



Two new subterranean *Typhlonesticus* (Araneae: Nesticidae) from the Alps with notes on their ecology, distribution and conservation

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Abstract

The spider genus *Typhlonesticus* Kulczyński, 1914 (Araneae: Nesticidae) includes seven species, most of which exhibiting strict preference for caves, abandoned mines and other subterranean habitats. In Italy the genus is represented by two species: *T. morisii* (Brignoli, 1975), an extremely narrow endemic species from SW-Alps with a very high level of subterranean adaptation and *T. idriacus* (Roewer, 1931), showing a much wider distribution in NE-Italy and poor adaptations to subterranean life. Our recent biospeleological surveys in the Alps lead to the discovery of new populations of highly troglomorphic *Typhlonesticus* that proved to belong to two new species based on morphological and molecular data. Considering the rarity of these new species, we provide general information on their ecology and distribution, including a comparative analysis of troglomorphic traits in *Typhlonesticus* in relation to biogeographic factors. Information on the conservation status, useful for assessing their extinction risk based on International Union for Conservation of Nature (IUCN) guidelines, is also provided.

Keywords

Alpine subterranean fauna, Biogeography, Endemic species, Extinction risk, Systematics, Troglomorphy

1. Introduction

The genus *Typhlonesticus* Kulczyński, 1914 (Araneae: Nesticidae) currently includes seven species of cave dwelling spiders, most of them characterised by a high level of adaptation to subterranean life. Given their habitat preferences, most of the species show narrow distributions, in some cases even point-like. The genus is primarily distributed in Europe with six species, plus

a W-American one that was recently transferred to *Typhlonesticus* by Ribera and Dimitrov (2023) (World Spider Catalog, 2023). As currently defined, the genus *Typhlonesticus* currently lacks an exhaustive morphological diagnosis, being the only available information found in Kulczyński (1914: 379) “Nestico valde affine, differt ab eo defectu oculorum”. A list of characters useful to

delimitate the genus was later provided by Lehtinen and Saaristo (1980: 50), who designated *T. absoloni* Kratochvíl, 1933 as type species.

According to the latest overview of European subterranean spiders (Mammola et al. 2018a), all European species of *Typhlonesticus* are obligate troglobionts, being exclusively found in subterranean habitats. This ecological preference parallels a suite of morphological and physiological adaptations to the subterranean environment (i.e., troglomorphism; see Christiansen 2012) pertaining, among others, size, leg elongation, loss of pigmentation and eye regression. As for *Typhlonesticus*, troglomorphism is particularly developed in *T. absoloni* (Kratochvíl, 1933), *T. obcaecatus* (Simon, 1907), *T. parvus* Kulczyński, 1914, *T. gocmeni* Ribera, Elverici, Kunt and Özkütük, 2014 and *T. morisii* (Brignoli, 1975), while in *T. idriacus* (Roewer, 1931) and *T. silvestrii* (Fage, 1929) troglomorphic characters are much less pronounced.

In the Alps the genus is represented by *T. morisii* and *T. idriacus*. *T. morisii* was described on material collected in the 1970s by Angelo Morisi in the military bunker of Vernante (Province of Cuneo, Piemonte, SW-Alps), an artificial subterranean site renowned among local biospeleologists for hosting a remarkable assemblage of highly specialised subterranean species and for being the type locality of several endemic species of troglobiont arthropods (Isaia et al. 2011). According to the original description (Brignoli 1975: 29), *T. morisii* exhibits fully depigmented and barely visible eyes (“occhi depigmentati, appena visibili”). The species remained unrecorded until the 2000s, when it was repeatedly re-collected at the type locality (Arnò and Lana 2005; Isaia et al. 2007, 2011). In 2017 we reported five new records (Isaia et al. 2017) in different localities across the SW-Alps, widening the species range to the Province of Imperia (Liguria) and to the Department of Alpes Maritimes (France). Compared to *T. morisii* and other European species, the other Italian *Typhlonesticus*, *T. idriacus*, shows less pronounced subterranean adaptations and a much wider range, covering an area from Garda Lake in the West to Trieste (NE-Italy), Idrija (NW-Slovenia) and Carinthia (Austria) in the East (Pantini and Isaia 2019). The species is currently recorded in 14 localities.

The finding of a female belonging to an undescribed species of *Typhlonesticus* in Central Alps and the first results of a phylogenetic analysis including material of *T. morisii* stimulated our thorough biospeleological searches in the Prealps of Bergamo (Lombardia) and a detailed re-examination of the material of *Typhlonesticus* available in MI and MCSNB collections.

As a result, we here provide the description of two new species of *Typhlonesticus* and a comparative analysis of troglomorphic traits in relation to range size and altimetric range within the genus. In parallel, considering the rarity of *Typhlonesticus* species in Italy, we also provide some general information about the ecology and conservation status of the new species, including details useful for the assessment of their extinction risk according to the Red List Categories and Criteria of the International Union for Conservation of Nature (IUCN 2001, 2012).

2. Methods

2.1. Molecular analysis

2.1.1. Sampling, DNA extraction, PCR amplification and sequencing

Our analysis includes all currently accepted species of the genus *Typhlonesticus* along with representatives of all European genera of Nesticids that were used as outgroups. All specimens of the new species were collected in the field, preserved directly in 96% or absolute ethanol and stored at -20°C . Total genomic DNA was extracted from legs or thorax tissue using the E.Z.N.A.® Tissue DNA Kit (V-Spin) (Omega Bio-tek) following the manufacturer protocol. DNA concentration was measured by fluorimetry (Qubit HS dsDNA Assay, Thermo Fisher Scientific, CA). Partial fragments of five genes, two mitochondrial – cytochrome c oxidase subunit I (cox1) and 16S rRNA (16S) – and three nuclear – 18S rRNA (18S), 28S rRNA (28S) and Histone H3 (H3) – were amplified and sequenced using the primers listed in Supplement Material Table S1.

PCR reactions were conducted at a final volume of 20 μL using MyRed Taq Polymerase (Bioline Inc). PCR products were cycle-sequenced in both directions using the same PCR primers. The sequencing was performed at the Macrogen sequencing centre in Madrid. Chromatograms were edited and assembled with Geneious v. 10.0.9 (<https://geneious.com>). All newly generated sequences were deposited in GenBank (see Supplement Material Table S2 for accession numbers).

2.1.2. Alignment and evolutionary model selection

The alignment of cox1 and H3 was trivial, given that the amplified fragments showed no insertions/deletions and hence the sequences were adjusted manually. We translated sequences into amino acids and checked for stop-codons to avoid sequencing/editing errors. For the ribosomal gene fragments, we performed alignments using the online version of Mafft (Katoh and Toh 2008) using the G-INS-i algorithm with default options.

The best partitioning schemes and substitution models for RAxML were explored using PartitionFinder v.1.0.1 (Lanfear et al. 2012). We partitioned data by codon position (in the case of the two protein coding genes) and explored the best partitioning scheme and substitution models simultaneously using the All heuristic algorithm. Bayesian information criterion was used to select the best partition scheme and the corresponding best fit models.

2.1.3. Phylogenetic analyses

Maximum likelihood analyses were carried out in the computer program RAxML 7.4.2 (Stamatakis 2006) through the graphical front-end RAxML-GUI 1.3 (Silvestro and Michalak 2012) applying the partition scheme

and models of molecular evolution selected by Partition-Finder. A GTR+G+I nucleotide substitution model was applied to each of the partitions corresponding to the best partition scheme selected. Nodal support was evaluated using the maximum likelihood plus thorough bootstrap in 10 runs with 1000 replicates. The graphical phylogenetic tree was generated and edited with FigTree v1.3.1 software (<http://tree.bio.ed.ac.uk/software/figtree>).

2.2. Taxonomy

2.2.1. Taxon sampling and morphological study

Specimens of the new *Typhlonesticus* used in the morphological study were collected in the field and preserved directly in 70% ethanol. Photographs are multifocus Z-stack images taken with a Flexacam C1 camera mounted on a Leica Stereozoom S8 APO stereoscopic binocular microscope. Specimens were examined and measured using a Leica M80 stereoscopic binocular up to 60x magnification connected to an EC3 camera. All measurements are given in mm.

The female vulva was removed and treated with 10% KOH prior to examination. After observation and drawings, the vulva was washed in acetic acid (5%) and successively stored in 70% ethanol in a micro-vial in the same tube containing the specimen. All illustrated male structures are from the left side. We follow Coyle and McGarity (1992) for describing the paracymbium and Huber (1993) and Agnarsson et al. (2007) for other parts of the male and female copulatory organs.

Measurements of troglomorphic traits (diameter of anterior median eyes and femur elongation) are issued from Mammola et al. (2022) and verified both on original publications and on original material available in MI, CRBA and MCSNB collections. Femur I elongation is measured as the ratio of the length of the femur I to the body size of females.

2.2.2. Depository

Holotypes are deposited at the Museo Civico di Scienze Naturali “E. Caffi” (Bergamo, Italy) (MCSNB), paratypes and other materials are stored at Marco Isaia’s collection at the Department of Life Science and Systems Biology of the University of Torino (CI). In addition, we examined the holotype of *T. morisii*, stored at Museo di Storia Naturale di Verona (MSNV), and material of *T. idriacus* stored at Centre de Recursos de Biodiversitat Animal (CRBA).

2.2.3. Conservation and biogeography

The description of the conservation status and the basic information for the IUCN Red List assessment follows for the Red List Categories and Criteria of the International Union for Conservation of Nature (IUCN 2001, 2012).

The calculation of the Extent of Occurrence (EOO) and altimetric range of all *Typhlonesticus* species (Table 1) is based on available occurrences found in literature (see table legend), successively elaborated in GIS environment. Speleological cadastral codes of the caves are shown in squared brackets [‘regional code’ and ‘number’], and coordinates of localities are given in decimal degrees (WGS84 datum). We refer to SOUISA (Marazzi 2005) for the geographic classification of the Alps.

The EOO and the altimetric range were both log-transformed to stabilise variance and related to troglomorphic traits (body size, Femur I elongation and diameter of anterior median eyes) via linear models in R environment.

2.2.4. Abbreviations used in text and illustrations

AER = anterior eye row; **ALE** = anterior lateral eyes; **AME** = anterior median eyes; **AOO** = Area of Occupancy; **CI** = Marco Isaia’s collection, University of Torino; **CRBA** = Centre de Recursos de Biodiversitat Animal, Universitat de Barcelona; **dp** = dorsal process of paracymbium; **e** = embolus; **EOO** = Extent of Occurrence; **FE** = femur; **id** = insemination duct; **ma** = median apophysis; **MCSNB** = Museo Civico di Scienze Naturali “E. Caffi” (Bergamo, Italy); **Me** = metatarsus; **MI** = Marco Isaia’s collection at the Department of Life Science and Systems Biology of the University of Torino; **MSNV** = Museo di Storia Naturale di Verona; **Pa** = patella; **PER** = posterior eye row; **PLE** = posterior lateral eyes; **PME** = posterior median eyes; **p** = paracymbium; **p1** = process 1 of the conductor; **p2** = process 2 of the conductor; **s** = spermatheca; **st** = subtegulum; **t** = tegulum; **Ta** = tarsus; **Ti** = tibia; **vp** = ventral process of paracymbium.

Illustrations were prepared by Alessandro Infuso directly on specimens observed under the stereomicroscope.

3. Results

3.1. Molecular data

Specimens, localities and GenBank accession numbers of the sequences used in this study are listed in Table S2. The concatenated matrix used in the analyses consisted of 18 terminals and 3430 aligned characters (cox1 = 930, 16S = 489, H3 = 317, 18S = 820 and 28S = 874).

The best partitioning scheme selected with Partition-Finder under the BIC criterion includes four partitions: cox1 1st and 2nd positions, 16S, 18S and 28S; cox1 3rd positions and H3 1st positions; H3 2nd positions; H3 3rd positions.

3.2. Maximum likelihood analysis

Figure 1 shows the maximum likelihood tree obtained with the combined data matrix. The new species cluster

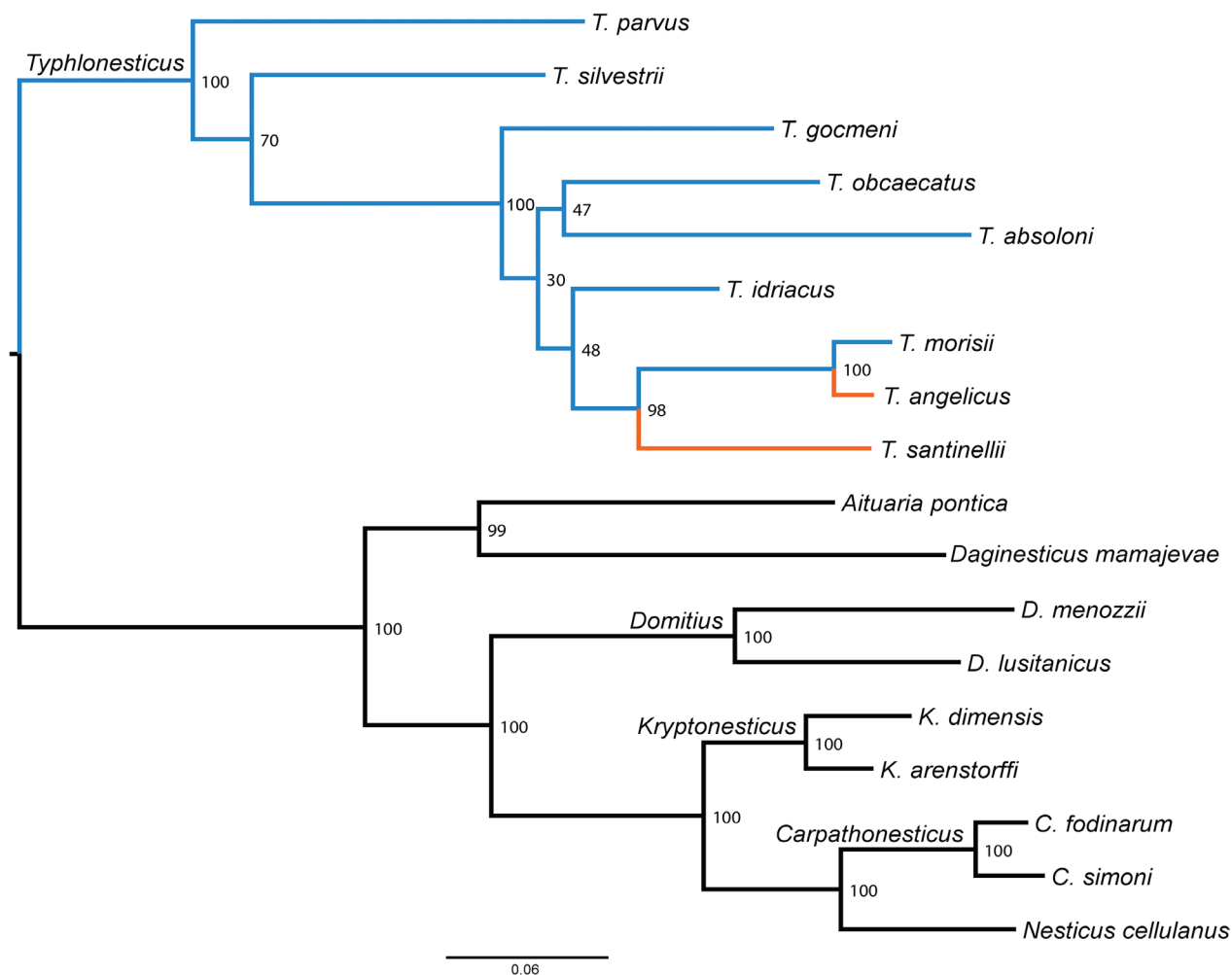


Figure 1. ML tree inferred using the concatenated data matrix of *cox1* and *rrnL* mtDNA, and H3, 18S and 28S nuDNA gene fragments. Numbers next to nodes correspond to bootstrap support values. Scale bar: units in nucleotide substitutions per site.

between the two geographically closest species, *Typhlonesticus morisii* and *T. idriacus*, being *T. angelicus* **sp. nov.** the sister species of *T. morisii* (bootstrap support 100%) and *T. santinellii* **sp. nov.** the sister species of both (with a bootstrap support of 98%). *T. absoloni*, *T. obcaecatus* and *T. idriacus* are poorly supported (low bootstrap supports), while the set of these seven species constitutes a highly supported evolutionary lineage (bootstrap support = 100).

3.3. Taxonomy

Family Nesticidae Simon, 1894

Genus *Typhlonesticus* Kulczyński, 1914

3.3.1. *Typhlonesticus angelicus* Isaia and Ribera, **sp. nov.**

<https://zoobank.org/3B2E78D4-7FC3-4006-932D-364240633BA0>

Figs 2A–E, 5A, C, 7A

Examined material. Holotype: ITALY, Piemonte, Province of Cuneo: Chiusa di Pesio, Grotta Superiore delle Camoscere [Pi 250], 44.2183°N, 7.6605°E (Fig. 7D), 1200 m a.s.l., 3.XII.2018, Isaia M. and Mammola S. leg. 1f (MCSNB) (sub “*Typhlonesticus morisii*” in Isaia et al. 2017: 323). — **Paratypes:** same locality as holotype, 15.VII.1987, Comotti G. and Baldan A. leg. 1f (MCSNB) • 3.XII.2018, Isaia M. and Mammola S. leg. 1 juv. (CI) • **Liguria, Province of Imperia:** Triora, Prospetto di Miniera del Monte Grai, 43.99650°N, 7.67296°E, 1878 m a.s.l., 12.VIII.2016, Beikes S. and Isaia M. leg. 1f (CI) • Roccaforte Mondovì, Grotta dei Partigiani della Tura [Pi 286], 44.24081°N, 7.73308°E, 1780 m a.s.l., 18.VIII.2016, Giachino P.M. and Lana E. leg. 1m • **FRANCE, Alpes Maritimes:** Brigue, Bunker Opera 34, Balconi di Marta, 44.01534°N, 7.64178°E, 1914 m a.s.l., 12.VIII.2016, Beikes S. and Isaia M. leg. 1m, 2juv. (CI) (all material cited (sub “*Typhlonesticus morisii*” in Isaia et al. 2017: 323)). — **Other material: ITALY, Piemonte, Province of Cuneo:** Roccaforte Mondovì, Grotta dei Partigiani della Tura [Pi 286], 44.24081°N, 7.73308°E, 1780 m a.s.l., 22.V.2016, Chesta M. and Lana E. leg. 1f (CI) • same locality, 7.VI.2016, Chesta M. and Lana E. leg. 1m, 2f, 1 juv. (CI) • same locality, 25.VI.2016, Chesta M. and Lana E. leg. 1f (CI) • same locality, 18.VIII.2016, Giachino P.M. and Lana E. leg. 3m, 1juv. (CI) • Roccaforte Mondovì, Grotticella della Tura [Pi n.c.], in the vicinity of Pi 286, 25.VI.2016, Chesta M. and Lana E. leg. 1f (CI) • same locality, 12.VIII.2016, Chesta M. and Lana E. leg. 2m, 1f, 2 juv. (CI) (all material cited sub “*Typhlonesticus morisii*” in Isaia et al. 2017: 323).

Diagnosis. Males of the new species differ from *Typhlonesticus morisii* by the shape and the arrangement of the paracymbial apophyses, mainly the ventral one, which is straight in *T. angelicus* **sp. nov.** (“vp” in Fig. 2D, E) and distally bent in *T. morisii* (Fig. 4D, E). Subtle differences are also found in the shape of p1 and p2 processes (Figs 2D, 5C) and the shape of the median apophysis (“ma” in Figs 2D, 5C). — Females of *T. angelicus* **sp. nov.** are best diagnosed by the orientation of the insemination ducts (Figs 2A, B, 5A), not twisted and connecting directly to

the spermathecae ventrally rather than dorsally, such as in *T. morisii* (Figs 4A, B, 5H).

Description of the female. Female holotype from Grotta superiore delle Camoscere [Pi 250], 3.XII.2018, Isaia M. and Mammola S. leg. — **Coloration:** Carapace pale yellow. Head region of the same color. Sternum yellow brown, without pattern. Opisthosoma grey-white without pattern. Legs same color as carapace. — **Prosoma:** Carapace 1.70 long, 1.42 wide, cephalic region not differen-

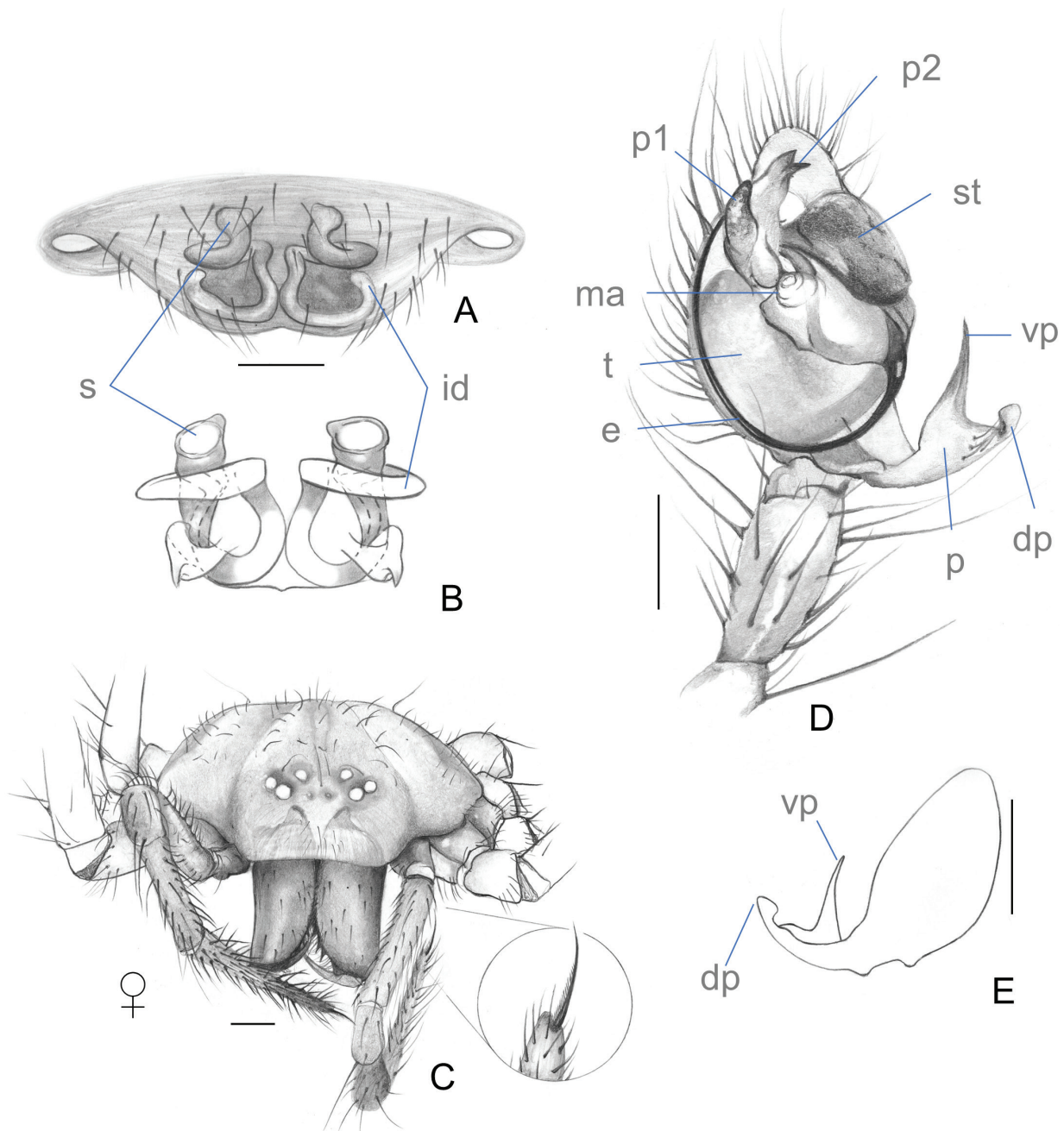


Figure 2. *Typhlonesticus angelicus* **sp. nov.**, holotype female from Chiusa di Pesio, Grotta Superiore delle Camoscere [Pi 250] (Isaia M. and Mammola S. leg., 3.XII.2018) (A, B, C) and paratype male from Grotta dei Partigiani della Tura [Pi 286] (Giachino P.M. and Lana E. leg., 18.VIII.2016) (D, E). Epigyne in ventral view (A), vulva in dorsal view (B), female cephalothorax in frontal view (C), left male pedipalp in ventral view (D) and male cymbium in dorsal view (E) (see section 2.2.3. for abbreviations). Scale bars 0.2 mm.

tiated from the rest of the carapace. Clypeus height under AME 0.302. Labium as long as wide or moderately wider than long. Sternum 0.97 long, 0.86 wide. Eyes reduced and depigmented, no corneal lenses are visible. Both eye rows recurved in dorsal view PER 0.52 wide, AER 0.36. AME reduced to a small spot of pigment. Eye diameter: AME 0.03, ALE 0.05; PME 0.05; PLE 0.05. — **Opisthosoma**: 2.37 long, 1.80 wide. Total length 4.07 (4.25 including spinnerets). Leg formula: I>II>IV>III. Epigynal plate 0.35 long, 0.47 wide. Epigyne convex and prominent, without sclerotized plates (Figs 2A, 5A). The posterior edge is slightly sclerotized. Spermathecae and insemination ducts can be observed through the tegument. Vulva simple (Figs 2B, 5A), consisting of two small spermathecae, insemination and fertilization ducts. Insemination ducts coiled, forming one lap around the fertilization ducts before reaching the spermatheca. Vulval pockets absent (Fig. 2A, B). — **Appendages**: Chelicerae 0.58 long, 0.31 wide, with 3 equally spaced teeth on promargin and 18–20 very small teeth grouped on retromargin. — **Leg measurements**: Leg I: femur 4.13, patella 0.82, tibia 4.01, metatarsus 3.82, tarsus 1.59, total 14.37. Leg II: femur 3.42, patella 0.68, tibia 3.03, metatarsus 2.99, tarsus 1.31, total 11.43. Leg III: femur 2.63, patella 0.59, tibia 2.05, metatarsus 2.17, tarsus 0.94, total 8.39. Leg IV: femur 3.63, patella 0.75, tibia 3.07, metatarsus 2.63, tarsus 1.13, total 11.21. Palp: femur 1.07, patella 0.32, tibia 0.63, tarsus 1.09, total 3.11.

Description of the male paratype. Paratype from Roccaforte Mondovì, Grotta dei Partigiani della Tura [Pi 286], 1780 m a.s.l., 44.24081°N, 7.73308°E, 18.VIII.2016, Giachino P.M. and Lana E. leg. — **Coloration**: Carapace yellow brown. Sternum yellow brown, without pattern. Head region of the same color. Opisthosoma grey-white without pattern. Legs same color as prosoma, all trochanters notched. — **Prosoma**: Carapace 1.66 long, 1.54 wide. Carapace approximately circular in dorsal view. Cephalic region not differentiated from the rest of the carapace. Clypeus height under AME 0.39. Labium as long as wide or moderately wider than long. Sternum 1.07 long, 1.06 wide. Reduced and depigmented eyes. No corneal lenses visible. Both eye rows recurved in dorsal view, PER 0.54 wide, AER 0.31. Eye diameter: AME 0.04, ALE 0.05; PME 0.05; PLE 0.05. — **Opisthosoma**: 2.63 long, 1.50 wide. Total length 4.29 (4.51 including spinnerets). Leg formula: I>II>IV>III. — **Appendages**: Chelicerae: 0.91 long, 0.33 wide, with 3 equally spaced teeth on promargin and 18–20 very small teeth grouped on retromargin. Leg formula: I>II>IV>III — **Male palp** (Fig. 2D, E): Paracymbium short, with a well-developed ventral process consists of a long and flattened lamella, curved towards the apex and tapering and a dorsal one consisting of a short laminar apophysis, ending in a rounded and enlarged lobe directed toward the ventral one (Figs 2D, E, 5C). Conductor complex with two well developed processes (p1 and p2): p1 longer than wide in lateral view (Fig. 5C), tapering in the distal third; p2 is in an apical position and ends with two convergent apical hooks running

as a conductor for the embolus. Embolus filamentous following a semi-circular course towards the apex and bordering the tegulum (Figs 2D, 5C). Large and well-developed median apophysis, ventrally directed, wider at its base and narrower at its medial-final part, cone-shaped with a blunt end (Fig. 2D). — **Leg measurements**: Leg I: femur 4.57, patella 0.79, tibia 4.66, metatarsus 4.42, tarsus 1.57, total 16.01. Leg II: femur 4.48, patella 0.81, tibia 4.52, metatarsus 4.35, tarsus 1.52, total 15.68. Leg III: femur 3.25, patella 0.74, tibia 2.74, metatarsus 2.92, tarsus 1.16, total 10.81. Leg IV: femur 4.42, patella 0.80, tibia 3.97, metatarsus 3.65, tarsus 1.39, total 14.23. Palp: femur 1.16, patella 0.28, tibia 0.44, tarsus 0.75, total 2.63.

Etymology. The species is dedicated to Angelo Morisi (1943–2016) beloved Piedmontese natural scientist, expert in reptiles, amphibians, cave-dwelling invertebrates, freshwater macroinvertebrates, fishes and lichens. Angelo was a kind-hearted, altruist and caring natural scientist, pioneer of the biospeleological research in Piedmont, and first collector of several rare troglonid species that still carry his name, including *Typhlonesticus morisii*, sister species of *T. angelicus* **sp. nov.** The specific epithet is in form of adjective and conjugates the name of Angelo and the features of benevolent immortal beings with a translucent body of light, barely visible to the human eye.

Distribution, sampling notes and ecology. *Typhlonesticus angelicus* **sp. nov.** is restricted to subterranean habitats of the central portion of Alpi del Marguareis, within the section of Alpi Liguri (Fig. 6). Five isolated populations are known so far, covering an area of approximately 100 km² spanning from the upper Maudagna Valley (two nearby populations) and mid-Pesio Valley (one population) in the north to the border of Italy and France in the south (two populations). The population of Balconi di Marta (Brigue), formerly assigned to *T. morisii* and now re-assigned to *T. angelicus* represents the only record of *Typhlonesticus* for France, leaving *T. morisii* as uniquely known from Italy (Vernante). All caves open in Alpine grasslands at 1,800–2,000 m a.s.l., with the exception of the designed type locality Pi 250 – Grotta Superiore delle Camoscere (Pesio Valley) (Fig. 7D) opening at the upper limit of a beech forest, approximately at 1,200 m a.s.l. — Several renowned Italian biospeleologists, including Angelo Morisi himself and Gianni Comotti, the first collector in history of a female specimen of this new species, visited this cave through time leading to the discovery of an extraordinary and highly specialized community of subterranean endemic arthropods, including *Troglohyphantes vignai* Brignoli, 1971 (Araneae: Linyphiidae), *Leptoneta crypticola* Simon, 1907 (Araneae: Leptonetidae), *Holoscotolemon oreophilum* Martens, 1978 (Opiliones: Travuniidae) (Isaia et al. 2011, 2022) and the rare subterranean beetle *Agostinia launoi* (Gestro, 1892) (Carabidae, Trechinae). — Individuals of *T. angelicus* **sp. nov.** were preferably found on cave walls and to a lesser extent on the cave ground, hanging upside down on their criss-cross web or walking on vertical rocks (Fig. 7A),

mostly hidden in rock fissures. Females may be found throughout the year, while males appear to be very rare. All caves inhabited by *T. angelicus* **sp. nov.** are characterised by constant high relative humidity close to saturation. — *Typhlonesticus angelicus* **sp. nov.** prefers the medium alpine montane belt (mean = 1,693 m; n = 4) and inhabits sites characterised by mean annual temperatures from 4.1 to 9.1°C (mean = 5.7°C; range: 5.0; n = 4), colder than *T. morisii* being uniquely found in Sotterranei di Vernante, where the temperature ranges from 8.3 to 9.8°C during the year.

Conservation status and basic information for an IUCN Red List assessment. Range description, Area of Occupancy (AOO) and Extent of Occurrence (EOO). The species is endemic to Alpi del Marguareis over an altimetric range of approximately 700 m (min–max elevation 1,200–1,914 m a.s.l.) (Fig. 6). Five populations are known so far (four if considering the two nearby localities in Roccaforte Mondovì as one), encompassing an estimated AOO of 16 km² and an EOO of 103 km². We infer a decline in both EOO and AOO because of global warming due to the sensibility of troglobionts to increasing global temperature (see Mammola et al. 2019a). Dispersal ability for this species is not known but given the high development of troglomorphic characters and the restricted range, it is assumed to be very low. — **Locations:** In the Red List criteria, “location” refers to a threat-based area and is different from the general notions of locality. In fact, the term ‘location’ defines a geographically or ecologically distinct area in which a single threatening event can rapidly affect all individuals of the taxon present. Global warming is expected to affect all known populations of *T. angelicus* **sp. nov.**, consequently, one single location should be considered for the assessment risk of this species. — **Threats:** The species is potentially exposed due to its extremely narrow geographic distribution range, its low dispersal capacity and its inferred low thermic tolerance related to the high level of troglomorphism. — **Conservation actions:** Considering the ongoing global warming, it is worth considering the extinction risk of *T. angelicus* **sp. nov.** As very little is known about the biology and life history of this species, to date it is not possible to provide any precise management actions. However, the inclusion of this species on the IUCN Red List represents an important starting point for its conservation. As seen for other subterranean systems of conservation concern for red listed and legally protected species, three out of five caves hosting *T. angelicus* **sp. nov.** benefit from being included in protected area or Sites of Conservation importance, namely IT1160056 (Alpi Marittime) and IT1160057 (Alta Valle Pesio and Tanaro). — **Note on the conservation status in France:** *T. morisii* has been cited in “CR” status in the Red List of Spiders in France since the beginning of 2023 (Bounias-Delacour et al., 2023). Its new distribution and its replacement at the French locality of Balconi di Marta by *T. angelicus*, would give the latter the same status in France.

3.3.2. *Typhlonesticus santinellii* Isaia and Ribera, **sp. nov.**

<https://zoobank.org/A5B798A7-3A15-4C06-AEC1-90FE8BF6F26F>

Fig. 3A–E, 5B, D, 7C

Examined material. Holotype: ITALY, Lombardia, Province of Bergamo: Oneta, Miniere di Gorno, Alpe Grem, Galleria del Pozzo (Fig. 7G), 45.8833°N, 9.8243°E, 1,208 m a.s.l., 14.X.2022, Falgari N., Isaia M., Nicolosi G., Pantini P., Scolari F. leg. 1m (MCSNB). — **Paratypes:** ITALY, Lombardia, Province of Bergamo: Oneta, Miniere di Gorno, Alpe Grem, Cantiere #7, 45.8862°N, 9.8235°E, 1,320 m a.s.l., 14.X.2022, Falgari N., Isaia M., Nicolosi G., Pantini P., Scolari F. leg. 1f (CI) • Oneta, Miniere di Gorno, Alpe Grem, Galleria del Pozzo (Fig. 7G), 45.8833°N, 9.8243°E, 1208 m a.s.l., 14.X.2022, Falgari N., Isaia M., Nicolosi G., Pantini P., Scolari F. leg. 1f (MCSNB) • Monasterolo Castello, Grotta Murdosso [LoBG 7407] (Fig. 7H), 45.7474°N 9.9542°E, 1092 m a.s.l., 12.V.2019, Santinelli R. leg. 1f, 1 m subadult (CI).

Diagnosis. Males of *Typhlonesticus santinellii* **sp. nov.** differ from *T. morisii* and *T. angelicus* **sp. nov.** by the shape of p1 and p2 processes, the shape of the median apophysis (Figs 3D, 5D) and by the shape and arrangement of the paracymbial apophyses, mainly the ventral one (“vp” in Fig. 3D, E), which is shorter and more tapering in *T. santinellii* **sp. nov.** than in *T. angelicus* **sp. nov.** and *T. morisii*. — Females are best diagnosed by the orientation of the insemination ducts, and the shape of the spermathecae (Figs 3A, B, 5B).

Description of the male. Male holotype from Miniere di Gorno, Galleria del Pozzo, 45.8833°N, 9.8243°E, 1208 m a.s.l., 14.X.2022, Falgari N., Isaia M., Nicolosi G., Pantini P., Scolari F. leg. — **Coloration:** Carapace yellow-brown. Head region of the same color. Sternum yellow brown, without pattern. Opisthosoma grey-white without pattern. Legs same color as prosoma, all trochanters notched. — **Prosoma:** Carapace 1.42 long, 1.30 wide. Cephalic region not differentiated from the rest of the carapace. Clypeus height 0.32 (under AME). Labium as long as wide or moderately wider than long. Sternum 0.88 long, 0.90 wide. Eyes reduced and lacking pigment, no corneal lenses are visible. Both eye rows recurved in dorsal view (PER 0.43 wide, AER 0.37), Eye diameter: AME 0.01, ALE 0.06; PME 0.05; PLE 0.06. — **Opisthosoma:** 1.84 long, 1.13 wide. Total length 3.26 (3.43 including spinnerets). Leg formula: I>II>IV>III. — **Appendages:** Chelicerae: 0.72 long, 0.30 wide, with three equally spaced teeth on promargin and 18–20 very small teeth grouped on retromargin. **Male palp** (Figs 3D, E, 5D): Paracymbium short. Ventral process consisting of a long and flattened lamella, straight, tapering in the distal part, ending in a thin spine, and a dorsal process scarcely developed consisting of a short laminar apophysis, perpendicularly bent at the apex, ending in a rounded lobe. Conductor complex with two well developed processes (p1 and p2): p1 is longer than wide, pointed,

tapering along its length in lateral view (Figs 3D, 5D); p2 is in an apical position and ending with two almost parallel small hooks, running as a conductor for the embolus (Figs 3D, 5D). Embolus filamentous following a semi-circular course towards the apex and bordering the tegulum (Figs 3D, 5D). Large and well-developed median apophysis, ventrally directed, wider at its base and narrower at its medial-final part, cone-shaped with a blunt end. (Figs 3D, 5D). — **Leg measurements:** Leg I: femur 5.23, patella 0.76, tibia 5.36, metatarsus 5.30, tarsus 1.80, total 18.45. Leg II: femur 3.98, patella 0.68, tibia 3.78, metatarsus 3.69, tarsus 1.39, total 13.52. Leg III: femur 3.96, patella 0.65, tibia 3.32, metatarsus 3.15, tarsus 1.20, total 12.28. Leg IV: femur 3.99, patella 0.66, tibia 3.77, metatarsus 3.64, tarsus 1.35, total 13.41. Palp:

femur 1.02, patella 0.24, tibia 0.31, tarsus 0.84, total 2.41.

Description of the female. Female paratype from Miniere di Gorno, Alpe Grem, Galleria del Pozzo, 45.8833°N, 9.8243°E, 1,208 m a.s.l., 14.X.2022, Falgari N., Isaia M., Nicolosi G., Pantini P., Scolari F. leg. — **Coloration:** Carapace pale yellow. Head region of the same color. Sternum yellow brown, without pattern. Opisthosoma grey-white without pattern. Legs same color as prosoma. — **Prosoma:** Carapace 1.51 long, 1.36 wide, approximately circular in dorsal view. Cephalic region not differentiated from the rest of the carapace. Clypeus height under AME 0.29, under ALE 0.298. Labium as long as wide or moderately wider than long. Sternum 0.79 long,

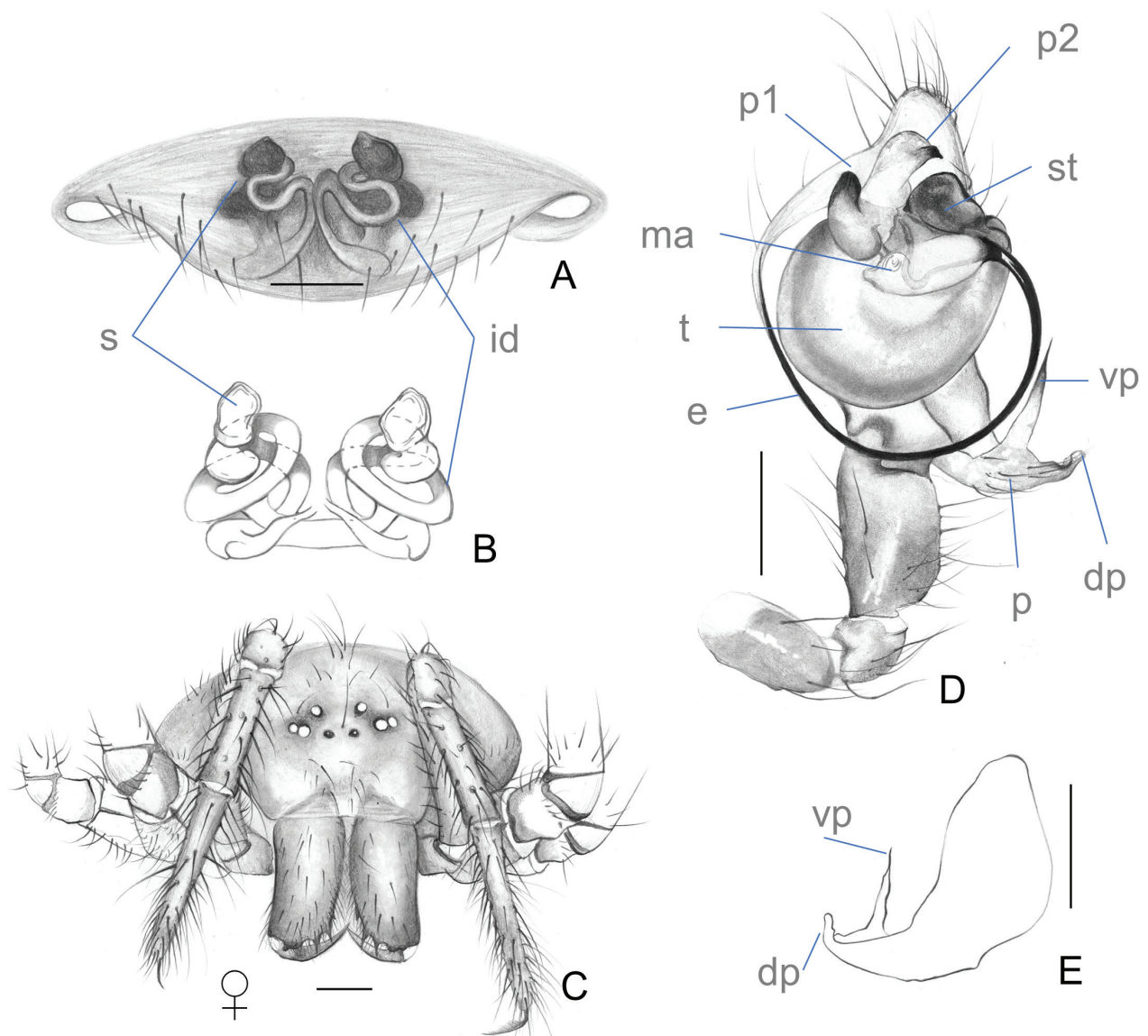


Figure 3. *Typhlonesticus santinellii* sp. nov., paratype female from Monasterolo Castello, Grotta Murdosso [LoBG 7407] (Santinelli R. leg., 12.V.2019) (A, B, C) and holotype male from Miniere di Gorno, Alpe Grem, Galleria del Pozzo (Falgari N., Isaia M., Nicolosi G., Pantini P., Scolari F. leg., 14.X.2022) (D, E). Epigyne in ventral view (A), vulva in dorsal view (B), female cephalothorax in frontal view (C), left male pedipalp in ventral view (D) and male cymbium in dorsal view (E) (see section 2.2.3. for abbreviations). Scale bars 0.2 mm.

0.96 wide. Eyes reduced and depigmented, no corneal lenses are visible. Both eye rows recurved in dorsal view, PER 0.49 wide, AER 0.36. AME reduced to a small spot of pigment. Eye diameter: AME 0.01, ALE 0.05; PME 0.06; PLE 0.06. — **Opisthosoma**: 2.01 long, 1.67 wide. Total length 3.52 (3.65 including spinnerets). Leg formula: I>II>IV>III. Epigynal plate 0.30 long, 0.55 wide, convex and prominent, without sclerotized plates (Figs 3A, 5B). The posterior edge is slightly sclerotized. Spermathecae and insemination ducts can be observed through the tegument. Vulva simple (Figs 3B, 5B), consisting of two small rhombohedral spermathecae, insemination and fertilization ducts. Insemination ducts coiled, forming three laps around the fertilization ducts before reaching the spermathecae (Fig. 3A, B). — **Appendages**: Chelicerae 0.76 long, 0.28 wide with three equally spaced teeth on promargin and 18–20 very small teeth grouped on retromargin. Legs same color as prosoma. — **Leg measurements**: Leg I: femur 4.48, patella 0.79, tibia 4.46, metatarsus 4.12, tarsus 1.59, total 15.44. Leg II: femur 3.52, patella 0.74, tibia 3.11, metatarsus 3.08, tarsus 1.26, total 11.71. Leg III: femur 2.72, patella 0.65, tibia 1.97, metatarsus 2.19, tarsus 0.99, total 8.52. Leg IV: femur 3.73, patella 0.78, tibia 3.02, metatarsus 2.74, tarsus 1.19, total 11.46. Palp: femur 1.09, patella 0.31, tibia 0.60, tarsus 1.14, total 3.14.

Etymology. The species is dedicated to the speleologist Roberto “Robertone” Santinelli, who first collected the female specimen in Murdosso cave, allowing the diagnosis of the new species. Noun in genitive.

Distribution, sampling notes and ecology. *Typhlonesticus santinellii* **sp. nov.** is restricted to subterranean habitats of the central portion of Alpi Orobie, within the section of Alpi e Prealpi Bergamasche (Marazzi 2005) (Fig. 6). Three populations are known so far (two of them very close to each other), covering an area of less than 5 km² in the Val del Riso, across the municipalities of Monasterolo Castello (Murdosso cave) and Oneta (mining complex of Gorno), both in the Province of Bergamo. The Murdosso cave is a wild cave opening in a beech forest in the nearby of Colli di San Fermo. The first female specimen was collected by Roberto Santinelli under big rocky debris, during the unblocking operations of the cave conducted by the local speleological groups “Underland” and “I Tassi”. Despite several attempts, no further specimens were collected at Murdosso cave after the collection of the first female. The two further occurrences refer to the galleries of the abandoned mining complex of Gorno, nowadays “Ecomuseo delle miniere di Gorno”, an ethnological museum dedicated to the cultural heritage of the mining activity in this area. The male and a few other specimens were collected during our biospeleological expedition in the mining complex led by Fabrizio Scolari (Ecomuseo delle miniere di Gorno) in October 2022. The galleries in which the new species was found open in alpine grasslands at 1,200–1,300 m asl. Individuals of *T. santinellii*

sp. nov. were preferably found on cave walls and among rocks near the ground, hanging upside down on their criss-cross web. Investigations at the mining complex of Gorno started following the suggestion of Marco Valle, Director of the Museo Civico di Scienze Naturali di Bergamo who conducted some biological investigations in the mining complex of Gorno in the early 1980s, together with the local biospeleologist Gianni Comotti. Marco Valle noticed that among the arachnological material collected during those explorations there was a remarkable nesticid, that was sent for examination to Konrad Thaler. The material was returned by Thaler to the Museum of Bergamo in 1984, except for one female identified as “*Nesticus* cf. *morisii*”, which was retained for further studies. Despite our search in the Thaler collection and elsewhere, we were not able to locate such material. However, our recent finding of the new species in the mining complex of Gorno confirmed Marco Valle’s and Konrad Thaler’s first impressions. — *Typhlonesticus santinellii* **sp. nov.** was collected in a natural cave and in the mining complex of Gorno at a mean altitude of 1,206 m (n = 3) and inhabits sites with mean annual temperatures from 6.3 to 7.8°C (mean = 7.0°C; range: 1.5; n = 3).

Conservation status and basic information for an IUCN Red List assessment. Range description, Area of Occupancy (AOO) and Extent of Occurrence (EOO). The species is endemic to a small sector of Alpi Orobie covering an altimetric range of approximately 250 m (min–max elevation 1,092–1,320 m) (Fig. 6). Three populations are known so far (two if considering the two nearby populations of the mining complex Gorno as one), encompassing an estimated EOO of 4 km² and an AOO of 8 km². Similarly to *T. angelicus* **sp. nov.**, we infer a decline in both EOO and AOO as a result of global warming. It is assumed that it has a very low dispersal capacity. — **Locations**: Global warming is expected to affect all known populations of *T. santinellii* **sp. nov.**, consequently, one single location should be considered for the assessment risk of this species. — **Threats**: The species is potentially exposed due to its extremely narrow geographic distribution range, its low dispersal capacity and its low thermic tolerance related to the high level of adaptation to subterranean life. — **Conservation actions**: In light of the ongoing global warming, it is worth considering the extinction risk of *T. santinellii* **sp. nov.** As for *T. angelicus* **sp. nov.**, it is not possible to provide any precise management actions, but the inclusion on the IUCN Red List represents an important starting point for its conservation. The subterranean sites of the mining complex of Gorno hosting *T. santinellii* **sp. nov.** benefit from being included in sites of Conservation importance, namely SC IT2060009 Val Nossana – Cima di Grem, managed by the Regional Park of Orobie Bergamasche. The current management of the subterranean site as tourist attractions does not seem to compromise the survival of this species.

3.3.3. *Typhlonesticus morisii* (Brignoli, 1975)

Figs 4A–E, 5F, H, 7B

Examined material. Holotype: ITALY, Piemonte, Province of Cuneo: Vernante, Sotterranei del Forte A di Vernante Opera 11, Tetto

Ruinas [Art. Pi CN] (Fig. 7E, F), 44.2524°N, 7.5276°E, 800 m a.s.l., 14.X.1972, Morisi A. leg. 1m (MSNV) (Brignoli 1975). — **Other material:** same locality as holotype, 15.VI.2009, Isaia M. and Paschetta M. leg. 1 juv. (CI) (Isaia et al. 2011); 26.IV.2004, Lana E. and Arnò C. leg. 1f (CI) (Isaia et al. 2011); 25/03/2022, Isaia M. and Nicolosi G. leg. 4 f (CI).

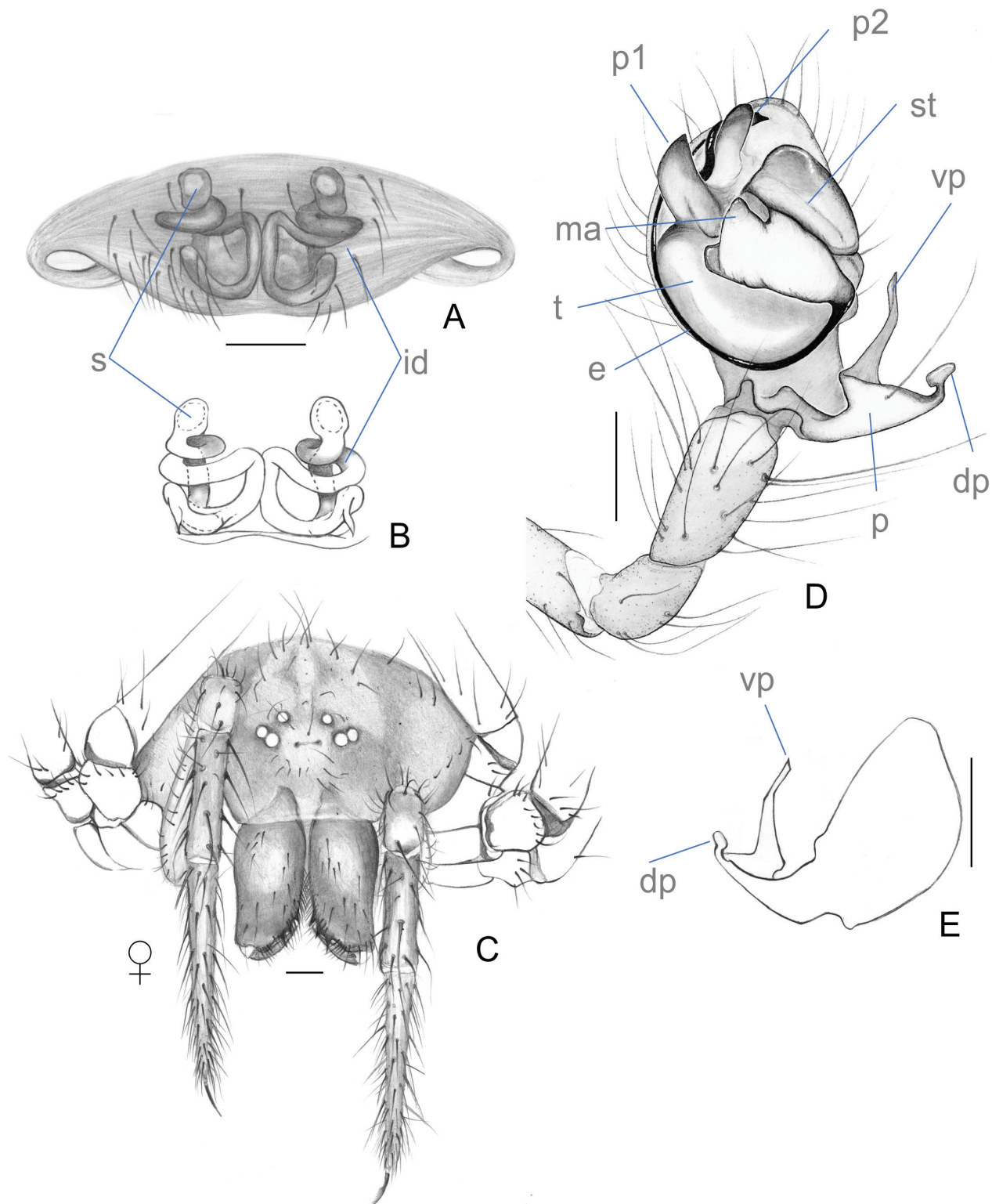


Figure 4. *Typhlonesticus morisii* (Brignoli), female from type locality, Vernante, Sotterranei del Forte A di Vernante Opera 11, Tetto Ruinas [Art. Pi CN] (Isaia M., Nicolosi G. leg., 25.III.2022) (CI) (A, B, C) and holotype male from same locality, Morisi A. leg., 14.X.1972 (D, E). Epigyne in ventral view (A), vulva in dorsal view (B), female cephalothorax in frontal view (C), left male pedipalp in ventral view (D) and male cymbium in dorsal view (E) (see section 2.2.3. for abbreviations). Scale bars 0.2 mm.

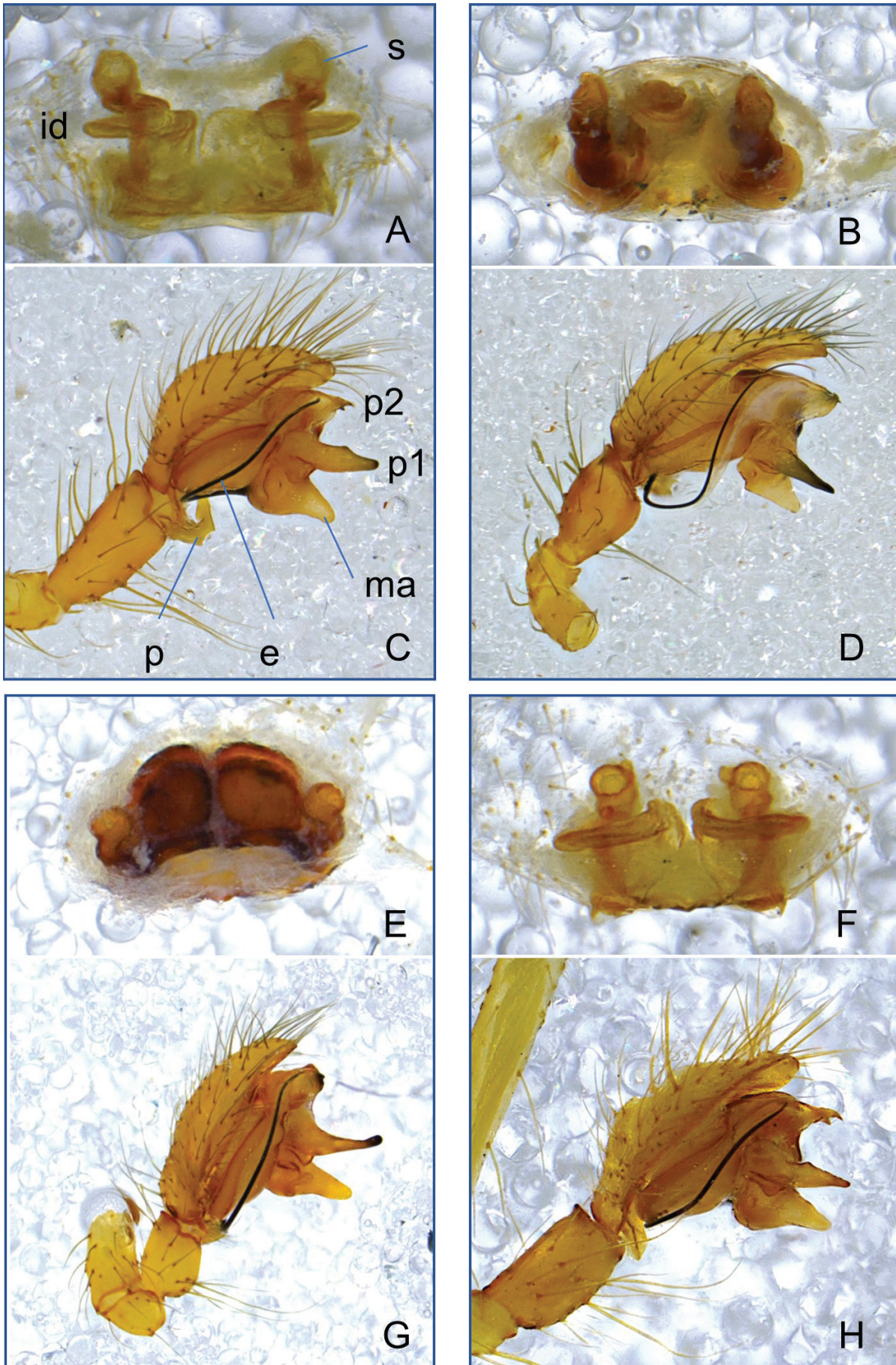


Figure 5. The four Italian species of *Typhlonesticus*: photographs of vulvae (A, B, E, F) and left male pedipalps in prolateral view (C, D, G, H). A, C *Typhlonesticus angelicus* sp. nov.; B, D *Typhlonesticus santinellii* sp. nov.; E, G *Typhlonesticus idriacus* (Roewer); F, H: *Typhlonesticus morisii* (Brignoli).

3.3.4. *Typhlonesticus idriacus* (Roewer, 1931)

Fig. 5E, G

Examined material. ITALY, Friuli Venezia Giulia, Province of Udine: Nimis, Grotta di Pre Oreak, 46,24054°N, 13,2920°E, 285 m a.s.l., 15.IX.2007, Lopez A. leg. 1f (CRBA).

3.4. Biogeography and troglomorphism in *Typhlonesticus*

A comparative analysis of troglomorphic traits and biogeographic features of *Typhlonesticus* spiders is presented in Table 1. *T. absoloni* (Montenegro) stands in terms of highest leg elongation (> 1), eye reduction (AME: 0.001 mm) and biggest body size (5.750 mm). *T. morisii* and *T. obcaecatus* are smaller in size but equally remarkable in terms of leg elongation (> 1) and eye reduction (0.001 mm). On the other end, *T. silvestrii* and *T. idriacus* exhibit the lowest level of troglomorphism, with bigger anterior median eyes (0.060 and 0.053, respectively) and lower leg elongation (0.56 and 0.70 respectively) within

the genus. As for the two new species, they show high values of leg elongation (> 0.90) and eye reduction (0.035 and 0.040).

When relating troglomorphic traits to biogeographic features (Fig. 8), we unravelled a significant positive relationship between the Extent of Occurrence and the diameter of anterior median eyes (Est: 0.0126; Std Err.: 0.0027; P: 0.0026**) and an inverse significant relation with leg elongation (Est: -0.0840 ; Std Err. 0.018; P: 0.0024**). The same trend was recovered when relating altimetric range to the diameter of anterior median eyes (Est: 0.0057; Std Err. 0.0020; P: 0.0275*). No significant relations were recovered when relating leg elongation to altimetric range (Est: -0.0298 ; Std Err. 0.0163; P: 0.1110 ns) or body size to both altimetric range (Est: 0.0379, Std Err. 0.1194; P: 0.7602 ns) and Extent of Occurrence (Est: -0.0160 ; Std Err. 0.2214; P: 0.9442 ns).

Interestingly, *T. absoloni* (Montenegro) deviates from the expected trends, showing higher leg elongation (Fig. 8A, B) and smaller eyes (Fig. 8C, D) than expected for the given range size (30 km²) and altimetric range (200 m). On the on the contrary, *T. santinellii* shows bigger eyes than expected for its range (8 km²).

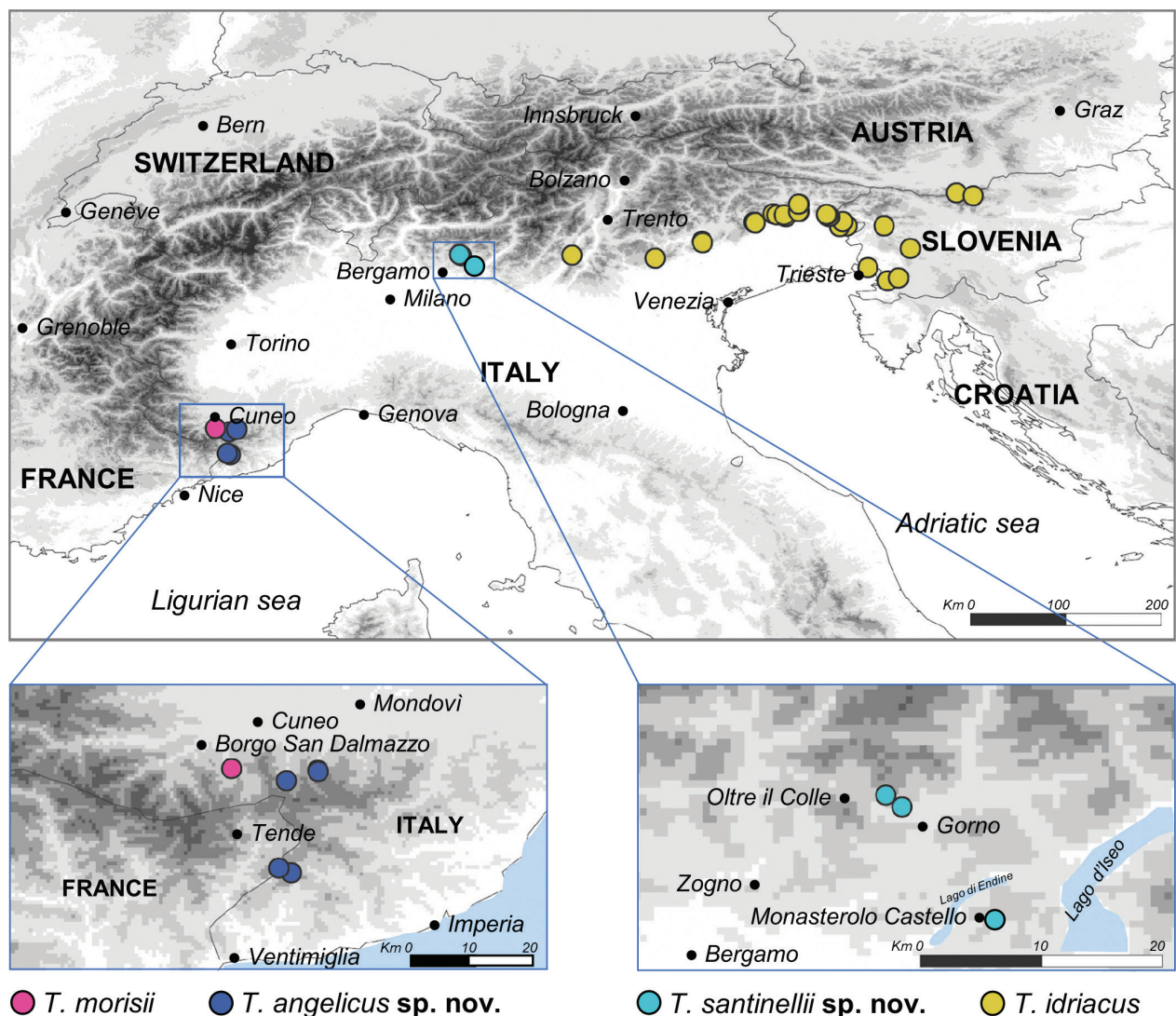


Figure 6. Distribution of *Typhlonesticus* in Italy.



Figure 7. Live specimens of *Typhlonesticus angelicus* sp. nov. (A male), *T. morisii* (Brignoli) (B female) and *T. santinellii* sp. nov. (C female) in their natural habitats. The entrance of Grotta superiore delle Camoscere [Pi 250], type locality of *T. angelicus* sp. nov. (D), the military bunker of Sotterranei di Vernante, type locality of *T. morisii* (Brignoli) (E, F); the walls of “Galleria del Pozzo” within the mining complex of Gorno, type locality of *T. santinellii* sp. nov. (G); the entrance of Murdosso cave [Lo BG 7407], where the first female of *T. santinellii* sp. nov. was collected during the unblocking operations of the cave conducted by the local speleological groups “Underland” and “I Tassi” (H).

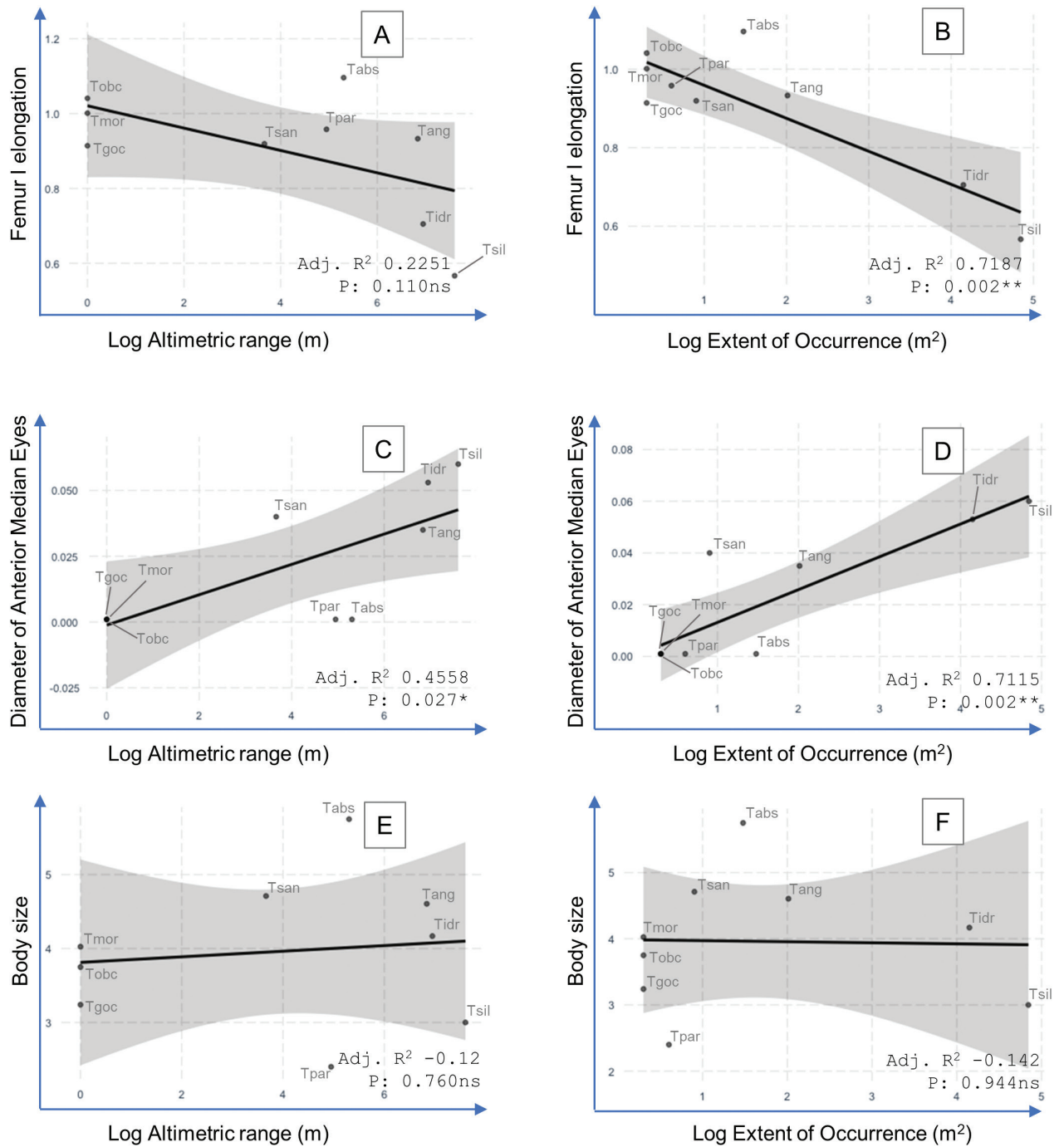


Figure 8. Predicted linear relationship (solid line) and 95% confidence interval (grey shading) between biogeographic features and troglomorphic traits in *Typhlonesticus* (all species), derived from the linear mixed model. Regression statistics for each regression are reported lower right. Femur I elongation is expressed as the ratio of the length of the femur I to the body size of females, the diameter of anterior median eyes is in mm. Extent of occurrence and altimetric range are Log transformed. Species abbreviations: Tobc = *T. obcaecatus*; Tmor = *T. morisii*; Tgoc = *T. gocmeni*; Tsan = *T. santinellii*; Tpar = *T. parvus*; Tabs = *T. absoloni*; Tang = *T. angelicus*; Tidr = *T. idriacus*; Tsil = *T. silvestrii*.

Table 1. Troglomorphic traits and biogeographic features of *Typhlonesticus* spiders. Body size, femur I elongation and diameter of anterior median eyes are issued from Mammola et al. (2022) and verified on original publications and/or on original material. Measures of the holotypes are taken as reference. Leg elongation is measured as the ratio of the length of the femur I to the body size of females. The calculation of the Extent Of Occurrence (EOO, km²) is based on occurrences found in literature (see corresponding reference). * Values of EOO and altimetric range are approximate values, based on non-exact occurrences.

		Troglomorphic traits			Biogeographic features			References
		Body size	Fem I elongation	Diameter of anterior median eyes	EOO	Alti-metric range	Number of occurrences	
<i>T. absoloni</i> (Kratohvil, 1933)*	Montenegro	5,750	1,096	0,001	30	200	5	Kulczyński (1914, 1933, 1934, 1939); Deeleman-Reinhold (1974)
<i>T. angelicus</i> sp. nov.	SW-Alps (Italy and France)	4,605	0,933	0,035	103	931	4	This work
<i>T. gocmeni</i> Ribera, Elverici, Kunt & Özkütük, 2014	Turkey	3,240	0,914	0,001	2	0	1	Ribera et al. 2014
<i>T. idriacus</i> (Roewer, 1931)	E-Alps (Italy, Slovenia, Austria)	4,168	0,705	0,053	14078	1040	36	Thaler (1981); Dresco (1966); Roewer (1931); Gasparo (1996, 1997); Gasparo et al. (2001); Paoletti (1978); Brignoli (1972); Kostanjšek (2010); Kranjc, Novak (1977)
<i>T. morisii</i> (Brignoli, 1975)	SW-Alps	4,025	1,001	0,001	2	0	1	Isaia et al. (2011)
<i>T. obcaecatus</i> (Simon, 1907)	Spain	3,750	1,041	0,001	2	0	1	Ribera (1979)
<i>T. parvus</i> Kulczyński, 1914	Bosnia and Herzegovina	2,400	0,958	0,001	4	140	3	Kratohvil (1933, 1934); Brignoli (1971)
<i>T. santinellii</i> sp. nov.	Orobic Alps (Italy)	4,710	0,919	0,040	8	38	3	This work
<i>T. silvestrii</i> (Fage, 1929)*	E-USA	3,000	0,567	0,060	70000	2000	38	Gertsch (1984)

4. Discussion

The new species are both diagnosed by morphological details of the epigyne and the male copulatory organs. In particular, the arrangement of spermathecae and the paracymbium apophysis proved to be diagnostic, with negligible overlap among species. As for *T. santinellii* sp. nov., the comparative examination of the epigyne of the female from Murdosso cave (Central Alps) with material of *Typhlonesticus* allowed a first preliminary identification of a putative new species, which was later corroborated by molecular evidence. At the same time, a careful morphological re-examination of non-topotypic material in CI and MCSNB collections previously identified as *T. morisii* (see Isaia et al. 2017: 323) and the corresponding phylogeny allowed the delimitation of *T. angelicus* sp. nov., leaving *T. morisii* uniquely reported at the type locality.

According to both the molecular and the morphological approach, *T. angelicus* sp. nov. is close to *T. morisii*, and to a lesser degree to *T. santinellii* sp. nov. and *T. idriacus*. The four Alpine species are distinctly grouped in the Alpine cluster, attesting their reciprocal affinity.

With the inclusion of *T. angelicus* sp. nov. and *T. santinellii* sp. nov., the evolutionary lineage of *Typhlonesticus* now includes nine species, eight of them distributed in the northern mountain ranges of the Mediterranean Basin, from Turkey to the Pyrenees, plus *T. silvestrii* from

western United States. According to Ribera and Dimitrov (2023) the genus *Typhlonesticus* originated 91.9 Mya (71.1–111.9) and constitutes the oldest lineage of European Nesticidae. With the exception of the two more basal species, *T. parvus* and *T. silvestrii*, the rest of the species originated during the Oligocene, approximately 22.4 Mya (15.4–30). At that period of time the climate in Europe gradually changed increasing seasonality and aridity (Bruch et al. 2011; Eronen et al. 2009) and the Aegean Sea was not yet open, enabling connections through mountain ranges in southern Europe, from Anatolia to the Iberian Peninsula (Ribera and Dimitrov 2023). In this scenario, the mountain ranges in the northern Mediterranean Basin acted as climatic refuges for *Typhlonesticus*. The two new species cluster with *T. morisii* and *T. idriacus*, forming the Alpine group of this genus.

Despite never being found in syntopy, all *Typhlonesticus* species are consistently found in the same habitat, i.e. in the dark zone of natural caves or artificial subterranean habitats, such as mines and bunkers. An exception to this pattern is provided by the American species *T. silvestrii*, recently transferred to the genus by Ribera and Dimitrov (2023), the only *Typhlonesticus* species recorded in both hypogean and epigean habitats.

The ability of *Typhlonesticus* spiders to maintain permanent subterranean populations (a “troglobiont” sensu Trajano and Carvalho 2017) is reflected in their high level of morphological adaptation to subterranean life (i.e. tro-

glomorph traits; Christiansen 2012). Our comparative analysis across the genus reveals a statistically significant relationship between troglomorphic characters (leg elongation and eye regression) and range size. Accordingly, species exhibiting higher level of troglomorphy have smaller distribution ranges and, presumably, minor dispersal ability. The trend relating geographical features and leg elongation is consistent across the genus representatives, except for *T. absoloni* (Montenegro) (Fig. 8A, B), which also deviates in respect to eye regression (Fig. 8C, D). Tentatively, this deviation would hint at the existence of overlooked diversity in this species, just as unravelled in this work for *T. morisii* and *T. angelicus* **sp. nov.** A similar, but opposite deviation is also apparent in *T. santinellii* **sp. nov.**, showing bigger eyes than expected for its range. Such deviating trend would support the occurrence of new records of this species outside of the currently known range.

Traits often associated with subterranean life in spiders are particularly notable in *Typhlonesticus*, being fully depigmented, showing non-functional eyes and greater leg elongation. If, from one side, the adaptive meaning of the lack of pigment and eye reduction is particularly consistent with a dark environment, the functional meaning of leg elongation is less straightforward (see Gertsch 1973; Mammola and Isaia 2017; Liu et al. 2017; Mammola et al. 2018b). Yet, all functional hypotheses remain untested experimentally (Isaia et al. 2022, 2023).

The small distribution and altimetric ranges observed in *Typhlonesticus* plausibly parallel a low thermal tolerance in these species, which is expected to align with the general pattern observed in Mammola et al. (2019b) for *Troglohyphantes* spiders and for other subterranean arthropods (e.g. Pallarés et al. 2019, 2020, 2021; Raschmanová et al. 2018; Jones et al. 2021; Colado et al. 2022). Accordingly, specialised subterranean species exhibit lower thermal tolerances in respect to related surface species, a condition that translates into an extremely poor dispersal ability and high genetic structuring of the populations, making these species particularly vulnerable to the ongoing global warming.

5. Conclusions

Combining morphological and genetic evidence, we revealed the existence of two new species of *Typhlonesticus*, the oldest evolutionary lineage of Nesticid spiders in Europe. The new species are characterised by a high level of adaptation to the subterranean environment, confirming the extraordinary richness of subterranean diversity in the Alps. Using a trait-based approach, we inferred how highly troglomorphic *Typhlonesticus* are particularly vulnerable to environmental changes, and in particular we interpreted their isolation and their small distribution ranges as critical conditions amplifying their extinction risk, especially from a global warming perspective. We particularly address this issue by recommending further

studies focusing on the thermal tolerance of these species, aiming at understanding their possible response to increasing global temperatures.

6. Acknowledgements

Special thanks are due to Roberto Santinelli and Nicolò Falgari for leading the expeditions to Murdosso cave. We warmly thank Fabrizio Scolarini (Ecomuseo di Gorno) and Paolo Pantini (Museo Civico di Scienze Naturali di Bergamo) for leading the expedition to the mining complex of Gorno in October 2022. Thanks to Sanne Beikes and Stefano Mammola for field assistance. Thanks to Martina Pavlek for providing occurrence data on the Balkan species of *Typhlonesticus*. We also thank Alba Enguidanos for technical support and sequencing.

This paper is dedicated to the memory of Angelo Morisi (1943–2016), esteemed zoologist, pioneer in biosepological research on Piedmont and good friend of Marco Isaia. We are grateful for the major contribution he gave to the knowledge of the Piedmontese fauna and for being a constant source of inspiration, especially for the young zoologists.

7. References

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Supplementary Material 1

Tables S1, S2

Authors: Isaia M, Nicolosi G, Infuso A, Ribera C (2023)

Data type: .pdf

Explanation note: **Table S1.** Primers used for PCR amplification and sequencing. — **Table S2.** Specimens, localities and sequences with corresponding GenBank accession numbers analyzed in the present study (sequences with * are new for this study).

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