The Genus Siro Latreille, 1796 (Opiliones, Cyphophthalmi, Sironidae), in North America with a Phylogenetic Analysis Based on Molecular Data and the Description of Four New Species

THE GENUS *SIRO* LATREILLE, 1796 (OPILIONES, CYPHOPHTHALMI, SIRONIDAE), IN NORTH AMERICA WITH A PHYLOGENETIC ANALYSIS BASED ON MOLECULAR DATA AND THE DESCRIPTION OF FOUR NEW SPECIES

GONZALO GIRIBET[1] AND WILLIAM A. SHEAR[2]

ABSTRACT. The North American fauna of the Laurasian family Sironidae is examined phylogenetically and compared with species from Europe and Japan. The North American clade is not resolved as monophyletic. The phylogenetic analyses and detailed morphological study identified four cryptic species of sironids in the western United States, formerly considered within the geographical and morphological range of *Siro acaroides* (Ewing, 1923). These four species are described as *Siro boyerae* sp. nov., *Siro calaveras* sp. nov., *Siro clousi* sp. nov., and *Siro shasta* sp. nov. We also provide new localities for the previously known species in the western United States. *Siro boyerae* sp. nov. forms a clade with *Siro kamiakensis* (Newell, 1943) and with the East Coast species *Siro exilis* Hoffman, 1963, characterized by the presence of narrow coxae III that do not meet along the midline. The affinities of *S. calaveras* sp. nov., *S. clousi* sp. nov., and *S. shasta* sp. nov. remain largely unresolved, but *S. clousi* sp. nov., is not related to *S. acaroides* despite being found sympatrically.

INTRODUCTION

The cyphophthalmid genus *Siro* currently includes a series of species found in North America and continental Western Europe (Giribet, 2000; Juberthie, 1970; Novak and Giribet, 2006; Shear, 1980). The status of the European members of the genus *Siro* has been recently revised, and the radiation of species related to *Cyphophthalmus duricorius* Joseph, 1868, in the Balkans and adjacent geographic areas seems to be unrelated to *Siro rubens* Latreille, 1804, and therefore considered a different genus (Boyer et al., 2005; Karaman, 2008; Murienne et al., 2010). In this article, we restrict the concept of *Siro* to a clade of recent Western European species composed of *S. rubens*; *Siro carpaticus* Rafalski, 1956; *Siro crassus* Novak & Giribet, 2006; and *Siro valleorum* Chemini, 1990, and to a clade of several North American species: *Siro acaroides* (Ewing, 1923); *Siro exilis* Hofman, 1963; *Siro kamiakensis* (Newell, 1943); and *Siro sonoma* Shear, 1980. The four previously known North American species were revised by Shear (1980) and profusely illustrated by de Bivort and Giribet (2004: figs. 10–39).

Siro acaroides was described in 1923 as the type of the new genus *Holosiro* Ewing, 1923, this species being the first cyphophthalmid discovered in the New World (Ewing, 1923). Later, it was recognized that the species could not be easily distinguished from the European *Siro* at the generic level, and *Holosiro* was considered a junior synonym of *Siro* (Newell, 1943). In the same article, Newell described a new species of American sironid in the new genus *Neosiro* Newell, 1943, for the species *Neosiro kamiakensis*. The new genus was based on the divided fourth tarsus of the male. Both species inhabit western North America, each originally described from single localities: *S. acaroides* from Benton County, southwestern Oregon, and *N. kamiakensis* from Whitman County in western Washington. An eastern North American species, *Siro*

[1] Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, Massachusetts 02138.

[2] Department of Biology, Hampden-Sydney College, Hampden-Sydney, Virginia 23943.

exilis, found in the Appalachian Mountains along the boundary between Virginia and West Virginia, was subsequently added to the list (Hoffman, 1963).

Meanwhile, Davis (1933) had described *Siro americanus* from northwestern Florida; after an unwarranted sojourn in the genus *Parasiro* Hansen and Sørensen, 1904 (Hinton, 1938), this species was made the type of the new genus *Metasiro* Juberthie, 1960 (Juberthie, 1960), within the family Sironidae (or Sironinae of Juberthie, 1970) (Giribet, 2000; Juberthie, 1970; Shear, 1980). Later on, Hoffman (1963) proposed the synonym genus *Floridogovea* Hoffman, 1963, for *Metasiro*. Based on ample morphological and molecular evidence, *Metasiro* is now considered a member of Neogoveidae (Boyer et al., 2007b; Giribet, 2007).

In 1980, Shear had access to a wide range of collections that had been assembled since 1947 (Shear, 1980). Contrary to the assertions of Ewing (1923) and Newell (1947), Shear (1980) proposed that *S. acaroides* was widely distributed in the Coast Ranges from northern California to Puget Sound and that *N. kamiakensis* occurred in at least one more locality in western Washington (Mt. Spokane) and at three places in Kootenai and Idaho Counties, in northern Idaho. Furthermore, Shear argued that the preponderance of characters of *N. kamiakensis* were consistent with a placement in the genus *Siro* and so synonymized Newell's genus *Neosiro*. Shear also added a distinctive fourth species of *Siro*, *S. sonoma* Shear, 1980, from Sonoma County in Northern California.

Some loose ends were mentioned in Shear's 1980 paper. In particular, a single female specimen from Calaveras County, California, in the Sierra Nevada Mountains, seemed clearly to be a new species, but Shear was reluctant to describe it from a single female example. Now additional material from that same collection has become available, and it is clear that this population represents a new, fifth species of American *Siro*. Additional material has also been recently collected by G. Giribet, S. Boyer, and R. M. Clouse in Calaveras Big Trees State Park, which was suitable for molecular work.

The map Shear published in 1980 did not correlate well with the list of localities given; a location for *S. acaroides* is shown significantly south of the California/Oregon border, but only Del Norte County records are listed in the text. This map symbol was added late in the preparation of the paper and referred to Shasta County specimens that were then considered *S. acaroides*. They are of a yet another new species.

A field trip through Idaho, Washington, Oregon, and Northern California by G. Giribet, S. Boyer, and R. M. Clouse in June 2005 yielded numerous collections of Cyphophthalmi, including all known species for the western United States, with the exception of the elusive *S. sonoma*. The aim of this trip was to obtain more specimens of the new species from Calaveras County and Shasta County, as well as to revisit other cyphophthalmid localities to obtain specimens suitable for molecular work for all the NW U.S. species. Two specimens of *S. sonoma* were collected by G. Giribet, T. Briggs, and D. Ubick in Monte Rio, December 2001. Phylogenetic analysis of the new specimens further revealed the presence of multiple cryptic lineages in the previously considered widespread species *S. acaroides*. Two of these species that could be characterized morphologically are described here. The new species double the number of known American sironids but also indicate that our knowledge of the American sironid fauna is still in its infancy.

California has not been intensively explored for cyphophthalmids. They are most easily collected from Berlese samples; the success of this method was demonstrated by the many specimens and new records of *S. acaroides* obtained by Ellen Benedict (Shear, 1980). We have also been successful collecting many live specimens by sifting with a 4-mm mesh size or via extraction with Winkler apparatus. But other than these

examples, most specimens have been obtained after occasional direct collecting. We predict that a thorough search of proper habitats in the Sierra Nevada, and both northern and southern Coast Ranges in California, will yield more new species of sironids. The distribution pattern of soil-dwelling organisms with species in the Appalachians in the east and the Coast Ranges and northern Idaho in the west often includes the central Rocky Mountains as well; *Siro* might be expected to turn up in Utah, Colorado, or New Mexico. With 43 extant species of sironids in Europe, it seems reasonable to expect that North America eventually could be shown to have more species than the 10 we know now.

MATERIALS AND METHODS

Abbreviations for Repository Institutions

AMNH	American Museum of Natural History, New York, New York, USA
BMNH	The Natural History Museum, London, United Kingdom
CAS	California Academy of Sciences, San Francisco, California, USA
CNHM	Field Museum of Natural History, Chicago, Illinois, USA (usually, FMNH for Field Museum of Natural History)
EME	Essig Museum of Entomology, U.C. Berkeley, Berkeley, California, USA
FMNH	Field Museum of Natural History, Chicago, Illinois, USA (in some labels, CNHM for Chicago Natural History Museum)
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA
MHNG	Muséum d'histoire naturelle, Geneva, Switzerland
SMF	Senckenberg Museum, Frankfurt am Main, Frankfurt, Germany

Morphological Methods

For each species, the male holotype and a female paratype were photographed using a JVC KY-F70B digital camera mounted on a Leica MZ 12.5 stereomicroscope. A series of images (ca. 10) were taken at different focal planes and assembled with the dedicated software package Auto-Montage Pro Version 5.00.0271 (Syncroscopy, Frederick, Maryland, USA). Each specimen was photographed in dorsal, ventral, and lateral views, and when available, the holotype was always photographed. Full body measurements of the holotype and a female paratype were then taken from these photographs in Adobe Photoshop CS3 with the "Analysis" menu and were recorded in a spreadsheet. Total body length refers to the distance between midpoint of anterior and midpoint of posterior margin of the dorsal scutum. Body width refers to the maximum width, whether recorded in the prosomal or in the opisthosomal region.

One male and one female specimen of each species were examined with a FEI Quanta 200 SEM (Peabody, Massachusetts, USA). Appendage and body part measurements were taken from the digital micrographs in Adobe Photoshop CS3 with the "Analysis" menu and were recorded in a spreadsheet. Measurements of the chelicera, palp, and leg articles were mostly taken on their dorsal side, from the midpoint of the anterior margin to the midpoint of the posterior margin. Depths were measured on the lateral side at the widest portion, except for tarsus IV of the male, which was measured behind the adenostyle. Tarsal length does not include the claw. The position of the adenostyle on tarsus IV is given at the more clearly marked distal point, where it abruptly rises from the dorsal surface of the tarsus.

Finally, some body measurements were taken with an ocular micrometer on an Olympus SZH dissecting microscope, at 50×. Measurements of appendages temporarily mounted on microscope slides were

Figure 1. Map of the NW United States with the sampled localities for *Siro acaroides* (red), *S. boyerae* sp. nov. (navy blue), *S. calaveras* sp. nov. (yellow), *S. clousi* sp. nov. (white), *S. kamiakensis* (black), *S. shasta* sp. nov. (orange), and *S. sonoma* (green). For details on the collecting localities, see Table 1 and Supplemental Appendix 1.

taken with an ocular micrometer on an Olympus BX50 compound microscope, at 100× with Nomarski differential interference contrast. Drawings were made using the latter microscope, equipped with a drawing tube; Nomarski contrast was used to clarify details of the spermatopositors.

Molecular Sampling

To evaluate the phylogenetic position of the new species and for testing the validity of the "widespread" species *S. acaroides*, we undertook phylogenetic analyses of molecular data from specimens of all American sironids (see distribution map in Fig. 1) and multiple representatives of other sironid genera, including *Suzukielus* from Japan, *Paramiopsalis* and *Parasiro* from the Iberian Peninsula, *Siro* from France and Italy, and *Cyphophthalmus* from multiple localities in the Balkans (Table 1).

Molecular data were obtained from freshly collected specimens preserved in 96% EtOH at −80° C. DNA from preserved tissues was extracted with the use of the Qiagen DNeasy® Tissue Kit following standard protocols described, for example, by Boyer et al. (2005). Three different loci were chosen for this study. Ribosomal sequence data of complete 18S rRNA and a ca. 2.1-kb fragment of 28S rRNA were

TABLE 1. LIST OF TAXA, MCZ ACCESSION NUMBERS, LOCALITIES, AND GENBANK ACCESSION NUMBERS FOR EACH SEQUENCED LOCUS.

Species	MCZ Accession	Country, State	Coordinates	18S rRNA	28S rRNA	COI
Suzukielus sauteri	MCZ DNA101543	Japan	35°38′03″N, 139°14′28″E	DQ513138	DQ513116	DQ513108
Cyphophthalmus sp. nov.	MCZ DNA101342	Bulgaria	N/A	AY918870	DQ513117	AY918878
Cyphophthalmus cf. *teyrovskyi*	MCZ DNA100910	Montenegro	N/A	AY639482	DQ513118	AY639571
Cyphophthalmus trebinjanus	MCZ DNA101038	Bosnia & Herzegovina	N/A	AY639483	DQ513119	AY639572
Cyphophthalmus duricorius	MCZ DNA100487	Slovenia	N/A	AY639461	DQ513120*	AY639556
Paramiopsalis ramulosus	MCZ DNA100459	Spain	42°18′54″N, 008°29′12″W	AY639489	DQ513121	DQ513109
Parasiro coiffaiti	MCZ DNA101383	Spain	42°09′09″N, 001°55′49″E	AY918872	DQ513122	DQ513110
Siro rubens	MCZ DNA100457	France	44°05′00″N, 003°34′53″E	AY428818	AY859602	DQ513111
Siro valleorum	MCZ DNA100461	Italy	N/A	AY639492	DQ513123	AY639580
Siro acaroides	MCZ DNA100488	USA, Oregon	N/A	AY639490	DQ513128	AY639578
Siro acaroides	MCZ DNA101616	USA, Oregon	44°40′00″N, 123°55′58″W	DQ513142*	DQ513129*	DQ513113
Siro acaroides	MCZ DNA101619	USA, Oregon	43°38′58″N, 123°53′41″W	DQ513143	DQ513130	DQ513114
Siro acaroides	MCZ DNA101620	USA, California	41°50′17″N, 124°08′39″W	DQ513144	DQ513131*	-
Siro acaroides	MCZ DNA101621	USA, California	41°18′10″N, 124°01′03″W	DQ513145	DQ513132*	-
Siro boyerae sp. nov.	MCZ DNA101614	USA, Washington**	46°59′32″N, 121°50′47″W	DQ513139	DQ513125	DQ513112
Siro boyerae sp. nov.	MCZ DNA101617	USA, Oregon	45°54′56″N, 123°57′52″W	DQ513141	DQ513127	-
Siro calaveras sp. nov.	MCZ DNA101623	USA, California**	38°16′38″N, 120°18′19″W	DQ513146	DQ513133*	-
Siro clousi sp. nov.	MCZ DNA101871	USA, Oregon**	44°40′00″N, 123°55′58″W	DQ513140	DQ513126	-
Siro exilis	MCZ DNA100489	USA, Maryland	N/A	AY639491	DQ513124	AY639579
Siro kamiakensis	MCZ DNA101611	USA, Idaho	47°44′47″N, 116°42′07″W	DQ513147	DQ513134*	DQ513115
Siro kamiakensis	MCZ DNA101613	USA, Washington**	46°52′04″N, 117°09′28″W	DQ513148	DQ513135*	-
Siro shasta sp. nov.	MCZ DNA101622	USA, California**	41°03′49″N, 122°21′37″W	DQ513149	DQ513136*	-
Siro sonoma	MCZ DNA100507	USA, California**	38°26′37″N, 122°59′19″W	DQ513150*	DQ513137*	-

* Indicates partial sequences.

** Denotes specimens from the type locality.

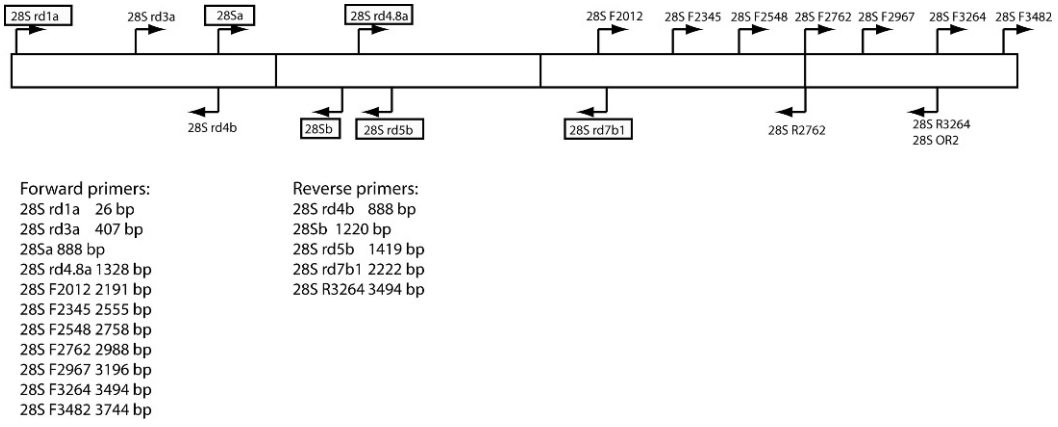

Figure 2. Schematic representation of a 28S rRNA locus with the primers listed in Supplemental Table 1 and used in this study. Primers used for amplification appear within a box. The position of each primer, in reference to the *Limulus polyphemus* 28S rRNA sequence AF212167 (Mallatt and Winchell, 2002), is provided. Information on primers not used in this study but included in the figure can be provided on request to G.G.

selected to resolve the deeper nodes in the trees, whereas the mitochondrial protein-coding gene cytochrome *c* oxidase subunit I (COI hereafter) was included to resolve more recent evolutionary events. The complete 18S rRNA loci were amplified in three overlapping fragments using the following primer pairs: 1F–4R, 3F–185sbi, and 18Sa2.0–9R. The 28S rRNA fragment was amplified in three overlapping fragments delimited by the following primer pairs: 28S rd1a–28Sb, 28Sa–28S rd5b, and 28S rd4.8a–28S rd7b1. COI was amplified with primer pair LCO1490–HCOoutout. Primer sequences, references, and annealing conditions are given in Supplemental Table 1. A map of the 28S rRNA primers used in this study is provided in Figure 2.

The amplified samples were purified using the QIAquick® PCR Purification Kit, labeled with BigDye® Terminator v3.0, and sequenced with an ABI 3730 genetic analyzer following manufacturer's protocols. Primers used in the sequencing reaction correspond to those used in the amplification step, with the addition of reverse primer 28S rd4b for the first fragment of 28S rRNA (see Table 1).

Chromatograms obtained from the automatic sequencer were read and "contig sequences" (assembled sequences) were assembled with the sequence editing software Sequencher™ 4.0 and further manipulated in MacGDE 2.4 (Linton, 2005). 18S rRNA measured between 1,762 and 1,763 bp and was divided into six fragments using defined primer regions. 28S rRNA was divided into 12 fragments defined by primer regions and conserved secondary structure motifs; two hypervariable fragments were deactivated from the analyses (but left in the raw data). The analyzed 28S rRNA segment (2,105–2,119 bp for the complete specimens) greatly expands previous cyphophthalmid datasets (e.g., Boyer et al., 2005; Giribet and Boyer, 2002; Schwendinger and Giribet, 2005). The protein-coding gene COI showed length variation. In addition, we combined published sequences with new sequences obtained with a primer located 3′ of the old HCO2198 (Boyer et al., 2005; Folmer et al., 1994), yielding sequences between 654 and 663 bp for the old fragment and between 811 and 814 for the new fragment. COI was divided into five fragments to accommodate published sequences on the basis of the Folmer et al. (1994) primers, with the new sequences using a primer downstream of HCO2198. In total, the amount of genetic data per complete taxon is ca. 4.7 kb, although COI did not amplify for many of

the North American sironids despite good DNA yield. All new sequences have been deposited in GenBank under the accession numbers DQ513108–DQ513150 (Table 1).

Molecular Data Analysis

The molecular data were analyzed with POY version 3.0 (Wheeler et al., 2004) according to the direct optimization method with parsimony as the optimality criterion (Wheeler, 1996). The data for all genes were analyzed independently and in combination. Tree searches were conducted in parallel (with PVM [Parallel Virtual Machine]) on a cluster of 30 dual-processor nodes (between 1 and 2.4 GHz) assembled at Harvard University (darwin.oeb.harvard.edu). Commands for load balancing of spawned jobs were, in effect, to optimize parallelization procedures (*-parallel -dpm -jobspernode 2*). Trees were built through a random addition sequence procedure (100 replicates) followed by a combination of branch-swapping steps (SPR [subtree pruning and regrafting] and TBR [tree bisection and reconnection]), and continuing with tree fusing (Goloboff, 1999, 2002) to further improve tree length. Discrepancies between heuristic and actual tree length calculations were addressed by adjusting slop values (*-checkslop 10*). While doing tree refinements with *tbr*, *-checkslop n* accepts all trees that are within *n* tenths of a percent of the current minimum value. For example, *-checkslop 10* accepts all trees up to 1% above the current minimum length while doing TBR.

POY facilitates efficient sensitivity analysis (Giribet, 2003; Wheeler, 1995). All data sets (individual genes and combinations) were analyzed under 10 parameter sets, for a range of indel-to-transversion ratios and transversion-to-transition ratios. The indel-to-transversion ratio refers to the opening gap cost, in that the extension gap cost was always fixed to 1. One parameter set follows the proposal of De Laet (2005), in which gaps are assigned a cost of 3, nucleotide transformations are assigned a cost of 2, and the gap extension cost is set to 1. Implied alignments—a topologically unique "alignment" or synapomorphy scheme (Giribet, 2005; Wheeler, 2003)—can be generated easily for each tree.

A character congruence technique, which is a modification of the ILD (Incongruence Length Difference) metric developed by Mickevich and Farris (1981; see also Farris et al., 1995), was used to select the most congruent parameter set, as proposed by Wheeler (1995; Table 2). The value is calculated for each parameter set by subtracting the sum of the scores of all partitions from the score of the combined analysis of all partitions, and normalizing it for the score of the combined length. This has been interpreted as a meta-optimality criterion for choosing the parameter set that best explains all partitions in combination, the one that maximizes overall congruence and minimizes character conflict among all the data (Giribet, 2003). This parameter set was given special consideration in the analysis of data from each individual gene and is referred to throughout this paper as the "optimal parameter set." Additionally, we discuss results from the strict consensus of all parameter sets explored, which has been interpreted as a measure of stability to model choice, as applied in statistical sensitivity analyses (Giribet, 2005; Wheeler, 2003), and the dependence on parameter set variation is shown graphically in the "Navajo rugs" at relevant nodes of our trees. Nodal support for all topologies was measured by parsimony jackknifing (Farris, 1997; Farris et al., 1996).

RESULTS

Sequence data were generated for all the known species of North American sironids, all of which have been recently collected by one of us (G.G.) and colleagues. However, *S. sonoma* has not been included in the analyses because it did not sequence well. After an initial collection of two specimens at the type locality in December 2001 (G.G., T. Briggs, D. Ubick), one male was used for SEM (de Bivort and Giribet, 2004), and a

TABLE 2. TREE LENGTHS FOR THE DIFFERENT PARTITIONS ANALYZED (18S = 18S RRNA; 28S = 28S RRNA; COI = CYTOCHROME *C* OXIDASE SUBUNIT I; MOL = THREE LOCI COMBINED) AND CONGRUENCE VALUE (ILD) FOR THE COMBINED ANALYSIS OF ALL THREE MOLECULAR LOCI COMBINED AT DIFFERENT PARAMETER SETS (LEFT COLUMN). THE FIRST NUMERAL USED IN THE PARAMETER SET COLUMN CORRESPONDS TO THE RATIO BETWEEN INDEL/TRANSVERSION, AND THE FOLLOWING TWO NUMBERS CORRESPOND TO THE RATIO BETWEEN TRANSVERSION/TRANSITION (E.G., 111 IS EQUAL WEIGHTS; 121 CORRESPONDS TO AN INDEL/TRANSVERSION RATIO OF 1:1 AND A TRANSVERSION/TRANSITION RATIO OF 2:1, SO INDELS HAVE A COST OF 2, TRANSVERSIONS HAVE A COST OF 2, AND TRANSITIONS HAVE A COST OF 1); 3221 CORRESPONDS TO THE PARAMETER SET ADVOCATED BY DE LAET (2005). (FOR A LIST OF SPECIFIC STEP MATRICES SEE GIRIBET ET AL., 2002: APPENDIX 4). OPTIMAL ILD VALUE IS INDICATED IN ITALICS.

	18S	28S	COI	MOL	ILD
111	70	445	1605	2132	0.00563
121	94	654	2453	3227	0.00806
141	140	1035	3924	5139	0.00778
211	72	499	1647	2234	0.00716
221	96	752	2511	3373	0.00415
241	144	1224	4150	5543	0.00451
411	74	586	1664	2331	0.00300
421	100	907	2532	3572	0.00924
441	152	1521	4211	5966	0.01374
3221	*143*	*912*	*3259*	*4324*	*0.00231*

female specimen was used for molecular work. After several attempts at amplifying its DNA, we were only able to obtain short amplifications, some from exogenous sources. Although we obtained sironid sequences for a fragment of 18S rRNA and 28S rRNA, the amount of information in such fragments is small, causing the species to become a wildcard. Subsequent collecting trips to the same locality in June 2005 and January 2006 and to additional localities in Sonoma County in June 2005 yielded no additional specimens of *S. sonoma*. Therefore, the results presented and discussed here are without *S. sonoma*.

Amplification of 18S rRNA and 28S rRNA was successful for all the specimens included in our analyses, although we had some difficulties amplifying the three 28S rRNA fragments employed here for all the North American species (indicated with an asterisk in Table 1). Likewise, COI amplifications were problematic for many of the North American specimens, despite numerous attempts (more than 20 PCR conditions, including three primer sets in some cases), often yielding double bands or no amplifications at all. Band excision yielded sequence data of possible pseudogenes. Therefore, the COI data set lacks data on *Siro clousi*, *S. calaveras*, and *S. shasta*. Conclusions about the relationships of these three species are thus based entirely on the ribosomal data sets. It is known that COI evolution in Cyphophthalmi in general, and sironids in particular, is odd compared with other arthropods because it presents very high evolutionary changes, including several amino acid indel events (Boyer et al., 2005), and this could be the cause for the difficulties in amplifying this marker.

Molecular Data Analyses

After sensitivity analysis, the parameter set that showed the lowest incongruence according to our modified ILD metric was parameter set 3221, wherein gap openings receive a cost of 3, gap extensions cost 1, and all nucleotide transformations cost 2 (Table 2). Therefore, the results under this parameter set are discussed in more detail and are presented for each analyzed partition (Figs. 3, 4). Additionally, the strict consensus of all trees obtained under all parameter sets is also presented (Fig. 4B). Parameter variation is shown in the form of sensitivity plots ("Navajo rugs"; Fig. 4).

The analysis of the complete 18S rRNA data set for the optimal parameter set yielded 18 trees of 143 weighted steps and found trees of minimal length in 100% of the replicates performed. The strict consensus of these 18 trees (Fig. 3A) identifies five lineages of North American sironids, one including the species *S. kamiakensis*, *S. exilis*, and *S. boyerae* sp. nov. and supported with 100% jackknife frequency (JF hereafter), and four other lineages corresponding to *S. acaroides*, *S. clousi* sp. nov., *S. calaveras* sp. nov., and *S. shasta* sp. nov. Within the kamiakensis–exilis–boyerae clade, the analyses also find support for a clade including the East Coast species *S. exilis* and the West

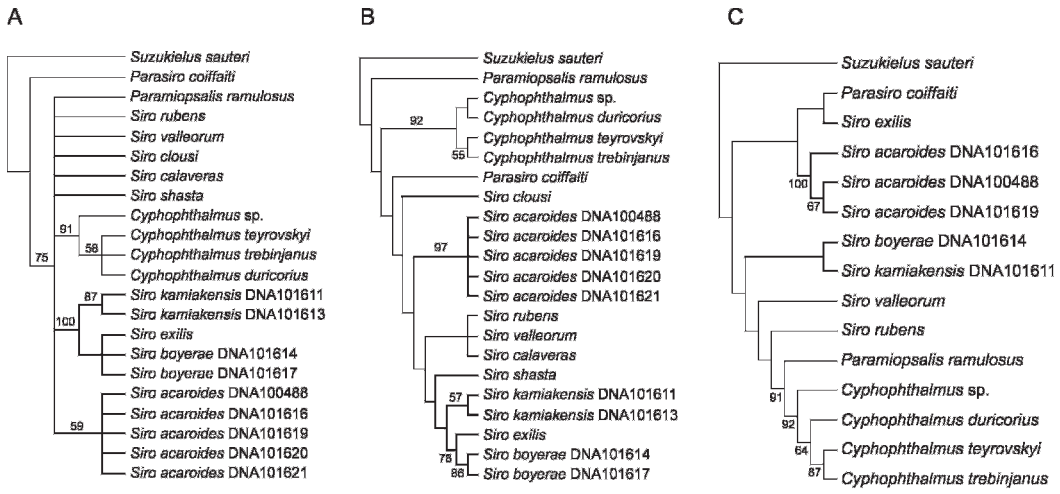

Figure 3. Partitioned analyses for the optimal parameter set. (A) Strict consensus of 18 trees at 143 weighted steps for the analysis of the 18S rRNA data set. (B) Strict consensus of seven trees at 912 weighted steps for the analysis of the 28S rRNA data set. (C) Shortest tree at 3,259 weighted steps for the analysis of the COI data set. Numbers on branches indicate jackknife support values above 50%. Branches in bold refer to the North American species.

Coast species *S. boyerae* sp. nov. The analysis did not find support for the genus *Siro* or for the monophyly of the North American species.

The analysis of the 2.1-kb fragment of 28S rRNA data set for the optimal parameter set yielded seven trees of 912 weighted steps and found trees of minimal length in 37% of replicates; tree-fusing did not find additional trees. The strict consensus of these seven trees (Fig. 3B) shows monophyly of the genus *Siro* (JF < 50%), but not for the North American species. As in the 18S rRNA analysis, 28S rRNA supports monophyly of each species, as well as the exilis–boyerae (78% JF) and the kamiakensis–exilis–boyerae (JF < 50%) clades. In this tree, *S. shasta* sp. nov. is sister group to the latter clade, and *S. calaveras* sp. nov. appears in a clade with the two Western European species *S. rubens* and *S. valleorum*. As in the 18S rRNA analysis, the genus *Cyphophthalmus* finds ample jackknife support.

The analysis of the COI data set for the optimal parameter set (we remind the reader that COI shows length variation within Cyphophthalmi and therefore requires indel events) yielded a single tree of 3,259 weighted steps (Fig. 3C) and was obtained in 40% of the replicates performed. Support from the COI analysis is only found for *Cyphophthalmus* + *Paramiopsalis* (91% JF), *Cyphophthalmus* (92% JF), or the monophyly of *S. acaroides* (100% JF). The tree also shows monophyly of *S. kamiakensis* + *S. boyerae* sp. nov., but these do not form a clade with *S. exilis*. When compared with the other partitions, COI contributes 3.5 times more than 28S rRNA and almost 23 times more than 18S rRNA, in terms of their tree length.

The combined analysis of the three markers for the optimal parameter set yielded eight trees at 4,324 weighted steps, and these trees were found in 30% of the replicates performed, without improvement after tree fusing. The strict consensus of these eight trees is presented in Figure 4A, as opposed to the strict consensus obtained under all parameter sets (Fig. 4B). The tree shows nonmonophyly of *Siro* or of the North American members of the genus. As in some of the partitioned analyses, the combined analysis of all data identifies the exilis–boyerae (63% JF) and the kamiakensis–exilis–boyerae (JF < 50%) clades. The latter clade, despite its low jackknife sup-

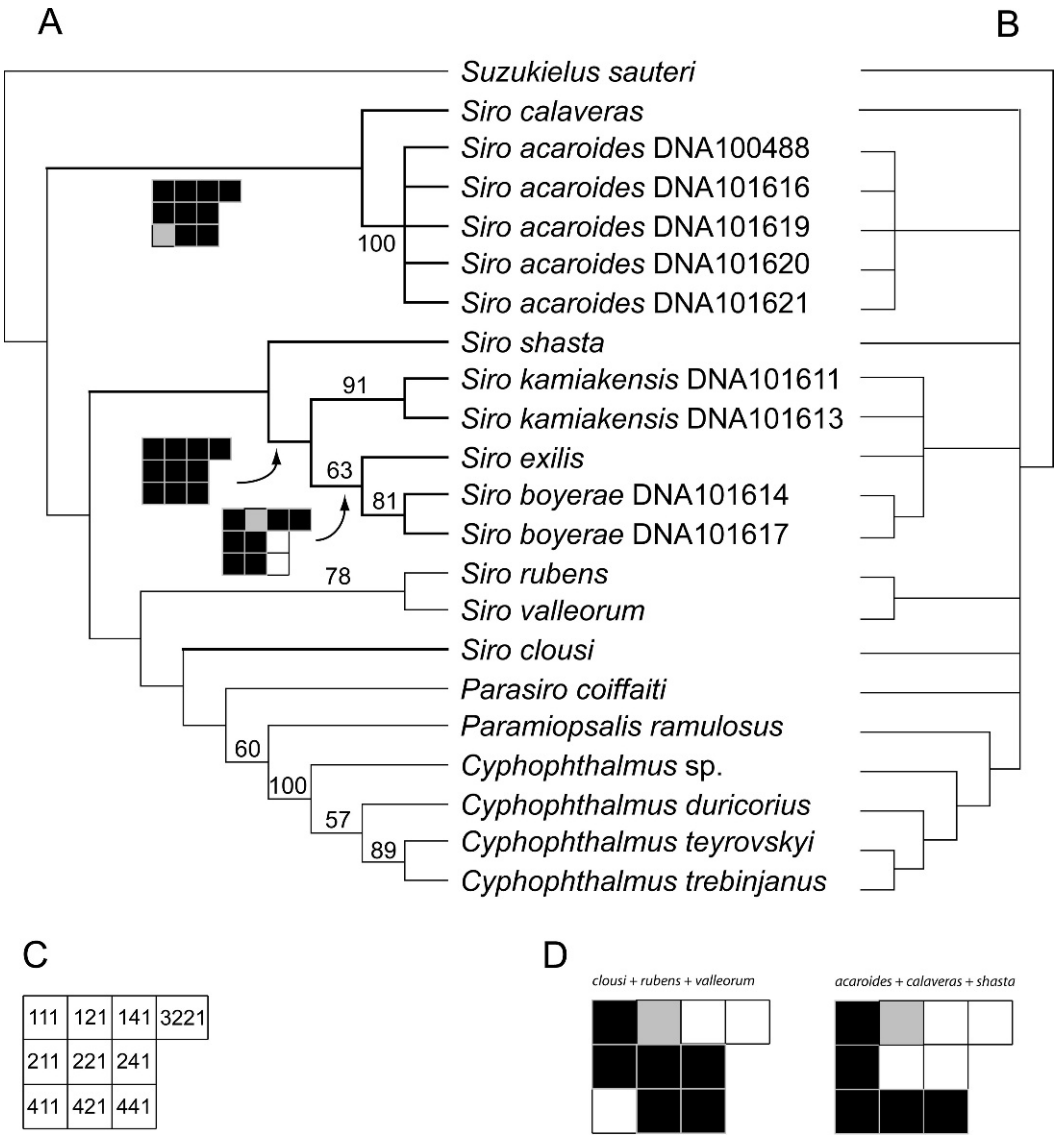

Figure 4. Combined analysis of all loci. (A) Strict consensus of the eight shortest trees at 4,324 weighted steps obtained under the optimal parameter set. Numbers on nodes indicate jackknife support values above 50%. Branches in bold refer to the North American species. (B) Strict consensus of all trees obtained under all the explored parameter sets. (C) Legend for the Navajo rugs depicted in Figures 4A, D, indicating the parameter sets for each square. (D) Navajo rugs for two of the topologies not depicted in the trees obtained under the optimal parameter set. Legends for Navajo rugs: black square indicates monophyly; white square indicates non-monophyly; gray square indicates monophyly under some but not all of the shortest trees.

port, appears under all analytical parameter sets (Fig. 4B and corresponding Navajo rug), indicating its stability. Likewise, the exilis–boyerae clade appears under most analytical parameters. The combined analysis also suggests a relationship of *S. calaveras* sp. nov. to *S. acaroides*, again, with JF $< 50\%$, but with enormous stability; only one parameter set suggests alternatives to the monophyly of the clade (see corre-

sponding Navajo rug in Fig. 4). A relationship of *S. shasta* sp. nov. to the kamiakensis–exilis–boyerae clade is obtained under the optimal parameter set only and shows JF < 50%. However, a number of parameter sets identifies *S. shasta* sp. nov. with the calaveras–acaroides clade (see corresponding Navajo rug, Fig. 4D). *Siro clousi* sp. nov. appears in alternative positions under the different parameter sets explored, but it mostly appears within a clade including the two Western European species *S. rubens* and *S. valleorum*.

Because of the lack of COI sequence data for some species and the enormous contribution by the COI locus, which has been shown to contain substantial homoplasy in previous analyses of cyphophthalmid relationships (Boyer et al., 2005; Schwendinger and Giribet, 2005), we undertook analysis of the two ribosomal genes alone. The combined analysis of the two ribosomal loci under the optimal parameter set yielded 14 trees of 1,066 weighted steps, and minimum tree length was found in 82% of replicates performed. The strict consensus of the 14 trees (Fig. 5) shows monophyly of *Siro*, but not monophyly of the North American species. As in the previous analyses, the data shows the kamiakensis–exilis–boyerae, exilis–boyerae, and calaveras–acaroides clades, but neither *S. shasta* sp. nov. nor *S. clousi* sp. nov. is unambiguously resolved. As in most previous analyses, the two European species of *Siro* and the four species of *Cyphophthalmus* form individual clades.

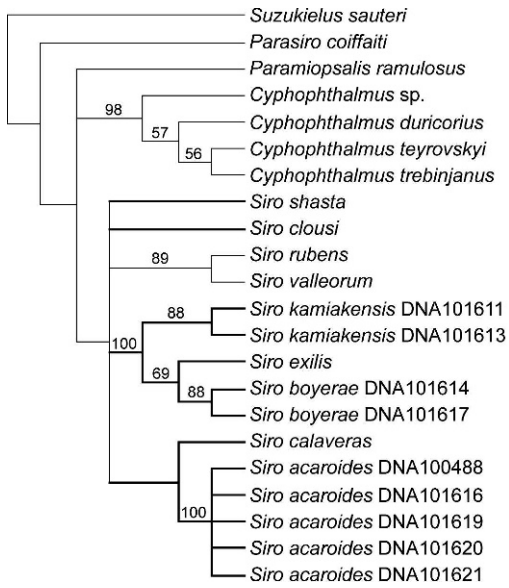

Figure 5. Strict consensus of the 14 trees at 1,066 weighted steps for the combined analysis of the two ribosomal loci. Numbers on branches indicate jackknife support values above 50%. Branches in bold refer to the North American species.

DISCUSSION

Phylogenetic analysis of the data analyzed in this study supports the presence of multiple lineages of North American sironids, although their relationships are not yet fully understood. One of the results of our analyses suggests that several specimens previously considered within the variation range of *S. acaroides* by Shear (1980) represent two independent lineages completely unrelated to *S. acaroides*. One of these, *S. clousi* sp. nov. is the largest North American sironid and is found sympatrically with *S. acaroides*. However, under several parameter sets, *S. clousi* sp. nov. appears to be related to the Western European instead of the North American species. This result surely deserves further scrutiny by adding more species of Western European sironids, as well as more individuals of *S. clousi* sp. nov.

A second species formerly considered within the variation of *S. acaroides*, *S. boyerae* sp. nov., is more closely related to *S. kamiakensis* from Washington and Idaho than to *S. acaroides* from nearby localities and, in fact, appears as the sister group to the East Coast species *S. exilis*. The relationship of these three species, the designated kamiakensis–exilis–boyerae clade, is well supported by our data in terms of stability to parameter variation, and the three of them share a unique position of coxae III, which do not meet along the midline (see Fig. 21). The presence of a clade constituted by three species separated by distances of over 2,000 km on both sides of

the Rocky Mountains is, to say the least, surprising, especially because genetic differences in the ribosomal genes between *S. exilis* and *S. boyerae* sp. nov. are extremely low. And again, this could be explained by the great age of the group; North American sironids have been estimated to have diversified in the Jurassic, whereas they separated from their European counterparts in the Triassic (Giribet et al., 2010).

Siro acaroides, a broadly distributed species from the redwood forests of coastal Oregon and Northern California forms a distinct clade with other redwood species from the Central region of California, including *S. shasta* sp. nov. and *S. calaveras* sp. nov., and perhaps with *S. sonoma* (data not shown), all of them with much narrower ranges. This species complex parallels the toad *Anaxyrus boreas* species group (Goebel et al., 2009), although in the case of the species in the genus *Siro*, divergences could be much older than in *Anaxyrus*.

Concluding Remarks

The four new species elevate the number of described North American Cyphophthalmi to a total of 10, nine of which occur in the continental United States (Shear, 1980) and one in Mexico (Shear, 1977). This diversity is considerably lower than that recorded for Europe. However, the ranges of some of the North American species (e.g., *Metasiro americanus*) are quite large and could include cryptic species, given the patchy distribution and the low dispersal ability of Cyphophthalmi (see, e.g., Boyer et al., 2007a). The large geographic gap without cyphophthalmid specimens for the kamiakensis–exilis–boyerae clade furthermore suggests that more species could be expected from some elevated humid forests in the center of the continental United States, although climatic conditions in the present and in the past, as well as large episodes of flooding, could constrain the current distributions of Cyphophthalmi species, which are not found at higher latitudes. An interesting parallel is seen in another putatively ancient arachnid group, the spider genus *Hypochilus*, with five species in the southern Appalachians, two in the southern Rocky Mountains, and three in California. But in this case, a morphological cladistic analysis showed that the three geographic areas also correspond to three clades (Catley, 1994). It remains clear that the North American cyphophthalmid fauna is still in serious need of further study, both at the faunistic and taxonomic levels, and indeed, single individuals, which could represent additional species, exist in museum collections.

TAXONOMY

Family Sironidae Simon, 1879

Genus *Siro* Latreille, 1796

Siro Latreille 1796: 185, Simon 1879: 144–145, Hansen and Sørensen 1904: 107–108, Juberthie 1970: 1383, Shear 1980: 3–5, Giribet 2000: 57 (complete references and previous synonymies).

***Siro boyerae* Giribet & Shear sp. nov.**

(Figs. 6–9)

Type Specimens

Holotype. Male (MCZ 92901 ex MCZ DNA101614) from Chenuis Fall (46°59′32″N, 121°50′47″W), Carbon River, Mount Rainier National Park, Pierce Co., WASHINGTON, collected 19.vi.2005 by *S. L. Boyer*, *R. M. Clouse*, *& G. Giribet* (Figs. 6A–C).

Paratypes. Three males (1for DNA work) and 8 females (MCZ 92902, MCZ DNA101614; one in Figs. 6D–F), same collecting data as holotype; 2 males, 1 juvenile (FMNH; CNHM(HD)#57-42; B_26) from Carbon River, Mount Rainier National Park, Pierce Co., WASHINGTON, collected 16 June 1957 by *H. S. Dybas*; 5 males (1 used for DNA work), 2 females, 1 juvenile (MCZ DNA101617) from Ecola State Park (45°54′56″N, 123°57′52″W), Clastop Co., OREGON, collected 20 June 2005 by *S. L. Boyer*, *R. M. Clouse*, *& G. Giribet*; 3 females, 9 juveniles (FMNH HD#68-149)

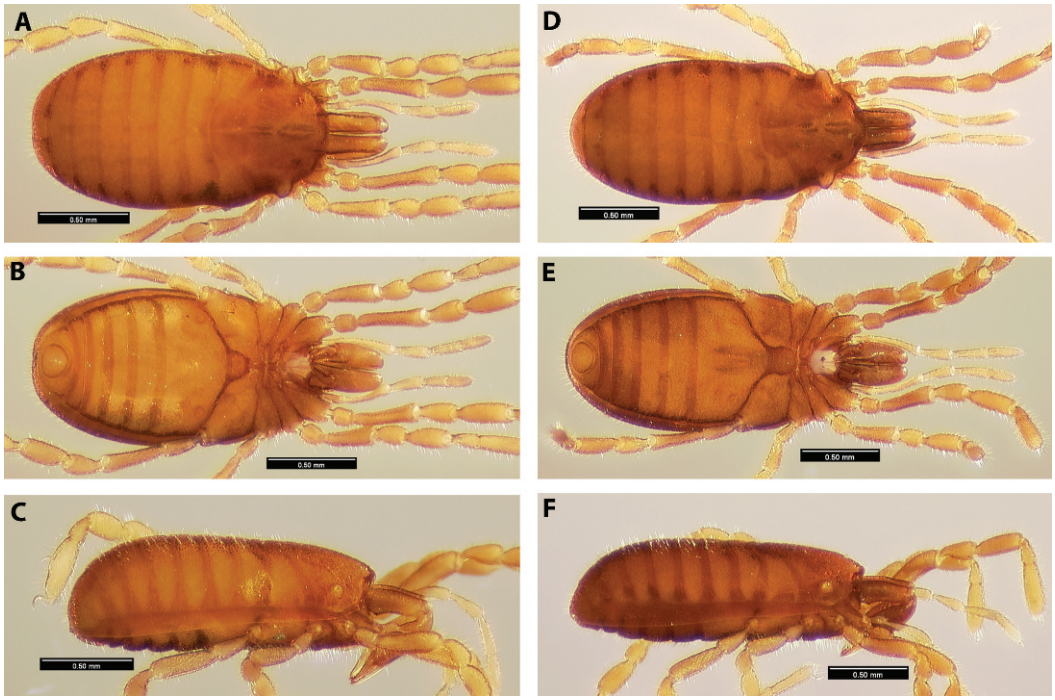

Figure 6. *Siro boyerae* sp. nov. (A–C) Holotype male (MCZ 92901) in dorsal (A), ventral (B), and lateral (C) view. (D–F) Paratype female (MCZ 92902) in dorsal (D), ventral (E), and lateral (F) view. Scale bars 500 μm.

from Ecola State Park, Clastop Co., OREGON, collected 21 July 1968 by *J. Wagner*.

Additional Material

WASHINGTON: One male (MCZ DNA104929) from Lower Hendrickson Canyon (46°22′158″N, 123°39′950″W, 27 m), Wahkiakum Co., collected 23.i.2004 by *W. Leonard*, *M. Leonard*, *C. Richart*, *B. Dyle*, & *K. Norose*; 1 male (MCZ DNA104928) from Gregory Creek, 5.2 mi N of SR4 (46°15′552″N, 123°08′038″W, 125 m), Cowlitz Co., collected 21.iii.2004 by *W. Leonard* & *C. Richart*; 1 male, 1 female (EME) from Amanda Park, Quinault, Olympic Peninsula, collected 9.vii.1959 by *L. M. Smith*; 1 male, 3 females, 2 juveniles (FMNH; CNHM(HD)#57-73; B_306) from Fairfax, Pierce Co., collected 16.vi.1957 by *H. S. Dybas* in floor debris in mixed maple–alder; 1 female (FMNH; CMNH(HD)#57-41; B_297) from Chenuis Fall, Carbon River, Mount Rainier National Park, Pierce Co., collected 16.vi.1957 by *H. S. Dybas*; 2 males (FMNH) from Olympic Hot Springs, Olympic National Park, collected 18–19.vi.1957 by *H. S. Dybas*; 4 males, 2 females, 1 juvenile (CAS) from 2.5 mi due N Swift Reserve Dam, Skamania Co., collected by *T. Briggs*, *V. Lee*, & *K. Hom*; 4 males, 8 females, 5 juveniles (CAS) from 2.5 mi due N Swift Reserve Dam, Skamania Co., collected by *T. Briggs*, *V. Lee*, & *K. Hom*.

Etymology. The species is named after cyphophthalmid biologist Sarah L. Boyer, who assisted collecting the type material of the species, for her dedication to these animals.

Diagnosis. *Siro boyerae* is similar to *S. acaroides*, although the former is more slender (length/with ratio [L/W] = 1.84 in *S. boyerae* and 1.5 in *S. acaroides*). The two species differ in the open circle spiracles of *S. acaroides*, which are almost circular in *S. boyerae*. The spiracles also differ from those of *S. shasta* sp. nov. It can be distinguished from *S. kamiakensis*, *S. sonoma*, and *S.*

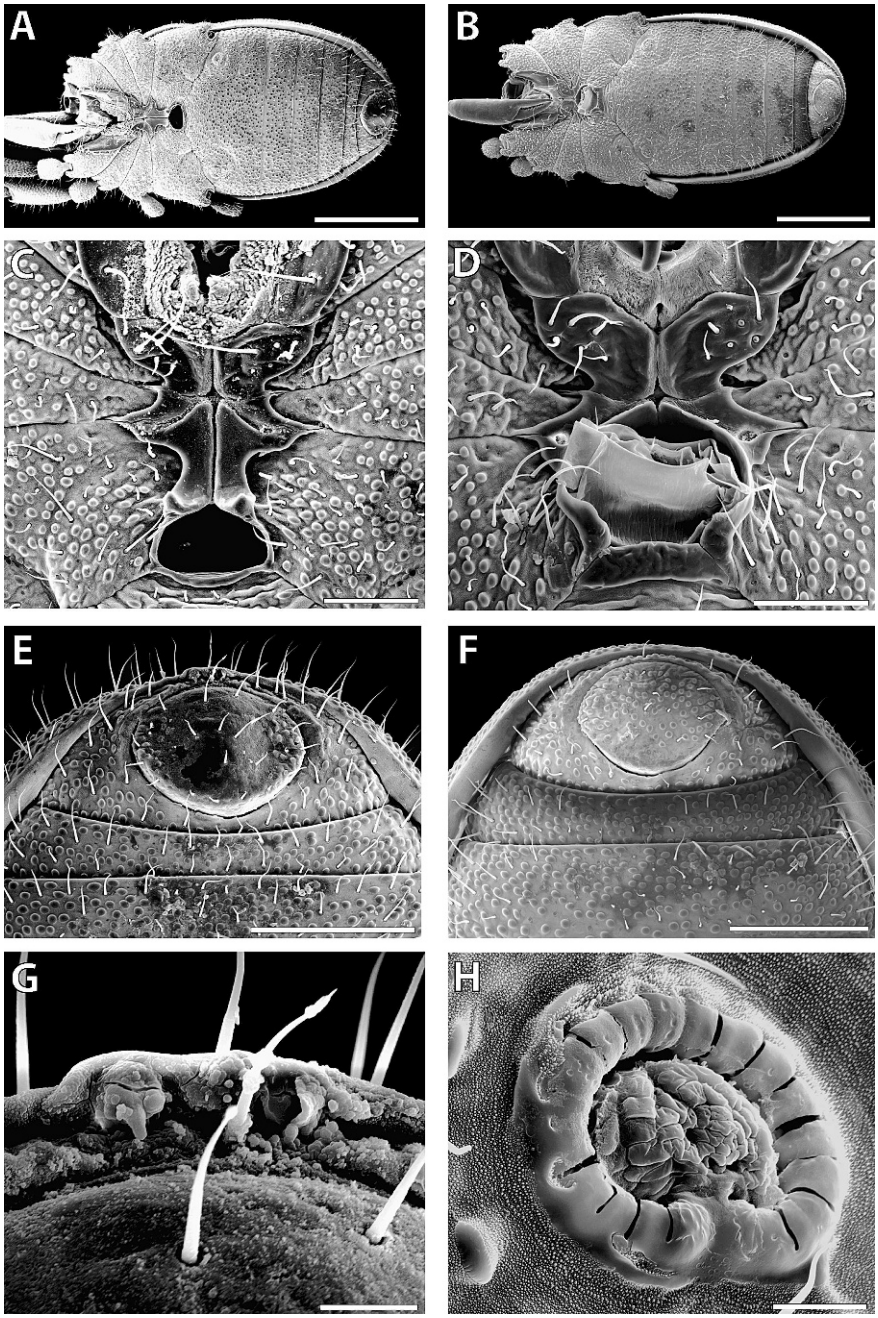

Figure 7. *Siro boyerae* sp. nov. paratype male and female (MCZ 92902). (A) Paratype male in ventral position. (B) Paratype female in ventral position. (C) Male ventral thoracic complex. (D) Female ventral thoracic complex. (E) Male anal region. (F) Female anal region. (G) Detail of anal gland openings. (H) Female spiracle. (A, B, Scale bars 500 μm; C–D, scale bars 100 μm; E, F, scale bars 200 μm; G, H, scale bars 20 μm.)

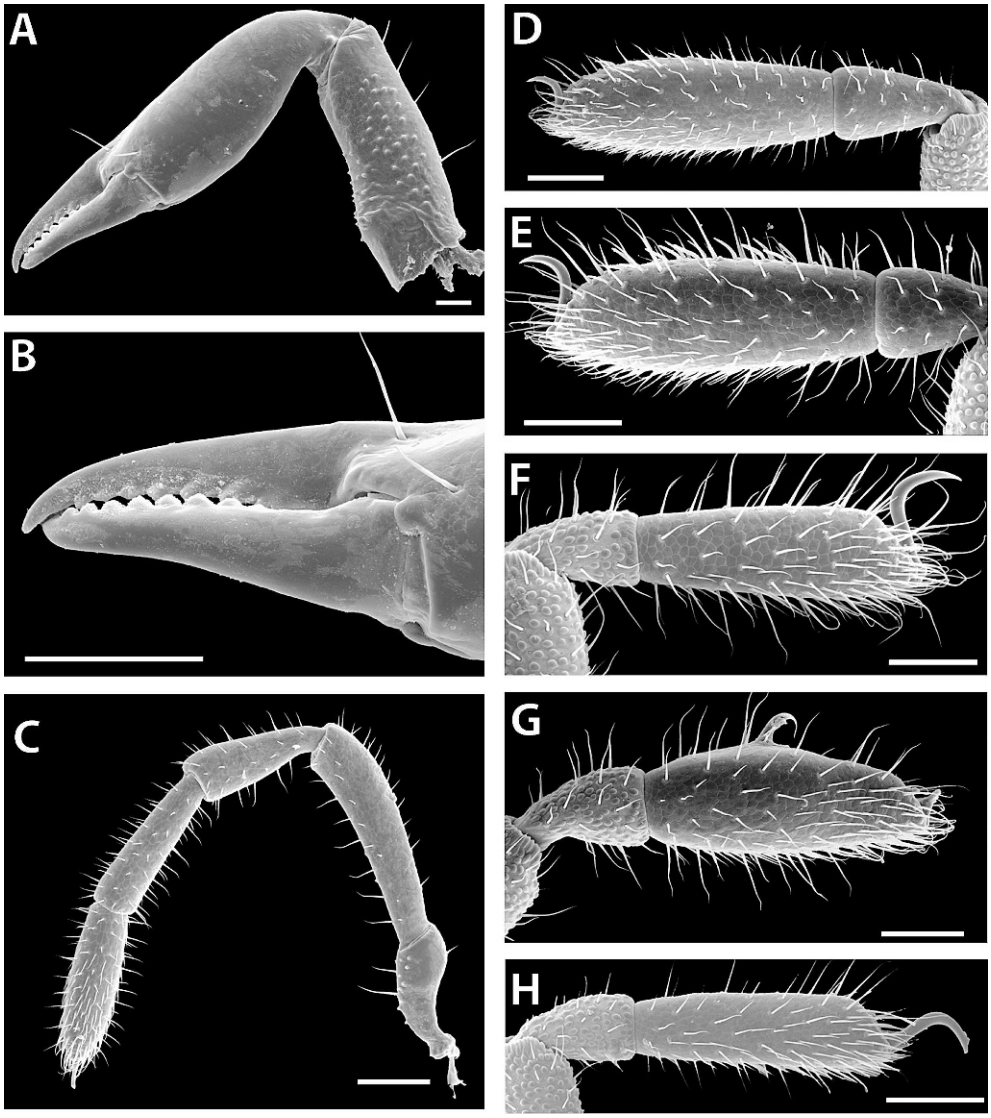

Figure 8. *Siro boyerae* sp. nov. paratype male and female (MCZ 92902). (A) Left chelicera of male. (B) Detail of the cheliceral pincer. (C) Left palp of male. (D) Metatarsus and tarsus I of male. (E) Metatarsus and tarsus II of male. (F) Metatarsus and tarsus III of male. (G) Metatarsus and tarsus IV of male. (H) Metatarsus and tarsus IV of female. (A, Scale bar 50 μm; B–H, scale bars 100 μm.)

clousi sp. nov. in lacking an anal keel in the male and from *S. shasta* sp. nov. because the latter has a depressed male anal plate (Fig. 7E). *Siro boyerae* shares with *S. exilis* (Fig. 21B), *S. kamiakensis* (Fig. 21C), and *S. sonoma* (Fig. 21D) endites of coxae III that do not meet along the midline (as opposed to *S. acaroides* [Fig. 21A], and the other three new species described herein), a character that might constitute a synapomorphy for them (see Figs. 3–5). However, the species can easily be separated from *S. sonoma* by the unmodified ventral surface of its male 4th tarsus and from *S. kamia-*

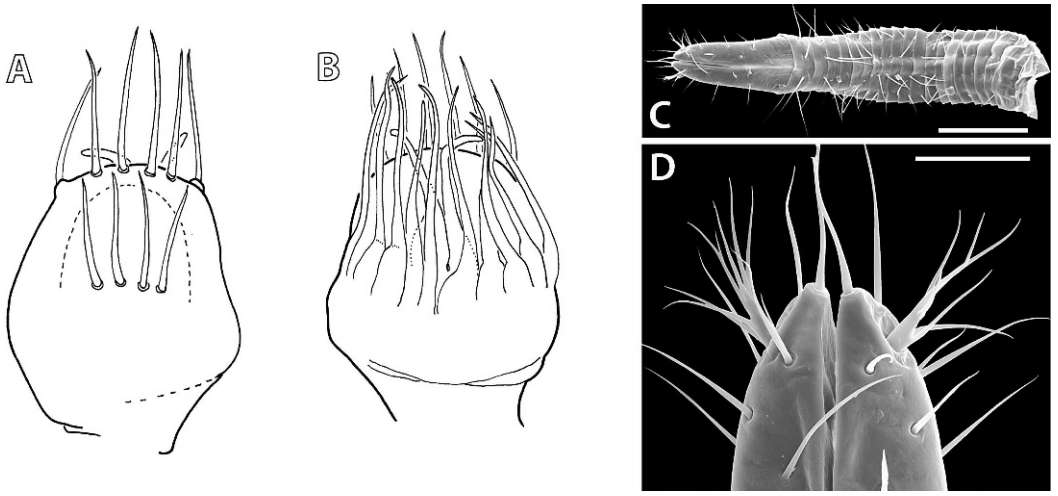

Figure 9. *Siro boyerae* sp. nov. (A) Spermatopositor, ventral view. (B) Spermatopositor, dorsal view. (C) Ovipositor. (D) Ovipositor sensory organ. (C, Scale bar 200 μm; D, scale bar 50 μm.)

kensis by its undivided male 4th tarsus. Likewise, it can easily be separated from *S. shasta* because the latter lacks ornamentation in the legs.

Description of Male. Small sironid of uniform chestnut brown color; total length of holotype 1.69 mm, maximum width at 3rd opisthosomal segment at 0.91 mm, body L/W = 1.84 (Fig. 6A). Anterior margin of dorsal scutum slightly convex; prosomal region almost semicircular. Ozophores conical, of type II (*sensu* Juberthie, 1970), with subterminal ozopore (*sensu* Novak and Giribet, 2006); maximum width across ozophores 0.75 mm. Eyes absent. Transverse prosomal sulcus little conspicuous; transverse opisthosomal sulci inconspicuous. Dorsal scutum with maximum height at around segments 4–5 (Fig. 6C).

Ventral prosomal complex (Figs. 6B, 7A, C) with coxae I–II free, coxae III–IV fused; coxae II and IV meeting along the midline, but not coxae III; coxae IV meeting along the midline for a distance greater than gonostome length; sternum absent; coxal pores clearly visible between coxae III and IV. Projections of coxae IV endites present in the anterior portion of gonostome wall (Fig. 7C). Male gonostome sub-semicircular, with slightly concave posterior margin, wider than long (0.12 × 0.07 mm), and delimited laterally and anterolaterally by the elevated endites of coxae IV. Spiracles (Fig. 7H) of circular type (*sensu* Giribet and Boyer, 2002), circular to oval in shape in male, with a maximum diameter of 0.07 mm.

Ventral opisthosomal region (Fig. 7A) without conspicuous modifications other than in the anal plate. Opisthosomal tergite IX and sternites 8 and 9 fused into a broad corona analis (Fig. 7E); tergite VIII without modifications. Anal plate oval, 0.21 × 0.15 mm, only ornamented in the sides, with a rather inconspicuous longitudinal central ridge that leaves two depressions laterally. Three anal gland pores on tergite VIII of males (Fig. 7G). Cuticle with tuberculate-microgranular surface (*sensu* Murphree, 1988; this is referred to as "ornamented" hereafter), nearly uniform in dorsal areas and in ventral areas, including coxae.

Chelicerae (Fig. 8A) relatively short and robust; basal article in males 0.40 mm long, 0.14 mm wide, without a ventral process or a dorsal crest; 2nd article 0.59 mm long, 0.15 mm wide; movable finger 0.20 mm long; all articles with few setae, the proximal one almost entirely granulated but with sparse granulation; 8 uniform denticles on

TABLE 3. LEG MEASUREMENTS (LENGTH/WIDTH, MM) IN *SIRO BOYERAE* SP. NOV. MEASUREMENTS REFER TO MALE PARATYPE MOUNTED FOR SEM.

Leg	Trochanter	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	0.14/0.11	0.43/0.12	0.22/0.12	0.28/0.12	0.18/0.10	0.38/0.13	1.65
II	0.11/0.10	0.33/0.11	0.19/0.11	0.22/0.12	0.15/0.09	0.33/0.11	1.35
III	0.14/0.09	0.23/0.11	0.16/0.10	0.19/0.11	0.09/0.07	0.29/0.08	1.14
IV	0.22/0.10	0.33/0.11	0.21/0.11	0.23/0.12	0.16/0.09	0.34/0.13	1.51

the cutting edge of each cheliceral finger (Fig. 8B). Second cheliceral segment not ornamented.

Palp (Fig. 8C) 1.11 mm long, smooth, slightly ornamented on trochanter. Measurements of palpal article length in SEM male paratype (mm): trochanter 0.165, femur 0.311, patella 0.205, tibia 0.212, tarsus 0.220; claw 0.037 mm long.

Legs relatively robust; leg formula I-IV-II-III (measurements in Table 3; Figs. 8D–G). Tarsus I with a concentration of setae, but not forming a distinct solea. Except for the tarsi I–IV and metatarsi I–II, all articles ornamented (Figs. 8D–G). Tarsus IV of male entire, with a narrow lamelliform adenostyle (Fig. 8G), subcylindrical at the base, with lateral pore; proximal margin at 40% of tarsal length. Claws hooked, smooth, without dentition or lateral pegs.

Spermatopositor (Figs. 9A, B) short, typical of sironids, smooth; with movable fingers, slightly curved outward, ending as hooks, longer than the membranous median lobe; microtrichial formula: 4, 6, 5+5 (n = 1). Gonopore complex not observed.

Description of Female. Total length 1.94 mm, maximum width 0.95 mm (L/W = 2.05; Fig. 6D). Ventral prosomal complex (Figs. 7B, D) only with coxae I–II meeting along the midline, coxae III delimiting the anterior part of gonostome. Female gonostome semicircular anteriorly, wider than long (Fig. 7D). Gonostome of female forming a tube. Corona analis not protruding or forming a tube (Fig. 7F). Female anal plate unmodified. Tarsus of leg IV (Fig. 8H) without modifications, narrower than that of males.

Ovipositor (Figs. 9C, D) 0.84 mm long, typical of *Siro* (see Juberthie, 1967), composed of two apical lobes and 20 circular articles (n = 1), each with 8 short setae equal in length; these setae slightly longer toward the terminus; most basal article without setae. Apical lobes (Fig. 9D) each with a long terminal seta and ca. 12 setae slightly increasing in length toward the tip; sensitive processes with multibranching setae with 6 endings. Because of SEM examination, we have not studied the receptaculum seminis.

Siro calaveras Giribet & Shear sp. nov. (Figs. 10–13)

Type Specimens

Holotype. Male (MCZ 92898, ex MCZ DNA101623) from North Grove (41°03′49″N, 122°21′37″W), Calaveras Big Trees State Park, Calaveras Co., CALIFORNIA, collected 23.vi.2005 by *S. L. Boyer*, *R. M. Clouse*, *& G. Giribet*; litter sifting (Figs. 10A–C).

Paratypes. Three males and 3 females (MCZ 92899, ex MCZ DNA101623); same collecting data as holotype; 1 male, 3 females, 1 juvenile (MCZ DNA101623), same collecting data as holotype (1 male and 1 female used for DNA extraction); 2 females, 2 juveniles (AMNH), from North Grove, Calaveras Big Trees State Park, Calaveras Co., CALIFORNIA, collected 5.iii.1958 by *L. M. Smith & R. O. Schuster*; 8 males, 11 females, 3 juveniles (AMNH), from North Grove, Calaveras Big Trees State Park, Calaveras Co., CALIFORNIA, collected 10.iii.1958 by *L. M. Smith & R. O. Schuster*, rotten log; 4 males (3 dissected for genitalia), 2 females (AMNH), from North Grove, Calaveras Big Trees State Park, Calaveras Co., CALIFORNIA, collected 5.iii.1958 by *L. M. Smith & R. O. Schuster*;

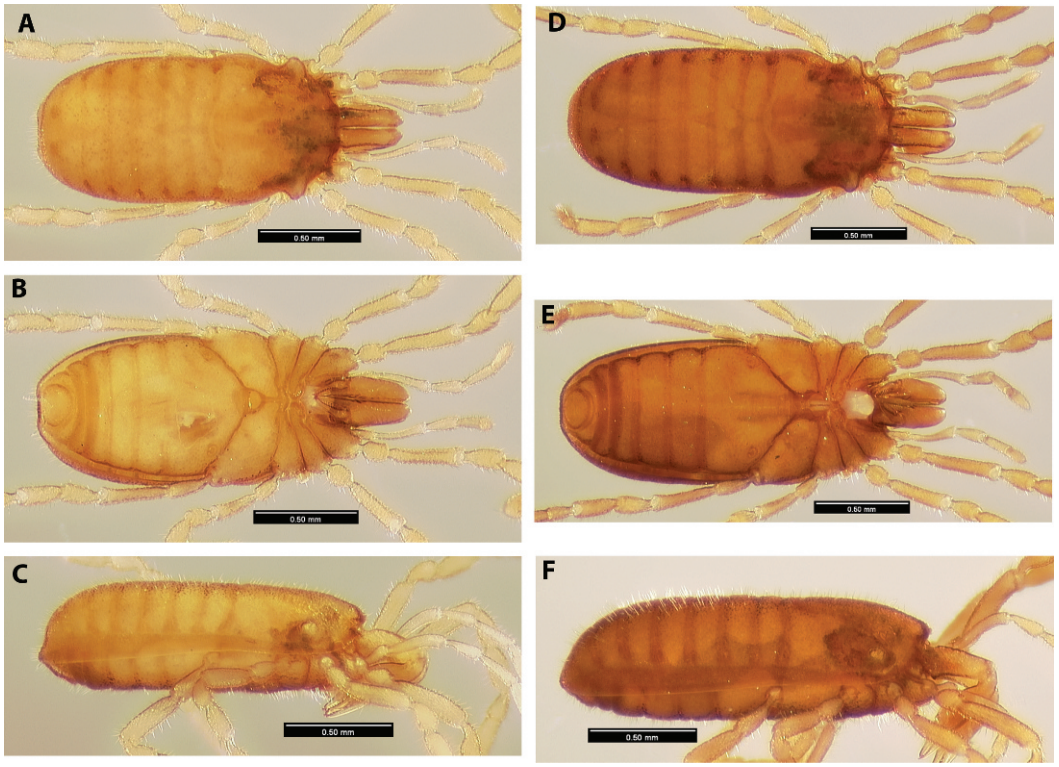

Figure 10. *Siro calaveras* sp. nov. (A–C) Holotype male (MCZ 92898) in dorsal (A), ventral (B), and lateral (C) view. (D–F) Paratype female (MCZ 92899) in dorsal (D), ventral (E), and lateral (F) view. Scale bars 500 μm.

1 male, 1 female in SEM stubs (MCZ, ex AMNH), from North Grove, Calaveras Big Trees State Park, Calaveras Co., CALIFORNIA, collected 5.iii.1958 by *L. M. Smith & R. O. Schuster*; 1 female (CAS), from South Grove, Calaveras Big Trees State Park, Calaveras Co., CALIFORNIA, collected 13.vi.1956 by *B. J. Adelson*.

Etymology. The species epithet is a noun in apposition, after Calaveras Co., California.

Diagnosis. *Siro calaveras* (Fig. 10) is a more slender species (L/W = 1.99) than *S. acaroides* (L/W = 1.50), to which it is similar in body length, and the legs are also proportionally shorter, as is male tarsus IV (L/W = 2.0 as opposed to 2.9 in *S. acaroides*); *S. acaroides* has a rather smooth palpal trochanter, whereas that of *S. calaveras* is ornamented; the number of spermatopositor microtrichiae of both species is the same. *Siro calaveras* is distinctly smaller than *S. shasta* (1.5 vs. 2.6 mm long), which lacks leg ornamentation and differs in spermatopositor microtrichiae. The species can easily be separated from *S. sonoma* by the unmodified ventral surface of its male tarsus IV and from *S. kamiakensis* by its undivided male tarsus IV. Unlike *S. exilis*, *S. calaveras* males have a concave 8th tergite. Finally, *S. calaveras* has a unique body profile (Fig. 10A) among North American species, with the body widest across the posterior opisthosomal part, rather than opisthosomal tergites 2 or 3.

Description of Male. Slender small sironid of uniform chestnut brown color; total length of holotype 1.53 mm, maximum width at prosoma at 0.77 mm, body L/W = 1.99 (Fig. 10A). Anterior margin of dorsal scutum slightly convex; prosomal region sub-semicircular. Ozophores conical, of

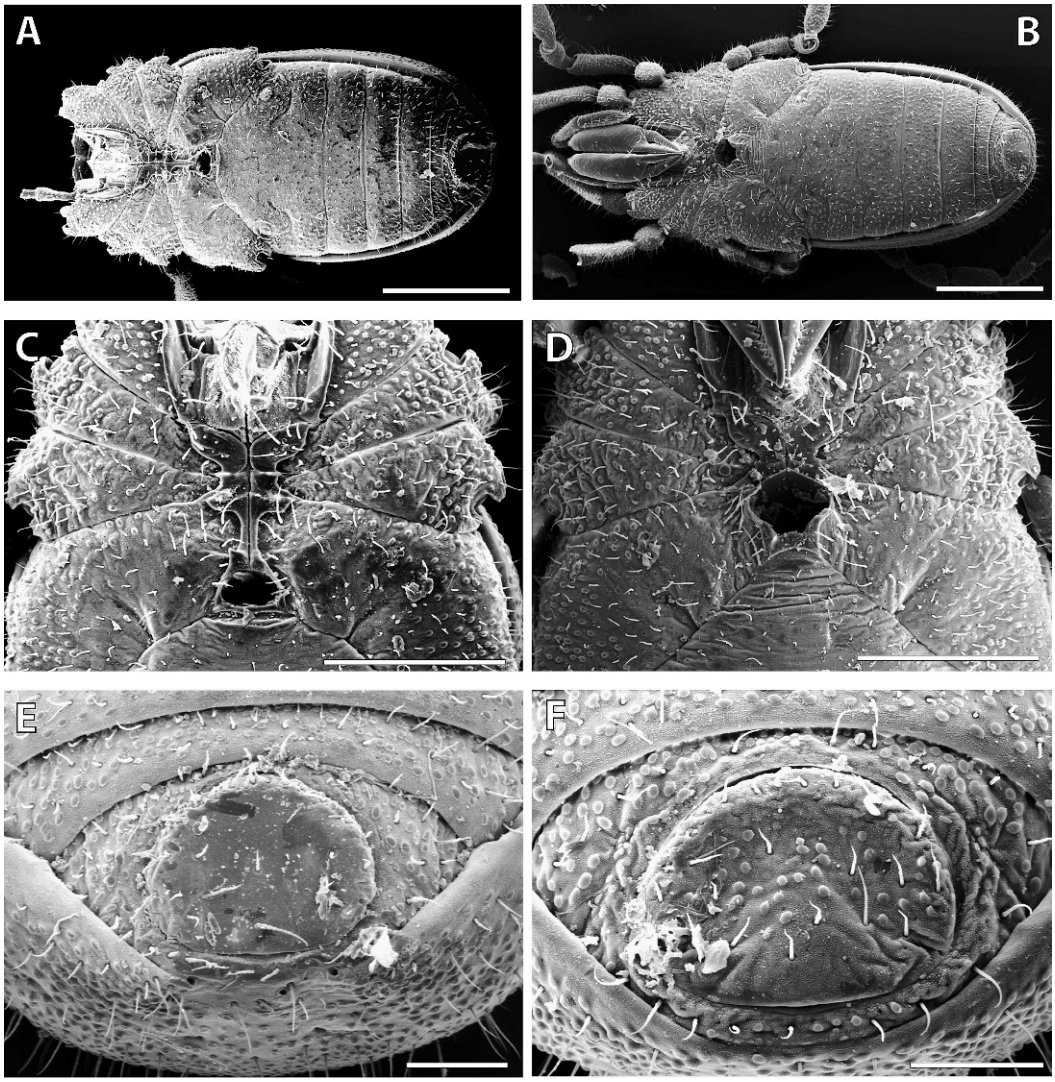

Figure 11. *Siro calaveras* sp. nov. paratype male (MCZ 92899) and female (MCZ 92899). (A) Paratype male in ventral position. (B) Paratype female in ventral position. (C) Male ventral thoracic complex. (D) Female ventral thoracic complex. (E) Male anal region. (F) Female anal region. (A, B, Scale bars 500 μm; C, D, scale bars 300 μm; E, F, scale bars 100 μm.)

type II (*sensu* Juberthie, 1970), with subterminal ozopore (*sensu* Novak and Giribet, 2006), and entirely ornamented; maximum width across ozophores 0.72 mm. Eyes absent. Transverse prosomal sulcus inconspicuous; transverse opisthosomal sulci inconspicuous. Dorsal scutum with maximum height posterior end, but very similar along the length of the animal (Fig. 10C).

Ventral prosomal complex (Figs. 10B, 11A, E) with coxae I–II free, coxae III–IV fused; coxae II, III, and IV meeting along the midline; coxae IV meeting along the midline for a distance greater than gonostome length; sternum absent; coxal pores clearly visible between coxae III and IV. Projections of coxae IV endites present in the anterior portion of gonostome wall

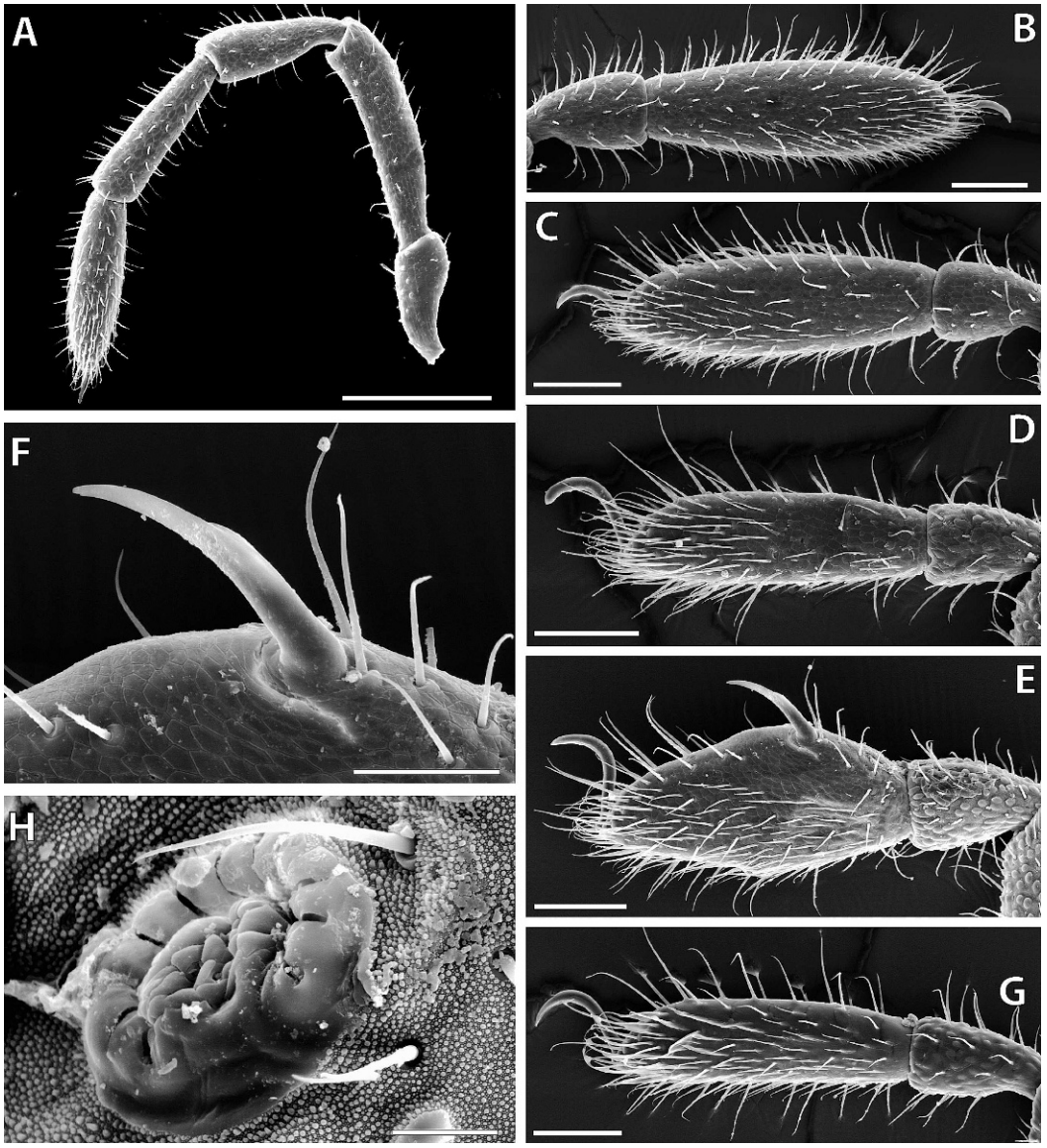

Figure 12. *Siro calaveras* sp. nov. paratype male (MCZ 92899) and female (MCZ 92899). (A) Left palp of male. (B) Metatarsus and tarsus I of male. (C) Metatarsus and tarsus II of male. (D) Metatarsus and tarsus III of male. (E) Metatarsus and tarsus IV of male. (F) Detail of adenostyle. (G) Metatarsus and tarsus IV of female. (H) Spiracle. (A, Scale bar 200 μm; B–E, G, scale bars 100 μm; F, scale bar 50 μm; H, scale bar 20 μm.)

(Fig. 11C). Male gonostome semicircular, with straight posterior margin, wider than long (0.10 × 0.06 mm), and delimited laterally and anterolaterally by the elevated endites of coxae IV. Spiracles (Fig. 12H) of circular type (*sensu* Giribet and Boyer, 2002), circular to oval in shape in male, with a maximum diameter of 0.06 mm.

Ventral opisthosomal region (Fig. 11A) without conspicuous modifications other than in the anal plate. Opisthosomal tergite IX and sternites 8 and 9 fused into a broad

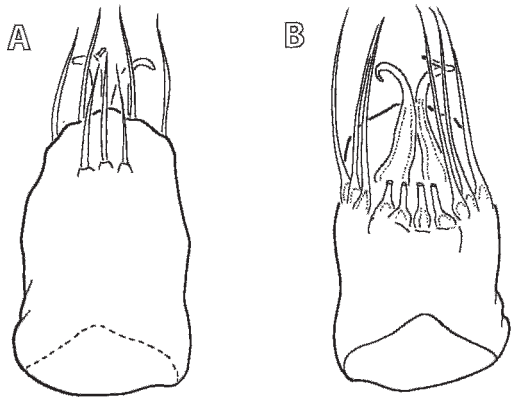

Figure 13. *Siro calaveras* sp. nov. (A) Spermatopositor, ventral view. (B) Spermatopositor, dorsal view.

corona analis (Fig. 11E); tergite VIII slightly bilobed. Anal plate oval, 0.22 × 0.17 mm, concave, mostly smooth, without ornamentation or a longitudinal carina. Three anal gland pores on tergite VIII of males (Fig. 11E). Cuticle with tuberculate–microgranular surface (*sensu* Murphree, 1988), nearly uniform in dorsal areas and in ventral areas including coxae.

Chelicerae robust; basal article in males 0.73 mm long, 0.17 mm wide, with a ventral process and a dorsal crest; 2nd article 0.60 mm long, 0.17 mm wide; movable finger 0.23 mm long; all articles with few setae, the proximal one almost entirely granulated with dense granulation; denticles on the cutting edge of each cheliceral finger uniform. Second cheliceral segment not ornamented.

Palp (Fig. 12A) 1.13 mm long, smooth, slightly ornamented on trochanter. Measurements of palpal article length in SEM male paratype (mm): trochanter 0.16, femur 0.31, patella 0.20, tibia 0.22, tarsus 0.24; claw 0.04 mm long.

Legs relatively robust; leg formula IV-I-II-III (measurements in Table 4; Figs. 12B–E). Tarsus I with a concentration of setae, but not forming a distinct solea. Except for tarsi I–IV and metatarsi I–II, all articles ornamented (Figs. 12B–E). Tarsus IV entire, globose, with a lamelliform adenostyle (Figs. 12E, F), subcylindrical at the base, with lateral pore (Fig. 12F); proximal margin at 31% of tarsal length. Claws hooked, smooth, without dentition or lateral pegs.

Spermatopositor (Figs. 13A, B) short, typical of sironids, smooth; with movable fingers, slightly curved outward, ending as hooks, longer than the membranous median lobe; microtrichial formula: 3, 4, 5+5 (n = 1). Gonopore complex not observed.

Description of Female. Total length 1.74 mm, maximum width 0.80 mm (L/W = 2.18; Fig. 10D). Ventral prosomal complex (Figs. 11B, F) only with coxae I–II meeting along the midline, coxae III delimiting the anterior part of gonostome. Female gonostome subtrapezoidal, wider than long. Gonostome of female forming a tube. Corona analis not protruding or forming a tube (Figs. 10D–F, 11B). Female anal plate (Fig. 11F) with modifications, slightly raised in the mid-posterior section, and forming two concave lateral areas; ornamentation is sparse. Tarsus of leg IV without modifications, narrower than that of males.

Ovipositor not studied.

Siro clousi Giribet & Shear sp. nov. (Figs. 14–16)

Type Specimens

Holotype. Male (MCZ DNA101871 ex DNA101616large; used for DNA study)

TABLE 4. LEG MEASUREMENTS (LENGTH/WIDTH, MM) IN *SIRO CALAVERAS* SP. NOV. MEASUREMENTS REFER TO MALE PARATYPE MOUNTED FOR SEM.

Leg	Trochanter	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	0.20/0.10	0.44/0.10	0.22/0.11	0.30/0.11	0.20/0.09	0.39/0.12	1.75
II	0.13/0.10	0.38/0.11	0.19/0.11	0.23/0.11	0.16/0.09	0.36/0.11	1.45
III	0.16/0.09	0.26/0.10	0.17/0.11	0.21/0.11	0.14/0.07	0.29/0.09	1.23
IV	0.25/0.10	0.40/0.11	0.24/0.12	0.28/0.11	0.28/0.10	0.33/0.16	1.78

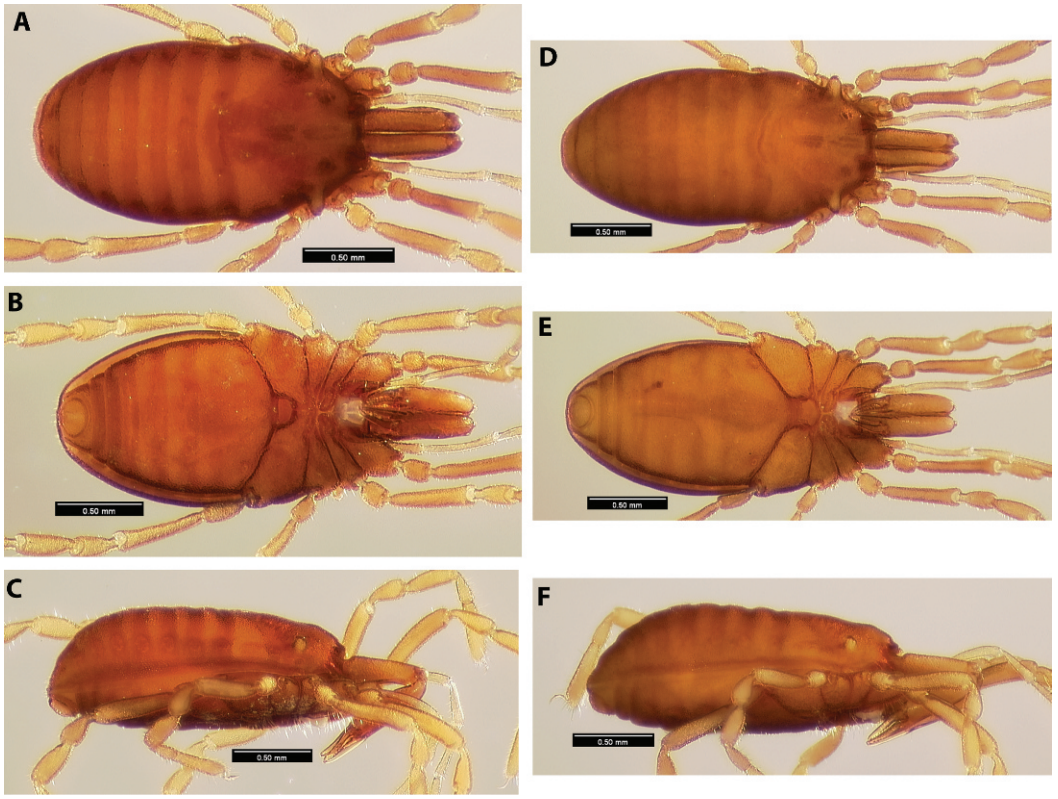

Figure 14. *Siro clousi* sp. nov. (A–C) Holotype male (MCZ DNA101871) in dorsal (A), ventral (B), and lateral (C) view. (D–F) Paratype female (MCZ DNA101871) in dorsal (D), ventral (E), and lateral (F) view. Scale bars 500 μm.

from Olalla Road, Lincoln Co., OREGON, collected 20.vi.2005 by *S. L. Boyer*, *R. M. Clouse*, & *G. Giribet*.

Paratypes. Two females (1 female for DNA work; MCZ DNA101871 ex DNA 101616large).

Etymology. The species is named after cyphophthalmid biologist Ronald M. Clouse, who assisted collecting the type material of the species.

Diagnosis. *Siro clousi* sp. nov. is easily distinguished from *S. kamiakensis* in that the latter has a divided male tarsus IV, from *S. sonoma* in that the latter has a mesal modification in the male tarsus IV, and from *S. shasta* in that the latter lacks ornamentation on the legs. The species is larger than *S. acaroides*, which live sympatrically and can also be distinguished from it by the spiracles, which are open in *S. acaroides*. The presence of the anal carina also distinguishes it from *S. acaroides*, *S. boyerae*, *S. calaveras*, and *S. shasta*. The presence of coxae III meeting along the midline also distinguishes it from *S. boyerae*, *S. exilis*, *S. kamiakensis*, and *S. sonoma*.

Description of Male. Medium-sized sironid of uniform chestnut brown color; total length of holotype 1.89 mm, maximum width at 3rd opisthosomal segment at 1.05 mm, body L/W = 1.80 (Fig. 6A). Anterior margin of dorsal scutum straight or slightly concave; prosomal region trapezoidal. Ozophores conical, of type II (*sensu* Juberthie, 1970), with subterminal ozopore (*sensu* Novak and Giribet, 2006), and entirely ornamented, with spiral ornamen-

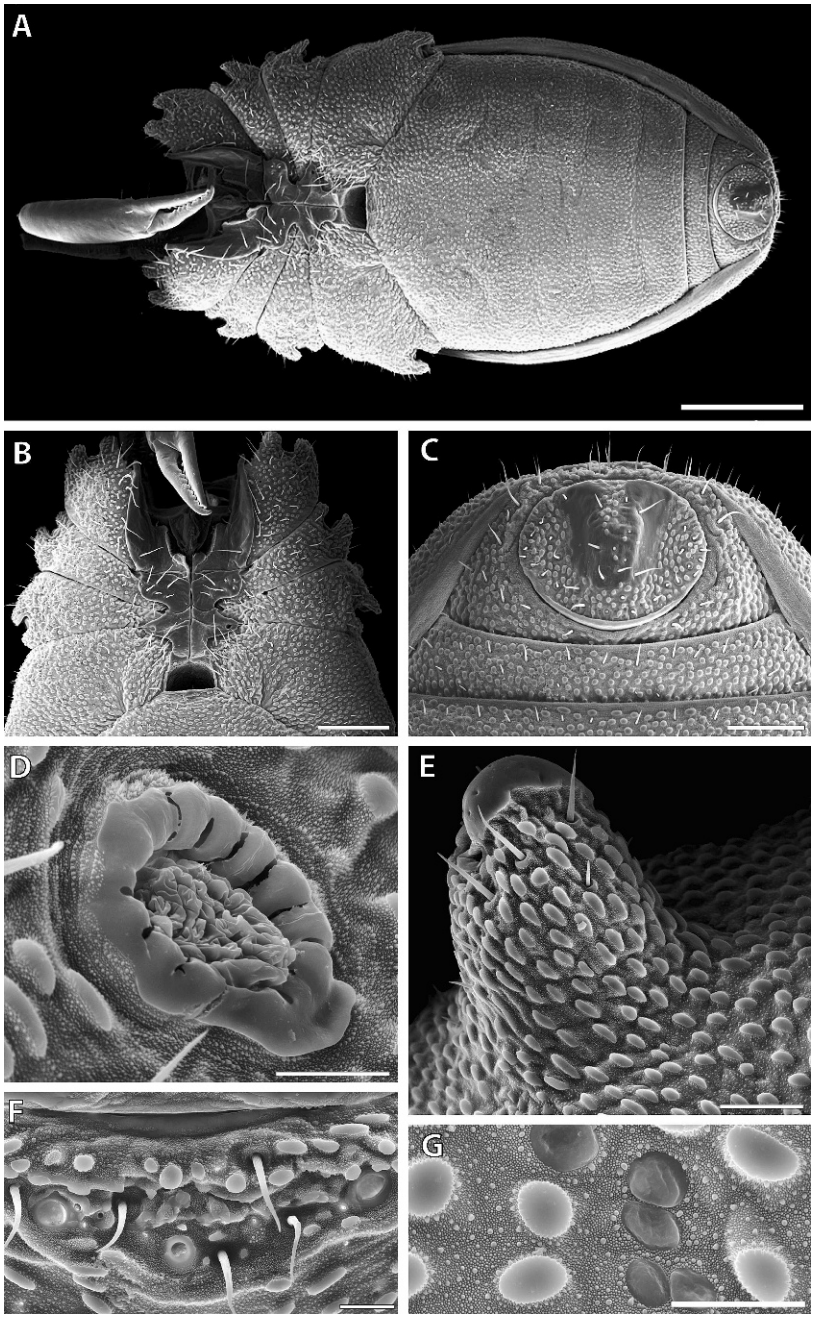

Figure 15. *Siro clousi* sp. nov. paratype male (MCZ DNA101871). (A) Paratype male in ventral position. (B) Male ventral thoracic complex. (C) Male anal region. (D) Spiracle. (E) Detail of the ozophore ornamentation. (F) Detail of the anal gland openings. (A, scale bar 400 μm; B, scale bar 200 μm, C, scale bar 100 μm; D, F, G, scale bars 20 μm; E, scale bar 40 μm.)

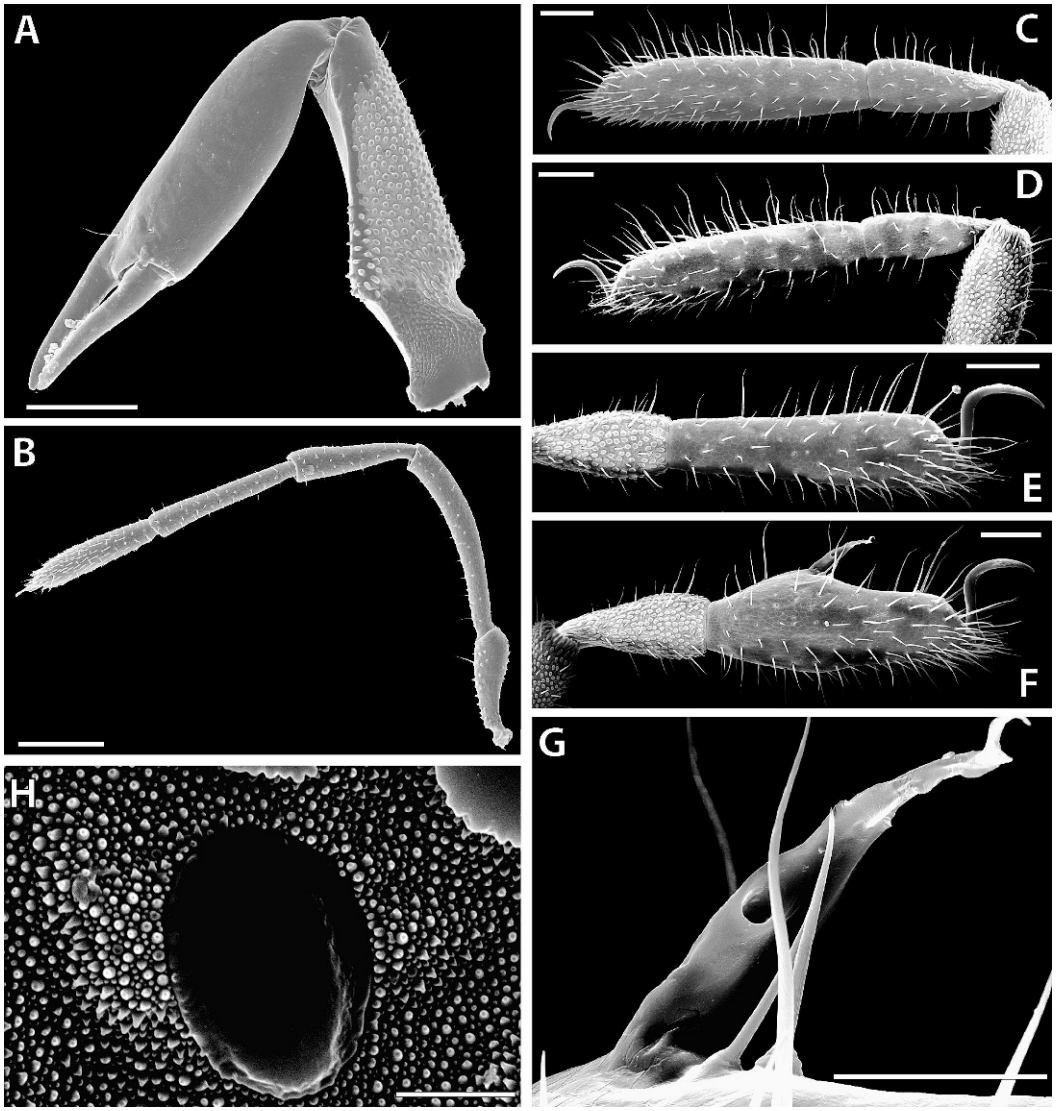

Figure 16. *Siro clousi* sp. nov. paratype male (MCZ DNA101871). (A) Left chelicera. (B) Left palp. (C) Metatarsus and tarsus I. (D) Metatarsus and tarsus II. (E) Metatarsus and tarsus III. (F) Metatarsus and tarsus IV. (G) Detail of the adenostyle. (H) Detail of leg I cuticle ornamentation. (A, B, scale bars 200 m; C–F, scale bars 100 μm; G, scale bar 50 μm; H, scale bar 5 μm.)

tation (*sensu* de Bivort and Giribet, 2004); maximum width across ozophores 0.95 mm. Eyes absent. Transverse prosomal sulcus inconspicuous; transverse opisthosomal sulci inconspicuous. Dorsal scutum with maximum height at around segments 4–5 (Fig. 14C).

Ventral prosomal complex (Figs. 14B, 15A, B) with coxae I–II free, coxae III–IV fused; coxae II, III, and IV meeting along the midline; coxae IV meeting along the midline for a distance greater than gonostome length; sternum absent; coxal pores clearly visible between coxae III and IV.

TABLE 5. LEG MEASUREMENTS (LENGTH/WIDTH, MM) IN *SIRO CLOUSI* SP. NOV. MEASUREMENTS REFER TO MALE PARATYPE MOUNTED FOR SEM.

Leg	Trochanter	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	0.17/0.14	0.62/0.13	0.31/0.12	0.41/0.12	0.26/0.10	0.51/0.12	2.28
II	0.15/0.13	0.57/0.13	0.36/0.13	0.35/0.13	0.23/0.09	0.46/0.11	2.12
III	0.16/0.13	0.42/0.12	0.24/0.13	0.28/0.13	0.23/0.09	0.42/0.10	1.75
IV	0.25/0.13	0.54/0.13	0.29/0.14	0.32/0.14	0.25/0.11	0.46/0.17	2.11

Projections of coxae IV endites present in the anterior portion of gonostome wall (Fig. 15B). Male gonostome semicircular, with straight posterior margin, wider than long (0.13 × 0.07 mm), and delimited laterally and anterolaterally by the elevated endites of coxae IV. Spiracles (Fig. 15D) of circular type (*sensu* Giribet and Boyer, 2002), circular to oval in shape in male, with a maximum diameter of 0.05 mm.

Ventral opisthosomal region (Fig. 15A) without conspicuous modifications other than in the anal plate. Opisthosomal tergite IX and sternites 8 and 9 fused into a broad corona analis (Fig. 15C). Anal plate oval, 0.24 × 0.18 mm, mostly ornamented, with a conspicuous longitudinal central ridge that leaves two lateral depressions deprived of ornamentation. Three anal gland pores on tergite VIII of males (Fig. 15F). Cuticle with tuberculate-microgranular surface (*sensu* Murphree, 1988), nearly uniform in dorsal areas and in ventral areas including coxae.

Chelicerae (Fig. 16A) robust; basal article in males 0.69 mm long, 0.21 mm wide, with a ventral process and a dorsal crest; 2nd article 0.84 mm long, 0.18 mm wide; movable finger 0.30 mm long; all articles with few setae, the proximal one almost entirely granulated with dense granulation; denticles on the cutting edge of each cheliceral finger uniform. Second cheliceral segment not ornamented.

Palp (Fig. 16B) 1.65 mm long, smooth, slightly ornamented on trochanter. Measurements of palpal article length in SEM male paratype (mm): trochanter 0.23, femur 0.46, patella 0.30, tibia 0.35, tarsus 0.30; claw 0.05 mm long.

Legs relatively robust, leg formula I-II-IV-III (measurements in Table 5; Figs. 16C–F). Tarsus I with a concentration of setae, but not forming a distinct solea. Except for the tarsi I–IV and metatarsi I–II, all articles ornamented (Figs. 16C–F). Tarsus IV of male entire, with a narrow lamelliform adenostyle (Fig. 16F), subcylindrical at the base, with lateral pore (Fig. 16G); proximal margin at 39% of tarsal length. Claws hooked, smooth, without dentition or lateral pegs.

Spermatopositor not studied.

Description of Female. Total length 2.04 mm, maximum width 1.02 mm (L/W = 1.99; Fig. 14D). Ventral prosomal complex (Fig. 14E) only with coxae I–II meeting along the midline, coxae III delimiting the anterior part of gonostome. Female gonostome semicircular anteriorly, wider than long. Gonostome of female forming a tube. Corona analis not protruding or forming a tube (Figs. 14D–F). Female anal plate unmodified. Tarsus of leg IV without modifications, narrower than that of males.

Ovipositor not studied.

Notes. *Siro clousi* sp. nov. is sympatric with the more widespread species *S. acaroides*, a considerably smaller species. Originally the specimens collected in the type locality, Olalla Road, were labeled as "large" and "small," but assigned the same MCZ DNA collection number, the vial containing the type material of the new species along with 4 males and 5 females of *S. acaroides*.

***Siro shasta* sp. nov.**
(Figs. 17–20)

Type Specimens

Holotype. Male (AMNH) from 8 mi south of Dunsmuir, Shasta Co., CALIFORNIA,

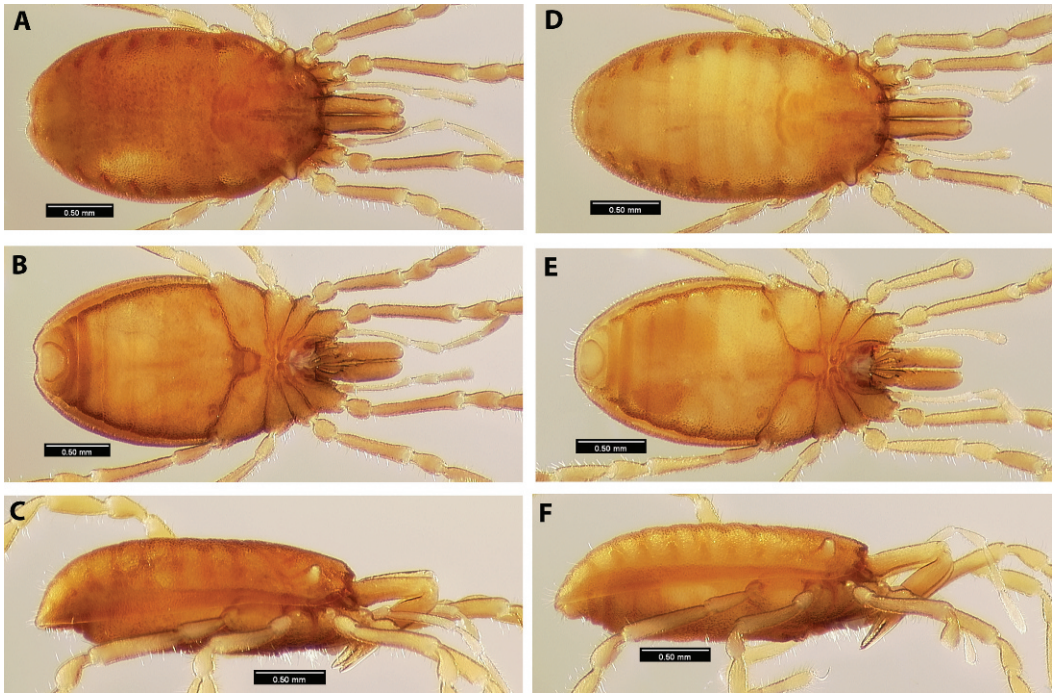

Figure 17. *Siro shasta* sp. nov. (A–C) Holotype male (AMNH) in dorsal (A), ventral (B), and lateral (C) view. (D–F) Paratype female (AMNH) in dorsal (D), ventral (E), and lateral (F) view. Scale bars 500 μm.

collected 11.vii.1954 by *R. O. Schuster & E. E. Gilbert* (Figs. 6A–C).

Paratypes. Eight males, 6 females (AMNH), same collecting data as holotype; 1 male (MCZ 92985, ex AMNH, on SEM stub), 1 female (MCZ 92986, ex AMNH, on SEM stub), same collecting data as holotype; 3 males, 5 females (AMNH) from North of Hazel Creek, Shasta Co., CALIFORNIA, collected 26.vi.1954 by *R. O. Schuster & B. Adelson*; 1 female (MCZ DNA101622) from Sims bridge, Shasta National Forest, Shasta Co., CALIFORNIA, collected 22.vi.2005 by *S. L. Boyer*, *R. M. Clouse*, & *G. Giribet*.

Etymology. The species epithet is a noun in apposition, after Shasta Co., California.

Diagnosis. *Siro shasta* (Fig. 17) is notably larger, with longer, thinner legs, than any of the other western North American *Siro* species, being about one-third longer than *S. acaroides* (2.6 vs. 1.5 mm); the body is more robust (L/W = 1.7) than that of *S. calaveras*, n. sp. (L/W = 1.9) and the 8th tergite of the male is noticeably more concave and bilobed than in any other North American species; in *S. exilis* the 8th tergite is convex. The entire 4th tarsus of the male differentiates the species from *S. kamiakensis*. The leg ornamentation differs considerably from all other North American species, being so sparse that it is barely noticeable elsewhere than on the trochanter and the dorsal part of the femur, whereas in all other species, legs I and II have a smooth tarsus and metatarsus only and legs III and IV have a smooth tarsus only. The ventral prosomal complex resembles mostly that of *S. acaroides*, *S. calaveras*, and *S. clousi*, in that the endites of coxae III meet along the midline, but not so in *S. boyerae*, *S. exilis*, or *S. kamiakensis*. The spiracles are similar to those of *S. acaroides*, in the form of an open circle, but differ from all other North American sironids, which have circular spiracles. The microtrichia of

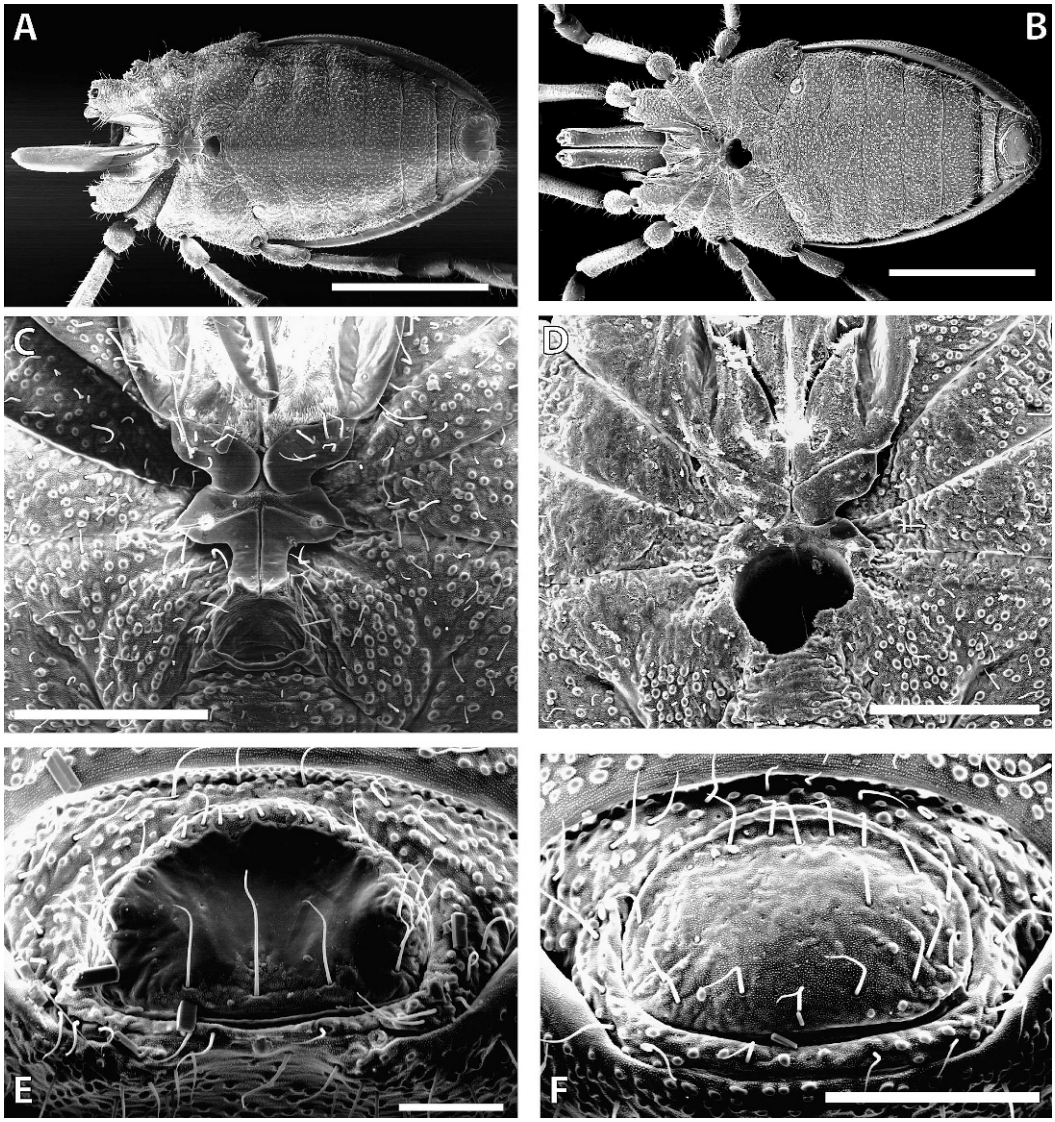

Figure 18. *Siro shasta* sp. nov. paratype male (MCZ 92985) and female (MCZ 92986). (A) Paratype male in ventral position. (B) Paratype female in ventral position. (C) Male ventral thoracic complex. (D) Female ventral thoracic complex. (E) Male anal region. (F) Female anal region. (A, B, scale bars 1 mm; C, D, scale bars 300 μm; E, scale bar 100 μm; F, scale bar 200 μm.)

the penis is unique among North American *Siro*, with 4 apical, 6 ventral, and 10 dorsal microtrichiae, whereas the unusually large movable fingers of the penis are like those of the western species group.

Description of Male. Large sironid of uniform chestnut brown color; total length of holotype 2.33 mm, maximum width at 3rd opisthosomal segment at 1.38 mm, body L/W = 1.69 (Fig. 17A). Anterior margin of dorsal scutum bilobed; prosomal region sub-semicircular. Ozophores conical, of type II (*sensu* Juberthie, 1970), with subterminal ozopore (*sensu* Novak and Giribet, 2006), and entirely ornamented; maximum width across ozophores 1.02 mm.

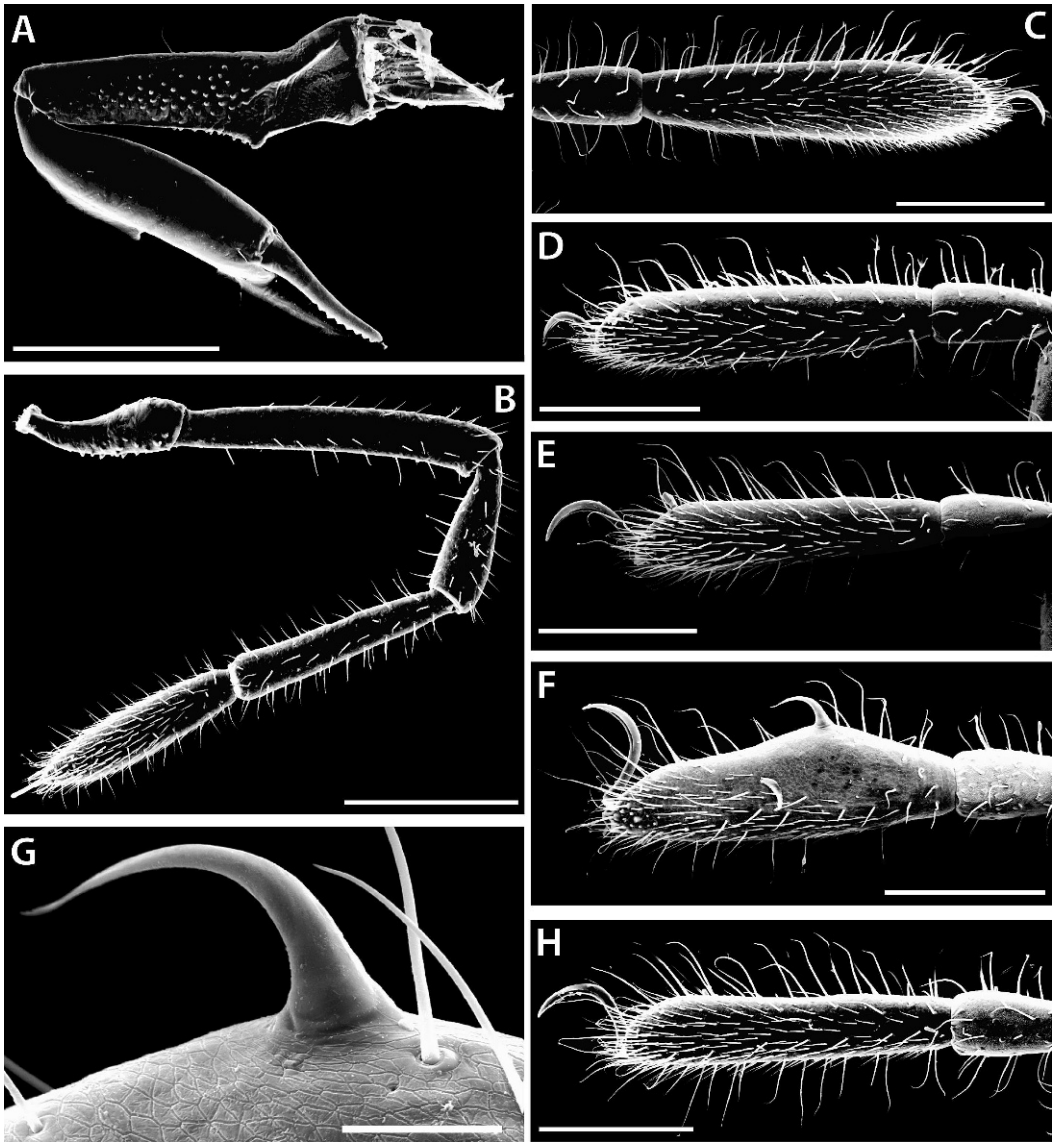

Figure 19. *Siro shasta* sp. nov. paratype male (MCZ 92985) and female (MCZ 92986). (A) Left chelicera of male. (B) Left palp of male. (C) Metatarsus and tarsus I of male. (D) Metatarsus and tarsus II of male. (E) Metatarsus and tarsus III of male. (F) Metatarsus and tarsus IV of male. (G) Detail of adenostyle. (H) Metatarsus and tarsus IV of female. (A, scale bar 500 μm; B–F, H, scale bars 300 μm; G, scale bar 50 μm.)

Eyes absent. Transverse prosomal sulcus inconspicuous; transverse opisthosomal sulci inconspicuous. Dorsal scutum with maximum height at around segments 4–5 (Fig. 17C).

Ventral prosomal complex (Figs. 18A, E) with coxae I–II free, coxae III–IV fused; coxae II, III, and IV meeting along the midline; coxae IV meeting along the midline for a distance slightly greater than gonos-

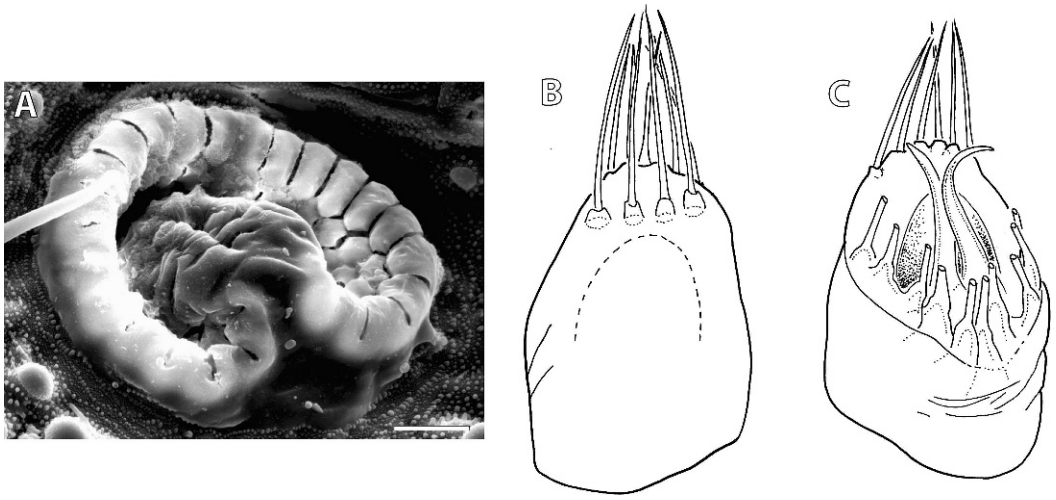

Figure 20. *Siro shasta* sp. nov. (A) Spiracle (scale bar 20 μm). (B) Spermatopositor, ventral view. (C) Spermatopositor, dorsal view.

tome length; sternum absent; coxal pores clearly visible between coxae III and IV. Projections of coxae IV endites present in the anterior portion of gonostome wall (Fig. 18C). Male gonostome almost oval in shape, with the posterior margin forming a concave lip, wider than long (0.14 × 0.10 mm), and delimited laterally and anterolaterally by the elevated endites of coxae IV. Spiracles (Fig. 20A) in the shape of an open circle (*sensu* Giribet and Boyer, 2002), with a maximum diameter of 0.10 mm.

Ventral opisthosomal region (Fig. 18A) without conspicuous modifications other than in the anal plate. Opisthosomal tergite IX and sternites 8 and 9 fused into a broad corona analis (Fig. 18E). Anal plate oval, 0.32 × 0.21 mm, completely depressed and smooth, except for the anterior and lateral rims. Three anal gland pores on tergite VIII of males. Tergite VIII depressed posteriorly, forming a bilobed posterior end. Cuticle with tuberculate-microgranular surface (*sensu* Murphree, 1988), nearly uniform in dorsal areas and in ventral areas including coxae.

Chelicerae (Fig. 19A) robust; basal article in males 0.85 mm long, 0.23 mm wide, with a ventral process but without a dorsal crest; 2nd article 1.07 mm long, 0.19 mm wide; movable finger 0.35 mm long; all articles with few setae, the proximal one almost entirely granulated but with sparse granulation; denticles on the cutting edge of each cheliceral finger uniform, triangular, with 8 denticles in the moveable finger. Second cheliceral segment smooth, not ornamented.

Palp (Fig. 19B) 1.91 mm long, smooth, slightly ornamented on trochanter. Measurements of palpal article length in SEM male paratype (mm): trochanter 0.27, femur 0.55, patella 0.29, tibia 0.40, tarsus 0.40; claw 0.06 mm long.

Legs slender, leg formula I-IV-II-III (measurements in Table 6; Figs. 19C–F). Tarsus I with a concentration of setae, but not forming a distinct solea. Tarsi I–IV and metatarsi I–II smooth, all other articles presenting sparse ornamentation, to the point that metatarsi III–IV are almost smooth, but present a few tuberculate structures (Figs. 19E, F). Tarsus IV of male entire, swollen, with a small lamelliform adenostyle (Fig. 19F), subcylindrical at the base, with lateral pore (Fig. 19G); proximal margin at 35% of tarsal length. Claws hooked, smooth, without dentition or lateral pegs.

Spermatopositor (Figs. 20B, C) short, typical of sironids, smooth; with movable

fingers, slightly curved outward, ending as hooks, not much longer than the membranous median lobe; microtrichial formula: 4, 6, 5+5 (n = 1). Gonopore complex not observed.

Description of Female. Total length 2.40 mm, maximum width 1.29 mm (L/W = 1.85; Fig. 17D). Ventral prosomal complex (Figs. 18B, F) only with coxae I–II meeting along the midline, coxae III delimiting the anterior part of gonostome. Female gonostome near circular, wider than long. Gonostome of female forming a tube. Corona analis not protruding or forming a tube, with most of its surface deprived of macrotuberculate ornamentation, only presenting microtuberculate one (Fig. 18F). Tarsus of leg IV without modifications (Fig. 19H), narrower than that of males.

Ovipositor not studied.

Siro acaroides (Ewing, 1923)

Holosiro acaroides Ewing 1923: 338.
Siro acaroides: Newell 1947: 354; Shear 1980: 10.

The following new records establish a new southern limit for the distribution of *S. acaroides*, by about 50 mi. The records for the locality, "18 miles south of Klamath," were labeled as being from Del Norte Co., but that distance south of Klamath would be well into Humboldt Co., and the records are so given here. It is possible that *S. acaroides* occurs much farther south; there is a single juvenile (CAS) known from Mendocino Co., 2 mi south of Rockport, collected by *C. W. O'Brien*, 2.ii.1962. However, the possibility that this is another species cannot be dismissed.

We have also found that *S. acaroides* exhibits at least one unique character when compared with other North American *Siro*: the palpal trochanter without tubercles (de Bivort and Giribet, 2002: fig. 16a).

CALIFORNIA: *Del Norte Co.*: One male (CAS) near Crescent City, Smith River, collected 9.xi.1956 by *J. Schuh*; 2 males, 1 female (CAS) 5 mi south of Crescent City, collected 9.ix.1958; 7 males (1 for DNA work) and 6 females (MCZ DNA101620) from Kings Valley, near Crescent City, collected 21.vi.2005 by *S. L. Boyer*, *R. M. Clouse*, & *G. Giribet*. *Humboldt Co.*: Thirteen males, 8 females (CAS) 18 mi south of Klamath, collected 13.viii.1953; 28 males, 28 females (CAS) collected 19.ix.1953; 5 males, 4 females (CAS) Big Lagoon, collected 13.viii.1953 by *G. A. Marsh & L. O. Schuster*; 3 males, 3 females (CAS) freshwater, collected 18.viii.1952 by *G. A. Marsh & L. O. Schuster*; 5 males (CAS) Prairie Creek Redwoods State Park, collected 8.ix.1958 by *L. M. Smith*; 7 males (1 for DNA work) and 9 females (1 for DNA work; MCZ DNA101621) from Lady Bird Johnson Grove, Redwood National and State Parks, collected 21.vi.2005 by *S. L. Boyer*, *R. M. Clouse*, & *G. Giribet*.

OREGON: *Benton Co.*: Thirteen males (1 for DNA work) and 19 females (MCZ DNA101618) from MacDonald State Forest, collected 20.vi.2005 by *S. L. Boyer*, *R. M. Clouse*, & *G. Giribet*. *Douglas Co.*: Eleven males (2 for DNA work) and 4 females (MCZ DNA101619) from Elliot State Forest, Umpqua State Scenic Corridor, collected 20.vi.2005 by *S. L. Boyer*, *R. M. Clouse*, & *G. Giribet*.

Key to Males of North American Sironidae

1a. Male 4th tarsus divided .....
.....*S. kamiakensis* (Newell, 1943)

Table 6. Leg measurements (length/width, mm) in *Siro shasta* sp. nov. Measurements refer to male paratype mounted for SEM.

Leg	Trochanter	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	0.27/0.18	0.83/0.14	0.38/0.16	0.58/0.16	0.31/0.12	0.70/0.16	3.07
II	0.18/0.15	0.68/0.16	0.28/0.16	0.45/0.16	0.26/0.11	0.62/0.13	2.47
III	0.25/0.16	0.51/0.15	0.26/0.17	0.41/0.16	0.25/0.11	0.57/0.13	2.25
IV	0.43/0.15	0.72/0.16	0.38/2.0	0.50/0.18	0.31/0.13	0.64/0.21	2.98

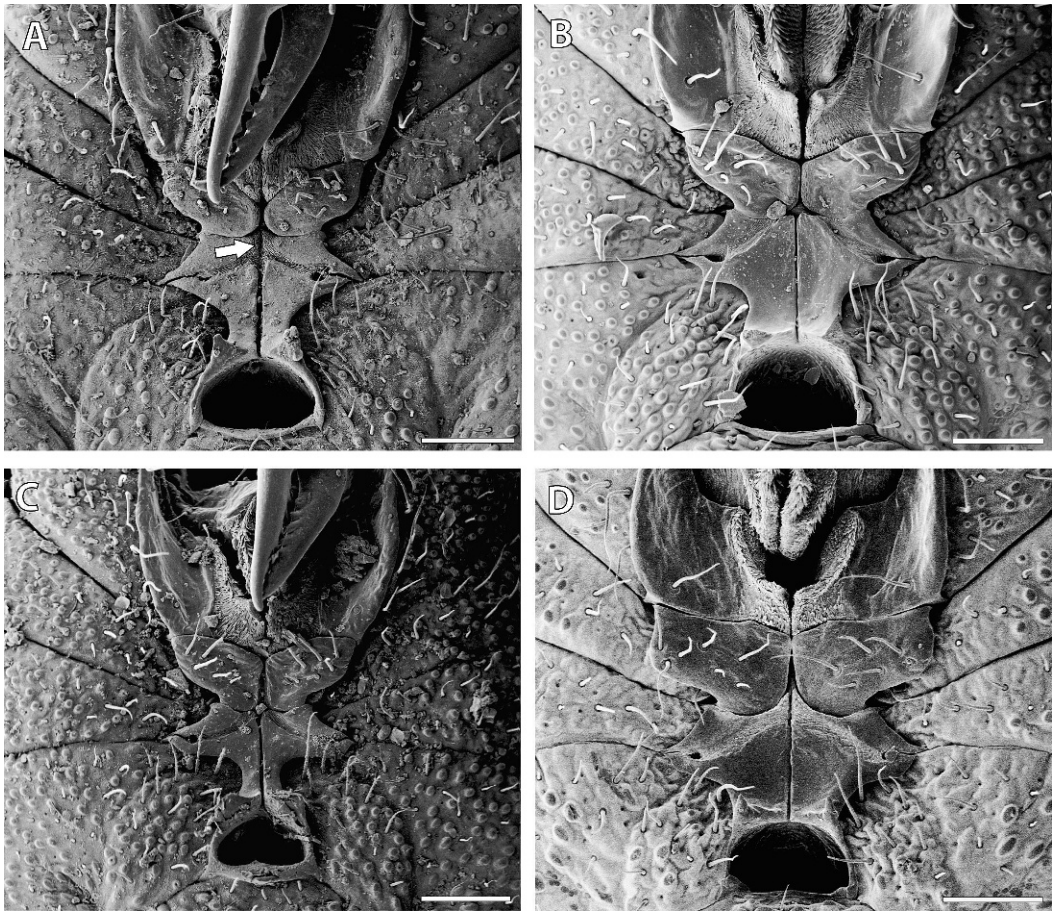

Figure 21. Male ventral thoracic complex of described North American sironids. (A) *Siro acaroides* (arrowhead indicates the area of contact of the endites of cozae III). (B) *S. exilis*. (C) *S. kamiakensis*. (D) *S. sonoma*. (A–D, scale bars 100 μm.)

1b. Male 4th tarsus not divided 2
2a. Male 4th tarsus with a ventral lobe mesally excavated .... *S. sonoma* Shear, 1980
2b. Male 4th tarsus without a ventral lobe 3
3a. Male tergite VIII convex, with small knob ....*S. exilis* Hoffman, 1963.
3b. Male tergite VIII concave, without a knob 4
4a. Legs with very sparse ornamentation ..... *S. shasta* sp. nov.
4b. Legs with basal articles ornamented until tibia I and II and until metatarsi III and IV 5
5a. Spiracles in the form of an open circle ... *S. acaroides* (Ewing, 1923).
5b. Spiracles circular 6
6a. Endites of coxae III not meeting along the midline .... *S. boyerae* sp. nov.
6b. Endites of coxae III meeting along the midline 7
7a. Anal carina present .... *S. clousi* sp. nov.

7b. Anal carina absent .....
..... *S. calaveras* sp. nov.

ACKNOWLEDGMENTS

We are indebted to many students in the Giribet laboratory that assisted with this research. Sarah Boyer and Tone Novak provided comments that helped to improve this article. Sarah Boyer participated in collecting trips to Japan and the NW United States and generated sequence data for some outgroups; Ron Clouse participated in the collecting trip to the NW United States; Prashant Sharma, Ligia Benavides, and Ben de Bivort assisted with SEM; Ligia Benavides generated the automontage images for the types; and Joey Pakes assisted with the molecular work. We are also indebted to Tom Briggs, Salvador Carranza, Michele Nishiguchi, Nobuo Tsurusaki, and Darrell Ubick for assisting with fieldwork in Sonoma Co. (California), Japan, and Spain. Marco Valle, Ivo Karaman, and Plamen Mitov provided specimens of *Cyphophthalmus* and *S. valleorum*. Finally, the arachnid curators and curatorial staff of the AMNH (Lorenzo Prendini and Norman Platnick), CAS (Charles Griswold and Darrell Ubick), FMNH (Petra Sierwald), and EME (Jerry Powell) are acknowledged for their support and long-term loans, which we promise will be returned some day. This material is based on work supported by the National Science Foundation under Grant 0236871.

LITERATURE CITED

Boyer, S. L., J. M. Baker, and G. Giribet. 2007a. Deep genetic divergences in *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi): a widespread 'mite harvestman' defies DNA taxonomy. Molecular Ecology **16:** 4999–5016.

Boyer, S. L., R. M. Clouse, L. R. Benavides, P. Sharma, P. J. Schwendinger, I. Karunarathna, and G. Giribet. 2007b. Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally distributed group of arachnids. Journal of Biogeography **34:** 2070–2085.

Boyer, S. L., I. Karaman, and G. Giribet. 2005. The genus *Cyphophthalmus* (Arachnida, Opiliones, Cyphophthalmi) in Europe: a phylogenetic approach to Balkan Peninsula biogeography. Molecular Phylogenetics and Evolution **36:** 554–567.

Catley, K. M. 1994. Descriptions of new *Hypochilus* species from New Mexico and California with a cladistic analysis of the Hypochilidae (Araneae). American Museum Novitates **3088:** 1–27.

Davis, N. W. 1933. A new opilionid from Florida (Arachnida, Cyphophthalmi). Journal of the New York Entomological Society **41:** 49–53.

de Bivort, B. L., and G. Giribet. 2004. A new genus of cyphophthalmid from the Iberian Peninsula with a phylogenetic analysis of the Sironidae (Arachnida : Opiliones : Cyphophthalmi) and a SEM database of external morphology. Invertebrate Systematics **18:** 7–52.

De Laet, J. E. 2005. Parsimony and the problem of inapplicables in sequence data, pp. 81–116. *In* V. A. Albert (ed.), Parsimony, Phylogeny, and Genomics. Oxford: Oxford Univ. Press.

Ewing, H. E. 1923. *Holosiro acaroides*, new genus and species—the only New World representative of the mite-like phalangids of the suborder Cyphophthalmi. Annals of the Entomological Society of America **16:** 387–390.

Farris, J. S. 1997. The future of phylogeny reconstruction. Zoologica Scripta **26:** 303–311.

Farris, J. S., V. A. Albert, M. Källersjö, D. Lipscomb, and A. G. Kluge. 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics **12:** 99–124.

Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1995. Constructing a significance test for incongruence. Systematic Biology **44:** 570–572.

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

Giribet, G. 2000. Catalogue of the Cyphophthalmi of the world (Arachnida, Opiliones). Revista Ibérica de Aracnología **2:** 49–76.

———. 2003. Stability in phylogenetic formulations and its relationship to nodal support. Systematic Biology **52:** 554–564.

———. 2005. Generating implied alignments under direct optimization using POY. Cladistics **21:** 396–402.

———. 2007. Neogoveidae Shear, 1980, pp. 95–97. *In* R. Pinto-da-Rocha, G. Machado, and G. Giribet (eds.), Harvestmen: The Biology of Opiliones. Cambridge, Massachusetts: Harvard Univ. Press.

Giribet, G., and S. L. Boyer. 2002. A cladistic analysis of the cyphophthalmid genera (Opiliones, Cyphophthalmi). The Journal of Arachnology **30:** 110–128.

Giribet, G., G. D. Edgecombe, W. C. Wheeler, and C. Babbitt. 2002. Phylogeny and systematic position of Opiliones: a combined analysis of chelicerate relationships using morphological and molecular data. Cladistics **18:** 5–70.

Giribet, G., L. Vogt, A. Pérez González, P. Sharma, and A. B. Kury. 2010. A multilocus approach to harvestman (Arachnida: Opiliones) phylogeny with

emphasis on biogeography and the systematics of Laniatores. Cladistics **26**. Available online only doi:10.1111/j1096-0031.2009.00296.x

GOEBEL, A. M., T. A. RANKER, P. S. CORN, and R. G. OLMSTEAD. 2009. Mitochondrial DNA evolution in the *Anaxyrus* boreas species group. Molecular Phylogenetics and Evolution **50:** 209–225.

GOLOBOFF, P. A. 1999. Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics **15:** 415–428.

———. 2002. Techniques for analyzing large data sets, pp. 70–79. *In* R. DeSalle, G. Giribet, and W. Wheeler (eds.), Techniques in Molecular Systematics and Evolution. Basel: Brikhäuser Verlag.

HINTON, B. E. 1938. A key to the genera of the Suborder Cyphophthalmi with a description and figures of *Neogovea immsi*, gen. et sp.n. (Arachnida, Opiliones). Annals and Magazine of Natural History Ser.11 **2:** 331–338.

HOFFMAN, R. L. 1963. A new phalangid of the genus *Siro* from eastern United States, and taxonomic notes on other American sironids (Arach., Opiliones). Senckenbergiana Biologica **44:** 129–139.

JUBERTHIE, C. 1960. Contribution a l'étude des opilions cyphophthalmes: description de *Metasiro* gen. n. Bulletin du Muséum National d'Histoire Naturelle **32:** 235–241.

———. 1967. *Siro rubens* (Opilion, Cyphophthalme). Revue d'Écologie et de Biologie du Sol **4:** 155–171.

———. 1970. Les genres d'opilions Sironinae (Cyphophthalmes). Bulletin du Muséum National d'Histoire Naturelle **41:** 1371–1390.

KARAMAN, I. M. 2008. Cyphophthalmi of Serbia (Arachnida, Opiliones), pp. 97–118. *In* D. Pavicevic and M. Perreau (eds.), Advances in the studies of the fauna of the Balkan Peninsula. Papers dedicated to the memory of Guido Nonveiller. Belgrade: Institute for Nature Conservation of Serbia.

LINTON, E. W [Internet]. MacGDE: Genetic Data Environment for MacOS X. Mt. Pleasant (MI): Central Michigan University; c2005 [updated 2009 Jun 3; cited 2010 June 09]. Available from: http://macgde.bio.cmich.edu/.

MALLATT, J., and C. J. WINCHELL. 2002. Testing the new animal phylogeny: first use of combined large-subunit and small-subunit rRNA gene sequences to classify the protostomes. Molecular Biology and Evolution **19:** 289–301.

MICKEVICH, M. F., and J. S. FARRIS. 1981. The implications of congruence in *Menidia*. Systematic Zoology **30:** 351–370.

MURIENNE, J., I. KARAMAN, and G. GIRIBET. (2010). Explosive evolution of an ancient group of Cyphophthalmi (Arachnida: Opiliones) in the Balkan Peninsula. Journal of Biogeography **37:** 90–102.

MURPHREE, C. S. 1988. Morphology of the dorsal integument of ten opilionid species (Arachnida, Opiliones). The Journal of Arachnology **16:** 237–252.

NEWELL, I. M. 1943. A new sironid from North America (Opiliones, Cyphophthalmi, Sironidae). Transactions of the American Microscopical Society **62:** 416–422.

———. 1947. The rediscovery and clarification of *Siro acaroides* (Ewing) (Opiliones, Cyphophthalmi, Sironidae). Transactions of the American Microscopical Society **66:** 354–365.

NOVAK, T., and G. GIRIBET. 2006. A new species of Cyphophthalmi (Arachnida, Opiliones, Sironidae) from Eastern Slovenia. Zootaxa **1330:** 27–42.

SCHWENDINGER, P. J., and G. GIRIBET. 2005. The systematics of the south-east Asian genus *Fangensis* Rambla (Opiliones : Cyphophthalmi : Stylocellidae). Invertebrate Systematics **19:** 297–323.

SHEAR, W. A. 1977. The opilionid genus *Neogovea* Hinton, with a description of the first troglobitic cyphophthalmid from the western hemisphere (Opiliones, Cyphophthalmi). The Journal of Arachnology **3:** 165–175.

———. 1980. A review of the Cyphophthalmi of the United States and Mexico, with a proposed reclassification of the suborder (Arachnida, Opiliones). American Museum Novitates **2705:** 1–34.

WHEELER, W. C. 1995. Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. Systematic Biology **44:** 321–331.

———. 1996. Optimization alignment: the end of multiple sequence alignment in phylogenetics? Cladistics **12:** 1–9.

———. 2003. Implied alignment: a synapomorphy-based multiple-sequence alignment method and its use in cladogram search. Cladistics **19:** 261–268.

WHEELER, W. C., D. GLADSTEIN, and J. DE LAET. 2004. POY Version 3.0. New York: American Museum of Natural History.

Suppl. Appendix 1. Voucher details for specimens used in molecular analyses.

***Suzukielus sauteri* (Roewer, 1916)**

MCZ DNA101543

Japan: Airin Camping Ground, Mt. Takao, Tokyo Prefecture, Honshu (35º38'03”N, 139º14'28”E, 294 m elevation); leg. S.L. Boyer, G. Giribet, Y. Minagoshi & N. Tsurusaki, 13.iv.2005

***Cyphophthalmus* sp.**

MCZ DNA101342

Bulgaria: Kljuch village, Distr. Petrich, Mt. Belasica; leg. P. Mitov, 22.v.2004

***Cyphophthalmus* cf. *teyrovskyi* (Kratochvíl, 1938)**

MCZ DNA100910

Montenegro: Ivanina (Velja) spila Cave, Donja Seoca, Virpazar; leg. I. Karaman, 2003

***Cyphophthalmus trebinjanus* Karaman, 2009**

MCZ DNA101038

Bosnia & Herzegovina: Trebinje-Vučja pećina Cave; leg. I. Karaman, vii.2003

***Cyphophthalmus duricorius* Joseph, 1868**

MCZ DNA100487

Slovenia: Podskokarjeva jama Cave, Zgornja Besnica; leg. M. Comatti, 7.x.2001

***Paramiopsalis ramulosus* Juberthie, 1962**

MCZ DNA100459

Spain: Valle del río Barragán, Moscoso, prov. de Pontevedra, Galicia (42º18'54”N, 008º29'12”W); leg. G. Giribet & M.K. Nishiguchi, 21.vii.2001

***Parasiro coiffaiti* Juberthie, 1956**

MCZ DNA101383

Spain: Font del Vidre, Berga, prov. de Barcelona, Catalunya (42º09'09”N, 001º55'49”E); leg. S. Carranza & G. Giribet, 2.vi.2004

***Siro acaroides* (Ewing, 1923)**

MCZ DNA100488

USA: 15 Km W of Philomath, Woods Creek Rd., Benton Co., Oregon; leg. A. Moldenke, 22.vii.1996

MCZ DNA101616

USA: Olalla Road, Lincoln, Co., Oregon (44º40'00"N, 123º55'58"W); leg. S.L. Boyer, R.M. Clouse & G. Giribet, 20.vi.2005

MCZ DNA101619

USA: Elliot State Forest, Umpqua State Scenic Corridor, Douglas Co., Oregon (43º38'58"N, 123º53'41"W); leg. S.L. Boyer, R.M. Clouse & G. Giribet, 20.vi.2005

MCZ DNA101620

USA: Kings Valley, near Crescent City, Del Norte Co., California (41º50'17"N, 124º08'39"W); leg. S.L. Boyer, R.M. Clouse & G. Giribet, 21.vi.2005

MCZ DNA101621

USA: Lady Bird Johnson Grove, Redwood National and State Parks, Humboldt Co., California (41º18'10"N, 124º01'03"W); leg. S.L. Boyer, R.M. Clouse & G. Giribet, 21.vi.2005

Siro boyerae **Giribet & Shear, sp. nov.**

MCZ DNA101614

USA: Chenuis Fall, Carbon River, Mt. Rainier Natl. Park, Pierce Co., Washington (46º59'32"N, 121º50'47"W); leg. S.L. Boyer, R.M. Clouse & G. Giribet, 19.vi.2005

MCZ DNA101617

USA: Ecola State Park, Clatsop Co., Oregon (45º54'56"N, 123º57'52"W); leg. S.L. Boyer, R.M. Clouse & G. Giribet, 20.vi.2005

Siro calaveras **Giribet & Shear, sp. nov.**

MCZ DNA101623

USA: North Grove, Calaveras Big Trees State Park, Calaveras Co., California (38º16'38"N, 120º18'19"W); leg. S.L. Boyer, R.M. Clouse & G. Giribet, 23.vi.2005

Siro clousi **Giribet & Shear, sp. nov.**

MCZ DNA101871

USA: Olalla Road, Lincoln, Co., Oregon (44º40'00"N, 123º55'58"W); leg. S.L. Boyer, R.M. Clouse & G. Giribet, 20.vi.2005

***Siro exilis* Hoffman, 1963**

MCZ DNA100489

USA: Swallow Falls State Park, Garrett Co., Maryland; leg. J.W. Shultz, vii.2000

***Siro kamiakensis* (Newell, 1943)**

MCZ DNA101611

USA: East of Hayden Lake on Hayden Lake Road, Coeur d'Alene National Forest, Kootenai Co., Idaho (47º44'47"N, 116º42'07"W); leg. S.L. Boyer, R.M. Clouse & G. Giribet, 17.vi.2005

MCZ DNA101613

USA: Kamiak Butte County Park, Whitman Co., Washington (46º52'04"N, 117º09'28"W); leg. S.L. Boyer, R.M. Clouse & G. Giribet, 18.vi.2005

***Siro rubens* Latreille, 1804**

MCZ DNA100457

France: Mont Aigoual, P.N. des Cévennes, Massif Central (44º05'00”N, 003º34'53”E); leg. G. Giribet, 26.vii.2001

***Siro shasta* Giribet & Shear, sp. nov.**

MCZ DNA101622

USA: Sims Bridge, Shasta National Forest, Shasta Co., California (41º03'49"N, 122º21'37"W); leg. S.L. Boyer, R.M. Clouse & G. Giribet, 22.vi.2005

***Siro sonoma* Shear, 1980**

MCZ DNA100507

USA: Monte Rio, Sonoma Co., California (38º26'37"N, 122º59'19"W); leg. T. Briggs, G. Giribet & D. Ubick, 20.xii.2001

***Siro valleorum* Chemini, 1990**

MCZ DNA100461

Italy: Colzate (BG), c/o Baite Sedernello, Lombardia; leg. Ferrario, Pantini, Pellizzoli & Valle, 2.viii.2001

Suppl. Table 1. List of primer sequences used for amplification and sequencing with original references of the primer sequences. Ribosomal genes were amplified at annealing temperatures ranging between 46 and 49 ºC. Protein-coding genes were amplified at annealing temperatures between 42 and 45 ºC.

18S rRNA

1F	5'- TAC CTG GTT GAT CCT GCC AGT AG – 3'	Giribet et al. (1996)
3F	5'- GTT CGA TTC CGG AGA GGG A – 3'	Giribet et al. (1996)
4R	5'- GAA TTA CCG CGG CTG CTG G – 3'	Giribet et al. (1996)
9R	5'- GAT CCT TCC GCA GGT TCA CCT AC – 3'	Giribet et al. (1996)
18Sa2.0	5'- ATG GTT GCA AAG CTG AAA C – 3'	Whiting et al. (1997)
18Sbi	5'- GAG TCT CGT TCG TTA TCG GA – 3'	Whiting et al. (1997)

28S rRNA

28Sa	5'- GAC CCG TCT TGA AAC ACG GA – 3'	Whiting et al. (1997)
28Sb	5'- TCG GAA GGA ACC AGC TAC – 3'	Whiting et al. (1997)
28S rd1a	5' - CCC SCG TAA YTT AGG CAT AT – 3'	Edgecombe and Giribet (2006)

28S rd4b	5’ – CCT TGG TCC GTG TTT CAA GAC –3’	Edgecombe and Giribet (2006)
28S rd4.8a	5’ – ACC TAT TCT CAA ACT TTA AAT GG – 3’	Schwendinger and Giribet (2005)
28S rd5b	5’ – CCA CAG CGC CAG TTC TGC TTA C – 3’	Schwendinger and Giribet (2005)
28S rd7b1	5’ – GAC TTC CCT TAC CTA CAT – 3’	Schwendinger and Giribet (2005)

COI

LCO1490	5’- GGT CAA CAA ATC ATA AAG ATA TTG G – 3’	Folmer et al. (1994)
HCOoutout	5’ - GTA AAT ATA TGR TGD GCT C - 3’	Prendini et al. (2005); Schwendinger and Giribet (2005)