European Journal of Taxonomy 733: 1–18
https://doi.org/10.5852/ejt.2021.733.1215
ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2021 · Ballarin F. & Yamasaki T.

This work is licensed under a Creative Commons Attribution License (CC BY 4.0).

Research article

urn:lsid:zoobank.org:pub:3675D40A-5DE0-49CE-9795-F4FCF13B17CB

Nihonella gen. nov., a new troglophilic genus of dwarf spiders from Japan with a discussion on its phylogenetic position within the subfamily Erigoninae (Araneae, Linyphiidae)

Francesco BALLARIN [1,*] & Takeshi YAMASAKI [2]

[1] Systematic Zoology Laboratory, Department of Biological Sciences, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji-shi, 192-0397, Tokyo, Japan.
[1] Department of Zoology, Museo Civico di Storia Naturale di Verona, Lungadige Porta Vittoria 9, I-37129, Verona, Italy.
[2] Institute of Nature and Environmental Sciences, University of Hyogo/Museum of Nature and Human Activities, Hyogo, Yayoigaoka 6, Sanda-shi, 669-1546, Hyogo, Japan.

[*] Corresponding author: ballarin.francesco@gmail.com
[2] Email: yamasaki@hitohaku.jp

[1] https://orcid.org/0000-0003-1417-2519
[2] https://orcid.org/0000-0002-2419-188X

[1] urn:lsid:zoobank.org:author:54F6F9C7-0385-48D4-AB09-52692BD05B53
[2] urn:lsid:zoobank.org:author:804886B5-C951-490C-95C4-D2CA7F4F94D7

Abstract. A new monospecific genus belonging to the family Linyphiidae Blackwell, 1859, *Nihonella* gen. nov., is described using an integrative taxonomic approach based on the species *N. chika* gen. et sp. nov. The new genus is endemic to Western Honshu, Japan, and it shows distinctive genitalic and somatic characters of other genera of the subfamily Erigoninae Emerton, 1882. *Nihonella* gen. nov. is found only in the twilight and transition zones of caves in Okayama and Nara Prefectures. The phylogenetic position of *Nihonella* gen. nov. within the subfamily Erigoninae, and its relationship as a sister clade of the species of the group of *Savignia* Blackwell, 1833 (sensu Millidge 1977), is discussed on the basis of both, morphological and molecular evidence.

Keywords. Caves, endemism, new species, phylogeny, subterranean environment.

Ballarin F. & Yamasaki T. 2021. *Nihonella* gen. nov., a new troglophilic genus of dwarf spiders from Japan with a discussion on its phylogenetic position within the subfamily Erigoninae (Araneae, Linyphiidae). *European Journal of Taxonomy* 733: 1–18. https://doi.org/10.5852/ejt.2021.733.1215

Introduction

Within the order Araneae, the spider family Linyphiidae Blackwall, 1859 is the second largest by number of species: currently 4638 species are known in 617 genera, with a worldwide distribution (World Spider Catalog 2020). Among them, the subfamily Erigoninae Emerton, 1882 is undoubtedly the most diverse

and species-rich (Tanasevitch 2020). At middle latitudes, these tiny spiders live in a wide range of habitats including forest leaf litter, empty spaces between rocks, and caves, where they build small sheet-webs. In Japan, 293 linyphiid species belonging to 109 genera are currently recorded (Tanikawa 2020). Although some Japanese species can colonize the entrances of caves or other semi-subterranean environments, the hypogean environment supports only a few species (Ono *et al.* 2009).

While collecting in caves in Central and Western Honshu, Japan, we found several specimens of spiders belonging to different families, including some Linyphiidae. A detailed morphological analysis of the collected material led to the discovery of an unknown linyphiid species belonging to the subfamily Erigoninae. The peculiar somatic characters, as well as the shape of the epigyne and male palp, excluded this species from the currently known genera. Thus, herein we describe this spider as a new species, and we establish a new genus on the basis of morphological and molecular evidence. To support this claim and to clarify the phylogenetic position of the new genus within the subfamily Erigoninae, we conducted a multi-locus phylogenetic analysis, the results of which are herein discussed.

Material and methods

Taxonomy

Specimens were collected inside caves in Okayama and Nara Prefectures (Western Honshu Island, Japan) and immediately preserved in 99% ethanol for morphological and molecular studies. Samples were studied at the Systematic Zoology Laboratory, Department of Biological Sciences, Tokyo Metropolitan University, Japan (TMU). A Nikon SMZ1270 stereo microscope and a Nikon Optiphot 2 microscope were used to observe habitus and genitalia of the specimens, and to take measurements. Photographs were taken using a Canon EOS60D digital camera mounted on the microscopes. Final images were merged with Helicon Focus 7 image stacking software. Additional scanning electron micrographs were taken using a Jeol JSM-6510LV scanning electron microscope at TMU. The male left palp was drawn and photographed. Epigyne was dissected using a sharp scalpel and cleared with lactic acid to show the inner structures. All measurements are reported in millimeters, leg measurements are given as total length (femur, patella, tibia, metatarsus, tarsus). The specified chaetotaxy (= tibial spine formula) refers to the number of dorsal spines on tibiae I–IV. All vouchers used in this study are preserved in the National Museum of Nature and Science, Tokyo, Japan (NMST).

Institutional abbreviations

NSMT = National Museum of Nature and Science, Tokyo, Japan
TMU = Tokyo Metropolitan University, Japan

Abbreviations used in the text and figures

AW = anterior wall of epigyne
C = cymbium
CD = copulatory duct
CO = copulatory opening
D = duct
DSA = distal suprategular apophysis
E = embolus
LW = lateral walls of epigyne
MM = median membrane
PC = paracymbium
PP = posterior median plate
PT = protegulum
PTA = prolateral tibial apophysis

R = radix
RA = radical apophysis
RE = receptacle
SDSA = secondary branch of the distal suprategular apophysis
ST = subtegulum
T = tegulum
TP = tailpiece of radix

Molecular analysis

The complete genomic DNA was extracted from four legs of each sample using a Qiagen DNeasy Blood & Tissue Kit, following the standard protocol suggested by the manufacturer. Extraction and Polymerase Chain Reaction amplification (PCR) of the samples were performed at TMU. Genomic DNA from voucher specimens was stored in a freezer at -20°C at the same institute. Five gene fragments were amplified using standard primers: cytochrome c oxidase subunit I (COI), 16SrRNA (16S), 18SrRNA (18S), 28SrRNA (28S), and histone 3 (H3). PCR amplifications were performed using a SimpliAmp Thermal Cycler (Thermo Fisher Scientific, USA) with a final volume of 11µl. All primers and protocols used in this study follow Arnedo *et al.* (2009). In addition, the following primers were also used to amplify the first segment of 18S: 18S-1F (forward) TACCTGGTTGATCCTGCCAGTAG (Giribet *et al.* 1996) and 18S-SSU (reverse) GTGGTGCCCTTCCGTCAATT (Balczun *et al.* 2005). Purified PCR products were submitted and sequenced by Eurofins Genomics Company, Tokyo branch. Sequences of other linyphiid spiders used in the analysis were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). In order to clarify the phylogenetic position of the new genus herein described, we reconstructed a simplified phylogenetic tree of Linyphiidae, including representative species of the five main subfamilies distributed in the Palearctic region: Stemonyphantinae (Wunderlich, 1986), Linyphiinae (Blackwell, 1859), Micronetinae (Hull, 1920), Ipainae Saaristo, 2007, and Erigoninae. A preliminary morphological study of our samples suggested that the new genus was closely related to the species of the *Savignia* group sensu Millidge, 1977. Thus, we focused the sampling especially on the Erigoninae subfamily, including distal Erigoninae, genera located in early branches, and species belonging to the *Savignia* group. *Pimoa rupicola* (Simon, 1884) from the family Pimoidae Simon, 1884 was preferentially selected as an outgroup to root the tree, due to the close relationship between Pimoidae and Linyphiidae (Wheeler *et al.* 2017; Fernandez *et al.* 2018). Fifty-eight taxa composed the final dataset. The complete list of sequences and related GenBank identification codes are reported in Table 1.

Final sequences were edited using Bioedit ver. 7.0.5 (Hall 1999) and aligned using the online version of MAFFT ver. 7.450 (Katoh *et al.* 2019) under the G-INS-I (COI, 18S, H3) and Q-INS-i (16S, 28S) algorithms. Protein coding genes (COI, H3) were translated to proteins using MEGA X ver. 10.0.5 (Kumar *et al.* 2018) to check for potential errors. A Bayesian Inference analysis was performed using the online version of MrBayes ver. 3.2.7 (Ronquist *et al.* 2012) available on the CIPRES Science Gateway ver. 3.3 (Miller *et al.* 2010). Two independent runs of four Markov Chain Monte Carlo algorithms were run for 20 million generations, sampled every 2000 generations with a burn-in of 25%. We used the partition scheme and substitution model suggested by PartitionFinder 2 ver. 2.1.1 (Lanfear *et al.* 2016) under a corrected Akaike information criterion (AICc). Tracer ver. 1.7.1 (Rambaut *et al.* 2018) was used to establish that the effective sample size was > 200 for all the parameters. The concatenated sequences in the final dataset were formed by a total of 3983 nucleotides divided as follows: COI = 672, 16S = 432, 18S = 1704, 28S = 848, H3 = 327.

In order to compare the genetic distance between the new genus and the main components of the *Savignia* group, partial fragments of the COI barcodes of nine species within the group were found on GenBank (see Table 1). The GenBank sequences were compared with the new genus sequences using an

Table 1. List of the species, gene fragments and related GenBank accession codes used to reconstruct the phylogenetic tree and in P-distance analyses. The new genus is highlighted in red color, asterisks refer to new sequences.

Species	COI	16S	18S	28S	H3	Notes
Agyneta ramosa	FJ838648	FJ838670	FJ838694	FJ838717	FJ838740	
Araeoncus crassipalpis	KY270223					only for P-distance
Asthenargus sp.	missing	missing	GU338493	GU338561	missing	
Bathyphantes gracilis	KM836935	KT003103	GU338464	FJ838719	FJ838742	
Bolyphantes alticeps	KY268546	AY078660	AY078667	AY078678	AY078700	
Centromerus trilobus	GU338656	GU338599	GU338468	GU338571	KT002817	
Ceratinopsis setoensis	JN817121	JN816488	JN816709	JN816919	missing	
Dicymbium sinofacetum	EF128167	GU338614	GU338487	GU338546	missing	
Dicymbium tibiale	KY268930	KT003114	KT002923	KT003021	KT002824	also for P-distance
Diplocentria bidentata	KM840840	GU338629	GU338494	GU338542	missing	
Diplocephalus cristatus	GU338696	GU338637	GU338490	missing	missing	also for P-distance
Diplostyla concolor	GU682473	GU338639	GU338467	GU338585	FJ838743	
Doenitzius pruvus	JN817116	GU338632	GU338474	KT003023	KT002826	
Drapetisca socialis	KY268428	FJ838674	FJ838698	FJ838721	FJ838744	
Erigone edentata	GU338686	missing	GU338486	GU338540	missing	
Erigone prominens	EF128171	missing	GU338498	GU338539	KT002828	
Erigonella ignobilis	KX039173					only for P-distance
Eskovina clava	JN817122	JN816489	JN816710	JN816920	missing	
Floronia bucculenta	KY270282	FJ838676	KT002928	FJ838723 +KT003026	FJ838746	
Frontinella communis	KY017766	KY015924	KY016500	KY017142	KY018271	
Glyphesis servulus	KY269551					only for P-distance
Gnathonarium dentatum	JN306340	GU338593	GU338477	GU338548	missing	
Gonatium rubellum	FJ838656	FJ838679	FJ838703	FJ838726	FJ838749	
Gonatium rubens	KY269351	KT003120	KT002930	KT003028	KT002831	
Grammonota sp.	HQ924393	missing	GU338491	missing	missing	
Helophora insignis	FJ838658	FJ838681	FJ838705	FJ838728	FJ838751	
Hylyphantes graminicola	KY270332	GU338595	GU338478	JN816917	KT002835	
Hylyphantes sp. ***irellus***	GU338668	GU338618	GU338481	GU338549	missing	
Janetschekia monodon	KJ363172					only for P-distance
Lepthyphantes sp.	GU338664	GU338610	GU338509	GU338562	missing	
Lepthyphantes minutus	KY270131	AY078663	AY078673	AY078681	AY078705	
Lin02 ***Nihonella chika***	MW177572*	MW192653*	MW192647*	MW192650*	MW177569*	♂ from Anatoyama cave
Lin04 ***Nihonella chika***	MW177573*	MW192654*	MW192648*	MW192651*	MW177570*	♀ from Uyamado cave, also for P-distance
Lin05 ***Nihonella chika***	MW177574*	MW192655*	MW192649*	MW192652*	MW177571*	♀ from Komorinoiwaya cave

Table 1. Continued.

Species	COI	16S	18S	28S	H3	Notes
Linyphia triangularis	AY078693	AY078664	EU003390 +AY078668	EU003410 +EU153170	AY078702	
Meioneta nigra	GU338662	GU338608	GU338504	GU338577	missing	
Micrargus herbigradus	KY270158	KT003135	KT002947	KT003042	KT002848	
Microctenonyx subitaneus	KX039262					only for P-distance
Microneta viaria	FJ838661	FJ838684	GU338502	GU338537	FJ838754	
Moebelia rectangula	missing	GU338591	GU338485	GU338557	missing	
Nematogmus sanguinolentus	KX039278	GU338635	GU338489	GU338544	missing	
Neriene macella	MG201053	MG200522	MG200699	MG200873	MG201230	
Neriene radiata	KM839120	KY467286	GU338463	JN816906	AY078709	
Nippononeta kantonis	GU338693	GU338634	GU338471	GU338530	missing	
Nippononeta sp.	GU338657	GU338602	GU338520	GU338531	missing	
Oedothorax apicatus	FJ838664	FJ838687	FJ838711	FJ838734 +KT003057	FJ838757	
Ostearius melanopygius	KX537231	FJ838688	FJ838712	FJ838735	FJ838758	
Paikiniana sp.	GU338647	GU338617	GU338495	GU338555	missing	
Parameioneta bilobata	GU338660	GU338605	GU338503	GU338533	missing	
Parasisis sp.	GU338650	GU338592	GU338500	GU338534	missing	
Pimoa rupicola	MG201051	MG200518	MG200697	MG200876	MG201228	
Porrhomma sp.	GU338661	GU338607	GU338466	GU338584	missing	
Saloca diceros	KY270378					only for P-distance
Savignia sp. 1	KT002778	KT003165	KT002977	KT003071	KT002879	
Savignia sp. 2	KT002779	KT003166	KT002978	KT003072	KT002880	also for P-distance
Sisicottus montanus	GU338673	GU338625	GU338479	GU338541	missing	
Solenysa mellotteei	KT002781	KT003168	KT002980	KT003076	KT002884	
Solenysa sp. 14	GU338667	GU338603	GU338507	GU338528	missing	
Sphecozone bicolor	GU338671	GU338622	GU338496	GU338553	missing	
Stemonyphantes sp.	KY017774	KY015933	KY016511	KY017153	KY018278	
Tenuiphantes sp.	GU338646	GU338612	GU338514	GU338568	missing	
Tenuiphantes tenuis	KC244266	FJ838693	FJ838716	FJ838739	FJ838763	
Walckenaeria clavicornis	MN680355	GU338596	GU338483	GU338554	missing	
Walckenaeria keikoae	GU338695	GU338636	GU338484	GU338556	missing	

uncorrected pairwise-distance genetic sequence divergence, run in MEGA X using a bootstrap method with 1000 replications.

Results

Taxonomy

Class Arachnida Cuvier, 1812
Order Araneae Clerck, 1757
Family Linyphiidae Blackwall,1859
Subfamily Erigoninae Emerton, 1882

Nihonella gen. nov.
urn:lsid:zoobank.org:act:05F43F8A-6FBC-4A95-98FF-56CEFACF73DB
Figs 1A–G, 2–4; Table 2

Type species

Nihonella chika gen. et. sp. nov.

Diagnosis

The new genus is distinguished from any other genera belonging to the distal Erigoninae clade by the following unique combination of somatic and genitalic characters: Femur I with 1 prolateral spine; Tibia I with 1 dorsal spine; tibial spine formula: 1.1.1.1; male palp with a well-developed distal suprategular apophysis and a hypertrophic 'secondary' DSA (Figs 2A–B, 3A–C, 4A–E) (usually presented in Erigoninae as a simple tooth and protruding form a different side of the DSA); a well-developed and uniquely-shaped prolateral tibial apophysis, the same length as the cymbium and partially covering it (Figs 2A–C, 3A–B, D, 4E–F). The unusual chaetotaxy and unique shape of the epigyne, with anteriorly converging lateral walls and two distinct, flat, ovoid inflations of the copulatory ducts, also distinguishes the female of this genus from females of any other genera in distal Erigoninae.

Etymology

The generic name is a combination of the word '*Nihon*' and the Latin suffix '*-ella*'. The former refers to the country of Japan where the genus is endemic; the latter is the feminine suffix of '*-ellus*' commonly used in Latin to form the feminine diminutive of a noun. Name in apposition, feminine in gender.

Species included

Only the type species *Nihonella chika* gen. et. sp. nov.

Taxonomic remarks

The morphology of *Nihonella* gen. nov. suggests it may be closely related to the species of the *Savignia* group. However, the presence of numerous differences in genitalia shape and somatic characters does not allow us to include the new genus within this group of species. A close but distinct relationship with the *Savignia* group is also supported by the molecular analysis (see Fig. 5).

Nihonella gen. nov. male palps have a general morphology similar to those found in some genera of the *Savignia* group (e.g., *Araeoncus* Simon, 1884 or *Diplocephalus* Bertkau, 1883). They share a similar shape in the embolic division: a long, modified palpal tibia, and a well-developed DSA. However, the new genus shows a distinct hypertrophy of the SDSA, which is extremely long and clearly protruding outside the frontal part of the palpal bulb (Figs 2A–B, D, 3A–C, 4A–E). Within the subfamily Erigoninae, some genera belonging to the *Savignia* group (sensu Millidge 1977) have a large DSA and a tooth-like SDSA (e.g., *Alioranus* Simon, 1926, *Dactylopisthes* Simon, 1884*, Delorrhipis* Simon 1885*, Savignia* Blackwall, 1833, etc., see Millidge 1977: figs 128, 135–136, 139). However, none of the *Savignia* group display a SDSA as strongly developed as in *Nihonella* gen. nov. Females of *Nihonella* gen. nov.

have an epigyne with two anteriorly converging lateral walls, which resembles the female genitalia, of most of genera within the *Savignia* group (e.g., *Araeoncus* Simon, 1884, *Diplocephalus* Bertkau, 1883, *Erigonella* Dahl, 1901, *Savignia* Blackwall, 1833, etc.). Nevertheless, both males and females

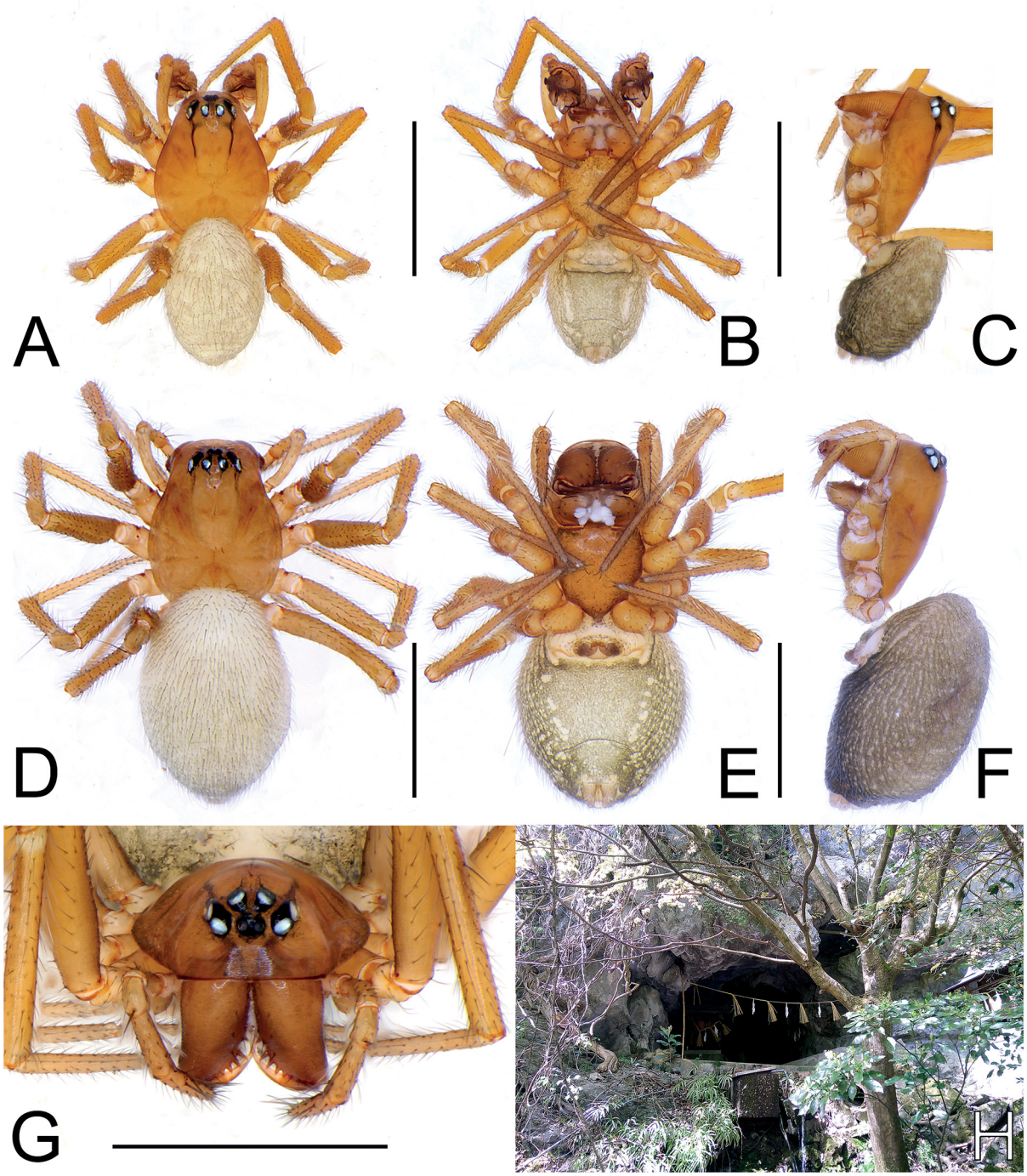

Fig. 1. Habitus and habitat of *Nihonella chika* gen. et. sp. nov. **A**. ♂, holotype (NSMT-Ar 20909), habitus, dorsal view. **B**. Ditto, ventral view. **C**. Ditto, lateral view. **D**. ♀, paratype (NSMT-Ar 20910), habitus, dorsal view. **E**. Ditto, ventral view. **F**. Ditto, lateral view. **G**. Ditto, cephalic region, frontal view. **H**. Entrance of Anatoyama Cave, type locality of the species. Scale bar = 1 mm.

Table 2. Uncorrected Pairwise-distance between the genus *Nihonella* gen. nov. (in bold) and the main genera included in the *Savignia* group based on the barcode COI partial sequence.

	Araeoncus	*Dicymbium*	*Diplocephalus*	*Erigonella*	*Glyphesis*	*Janetschekia*	*Microctenonyx*	*Saloca*	*Savignia*
Araeoncus									
Dicymbium	0.115								
Diplocephalus	0.112	0.101							
Erigonella	0.147	0.125	0.112						
Glyphesis	0.140	0.118	0.140	0.140					
Janetschekia	0.139	0.147	0.150	0.189	0.156				
Microctenonyx	0.156	0.147	0.144	0.155	0.143	0.143			
Saloca	0.161	0.137	0.141	0.147	0.138	0.147	0.158		
Savignia	0.124	0.112	0.122	0.118	0.135	0.164	0.150	0.155	
Nihonella	**0.143**	**0.129**	**0.140**	**0.141**	**0.131**	**0.139**	**0.156**	**0.144**	**0.132**

of *Nihonella* gen. nov. have a highly distinctive chaetotaxy which strongly differ from the chaetotaxy usually found in species belonging to the *Savignia* group (1.1.1.1 vs 2.2.1.1). Although some species included in this group may occasionally have a tibial spine formula of 1.1.1.1, this usually only occurs in males and as a consequence of the reduction of the distal spines in tibia I and II (e.g., *Araeoncus crassipes* Heimer & Nentwig, 1991 = 1.1.1.1, *A. humilis* (Blackwall, 1841) = 0.0.1.1: Tanasevitch, in litteris). An exception is the genus *Microctenonyx* Dahl, 1886, the female of which has a tibial spine formula of 1.1.1.1. However, *Microctenonyx* can be easily distinguished from *Nihonella* gen. nov. by the large genetic distance between the two genera (see Table 2), and by the shape of the epigyne and male palp (short SDSA, different shape of epigyne and internal ducts, see Figs 2A–H, 3A–D vs Millidge 1977: fig. 140 and Bosmans 2007: figs 111–115).

Distribution

Endemic to Western Honshu, Japan. Currently known from three caves only (Figs 1H, 6).

Nihonella chika gen. et. sp. nov.
urn:lsid:zoobank.org:act:87924F73-CCDB-4872-99A3-20A5A3A4EBE9
Figs 1A–G, 2–4; Table 1

Diagnosis

Male *Nihonella chika* gen. et. sp. nov. can easily be distinguished from males of species of the *Savignia* group by the clearly visible hypertrophic secondary DSA apophysis, long and hooked, which instead is absent or much shorter in species of *Savignia* and usually straight and tooth-shaped (see Figs 2A–B, D, 3A–C, 4A–E vs Millidge 1977: figs 122–144). Another distinct character of the male *Nihonella chika* gen. et. sp. nov. is the shape of the prolateral tibial apophysis of the palp: long, partially covering the middle line of the cymbium, and ending with a triangular structure covered with short, stocky spikes (Figs 2A–C, 3A–B, D, 4E–F). Female *Nihonella chika* gen. et. sp. nov. are easily recognized by the general shape of the epigyne, which has two ovoid, flat inflations of the copulatory ducts where the lateral walls of the epigyne join to each other; further, the anterior wall protrudes slightly (Figs 2E, 3E). The epigyne also has a trapezoidal posterior median plate (Figs 2F–G, 3F), and a twisted course of the copulatory ducts (Figs 2H, 3G).

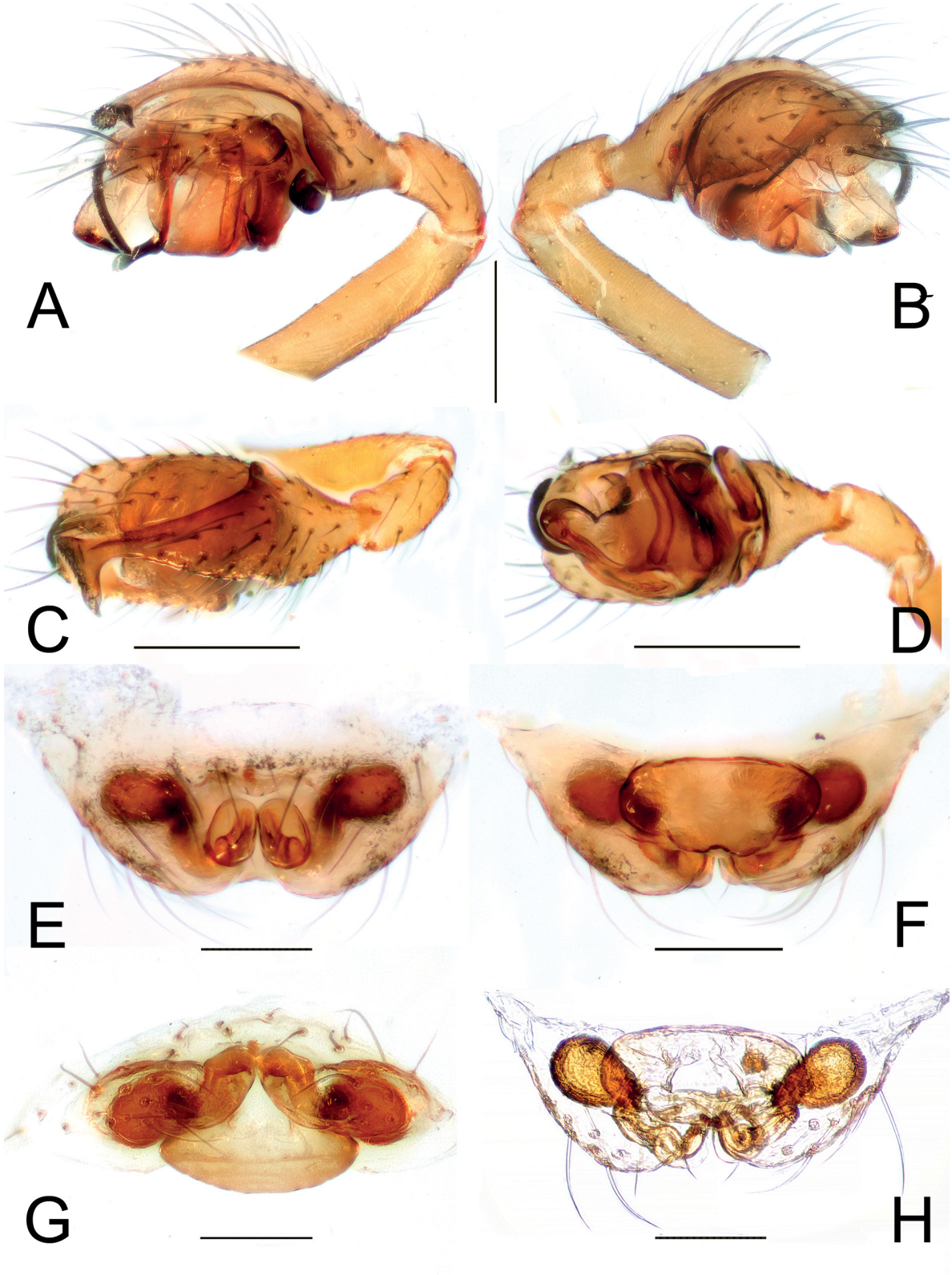

Fig. 2. Genitalia of *Nihonella chika* gen. et. sp. nov. **A**. ♂, holotype (NSMT-Ar 20909), palp, retrolateral view. **B**. Ditto, prolateral view. **C**. Ditto, dorsal view. **D**. Ditto, ventral view. **E**. ♀, paratype (NSMT-Ar 20910), epigyne, ventral view. **F**. Ditto, dorsal view. **G**. Ditto, posterior view. **H**. Ditto, vulva after being cleared, ventral view. Scale bars: A–D = 0.2 mm; E–H = 0.1 mm.

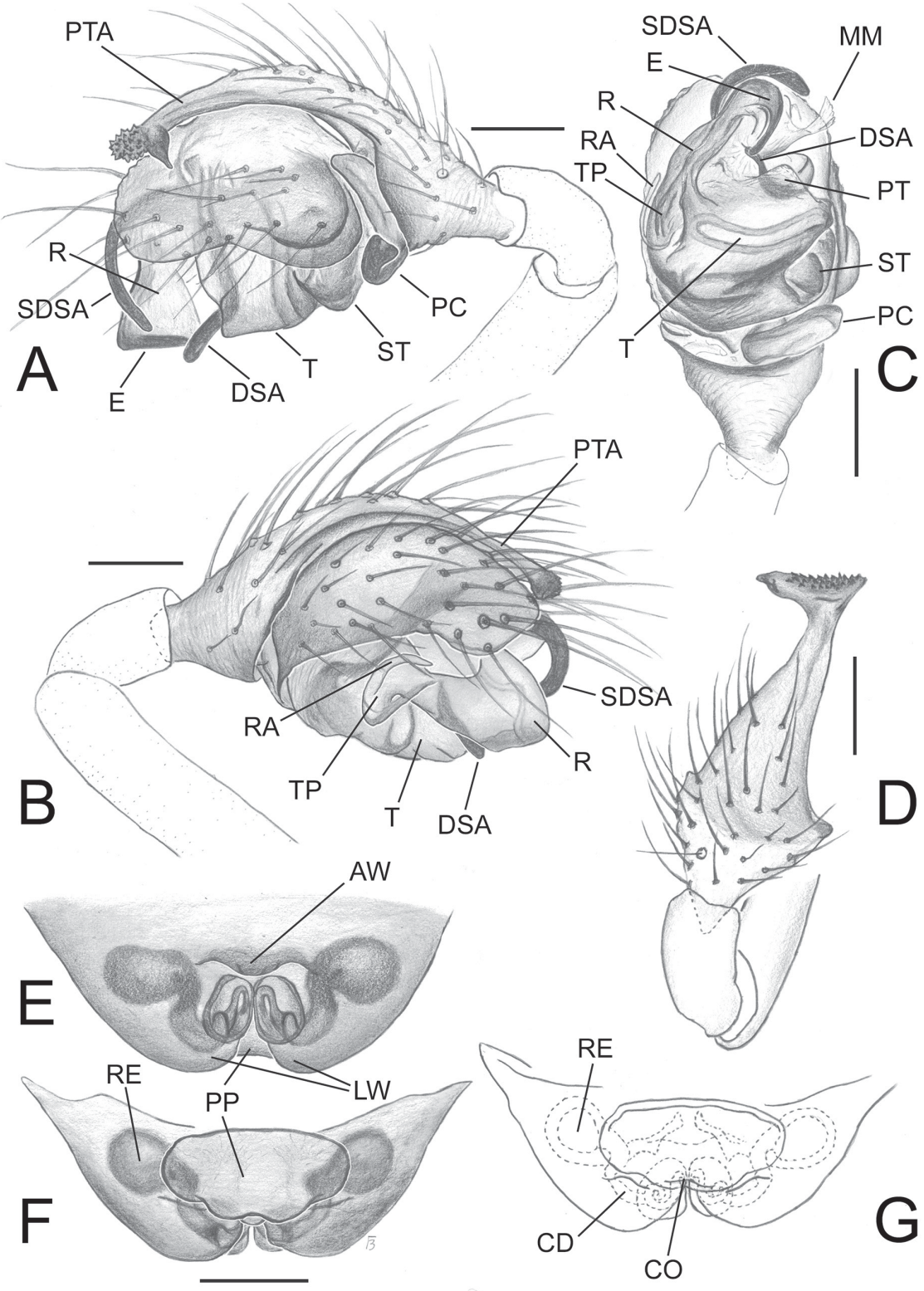

Fig. 3. *Nihonella chika* gen. et. sp. nov. **A**. ♂, holotype (NSMT-Ar 20909), palp, retrolateral view. **B**. Ditto, prolateral view. **C**. Ditto, ventral view. **D**. Ditto, palpal tibia, dorsal view. **E**. ♀, paratype (NSMT-Ar 20910), epigyne, ventral view. **F**. Ditto, dorsal view. **G**. Ditto, vulva, dorsal view. Abbreviations: see Material and methods. Scale bar = 0.1 mm.

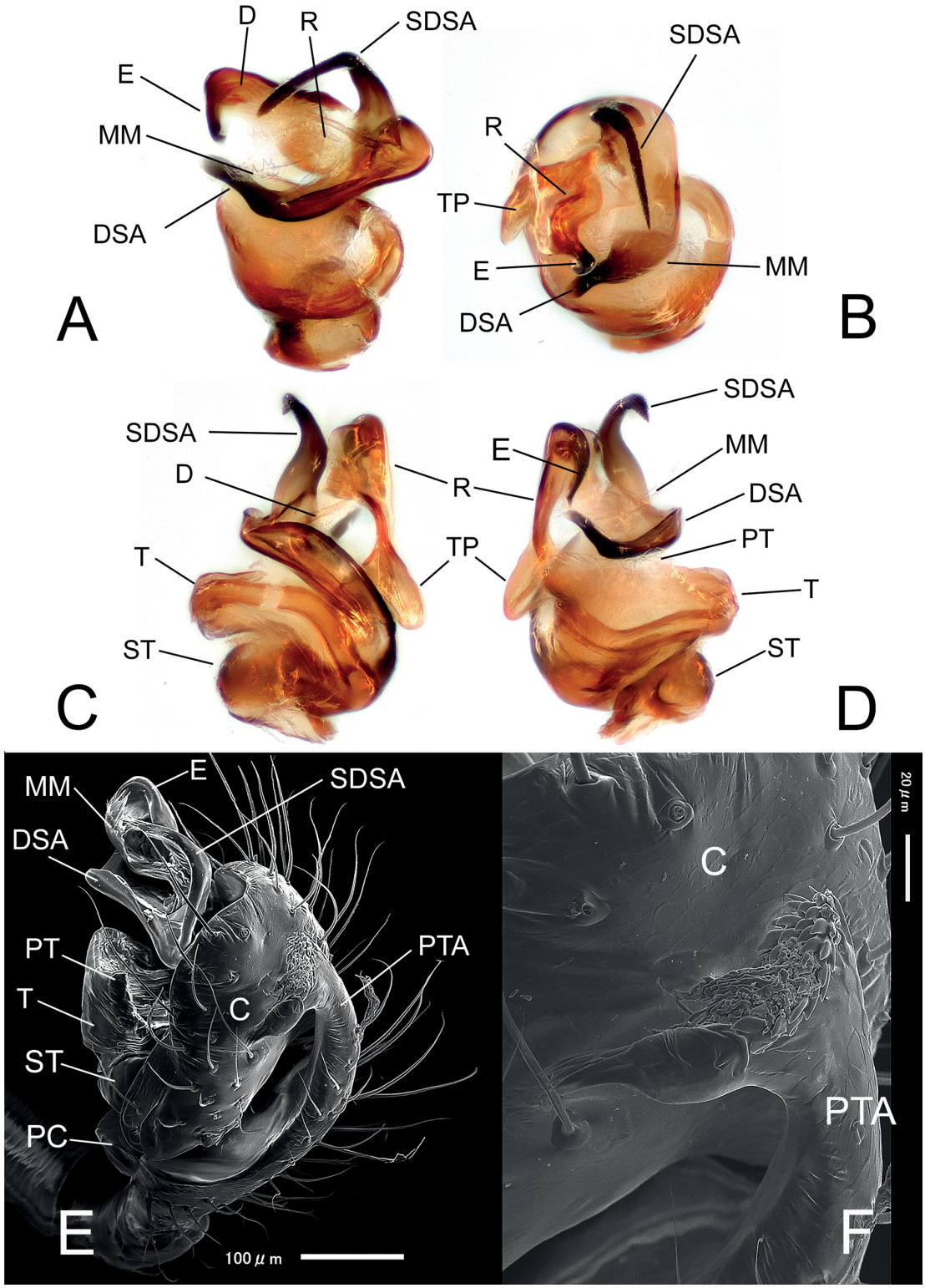

Fig. 4. Embolic division and details of the male palp of *Nihonella chika* gen. et. sp. nov. **A**. ♂, paratype (NSMT-Ar 20911), embolic division, ventro-retrolateral view. **B**. Ditto, frontal view. **C**. Ditto, dorsal view. **D**. Ditto, ventral view. **E**. ♂, paratype (NSMT-Ar 20910), palp under SEM microscope, antero-retrolateral view. **F**. Ditto, detail of the tip of the prolateral tibial apophysis. Abbreviations: see Material and methods.

Etymology

The specific name is derived from the Japanese word '*chika*' (地下) meaning 'underground, subterranean' and thus refers to the habitat of the species, but it is also the pronunciation of a feminine given name in the Japanese language. Name in apposition.

Material examined

Holotype

JAPAN • ♂; Honshu Island, Okayama Prefecture, Takahashi-shi, Kawakamichō, Kōyamaichi, Anatoyama Shrine, Anatoyama cave (穴門山洞窟); 34.7440° N, 133.3918° E; 480 m a.s.l.; 22 Apr. 2019; Ballarin F. and Yamasaki T. leg.; narrow and long cave behind a Shinto shrine; NSMT-Ar 20909.

Paratypes

JAPAN • 1 ♂, 14 ♀♀; same collection data as for holotype; NSMT-Ar 20910 • 1 ♂, 10 ♀♀; Niimi-shi, Toyonagauyama, Uyama-do cave (宇山洞); 34.94226° N, 133.57499° E; 423 m a.s.l.; 21 Apr. 2019; Ballarin F. and Yamasaki T. leg.; large and deep humid cave with a subterranean creek; NSMT-Ar 20911.

Other material

JAPAN • 1 ♀; Honshu Island, Nara Prefecture, Yoshino District, Tenkawa-shi, Dorogawa, Komorinoiwaya cave (蝙蝠の窟); 34.2686° N, 135.8906° E; 06 Oct. 2019; Ballarin F. and Tanikawa A. leg.; NSMT-Ar 20912.

Description

Male (holotype)

Habitus. As shown in Fig. 1A–C.

Measurements. Total length: 1.79, carapace 0.97 long, 0.75 wide.

Prosoma. Carapace, chelicerae, labium, and sternum uniformly light brownish. Head distinctly raised, AME = 0.04, PME, ALE, PLA = 0.06. Anterior margin of cheliceral groove bearing 5 robust teeth.

Opisthosoma. Opisthosoma uniformly grayish, lacking any pattern, covered with numerous short hairs. Central area of ventral side of opisthosoma slightly lighter.

Legs. Legs uniformly light brownish. Femur I with 1 prolateral spine. Patella I and Tibia I with 1 dorsal spine. Tibial spine formula = 1.1.1.1. One trichobothrium on metatarsi I–III, absent on metatarsus IV. TmI = approx. 0.55. Leg measurements as follows: Leg I: 3.02 (0.84, 0.23, 0.80, 0.71, 0.44); Leg II: 2.92 (0.81, 0.24, 0.73, 0.66, 0.48); Leg III: 2.44 (0.68, 0.23, 0.58, 0.56, 0.39); Leg IV: 3.15 (0.87, 0.26, 0.82, 0.69, 0.51).

Palp. As shown in Figs 2A–D, 3A–D, 4E–F. Embolic division as in Fig. 4A–D. Palpal tibia bearing 1 trichobotrium and long prolateral tibial apophysis approximately as long as the cymbium and partially covering it along its median line. PTA triangle-shaped when observed dorsally, ending with triangular spiked structure. Cymbium ovoid when observed dorsally, covering whole bulb with exception of the tip of secondary branch of distal suprategular apophysis. Deep groove along middle line of cymbium, in which rests ventral part of PTA. Paracymbium stumpy and simple, lacking in apophyses. Tailpiece of radix slightly protruding, with well-developed, thin apophysis, hook-like and frontally-oriented. DSA well-developed, hook-like and ending with blunt tip. Secondary branch of distal suprategular apophysis hypertrophic, long and thin, strongly protruding frontally and ventrally, ending with sharp tip. Median membrane transparent and barely visible, protruding retrolaterally. Embolus hook-like, initial trait oriented frontally then ventro-posteriorly, stumpy, ending with blunt end near tip of DSA.

Female (based on three paratypes)

Habitus. As shown in Fig. 1D–G.

Measurements. Total length: 2.10–2.47, carapace 1.00–1.06 long, 0.80–0.83 wide.

Prosoma and opisthosoma. Coloration and other details of carapace, chelicera, and opisthosoma as in male. Head only slightly raised.

Legs. Legs coloration, tibial spine formula, trichobothria and TmI as in male. Leg measurements as follows (based on one paratype): Leg I: 3.59 (1.02, 0.3, 0.95, 0.77, 0.55); Leg II: 3.36 (0.95, 0.28, 0.87, 0.75, 0.51); Leg III: 2.87 (0.81, 0.25, 0.71, 0.68, 0.42); Leg IV: 3.65 (1.05, 0.3, 1.01, 0.82, 0.47).

Epigyne and vulva. As shown in Figs 2E–H, 3E–G. Lateral walls converging anteriorly. Anterior wall of epigyne with small projection. End of copulatory ducts with inflation, forming two small, transparent,

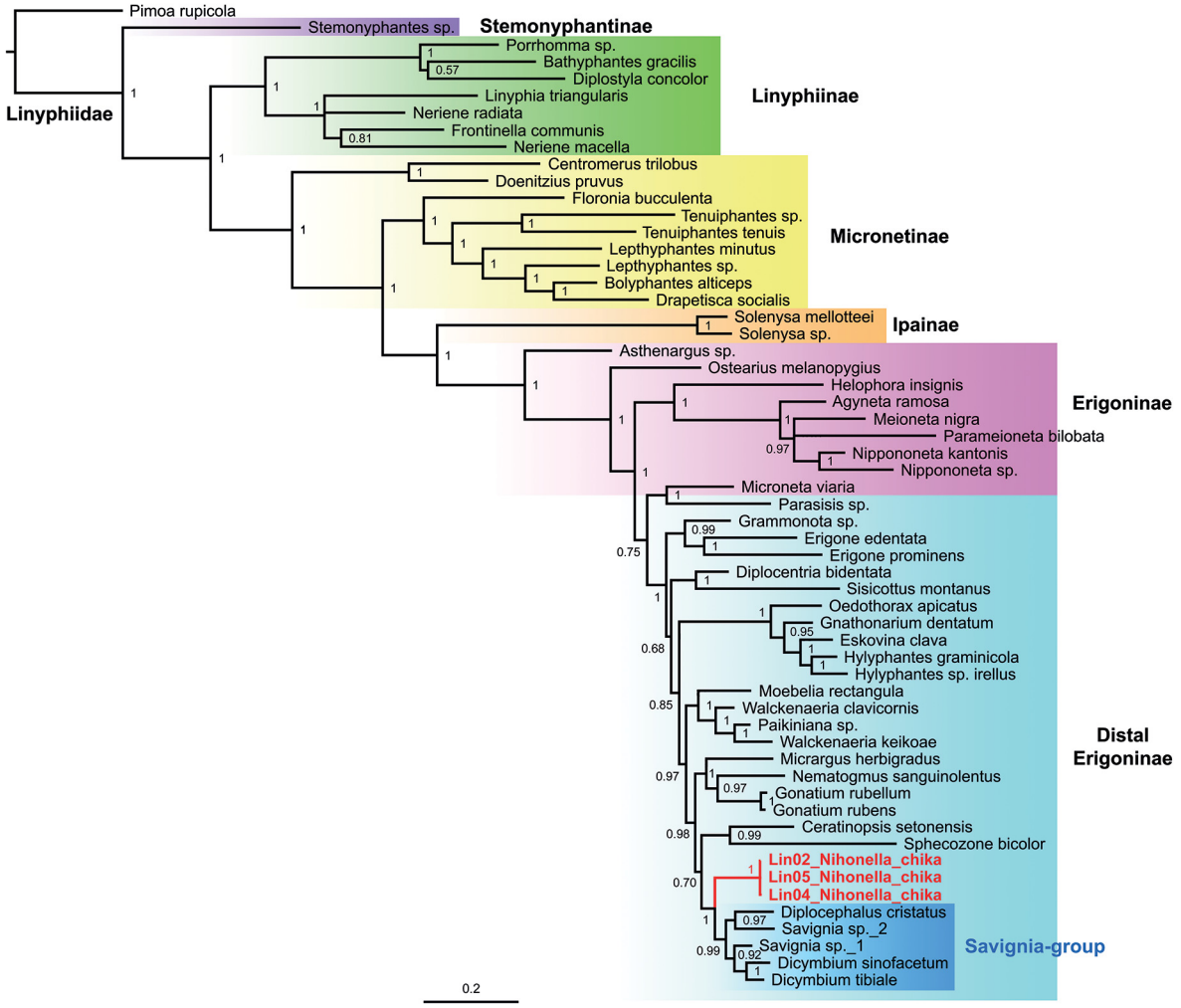

Fig. 5. Bayesian inference phylogenetic tree of Erigoninae and other subfamilies of Linyphiidae based on the five concatenated genes discussed in the text. Each color denotes a different subfamily while the *Savignia* group is indicated in blue. The phylogenetic position of the genus *Nihonella* gen. nov. is highlighted in red. Numbers at each node denote posterior probability support. Branch lengths are scaled in relation to the number of substitutions per site.

ovoid plates in central part of the epigyne. Internal ducts visible through epigyne by transparency. Posterior medium plate larger than wide, approximately trapezoidal when epigyne is observed dorsally. Receptacles subspherical, located lateral to the PP. Copulatory ducts starting from posterior/inner side of receptacles, initial trait oriented towards posterior part of the epigyne, then turning frontally before reaching copulatory opening with twisted course. Copulatory openings located under ovoid plates, approximately at joining point of lateral walls.

Ecology and habitat

Although lacking extreme troglomorphic characters (e.g., eye loss), *N. chika* gen. et. sp. nov. shows troglophilic adaptations, such as body depigmentation. This species has only been found inside caves, several meters from the entrance, in the twilight and transition zones where the light is strongly reduced or absent. *N. chika* gen. et. sp. nov. builds small sheet-webs inside cracks or empty spaces between rocks on the cave floor.

Distribution

Endemic to Western Honshu, Japan. Currently known only for few caves in Okayama and Nara Prefectures (Fig. 6). Type locality: Anatoyama cave (穴門山洞窟) in Okayama Prefecture (Fig. 1H).

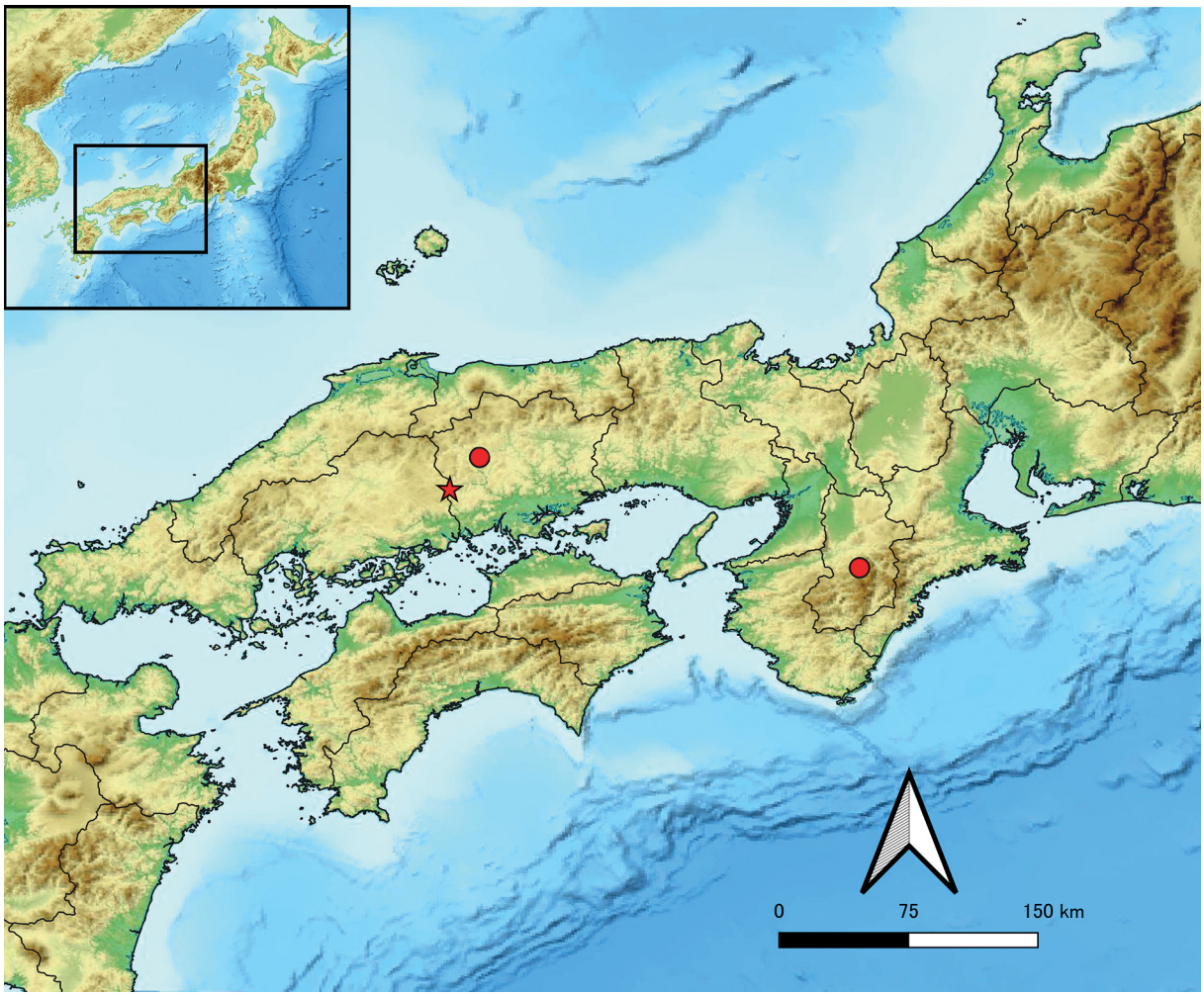

Fig. 6. Distribution of *Nihonella chika* gen. et. sp. nov. in Western Japan. The star refers to the type locality.

Molecular analysis

The general structure of our Bayesian phylogeny (Fig. 5) shows strong similarity to phylogenies reported in previous studies on Linyphiidae (Miller & Hormiga 2004; Arnedo *et al.* 2009; Zhao & Li 2017). In our tree, the nodes of clades forming the main linyphiid subfamilies are strongly supported (PP = 1) and show monophyly. Some genera of Micronetinae appear mixed together with the basal Erigoninae; this is in line with previous research and confirms that these two taxa, as they are currently recognized, are not monophyletic. Within the distal Erigoninae, the node support is sometimes low (PP ≤ 0.85) and thus the phylogenetic positions of some genera are uncertain. Nevertheless, most of the main clades, including the *Savignia* group, are well-defined and with high node support (PP > 0.95). In particular, the genera *Dicymbium* Menge, 1868, *Diplocephalus* Bertkau, 1883, and *Savignia* Blackwall, 1833 form a well-supported clade, as hypothesised by Frick *et al.* (2014). Our analysis highly supports (PP = 1) *Nihonella* gen. nov. as a distinct and well-defined genus within the subfamily Erigoninae, and in particular as part of the distal Erigoninae clade. Such results further suggest a close relationship between the new genus and the species forming the *Savignia* group *sensu* Millidge (1977).

The pairwise distance analysis (Table 2) shows a large genetic distance (13–15%) between *Nihonella* gen. nov. and the *Savignia* group. Genera within the *Savignia* group are also separated to each other by between 10% and 19% genetic distance.

Discussion

Both the molecular results and the genital morphology support a close relationship of *Nihonella* gen. nov. with the main genera forming the *Savignia* group. Thus, we reject the hypothesis of morphological similarities in palp and epigyne between *Nihonella* gen. nov. and the *Savignia* group as the result of evolutionary convergence. Nevertheless, despite this affinity, well-defined genetic differences and distinctive traits in somatic characters and genital morphology clearly show that the new genus belongs to an independent monophyletic clade, separate from the *Savignia* group. In particular, a tibial spine formula of 1.1.1.1, as shown in the new genus, is unusual and in contrast with the standard chaetotaxy of the species forming the *Savignia* group. According to our phylogenetic results, *Nihonella* gen. nov. can be considered a sister clade to the *Savignia* group to which it shares a common ancestor.

The *Savignia* group remains an unresolved taxon, in need of further study. Although the wide majority of its species seem to share the same common origin, recent phylogenetic studies suggest that this group, as was originally defined by Millidge in 1977, is probably not monophyletic (Frick *et al.* 2010). Thus, more comprehensive analyses are necessary to evaluate the correct composition and systematic position of the *Savignia* group within the distal Erigoninae clade. Due to its probable close relationship and potential common ancestry with the *Savignia* group, *Nihonella* gen. nov. might be a promising genus to shed further light on the phylogeny of this group of species.

As far as we know, Linyphiidae are not commonly found in caves within Japan or other Asian countries (Zhao & Li 2017). Some Japanese genera (e.g., *Arcuphantes* Chamberlin & Ivie, 1943) inhabit the entrance and inner areas of natural large cavities, but their species are not endemic to the subterranean environment. Such limited affiliation with the hypogean habitat is also highlighted by the retention of cuticle pigmentation in these spiders. Among other linyphiid genera, in Japan only *Porrhomma* Simon, 1884 and *Micrargus* Dahl, 1886 seem to contain species which are only found in caves. Among endemic linyphiid cave species, only *P. ohkawai* Saito, 1977 and *P. rakanum* Yaginuma & Saito, 1981 share marked troglobitic adaptations. No other obligate cave-dwelling linyphiid species were known in Japan until our research. With the present study, we add one more genus with clear troglophilic habits to the Japanese endemic fauna. Although *Nihonella* gen. nov. does not exhibit distinct troglobitic traits (e.g.,

eye loss), its depigmentation and preference for the inner traits of caves, as well as the lack of any known epigean record, suggest a strong affiliation with the subterranean environment.

Nihonella gen. nov. is currently monospecific, and has only been found in three localities. Nevertheless, the collection of specimens in caves in Okayama and Nara Prefectures, approximately 240 km away from each other, suggests that the new genus might be more widely distributed than currently known. Such lack of data is probably related to the scarcity of recent taxonomic studies on the Japanese cave spider fauna, with the last extensive surveys conducted in the 1970s. Further collections in Japanese caves, or other Asian karst areas, will probably lead to the discovery of new records of *Nihonella chika* gen. et. sp. nov. and, possibly, new congeners. The recent discovery of cave-adapted linyphiid genera in China (Zhao & Li 2017), and now Japan, further indicates that the study of the troglophilic linyphiid fauna is in general still largely unexplored in East Asia. Specific taxonomic studies on these small spiders in Asian caves may lead to new and interesting discoveries.

Acknowledgments

We are particularly grateful to Andrei V. Tanasevitch (Russian Academy of Sciences, Russia) for his precious suggestions and advises on the taxonomy of Erigoninae, his help has been essential to correctly identify the new genus and define the diagnostic characters. We are equally grateful to Katsuyuki Eguchi and Noriaki Murakami (Tokyo Metropolitan University, Japan) for allowing us the use of microscopes and equipment for molecular analysis at TMU. Many thanks to Akio Tanikawa (University of Tokyo, Japan) for kindly helping with the field work in Nara Prefecture. We further thank Yamada Aiki (TMU, Japan) for his help on taking pictures with the SEM microscope. Victoria Smith (Canterbury Museum, New Zealand) kindly revised the English text of the manuscript. We are grateful to two anonymous referees who helped to improve the paper with their comments and suggestions. This work was supported by the YF 2018 and YF 2019 Japan Society for the Promotion of Science Postdoctoral Fellowships, Japan (JSPS KAKENHI n°18F18380 and n°19K16216).

References

Arnedo M.A., Hormiga G. & Scharff N. 2009. Higher level phylogenetics of linyphiid spiders (Araneae, Linyphiidae) based on morphological and molecular evidence. *Cladistics* 25 (3): 231–262. https://doi.org/10.1111/j.1096-0031.2009.00249.x

Balczun C., Bunse A., Hahn D., Bennoun P., Nickelsen J. & Kück U. 2005. Two adjacent nuclear genes are required for functional complementation of a chloroplast trans-splicing mutant from *Chlamydomonas reinhardtii*. *The Plant Journal* 43: 636–648. https://doi.org/bmhq4z

Bosmans R. 2007. Contribution to the knowledge of the Linyphiidae of the Maghreb. Part XII. Miscellaneous erigonine genera and additional records (Araneae: Linyphiidae: Erigoninae). *Bulletin & Annales de la Société entomologique de Belgique* 143: 117–163.

Fernandez R., Kallal R.J., Dimitrov D., Ballesteros J.A., Arnedo M.A., Giribet G. & Hormiga G. 2018. Phylogenomics, diversification dynamics, and comparative transcriptomics across the spider tree of life. *Current Biology* 28 (9): 1489–1497. https://doi.org/10.1016/j.cub.2018.03.064

Frick H., Nentwig W. & Kropf C. 2010. Progress in Erigonine spider phylogeny – the *Savignia*-group is not monophyletic (Araneae: Linyphiidae). *Organisms Diversity & Evolution* 10 (4): 297–310. https://doi.org/10.1007/s13127-010-0023-1

Giribet G., Carranza S., Baguñà J., Riutort M. & Ribera C. 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. *Molecular Biology and Evolution* 13: 76–84. https://doi.org/fp2w

Hall T.A. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* Series 41: 95–98.

Katoh K., Rozewicki J. & Yamada K.D. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166. Available from https://mafft.cbrc.jp/alignment/software [accessed 5 Jan. 2021]. https://doi.org/10.1093/bib/bbx108

Kumar S., Stecher G., Li M., Knyaz C. & Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35 (6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Lanfear R., Frandsen P.B., Wright A.M., Senfeld T. & Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34 (3): 772–773. https://doi.org/10.1093/molbev/msw260

Miller M.A., Pfeiffer W. & Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov. 2010, New Orleans: 1–8. Available from https://www.phylo.org/ [accessed 6 Jan. 2021]. https://doi.org/10.1109/GCE.2010.5676129

Miller J.A. & Hormiga G. 2004. Clade stability and the addition of data: a case study from erigonine spiders (Araneae: Linyphiidae, Erigoninae). *Cladistics* 20 (5): 385–442. https://doi.org/10.1111/j.1096-0031.2004.00033.x

Millidge A.F. 1977. The conformation of the male palpal organs of linyphiid spiders, and its application to the taxonomic and phylogenetic analysis of the family (Araneae: Linyphiidae). *Bulletin of the British arachnological Society* 4 (1): 1–60.

Ono H., Matsuda M. & Saito H. 2009. Linyphiidae, Pimoidae. *In*: Ono H. (ed.) *The Spiders of Japan with Keys to the Families and Genera and Illustrations of the Species*. Tokai University Press, Kanagawa.

Rambaut A., Drummond A.J., Xie D., Baele G. & Suchard M.A. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67 (5): 901–904. https://doi.org/10.1093/sysbio/syy032

Ronquist F., Teslenko M., Van Der Mark P., Ayres D.L., Darling A., Höhna S. & Huelsenbeck J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61 (3): 539–542. https://doi.org/10.1093/sysbio/sys029

Tanasevitch A.V. 2020. *Linyphiid Spiders of the World*. Available from http://old.cepl.rssi.ru/bio/tan/linyphiidae/ [accessed 25 Jul. 2020].

Tanikawa A. 2020. *A Check List of Japanese Spiders ver. 2020 R1*. Available from http://www.asahi-net.or.jp/~dp7a-tnkw/japan.pdf [accessed 25 Jul. 2020].

Wheeler W.C., Coddington J.A., Crowley L.M., Dimitrov D., Goloboff P.A., Griswold C.E., Hormiga G., Prendini L., Ramírez M.J., Sierwald P., Almeida-Silva L., Alvarez-Padilla F., Arnedo M.A., Silva L.R.B., Benjamin S.P., Bond J.E., Grismado C.J., Hasan E., Hedin M., Izquierdo M.A., Labarque F.M., Ledford J., Lopardo L., Maddison W.P., Miller J.A., Piacentini L.N., Platnick N.I., Polotow D., Silva-Dávila D., Scharff N., Szűts T., Ubick D., Vink C.J., Wood H.M. & Zhang J. 2017. The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling. *Cladistics* 33 (6): 574–616. https://doi.org/10.1111/cla.12182

World Spider Catalog (2020) *World Spider Catalog. Version 21.5*. Natural History Museum Bern. Available from http://wsc.nmbe.ch [accessed 6 Jan. 2021]. https://doi.org/10.24436/2

Zhao Q. & Li S. 2017. *Callosa* gen. n., a new troglobitic genus from southwest China (Araneae, Linyphiidae). *ZooKeys* 703: 109–128. https://doi.org/10.3897/zookeys.703.13641

Manuscript received: 4 September 2020
Manuscript accepted: 10 November 2020
Published on: 26 January 2021
Topic editor: Rudy Jocqué
Desk editor: Eva-Maria Levermann

Printed versions of all papers are also deposited in the libraries of the institutes that are members of the *EJT* consortium: Muséum national d'histoire naturelle, Paris, France; Meise Botanic Garden, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; Royal Belgian Institute of Natural Sciences, Brussels, Belgium; Natural History Museum of Denmark, Copenhagen, Denmark; Naturalis Biodiversity Center, Leiden, the Netherlands; Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain; Real Jardín Botánico de Madrid CSIC, Spain; Zoological Research Museum Alexander Koenig, Bonn, Germany; National Museum, Prague, Czech Republic.