BREVIORA

Museum of Comparative Zoology

US ISSN 0006-9698

CAMBRIDGE, MASS. 9 FEBRUARY 2015 NUMBER 543

CYPHOPHTHALMUS SOLENTIENSIS SP. NOV. (CYPHOPHTHALMI, SIRONIDAE), A NEW ENDOGEAN MITE HARVESTMAN SPECIES FROM CROATIA, WITH AN APPLICATION OF CONFOCAL LASER MICROSCOPY TO ILLUSTRATE GENITALIA IN OPILIONES

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ABSTRACT. The genus *Cyphophthalmus* is one of the most diverse genera of Cyphophthalmi and has been used as a model to study diversification in the Balkan region. However, the taxonomy of the group is deficient and type material is not available for study. Here we describe a new species, *Cyphophthalmus solentiensis* **sp. nov.**, from the coastal region of Croatia using state-of-the-art techniques for illustrating species of Cyphophthalmi. The species, phylogenetically close to *C. gjorgjevici* on the basis of a molecular data analysis of four markers, is illustrated by means of stereomicroscopy and scanning electron microscopy, and the genitalia are imaged using confocal laser microscopy and three-dimensional reconstruction techniques, allowing unparalleled visualization of Opiliones genitalia. We hope that this description stimulates research in this diverse but still obscure genus of Cyphophthalmi.

KEY WORDS: Opiliones; Arachnida; genitalia; phylogeny; Balkans; Mediterranean region; confocal laser microscopy; molecular data

INTRODUCTION

Among the most diverse genera of Cyphophthalmi is the sironid *Cyphophthalmus* Joseph, 1868, with 32 species currently recognized (Karaman, 2009), distributed from Austria to Turkey along the Mediterranean region. The group has undergone an interesting biogeographical history due to its ancient age and because it diversified explosively in the Balkan region, giving origin to at least three phylogenetic lineages (Boyer *et al.*, 2005; Murienne *et al.*, 2010) whose evolution could be related to the paleogeographic history of the Adria microplate (Murienne *et al.*, 2010). Among these three clades, the *gjorgjevici* lineage is one of the poorest-known lineages, and includes *C.*

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gjorgjevici (Hadži, 1933) and undescribed species from Macedonia (Karaman, 2009; Murienne *et al.*, 2010). However, despite the large diversity of this clade and its importance for understanding the biogeography of the Balkan region, the group has a large number of missing types for the older species (see Giribet, 2000), and has substandard descriptions of recent species (Karaman, 2008, 2009), with types kept in a private collection not broadly available to other researchers. Fortunately, advances in molecular phylogenetics and species delimitation techniques allow assessment of species molecularly, which has helped to identify a new species of *Cyphophthalmus* recently collected in endogean habitats in Croatia. The species, closely related to other endogean species, *C. gjorgjevici*, is here described and fully illustrated by means of light stereomicroscopy, scanning electron microscopy (SEM), and confocal laser microscopy to highlight informative characters of interest in describing new diversity of *Cyphophthalmus*.

MATERIALS AND METHODS

Stereomicroscope imaging

The male holotype and one female paratype were cleansed with ultrasounds for 5 min and imaged using a JVC KY-F70B digital camera mounted on a Leica MZ 12.5 stereoscope with the 0.5–4.0× objective. A series of 10–20 images was taken at different focal lengths, and then assembled using the software package Auto-Montage Pro Version 5.00.0271. Each specimen was photographed in dorsal, ventral, and lateral positions.

SEM imaging

Two males and one female paratype were cleansed as explained above and mounted on SEM stubs with biadhesive carbon tape. These samples were sputter coated with an EMS 300T D dual-headed sputter coater at the Harvard Center for Nanoscale Systems. A 5-nm platinum/palladium layer was applied. Samples were imaged with a Carl Zeiss Ultra Plus FESEM using the SE2 detector. Images were then edited using Adobe Photoshop CS5.

Autofluorescence imaging

Following Murienne and Giribet (2009), we took advantage of the autofluorescence of the arthropod cuticle (Klaus *et al.*, 2003, 2014) to image the spermatopositor and ovipositor organs of the new species. Three spermatopositors and one ovipositor were dissected out and placed in lactic acid for 1–24 hours. Subsequently, the organs were mounted in glycerin on microscope slides. The specimens were imaged using the Zeiss Elyra microscope at the Harvard Center for Biological Imaging, set to the Plan Apochromat 20×/0.8 Ph2 objective. The images recorded the autofluorescence of the samples by laser excitation. A filter prevented laser light from reaching the detector but allowed fluorescence. A laser wavelength of 561 nm was used, and autofluorescence of all wavelengths above that were recorded. Gain was adjusted for maximum clarity. The spermatopositor was imaged using a gain of 565, and the ovipositor was imaged using a gain of 614.

Images were recorded as stacks in the *z*-axis. This was done by imaging the same sample 50–150 times at different focal planes. Carl Zeiss Zen software (Black Edition v. 2010) was then used to create a three-dimensional automontage of the images.

Molecular methods

To test the phylogenetic position of the new species, we conducted a standard

Table 1. Taxa and Markers Used in This Study with MCZ and GenBank Accession Numbers. GenBank Accession Numbers in Bold Indicate New Sequences for this Study.

	MCZ #	18S rRNA	28S rRNA	COI	16S rRNA
Metasiro americanus	IZ-133799	DQ825542	DQ825595	JF786394	DQ825616
Cyphophthalmus duricorius	IZ-135009	**KJ857509**	**KJ857512**	**KJ857527**	**KJ857515**
Cyphophthalmus ere	IZ-135018	AY639462	DQ825593	AY639557	AY639527
Cyphophthalmus gjorgjevici	IZ-135017	AY639464	DQ825587	AY639559	AY639529
Cyphophthalmus gordani	IZ-135014	AY639467	DQ825592	-	AY639532
Cyphophthalmus hlavaci	IZ-135052	-	-	-	**KJ857544**
Cyphophthalmus markoi	IZ-135016	AY639469	AY639504	AY639561	AY639534
Cyphophthalmus martensi	IZ-135013	AY639471	DQ825589	AY639563	AY639536
Cyphophthalmus minutus	IZ-135012	AY639473	DQ825591	AY639565	AY639537
Cyphophthalmus ognjenovici	IZ-135027	AY639475	DQ825594	AY639567	-
Cyphophthalmus rumijae	IZ-135011	AY639477	DQ825588	AY639569	AY639539
Cyphophthalmus teyrovskyi	IZ-135025	AY639482	DQ513118	AY639571	AY639544
Cyphophthalmus trebinjanum	IZ-135026	AY639483	DQ513119	AY639572	-
Cyphophthalmus zetae	IZ-135022	AY639485	AY639515	AY639574	AY639546
Cyphophthalmus solentiensis **sp. nov.**	IZ-129787	**KJ857518**	**KJ857522**	**KJ857528**	**KJ857532**
Cyphophthalmus solentiensis **sp. nov.**	IZ-135079	**KJ857519**	**KJ857523**	**KJ857529**	**KJ857533**
Iberosiro sp.	IZ-135072	**KJ857520**	**KJ857524**	**KJ857530**	**KJ857534**
Paramiopsalis eduardoi	IZ-135034	EU638284	EU638287	EU638288	EU638281
Paramiopsalis ramulosus	IZ-135006	AY639489	DQ513121	DQ825641	AY639550
Paramiopsalis sp.	IZ-135070	JF934957	JF934991	JF786390	JF935024
Parasiro coiffaiti	IZ-132372	AY918872	DQ513122	DQ825642	AY918877
Parasiro minor	IZ-132374	JF934958	JF934992	JF786391	JF935025
Siro acaroides	IZ-134454	AY639490	DQ513128	DQ825643	AY639551
Siro carpaticus	IZ-135071	**KJ857536**	**KJ857539**	**KJ857542**	**KJ857545**
Siro clousi	IZ-130003	**KJ857537**	**KJ857540**	**KJ857543**	-
Siro exilis	IZ-134551	AY639491	DQ825585	AY639579	-
Siro kamiakensis	IZ-132388	**KJ857538**	**KJ857541**	-	-
Siro rubens	IZ-132391	AY428818	DQ825584	DQ513111	-
Siro shasta	IZ-130004	**KJ857521**	**KJ857525-6**	**KJ857531**	**KJ857535**
Siro valleorum	IZ-135008	AY639492	DQ513123	AY639580	AY639552
Suzukielus sauteri	IZ-132256	DQ513138	DQ513116	DQ513108	DQ518086
Suzukielus sauteri	IZ-132263	DQ825541	DQ825583	DQ825640	DQ825615

phylogenetic analysis using four polymerase chain reaction (PCR)-amplified markers, the nuclear ribosomal genes 18S ribosomal RNA and 28S rRNA, and two mitochondrial genes, the ribosomal 16S rRNA and the protein-encoding cytochrome *c* oxidase subunit I. DNA extraction, PCR amplification, and sequencing follow previous work on Cyphophthalmi (e.g., Giribet and Shear, 2010; Giribet *et al.*, 2012). Single-step (i.e., direct optimization) and two-step (alignment + tree inference) phylogenetic analyses were conducted on a sironid data set (Table 1), rooted with the neogoveid *Metasiro americanus*, mostly following published recent analyses of centipede and arachnid data sets of similar characteristics (Giribet and Edgecombe, 2013; Giribet *et al.*, 2014). For the direct optimization analyses (Wheeler, 1996) we used POY v.5.1.1 (Wheeler *et al.*, 2014), exploring six parameter sets (Table 2). All input files were unaligned and sequences were treated as a single unpartitioned fragment. Tree searches were conducted using the timed search function in POY, i.e., multiple cycles of (a) building Wagner trees,

TABLE 2. WEIGHTED STEPS FOR THE ANALYSIS OF THE SIX PARAMETER SETS (FIRST COLUMN) FOR THE DIRECT OPTIMIZATION ANALYSES FOR THE FOUR MARKERS AND THE COMBINED ANALYSES (MOL), WITH $_{W}$ILD VALUES. ITALICIZED NUMBERS INDICATE VALUES FOR PARAMETER SET THAT MINIMIZE INCONGRUENCE AMONG DATA PARTITIONS.

	18S	28S	16S	COI	MOL	$_{W}$ILD
111	*85*	*748*	*1654*	*2641*	*5192*	*0.01233*
121	111	1133	2650	4020	8019	0.01309
211	87	893	1878	2696	5625	0.01262
221	115	1406	3055	4101	8807	0.01476
3211	113	1202	2761	4072	8253	0.01272
3221	172	1576	3464	5383	10728	0.01240

(b) subtree pruning and regrafting, (c) tree bisection and reconnection, (d) ratcheting (Nixon, 1999), and (e) tree-fusing (Goloboff, 1999, 2002) [command: search (max_time: 00:01:00, min_time:00:00:10, hits:20, memory:gb:2)]. For the individual partitions, timed searches of 1 hour were run on four processors under six parameter sets, as in Giribet *et al.* (2012) (see Table 2). For the combined analysis of the four markers we started with the same search strategy, giving a preliminary tree as input, and the resulting trees were given as input for a second round of analyses (sensitivity analysis tree fusing, SATF), as described by Giribet (2007), and continued until the tree lengths stabilized (Giribet *et al.*, 2012). The optimal parameter set was estimated using the modified $_{W}$ILD metrics (Wheeler, 1995; Sharma *et al.*, 2011) as a proxy for the parameter set that minimizes overall incongruence among data partitions (Table 2). Nodal support for the optimal parameter set was estimated via jackknifing (100 replicates), with a probability of deletion of e^{-1} (Farris *et al.*, 1996) using auto_sequence_partition, as discussed in earlier work (Giribet *et al.*, 2012).

Maximum likelihood (ML) analyses were conducted on static multiple sequence alignments inferred in MUSCLE v. 3.6 (Edgar, 2004) through the EMBL-EBI server (http://www.ebi.ac.uk/Tools/msa/muscle/). The MUSCLE alignments were conducted for each gene independently and hypervariable regions in the data set were subsequently trimmed with Gblocks v. 0.91b (Castresana, 2000; Talavera and Castresana, 2007) to cull positions of ambiguous homology. Data sets were concatenated with SequenceMatrix (Vaidya *et al.*, 2011).

Maximum likelihood analyses were conducted using RAxML ver. 7.2.7 (Stamatakis *et al.*, 2008b) in the CIPRES server (Miller *et al.*, 2010). For the searches, a unique general time reversible (GTR) model of sequence evolution with corrections for a discrete gamma distribution (GTR + Γ) was specified for each independent data partition, and 100 independent searches were conducted. Nodal support was estimated via the rapid bootstrap algorithm (1,000 replicates) using the GTR-CAT model (Stamatakis *et al.*, 2008a). Bootstrap resampling frequencies were thereafter mapped onto the optimal tree from the independent searches.

RESULTS

The SATF analyses with POY stabilized after two to five rounds, depending on the parameter set. The parameter set that minimized $_{W}$ILD was 111, where all nucleotide and indel transformations are equally weighted. The resulting tree was nearly identical to those found under the other explored parameter sets. This tree (Fig. 1) shows monophyly of *Cyphophthalmus* and a

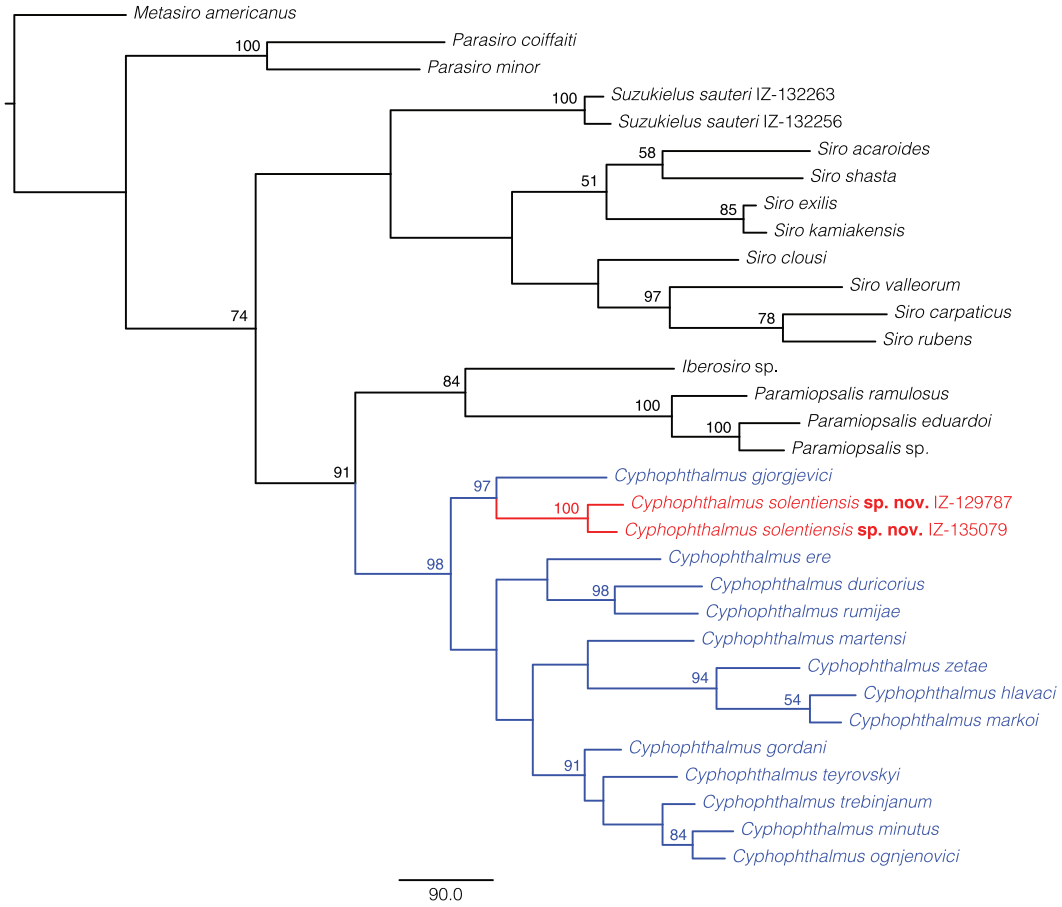

Figure 1. Direct optimization tree under parameter set 111 for the combined analysis of all four markers (5,192 steps; wILD = 0.01233). Numbers on branwches represent jackknife support values.

basal split between the clade containing *C. gjorgjevici* and *C. solentiensis* **sp. nov.** and the other *Cyphophthalmus* species. This result is also obtained with the static alignment analyzed under ML (Fig. 2). Both trees find *Cyphophthalmus* as the sister group to the clade including *Iberosiro* and *Paramiopsalis* (an Iberian clade), and mostly differ in some of the unsupported internal relationships of *Cyphophthalmus*. Interestingly, *C. solentiensis* **sp. nov.** does not group with *C. hlavaci*, the closest species geographically. Although sequence data for *C. hlavaci* are restricted to the 16S rRNA, individual analysis of this gene continues to place these two species in clearly separate clades.

TAXONOMY

Family Sironidae Simon, 1879
Genus *Cyphophthalmus* Joseph, 1868
Cyphophthalmus solentiensis Dreszer, Rađa & Giribet **sp. nov.**
Figures 3–7

Type specimens

Holotype. Male (Museum of Comparative Zoology [MCZ] IZ-135079; ex DNA107119) from cave Bratska jama (43.34475, 16.3410),

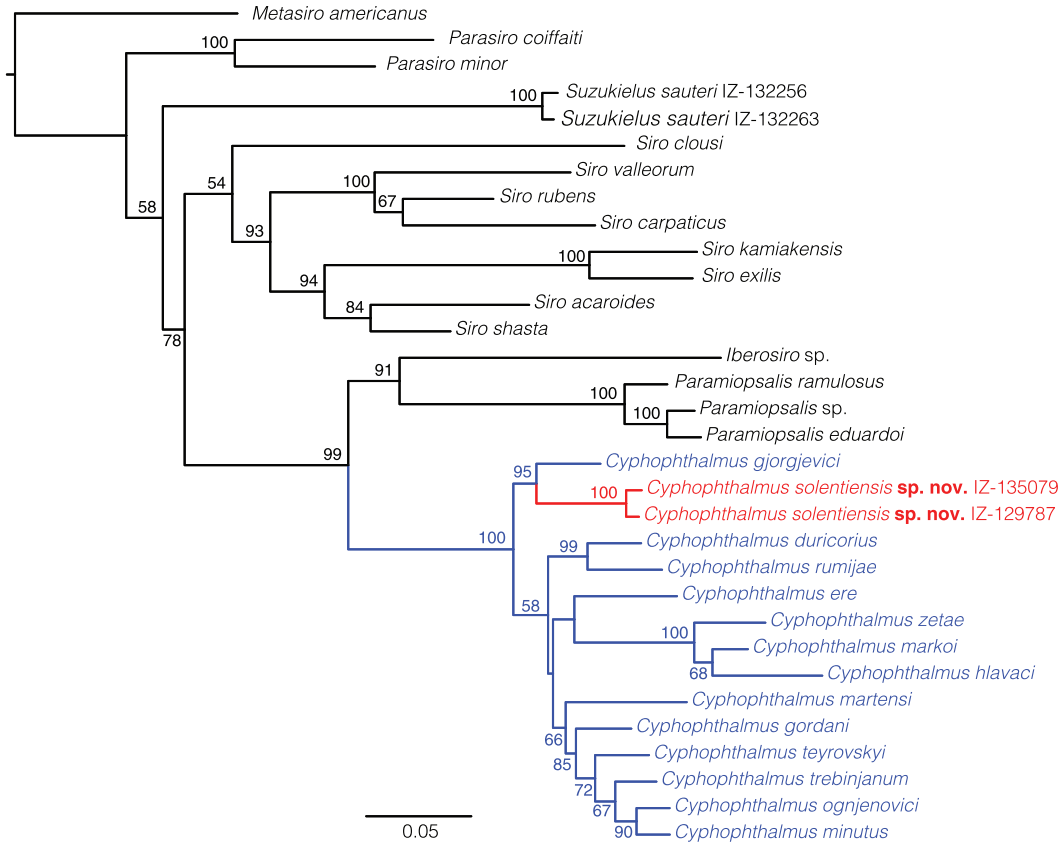

Figure 2. Maximum likelihood phylogenetic hypothesis of the concatenated trimmed aligned data (−ln L = 22,118.686904). Numbers on nodes indicate bootstrap support values.

Gornje Selo, Šolta Island, Middle Dalmatia, Croatia, Leg. Tonći Rađa, 20.iii.2012 (Figs. 3A–C).

Paratypes. Two males, one female mounted for SEM (MCZ IZ-135079); one female imaged (Figs. 3D–F), two males dissected for genitalia, eight males, five females in 96% EtOH (MCZ IZ-135079); same collecting data as holotype. Nineteen specimens from Podašpilje village, near Omiš, Middle Dalmatia, Croatia (MCZ IZ-129787), Leg. Tonći Rađa, 24.iv.2013. One male and one female mounted for SEM (MCZ IZ-129787); one male dissected for genitalia (MCZ IZ-129787).

Etymology. The species is named after the island of Šolta, its type locality, on the basis of its Latin name, Solent, Solentia, Solentium.

Diagnosis. Cyphophthalmus with a longitudinal carina of the male anal plate low and without ornamentation, and without the heavily granulated "rostrum" of *C. gjorgjevici*, its closest species phylogenetically. Spermatopositor with three microtrichiae ventrales positioned in the edges of a V, the central one being more basal, a character not yet described in any other *Cyphophthalmus*.

Description of Male. Total length of male holotype (in mm): 2.09; largest width at

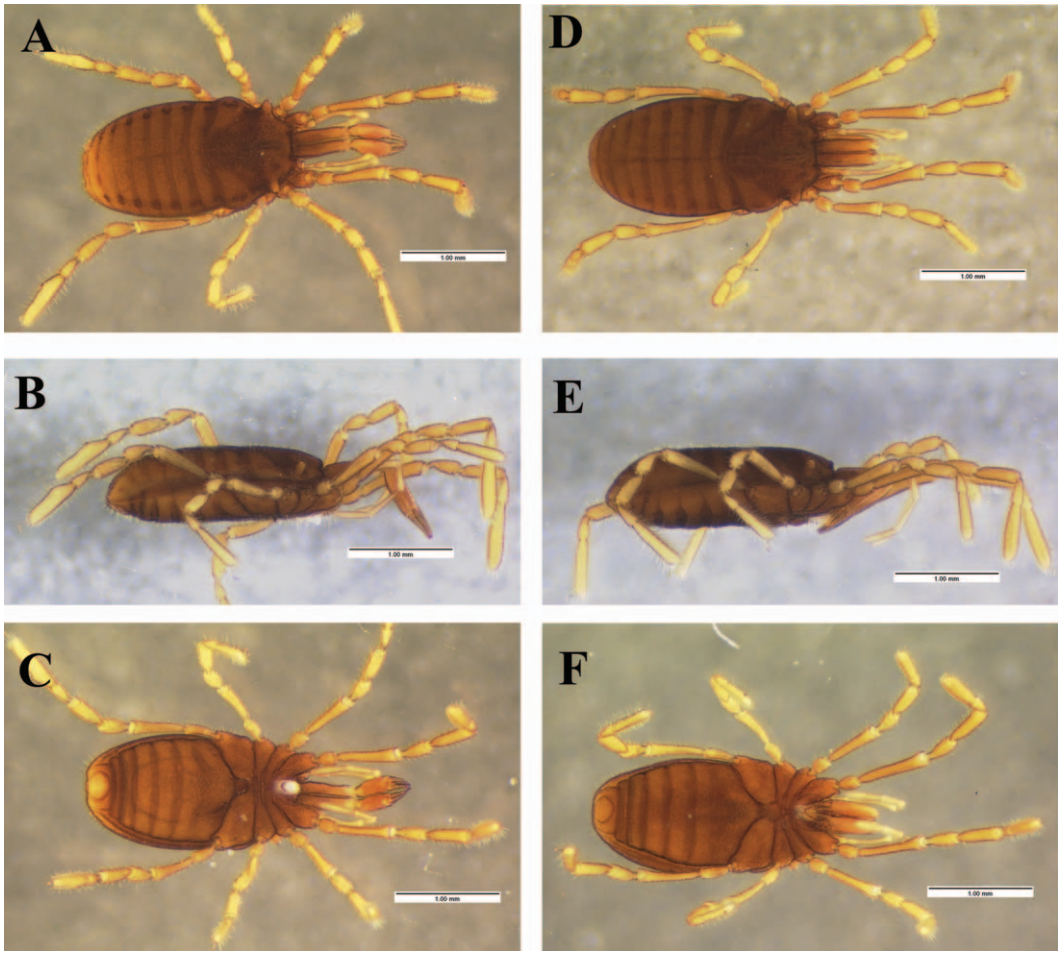

Figure 3. *Cyphophthalmus solentiensis* **sp. nov.** IZ-135079. A–C, male holotype in A, dorsal, B, lateral, and C, ventral views. D–F, female paratype in D, dorsal, E, lateral, and F, ventral views. Scale bars = 1 mm.

second opisthosomal segment: 1.13; length/width ratio 1.85; width across tip of ozophores: 0.91; prosomal width: 1.11. Body brown-orange and legs slightly lighter (in ethanol). Cuticle with light tuberculate–microgranulate surface (Figs. 4–6) (*sensu* Murphree, 1988).

Ozophore conical of type 2 (*sensu* Juberthie, 1970), completely ornamented (Fig. 5F); with a subterminal ozopore. Eyes absent. Ventral prosomal complex (Figs. 4A, B, 5A, B) with coxae I and II free, coxae III and IV fused; gonostome semicircular (130 μm wide × 90 μm long), with two triangular projections on its posterior angles (Fig. 5A); sternum absent. Proximal end of coxae I to IV all meeting along the midline. Endites of coxae of legs II and III and of legs III and IV running along their suture; coxal pores present in endites between coxae III and IV, with two projections of the coxae IV endite near the coxal pore (Fig. 5A); endites of coxae IV running adjacent to midline suture for a length approximate to that of

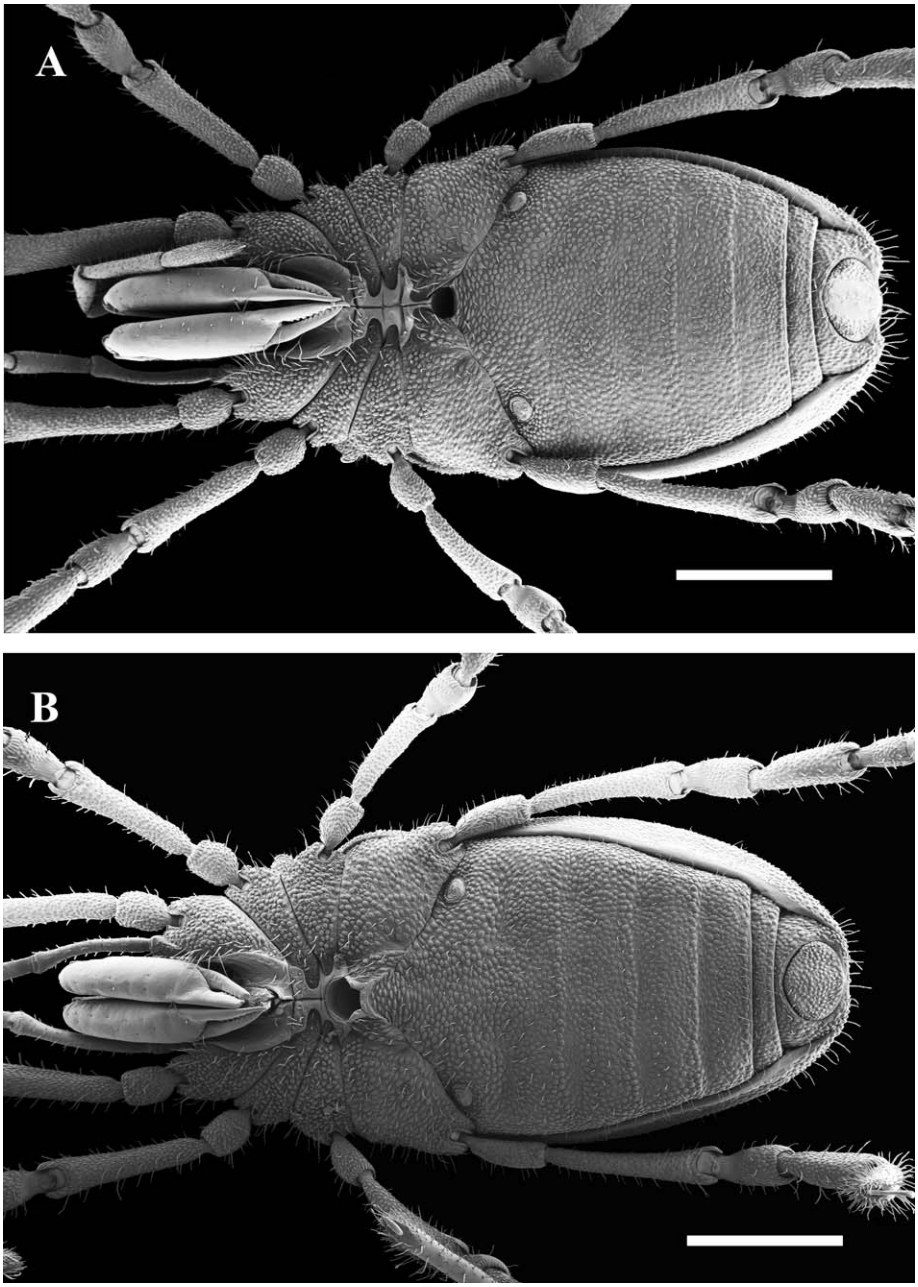

Figure 4. *Cyphophthalmus solentiensis* **sp. nov.** IZ-135079. A, paratype male in ventral position; B, paratype female in ventral position. Scale bars = 0.5 mm.

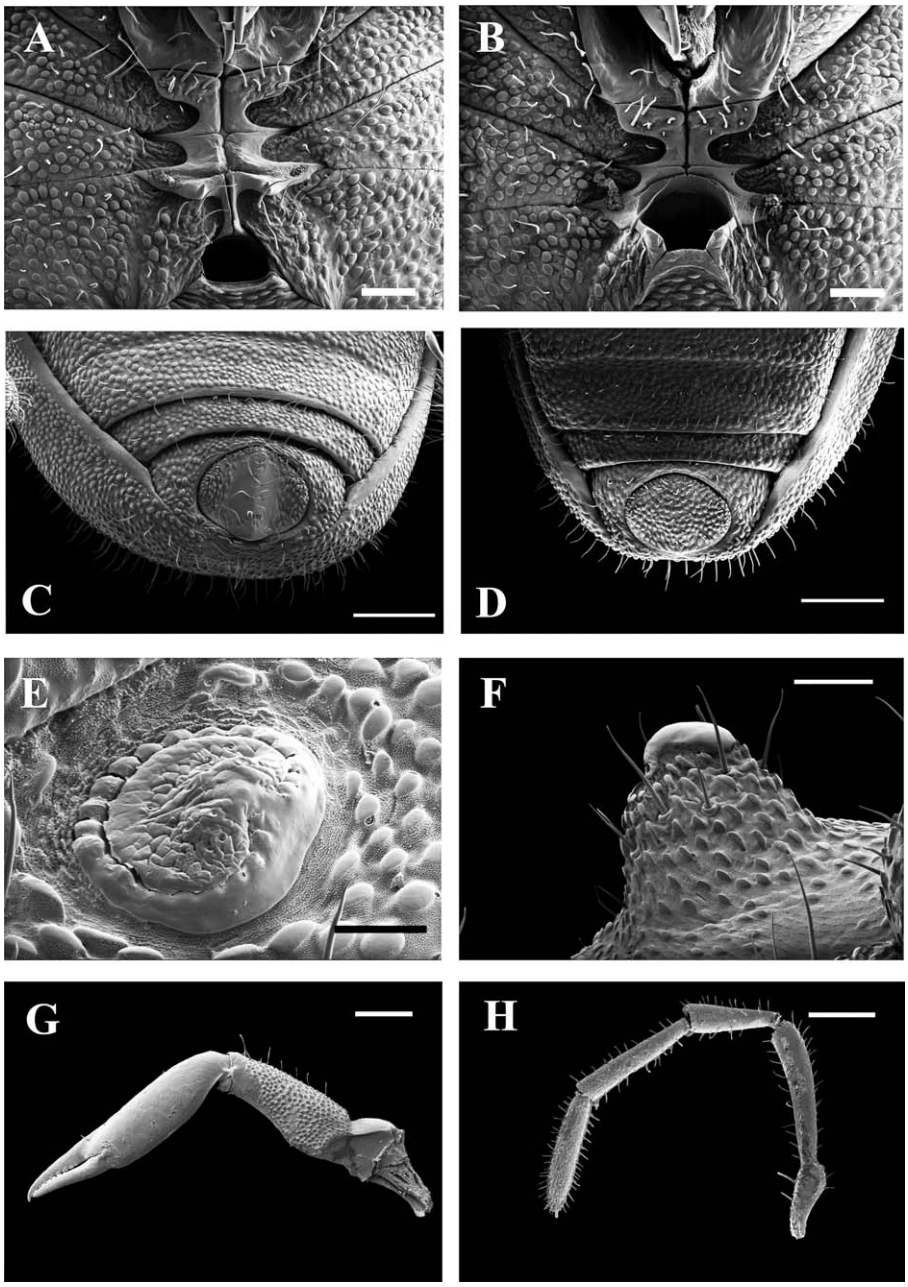

Figure 5. *Cyphophthalmus solentiensis* **sp. nov.** paratypes IZ-135079. A, male and B, female thoracic complex, scale bars = 80 μm. C, male and D, female anal region, scale bars = 200 μm. E, male spiracle, scale bar = 30 μm. F, male ozophore, scale bar = 50 μm. G, male chelicer and H, male palp, scale bars = 200 μm.

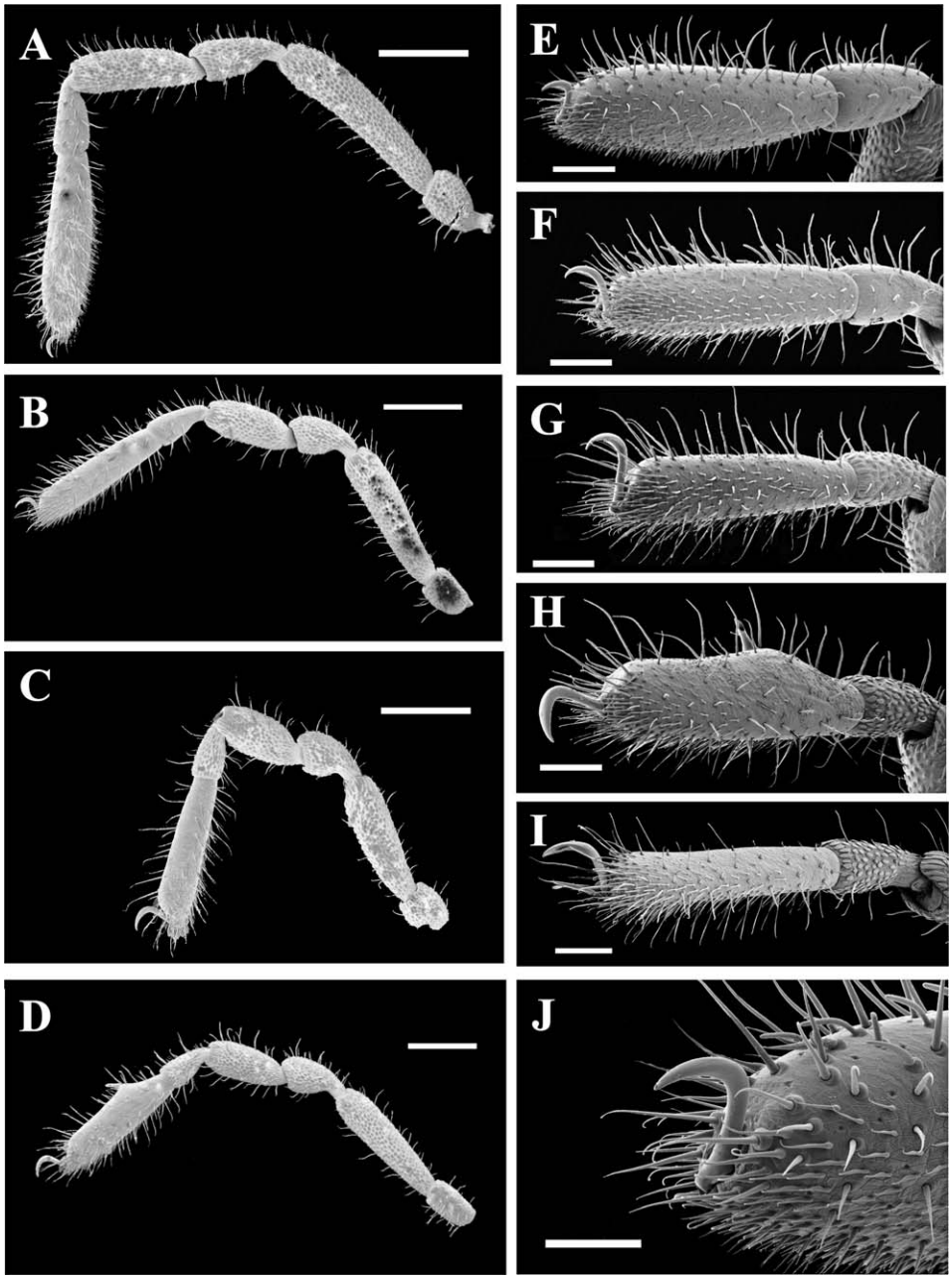

Figure 6. A–H, J, *Cyphophthalmus solentiensis* **sp. nov.** paratype male IZ-135079 and I, female IZ-135079. A, leg I of male; B, leg II of male; C, leg III of male; D, leg IV of male; E, metatarsus and tarsus I; F, metatarsus and tarsus II; G, metatarsus and tarsus III; H, metatarsus and tarsus IV of male; I, metatarsus and tarsus I of female; J, detail of claw I. (A–D, scale bars = 300 μm. E–I, scale bars = 100 μm. J, scale bar = 50 μm.)

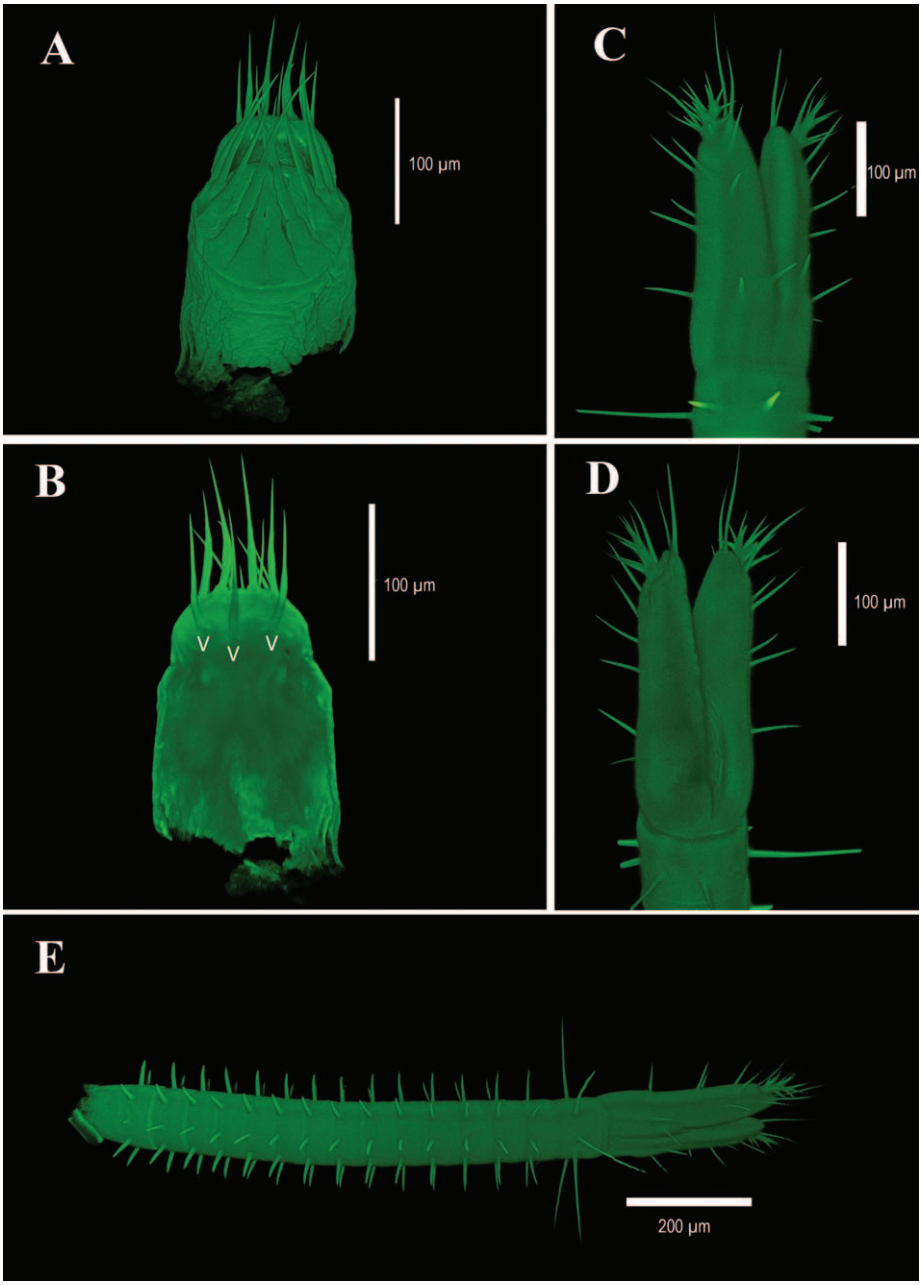

Figure 7. *Cyphophthalmus solentiensis* **sp. nov.** A, B, spermatopositor of paratype male IZ-135079. A, dorsal view; B, ventral view (v indicates microtrichiae ventrales). C–E, ovipositor of paratype female IZ-135079. C, detail of ovipositor tip, dorsal view; D, detail of ovipositor tip, ventral view; E, whole ovipositor. (A–D, scale bars = 100 µm. E, scale bar = 200 µm).

Table 3. Leg Measurements (Length/Width, mm) of Male Paratype Mounted for SEM.

Leg	Trochanter	Femur	Patella	Tibia	Metatarsus	Tarsus	Total Length
I	0.27/0.15	0.67/0.15	0.33/0.14	0.46/0.14	0.27/0.12	0.61/0.15	2.61
II	0.20/0.13	0.58/0.15	0.28/0.15	0.37/0.14	0.23/0.11	0.54/0.13	2.20
III	0.16/0.14	0.45/0.13	0.25/0.15	0.33/0.14	0.21/0.10	0.49/0.12	1.89
IV	0.23/0.13	0.60/0.15	0.31/0.16	0.38/0.16	0.23/0.11	0.56/0.18	2.31

gonostome. Opisthosomal mid-dorsal longitudinal sulcus conspicuous (Figs. 3A, B). Spiracles circular (Fig. 5E). Ventral opisthosomal area without exocrine glands; opisthosomal exocrine glands with a pair of openings on tergite VIII (Fig. 5C). Opisthosomal tergite IX and sternites 8 and 9 fused into a corona analis (Fig. 5C). Anal plate oval, with a smooth mid-longitudinal ridge with setae (Fig. 5C).

Proximal cheliceral segment 505 μm long, ornamented, with a tuberculate surface for most of its length, but smooth near the distal tip, without a dorsal crest or a ventral process (Fig. 5G). Second cheliceral segment 893 μm long; mobile digit 301 μm. Widest part of the second cheliceral segment near articulation with mobile digit; cheliceral distal segments with uniform dentition (Fig. 5G). Pedipalp (Fig. 5H) 2.709 mm long; trochanter without ventral apophysis. Pedipalp measurements of male paratype (in μm): trochanter 229; femur 448; patella 296; tibia 389; tarsus 374.

Legs slender (Figs. 6A–D; measurements in Table 3); leg formula I, II, IV, III. Except for tarsi I–IV and metatarsi I–II, all articles ornamented (Figs. 6A–D). All legs with setae, the highest concentration occurring along the ventral side of the tarsus of all four walking legs (Figs. 6E–I). Tarsus I without a distinct solea (Figs. 6A, E). Tarsus of leg IV entire (Figs. 6D, H), with a lamelliform adenostyle positioned toward the first third of the dorsal side on tarsus IV. All claws smooth, without dentition or lateral pegs.

Spermatopositor ($n = 3$; Fig. 7A; see suppl. videos in http://mczbase.mcz.harvard.edu/guid/MCZ:IZ:135079 and http://mczbase.mcz.harvard.edu/guid/MCZ:IZ:129787). Distal margin of terminal lobe semicircular, ca. 80 μm in diameter, terminally with small denticles and two pairs of microtrichiae[3] terminales, evenly spaced, not touching at the base, the two median ones longer than the lateral ones. Lateral movable fingers very broad at the base (ca. 20 μm) and clearly hooked, not surpassing the terminal lobe. Dorsal microtrichiae in two symmetrical groups, a lateral one with three short microtrichiae (the longest 106 μm) coming from a common lateral lobe, and a dorsal one with two long microtrichiae (ca. 130 μm), with enlarged bases, touching at the base, up to ca. 20 μm wide × 50 μm high. With three short microtrichiae ventrales, ca. 50 μm long, the central one in a more basal position than the lateral ones (Fig. 7B).

Description of Female. Total length 2.22 mm long, 1.14 mm maximum wide, at second opisthosomal segment. Ventral prosomal complex (Fig. 5B) with coxal lobes II narrower (at their narrowest part) than long (*sensu* Karaman, 2009). Anal plate without conspicuous modifications (Fig. 5D). Tarsus IV narrow and elongate (Fig. 6I), without glandular pores or other modifications.

[3] We follow here Schwendinger & Giribet (2005) in using microtrichiae instead of setae for the spermatopositor organ. Thus, instead of setae terminales we use microtrichiae terminales, and so on.

Ovipositor (Figs. 7C–E; see suppl. videos in http://mczbase.mcz.harvard.edu/guid/MCZ:IZ:135079) composed of 15 annular segments plus apical lobes, measured at 1.1 mm long extended. Each annulus with eight simple setae, equidistant, the three lateral ones longer than the ventral ones, and with the ventral setae being shorter than the dorsal ones. Setae of annulus 15 much longer than the others (150 vs. 55 μm long between the lateral setae of annuli 15 and 14, respectively). Apical lobes 270 μm long, with saccate receptacles occupying ca. 100 μm. Each apical lobe bears one multibranched sensorial process with a very wide base, opening from a lateral depression, and two long terminal simple setae, ca. 100 μm long, and eight to nine shorter simple setae.

Distribution. Known only from two localities in Croatia.

Notes. Cyphophthalmus solentiensis **sp. nov.** is phylogenetically closely related to *C. gjorgjevici* (Hadži, 1933), although they differ in the dorsal side of the prosoma, and overlaps geographically with *C. hlavaci* Karaman, 2009. Unfortunately the latter species is poorly illustrated. However, there seems to be major differences in several characters, including the longitudinal carina of the male anal plate, which is narrow and pronounced in *C. hlavaci* (although it is not illustrated in the original description), but low and without ornamentation in *C. solentiensis* **sp. nov.** The new species seems to be larger and has much more slender appendages than *C. hlavaci* (Karaman, 2009: figure 33).

DISCUSSION

Molecular phylogenetic analysis of a sironid data set clearly places *C. solentiensis* **sp. nov.** with *C. gjorgjevici*, a species described from Skopje, more than 500 km away, and not with its geographically close species *C. hlavaci*. The phylogenetic results place these two species in separate clades with high support. Although we think that a diagnostic character of our species is the V-shaped disposition of the three microtrichiae ventrales from the spermatopositor organ, most authors did not illustrate the spermatopositor in *Cyphophthalmus*, and some have only illustrated spermatopositors in dorsal view (Juberthie, 1970; Karaman, 2008, 2009). Gruber (1969) illustrated the ventral side of the spermatopositor of several species he described as subspecies of *C. duricorius,* and in these species the microtrichiae ventrales are lined up in a row, or the central one is more distal than the lateral ones, contrary to *C. solentiensis* **sp. nov.**, but no other proper illustrations are available for the ventral side of any other *Cyphophthalmus* species. Unfortunately, no information on the genitalia of *C. gjorgjevici* is available.

Here we provided state-of-the-art images and accessory videos for a new species of *Cyphophthalmus* with the aim to improve the deficient taxonomy of this group of biogeographical importance. We hope that the new imaging techniques for Opilones genitalia can be rapidly applied to many other species to generate data sets on par with the technologies available to many researchers.

ACKNOWLEDGMENTS

Erin McIntyre assisted with the molecular work, which was funded by the MCZ. The Harvard Center for Biological Imaging provided access to the Zeiss Elyra laser confocal microscope. The Division of Science funded the usage fees through its undergraduate imaging account. Douglas Richardson provided training and subsequent assistance with the Elyra microscope. The Harvard Center for Nanoscale Systems provided access to the Zeiss FESEM Ultra

Plus scanning electron microscope, and the usage fees were funded by a Grant for Undergraduate Research through the MCZ. David Lange provided training on this microscope. A Harvard College Research Program grant provided a stipend for the imaging work done for this paper. Three anonymous reviewers provided comments that helped to improve this paper.

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