Advances in the systematics of the spider genus Troglohyphantes (Araneae, Linyphiidae)

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ABSTRACT

With 128 described species and 5 subspecies, the spider genus *Troglohyphantes* (Araneae, Linyphiidae) is a remarkable example of species diversification in the subterranean environment. In this paper, we conducted a systematic revision of the *Troglohyphantes* species of the Italian Alps, with a special focus on the *Lucifuga* complex, including the description of two new species (*T. lucifer* n. sp. and *T. apenninicus* n. sp). In addition, we provided new diagnostic drawings of the holotype of *T. henroti* (*Henroti* complex) and established three new synonymies within the genus. The molecular analysis of the animal DNA barcode confirms the validity of this method of identification of the Alpine *Troglohyphantes* and provides additional support for the morphology-based species complexes. Finally, we revised the known distribution range of additional *Troglohyphantes* species, as well as other poorly known alpine cave-dwelling spiders.

Keywords: cave-dwelling fauna, endemism, taxonomy, Italian spiders, species complexes, DNA barcoding

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INTRODUCTION

The linyphiid spider genus *Troglohyphantes* Joseph, 1881 is presently known to include 128 species and 5 subspecies (World Spider Catalog, 2016), which are generally found in a variety of habitats such as caves, mines, soil litter, rocky debris, and other moistly and shaded retreats (Fage, 1919; Deeleman-Reinhold, 1978; Isaia et al., 2011; Isaia, Lana, & Pantini, 2010). The genus is primarily distributed in the European mountain range, from the Cantabric Mountains in the West to the Balkans and Caucasus in the East. Four additional species are found in Northern Africa (Atlas) and two in the Canary Islands (World Spider Catalog, 2016). The systematics and the distribution of the genus has been studied in a variety of works, with major focus on the Balkan peninsula (Deeleman-Rehinold, 1978), the Pyrenees (Fage, 1919; 1931) and the Alps (Deeleman-Rehinold, 1978; Isaia et al., 2011; Isaia & Pantini, 2010; Pesarini, 1988a, 1988b, 1989, 2001). In general, *Troglohyphantes* species are rare and show narrow distributions. In several cases, they have been reported just from a single or few localities.

Because of the high speciosity of this genus, several authors have proposed to assemble the different species in groups based on either phenetic grounds—overall similarity—or authoritative, non-quantitative, phylogenetic hypotheses. The first classification was proposed by Fage (1919) who sorted 21 species—mainly pyrenaic and alpine—into 5 "*Groupes*" (I–V). Diagnoses were based on morphological characters, mostly pertaining to the morphology of male palps and epygines. In 1978 Deeleman-Reinhold revisited Fage's classification and included 101 species—96 of which are presently valid species—which were classified in three series (A, B and C) according to the epyginal morphology. Each series was further subdivided into 12 groups named after the more representative species and based on male palp morphology. However, especially in series B, boundaries between groups remained vague, sometimes including species of difficult placement. In spite of that, most of the authors describing new *Troglohyphantes* species after 1978, classified them following Deeleman-Reinholds's criteria.

A preliminary attempt to classify Italian species was proposed by Thaler (1967) and Brignoli

(1971). Stemming from these early works, Pesarini (2001) retrieved Deeleman-Reinhold's classification and used it as a baseline to sort the Italian species into 11 "*Complexes*", partly overlapping with the extant classifications. Because of the high diversity of the genus in Italy, Pesarini (2001) further created some specific complexes for the Italian fauna, which included Italian species only.

When considering these three classifications and the work of further authors, more than 80% (109 out of 132) of the genus diversity is indeed classified within at least one of the available diagnostic criteria. The highest number of species is classified according to Deeleman-Reinhold's criteria (99 species, 75%) and covers mostly Alpine and Dinaric species. Fage's classification follows, covering a similar geographic range (88 species, 66%), while Pesarini's mostly focuses on Alpine species (38 species, 28%). Overlaps and geographic coverages of the three classifications are illustrated in Fig. 1 and detailed in Supplementary material Table S1.

However, in the absence of a proper morphological or molecular phylogenetic quantitative evaluation, the delimitations of the species groups remains speculative.

Knowledge of the genus in Italy has grown considerably in the last decades, mainly due to the contributions of Pesarini (1988a, 1988b, 1989, 2001) and studies conducted by our research team (Isaia & Pantini, 2008, 2010; Isaia et al., 2010, 2011; Mammola, Isaia, & Arnedo, 2015; Mammola & Isaia, 2016). However, even from a merely taxonomic standpoint, knowledge of *Troglohyphantes* spiders is far from being exhaustive and it is probable that more species have yet to be described. One of the most speciose group in Italy is Deeleman-Reinhold's *Orpheus* group, which includes nine species from the Alps, one from the Massif Central and two from the Pyrenees. Pesarini (2001) splitted the *Orpheus* group in two complexes—*Lucifuga* and *Orpheus*. The two complexes differ mainly in the general shape of the *lamella characteristica* and have subtle differences in the epyginal structure. The *Orpheus* complex includes four species that exhibit troglomorphic adaptations, such as depigmentation and eye reduction.

In the present study, we focused on the *Lucifuga* complex. We described two new species—*T. lucifer* n. sp. and *T. apenninicus* n. sp.—, provided a detailed overview of the remaining species included in the group to facilitate their identificaiton and proposed several nomenclatural changes. Furthermore we provided new data on species belonging to other complexes (*Caporiaccoi*, *Diurnus*, *Henroti*, *Microcymbium*, *Orpheus*, *Polyophtalmus*, *Ruffoi* and *Sordelli*) and refined their distribution ranges, illustrated the holotype of *T. henroti* and provided new faunistic data on rare, stenoendemic cave-dwelling spiders collected during our recent surveys. Finally, we used a DNA barcoding approach to help in species delimitation and facilitate identification of Italian species.

MATERIAL AND METHODS

Molecular methods

Sequences for the mitochondrial cytochrome *c* oxidase subunit I gene—hereinafter cox1, the Animal DNA barcode —were obtained following the protocols described in Mammola et al. (2015). We were able to sample half of the known diversity of *Troglohyphantes* in Italy (17 out of 37 species). For each species considered in the molecular analysis, we have reported the relative DNA code in Supplementary Material Table S2.

Sequences were edited and managed using Geneious R9 (Kearse et al., 2012). The alignment of the sequences was trivial, as they showed no evidence of indel mutations.

Parsimony analysis of the *cox1* matrix was conducted with TNT v.1.1 (Goboloff, Farris, & Nixon, 2008) using 1,000 iterations of Wagner trees, followed by TBR branch swapping, and clade support assessed with 1,000 Jackknife resampling replicates—removal probability 36%. The best partitioning schemes and substitution models were assessed simultaneously with PartitionFinder v.1.0.1 (Lanfear, Calcott, Ho, & Guindon, 2012) under a Bayesian information criterion (BIC). Maximum Likelihood (ML) analysis was conducted in RAxML v.7.4.2 (Stamatakis, 2006). We inferred the best ML tree and bootstrap support, automatically determining a sufficient number of

bootstrap replicates, using the MRE convergence criteria. Bayesian (BI) analysis was conducted in MrBayes v.3.2 (Ronquist et al., 2012) with two independent runs of 2 million generations with four Markov chains (one cold, three heated), sampling every 1,000 generations. The chain convergence (ASDSF), the correct mixing (EES) and the number of generation to discard as burn-in were monitored with Tracer v.1.6 (Rambaut, Suchard, Xie, & Drummond, 2014). The first 25% of trees in each run were discarded as burn-in. In all analyses the Canarian species *Troglohyphantes oromii* (Ribera & Blasco, 1986) was used as an outgroup to root the trees.

The ability of the animal barcode to identify species in Italian *Troglohyphantes* was investigated by means of distance metrics (Meier, Shiyang, Vaidya, & Ng, 2006), as implemented in the R package SPIDER 1.3 (Brown et al., 2012). Genetic distances were corrected using the Kimura 2 parameters model, as widely applied in Barcoding analyses, and using the R package APE 3.4 (Paradis, Claude, & Strimmer, 2004). First, we used the nearest neighbour criterion to assign a query sequence to the same species as its closest sequences in the reference library. We then inferred the threshold values that minimised the identification error rates—ie. false negatives or false positives—by optimising the SPIDER function *thereshOptm* testing threshold divergence values from 0.1 to 15%. The presence of a barcoding gap was visualized by ploting the maximum intraspecific divergence to the smallest interspecific divergence.

Taxonomy

Except otherwise stated, specimens are stored in 75% ethanol at the Museo Civico di Scienze Naturali “E. Caffi” of Bergamo (Italy). Additional materials are stored in:

i) Marco Isaia’s collection (CI) at Dipartimento di Scienze della Vita e Biologia dei Sistemi, University of Torino, Italy;

ii) Fulvio Gasparo's private collection (CG);

iii) the Natural History Museum of Bern (NHMB);

iv) the Museo Civico di Storia Naturale di Milano (MCSNM);

v) the Muséum National d'Histoire Naturelle de Paris (MNHN).

All specimens were hand collected, except otherwise stated.

We studied materials using a Leica M80 stereoscopic binocular microscope. Illustrations of pedipalps and epigynes were made by Elena Pelizzoli using a *camera lucida*. Paolo Pantini provided the diagrams of the female internal genitalia, using *camera lucida* on cleared epygines. All measurements are in mm. Anatomical terms follow Deeleman-Reinhold (1978).

The summary bibliography, the relevant literature and the synonyms for each of the species here presented refers to the World Spider Catalog (2016).

The toponomastics and classification of the different sectors and sub-sectors of the Alps follows the standard partition of the alpine chain (SOIUSA; Marazzi, 2005). Whenever appropriate/applicable, the speleological cadastrial codes of the caves are given in squared brackets [regional code and number].

The following abbreviations are used in the text: ALE = anterior lateral eyes; AME = anterior median eyes; MSS = *Milieu Souterrain Superficiel* (*sensu* Mammola et al., 2016); PLE = posterior lateral eyes; PME = posterior median eyes; SA = Suprategular apophysis; SSD = Subterranean Sampling Device; SSH = Shallow Subterranean Habitat (*sensu* Culver & Pipan, 2014); TLL = total leg length; TmI = position of trichobothrium on metatarsus of first leg.

RESULTS

Molecular analyses

The new sequences obtained in the present study are available in GenBank® (KT831559–KT831596; see Supplementary materials Tables S2).

We generated 48 sequences of 676 bp of the cox1 DNA barcode, corresponding to 43 unique haplotypes. The mean interspecific genetic divergence (K2P) across the Italian *Troglyphantes* individuals was 16.8% (sd=0.023). The comparison of the minimum interspecific divergences, with the maximum intraspecific divergences revealed the existence of a barcoding gap (Supplementary

Material Fig. S1), with an optimum threshold divergence estimated beetwen 7 and 7.6%. The nearest neighbour criterion resulted in a 100% identification success in species represented by more than one sequence.

The parsimony analysis yielded three trees of 1132 steps. Partitionfinder selected the simple codon—i.e. two partitions 1st and 2nd codon partitions together and 3rd partition apart—as the best partition scheme. The preferred evolutionary model for the 1st + 2nd codon partition was HKY+I+G and for the 3rd codon partition the TrN+G—a GTR was implemented in MrBayes instead. We used unlinked GTR+G models for the RAxML analysis. The results of the analyses conducted under the different phylogenetic inference methods are summarized in Fig. 2. All species showed exclusive haplotypes that formed supported clades. As expected by using a single, highly variable gene, deep relationships were poorly supported and some differed across methods. However, all complexes proposed by Pesarini (2001) were recovered and mostly supported as monophyletic, except for the *Microcymbium* complex, which was recovered as paraphyletic with regards to the *Caporiaccoi*, *Sordellii* and *Henroti* complexes.

TAXONOMIC ACCOUNT

***Lucifuga* complex**

The *Lucifuga* complex so far comprises seven species (Pesarini, 2001, Isaia and Pantini, 2010): *Troglohyphantes albopictus* Pesarini, 1989, restricted to Colli Euganei and Colli Berici—formally outside of the alpine chain, a few km south of the Prealps of Veneto; *T. aldae* Pesarini, 2001 only recorded in the type locality—Asiago plateau, Prealps of Veneto; *T. lucifuga* (Simon, 1884) —from Lanzo Valley to Tessin and Wallis; *T. pluto* di Caporiacco, 1938, endemic to the Corsaglia Valley and high Tanaro Valley—Ligurian Alps; *T. sarae* Pesarini, 2011, endemic to Val d'Aosta; *T. sciakyi* Pesarini, 1989, endemic to the Central Lombard Prealps; *T. subalpinus* Thaler, 1967, restricted to Northern Tyrol and Lower Austria (Fig. 3). Together with the species of the *Orpheus* complex, all species included in the *Lucifuga* complex belong to the *Orpheus* group *sensu* Deeleman-Reinhold

(1978). With *T. subalpinus* (Austria) and *T. lucifuga* (Italy and Switzerland) as the only exceptions, all species included in this complex are Italian endemic.

The complex is characterized by the peculiar dorso-flattened *lamella characteristica*, with the two branches (*sensu* Deeleman-Rehinold, 1978) fused for most of their length. In this complex, the *lamella characteristica* is not entirely visible from a lateral view and requires detachment—or at least bulb expansion. The scape of the epygine is always pedunculated —"*palette*" sensu Fage, 1919. All the species belonging to this group present an abdominal pattern, which is rather reduced in *T. pluto*.

Here we provided comparative plates illustrating the diversity of the species belonging to this complex (Figs. 4, 5, 6, 7, 8, 9). Moreover, we described two new species and proposed two synonymies within the complex. Accordingly, the complex still includes seven species: *T. albopictus*, *T. apenninicus* n. sp., *T. lucifer* n. sp., *T. lucifuga*, *T. pluto*, *T. sciakyi* and *T. subalpinus.*

Troglohyphantes albopictus Pesarini, 1989 (= _T. aldae_ Pesarini 2001, new synonymy)

Figs. 4.1, 5.1, 6.1, 6.2, 7.1, 8.1, 9.1

Material examined

Italy, Veneto, Province of Padova: Rovolon, Colli Euganei, 22.IV.l988, leg. Zanon (male holotype of *T. albopictus;* MCSNM). Province of Vicenza: Valle Scalon (Altopiano di Asiago), in military bunkers, 30.V.1989, leg. Comotti e Baldan (male holotype of *T. aldae*; MCSNM)*;* Mt. Grappa, 10.X.1969 Buche leg. 1♀ 2juv; same locality, 28.IX.1977, Buche leg. 2♂♂; Recoaro, Monti Lessini, Fongara, m 850, 28.IV.1984, Thaler leg. 1♂ 1♀ 1juv; Campodalbero, m 1200, 27.IV.1984 Thaler leg. 2♂♂ 3♀♀ 1juv; Nanto, Colli Berici, 25.IV.1985, Thaler leg. 1♂ 1♀; Schio, Sant'Antonio, Pian delle Fugazze, Rifugio Balasso (1000m), 10.X.1969, 1♂. Trentino Alto Adige, Province of Trento: Borgo Valsugana, Val di Sella, m 900-1200, 30.IX.1977, 2♂♂ 3♀♀ 3juv

Notes

The examination of the holoytype of *Troglohyphantes albopictus*, the material from other localities and the holotype of *T. aldae*, allowed the comparison of *lamella characteristica* (Figs. 6.1, 6.2), suggesting that *T. aldae* Pesarini 2001 is a junior synonym of *T. albopictus* Pesarini 1989.

Accordingly, the range of distribution of *T. albopictus* now comprises Colli Euganei, Colli Berici and the Prealpi Venete—Asiago plateau.

Troglohyphantes apenninicus **Isaia, Mammola & Pantini new species**

Figs. 4.2, 5.2, 6.3, 7.2, 8.2, 9.2, 10

Type series

Holotype. Italy, Toscana, Province of Pistoia: Abetone, 10.66270 E 44.14500 N, 15.X.1975, Thaler leg. 1♂.
Paratypes. Italy, Toscana, Province of Massa: Apuane Alps, Mount Sumbra, 10.27900 E 44.07800 N, 04.VI.1988, Thaler leg. 2 juv Province of Pistoia: Abetone, 10.662700 E 44.14500 N, 15.X.1975, Thaler leg. 4♂♂ 6♀♀ 6juv

Diagnosis

Males of *Troglohyphantes apenninicus* are primarily distinguished from other species of *Troglohyphantes* by the shape of the *lamella characteristica* —better viewed if extracted (Fig. 6.3),—flattened dorso-ventrally, with the external branch ending with a sharp tooth pointed upwards, almost parallel to the longer axis of the external branch. In comparison with the sister species, in *T. albopictus* (Fig. 6.1)*, T. lucifer* n.sp. (Fig. 6.4), and *T. lucifuga* (Figs. 6.5, 6.6) the external branch tapers in a subtriangular apex, bent at the top towards the internal branch. Conversely in *T. apenninicus* (Fig. 6.3)*, T. sciaky* (Fig. 6.8)*, T. pluto* (Fig. 6.7) and *T. subalpinus* (Fig. 6.9) the apex is not bent, being smaller in *T. subalpinus,* pointed and slender in *T. pluto* and enlarged at its base in *T. sciaky*. The shape of the cymbium (Fig. 5) is also diagnostic, ending proximally with three stout apophysis: the internal apophysis is similar to *T. albopictus* (Fig. 5.1) but stouter, the median apophysis is subtriangular—smaller and more pointed than *T. albopictus* and bigger than the other species of the complex,—and the external apophysis is similar to *T. albopictus*.

Females are best diagnosed by the epyginum viewed ventrally, by combining the shape of the scape—rhomboidal—with the margins of the pedunculated part—curved,—although differences

across species of the *Lucifuga* complex can be very subtle (Fig. 7). In *T. apenninicus* n.sp. the epyginal plate forms a rhomboidal scape, narrowed at its base and enlarged distally (Figs. 7.2, 10.3). In comparison with other species, the scape is almost round in *T. albopictus* (Fig. 7.1) and trapeizoidal—more enlarged at the base—in *T. pluto* (Fig. 7.5) and *T. sciaky* (Fig. 7.6). The margins of the proximal part of the scape—connecting the scape to the upper part of the epygyne—are curved, unlike the parallel margins found in *T. albopictus* (Fig. 7.1), *T. lucifer* n. sp. (Fig. 7.3) and *T. lucifuga* (Fig. 7.4). Viewed laterally, the scape appears similar to *T. sciaky* (Fig. 8.6), more arched than in the other species of the complex—especially *T. albopictus* (Fig. 8.1)*, T. lucifer* n. sp. (Fig. 8.3) and *T. lucifuga* (Fig. 8.4). The identification of females in absence of males remains doubtful in most cases.

Description

Male holotype: prosoma 1.37 long, 1.10 wide, yellowish. Thoracic region slightly swollen, yellowish. Cephalic region slightly elevated, interspersed with black bristles between the eyes. Clypeus slightly indented under the eyes, then convex, 0.31 long. Eyes normally developed, with pigment and black margins. AME smallest. PLE as large as PME, ALE slightly larger than PLE. ALE and PLE almost contiguous. PLE –PME distance = 0.03, ALE–AME distance = 0.04, PME–PME distance = 0.06. Eye diameters AME 0.06, PME and PLE 0.07, ALE 0.10.

Sternum heart-shaped, brownish with flimsy darkened anterior edges. Chelicerae 0.65 long, brownish, with ca. 30 lateral stridulatory ridges and armed with three teeth on the anterior side of the chelicerae, and five small, aligned teeth on the internal side. Legs brownish, uniform in colour. Leg I: femur 2.88, patella 0.34, tibia 3.13, metatarsus 2.66, tarsus 1.66, TLL 10.66; leg II: femur 2.69, patella 0.31, tibia 2.72, metatarsus 2.50, tarsus 1.44, TLL 9.66; leg III: femur 2.03, patella 0.31, other articles missing; leg IV: femur 2.81, patella 0.31, tibia 2.69, metatarsus 2.50, tarsus 1.38, TLL 9.69. Abdomen 1.90 long, 1.35 wide; greyish with a dark pattern (Fig. 10.2). Palp (Fig. 10.1): femur 1.28, patella 0.38, tibia 0.31, total palpal length 1.97. Cymbium faintly convex, roughly rectangular when

seen from above, ending proximally with three stout apophysis, the external and the medial subtriangular, the internal subrectangular (Fig. 5.2). Posterior part of paracymbium subtriangular, the apical part gradually narrowed anteriorly. *Lamella characteristica* flattened dorso-ventrally, better visible after extraction (Fig. 6.3). External and internal branches fused over most of their length. The internal branch is attached to the radix, made up of two lobes, the smaller branch is short and rounded and the longer one is enlarged distally and separated from the external branch by a depression. Fickert's gland absent. External branch of the lamella, ending with a sharp tooth, darkened at its end, pointed upwards, almost parallel to the longer axis of the external branch. Distal suprategular apophysis (median apophysis *sensu* Deelman-Reinhold, 1978) directed upwards, with a sharp end. Tip of the embolus spiculate. Spination (Tibia and Metatarsus III absent): femur I–II with one dorsal spine; femur III–IV with no spine. Patella I–IV with one dorsal spine. Tibia I with two dorsal, two prolateral, two retrolateral and two ventral spines. Tibia II with two dorsal, two retrolateral spines, one ventral and one prolateral; Tibia III absent; tibia IV with two dorsal, one retrolateral and one prolateral spine. Metatarsus I, II, IV with one dorsal spine. Patella of the Palp with one long, curved spine. TmI: 0.21. Trichobothrium on Mt IV absent.

Female (paratype from same locality as holotype): prosoma yellowish, 1.31 long, 1.06 wide. Cephalic region grey-yellowish. Carapace, ocular area, clypeus, and sternum similar to the male in all features. Clypeus 0.25 long, chelicerae 0.50 long. Anterior margin of the chelicerae armed with three teeth on the anterior side of the chelicerae, and five small, aligned teeth on the internal side. PLE–PME distance = 0.03, ALE–AME distance = 0.04, PME–PME distance = 0.06, AME–AME almost contiguous, ALE–PLE contiguous. Eye diameters: AME 0.06, PME 0.07, ALE 0.10, PLE 0.09. Abdomen greyish, 1.53 long, 1.18 wide, with a dark pattern. Leg I: femur 2.69, patella 0.38, tibia 2.97, metatarsus 2.22, tarsus 1.41, TLL 9.66; leg II: femur 2.47, patella 0.34, tibia 2.25, metatarsus 2.06, tarsus 0.91, TLL 8.03; leg III: femur 2.06, patella 0.34, tibia 1.56, metatarsus 1.63, tarsus 0.81, TLL 6.50; leg IV: femur 2.59, patella 0.34, tibia 2.50, metatarsus 2.00, tarsus 1.22, TLL 8.66. Female

palp: femur 1.13, patella 0.25, tibia 0.75, tarsus 0.44, total palp length 3.50. Spination: Patella of the palp with one dorsal spine, pedipalpal claw present; Tibia of the palp with one dorsal, two prolateral and one retrolateral spine; Tarsus of the palp with three ventral and two prolateral spines, and one retrolateral spine. Femur I–II with one dorsal spine; femur III–IV with no spine. Patella I–IV with one dorsal spine. Tibia I with two dorsal, two prolateral, two retrolateral and two ventral spines. Tibia II with two dorsal, one ventral, two retrolateral and one prolateral spine; Tibia III with two dorsal, one prolateral and one retrolateral spine; tibia IV with two dorsal, one retrolateral and one prolateral spine. Metatarsus I–IV with one dorsal spine. Position of TmI: 0.21. Trichobothrium on Mt IV absent. Epigynum strongly protruding (Figs. 7, 8). Epyginal plate strongly incised, forming a rhomboidal scape, narrowed at its base, enlarged medially and converging distally. Lateral lobes emerging at the posterior end of the epygine (Figs. 7.2, 10.3). Scape arched from a lateral view (Figs. 8.2, 10.4), covering entirely the inner part of the epygine ("*languette interne*" according to Fage, 1919); stretcher tongue-shaped bent upwards toward the scape, bearing a pitted knob at its end, clearly visible from a ventral point of view. Internal genitalia as in Fig. 9.2.

Etymology

The species epithet derives from the Latin *Apenninum,* the Apennine mountain range, in which the type series was collected.

Distribution

The species is currently known to occur in two localities of the Tuscan Apennines: Abetone (Pistoiese mountains) and Mount Sumbra (Apuan Alps). The species was collected by Konrad Thaler in epigean localities. No indications about the habitat were given.

Troglohyphantes lucifer Isaia, Mammola & Pantini new species

Figs. 4.3, 5.3, 6.4, 7.3, 8.3, 9.3, 11, 12

Type series

Holotype. Italy, Piemonte, Province of Torino: Roure, Tana del Diavolo [Pi 1591], 7.1220669 E 45.0263401 N, 12.IX.2014, Isaia & Mammola leg. 1♂ (CI 2566).

Paratypes. Italy, Piemonte, Province of Torino: Almese, Viù, Colle del Lys, 7.36146 E 45.17751 N, 9.06.2016, Isaia, Mammola & Palermo leg. 3♂♂ 6♀♀ (CI 2883-2884); Gravere, Balma Fumarella [Pi 1597], 7.034559 E 45.125928 N, 13.VI.2014, Mammola & Piano leg. 1♀ (CI 2727).

Material examined

Italy, Piemonte, Province of Torino: Almese, Viù, Colle del Lys, 10.X.1972, Thaler leg. 7♂♂ 9♀♀ 6juv; same locality, same data, Thaler leg. 3♂♂ 5♀♀; same locality, same data, Thaler leg. 1♂ 1♀; same locality, same data, Thaler leg. 7♂♂ 8♀♀ 2juv; same locality, same data, Thaler leg. 4♂ 9♀; same locality (in an abandoned house), 9.VI.2016, Isaia, Mammola & Palermo leg. 1♂ 2♀♀ (CI); same locality (in rocky debris), same data, Isaia, Mammola & Palermo leg. 2♂♂ 4♀♀ (CI); Giaveno, W Forno, 09.X.1972, Thaler leg. 2♂ 7♀ 4juv; Bruzolo, Seinera mineshaft, 30.IX.2016, Isaia & Mammola leg. 1♀ (CI); Novalesa, Grotta del Ghiaccio di Bosconero [Pi 1580], 15.VII.2006, Lana E. leg. 1♂; same locality, 18.II.2016, Isaia & Mammola leg. 2♂♂ 6♀♀ (CI); Mezzenile, Borna Maggiore del Pugnetto [Pi 1501], 17.VI.2006, Isaia leg. 1juv (CI 1033); same locality, 29.I.2010, Isaia leg. 1♀ (CI 1057); Same locality, 17.VII.2015, Mammola & Piano leg. 1♂ 2♀♀ (CI 2725); Mezzenile, Tana del Lupo [Pi 1502], 17.XII.14, Isaia & Mammola leg. 2♂♂ 1♀ (CI 2613); Mezzenile, "Cavernetta 5" [Pi], 11.III.2016, Mammola & Isaia leg. 1♀; Mezzenile, Pugnetto beech forest, 1.VII.2012–1.VII.2013, SSD in MSS 0.60 m deep, Isaia & Piano leg. 4 juv (CI 2381); same locality, same data, SSD in MSS 0.80 m deep, Isaia & Piano leg. 1♀ (CI); Mezzenile, Pugnetto beech forest (leaf litter), 12.IX.2013, Isaia leg. 3♀♀ (CI 2561).

Other material

Italy, Piemonte, Province of Torino: Mezzenile, Borna Maggiore del Pugnetto [Pi 1501] (Isaia *et al.,* 2010, 2011 sub *T. lucifuga*); Mezzenile, Borna Inferiore del Pugnetto [Pi 1502] (Isaia *et al.,* 2010, 2011 sub *T. lucifuga*); Novalesa, Grotta del Ghiaccio di Bosconero [Pi 1580] (Isaia *et al.,* 2010, 2011 sub *T. lucifuga*); Novalesa, Boira dal Farfujet o Balma dei Folletti [Pi 1620] (Arnò & Lana, 2005 sub Linyphiidae indet.; Isaia *et al.,* 2010, 2011 sub *T. lucifuga*).

Diagnosis

Males of *Troglohyphantes lucifer* n. sp. are primarily distinguished from other species of *Troglohyphantes* by the shape of the *lamella characteristica*, better viewed if extracted (Fig. 6.4). The new species is close to *T. lucifuga* and other species of the *Lucifuga* complex, from which it is

distinguishable by the presence of three teeth-like apophysis on the *lamella characteristica* (Fig. 6.4), two on the external branch and one on the internal branch. Compared to the other species, the teeth-like apophysis on the external branch are unique to *T. lucifer* n.sp. and are absent in other species of the complex. The shape of the cymbium, ending proximally with three stout apophysis and rounded at the proximal border (Fig. 5.3), is also diagnostic. Compared to other species, the internal apophysis is long and slender, with parallel margins—i.e. almost rectangular if compared to other species within the group,—with a rounded apex. Females are best diagnosed by the epyginum viewed ventrally (Figs. 7.3, 11.3), although differences across species of the *Lucifuga* complex can be very subtle (Figs. 7, 8). Compared to other species, the epyginal plate is strongly incised, forming a trapezoidal scape (Fig. 7.3). The margins of the proximal part of the scape—connecting the scape to the upper part of the epygyne—are parallel, similar to *T. albopictus* (Fig. 7.1), *T. apenninicus* n. sp. (Fig. 7.2) and *T. lucifuga* (Fig. 7.4). Viewed laterally, the scape appears similar to *T. albopictus* (Fig. 8.1) and *T. lucifuga* (Fig. 8.4), and less arched than the other species of the complex. The identification of females in absence of males remains doubtful in most cases—see also diagnosis of *T. apenninicus*.

Description

Male holotype (CI2566): prosoma 1.25 long, 1.12 wide, yellowish. Thoracic region slightly swollen, yellowish with grey shades. Cephalic region elevated, interspersed with black bristles between the eyes, with few small black bristles forming the eye region and continuing backwards in three longitudinal rows converging at the thoracic furrow. One bristle just below AME. Carapace with darker margins. Clypeus slightly indented under the eyes, then convex, 0.59 long. Eyes normally developed, with pigment and black margins. AME smallest. ALE slightly bigger than PME and PLE. ALE and PLE contiguous. PLE–PME distance = 0.04, ALE–AME distance = 0.04, PME–PME distance = 0.04. Eye diameters AME 0.06, PME 0.07, ALE 0.09, PLE 0.07. Sternum heart-shaped, yellowish with flimsy darkened anterior edges. Chelicerae light brownish, 0.62 long, with ca. 30 lateral stridulatory ridges and armed with three anterior teeth. Legs yellowish, uniform in colour. Leg

I: femur 3.19, patella 0.72, tibia 3.28, metatarsus 2.91, tarsus 1.72, TLL 11.81; leg II: femur 3.00, patella 0.75, tibia 3.13, metatarsus 2.81, tarsus 1.56, TLL 11.25; leg III: femur 2.19, patella 0.41, tibia 1.97, metatarsus 1.72, tarsus 1.09, TLL 7.38; leg IV: femur 2.94, patella 0.66, tibia 2.78, metatarsus 2.66, tarsus 1.38, TLL 10.41. Abdomen greyish with faint pattern (Fig. 11.2), 2.03 long, 1.31 wide. Palp (Fig. 10a): femur 0.63, patella 0.19, tibia 0.19. Cymbium faintly convex, roughly rectangular when observed from above, ending proximally with three stout apophysis, rounded at the proximal border (Fig. 5.3).

Posterior part of paracymbium subtriangular, apical part gradually narrowed anteriorly (Fig. 11.1).

Lamella characteristica similar to *T. lucifuga* (Fig. 6.5, see Isaia *et al.,* 2011: p. 132, fig. 2.51a), flattened dorso-ventrally, better visible from a dorsal view after extraction (Fig. 6.4). External and internal branch fused over most of their length. The internal branch attached to the radix, made up of two lobes, the smaller branch short and rounded and the longer one enlarged distally, bearing on the outer margin two characteristic teeth-like apophysis, darkened at their tips —see also the paragraph on the synonymy *T. sarae* = *T. lucifuga*—: one placed medially, sharp and well defined, tapering, and pointing towards the internal branch; the other shorter, smaller and less pointed, placed in the notch between the latter and the distal apex of the external branch of the lamella. Fickert's gland absent. External branch of the lamella, ending with a sharp tooth, darkened at its end, pointed towards the internal branch, nearly perpendicular to the longer axis of the external branch. Suprategular apophysis directed upwards, with a sharp end (Fig. 11.1). Tip of the embolus spiculate. Spination: femur I with two prolateral spines; Femur II–IV with no spine. Patella I–IV with one dorsal spine. Tibia I with one dorsal, two ventral , and one retrolateral spine; Tibia II with one dorsal, one ventral, one retrolateral and one prolateral spine; tibia III with one prolateral, one dorsal and one retrolateral spine; Tibia IV with one dorsal, two prolateral, and three retrolateral spines. Metatarsus I–IV with one dorsal spine. Patella of the palp with one long, curved spine. TmI: 0.2. Trichobothrium on Mt IV absent.

Female (paratype from Pugnetto CI 2727): prosoma 1.23 long, 1.00 wide, slightly darker than male.

Cephalic region ligh-brownish. Carapace, ocular area, clypeus, and sternum are similar in all features to the analogous male body parts. Dark brown sternum with dark margins. Anterior margin of the chelicerae armed with three teeth. Clypeus 0.23 long, chelicerae 0.47 long. PLE–PME distance = 0.06, ALE–AME distance = 0.04, PME–PME distance = 0.06, AME–AME distance = 0, ALE–PLE distance = 0. Eye diameters: AME 0.04, PME, AME and PLE = 0.07. Abdomen greyish with black pattern (Fig. 12), 2.5 long, 1.87 wide. Leg I: femur 2.50, patella 0.50, tibia 3.22, metatarsus 2.72, tarsus 1.53, TLL 10.47; leg II: femur 2.66, patella 0.56, tibia 2.81, metatarsus 2.50, tarsus 1.38, TLL 9.91; leg III: femur 2.19, patella 0.34, tibia 1.88, metatarsus 1.88, tarsus 0.81, TLL 7.09; leg IV: femur 2.72, patella 0.38, tibia 2.50, metatarsus 2.41, tarsus 1.25, TLL 9.25. Female palp: femur 0.66, patella 0.13, tibia 0.38, tarsus 0.78, total palp length 1.94. Spination (CI 2381): Femur I with one prolateral spine; Femur II–IV with no spine. Patella I–IV with one dorsal spine. Tibia I–II with two dorsal, one prolateral, two ventral and three retrolateral spines; Tibia III with two dorsal, one prolateral and one retrolateral spine. Tibia IV with two dorsal, two ventral and one retrolateral spine. Metatarsus I–IV with one dorsal spine. Patella of the palp with one dorsal spine, pedipalpal claw present; Tarsus of the palp with four dorsal, three retrolateral and three prolateral spines. TmI: 0.2. Trichobothrium on Mt IV absent.

Epigynum strongly protruding, with trapezoidal scape (Fig. 11.3), arched from a lateral view (Fig. 11.4). Scape diverging distally, with a short stem. Tips of the lateral lobes visible in normal position (Fig. 11.3). Epyginal plate incised, forming a trapezoidal scape, enlarged medially and converging distally. Lateral lobes emerging at the posterior end of the epygine (Fig. 11.3). Scape arched from a lateral view (Fig. 11.4), covering entirely the inner part of the epygine ("*languette interne*" according to Fage, 1919); stretcher tongue-shaped almost straight, abruptly bent upwards toward the scape, bearing a pitted knob at its end clearly visible from a ventral point of view. Internal genitalia as in Fig. 9.3.

Etymology

The species epithet derives from the name of the type locality *Tana del Diavolo*—Devil's lair. Lucifer is the classical traditional Jewish-Christian name assigned to the Devil after the interpretation of a Bible verse from Isaiah. More precisely, Lucifer is the name of the Devil before the Fall from heaven "*to the depth of the pit*" (Isaiah 14: 15). The epithet also recalls the previous misidentifications of *Troglohyphantes lucifer* with *T. lucifuga.*

Distribution

The species is known to be found in several localities of a small sector of the Northern Cottian Alps —Viù, Susa and Chisone Valley.

Notes

This species was firstly collected in an unspecified epigean habitat by Konrad Thaler in 1972, in the nearby of Colle del Lys and Giaveno—Cottian Alps. The majority of the recent material listed in this contribution was collected on floors and walls of the twilight zone of natural caves as well as in block fields in beech forests. Further specimens were collected in MSS during recent biospeleological investigations at the hypogean complex of Pugnetto—Mezzenile, Lanzo valley, Graian Alps, North-western Alps. MSS was sampled using Subterranean Sampling Devices (SSD; Domingo-Quero & Alonso-Zarazaga, 2010; López & Oromí, 2010) installed at a depth of 0.40–0.80 m.

Troglohyphantes lucifuga (Simon, 1884) (= _T. sarae_ Pesarini, 2011 new synonymy)

Figs. 4.4, 5.4, 6.5, 6.6, 7.4, 8.4, 9.4

Material examined

<u>Italy, Valle d'Aosta, Province of Aosta:</u> Val di Rhêmes, Vandalettaz, 15.X.2006, Fantoni & De Angelis leg. 1♂ (Paratype of *T. sarae*; MCSNM); Brusson, 09.V.1995, Lana leg. 1♂ 4♀♀ (CI 1055); La Salle, Borna d'la Glace [Ao 2001], 09.IX.1995, Lana leg. 5♂♂, 7♀♀ (CI 1062); same locality, 16.IX.2014; Isaia & Mammola leg 3♂♂ (CI 2567); Petosan, 21.X.2009, Lana leg. 2♂♂, 4♀♀ (CI 1063); St. Rhemy, Fortino presso St.Rhemy, 07.IX.2008, Lana leg. 1♂ 1♀ (CI

1067); Verrogne, Fessura di Verrogne [Ao 2017], 09.IX.1995, Lana leg. 4♂♂ 4♀♀ 1juv (CI 1069); same locality, 16.IX.2014; Isaia & Mammola leg 1♂ (CI 2568); Sarre, Mezz'Abisso [Ao 2071], 26.X.2008, Lana leg. 1♂ 1♀ (CI 1073); Sarre, Grotta della Soldanella [Ao 2072], 26.X.2008, Lana leg. 1♂ 3♀♀ (CI 1074). Piemonte, Province of Verbania: Macugnaga, Moraine of Belvedere glacier (Mount Rosa), 24/09–12.X.2014, pitfall trap, Tampucci leg. 1♂ 3♀♀; Sambughetto, Caverna delle Streghe di Sambughetto [Pi 2051], 01.X.2013, Isaia & Mammola leg. 2♂♂ (CI 2573). Province of Vercelli: Borgosesia, Buco della Bondaccia [Pi 2505], 27.I.2008, Isaia leg. 1♂ 2♀♀ (CI 1054); Valduggia, Bell'Ingresso [Pi 2539], 17.VI.2009, Lana leg. 1♂ (CI 1071); Valduggia, Bocc d'la Mocia [Pi 2541], 17.VI.2009, Lana leg. 1♂ (CI 1070). Province of Novara: Alagna, Alpi Pile, 03.X.1971, Thaler leg. 2♂♂ 3♀♀ 1juv; same locality, same data, Thaler leg. 1♀; same locality, same data, Thaler leg. 3♂♂ 10juv; Alagna, Quarone (Roccapietra), 03.X.1971 Thaler leg. 1♀ 6juv; Province of Biella: Biella, Santuario di Oropa, 11.X.1972, Thaler leg. 5♂♂, 3♀♀. Province of Torino: Brosso, Buca del Ghiaccio della Cavallaria [Pi 1609], 07.XI.2014, Isaia & Mammola leg. 3♂♂ 2♀♀ 3juv (CI 2607); Sparone, Grotta la Custreta [Pi 1593], 07.XI.2014, Isaia & Mammola leg. 2♂ 3♀ (CI 2610); Ceres, Borna del Servais B, 24.IX.2014, Mammola & Paschetta leg. 1♂ 1♀ 2juv (CI 2571); same locality, 14.X.2009, Isaia & Paschetta leg. 1♂ (CI 1061); same locality, 29.IX.2002, Lana leg. 1♂ 1♀ (CI 1077); Ingria, Torrente Soana, 10.X.1972, Thaler leg. 3♂♂, 5♀♀ 1 juv; same locality, same data, Thaler leg. 2♂♂ 5♀♀ 3 juv

Notes

The comparison of specimens of *T. lucifuga* from different localities of Val d'Aosta and Piemonte with type material of *T. sarae* Pesarini 2011—described on specimens from Val di Rhèmes and Valsavaranche, Val d'Aosta—suggests that the latter is a junior synonym of *T. lucifuga*. Specifically, the observation of the detached *lamella characteristica* revealed a clear correspondence between the two species (Figs. 6.5, 6.6). The diagnosis of *T. sarae* was based on the presence of two small and squat teeth on the medial part of the outer margins of the internal and the external branches of the lamella (Pesarini, 2011, p. 65, f. 3). By examining the detached lamella (Fig. 6.5) of different specimens of *T. lucifuga* and comparing them with the type material of *T. sarae* (Fig. 6.6), two weak longitudinal bumps were observed on the dorsal part of the branches of the internal and external lamella, which determine two apparent discontinuities in the profiles of the distal margins of the lamella. Such discontinuities, identical in *T. sarae*, appear like small teeth from a lateral point of view, which may explain Pesarini's wrong diagnosis. On this base, we propose *T. sarae* Pesarini, 2011 as junior synonym of *T. lucifuga* (Simon, 1884).

Troglohyphantes lucifuga is generally found in the outer part of caves, but also in epigean habitats, such as moist shaded places, deep leaf litter and other SSHs. The species is widely distributed in the North-Western Italian Alps—Val d'Aosta and Northern Piemonte. The presence of this species in Switzerland is testified by historical records (Dresco, 1959; Lessert, 1910; Schenkel, 1933) in Wallis (Bourg Saint Pierre and Zermatt) and Tessin (Frasco).

***Troglohyphantes pluto* di Caporiacco, 1938**

Figs. 4.5, 5.5, 6.7, 7.5, 8.5, 9.5

Material examined

Italy, Piemonte, Province of Cuneo: Frabosa Sottana, Balma Ghiacciata del Mondolè [Pi 102], 22.IX.2015, Isaia, Mammola & Ladame leg. 5♀♀ 9 juv (CI 2644); Roccaforte Mondovì, Grotta dei Partigiani della Tura [Pi 286], 11.X.2015 Lana leg. 1♀ (CI); same locality, 22.V.2016, Chesta & Lana leg. 1♂ 1♀; same locality, 9.VIII.2016, Lana leg 1♀ (CI); same locality, 22.VIII.2016, Lana leg 1♀ (CI); same locality, 18.VIII.2016, Giachino & Lana leg 1♂ 2juv (CI); Roccaforte Mondovì, Grotticella della Tura [Pi n.c.], 12.VIII.2016, Chesta & Lana leg. 1♀ (CI); same locality, 22.VIII.2016, Chesta & Lana leg. 1♂ (CI); Roccaforte Mondovì, Plutonis Antrum [Pi n.c.], 16.VII.2016, Chesta & Lana leg 2♂♂ 2♀♀ (CI); same locality, 11.VI.2016, Chesta & Lana leg. 1♂ (CI).

Notes

The species presence was previously recorded in four localities in the Corsaglia valley (Isaia et al., 2011). Remarkbaly, the population of Balma Ghiacciata del Mondolè [Pi 102] is found in the twilight zone of the cave, opening in mount Mondolé at 2,071 m asl—Artesina, Province of Cuneo, Italy. The cave is characterized by a very cool microclimatic condition, sustaining a perennial snowfield near the entrance.

Troglohyphantes sciakyi Pesarini, 1989

Figs. 4.6, 5.6, 6.8, 7.6, 8.6, 9.6

Material examined

Italy, Lombardia, Province of Bergamo: Roncobello, Pozzo del Castello [Lo 1310], 31.XII.1989, Comotti & Baldan leg. 2♂♂ 2♀♀ 2juv; Castione della Presolana, Passo della Presolana, Monte Scanapa (1600m), 29.IX.1971, 4♂♂ 4♀♀ 7juv

Notes

New records for this rare species, previously known to be found in eight localities (Isaia & Pantini, 2010; Pesarini, 1989).

***Caporiaccoi* complex**

The *Caporiaccoi* complex comprises seven Italian species, of which distributions are mostly centred in the Central Lombardian Prealps: *T. caligatus* Pesarini, 1989, *T. caporiaccoi* Brignoli, 1971, *T. comottii* Pesarini, 1989, *T. dominici* Pesarini, 1988, *T. iulianae* Brignoli, 1971, *T. spatulifer* Pesarini, 2001 and *T. zanoni* Pesarini, 1988. *T. caporiaccoi* is the only species in this complex showing troglobiomorphic features (Isaia & Pantini, 2010).

The complex is characterized by small species (total length around 3 mm) bearing a well developed dorsal median process on the cymbium. The epygine, which is very enlarged at the base, has a subtriangular scape ("*clavus*" *sensu* Brignoli, 1971).

Despite its affinity with Deeleman-Reinhold's *Diurnus* group, the complex does not overlap with any of the previous classifications and the species included herein are all Italian endemic.

***Troglohyphantes caligatus* Pesarini, 1989**

Material examined

Italy, Lombardia, Province of Como: between Lasnigo and Barni, m 600, 1.X.1971, 2♂♂ 6♀♀ 3juv; Grotta Tacchi [Lo 2029], Zelbio, 19.V.1985, Comotti & Baldan leg. 6♀♀; Pian del Tivano, Grotta Tacchi, 7.V.2016, Isaia, Mammola, Barzaghi, Manenti & Santinelli leg. 2♂♂ 3♀♀ (CI).

Notes

A rare species for which we provide new records collected in Triangolo Lariano—Province of

Como. The species was known to be found in Monte San Primo (Triangolo Lariano, Italy) (Pesarini, 1989) and Monte Generoso (Switzerland) (Hänggi, 1990).

Troglohyphantes dominici **Pesarini, 1988**

Material examined

Italy, Lombardia, Province of Bergamo: Colzate, pendici Monte Alben, sopra Baite del Sedernello, m 1300, 13.VI.1990, Valle leg. 1♂ 1♀; Gazzaniga, Valle Platz, m 850, XI.1984 Comotti & Valle leg. 1♂.

Notes

New records of this species, of which distribution is centred on the Alps and Prealps of Bergamo.

Troglohyphantes iulianae **Brignoli, 1971**

Material examined

Italy, Liguria, Province of Savona: between Pontinvrea and Giusvalla, m 500, 1.X.1972, 1♂ 5♀♀ 3juv (NHMB). Toscana, Province of Lucca: Castelnuovo, Torrente Turrite Secca, 10.406700 E, 44.108500 N., 16.X.1975, Thaler leg. (NHMB).

Notes

New records of the presence of this species in the Tuscanian Apennines. Previously observed in a few localities in the Ligurian Alps (Brignoli, 1971; Gasparo, 2001; Isaia et al., 2011) and in the Apuan Alps (Pesarini, 2001).

Troglohyphantes zanoni **Pesarini, 1988**

Material examined

Italia, Lombardia, Province of Bergamo: Costa Imagna, Pozzo delle Pozzette [Lo 1372], 25.III.1984, Comotti leg. 1♂ 1♀; Rota d'Imagna, Tomba dei Polacchi [Lo 1003], 1.X.1987, Comotti & Valle leg. 1♂; Villa d'Ogna, m 550, wood, VII-VIII.1985, Pisoni & Valle leg. 2♂♂; Val Taleggio, between Taleggio and Sottochiesa, m 700, 30.IX.1971, Buche leg. 2♂♂ 2♀♀; Treviglio, Parco del Roccolo, m 155, wood, 3.X – 6.XI.2011, pitfall trap, Leoni leg.1♂; same locality, 6.XI – 15.XII.2011, pitfall trap, Leoni leg. 1♂; same locality, 15.III – 19.IV.2012, Leoni leg. 1♂; same locality, 26.VII – 21.X.2012, Leoni leg. 1♀; Province of Lecco: Rongio, Grotta Ferrera [Lo 1502], Barzaghi, Isaia, Mammola & Santinelli leg. 1♂ 4♀♀ (CI).

Notes

This epigean species shows a disjunct distribution in the Prealps of Bergamo-Brescia and Colli Euganei, possibly related to the fragmentation of the original forest of the Po plain (*Quercus-Carpinetum*) (Isaia & Pantini, 2010). This hypothesis is supported by the findings of new populations in a small patch of residual forest in the Po Plain near Treviglio—Parco del Roccolo. Interestingly, there are records of the species presence in the Tomba dei Polacchi cave [Lo 1003], the type locality of *T. caporiaccoi* (*Caporiaccoi* complex). The coexistence of two species of *Troglohyphantes* seems to occur only in distantly related groups, as hypothesized by Deeleman-Reinhold (1978).

***Diurnus* complex**

In Italy, the *Diurnus* complex comprises the troglobiomorphic species *T. sbordonii* Brignoli, 1975 which is found in several localities in Giulie Alps, Giulie Prealps and Carnic Prealps and *T. juris* Thaler, 1982, endemic to the Carnic Prealps. The species of this complex share the features of the homonym Deeleman-Reinhold's group (including three Slovenian species, see Supplementary Material, Table S1), namely the presence of a remarkable dorsal medial apophysis and the simple structure of the cymbium, lacking the two basal divergent processes.

***Troglohyphantes juris* Thaler, 1982**

Material examined

Italy, Friuli Venezia Giulia, Provice of Pordenone: Montereale Valcellina, inghiottitoio Val di Pai [Fr 469], 20.VIII.1987, Comotti leg. 1♂.

Notes

A new record of this rare species, previously found in very few localities (Thaler, 1982; Pesarini, 1989).

***Henroti* complex**

The *Henroti* complex comprises *Troglohyphantes vignai* Brignoli, 1971 (Cottian and Ligurian Alps), *T. nigreaerosae* Brignoli, 1971 (Graian Alps, from Gran Paradiso massif to the Lanzo valleys) and *T. henroti* Dresco, 1956, a French endemic species from Isère and Drôme. The complex entirely overlaps the homonym Deeleman-Reinhold's group. Species within this complex are characterized by a well-developed, simple structure of the external branch of the *lamella characteristica* and by the triangular scape of the epygine, wide and enlarged at the base.

***Troglohyphantes henroti* Dresco, 1956**

Fig. 13

Material examined

France, Drôme Department: Maison Forestiere, Lente, 24 .IX.1947 Negre & Henrot leg. (male holotype; MNHN); Grotté des Feès, Col de la Machine, 02.07.1950 Henrot leg. (female paratype; MNHN).

Notes.

The species can be found in a few localities of the municipalities of Presles (Isère Department) and Bouvante (Drôme Department), both within the Auvergne-Rhône-Alpes region (Dresco, 1956). The species is illustrated partially in Dresco (1956) and Deeleman-Reinhold (1978). Given the lack of complete diagnostic drawings, we provided new illustrations of the male holotype (Fig. 13.1) and the female paratype (Figs. 13.2, 13.3), for future comparative aims.

***Troglohyphantes vignai* Brignoli, 1971**

Material examined

Italy, Piemonte, Province of Cuneo: Frabosa Soprana, Grotta Beppe Bessone (=lo Zucco) [Pi 3303], 22.V.2015, Isaia & Mammola leg. 1♂, 1♀, 1juv (CI).

Notes

The species can be found in Cottian and Ligurian Alps with a remarkable distribution gap in the

Marittime Alps. We here provided a new record of the species presence in the Ligurian Alps—subsection Alpi del Marguareis,—that slightly widens the range of the distribution of this species eastwards. It is worth noticing that the district of Alpi del Marguareis shows the highest diversity of *Troglohyphantes* within the Western Alps—*T. konradi, T. vignai, T. pluto, T. pedemontanus, T. iulianae* and *T. bolognai.*

***Microcymbium* complex**

The *Microcymbium* complex comprises four isolated species, all characterized by very small distribution ranges: *T. microcymbium* Pesarini, 2001 (two caves in Prealps of Bergamo), *T. bornensis* Isaia & Pantini, 2008 (Pugnetto cave complex, Graian Alps), *T. lanai* (Fenera massif, Pennine Alps), and *T. cavadinii* Pesarini, 1989 (two caves in the Prealps of Bergamo).

***Troglohyphantes microcymbium* Pesarini, 2001**

Material examined

Italy, Lombardia, Province of Bergamo: Sant'Omobono, Grotta di Nala di Cà Maquela [Lo 1135], 10.VI.2016, Santinelli, Manenti, Barzaghi leg. 2♀♀, 1 juv (CI); Province of Lecco: Mandello del Lario, Grotta I Ching [Lo 5079], 20.VIII.2008, Aimar leg. 1♂.

Notes

Previously found exclusively in the type locality—Sant'Omobono (BG), Grotta Nala di Ca' Maquela [Lo 1135].

***Orpheus* complex**

The *Orpheus* complex comprises four troglobiomorphic species distributed in SW-Alps: *Troglohyphantes bolognai* Brignoli, 1975 (one cave in Ligurian Alps), *T. bonzanoi* Brignoli, 1979 (one cave in Ligurian Alps), *T. konradi* Brignoli, 1975 (seven caves in Maritime Alps) and *T. pedemontanus* (Gozo, 1908) (three caves in Ligurian Alps). The whole complex is part of

Deeleman-Reinhold's *Orpheus* group, which includes species from the Alps, the Pyrenees, one species from Massif Central and one from Caucasus. The complex gets its name from *T. orpheus* (Simon, 1884), a French endemic species of the departments of Aude, Ariège and Pyrénées-Orientales (Simon, 1929). The distal portion of the *lamella characteristica* is not—or only partially—flattened dorso-ventrally.

According to the nomenclature change here provided, the complex now includes three species: *T. bolognai*, *T. konradi* and *T. pedemontanus.*

Troglohyphantes bolognai Brignoli, 1975 (= _T. bonzanoi_ Brignoli, 1979 new synonymy)

Material examined

Italy, Liguria, Province of Imperia: Pieve di Teco, Sgarbu du Ventu [Li 619], (type locality of *T. bonzanoi*), 27.XII.2014, Isaia & Mammola leg. 1♀ (CI 2600); same locality, 09.XII.2015, Isaia & Mammola leg. 4♂♂, 5♀♀, 3 juv (CI 2645); Badalucco, Tana Bertrand [Li 104] (type locality of *T. bolognai*), 04.IV.2014, Isaia & Mammola leg. 2♀♀, 5 juv (CI 2564); same locality, 27.XII.2014, Isaia & Mammola leg. 1♂ 1♀ (CI 2609).

Notes

This troglobiomorphic species description was based on one female, collected in the Tana di Bertrand cave [Li 104] in Badalucco, Province of Imperia, Liguria (holotype stored at Museum of Verona, P.M. Brignoli's collection, not examined here). Our collection of topotypic material—including the so far unknown male—allowed the comparison with topotypic males of *T. bonzanoi,* a troglobiomorphic species from Sgarbu du Ventu cave [Li 619] (holotype stored at Museum of Verona, P.M. Brignoli's collection, not examined here), a few kilometres away from the type locality of *T. bolognai*. The *lamella characteristica* of the two species was found to be identical. On this base we propose the synonymy *Troglohyphantes bonzanoi* Brignoli, 1979 = *T. bolognai* Brignoli, 1975. The synonymy is also supported by molecular base (see Fig. 2).

***Troglohyphantes konradi* Brignoli, 1975**

Material examined

France, Alpes Maritime: Brigue, Blockhouses in the nearby of Balconi di Marta, 12.VIII.2016, Beikes & Isaia leg. 1♀ (CI).

Notes

This record attest for the first time the presence of this species in France.

***Polyophtalmus* complex**

The *Polyophtalmus* complex includes *Troglohyphantes fagei,* which is widely distributed in the Eastern Alps, the troglobiomorphic *T. scientificus* Deeleman-Rheinold, 1978, endemic to the Giulie Prealps and Giulie Alps, and *T. poleneci* Wiehle, 1964, doubtfully recorded by Pesarini (2001) in Monte Matajur (Giulie Prealps). The complex gets its name from Deeleman-Reinhold's homonym group, which mostly includes Balkanic species (see Supplementary materials Table S1). The female is characterised by the shape of the scape, squat and enlarged at the base. The male paracymbium bears a peculiar "pocket" (*sensu* Deeleman-Reinhold, 1978) and the cymbium lacks dorsal processes.

***Troglohyphantes fagei* Roewer, 1931**

Material examined

Italy, Veneto, Province of Treviso: Susegana, Bus de le Fade [V 1271], m 215, 28.IV.1990, Gasparo leg. 1♂ 1♀ (CG).
Friuli Venezia Giulia, Province of Udine: Torreano, Foran di Landri [Fr 46], m 425, 19.V.1993 Gasparo leg. 1♀ (Gasparo 1997: 20, sub *Troglohyphantes* sp.) (CG).

Notes

Mostly found in epigean habitats in the Austrian and Eastern Italian Alps. Specimens found in Bus de le Fade [V 1271] are characterized by pronounced microphthalmy.

***Ruffoi* complex**

The *Ruffoi* complex comprises *Troglohyphantes ruffoi* di Caporiacco, 1936 (Prealps of Garda Lake, Prealps of Vicenza and Colli Berici) and *T. fatalis* Pesarini, 1988 (Prealps of Belluno and Colli Euganei). Deeleman-Reinhold's included *T. ruffoi* in the *Polyophtalmus* group, but according to Pesarini (2001), *T. ruffoi* and *T. fatalis* have to be considered in a separate complex. Species belonging to this group are characterized by the narrowed epyginal scape of the female and by the presence of two robust spines on the pedipalp tibia of the male. The *lamella characteristica* bears well-developed apical apophysis.

***Troglohyphantes ruffoi* di Caporiacco, 1936**

Material examined

Italy, Veneto, Province of Vicenza: Arsiero, Riofreddo Valley, 4.VI – 27.VI.2001, pitfall trap, Busato leg. 1♂ 2♀♀ (CI).

Notes

New record of this species, found in both epigean and hypogean localities of Southern Trentino and Monti Lessini (Pesarini, 2001).

***Sordellii* complex**

The *Sordellii* complex was firstly defined by Thaler (1967) and then retrieved by Pesarini (2001). It includes four species: *Troglohyphantes sordellii* (Pavesi, 1875) and *T. gestroi* Fage, 1933 (both endemic to the Prealps of Lugano and Lombardian Prealps), *T. lessinensis* (endemic to Lessini Mountains), and *T. regalini* Pesarini, 1989 (recorded in a few caves in the area of the Sebino Bergamasco), the latter being the only troglobiomorphic species within the complex. The complex only include Italian species showing affinities with Deeleman-Reinhold's *Polyophtalmus* group.

This complex is characterized by a sub-rectangular epyginal scape and by the peculiar wrench-shaped *lamella characteristica* and the absence of dorsal processes on the cymbium.

***Troglohyphantes gestroi* Fage, 1933**

Material examined

Italy, Lombardia, Province of Brescia: Altopiano di Cariadeghe, Bus del Budrio [Lo 71], 10.III.2016, Santinelli leg. 1♀.

***Troglohyphantes lessinensis* di Caporiacco, 1936**

Material examined

Italy, Veneto, Province of Vicenza: Cornedo Vicentino, Cereda, Grotta del Cameron, 5.VII.1987, Comotti & Baldan leg. 1♂ 1♀; Monte di Malo, Bus del Soglio [V 172], 5.VII.1987, 2♀♀ 2juv; same locality, 14.VIII.1988, Comotti & Baldan leg. 2♂♂ 1♀ 1juv

Notes

Specimens examined are characterized by a pronounced microphthalmy.

***Troglohyphantes regalini* Pesarini, 1989**

Material examined

Italy, Lombardia, Province of Brescia: Marcheno, Caregno, Caia dell'Angilì [Lo 405], 1.I.1990, Comotti & Baldan leg. 1♂ 4♀♀.

Notes

New record of this eyeless species, previously found in a few caves in the Sebino Bergamasco.

New data on other subterranean spiders

Given the rarity of these species and the general lack of information available in literature, we here provide new data of occurrence of *Turinyphia clairi* (Simon, 1884) (Linyphiidae) and *Typhlonesticus morisii* (Brignoli, 1975) (Nesticidae) gathered during our recent surveys.

***Turinyphia clairi* (Simon, 1884) (Araneae, Linyphiidae)**

Material examined

Italy, Piemonte, Province of Cuneo: Becetto, Pertus dei Drai [Pi 1017], 16.VIII.2015, Isaia & Mammola leg. 1♂ (CI). Liguria, Province of Imperia: Coscio di Arroscia, Garbo di Pian Cavallo [Li 851], 09.I.2016, Isaia & Ladame leg. 1♂ (CI); Monte Ceriana, Military blockhouse, 12.VIII.2016, Beikes & Isaia leg. 4♀♀ 1♂ (CI).

Notes

Rare species, found in caves and other shaded, damp habitats. In the Western Italian Alps, the species was previously found in only six localities (Arnò & Lana, 2005; Isaia et al., 2011). The Palearctic genus of *Turinyphia* includes four species worldwide: *T. clairi* (Southern Europe), *T. maderiana* (Schenkel, 1938) from Madeira, *T. cavernicola* Wunderlich 2008 from Azores and *T. yunohamensis* (Bösenberg & Strand, 1906) from Japan. *Turinyphia* species are regarded by Borges and Wunderlich (2008) as relict taxa and palaeoendemic, which survived in mountain areas and islands.

***Typhlonesticus morisii* (Brignoli, 1975) (Araneae, Nesticidae)**

Material examined

Italy, Piemonte, Province of Cuneo: Chiusa di Pesio, Grotta Superiore delle Camoscere [Pi 250], 15.VII.1987, Comotti & Baldan leg 1♀; Roccaforte Mondovì, Grotta dei Partigiani della Tura [Pi 286], 22.V.2016, Chesta & Lana leg. 1♀ (CI); same locality, 7.VI.2016, Chesta & Lana leg. 2♀♀ 1juv (CI); same locality, 25.VI.2016, Chesta & Lana leg. 1♀ (CI); same locality, 18.VIII.2016, Giachino & Lana leg. 4♂♂ 1♂ (CI); same locality, 22.VIII.2016, Lana leg. 1♂ (CI); Roccaforte Mondovì, Grotticella della Tura [Pi n.c.], 25.VI.2016, Chesta & Lana leg. 1♀; same locality, 12.VIII.2016, Chesta & Lana leg. 2♂♂ 1♀ 2juv (CI). Liguria, Province of Imperia: Triora, Prospetto di Miniera del Monte Grai, 12.VIII.2016, Beikes & Isaia leg. 1♀ (CI). France, Alpes Maritime: Brigue, Balconi di Marta, 12.VIII.2016, Beikes & Isaia leg. 2♂♂ 2♀♀ (CI).

Notes

Troglobiomorphic species previously found exclusively in the type locality—Sotterranei del forte A di Vernante, Opera 11 Tetto Ruinas, Vernante. *Typhlonesticus morisii* shares sister species in Spain (*T. obcaecatus*), south-eastern Alps (*T. idriacus*), Montenegro (*T. absoloni*), and Turkey (*T. gocmeni*) (Nentwig, Blick, Gloor, Hänggi, & Kropf, 2016; Ribera, Elverici, Kunt, & Özkütük, 2014). We here provide several new records from different localities in the Ligurian Alps, including the first French record of this species.

DISCUSSION

DNA barcode analysis

The large diversity of the genus *Troglohyphantes* in Europe prompted the categorization of the species in different phenetic groups, aiming to achieve a better understanding of the distribution patterns and relationships within the genus. Despite being preliminary, our inferred gene tree, based on the Animal DNA barcode, recovers most of the species complexes concerning the Italian species proposed by Pesarini (2001) (Table 1). These results suggest that the morphological characters used to define those complexes are phylogenetically relevant. Moreover, we have shown that the species can be diagnosed based on their genetic barcode, and that DNA barcoding is an effective tool for species identification in this group. The use of DNA barcodes may greately help to increase our taxonomic information on the group and better delimit the distribution range of the species by assigning juveniles or by identifying the presence of the species in environmental samples (Thomsen & Willerslev, 2015).

The gene tree affinities between lineages and the geographic distribution of the species therein, suggest a pattern of multiple cave colonization processes. This hypothesis is supported by the co-occurrence of representatives of different complexes at a sub-regional scale—sometimes also at the same locality. For example, *T. bornensis* (*Microcymbium* complex), *T. lucifer* n. sp. (*Lucifuga*

complex) and *T. nigraerosae* (*Henroti* complex) can be found at short distance from each other within the same valley—Lanzo. Similarly, multiple genetically distant species are present in the Prealps of Bergamo (see Isaia & Pantini, 2010) and in the SW-Alps—Ligurian Alps and Maritime Alps. Therefore, we hypothesize that species communities in *Troglohyphantes* are the result of multiple, independent colonizations, rather than a consequence of local diversification of a single common ancestor . The extant lineages could be interpreted as the result of range contractions and isolation during past climatic changes, followed by population expansions of certain species—i.e. the less troglobiomorphic—during interglacials and other favourable periods. A recent phylogeographic study on *T. vignai* (Mammola et al., 2015) revealed similar patterns, and suggested that long-term climatic changes have been the main cause of the diversification of the genus in the Western Alps.

Because of the high diversity of this genus, the rarity of the species and the use of a single, maternally inherited marker, our conclusions based on the molecular analysis should be considered tentative. A more thorough sampling of the species representing the diversity of the genus, and the use of multiple, unlinked DNA markers will be required to provide a well-supported hypothesis of the phylogenetic relationships within the genus. In this regards, a collaborative project aiming to provide a phylogenetic structure of European *Troglohyphantes* and to investigate the origins and diversity of this remarkable group, is currently under way.

Ecology and natural history of the Italian species

Troglohyphantes spiders are generally associated with dark and humid habitats, such as caves, artificial subterranean habitats and SSHs. Except for the recent study on the ecological niche of *T. vignai* by Mammola and Isaia (2016) and the information provided by Deeleman-Rehinold (1978), little is known about the specific preferences of *Troglohyphantes* spiders.

In this study, we attested for the first time in Italy the presence of *Troglohyphantes* in MSS—i.e., *T.*

lucifer n.sp. This is not surprising, especially considering that troglobiomorphic spiders have been recently documented in a variety of MSS in Europe (e.g., Arnedo, Oromi, Múrria, Macías-Hernández, & Ribera, 2007; Deltshev, Lazarov, Naumova, & Stoev, 2011; Jiménez-Valverde *et al.,* 2015; Laška et al., 2011; Mammola et al., 2016; Nae, 2008; Růžička & Dolanský, 2016; Růžička & Klimeš, 2005; Růžička, Šmilauer, & Mlejnek, 2013; Růžička & Thaler, 2002). Unfortunately, spider research on the MSS in Italy is still in its infancy. It is most likely that extensive investigations will lead to the discovery of additional hidden biodiversity within the genus.

The two new species described in this paper, *T. lucifer* n.sp. and *T. apenninicus* n.sp., can be classified as troglophiles (*sensu* Sket, 2008), having fully developed eyes and abdominal pattern (Figs. 8b, 9b). Troglophile species of *Troglohyphantes* are usually cold adapted (Isaia et al., 2010; Novak et al., 2014), and possess other exaptations to the subterranean conditions—e.g. lucifugous or hygrophilic species.

T. lucifer n.sp. shows a wide ecological plasticity, being able to colonize a variety of habitats, including the twilight zone of caves and various SSHs—leaf litter, deep soil strata, MSS, rocky accumulations. The species is very similar to—and has been frequently confused with—*T. lucifuga,* both regarding morphology and ecological requirements (Isaia et al., 2010, 2011; Isaia & Pantini, 2010). It seems likely that *T. lucifer* represents the ecological vicariant of *T. lucifuga* in the Cottian and Southern Graian Alps. According to our records, in certain localities *T. lucifer* n. sp. is able to coexist with other *Troglohyphantes* spiders. In this regard, Deeleman-Rehinold (1978) suggested that the co-occurrence of more species of *Troglohyphantes* is rare, and may occur exclusively in phylogenetically distant lineages. The coexistence of two unrelated congeneric species in the same cave was already observed in few caves in Slovenia (Deeleman-Rehinold, 1978) and in Croatia (Martina Pavlek, personal communication, June 18, 2016). In the Western Italian Alps, the co-occurrence of species of the *Lucifuga* complex and others is documented for *T. lucifuga* - *T. nigraerosae* (*Henroti* complex), *T. lucifer* n.sp. - *T. vignai* (*Henroti* complex), *T. lucifer* n.sp. - *T. bornensis* (*Microcymbium* complex) and *T. lucifuga* - *T. lanai* (*Microcymbium* complex) (Isaia et al.,

2010, 2011; Isaia & Pantini, 2010; this study).

Since the only known records of *T. apenninicus* are uniquely represented by the type series—collected in unspecified epigean habitats in 1975 and 1988 by Konrad Thaler,—the ecology of *T. apenninicus* n.sp. is still unknown. Unfortunately, no information about the habitat are provided on the original labels.

Supplementary Materials

Table S1. *Troglohyphantes* species listed in WSC (2016). For each species, we report the current taxonomic status, the placement—if any—in the phenetic classifications according to Fage (1919), Deeleman-Reinhold (1978) and Pesarini (2001).

Table S2. List of specimens sequenced in this study with voucher information, DNA code and GenBank® access code.

Figure S1. Barcoding analysis of the Italian species of *Troglohyphantes*. Plot of the maximum intraspecific K2P distance against the minimum interspecific K2P distance (left). Values above the 1:1 line indicate the presence of a barcode gap. Plot of false positives (in blue) and false negatives (in red) against genetic divergences, the optimal thereshold laid between 7 and 7.6% K2P divergence (right).

ACKNOWLEDGEMENTS

This work is dedicated in memory of our estimated colleague and extraordinary natural scientist Angelo Morisi, who recently passed away after a long illness. A special thank goes to Alexandra Jones for proof-reading the paper and to Elena Pelizzoli for the illustrations. The authors would like to thank Benedetta Barzaghi, Sanne Beikes, Mike Chesta Pier Mauro Giachino, Fabio Ladame, Enrico Lana, Raul Manenti, Jacopo Orlandini, Mauro Paschetta, Elena Piano and Roberto Santinelli for fieldwork assistance. We are sincerely grateful to Francesco Tomasinelli for the photographs.

We are indebted to Franco Bianchi, and Martin, Giovanna, Tsojikie, Gerd, and Sanne Beikes for helping us in finding the Tana di Bertrand. Thanks to Bartolomeo Vigna for guiding us to the Balma Ghiacciata del Mondolè. A warm thank goes to Barbara Knoflach, for providing to us specimens of *Troglohyphantes* stored in Thaler's collection. Thanks to Andrea Sabbadini for providing materials stored in the Museo Civico di Storia Naturale (Milano) and to Enrico Lana, Mike Chesta, Sanne Beikes and Gianni Comotti for the craved elusive discovery of the new localities of *Typhlonesticus morisii* in the Ligurian Alps. We are grateful to Gustavo Hormiga, Dimitar Dimitrov and an anonymous referee for helping us for helping with improving the quality of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

REFERENCES

Arnedo, M. A., Oromi, P., Múrria, C., Macías-Hernández, N., & Ribera, C. (2007). The dark side of an island radiation: systematics and evolution of troglobitic spiders of the genus *Dysdera* Latreille (Araneae: Dysderidae) in the Canary Islands. *Invertebrate Systematics, 21*, 623–660. doi: 10.1071/IS07015

Arnò, C., & Lana, E. (2005). *Ragni cavernicoli del Piemonte e della Valle d'Aosta*. Torino: Associazione Gruppi Speleologici Piemontesi.

Borges, P. A. V. & Wunderlich, J. (2008). Spider biodiversity patterns and their conservation in the Azorean archipelago, with descriptions of new species. *Systematics and Biodiversity*, *6*, 249–282. doi: 10.1017/S1477200008002648

Brignoli, P. M. (1971). Note su ragni cavernicoli italiani (Araneae). *Fragmenta entomologica, 7,* 121–229.

Brown, S.D.J., Collins, R.A., Boyer, S., Lefort, M.-C., Malumbres-Olarte, J., Vink, C.J., & Cruickshank, R.H. (2012). Spider: An R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. *Molecular Ecology Resources 12*, 562-565.

Culver, D. C., & Pipan, T. (2014). *Shallow Subterranean Habitats. Ecology, Evolution, and Conservation.* Oxford: University Press.

Deeleman-Reinhold, C. L. (1978). Revision of the cave-dwelling and related spiders of the genus *Troglohyphantes* Joseph (Linyphiidae), with special reference to the Yugoslav species. *Opera Academia Scientiarum et Artium Slovenica (Classis IV) Ljubljana, 23*(6), 1–221.

Deltshev, C., Lazarov, C., Naumova, C., & Stoev, C. (2011). A survey of spiders (Araneae) inhabiting the euedaphic soil stratum and the superficial underground compartment in Bulgaria. *Arachnologische Mitteilungen, 40*, 33–46. doi: 10.5431/aramit4005

Dresco, E. (1956). Note sur quelques araignées cavernicoles du genre *Troglohyphantes* et description d'espèces nouvelles. *Congrès International de Spéléologie I, 3*, 295–300.

Dresco, E. (1959). Catalogue raisonné des araignées et des opilions des grottes du Canton du Tessin (Suisse). *Annales de Spéléologie, 14*, 359–390.

Domingo-Quero, T., & Alonso-Zarazaga, A. M. (2010). Soil and litter sampling, including MSS. *Abc Taxa, 8*(1), 173–212.

Fage, L. (1919). Etudes sur les araignées cavernicoles. III. Sur le genre *Troglohyphantes*. Biospeleologica XL. *Archives de Zoologie Expérimentale et Générale (Biospelogica XL), 58*, 55–148.

Fage, L. (1931). Araneae, 5e série, précédée d'un essai sur l'évolution souterraine et son déterminisme. *Biospeologica, LV. Archives de Zoologie Expérimentale et Générale, 71*, 91–291.

Gasparo, F. (1997). Miscellanea biospeologica. Parte I: Friuli. *Atti e Memorie della Commissione Grotte "E. Boegan", 34*, 17–48.

Gasparo, F. (2001). Note su *Troglohyphantes iulianae*, con descrizione del maschio (Araneae, Linyphiidae). *Fragmenta Entomologica*, *33*, 1–7.

Goboloff, P. A., Farris, J. S. & Nixon, K. C. (2008). TNT, a free program for phylogenetic analysis. *Cladistics, 24*, 774–786. doi: 10.1111/j.1096-0031.2008.00217.x.

Hänggi, A. (1990). Beiträge zur Kenntnis der Spinnenfauna des Kt. Tessin III-Für die Schweiz neue und bemerkenswerte Spinnen (Arachnida: Araneae). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft, 63*, 153–167.

Isaia, M., & Pantini, P. (2008). A new species of *Troglohyphantes* (Araneae, Linyphiidae) from the western Italian Alps. *Journal of Arachnology, 35*, 427–431. doi: 10.1636/H07-16.1

Isaia, M., & Pantini, P. (2010). New data on the spider genus *Troglohyphantes* (Araneae, Linyphiidae) in the Italian Alps, with the description of a new species and a new synonymy. *Zootaxa, 2690*, 1–18. doi: 10.11646/%25x

Isaia, M., Lana, E. & Pantini, P. (2010). Ecology and distribution of the genus *Troglohyphantes* Joseph, 1881 in the Western Italian Alps. In: W. Nentwig, M. Schmidt-Entling, & C. Kropf (Ed.), *European Arachnology 2008* (pp. 89−97). Bern: Natural History Museum.

Isaia, M., Paschetta, M., Lana, E., Pantini, P., Schőnhofer, A. L., Christian, E., & Badino, G. (2011). *Subterranean arachnids of the Western Italian Alps (Arachnida: Araneae, Opiliones, Palpigradi, Pseudoscorpiones).* Torino: Museo di Scienze Naturali.

Jiménez-Valverde, A., Gilgado, J. D., Sendra, A., Pérez-Suàrez, G., Herrero-Borgoñón, J. J., & Ortuño, V. M. (2015). Exceptional invertebrate diversity in a scree slope in Eastern Spain. *Journal of Insect Conservation, 19*, 713−728. doi: 10.1007/s10841-015-9794-1

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., ... Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647-1649. doi: 10.1093/bioinformatics/bts199

Lanfear, R., Calcott, B., Ho, S. Y. W., & Guindon, S. (2012). PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. *Molecular Biology and Evolution, 29*, 1695−1701. doi: 10.1093/molbev/mss020.

Laška, V., Kopecký, O., Růžička, V., Mikula, J., Véle, A., Šarapatka, B., & Tuf, I. H. (2011). Vertical distribution of spiders in soil. *Journal of Arachnology, 39*(3), 393-398. doi: 10.1636/P09-75.1

Lessert, R. de (1910). *Catalogue des invertebres de la Suisse. Fasc. 3, Araignées*. Geneva: Musée d'Histoire Naturelle de Genève.

López, H., & Oromí, P. (2010). A pitfall trap for sampling the mesovoid shallow substratum (MSS) fauna. *Speleobiology Notes, 2*, 7−11.

Mammola, S., & Isaia, M. (2016). The ecological niche of a specialized subterranean spider. *Invertebrate biology, 135*(1), 20−30. doi: 10.1111/ivb.

Mammola, S., Isaia, M., & Arnedo, M. A. (2015). Alpine endemic spiders shed light on the origin and evolution of subterranean species. *PeerJ, 3*, e1384. doi: 10.7717/peerj.1384

Mammola, S., Giachino, P. M., Piano, E., Jones, A., Barberis, M., Badino, G., & Isaia, M. (2016). Ecology and sampling techniques of an understudied subterranean habitat: the Milieu Souterrain Superficiel (MSS). *The Science of Nature*, *103*, 88. doi:10.1007/s00114-016-1413-

9

Marazzi, S. (2005). *Atlante orografico delle Alpi. SOIUSA. Suddivisione orografica internazionale unificata del Sistema Alpino*. Scarmagno: Priuli & Verlucca.

Meier R., Shiyang K., Vaidya G., Ng PK. DNA barcoding and taxonomy in diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology,* 55, 715–728

Nae, A. (2008). Data concerning the Araneae fauna from the aninei mountains Karsik Area (Banat, Romania). *Travail Institutul de Speologie "E. Racovitza"*, *47*, 53−63.

Nentwig W., Blick, T., Gloor, D., Hänggi, A., & Kropf, C. (2016). Spiders of Europe. Retrieved from: www.araneae.unibe.ch

Novak, T., Šajna, N., Antolinc, E., Lipovšek, S., Devetak, D., & Janžekovič, F. (2014). Cold tolerance in terrestrial invertebrates inhabiting subterranean habitats. *International Journal of Speleology, 43*(3), 265−272. doi: 10.5038/1827-806X.43.3.3.

Pantini, P., & Isaia, M. (2016). Checklist of the Italian spiders (Version April 2016). Retrieved from: http://www.museoscienzebergamo.it/

Paradis E., Claude J. & Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289-290

Pesarini, C. (1988a). Due nuove specie di *Troglohyphantes* delle Prealpi Lombarde (Araneae Linyphiidae). *Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, 129*, 89−100.

Pesarini, C. (1988b). Osservazioni su alcuni *Troglohyphantes* della fauna Italiana, con descrizione di due nuove specie (Araneae Linyphiidae). *Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, 129*, 237−247.

Pesarini, C. (1989). Note su alcune specie italiane di *Troglohyphantes* Joseph, con descrizione di sei nuove specie (Araneae Linyphiidae). *Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, 130*, 229−246.

Pesarini, C. (2001) Note sui *Troglohyphantes* italiani, con descrizione di quattro nuove specie (Araneae Linyphiidae). *Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, 142*, 109−133.

Pesarini, C. (2011). *Troglohyphantes sarae*, nuova specie della Val d'Aosta (Araneae, Linyphiidae). *Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, 152*, 63−68.

Rambaut A, Suchard MA, Xie D & Drummond AJ (2014) Tracer v1.6. Retrieved from: http://beast.bio.ed.ac.uk/Tracer

Ribera, C., Elverici, M., Kunt, K. B., & Özkütük, R. S. (2014). *Typhlonesticus gocmeni* sp. n., a new cave-dwelling blind spider species from the Aegean region of Turkey (Araneae, Nesticidae). *ZooKeys, 419*, 87−102. doi: 10.3897/zookeys.419.5739.

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*, 539−542. doi: 10.1093/sysbio/sys029

Růžička, V., & Thaler, K. (2002). Spiders (Araneae) from Deep Screes in the Northern Alps (Tyrol, Austria). *Verein Innsbruck, 89*, 137−141.

Růžička, V., & Klimeš, L. (2005). Spider (Araneae) communities of scree slopes in the Czech Republic. *Journal of Arachnology, 33*, 280−289. doi: 10.1636/04-44.1.

Růžička, V., & Dolanský, J. (2016). Catching of spiders in shallow subterranean habitats in the Czech Republic. *Arachnologische Mitteilungen, 51*, 43−48. doi: 10.5431/aramit5106.

Růžička, V., Šmilauer, P., & Mlejnek, R. (2013). Colonization of subterranean habitats by spiders in Central Europe. *International Journal of Speleology, 42*, 133−140. doi: 10.5038/1827-806X.42.2.5.

Schenkel, E. (1933). Beitrag zur Kenntnis der schweizererischin Spinnenfauna. V. Teil. Spinnen aus

dem Saas-Tal (Wali) und von der Gegend zwischen Trins und Flims (Graubünden). *Revue Suisse de Zoologie, 40*(2), 11−29.

Simon, E. (1929). Les arachnides de France. Synopsis générale et catalogue des espèces françaises de l'ordre des Araneae, 3e partie. *Paris, 6*, 533−772.

Sket, B. (2008). Can we agree on an ecological classification of subterranean animals? *Journal of Natural History*, *42*, 1549−1563. doi: 10.1080/00222930801995762.

Stamakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics, 22*, 2688−2690. doi: 10.1093/bioinformatics/btl446

Thaler, K. (1967). Zum Vorkommen von *Troglohyphantes*-Arten in Tirol und dem Trentino (Arachn., Araneae, Linyphiidae). *Berichte des Naturwissenschaftlich-Medizinischen Vereins in Innsbruck*, *55*, 155−173.

Thaler, K. (1982). Zwei weitere Deckennetzspinnen der Ostalpen: *Troglohyphantes tauriscus* n. sp. und *T. juris* n. sp. (Arachnida: Aranei, Linyphiidae). *Archives des Sciences, Genève, 35*, 161−172.

Thomsen, P.F. & Willerslev, E. 2015. Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation,* 183, 4-18.

World Spider Catalog, (2016). World Spider Catalog. Natural History Museum Bern (Version 16.5). Retrieved from: http://wsc.nmbe.ch

Tables

Table 1. The Italian species of *Troglohyphantes* and their affinities to Pesarini's complexes (2001).

Species	**Pesarini's complexes (2001)**
Troglohyphantes bolognai, T. konradi, T. pedemontanus	*Orpheus*
T. albopictus, T. lucifuga, T. pluto, T. sciakyi, T. apenninicus n.sp., *T. lucifer* n.sp., *T. subalpinus*	*Lucifuga*
T. nigraerosae, T. vignai	*Henroti*
T. juris, T. sbordonii	*Diurnus*
T. bornensis, T. lanai, T. microcymbium, T. cavadinii	*Microcymbium*
T. caligatus, T. caporiaccoi, T. comottii, T. dominici, T. iulianae, T. spatulifer, T. zanoni	*Caporiaccoi*
T. gestroi, T. lessinensis, T. regalini, T. sordellii	*Sordellii*
T. fatalis, T. ruffoi	*Ruffoi*
T. fagei, T. poleneci, T. scientificus	*Polyophtalmus*
T. exul, T. pavesii	*Exul*
T. excavatus	*Croaticus*

Figure captions

Fig. 1. Venn diagram illustrating the extant classifications of the species of *Troglohyphantes* according to Fage (1919), Deeleman-Reinhold (1978) and Pesarini (2001). Numbers in sets indicate the number of species classified according to the different authors and pie charts refer to the geographic coverages of the species included therein. Data used to generate the figure are reported in Supplementary Material, Table S1.

Fig. 2. Maximum likelihood tree. Circles on internal nodes denote support values as follow: upper left: Bayesian posterior probabilities (PP); upper right: maximum likelihood bootstraps (BS), bottom: parsimony jackknifing (PJ). Filled box: PP>95% or BS>75 or PJ>0.75. Grey boxes: clades recovered with support values below former thresholds. Empty sectors: clades not recovered. The tree was rooted using *Troglohyphantes oromii* (Ribera & Blasco, 1986). Pesarini's species groups recovered as monophyletic in dotted boxes.

Fig. 3. Distribution map of the *Lucifuga* complex.

Fig. 4. Male pedipalp—*Lucifuga* complex (E = Embolus; LC = Lamella characteristica; PC = Paracymbium; SA = Suprategular apophysis; Te = Tip of the embolus). (4.1) *Troglohyphantes albopictus.* Male from Arcugnano (VI) (27.3–12.04.2003 Pantini legit); (4.2) *T. apenninicus* n. sp. Holotype male (15.X.1975 Thaler legit); (4.3) *T. lucifer* n. sp. Male from Col del Lys, Almese (TO) (10.X.1972 Thaler legit); (4.4) *T. lucifuga*. Male from Ex-miniera di Cudine, Corio (TO) (Isaia et al., 2011 fig. 2.51A, modified); (4.5) *T. pluto.* Male from [197 Pi/CN] Abisso Artesinera, Frabosa Sottana (CN) (Isaia et al., 2011 fig. 2.63A, modified); (4.6) *T. sciakyi*. Male from Camerata Cornello (BG) (27/8–08.X.2010 Massareo & Zucchelli legit); (4.7) *T. subalpinus.* Male from Styria, Muraztal S Frein (10.X.1973 Buche *legit*). Scale: 0.5 mm. Illustration by Elena Pelizzoli.

Fig. 5. Cymbium of the right palp—*Lucifuga* complex. (5.1) *Troglohyphantes albopictus*. Male from Altopiano d'Asiago (VI) (05–06.1989 Comotti & Baldan legit); (5.2) *T. apenninicus* n. sp. Holotype; (5.3) *T. lucifer* n. sp. Male from Col del Lys, Almese (TO) (10.X.1972 Thaler legit); (5.4) *T. lucifuga*. Male from [2057 Ao/AO] Grotta VM1, Chatillon (15.V.2006 Lana *legit*); (5.5) *T. pluto.* Male from [197 Pi/CN] Abisso Artesinera, Frabosa Sottana (28.VI.2008 Isaia *legit*); (5.6) *T. sciakyi.* Male from Alpe Arera (BG) (17.07.2002 Fabbri, Lodovici & Valle *legit*); (5.7) *T. subalpinus.* Male

from Styria, Muraztal S Frein (10.X.1973 Buche *legit*). Scale: 0.2mm. Illustration by Elena Pelizzoli.

Fig. 6. *Lamella characteristica* (extracted)—*Lucifuga* complex (EB = External branch; IB = Internal branch; Rad= Radix). (6.1) *Troglohyphantes albopictus*. Male from Arcugnago (VI) (27.03–12.IV.2003 MSNB *legit*); (6.2) *T. aldae* = *T. albopictus.* Holotype; (6.3) *T. apenninicus* n. sp. Holotype; (6.4) *T. lucifer* n. sp. Male from Col del Lys, Almese (TO) (10.X.1972 Thaler *legit*); (6.5) *T. lucifuga*. Male from [2057 Ao/AO] Grotta VM1, Chatillon (15.V.2006 Lana *legit*); (6.6) *T. sarae* = *T. lucifuga.* Holotype; (6.7) *T. pluto.* Male from [197 Pi/CN] Abisso Artesinera, Frabosa Sottana (28.VI.2008 Isaia *legit*); (6.8) *T. sciakyi.* Male from Alpe Arera (BG) (17.07.2002 Fabbri, Lodovici & Valle *legit*); (6.9) *T. subalpinus.* Male from Styria, Muraztal S Frein (10.X.1973 Buche *legit*). Scale: 0.25mm. Illustration by Elena Pelizzoli.

Fig. 7. Epigyne, ventral view—*Lucifuga* complex. (7.1) *Troglohyphantes albopictus*. Female from Monti Berici, Nanto (I) (Thaler legit); (7.2) *T. apenninicus* n. sp. Female from S. Abetone (09.1975–10.1975); (7.3) *T. lucifer* n. sp. Female from Col del Lys, Almese (TO) (10.X.1972 Thaler legit); (7.4) *T. lucifuga*. Female from [art. Pi/TO] *ex miniera di Cudine*, Corio (15.V.2006 Lana *legit*) (Isaia et al., 2011: fig. 2.51B, modified); (7.5) *T. pluto.* Female from [197 Pi/CN] Abisso Artesinera, Frabosa Sottana (28.VI.2008 Isaia *legit*) (Isaia et al., 2011: fig. 2.63B, modified); (7.6) *T. sciakyi.* Female from Mare di Burrasca, Colere (BG) (09 .IX.2015 Massaro, Mazzo, Oneto & Pantini *legit*); (7.7) *T. subalpinus.* Female from Styria, Muraztal S Frein (10.X.1973 Buche *legit*). Scale: 0.2mm. Illustration by Elena Pelizzoli.

Fig. 8. Epigyne, lateral view—*Lucifuga* complex. (8.1) *Troglohyphantes albopictus*. Female from Monti Berici, Nanto (I) (Thaler legit); (8.2) *T. apenninicus* n. sp. Female from S. Abetone (09.1975–10.1975); (8.3) *T. lucifer* n. sp. Female from Col del Lys, Almese (TO) (10.X.1972 Thaler legit); (8.4) *T. lucifuga*. Female from [art. Pi/TO] *ex miniera di Cudine*, Corio (15.V.2006 Lana *legit*) (Isaia et al., 2011: fig. 2.51C, modified); (8.5) *T. pluto.* Female from [197 Pi/CN] Abisso Artesinera, Frabosa Sottana (28.VI.2008 Isaia *legit*) (Isaia et al., 2011: fig. 2.63C, modified); (8.6) *T. sciakyi.* Female from Mare di Burrasca, Colere (BG) (09.IX.2015 Massaro, Mazzo, Oneto & Pantini *legit*); (8.7) *T. subalpinus.* Female from Styria, Muraztal S Frein (10.X.1973 Buche *legit*). Scale: 0.2mm. Illustration by Elena Pelizzoli.

Fig. 9. Diagrams of internal female genitalia, aboral view—*lucifuga* group (s = spermathecae; cg =

copulatory groove; fg = fertilization groove). (9.1) *Troglohyphantes albopictus*. Female from Campodalbero, Vicenza (V) (Thaler legit); (9.2) *T. appenninicus* n. sp. Female from S. Abetone (09.1975–10.1975); (9.3) *T. lucifer* n. sp. Female from Col del Lys, Almese (TO) (03.X.1972 Thaler legit); (9.4) *T. lucifuga*. Female from Alpi Pile, Alagna (NO) (03.X.1971 Thaler *legit*); (9.5) *T. pluto.* Female from [197 Pi/CN] Abisso Artesinera, Frabosa Sottana (28.VI.2008 Isaia *legit*) (Isaia et al., 2011: fig. 2.63C, modified); (9.6) *T. sciakyi.* Female from Mare di Burrasca, Colere (BG) (09.IX.2015 Massaro, Mazzo, Oneto & Pantini *legit*). Scale: 0.2mm. Illustration by Paolo Pantini

Fig. 10. *Troglohyphantes apenninicus* n. sp. Holotype male and paratype female (15.X.1975 Thaler *legit*). (10.1) Retrolateral view of male pedipalp (E = Embolus; LC = Lamella characteristica; PC = Paracymbium; SA = Suprategular apophysis; Te = Tip of the embolus). (10.2) Abdominal pattern. (10.3) Epigyne, ventral view. (10.4) Epigyne, lateral view; Scales: a=0.5 mm; b=1 mm; c–d=0.2 mm. Illustration by Elena Pelizzoli.

Fig. 11. *Troglohyphantes lucifer* n. sp. Male and female from Col del Lys, Almese (TO) (10.X.1972 Thaler *legit*). (11.1) Retrolateral view of male pedipalp (E = Embolus; LC = Lamella characteristica; PC = Paracymbium; SA = Suprategular apophysis; Te = Tip of the embolus). (11.2) Abdominal pattern. (11.3) Epigyne, ventral view. (11.4) Epigyne, lateral view. Scales: a=0.5 mm; b=1 mm; c–d=0.2 mm. Illustration by Elena Pelizzoli.

Fig. 12. *Troglohyphantes lucifer* n. sp. Female from [1502 Pi/TO] Borna inf. del Pugnetto, Pugnetto (TO) (11.III.2016 Isaia, Mammola, Piano, Tomasinelli *leg.*). (12.1) Abdominal pattern, dorsal view (12.2–12.4). Live specimen. Photo by Francesco Tomasinelli.

Fig. 13. *Troglohyphantes henroti* Dresco, 1956. Holotype male and paratype female (02.VII.1950 Henrot *legit*). (13.1) Retrolateral view of male pedipalp (E = Embolus; LC = Lamella characteristica; PC = Paracymbium; SA = Suprategular apophysis; Te = Tip of the embolus); (13.2) Epigyne, ventral view. (13.3) Epigyne, lateral view. Scales: 2.1 = 0.5 mm; 2.2–2.3 = 0.2 mm. Illustration by Elena Pelizzoli.