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A new *Loureedia* species on overgrazed former cork oak forest in Morocco (Araneae: Eresidae)

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Abstract

In this paper a new velvet spider species from Morocco is described from an overgrazed former cork oak [*Quercus suber* (*Linné* 1753)] forest. It is the second known species of the hitherto monotypic genus *Loureedia*. *Loureedia maroccana* **sp. n.** is distinguished from *L. annulipes* (*Lucas*, 1857) by the morphology of the conductor, the anteriorly widening cephalic region of the prosoma and opisthosoma decorated with a lobed, bright red marking on the dorsal side. Furthermore, three partial gene fragment sequences (histone 3, 28S ribosomal and cytochrome c oxidase) are also given, supporting the establishment of the new species.
Keywords: *Loureedia,* velvet spiders, cork oak, Morocco

Introduction

Velvet spiders (Eresidae) contains nine genera and 96 described species worldwide (*World Spider Catalog* 2017). According to the present knowledge, the monotypic genus *Loureedia* was established by *Miller et al*, (2012) based on *L. annulipes,* the type species, which described in Israel. Former publications mentioned two synonyms of *L. annulipes*: *Eresus semicanus Simon*, 1908 and *Eresus jerbae El-Hennawy*, 2005 (*Simon* 1908; *El-Hennawy* 2005).

Loureedia annulipes was originally described as *Eresus annulipes Lucas*, 1857. The genus *Loureedia* mainly differs from the other velvet spider genera in having a strongly bifid apical region of the conductor, in the shape of the cephalic region of the prosoma and also in the extremely bright pattern of the dorsal side of the opisthosoma. At present, *L. annulipes* is known from Algeria, Tunisia, Egypt, Israel (*Miller et al*, 2012) and Spain (*Nentwig et al*, 2017).

Zakkak et al, (2014) found a positive correlation between the ground spider richness and low intensity grazing. *Horváth et al,* (2013) found that the spiders are less diverse in overgrazed grasslands and the negative effect is minimal in small and isolated grasslands.

In this paper, we present a species belonging to the hitherto monotypic genus *Loureedia*, collected in an overgrazed cork oak forest in Morocco. Thorough examination of these specimens showed coherent morphological characteristics clearly different from those of *L. annulipes*, and the species is described here as new to science.

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Materials and methods

Specimens were collected individually and stored in 70 % ethyl-alcohol.
Three males and the palps of one additional specimen partially destroyed during transportation were studied. All the measurements are given in millimetres (mm).

The holotype and paratypes have been deposited in the Soil Zoological Collection (former Arachnoidea Collection) of the Department of Zoology, Hungarian Natural History Museum (collection number of holotype: HNHM Araneae-8869 and collection number of paratype: HNHM Araneae-9007) Budapest (curator Dr. László Dányi).

Specimens and copulatory organs were studied using a Leica MZ FL III stereomicroscope and photographed by Canon Q Imaging Micro 5.0 RTV at the Institute of Genetics, BRC. Scanning electron micrographs were taken with a Hitachi S-4700 microscope at the Department of Applied and Environmental Chemistry, University of Szeged, Hungary.
One segment of a spider leg was used to extract total genomic DNA after the modified Drosophila DNA extraction protocol (Engels et al. 1990). One µl of extracted DNA was used as template in the total amount of 25 µl polymerase chain reaction (PCR) following the manufacturer's instructions (Promega GoTaq® Hot Start Kit). Reactions were conducted with two set of nuclear primers (for histone 3-H3 and 28S rRNA partial genes) and one set of mitochondrial primer pair (for cytochrome c oxidase subunit I – COX1 partial gene). Primer sequences are listed in Supplementary file, *Table 1*. PCR products were controlled on agarose gel and purified after gel electrophoresis following the manufacturer's protocol (Zymoclean™ Gel DNA Recovery Kit) and were sequenced by Macrogen Inc.

Raw sequences were assembled in Staden Package 2.0 (*Staden et al,* 2000). Each base call and any discrepancies of the sequences were corrected according base confidence values (*Bonfield et al,* 2010). Sequences used in this study were obtained from GenBank with the accession numbers shown in supplementary material (see *Table 2*.). Accession numbers of the newly sequenced taxa are the following: *Loureedia maroccana* sp. n. isolate LIV, KX443580 (28S rDNA), KX443586 (H3), KX443583 (COX1); *Eresus* sp. isolate C4d, KX443581 (28S rDNA), KX443587 (H3), KX443584 (COX1); *Eresus sandaliatus* isolate JL-1589, KX443582 (28S rDNA), KX443588 (H3), KX443585 (COX1).

Consensus sequences were aligned using the MUSCLE (Edgar 2004) algorithm in MEGA 6.06 (*Tamura* 2013). The alignment was further curated in BioEdit 7.0.9.0 (*Hall* 1999). The genetic distances between taxa were assessed by MEGA 6.06.

***Table 1*: List of primer pairs used in this study**

Primer		Sequence	Reference
Forward	LC01490-oono	CWA CAA AYC ATA RRG ATA TTG G	Modified from Folmer et al. (1994) in Miller et al. (2010)
Reverse	HC02198	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer et al. (1994)
Forward	H3nF	ATG GCT CGT ACC AAG CAG AC	Colgan et al. (1998)
Reverse	H3aR	ATA TCC TTR GGC ATR ATR GTG AC	Colgan et al. (1998)
Forward	28S0	GAA ACT GCT CAA AGG TAA ACG G	Hedin and Maddison (2001)
Reverse	28SC	GGT TCG ATT AGT CTT TCG CC	Hedin and Maddison (2001)

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***Table 2*: GenBank accession numbers obtained from GenBank. New sequences generated for this study are shown in bold.**

Species	Code	28S	H3	COI
Eresus cf. *kollari* 14_04	14_04	FJ948958	FJ949036	FJ948998
Eresus sandaliatus	**JL-1589**	**KX443582**	**KX443588**	**KX443585**
Eresus sp. 13_06	13_06	FJ948957	FJ949035	FJ948997
***Eresus* sp. C4d**	**C4d**	**KX443581**	**KX443587**	**KX443584**
Eresus walckenaeri 14_05	14_05	FJ948959	FJ949037	FJ948999
Gandanameno fumosa 09_05	09_05	FJ948963	FJ949041	FJ949003
Gandanameno fumosa 14_6	14_06	FJ948964	FJ949042	FJ949004
Gandanameno sp. 09_02	09_02	FJ948962	FJ949040	FJ949002
Gandanameno sp. 13_10	13_10	FJ948961	FJ949039	FJ949001
Loureedia (former *Stegodyphus*) *annulipes* 15_10	15_10	FJ948960	FJ949038	FJ949000
***Loureedia maroccana* sp. n. LIV**	**LIV**	**KX443580**	**KX443586**	**KX443583**
Paradonea variegata	14522	-	-	JQ026517
Paradonea variegata	14512	JQ026518	-	JQ026516
Stegodyphus lineatus 14_02	14_02	FJ948976	FJ949053	FJ949016
Stegodyphus mimosarum 09_06	09_06	FJ948977	FJ949054	FJ949017
Stegodyphus tentoriicola 14_12	14_12	FJ948975	FJ949052	FJ949015

Abbreviations

Standard abbreviations of morphological terms follow *Miller et al,* (2012). Further abbreviations: **PME** = posterior median eyes, **PLE** = posterior lateral eyes.
BRC Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary;
HNHM Hungarian Natural History Museum, Budapest, Hungary;

Results and discussion

Taxonomy

***Loureedia maroccana* sp. n.**

Material examined. Holotype: Male. Morocco, near the locality of Sidi Boukhalkhal, N 34° 05' 57,70'', W 6°24' 23,22'', singled, 04.11.2013., J. Gál (HNHM, collection number: HNHM Araneae-8869).
Paratypes: 2 Males. Morocco, close to Sidi Boukhalkhal, N 34°07'16,65'', W 6°25'36,44", singled, 28.10.2015., R. Bagyó (HNHM, collection number: HNHM Araneae-9007)
Etymology. The species is named after the country of the type locality, Morocco.
Generic placement. This species has a wider than long cephalic region (*Fig. 1*), a median eye group with the PME clearly larger than the AME, it lacks tubercles associated with ALE, has a palpal conformation with a proximal-distal axis, a helical embolus encircling the distal part, and a strongly bifid (doubly pronged) conductor. These features together unambiguously place this species within the heretofore monotypic genus *Loureedia*.

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***Figure 1*: Habitus of living adult male specimen of *Loureedia maroccana* sp. n. (HNHM, collection number: HNHM Araneae-8869)**

Diagnosis. Distinguished from males of the only other member of the genus *Loureedia*, *L. annulipes*, by the cephalic region, which is subtrapezoidal when viewed from above, clearly bulging laterally with doubly arched lower margin above chelicerae in frontal view; the clypeal hood, which is acutely angled with concave sides and the apical palpal complex with embolic division longer than tegular division. By contrast, *L. annulipes* males are characterized by a cephalic region with subrectangular outline when viewed from above, with nearly parallel sides and almost flat lower margin at the base of chelicerae in frontal view; clypeal hood forming a nearly 90° angle with strait sides and an apical palpal complex with embolic division shorter than tegular division. In addition, the edge of the dorsal prong of the conductor is evenly curved in the case of the *L. maroccana* while it is clearly S-shaped in *L. annulipes* (shown by *Miller et al,* 2012). Carapace and opisthosoma of *L. maroccana* are predominantly black and red, as opposed to the variable, but usually white-decorated (often in combination with orange yellow) body of *L. annulipes.*

Description. Male. *Prosoma* (*Fig. 2*): Lengths: 4.5; 3.95; 3.1. Carapace dark blackish brown, cephalic region dorsally covered by short red setae on the front and the centre, with some scattered red hairs on the flanks, scattered white hairs restricted to the posterior and to the extreme anterior edge; remaining area covered by black setae. Carapace covered by red setae, except for a short longitudinal, black bar running through the moderately deep fovea, and a dark blackish-brown posterior triangle mostly devoid of hairs. Cephalic region steeply ascending posteriorly, then evenly rounded until about PLE, followed by a region gradually decreasing towards PME. AME distinctly smaller than PME, ALE not associated with tubercle. Viewed from above, cephalic part somewhat wider than thoracic part, clearly wider than long, subtrapezoidal, widening towards anterior third; posteriorly arcuate, broadly rounded laterally, and with a shallow, longitudinal depression along the midline most obvious at the posterior third. In frontal view, lower margin of carapace arched above the articulation of each chelicera, flanks slightly, but clearly bulging laterally. Clypeal hood acute-angled is with slightly concave sides.

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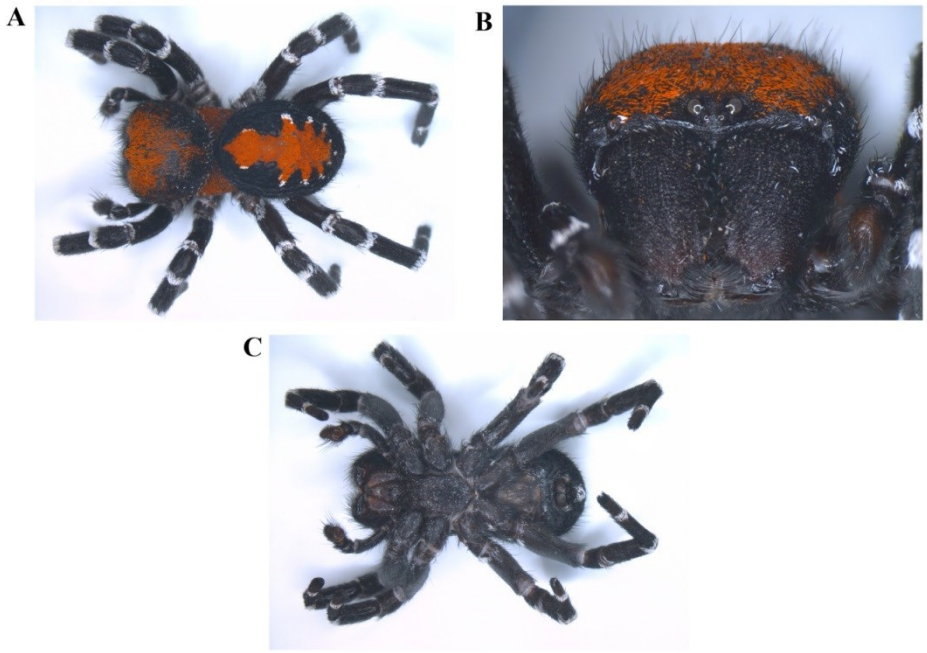
***Figure 2*: A-C. Habitus of adult male specimen of *Loureedia maroccana* sp. n.: A. dorsal view, B. frontal view, C. ventral view. (HNHM, collection number: HNHM Araneae-9007).**



Chelicerae (*Fig. 2*): black, covered by long, nearly adpressed black hairs.

Legs and palps (*Figs. 2 and 3-4*): black to dark grey, white striped dorsally at joints. Palps with a proximal-distal axis, apical complex making slightly more than one helical turn. Embolic division somewhat longer than tegular division, membranous conductor abruptly transitioning just before a deep cleft dividing the conductor dorsally-retrolaterally into a heavily sclerotized, two-pronged structure with the dorsal prong flatly and evenly curved at the edge facing the cleft.
Opisthosoma (*Figs. 1, 2*): dark blackish brown, covered by black/dark grey setae, decorated with a narrow crescent covered by white hairs at the lower anterior edge and with a roughly almond-shaped red area along the dorsal midline with white-tipped lateral lobes. In contrast *L. annulipes* (see *Miller et al,* 2012); *L. maroccana* possesses a fig leaf shaped dorsal colour pattern of fire red colour. It lacks a dark medieval centre line.
Remark. One of the collected specimens lacks white spots at the tips of the anterior-most pair of lateral lobes.
Female: unknown.

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***Figure 3*: A-C. Photomicrographs of *Loureedia maroccana sp. n.* male right palp: A. prolateral view, B. ventral view; C. retrolateral view.**

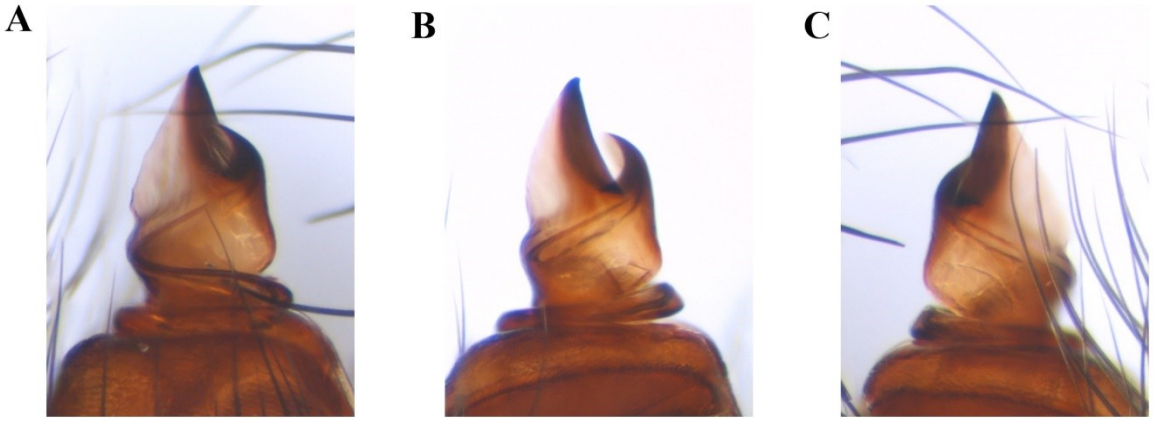
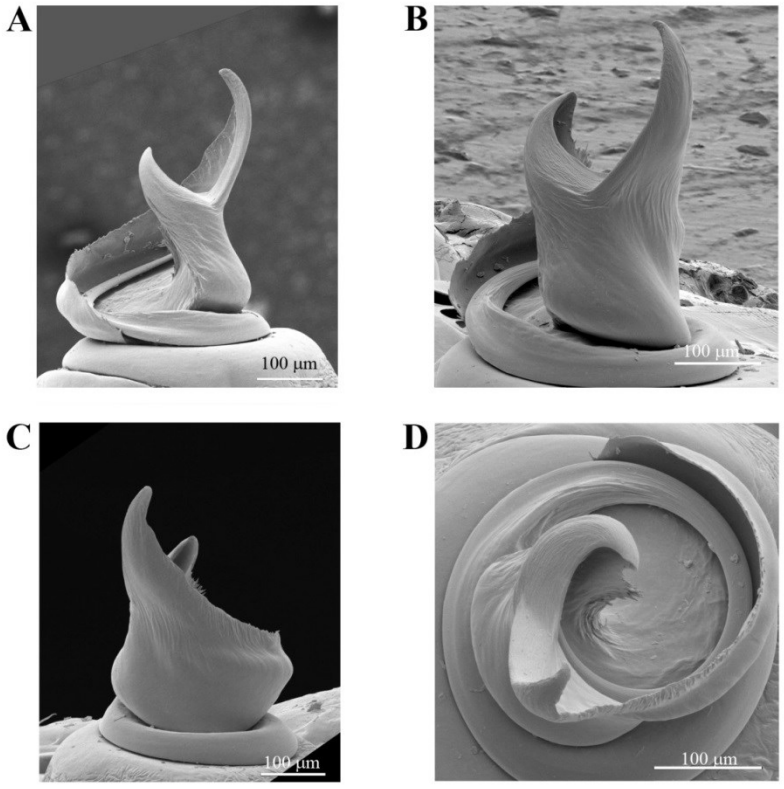


***Figure 4*: A-D. Scanning electron micrographs of *Loureedia maroccana* sp. n. adult male left palp: A. prolateral view, B. ventral view, C. retrolateral view, D. apical view.**



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Distribution. At the time of manuscript submission known only from the type locality, close to Sidi Boukhalkhal in overgrazed former cork oak forest.
Habitat. Collected specimens have been found from glades of semi-natural *Q. suber* woods on the southern dry slopes of the western foothills of the Moroccan Middle Atlas Mts. The habitat was strongly overgrazed by sheep and goat.
Phenology. Males were found wandering on the surface of soil between September and November, indicating a late autumnal copulation period.
Note. The finding that males of *L. maroccana* have a subtrapezoidal cephalic region requires a slight modification of the circumscription of the genus *Loureedia*, as the subrectangular shape of cephalic region can no longer be considered as a distinguishing character. However, this in no way affects the stability of the genus, since numerous other characters (see *Miller et al,* 2012) set *Loureedia* apart from the other genera of family Eresidae.

Genetic examination
318, 399 and 725 base pair long partial gene fragments were obtained by H3, COX1, and 28S primer pairs respectively. The mitochondrial sequences differ by 10.27 % between *L. annulipes* and *L. maroccana* specimens, similarly to other interspecific sequence divergence estimates of mitochondrial markers among Eresidae (*Johannesen et al,* 2005; *Johannesen et al,* 2007; *Robinson et al,* 2009). The sequence diversity of 28S rRNA nuclear gene fragment is 1.2 % between the two *Loureedia* species. The variability of 28S rRNA gene fragment between these species is higher than the average interspecific sequence divergence among the examined *Eresus* species, which is 0.7 %. The H3 gene fragments of the two *Loureedia* species were compared and no gaps were found, but sequence polymorphisms were identified at 12 different positions (see the alignment of supplementary material).

Table 3. shows estimates of evolutionary divergence over partial COX1 sequence pairs for intra- and intergeneric level (within and between groups) of some Eresidae genera. The estimates of average genetic distances within the genera were lower than between the examined genera, as expected. The average genetic distance detected between the genera *Loureedia* and *Eresus* is low (0.157), which confirms the findings of *Miller et al,* (2010) in that the genus *Loureedia* (as *Stegodyphus annulipes*) together with genera *Stegodyphus* constitute a sister group of the *Eresus* clade.

***Table 3*: Estimates of Average Evolutionary Divergence over Sequence Pairs of partial COX1 gene at intra-and intergeneric level.**

A			B				
Taxon name	**d.**	**S.E.**	*Paradonea*	*Gandanameno*	*Loureedia*	*Eresus*	*Stegodyphus*
Paradonea	**0**	0		0.027	0.024	0.02	0.024
Gandanameno	**0.075**	0.013	**0.23**		0.022	0.019	0.02
Loureedia	**0.13**	0.023	**0.188**	**0.189**		0.016	0.018
Eresus	**0.101**	0.012	**0.163**	**0.183**	**0.157**		0.015
Stegodyphus	**0.143**	0.018	**0.195**	**0.193**	**0.173**	**0.154**	

The average number of base substitutions per site for each sequence pairs (d.) within a given genus (A) and between genera are given (B). Standard error estimates (S.E.) are shown above the diagonal on part B. Analyses were conducted using the LogDet model (*Lockhart et al*. 1994). The analysis involved 16 nucleotide sequences. All positions with less than 95 % site coverage were

eliminated. A total of 399 positions were retained in the final dataset. The analysis was conducted in MEGA6 (*Tamura et al*, 2013).

It is worth noting that one change of the 309 position in the COX1 DNA alignment results in the alteration of a predicted Ser of *L. annulipes* into a predicted Lys in *L. maroccana* using 'in silico' translated (*Stothard* 2000) COX1 protein sequences (see the amino acid alignment of supplementary material), also supporting the notion that *L. maroccana* and *L. annulipes* are distinct species.

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