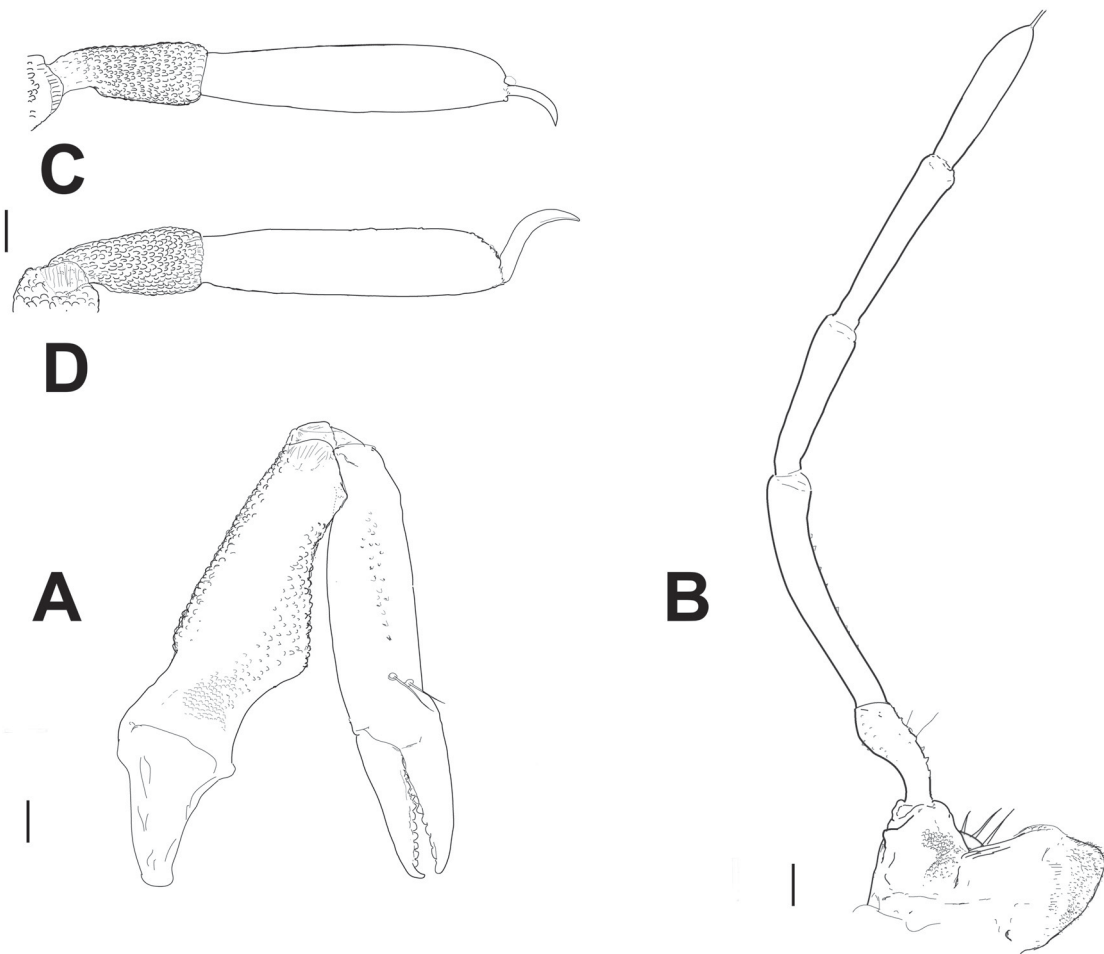


Fig. 9. *Siro ozimeci* Karaman sp. nov., holotype, ♀ (GMV 100066). **A.** Chelicerae, medial view. **B.** Pedipalp, medial view. **C.** Basitarsus and telotarsus, leg I. **D.** Basitarsus and telotarsus, leg IV. Scale bars = 100 µm.

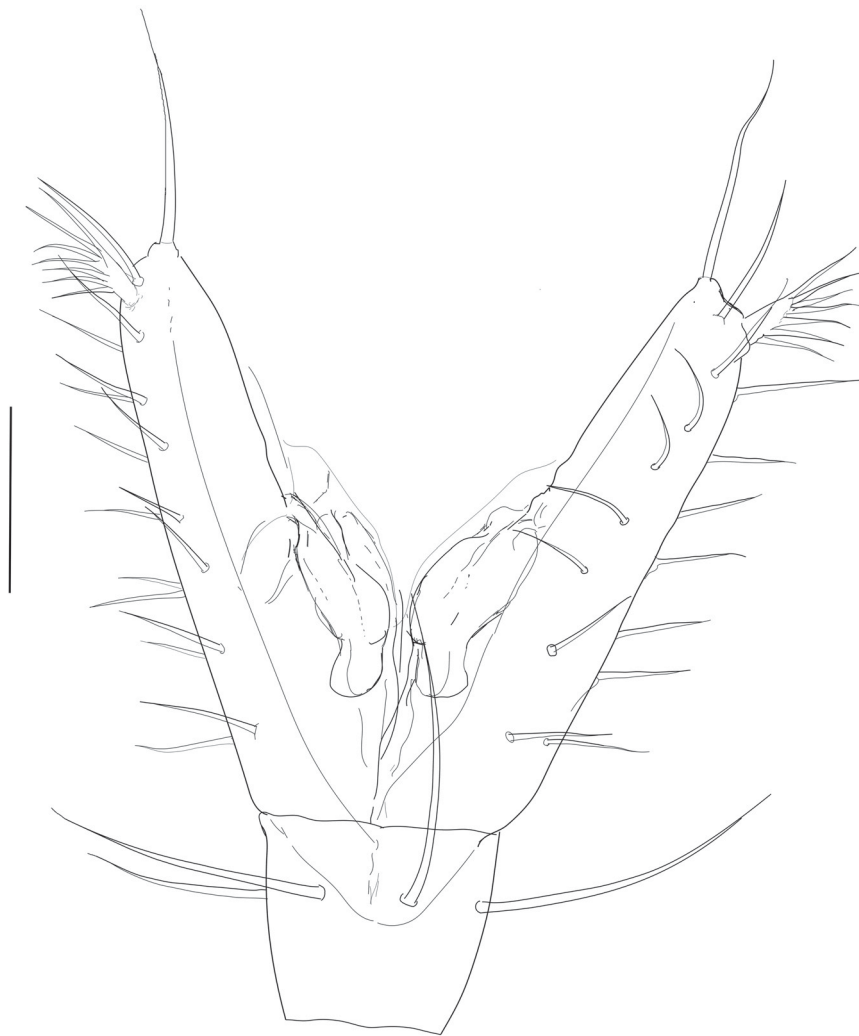


Fig. 10. *Siro ozimeci* Karaman sp. nov., holotype, ♀ (GMV 100066). Distal portion of ovipositor, ventral view. Scale bar = 100 μ m.

The species is distributed in a Peripannonian region in the border zone between the Pannonian Basin, Prealps and Dinarides of the Balkan. It is the only representative of the genus *Siro* present on the Balkan Peninsula (albeit only partially, in its extreme northwestern rim).

Discussion

The small range of *S. franzi* sp. nov. (south slopes of the Koralpe) reflects its relict distribution and indicates its low vagility and probably more specific requirements regarding the substrate and humidity regime when compared to other sironids of this region. The closely related *S. crassus* and *S. ozimeci* sp. nov. are distributed further southwards (Fig. 1), have a wider range of distribution (also of relictual character), reflecting their higher vagility which, along with less specific environmental requirements, probably enabled them to expand their areas into the south. Long-legged *S. crassus* and *S. ozimeci* significantly differ by their larger size and proportions from other species of the genus *Siro*. We believe that these specific differences represent a synapomorphy resulting from preadaptation from endogean

to a more terricole lifestyle under conditions of reduced competition and a more humid climate during the Tertiary.

Our molecular results indicate a close relationship among *S. franzi* sp. nov., *S. crassus*, and *S. valleorum*, as do those of Giribet *et al.* (2017) for the two latter species. However, the morphology of *S. crassus* (and *S. ozimeci* sp. nov.) differs significantly from that of *S. valleorum* and *S. franzi*. Nonetheless, we believe that these differences are driven by strong selection pressure by novel habitats and the lifestyle of the *S. crassus* and *S. ozimeci* ancestor, rather than large phylogenetic distances from the remaining two alpine species. Therefore *S. valleorum*, *S. franzi*, *S. crassus* and *S. ozimeci* are treated here as a monophyletic, alpine *Siro* group distinct from the remaining two paleoeuropean species *S. rubens* and *S. carpaticus* (palaeoeuropean *Siro* group).

In the second half of the Cretaceous, Europe transformed into an extensive island archipelago by tectonic movements (Csiki-Sava *et al.* 2015). The history of the alpine *Siro* group is related to the history of a part of the dynamic European archipelago in the Mediterranean Tethys area, which became part of the Alpine orogeny in a long-term alpine process. The diversification of the alpine *Siro* group is hence linked to the orogenic evolution of the Alps, resulting from the collision of the African and Eurasian tectonic plates. The distribution of the alpine *Siro* group is linked to the Austroalpine and South Alpine tectonic units, the uppermost units of the Alps which are widely exposed in the Eastern Alps. Both consist of material from the Apulian Plate (Schmid *et al.* 2004), which was thrust over the European Plate during Alpine orogeny. As the Austroalpine and South Alpine units are part of the Apulian Plate (that broke away from the African Plate), the origin of the alpine *Siro* group cannot be linked to them but to the European paleocontinental margin. Its continental crust slivers split off the major continental landmass during the Cretaceous. Such could also be the Briançonnais ribbon continent (Briançonnais terrane) (Fig. 11) which was close to and possibly attached to the European paleocontinental margin (Platt *et al.* 1989), or of which the Briançonnais terrane was a part. It was separated from the European paleocontinental margin with the opening of the Valais Ocean in Early Cretaceous times (Schmid *et al.* 2004). The northern margin of the Apulian Plate formed by the Austroalpine units collided first with the Briançonnais terrane during the earliest Tertiary (Plašenka 2009) before the collision with the European paleocontinental margin. If we trace the origin of the alpine *Siro* group to the Briançonnais terrane, it separated from the palaeoeuropean *Siro* group by the event of opening of the Valais Ocean. In that case, the ancestor of the alpine *Siro* group was located in the northern part of the Briançonnais terrane, well corresponding to the current picture of the distribution of the group exclusively in the eastern part of the Alps. The dynamic diversification of the alpine *Siro* group most likely began by the collision of two landmasses (Briançonnais terrane and Austroalpine units) in the early Tertiary.

In the second half of the Cretaceous the old cratonic areas of Europe were covered by epicontinental seaways dividing Europe into an archipelago of uplifted pre-Alpine massifs (Csiki-Sava *et al.* 2015). Palaeoeuropean species, *S. rubens* and *S. carpaticus*, were separated and survived thanks to the old orogens, Massif Central and Bohemian Massif, respectively, being relicts of that period. They also survived due to the geographical positions of these massifs (their parts) during the Pleistocene.

The long-term and dynamic Alpine orogenic process has certainly led to a significant diversification of the alpine *Siro* group. However, events during the Pliocene and especially in the Pleistocene reduced the alpine *Siro* fauna to the peripheral southern and eastern parts of Alps (Fig. 1). In this area are likely additional, yet undiscovered, species of *Siro*.

The area inhabited by *Siro crassus*, *S. ozimeci* sp. nov. and *S. franzi* sp. nov. has secondarily been occupied by *Cyphophthalmus duricorius* Joseph, 1868, which is dominant and very numerous in all localities where these species are present (the distributions of European species of *Siro* do not overlap).

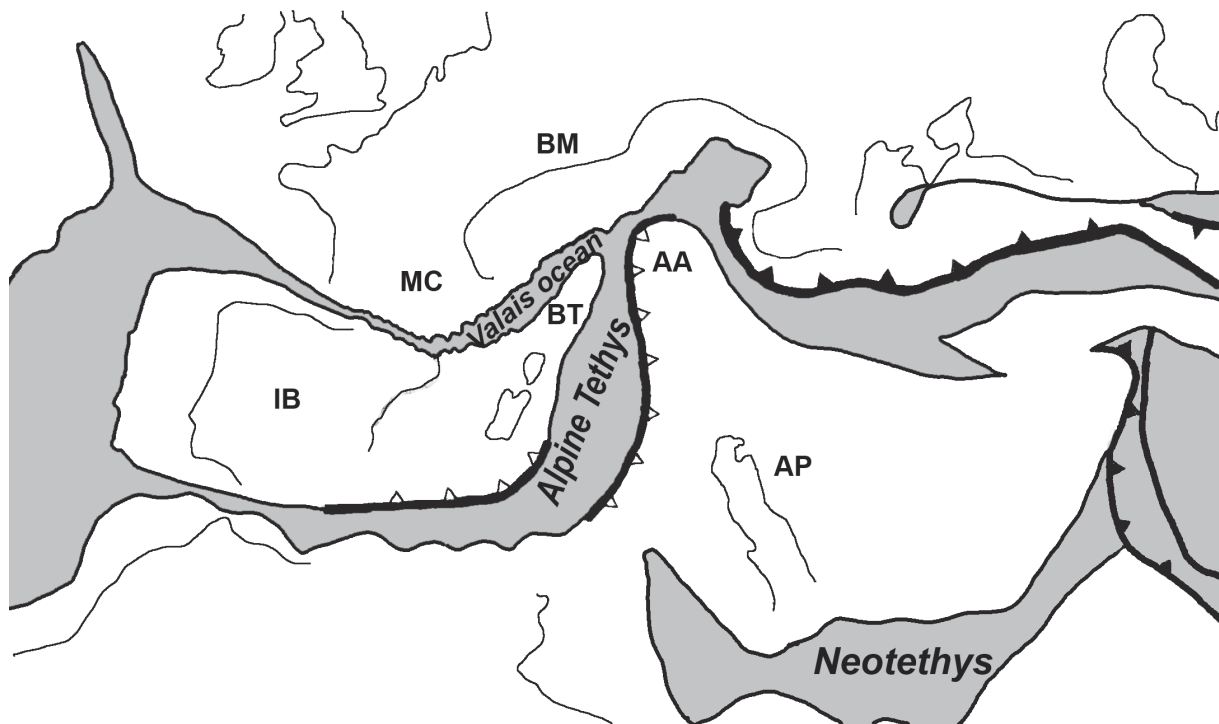


Fig. 11. Paleogeographical reconstruction of southern Europe for the Late Cretaceous (after Schmid *et al.* 2004, modified). AA = Austroalpine; AP = Apulian Plate; BM = Bohemian Massif; BT = Briançonnais terrane; IB = Iberia; MC = Massif Central.

Cyphophthalmus duricorius spread over into the areas inhabited by these species of *Siro* from the south, from the extreme western parts of the Dinarides. Quaternary events and climatic conditions have favored this species to expand its range, now representing one of the species of Cyphophthalmi with the largest known area of distribution. We assume that competition and inferiority of the regarded species of *Siro* are the reason for the small number of individuals hitherto found, along with the fact that they are obviously rare in the areas where they are present. This is not the case with the remaining European species of *Siro* (*S. rubens*, *S. carpaticus* and *S. valleorum*) which are often numerous in their characteristic habitats. The reason for this is most likely the lack of competition by other Cyphophthalmi.

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