DOMITIUS CULSU SP. NOV. (ARANEAE, NESTICIDAE), A NEW TROGLOBIONT SPIDER FROM ITALY WITH NOTES ON ITALIAN NESTICIDS OF THE GENUS DOMITIUS RIBERA, 2018

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Abstract

Seven species from the spider family Nesticidae are currently known for the Italian fauna. Three Italian nesticids belong to the newly-established genus Domitius Ribera, 2018. All these species show a restricted distribution along the Apennine mountain chain and deep adaptation to cave life. Herein, a fourth species, D. culsu sp. nov. from a single cave in Northern Apennines is described. Detailed illustrations and diagnosis of the new species are provided. Molecular and morphological analysis of both sexes of D. culsu sp. nov. supports the validity of the new species and its close relationship with the other Domitius species from the same geographical area. A close affinity with the species distributed in the Iberian Peninsula is also observed. The potential susceptibility of D. culsu sp. nov. to external disturbance, and its extremely limited distribution, makes this spider of interest for conservation.

INTRODUCTION

Nesticidae Simon, 1894 is a small family of spiders with a worldwide distribution. Currently, 278 species and 16 genera are recognized (World Spider Catalog, 2020). At temperate latitudes, nesticids mostly occur in dark, damp environments such as caves, often showing high levels of endemism. Previously, the majority of nesticid species were included in the genus Nesticus Thorell, 1869. Nesticus has recently been partially revised (Lin et al., 2016; Pavlek and Ribera, 2017; Ribera, 2018) and several of its European species moved to different genera (e.g. Typhlonesticus Kulczyński, 1914, Kryptonesticus Pavlek and Ribera, 2017, Domitius Ribera, 2018).

Eight nesticid species belonging to five genera are currently known in Italy: Domitius menozzii (di Caporiacco, 1934), D. sbordonii (Brignoli, 1979), D. speluncarum (Pavesi, 1873), Eidmannella pallida (Emerton, 1875), Kryptonesticus eremita (Simon, 1880), Nesticus cellulanus (Clerck, 1757), Typhlonesticus idriacus (Roewer, 1931), and T. morisi (Brignoli, 1975) (Pantini and Isaia, 2019). The Italian Domitius species are all considered troglobionts (Mammola and Isaia, 2017) showing extreme adaptations to the subterranean environment (e.g. reduction of eyes, depigmentation, and elongation of legs). Such spiders are characterized by a limited distribution, and are endemic to a small number of caves in Northern (D. menozzii, D. speluncarum) or Central (D. sbordonii) Apennines mountains (Brignoli, 1979, Ribera, 2018). Initially, the taxonomy and geographic range of D. menozzii and D. speluncarum were uncertain: the two species were often mistaken for each other, or considered as subspecies or a synonymy of K. eremita (see Brignoli, 1971 202–205, in Italian). Dresco (1966) and Brignoli (1971) revised the taxonomy of D. menozzii and D. speluncarum, pinpointing their differences. Nevertheless, there is still occasional confusion regarding their taxonomic status: for example, D. speluncarum is sometimes wrongly attributed to the Dinaric Alps (Pavlek and Ribera, 2017).

Individuals of D. speluncarum and D. menozzii were found by the author while collecting in caves in Liguria and Toscanca Regions (Italy). Specimens from one cave appeared to show distinct morphological differences. A more detailed examination of genitalia revealed that those specimens represented a new species. In this paper, the new species is described. The morphological differences between the Italian Domitius species are explored in detail, and the precise geographical distribution of the genus in the Italian peninsula is illustrated. To better establish the systematic position of the species, a phylogenetic tree of the genus Domitius, in relation with the other main European nesticid genera, is carried out.

MATERIAL AND METHODS

Taxonomy

Fresh specimens were hand-collected in caves and fixed in 96 % ethanol for molecular and morphological analysis. Photographs and measurements of the samples were taken at the Museo Civico di Storia Naturale of Verona, Italy, using a Leica DFC450 digital camera mounted on a Leica M165C stereomicroscope. A Leitz Diaplan microscope was used to photograph the vulvae. Images were subsequently combined using Helicon Focus 6 image stacking software. The left palps of males were photographed. Epigynes were dissected using a sharp needle and boiled for a few minutes in a 20 % KOH solution to show the vulval internal structures. Leg measurements are given as following: total length (femur, patella,
tibia, metatarsus, tarsus). All measurements are reported in millimeters. Unless otherwise specified, type descriptions are based on wet specimens in ethanol. Specimens used in this study are stored in the collections of the Museo Civico di Storia Naturale di Verona (MSNV) and Museo Civico di Storia Naturale “E. Caffi” of Bergamo (MSNB). The following abbreviations are used in the text and figures: AM = anterior median eyes; AL = anterior lateral eyes; C = conductor complex; Cd = copulatory duct; Co = copulatory opening; Da 1-3 = dorsal apophyses of the paracymbium; Di = distal apophysis of the paracymbium; E = embolus; Id = insemination duct; Ma = median apophysis; Ms = median septum; P = paracymbium; Pc 1-3 = processes of the conductor complex; PM = posterior median eyes; PL = posterior lateral eyes; S = spermatheca; St = subtegmentum; T = tegulum; Va = ventral apophysis of the paracymbium; Vp = vulval pocket.

**Breeding**

To increase the available number of adult specimens and to obtain information about the life-cycle of the species for further research, juveniles of *D. culsu* sp. nov. at different growth stages were collected and raised in captivity in the basement of the Museo Civico di Storia Naturale di Verona. Each specimen was kept in a box (size 10 × 5 × 3 cm) made of plaster, with a glass lid and a layer of cave mud on the bottom. All boxes were placed in a large plastic tray with a transparent plexiglass lid and a layer of plaster on the bottom. To maintain the correct degree of humidity, the bottom of the tray was moistened with water every two weeks. Specimens were frequently checked and fed with fruit flies or mosquitoes approximately once or twice per month.

**Molecular Analysis**

Sequences of *Domitius* species and other European nesticids were obtained from freshly collected specimens or acquired from the GenBank online database (GenBank, 2018). Since preliminary phylogenetic analysis of the family suggests *Gaucelmus* is a sister clade to all Nesticidae *sensu stricto* (Ballarin and Li, in prep.), *Gaucelmus augustinus* Keyserling, 1884 from North America was set as an outgroup to root the tree. Extraction of DNA, and PCR amplification, were performed in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (IZCAS). All fresh specimens used for the molecular analysis were identified at species level using morphology, before storing at −20 °C at IZCAS. For each species, total genomic DNA was extracted from two legs of an adult specimen using a TIANamp Genomic DNA Kit (TIANGEN) following protocols and primers as indicated in Ballarin and Li (2018). Raw sequences were aligned using the online version of MAFFT v.7.0 (Katoh and Standley, 2013) under the algorithms G-INS-i for COI and H3 and Q-INS-i for 16S. Aligned sequences were subsequently visually inspected for mismatching and edited with BioEdit v.7.2.5 (Hall, 1999). A Maximum Likelihood (ML) analysis under a GTR+Gamma nucleotide substitution model was performed using the online version of RAxML v.8.2.0 (Stamatakis, 2014) on CIPRES Science Gateway V. 3.3 (Miller et al. 2010, available at: https://www.phylo.org/). One thousand replicates of rapid bootstrap were performed twice, using an individual gene partition scheme. Uncorrected pairwise-distance genetic divergences between the species was performed using MEGA v.7.0.14 (Tamura et al., 2013). The list of species used in the analysis and related GenBank accession numbers of the sequences are reported in Table 1.

**RESULTS**

**Taxonomy**

*Class Arachnida Cuvier, 1812*

*Order Araneae Clerck, 1757*

*Family Nesticidae Simon, 1894*

*Genus Domitius Ribera, 2018*

*Domitius culsu* Ballarin sp. nov.

Figures 1A–G, 2A–E, 3A–D.

*Nesticus speluncarum* Brignoni, 1979: 214 (misidentification)

Type material. Holotype ♀. ITALY, Toscana: Garfagnana area, Lucca Province, Coreglia Antelminelli Municipality, Tana delle Fate di Coreglia Antelminelli cave, 141/T/LU, 260m a.s.l., 44.046336°N 10.523525°E, 21.VIII.2018, leg. F. Ballarin and D. Avesani (MSNV). Paratypes. Same locality as the holotype, 1♀, 04.IX.1967, leg. A. Vigna Taglianti (MSNV) (Brignoli 1979, sub *Nesticus speluncarum*); 1♂, 15.VIII.2015, leg. F. Ballarin and M. Gaiga (MSNV); 4♀♀, 24.VI.2017, (3♀♀ collected as juveniles and raised in captivity, adults: 20.VIII.2017, 15.IX.2017 and 28.VI.2018 respectively), leg. F. Ballarin and R. Ballarin (MSNV); 4♀♀, 21.VIII.2018, leg. F. Ballarin and D. Avesani (MSNV, MSNB).
Examined comparative material. 

**Domitius speluncarum** (Pavesi, 1873): **ITALY, Liguria**: 1♂, 1♀, (topotypes), La Spezia Province, Grotta Bocca Lupara cave, 74/Li/SP, 120m a.s.l., 05.III.1969, leg. P.M. Brignoli (MSNV) (Brignoli, 1971); **Toscana**: 1♂, Lucca Province, Garfagnana area, Villa Collemandina Municipality, Canigiano village, Tana di Magnano cave, 162/T/LU, 653m a.s.l., 44.177285°N, 10.38803°E, 03.XI.1967, leg. A. Vigna Taglianti (MSNV) (Brignoli, 1971); 1♀, 01.VIII.1975, leg. unknown (MSNV) (Brignoli, 1985).

**Domitius menozzii** (di Caporiacco, 1934): **ITALY, Liguria**: 1♂, 1♀, (topotypes), La Spezia Province, Grotta Bocca Lupara cave, 74/Li/SP, 120m a.s.l., 05.III.1969, leg. P.M. Brignoli (MSNV) (Brignoli, 1971); **Toscana**: 1♂, Lucca Province, Garfagnana area, Villa Collemandina Municipality, Canigiano village, Tana di Magnano cave, 162/T/LU, 653m a.s.l., 44.177285°N, 10.38803°E, 03.XI.1967, leg. A. Vigna Taglianti (MSNV) (Brignoli, 1971); 1♀, 01.VIII.1975, leg. unknown (MSNV) (Brignoli, 1985).

**Domitius sbordonii** (Brignoli, 1979): **ITALY, Lazio**: 1♂ (holotype), Frosinone Province, Supino, Valle Serena, Grotta della Croce cave, 01.IX.1977 leg. V. Sbordoni (MSNV) (Brignoli, 1979); 1♀ (paratype), 08.II.1976, leg. V. Sbordoni (MSNV) (Brignoli, 1979).

**Kryptonesticus eremita** (Simon, 1880): **ITALY, Liguria**: 2♂♂ (sub. Nesticus menozzii), Creto, Tanna de Fate cave, 17/Li/GE, 30.X.1971, leg. G. Gardini (MSNV); **Emilia-Romagna**: 2♀♀ Ravenna Province, Riole Terme, Borgo Rivola, Grotta del Re Tiberio cave, 36/Er/RE. 19.I.1951, leg. Denis (MSNV) (Zangheri, 1966, sub. Nesticus speluncarum); **Toscana**: 1♀ Garfagnana area, Lucca Province, Coreglia Antelminelli Municipality, Tana delle Fate di Coreglia Antelminelli, Coreglia Antelminelli, Toscana, Italy

**Table 1.** Species, GenBank accession numbers, and locality of the specimens used in the phylogenetic analysis.

| Species Code | COI | 16S | H3 | Locality |
|--------------|-----|-----|----|----------|
| Dbae MF693114 | MF693118 | MF693106 | Cueva del Castillo. Siles, Jaén. Spain |
| Dlq MF693112 | EU746439 | MF693104 | Cueva de la Picona, San Pedro de Carmona, Cabuérniga, Cantabria, Spain |
| Dlus MF693113 | EU746429 | MF693105 | Algar de Marradinhas II, Concelho de Alcanena, Portugal |
| D213 MK860151 | MK860133 | MK860142 | Tana da Suja, Prati di Bavarri, Liguria, Italy |
| D555 MK860152 | MK860134 | MK860143 | Tana delle Fate di Coreglia Antelminelli, Coreglia Antelminelli, Toscana, Italy |
| Dsbo MF693110 | MF693116 | MF693102 | Tana degli orchetti, Supino, Lazio, Italy |
| D557 MK860153 | MK860135 | MK860144 | Tana di Magnano, Canigiano, Lucca, Toscana, Italy |
| N214 MK860154 | MK860136 | MK860145 | Cave of Koufovouno, Didimoticho, Thrace, Greece |
| K566 MK860155 | MK860137 | MK860146 | Dim cave, Antalya, Turkey |
| K211 MK860156 | MK860138 | MK860147 | Grotta di Ponte Subiolo, Mori, Veneto, Italy |
| C162 MK860157 | MK860139 | MK860148 | Small cave along the river, Sighistel, Bihor, Romania |
| C166 MK860158 | MK860140 | MK860149 | Humid and shadowed cliff near Lazaret village, Sibiu, Romania |
| T0bc KF939309 | EU746437 | MF693109 | Cueva del Molino de Aso, Boltana, Huesca, Spain |
| T167 MG201050 | MG200521 | MG201227 | Grotta Pre Oreak, Nimis, Udine, Friuli Venezia Giulia, Italy |
| Tmor KF939311 | KF939308 | ∙∙∙ | Sotterranei del Forte di Vernante, Vernante, Cuneo, Italy |
| G601 MK860159 | MK860141 | MK860150 | Climax cave, Bainbridge, Georgia, USA |

* New Sequences.

Etyymology

The name of the new species is derived from the Etruscan goddess Culsu who, according to the Etruscan mythology, ruled the cave-like entrance of the underworld. Noun in apposition.
Diagnosis

Species closely related to *D. speluncarum* and *D. menozzii*. Males of *D. culsu* sp. nov. can be separated from males of all other Italian species of the genus *Domitius* by the different shape of the apophyses of the para-cymbium (Figs. 1A–C, 2B, C vs. Fig. 4A–I). *D. culsu* sp. nov shows a robust, S-shaped dorsal apophysis 1 in contrast with a large, flat and axe-like Da 1 in *D. speluncarum* (Figs. 1A–C, 2B, C vs. Fig. 4A–C); a short and stumpy Da 1 in *D. menozzii* (Figs. 1A–C, 2B, C vs. Fig. 4D–F) or a long and thread-like Da 1 in *D. sbordonii* (Figs. 1A–C, 2B, C vs. Fig. 4G–I). Additionally, males of *D. culsu* sp. nov. have a well-developed, triangular median apophysis, absent in males of the other three species (Figs. 1A, 2A vs. Fig. 4A, D, G).

Female *D. culsu* sp. nov. can be easily distinguished from female *D. speluncarum* by the narrower, trapezoid-shaped median septum with slanting edges, in contrast with the larger, lobate Ms with rounded edges in *D. speluncarum* (Figs. 1E, 2D vs. Fig. 5A). Female *D. culsu* sp. nov. are separated from female *D. morisii* by the absence of a bulge on the Ms (clearly visible in *D. morisii*, Figs. 1E, 2D vs. Fig. 5C). Additionally, they can be distinguished by the different shape of copulatory ducts when the vulva is observed dorsally: with a rather uniform diameter in *D. culsu* sp. nov. and bearing a large, flattened middle trait in *D. morisii* (Figs. 1G, 2E vs. Fig. 5D). Female *D. culsu* sp. nov. are easily separated from those of *D. sbordonii* by the trapezoid-shaped Ms with slanting edges, in contrast with the squared Ms with vertical edges in *D. sbordonii* (Figs. 1E, 2D vs. Fig. 5E). They can further be distinguished by the different position of spermathecae, located in the lower half of the vulva and below the vulval pockets in *D. culsu* sp. nov., in contrast with S located in the upper half of vulva and above Vp in *D. sbordonii* (Figs. 1G, 2E vs. Fig. 5F).

Figure 1. *Nesticus culsu* sp. nov. Male palp: A. ventral view; B. dorsal view; C. retrolateral view; D. female, cephalic region showing the eye pattern; E. epigyne, ventral view; F. epigyne after clearing, ventral view, the schematic course of internal ducts is outlined with a white line; G. vulva after clearing, dorsal view.
Description. Male (holotype). Total length 4.19. Carapace: 1.81 long, 1.56 wide.

Habitus as in Fig. 3A. Carapace uniformly pale yellow with some sparse setae (more reddish while alive, see Fig. 3A). Cephalic region not clearly differentiated from the rest of carapace. Eyes reduced, AM missing, reduced to black maculae. Eye diameters: AM -, AL 0.079, PM 0.080, and PL 0.078. Thoracic grooves and fovea distinct. Mouthparts and sternum uniformly colored as the carapace. Promargin of chelicera with three teeth approximately of the same size, retromargin with several small denticles. Legs uniformly light yellowish. Legs measurements as follows: I 17.40 (4.89, 0.90, 4.78, 4.90, 1.93), II 14.03 (3.92, 0.80, 3.67, 3.76, 1.88), III 10.35 (3.19, 0.69, 2.53, 2.79, 1.15), IV 13.47 (4.34, 0.84, 3.53, 3.49, 1.27). Leg formula: I, II, IV, III. Opisthosoma gray-yellowish colored (lighter than carapace while alive, see Fig. 3A), covered with long hairs.

Palp as in Figs. 1A–C, 2A–C. Cymbium oval, covered with short, sparse setae, with a tuft of longer hairs in the pro-lateral distal area near the tip. Embolus filamentous, slender in the terminal part. Conductor complex with three distinct processes: Cp 1-3. Cp 1 stocky and roughly triangularly shaped, Cp 2 and Cp 3 located at the distal part of the bulb and diagonally protruding (approx. 2 o'clock seeing the left palp ventrally), their tips curved towards each other (Figs. 1A, C, 2A, C). Median apophysis well-developed, shaped as a long, sharp triangle, heading prolaterally (Figs. 1A, 2A). Paracymbium large with well-developed, sclerotized dorsal, distal and ventral processes. Two dorsal apophyses, Da 1-2: Da 1 robust and long, ending sharply, S-shaped when the palp is observed dorsally, Da 2 stocky, triangularly-shaped. Distal apophysis triangularly shaped. Ventral apophysis lobate, dorso-ventrally flattened and heading toward the cymbium (Figs. 1A–C, 2A–C).

Female (based on 4 paratypes). Total length 3.65–5.27. Carapace: 1.71–1.98 long, 1.54–1.64 wide.

Habitus as in Fig. 3B, C. Carapace uniformly yellowish with some sparse setae (often more reddish while alive, see Fig. 3B). Cephalic region not clearly differentiated from the rest of the prosoma. Eyes reduced, AM strongly reduced and barely visible, reduced to small, dark maculae in some specimens. Eye diameters: AM (when present): 0.032, AL: 0.087, PM: 0.078, and PL: 0.77. Thoracic grooves and fovea distinct. Mouthparts and sternum uniformly colored as in the carapace. Teeth of chelicera as in the male. Legs uniformly light yellowish. Leg measurements as follows: I 17.07 (4.38, 1.01, 4.95, 4.90, 1.83), II 13.69 (4.04, 0.92, 3.57, 3.64, 1.52), III 10.46 (3.43, 0.80, 2.50, 2.56, 1.17), IV 13.89 (4.64, 0.92, 3.59, 3.41, 1.27).
Figure 3. Habitus and type locality of *Domitius culsu* sp. nov. A. Habitus of male; B. habitus of female; C. female with prey; D. juvenile in captivity; E. entrance of Tana delle Fate di Coreglia Antelminelli cave; F. map of the cave and detail of the entrance, showing the spatial distribution of the two co-existing nesticid species living inside: green = *D. culsu* sp. nov., orange = *Kryptonesticus eremita*, arrow = entrance of the cave.
Leg formula: I, IV, II, III. Opisthosoma yellowish-gray (often lighter colored than carapace while alive, see Fig. 3B), covered with long hairs.

Epigyne as in Figs. 1E, F, 2D, E. Median septum short, not protruding, shaped as an inverted trapezoid with a narrower base. Vulval pockets and copulatory ducts externally visible by transparence through the tegument. Copulatory openings at the lower, lateral side of median septum. Vulva as in Figs. 1G, 2E. Spermathecae small and round, located in the lower-half of the vulva, below vulval pockets and being partially covered by them. Vulval pockets wide and rounded, sac-shaped, located above spermathecae. Copulatory ducts with a wider diameter in the ventral trait and narrower in the dorsal trait, rolling up around the lower part of vulval pockets and reaching spermathecae with some turns (Figs. 1F, 2E). Insemination ducts beginning from the lower part of spermathecae and following the same course of copulatory ducts.

Distribution

Italy, endemic to the northern Apennines. Known only from the type locality; Tana delle Fate di Coreglia Antelminelli cave (Fig. 6).
The entrance of Tana delle Fate di Coreglia Antelminelli cave (Italian National Caves Registry number: 141/T/LU; Fig. 3E) opens in the left bank of the narrow valley of Segone Creek in the Province of Lucca (Toscana region) at an elevation of 260 m a.s.l. The cave occurs in the limestone of the Maiolica formation (lower Tithonian–lower Aptian, ~150–120 Ma), which is particularly rich in flint nodules. After an initial steep slope (approximately 10 m deep), the cave continues with a long and sub-horizontal spatial development and a general NW–SE orientation (Fig. 3F). It branches with several, sub-circular tunnels as a result of ancient groundwater flows. The cave has an estimated total extension of 1100 m, although the deeper segments are still unexplored, as they are either filled with water or ending with sumps. The inner section is generally humid, with mud often covering the bottoms of the tunnels. The cave hosts a rich subterranean fauna including some endemic or locally protected species, e.g. the carnivorous land snail *Oxychilus* sp. (Gastropoda, Oxychilidae), the cave cricket *Dolichopoda laetitiae* Minozzi, 1920 (Orthoptera, Rhaphidophoridae), the
blind subterranean beetle *Duvalius apuanus lanzai* Straneo, 1943 (Coleoptera, Trechinae), the Italian cave salamander *Spelomantes italicus* (Dunn, 1923) (Amphibia, Plethodontidae), and three species of bats: the greater horseshoe bat *Rhinolophus ferrumequinum* (Schreber, 1774), the lesser horseshoe bat *Rhinolophus hipposideros* (Bechstein, 1800) (Chiroptera, Rhinolophidae), and the common bent-wing bat *Miniopterus schreibersii* (Kuhl, 1817) (Chiroptera, Miniopteridae). Other animals known from the cave from the literature (Lanza, 1961) or directly observed by the author include: *Octodrilus complanatus* (Dugès, 1828), *O. hemiandrus* (Cognetti, 1901), *O. transpadanus* (Rosa, 1884), and *Aporrectodea rosea* (Savigny, 1826) (Anellida, Lumbricidae); *Chaetophiloscia cellaria* (Dollfus, 1884) (Isopoda, Philosciidae), *Androniscus dentiger* Verhoeff, 1908, and *Spelaenethes mancinii* (Brian, 1913) (Isopoda, Trichoniscidae); *Euscorpius carpathicus* (Linnaeus, 1767) (Scorpiones, Euscorpiidae); *Trogulus* sp. (Opiliiones, Trogulidae), *Ischyropsalis adamii* Canestrini, 1873 (Opiliiones, Ischyropsalididae); *Lithobius tylopus* Latzel, 1882 (Chilopoda, Lithobiidae), *Grillomorpha dalmatina* (Ocskay, 1832) (Orthoptera, Gryllidae), *Hypaena* sp. (Lepidoptera, Noctuidae), *Stenophylax permistus* McLachlan, 1895 (Tricoptera, Limnephilidae), and a large population of limoniid crane flies (Diptera, Limoniidae). Near the entrance and in the early section of the cave, numerous species were also observed: *Amaurobius ferox* (Walckenaer, 1830), *A. pesarinii* Ballarin and Pantini, 2017 (Amaurobiidae), *Kryptonesticus eremita* (Simon, 1880) (Nesticidae), *Meta menardi* (Latreille, 1804), *Metellina meriana* (Scopoli, 1763) (Tetragnathidae), *Pholcus phalangioides* (Fuesslin, 1775) (Pholcidae), and *Tegenaria* sp. (Agelenidae). The new species was found in the initial segments of the cave, but at some distance from the entrance (Fig. 3F). During summer, when the cave was visited, adults, subadults, and juveniles of *D. culsu* sp. nov. were observed together, with a substantially higher number of adults and subadults during the month of August. Most of the juveniles collected in the cave and bred in captivity became adults after 2–3 months of captivity, while it took approximately one year for the youngest specimens to reach sexual maturity.
Two different species of nesticid spiders, *K. eremita* and *D. culsu* sp. nov., were collected together in the Tana delle Fate di Coreglia Antelminelli cave. These species cover a different spatial distribution within the cave (Fig. 3F), coexisting without overlapping despite occupying approximately the same ecological niche. Cohabitant nesticids, in particular involving *D. menozzii* or *D. speluncarum* together with *K. eremita*, have been previously observed in several occasions in Italian caves, and sometimes collected at short distances from each other (Brignoli, 1971). However, no clear species overlap are reported within the same cave. Such distinct spatial partition can be explained by the different grade of adaptation to the hypogean environment showed by these arachnids. In fact, *K. eremita* appears to be a less specialized cave-dweller, lacking extreme morphological adaptations to subterranean life. Therefore, it mostly occurs near the entrance of caves or inside artificial tunnels, including, occasionally, shadowed epigean habitats with constant temperature and high relative humidity (Brignoli, 1971 and personal observations by the author). On the other hand, all *Domitius* species show a greater degree of adaptation to the subterranean habitat, as suggested by reduction of the eyes and body depigmentation. Such strong adaptation allows *Domitius* to occupy deeper segments of the caves, thus avoiding direct competition with *K. eremita*.

Conservation Notes

Since caves are a unique and delicate ecosystem, they are highly susceptible to external disturbance (Culver and Pipan, 2009). Its visible entrance and sub-horizontal extension makes Tana delle Fate di Coreglia Antelminelli cave easily accessible to visitors even with limited experience in speleology, and the cave is often used for training purposes by local speleological clubs. Although not threatened, *D. culsu* sp. nov. should be considered potentially at risk in case of frequent and long-lasting human disturbance due to its strict habitat requirements, its reduced population, and its extremely limited distribution, which appears to be confined to a single cave. Therefore, the new species is a good candidate for species conservation, deserving a place in the list of locally protected species.
Phylogenetic Analysis

A total of 16 nesticid species were used in this study, including representatives of the main nesticid genera present in Europe and all the species distributed in the Italian peninsula. Taxon sampling comprised the wide majority of Domitius species. Only D. murgis (Ribera and De Mas, 2003) from Spain was excluded from the analysis due to the absence of available sequences and fresh samples. The final dataset was formed by 1975 pair bases (bp) distributed as; COI = 1197 bp, 16S = 469 bp, and H3 = 309 bp. The resulting phylogenetic tree is illustrated in Figure 7, and the uncorrected pairwise distance between the species is reported in Table 2. The European nesticids cluster into five different clades corresponding to the main genera Carpathonesticus, Domitius, Kryptonesticus, Nesticus, and Typhlonesticus, each of them highly supported (bootstrap support value = 100%). Each lineage represents a different and well-defined evolutionary line. These results concur with the outcomes of recent morphological and phylogenetic studies on the family Nesticidae (Pavlek and Ribera, 2017; Ribera, 2018; Ballarin and Li, in prep.), supporting the validity of the newly-established genera. According to these results, Domitius represents the sister lineage of the monophyletic clade formed by the genera Carpathonesticus, Kryptonesticus, and Nesticus, with which it shares a common ancestor. The analysis supports Typhlonesticus as a basal clade within the European Nesticidae, as also suggested by recent molecular studies (Ballarin and Li, 2018; Ribera, 2018). Within Domitius, D. culsu sp. nov. shows a closer affinity with the species from the same geographic area; particularly D. speluncarum, but also D. menozzi. Its position at the far end of the phylogenetic tree of the genus suggests a more recent origin in comparison with the other congeneric species.

All the Domitius species distributed in the Northern Apennines share a close affinity with species from the Iberian Peninsula. Such close relations also reflected in genital morphology. For instance, all these species share a similar position of spermathecae, located in the lower-half of the vulva, and below the vulval pockets (see Figs. 1G, 5B, D and Figs. 4A–E in Ribera, 2018). On the other hand, D. sbordonii from the Central Apennines appears to be morphologically and genetically separated from all the other species of the genus, including those from Northern Apennines. The difference is highlighted in the peculiar shape of the vulva, being the only Domitius species showing simple-coiled internal ducts and spermathecae located in the upper-half of the vulva, over the vulval pockets (see Fig. 5F vs. Figs. 1G, 5B, D and Figs. 4A–E in Ribera, 2018). Upper-positioned spermathecae are also present in several other European nesticid genera such as Carpathonesticus (sensu stricto), Kryptonesticus, and Nesticus (sensu stricto). Based on these results

Table 2. Uncorrected genetic p-distance of the COI partial sequence of the nesticid species discussed in the text. The newly described species is in bold.

| No. | Species                        | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 |
|-----|--------------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1   | Dbae_Domitius_baeticus         | 0.142 |
| 2   | Dluq_Domitius_luquei           | 0.142 |
| 3   | Dlus_Domitius_lusitanicus      | 0.135 0.135 |
| 4   | D213_Domitius_menozzii         | 0.140 0.146 0.119 |
| 5   | D555_Domitius_culsu sp. nov.  | 0.167 0.144 0.133 0.121 |
| 6   | Dsbo_Domitius_sbordonii        | 0.181 0.176 0.167 0.181 0.185 |
| 7   | D557_Domitius_speluncarum      | 0.162 0.144 0.121 0.121 0.071 0.190 |
| 8   | N214_Nesticus_cellulanus       | 0.172 0.174 0.151 0.190 0.181 0.176 0.172 |
| 9   | K566_Kryptonesticus_dimensis   | 0.156 0.151 0.135 0.144 0.165 0.172 0.146 0.121 |
| 10  | K211_Kryptonesticus_eremita    | 0.149 0.156 0.142 0.156 0.169 0.169 0.162 0.117 0.078 |
| 11  | C162_Carpathonesticus_fodinarum| 0.169 0.153 0.153 0.176 0.178 0.172 0.167 0.085 0.089 0.108 |
| 12  | C166_Carpathonesticus_lotriensis| 0.144 0.160 0.146 0.162 0.190 0.176 0.190 0.112 0.094 0.101 0.069 |
| 13  | Tobc_Typhlonesticus_obcaecatus | 0.197 0.178 0.167 0.165 0.178 0.176 0.181 0.149 0.156 0.158 0.140 0.142 |
| 14  | T167_Typhlonesticus_idriacus   | 0.181 0.174 0.167 0.183 0.192 0.197 0.185 0.142 0.156 0.165 0.149 0.156 0.096 |
| 15  | Tmor_Typhlonesticus_morisi     | 0.174 0.174 0.174 0.181 0.199 0.208 0.181 0.183 0.160 0.183 0.176 0.174 0.117 0.124 |
| 16  | G601_Gaucelmus_augustinus      | 0.229 0.229 0.227 0.222 0.245 0.243 0.236 0.252 0.211 0.238 0.240 0.233 0.249 0.254 0.247 |
it is possible to speculate that D. sbordonii represents a basal element within the genus Domitius, possibly still carrying the ancestral characters of the older forebear of the European nesticids.

Conclusions

All Italian species of the genus Domitius appear to be highly adapted to a permanent life in the subterranean environment, showing eye reduction and lack of body pigmentation. They further present a localized distribution, with distinct genetic and morphological differences between the species living in the Northern and Central areas of the Apennines. At the same time a close affinity with the species distributed in the Iberian Peninsula is observed. Such features, together with a high genetic p-distance among the species (Table 2), suggests a potentially complex evolutionary history of the genus Domitius that still needs to be properly explored (see also Ribera, 2018).

Because of their apparent similarities in habitus and female genitalia, D. culsu sp. nov has previously been mistaken for D. speluncarum and ignored as a distinct species by previous arachnologists. A detailed molecular and morphological analysis of both sexes of D. culsu sp. nov carried out in this work supports the validity of the new species and its close relationship with the other Domitius species from the same geographical area. Its potential susceptibility to external disturbance, and extremely limited distribution, makes D. culsu sp. nov of interest for conservation.

Finding a new nesticid species in Italy further suggests that our knowledge on the diversity of the family Nesticidae in Southern Europe is still far from complete. Further collections along the Italian peninsula will probably lead to the discovery of other highly-specialized nesticid species allowing a deeper and more precise understanding of the spider cave fauna in Italy and in the Mediterranean area.

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