

Research article

[urn:lsid:zoobank.org:pub:B2675040-8D9E-4B6C-8616-3EA095550C88](https://zoobank.org/pub:B2675040-8D9E-4B6C-8616-3EA095550C88)

**Two new sympatric species of the pirate spider genus  
*Ero* C.L. Koch, 1836 from the cloud forest of Saint Helena Island,  
 South Atlantic Ocean (Araneae: Mimetidae)**

Danniella SHERWOOD<sup>1,\*</sup>, Arnaud HENRARD<sup>2</sup>, Martina PETERS<sup>3</sup>,  
 Benjamin W. PRICE<sup>4</sup>, Andie C. HALL<sup>5</sup>, Oliver W. WHITE<sup>6</sup>,  
 Virginie GRIGNET<sup>7</sup> & Vicky WILKINS<sup>8</sup>

<sup>1</sup>Arachnology Research Association, 124 City Road, London, EC1V 2NX, UK.

<sup>1</sup>Fundación Ariguanabo, 4111, Calle 58, e/ ave. 41 y ave.43, San Antonio de los Baños,  
 Provincia Artemisa c.p. 18100, Cuba.

<sup>1,8</sup>IUCN Species Survival Commission, Mid-Atlantic Islands Invertebrate Specialist Group,  
 c/o Species Recovery Trust, 37 Albany Road, Salisbury SP1 3YQ, UK.

<sup>2,7</sup>Royal Museum for Central Africa, Leuvensesteenweg 13, 3080 Tervuren, Belgium.

<sup>3</sup>Saint Helena National Trust, Broadway House, Jamestown, STHL 1ZZ, Saint Helena.

<sup>4,5,6</sup>Natural History Museum, Cromwell Road, South Kensington, London, SW7 5BD, UK.

<sup>8</sup>Species Recovery Trust, 37 Albany Road, Salisbury SP1 3YQ, UK.

\* Corresponding author: [danni.sherwood@hotmail.com](mailto:danni.sherwood@hotmail.com)

<sup>2</sup> Email: [arnaud.henrard@africamuseum.be](mailto:arnaud.henrard@africamuseum.be)

<sup>3</sup> Email: [martina.peters@trust.org.sh](mailto:martina.peters@trust.org.sh)

<sup>4</sup> Email: [b.price@nhm.ac.uk](mailto:b.price@nhm.ac.uk)

<sup>5</sup> Email: [a.hall@nhm.ac.uk](mailto:a.hall@nhm.ac.uk)

<sup>6</sup> Email: [o.white@nhm.ac.uk](mailto:o.white@nhm.ac.uk)

<sup>7</sup> Email: [virginie.grignet@africamuseum.be](mailto:virginie.grignet@africamuseum.be)

<sup>8</sup> Email: [vicky.wilkins@speciesrecoverytrust.org.uk](mailto:vicky.wilkins@speciesrecoverytrust.org.uk)

<sup>1</sup>[urn:lsid:zoobank.org:author:0E91B200-D748-4EF3-A93D-68880F2D5CC6](https://zoobank.org/author:0E91B200-D748-4EF3-A93D-68880F2D5CC6)

<sup>2</sup>[urn:lsid:zoobank.org:author:E1B02E6E-D91C-43FE-8D8C-CD102EFEE3B4](https://zoobank.org/author:E1B02E6E-D91C-43FE-8D8C-CD102EFEE3B4)

<sup>3</sup>[urn:lsid:zoobank.org:author:91A3FE30-DD41-43EF-947F-FD0F8BB87127](https://zoobank.org/author:91A3FE30-DD41-43EF-947F-FD0F8BB87127)

<sup>4</sup>[urn:lsid:zoobank.org:author:AD44475A-AEAB-4659-A70F-6C5E90DD9056](https://zoobank.org/author:AD44475A-AEAB-4659-A70F-6C5E90DD9056)

<sup>5</sup>[urn:lsid:zoobank.org:author:81DAE41E-0005-4295-B92D-BA093F240229](https://zoobank.org/author:81DAE41E-0005-4295-B92D-BA093F240229)

<sup>6</sup>[urn:lsid:zoobank.org:author:3B206AC7-1981-469E-80C7-520B35DB806C](https://zoobank.org/author:3B206AC7-1981-469E-80C7-520B35DB806C)

<sup>7</sup>[urn:lsid:zoobank.org:author:A2D14150-D0FF-49A4-8133-A8FA8769BBB0](https://zoobank.org/author:A2D14150-D0FF-49A4-8133-A8FA8769BBB0)

<sup>8</sup>[urn:lsid:zoobank.org:author:A1CF0CC3-A933-403E-B433-B0D5F853E5D6](https://zoobank.org/author:A1CF0CC3-A933-403E-B433-B0D5F853E5D6)

**Abstract.** A remarkable morphologically and genetically distinct species of the genus *Ero* C.L. Koch, 1836 is described based on both sexes from the cloud forest of the island of Saint Helena: *Ero lizae* sp. nov. Another new species, *Ero natashae* sp. nov., is also described on the basis of morphological differences in the male and female genitalia. Both species were initially reported a single species, *Ero aphana* (Walckenaer, 1802), from the island by Unzicker (1977).

**Key words.** Barcoding, mitogenome, morphology, Peaks National Park, South Atlantic Ocean, United Kingdom Overseas Territory.

Sherwood D., Henrard A., Peters M., Price B.W., Hall A.C., White O.W., Grignet V. & Wilkins V. 2024. Two new sympatric species of the pirate spider genus *Ero* C.L. Koch, 1836 from the cloud forest of Saint Helena Island, South Atlantic Ocean (Araneae: Mimetidae). *European Journal of Taxonomy* 921: 76–97. <https://doi.org/10.5852/ejt.2024.921.2417>

## Introduction

Saint Helena is situated in the South Atlantic Ocean, part of the United Kingdom Overseas Territory of Saint Helena, Ascension and Tristan da Cunha, and widely recognised as one of the world's most remote inhabited islands. The invertebrate fauna of the island is endemic-rich and highly diverse (Ashmole & Ashmole 2000) and recent revisions of its spider fauna are providing interesting results (Sherwood & Fowler 2023). The unique position of this volcanic island and its geology and varied flora, including many charismatic endemic species, has resulted in several unique habitats on this small island. The cloud forest, occupying the central ridge of the island, including Diana's Peak (818 m) as its highest point of elevation, is now a severely fragmented habitat occupying only 16 hectares today. It hosts important endemic flora (e.g., Black Scale Fern, Tree Fern, He Cabbage, and She Cabbage, amongst many others), and is the catchment area for more than half of the island's fresh drinking water. Due to its unique resulting habitat, it hosts more than one hundred and twenty endemic invertebrate species (Key *et al.* 2021).

The genus *Ero* C.L. Koch, 1836 contains 40 valid species (World Spider Catalog 2023) of which six are known to occur in the Afrotropical region: *Ero aphana* (Walckenaer, 1802), *E. capensis* Simon, 1895, *E. awrencei* Unzicker, 1966, *E. eburnea* Thaler, 2004, *E. comorensis* Emerit, 1996, *E. lokobeana* Emerit, 1980 and *E. madagascariensis* Emerit, 1980, the latter three occurring on Comoros and the Seychelles, and Madagascar respectively (Emerit 1980, 1996). It is also pertinent to mention *Ero quadrituberculata* Kulczyński, 1905, a species endemic on Madeira which, like Saint Helena, is an island situated to the west of the African continent.

Unzicker (1977) recorded *E. aphana* as an introduced species on Saint Helena. However, the examination of specimens from the cloud forest in Natural History Museum, London, Royal Museum for Central Africa (i.e., all of the material mentioned and examined by Jack Unzicker), and the Saint Helena National Trust by DS and AH showed morphological differences with *E. aphana* and revealed that at least two species occur on the island. There were clear differences in the male palp and female epigyne, and both sexes possess large spike-like tubercles, in contrast to what is known for *E. aphana*, but had been previously determined as that species by Unzicker (1977). Furthermore, DS took DNA samples of one male, which were sequenced by BP, showing this species had a significant (> 11%) genetic distance from *E. aphana*, and indeed from other species of *Ero* with sequences available on the Barcode of Life Database (BOLD 2023).

In this work, two new species of *Ero* are thus described based on both sexes, representing fascinating new species from the cloud forest, previously misidentified as a common European species. Furthermore, the complete mitogenome of *Ero lizae* sp. nov. could be sequenced, and its phylogenetic distinction was inferred using the barcode region (COI). *Ero lizae* is remarkable considering its large genetic distance from *E. aphana* and the presence of large spike-like tubercles, not found in the previous species.

## Material and methods

### Morphological examination

Specimens were examined using a stereomicroscope, images of type material were taken by DS using a Leica M205C auto-montage with images stacked using Helicon Focus, and AH using a Leica DMC500 digital camera mounted on a Leica MZ16A, stacked using the Leica Application Suite (LAS) ver. 4.13. Photographs of specimens of the Royal Museum for Central Africa (RMCA) are accessible through the RMCA Virtual Collection website (<https://virtualcol.africamuseum.be>). Drawings were produced by VG using images taken, as discussed above, by DS and AH.

Genital organ terminology follows Thaler, van Harten & Knoflach (2004) except for their term “hump” which we replace with dorsal extension. We additionally differentiate them as the upper and lower dorsal extensions. Leg measurements are given as total length, followed by individual segment lengths in brackets (i.e., femur, patella, tibia, metatarsus, tarsus). Authors’ emphases in [ ].

### Institutional abbreviations

NHMUK = Natural History Museum, London  
RMCA = Royal Museum for Central Africa, Tervuren  
SHNT = Saint Helena National Trust, Jamestown

### Abbreviations for morphological terms

ALE = anterior lateral eyes  
AME = anterior median eyes  
C = conductor  
CO = copulatory openings  
E = embolus  
LE = lower dorsal extension  
MS = median septum  
PLE = posterior lateral eyes  
PME = posterior median eyes  
RP = retrolateral process  
SR = spermathecal receptacles  
UE = upper dorsal extension  
VB = ventral blade

### Molecular analyses

DNA was extracted from two legs following Hall & Price (2023), using the ancient DNA protocol from Rohland *et al.* (2018) modified to work at smaller volumes on a Kingfisher™ Flex robot. The leg was lysed overnight at 56°C in 90 µl of lysis buffer C (Korlević *et al.* 2021), after which the lysate was transferred to deep well plates with 900 µl of binding buffer (Dabney *et al.* 2013) and 10 µl of silica bead suspension, prepared following Rohland *et al.* (2018). Following extraction, the DNA was quantified using a Qubit fluorimeter and the Qubit HS dsDNA assay kit (ThermoFisher Scientific). A sequencing library was prepared using the IDT xGen™ ssDNA kit, and uniquely indexed using 10 bp indexes, before being pooled with other museum specimens and sequenced on a single lane of a NovaSeq S4 2\*150 bp flow cell. A total of 88.5 M reads were sequenced (raw data ENA accession: ERS15441084) and used for mitogenome recovery with the following pipeline: mitochondrial reference sequences were downloaded from NCBI and formatted for use with GetOrganelle using the custom python script “go\_fetch.py” ([https://github.com/o-william-white/go\\_fetch](https://github.com/o-william-white/go_fetch)), targeting the lowest taxonomic rank until at least 10 mitochondrial genomes are available.

Tandem repeat sequences were masked using *trf* (Benson, 1999) and annotated gene sequences were then extracted using the python script “*get\_annotated\_regions\_from\_gb.py*” (<https://github.com/Kinggerm/PersonalUtilities/blob/master/>). Adapter sequences were removed for each sample using *fastp* with quality filtering disabled (Chen *et al.* 2018), then the mitochondrial genome was assembled using *GetOrganelle* (Jin *et al.* 2020) with the sample specific reference data described above, and the additional settings “--reduce-reads-for-coverage inf” and “--max-reads inf”. Assembly quality was assessed using *blobtools2* (Laetsch *et al.* 2017; Challis *et al.* 2020) using read mapping produced with *minimap2* (Li 2018) and taxonomic inference based on a *blastn* search against the NCBI nucleotide database (Camacho *et al.* 2009). Contigs with a taxonomic identification from non-target taxa (e.g., fungi) were removed from downstream analyses. Assembled mitochondrial genome data were then annotated using *MITOS2* (Donath *et al.* 2019) and each protein coding gene was extracted using a custom python script. The COI sequence was aligned to data from BOLD (<https://www.boldsystems.org/>) in MEGA (Tamura *et al.* 2021) and translated to confirm the absence of stop codons, then uploaded to BOLD (Process ID: HELEN069-23). A neighbour joining tree was produced using default options on BOLD, including the Kimura 2 parameter distance model and 100 nearest sequences in the full database (Fig. 1).

## Results

### Phylogenetics

The holotype male of *E. lizae* sp. nov. was sequenced and the pipeline recovered 15 607 bp of the mitochondrial genome in a complete single circular contig (ENA Accession: ERS15441084), including the entire COI gene. Genetic comparison on BOLD showed that this species has a significant (> 11%) genetic distance between *E. aphana*, and indeed from other species of *Ero* with sequences available on the Barcode of Life Database (BOLD 2023). However, BOLD only contains a handful of identified species and a multitude of specimens only identified to species level. Therefore, the tree presented here (Fig. 1) is intended only to represent the genetic distance between *E. aphana* and *E. lizae* to show their distinctness and should not be used to infer broader phylogenetic relationships.

### Taxonomy

Class Arachnida Cuvier, 1812  
 Order Araneae Clerck, 1757  
 Family Mimetidae Simon, 1881  
  
 Genus *Ero* C.L. Koch, 1836

### Type species

*Ero tuberculata* (De Geer, 1778).

*Ero lizae* sp. nov.

[urn:lsid:zoobank.org:act:8AF98DA2-DF2C-4C55-9823-294E4A99EFAB](https://zoobank.org/urn:lsid:zoobank.org:act:8AF98DA2-DF2C-4C55-9823-294E4A99EFAB)

Figs 1–9

*Ero aphana* – Unzicker 1977: 127–129 (misidentification).

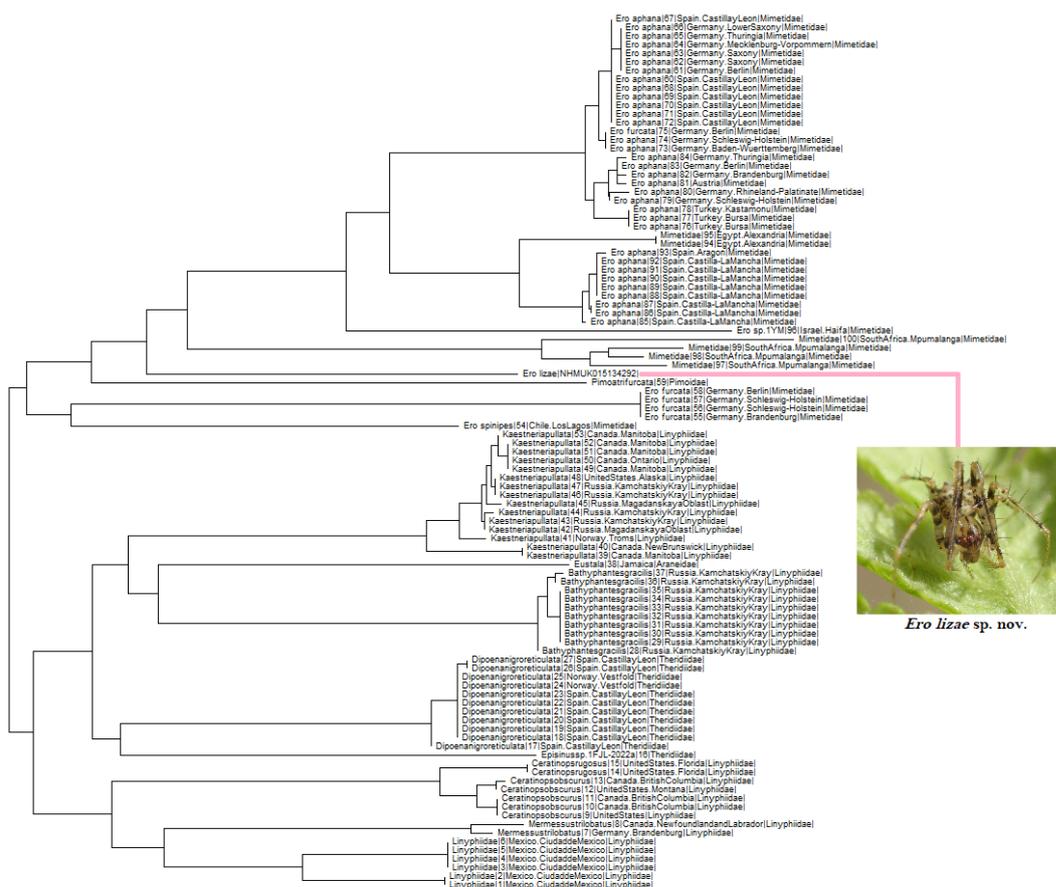
### Diagnosis

*Ero lizae* sp. nov. can be distinguished from all presently known congeners based on the presence of two large spike-like protuberances on the dorso-posterior opisthosoma (Figs 2, 4A–D, 6A) (dorso-posterior opisthosoma without two large spike-like protuberances in all other known congeners).

Further distinguished from the male of *E. aphana* by palp morphology, with the ventral blade twice the length of the dorsal extensions and lower dorsal extension little more than half the length of upper dorsal extension (Figs 3, 5A–B, 6F, 7) (ventral blade less than twice the length of the dorsal extensions and lower dorsal extension more than half the length of upper dorsal extension in *E. aphana*). Also distinguished further from males of *E. natashae* sp. nov. by non-hooked retrolateral cymbial process and the wider and less developed conductor (retrolateral cymbial process hooked and conductor thinner and more developed in *E. natashae*). The female can be distinguished from that of *E. aphana* by the presence of spike-like protuberances (see above) and further by epigynal morphology, with a smaller atrium and the scape ending beyond the anterior atrium (Figs 4E–F, 5C–D, 6C–E, 7) (atrium larger and scape ending before the anterior atrium in *E. aphana*). Further distinguished from those of *E. natashae* by the wider copulatory openings and anteromedian plate slightly protruding, not medially crossed by a thin septum (copulatory openings narrower and anteromedian plate strongly protruding, medially crossed with a thin septum in *E. natashae*). Additionally, *Ero lizae* is genetically distinct from those taxa for which COI barcodes exist on BOLD by a genetic difference of 11.7% (Fig. 1).

### Etymology

The specific epithet is a matronym honouring Saint Helenian conservationist Liza Fowler (Saint Helena National Trust) in recognition of her more than ten years of service to the protection of endemic



**Fig. 1.** Neighbour joining tree formed from COI barcode data on BOLD, showing relationship of *E. aphana* s.s. and *E. lizae* sp. nov.

invertebrates on the island. The senior author's expedition to Saint Helena would not have been nearly as successful without her hard work, knowledge, and encouragement.

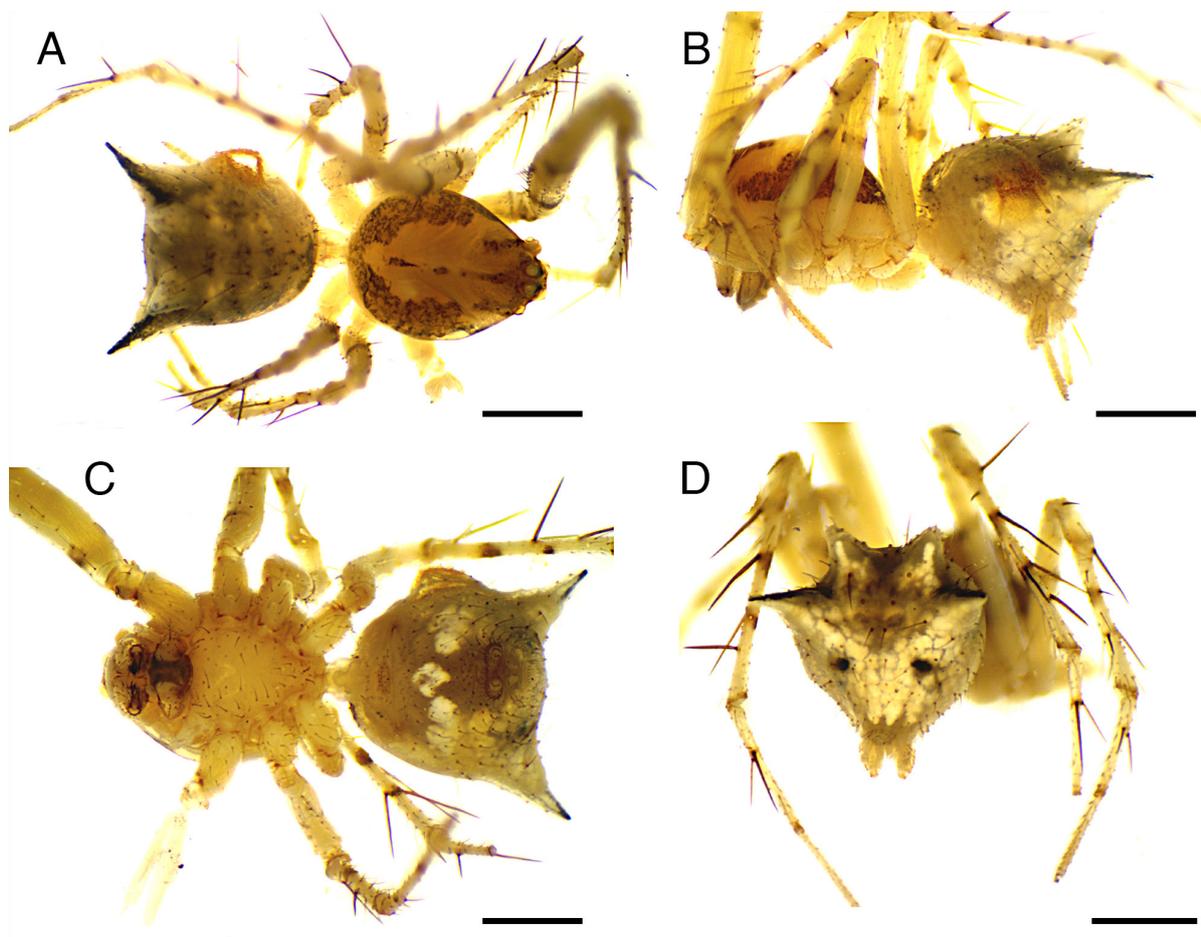
### Material examined

#### Holotype

UNITED KINGDOM – **Saint Helena, Ascencion and Tristan da Cunha** • ♂; Actaeon Peak [= Mt Actaeon], Saint Helena; 16 Dec. 2005–9 Mar. 2006; P. Ashmole and M. Ashmole leg.; B3; 6312/C; [NHMUK AQ ZOO-2022-84]; NNHMUK 015134292.

#### Paratypes

UNITED KINGDOM – **Saint Helena, Ascencion and Tristan da Cunha** • 1 ♀; same collection data as for holotype; [NHMUK AQ ZOO-2022-84]; NNHMUK 015134292 • 1 ♀; Cuckhold's Point, Saint Helena; 15°58'15.5" S, 5°42'11.6" W; alt. 221 m; 9 Feb. 2006; P. Ashmole and M. Ashmole leg.; P. Ashmole and M. Ashmole coll.; night visit; 6298; NHMUK AQ ZOO 2022-84 • 1 imm. ♂; Deep Valley Head, Saint Helena; off Black Scale Fern; 12 Jan. 2006; H. Mendel, P. Ashmole and M. Ashmole leg.; P. Ashmole and M. Ashmole coll.; 6285/C; NHMUK AQ ZOO 2022-84 • 1 ♀; High Central Ridge, Cabbage Tree Road, Saint Helena; alt. 701–792 m; 6 Feb. 1967; J. Decelle and N. Leleup leg.; BE\_RMCA\_ARA. Ara.133384 • 1 ♂, 1 ♀; same collection data as for preceding; Mar. 1967; BE\_RMCA\_ARA. Ara.133283



**Fig. 2.** *Ero lizae* sp. nov., holotype, ♂ (NHMUK 015134292), habitus. **A.** Dorsal view. **B.** Lateral view. **C.** Ventral view. **D.** Posterior view. Scale bars = 1 mm.

• 1 ♀; same collection data as for preceding; 22 Jan. 1967; BE\_RMCA\_ARA.Ara.133370 • 1 ♂; New Restoration, Diana's Peak, Saint Helena; 15°58'13.2" S 5°42'11.5" W; Malaise trap; SHNT.

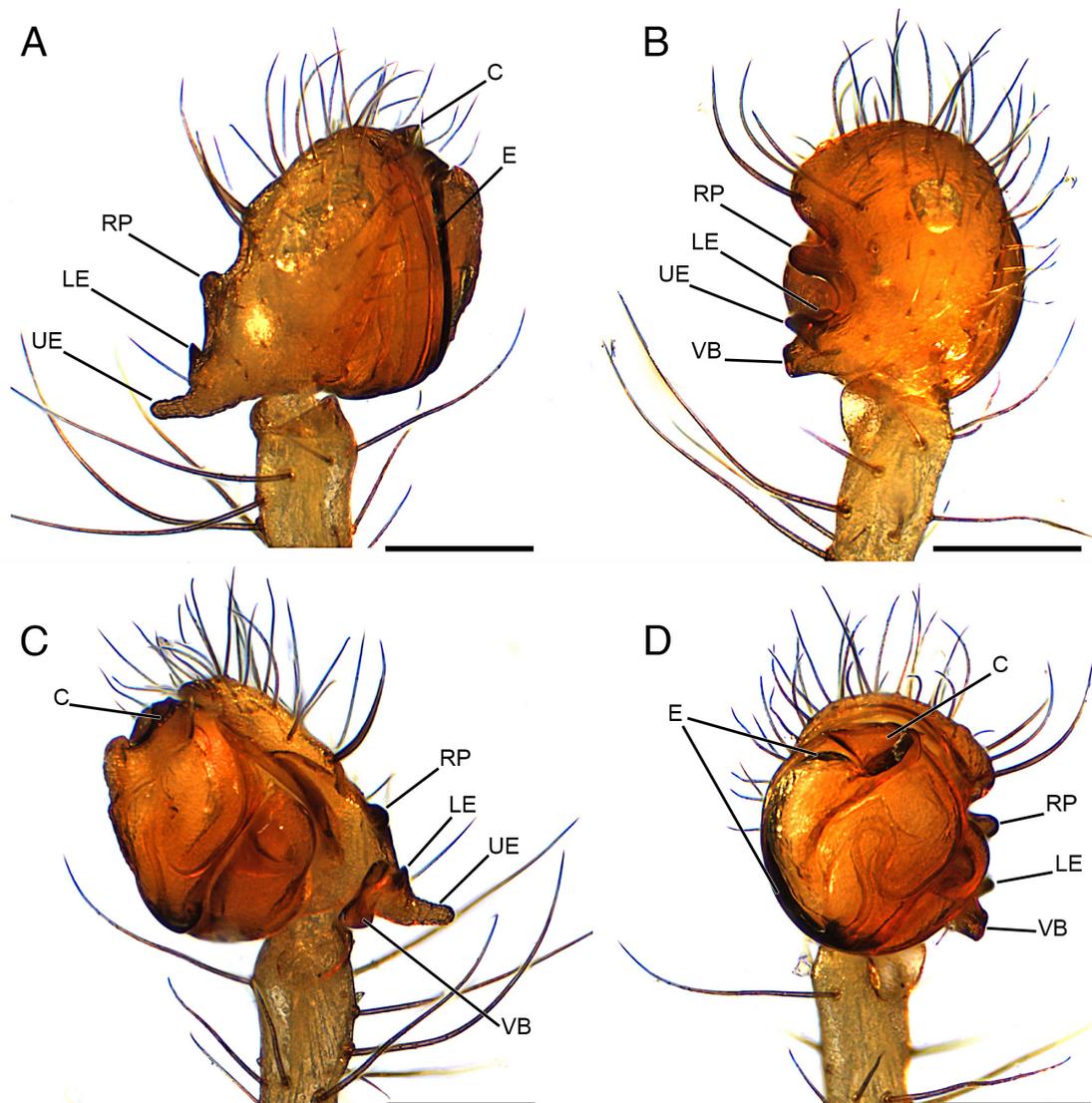
#### Other material

UNITED KINGDOM – **Saint Helena, Ascencion and Tristan da Cunha** • 1 imm.; High Central Ridge, Cabbage Tree Road, Saint Helena; alt. 701–792 m; Mar. 1967; BE\_RMCA\_ARA.Ara.133286 • 1 imm. Diana's Peak, Saint Helena; 15°58' S, 5°42' W; 9 Feb. 1967; J. Decelle and N. Leleup leg.; Cuvette; BE\_RMCA\_ARA.Ara.133448.

#### Description

##### Male holotype

MEASUREMENTS. Total length including chelicerae: 4.01. Carapace: 2.06 long, 1.56 wide. Ocular tubercle: 0.23 long, 0.78 wide. PLE distinctly projecting over outer edge of carapace, ALE distinctly projecting



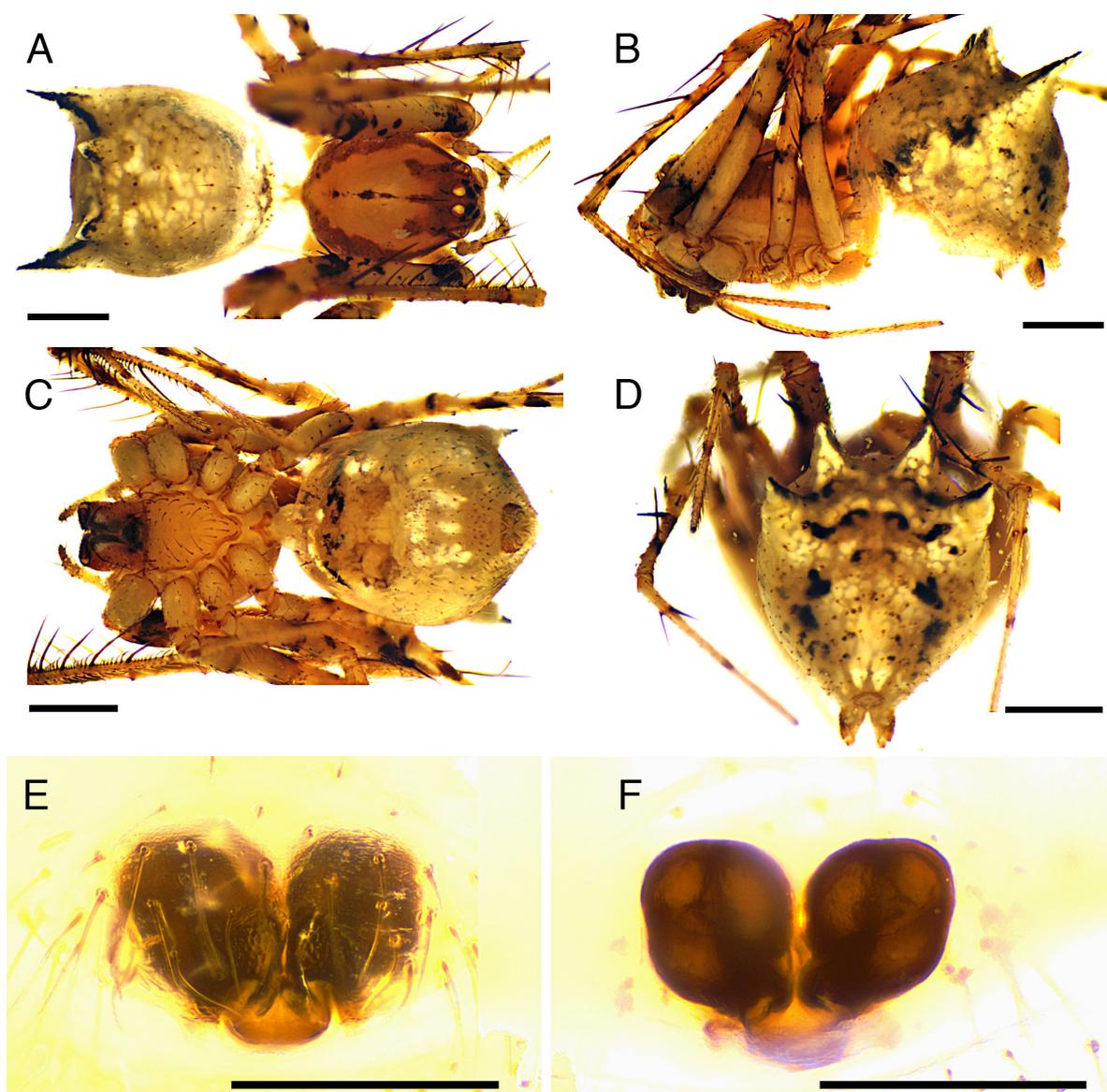
**Fig. 3.** *Ero lizae* sp. nov., holotype, ♂ (NHMUK 015134292), palp. **A.** Prolateral view. **B.** Dorsal view. **C.** Retrolateral view, slightly ventral. **D.** Ventral view. Abbreviations: see Material and methods. Scale bars = 0.2 mm.

over front of ocular tubercle. Chelicerae with 8 peg teeth. Stridulatory ridges absent. Opisthosoma: 1.91 long, 1.78 wide.

LEGS (femur + patella + tibia + metatarsus + tarsus). I 14.49 (4.13 + 1.05 + 4.31 + 3.37 + 1.63), II 8.51 (2.56 + 0.57 + 2.00 + 2.75 + 0.63), III 5.05 (1.97 + 0.43 + 1.00 + 0.85 + 0.80), IV 6.39 (2.29 + 0.64 + 1.74 + 0.93 + 0.79). Metatarsus I with 5 strong spines.

OPISTHOSOMA. With two pairs of spike-like protuberances, anterior pair smaller than posterior pair, which are profoundly enlarged (Fig. 2A–D).

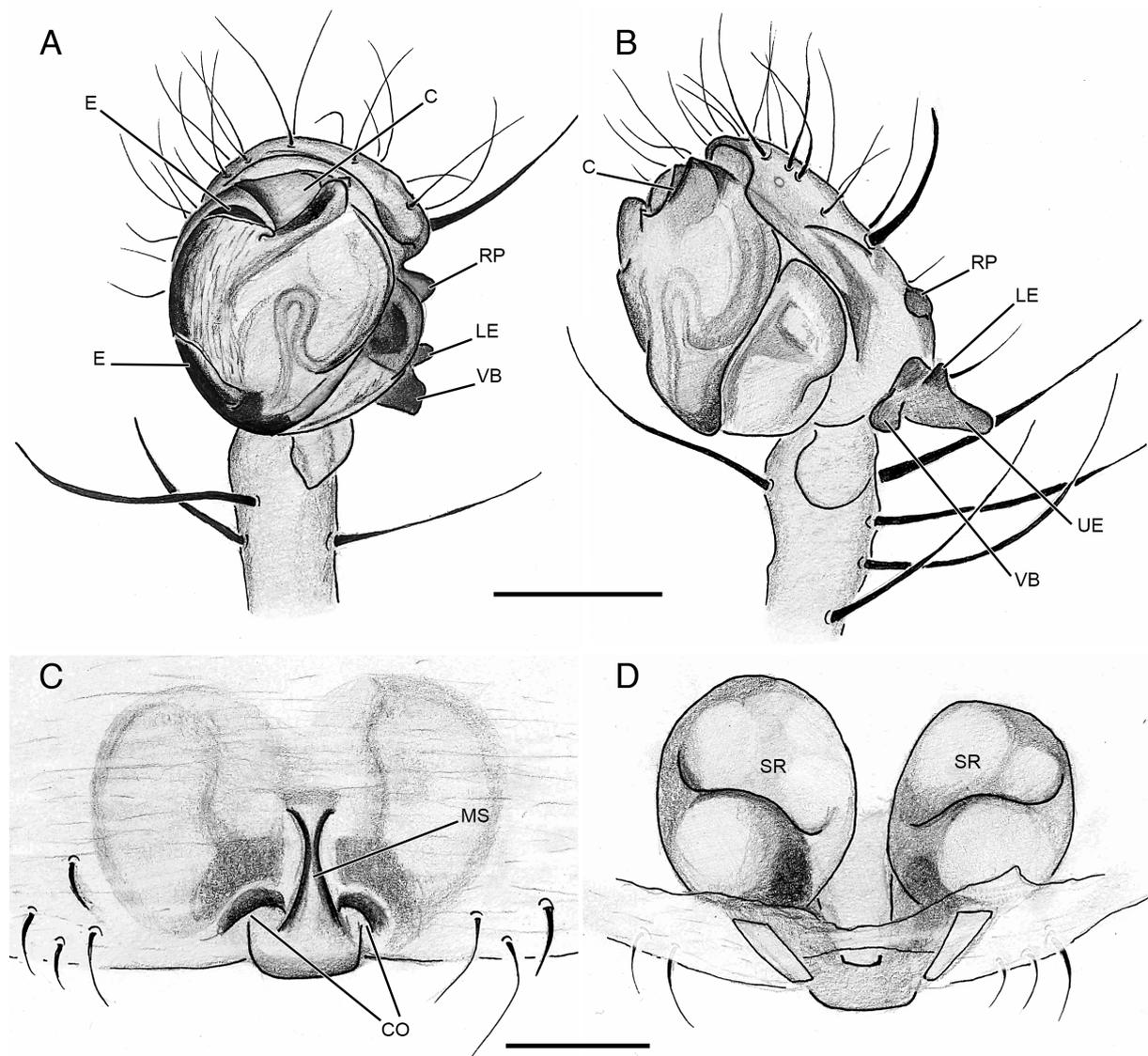
PALP (Figs 3, 5A–B, 6F, 7). Tibia distally with retrolateral circular depression, cymbium medially with blunt retrolateral process (RP), paracymbium with two dorsal extensions and one ventral blade (VB),



**Fig. 4.** *Ero lizae* sp. nov., paratype, ♀ (NHMUK 015134292). **A–D.** Habitus. **A.** Dorsal view. **B.** Lateral view. **C.** Ventral view. **D.** Posterior view. **E.** Epigyne, cleared, ventral view. **F.** Vulva, cleared, dorsal view. Scale bars: A–D = 1 mm; E–F = 0.5 mm.

upper dorsal extension conical, tip rounded (UE), lower dorsal extension triangular (LE), ventral blade elongate (twice the length of dorsal extensions), rounded, embolus emergent proximally, twisted distally, conductor distally rounded (Figs 3, 5A–B).

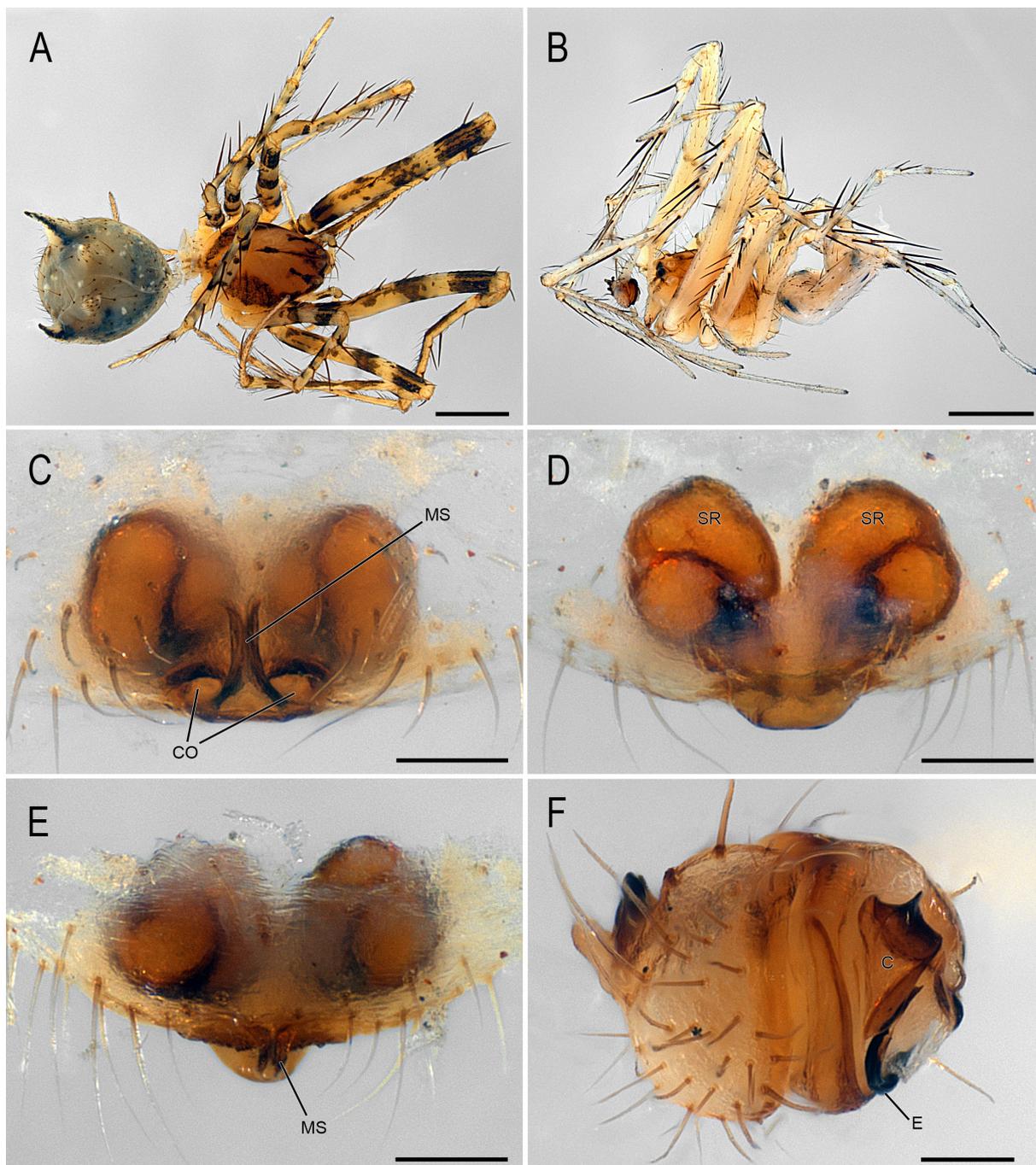
COLOUR (in alcohol; Fig. 2). Carapace brown, with brown markings on lateral and posterior edges, brown blotches forming broken line medially behind ocular tubercle, and single, broken longitudinal brown line extending entire length of carapace medially; legs annulated; opisthosoma brown with black and cream blotches in posterior half; opisthosomal spike-like protuberances black on anterior faces and cream on posterior faces.



**Fig. 5.** *Ero lizae* sp. nov., genitalia illustrations. **A.** Holotype, ♂ (NHMUK 015134292), palp, retrolateral view. **B.** Idem, ventral view. **C.** Paratype, ♀ (BE\_RMCA\_ARA.Ara.133384), epigyne, ventral view. **D.** Vulva, dorsal view. Abbreviations: see Material and methods. Scale bars: A–B = 0.2 mm; C–D = 0.1 mm.

**Female paratype** (specimen from same tube as holotype)

MEASUREMENTS. Total length including chelicerae: 5.68. Carapace: 2.44 long, 1.77 wide. Ocular tubercle: 0.40 long, 1.04 wide. PLE projecting over outer edge of carapace, ALE slightly projecting over front of

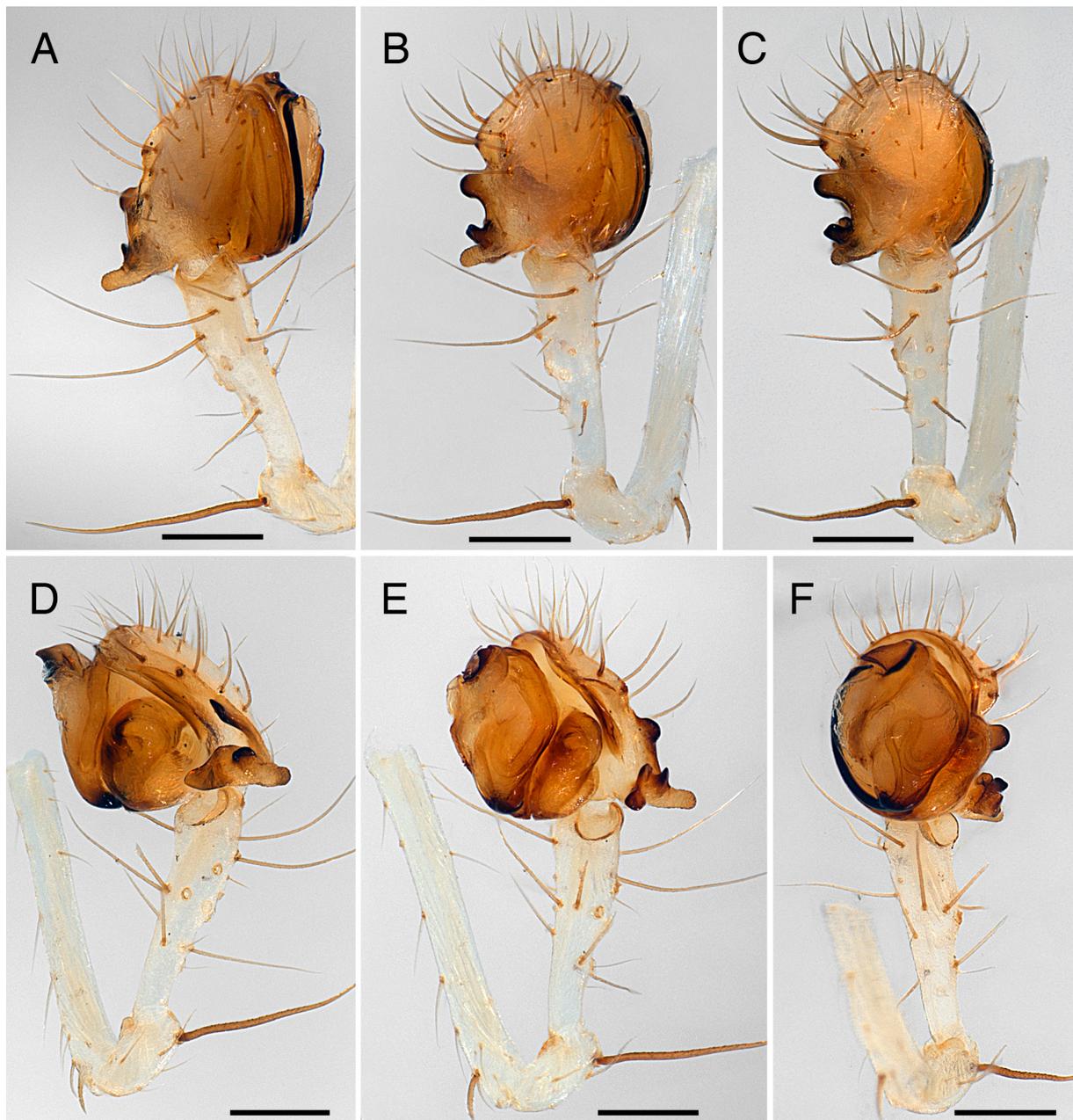


**Fig. 6.** *Ero lizae* sp. nov., additional illustrations. **A.** Paratype, ♀ (BE\_RMCA\_ARA.Ara.133283), habitus, dorsal view. **B.** Paratype, ♂ (BE\_RMCA\_ARA.Ara.133283), habitus, lateral view. **C.** Paratype, ♀ (BE\_RMCA\_ARA.Ara.133283), epigyne, ventral view. **D.** Idem, dorsal view. **E.** Idem, anterior view. **F.** Paratype, ♂ (BE\_RMCA\_ARA.Ara.133283), palp, anterior view. Abbreviations: see Material and methods. Scale bars: A–B = 1 mm; C–F = 0.1 mm.

ocular tubercle. Chelicerae with 8 peg teeth. Stridulatory ridges absent. Opisthosoma: 2.78 long, 2.63 wide.

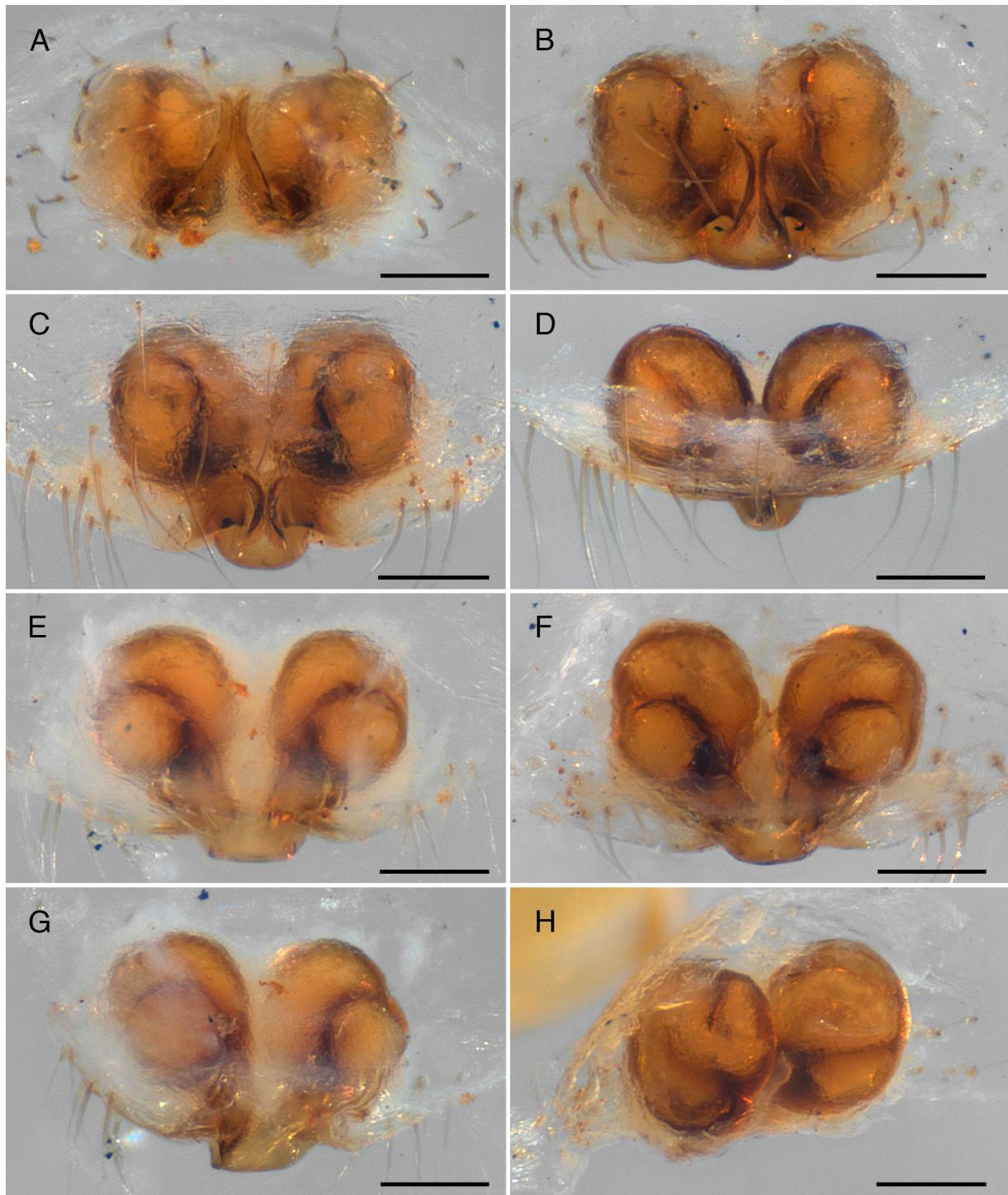
LEGS. I 14.75 (4.47 + 1.14 + 4.10 + 4.16 + 0.88), II 11.67 (3.00 + 0.92 + 2.63 + 3.41 + 1.71), III 6.55 (2.29 + 0.37 + 1.83 + 1.48 + 0.58), IV 7.78 (2.63 + 0.82 + 2.13 + 1.37 + 0.83). Metatarsus I with 5 strong spines.

OPISTHOSOMA. With two pairs of spike-like protuberances, anterior pair smaller than posterior pair (Fig. 4A–D).

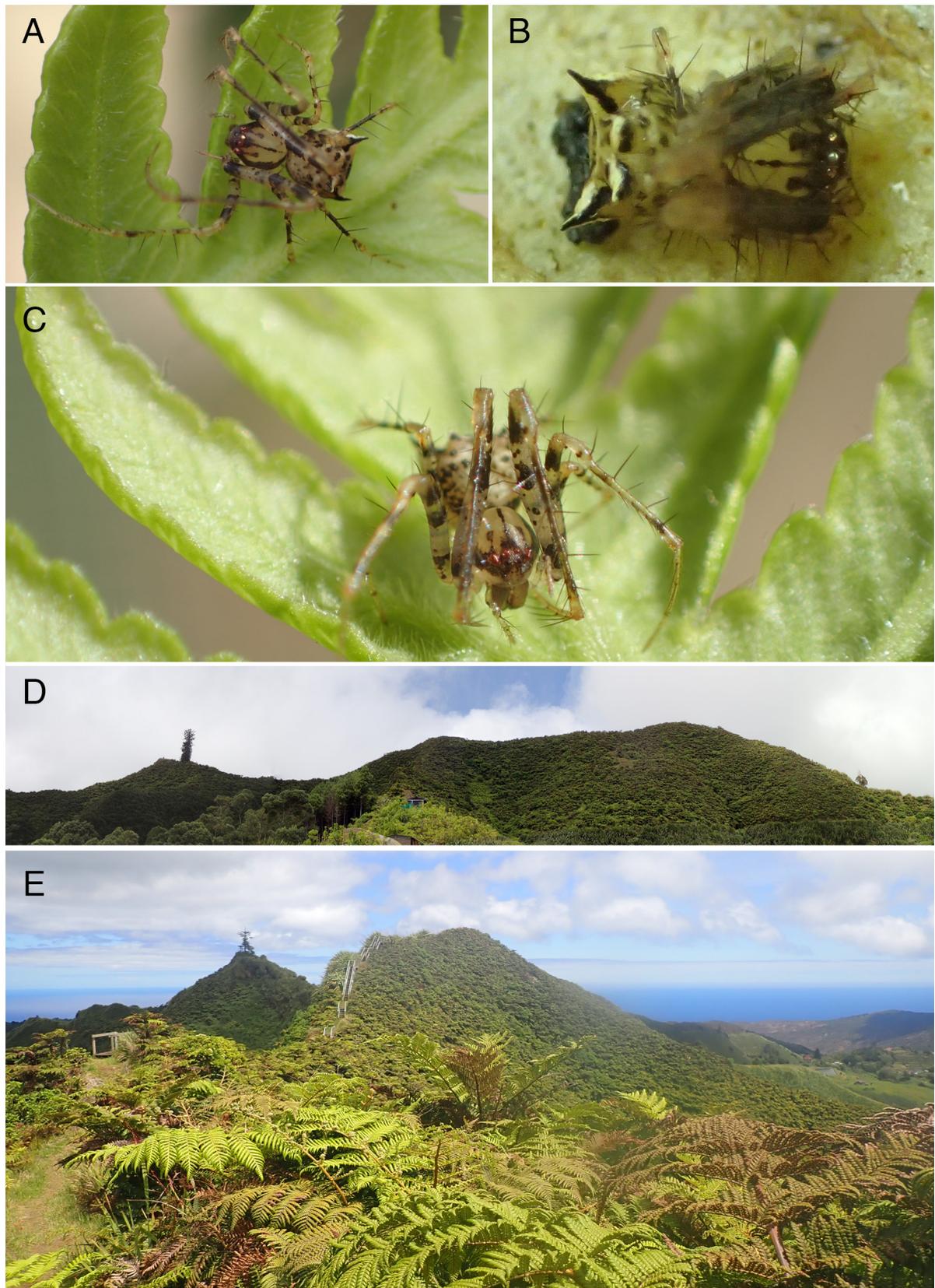


**Fig. 7.** *Ero lizae* sp. nov., additional illustrations of male palp, paratype (BE\_RMCA\_ARA.Ara.133283). **A.** Prolateral view. **B.** Idem, slightly dorsal. **C.** Dorsal view. **D.** Retrolateral view. **E.** Idem, slightly ventral. **F.** Ventral view. Scale bars = 0.2 mm.

EPIGYNE AND VULVA (Figs 4E–F, 5C–D, 6C–E, 8). Epigyne with thin median septum, outer edges of septum sclerotised, longitudinally concave, anteriorly outstripping copulatory openings, copulatory openings



**Fig. 8.** *Ero lizae* sp. nov., additional illustrations of female genitalia. **A, E, G.** Paratype (BE\_RMCA\_ARA.Ara.133370). **B–D, F, H.** Paratype (BE\_RMCA\_ARA-Ara.133384). **A.** Epigyne, ventral view, slightly posterior. **B.** Idem, ventral view. **C.** Idem, slightly anterior. **D.** Idem, anterior view. **E.** Vulva, ventral view. **F.** Idem. **G.** Idem, slightly lateral. **H.** Idem, slightly anterior. Scale bars = 0.1 mm.



**Fig. 9.** A–C. *Ero lizae* sp. nov., non-type, ♀ in vivo. D. Panorama of Peaks showing cloud forest habitat. E. Ground-view from type locality.

circular, with thick and strongly sclerotized anterior margins; vulva with two globular spermathecal receptacles (SR), each dorsally with antero-medial groove (Fig. 4E–F).

COLOUR (in alcohol; Fig. 4A–D). Carapace brown, with brown markings on lateral and posterior edges, brown blotches forming broken line medially behind ocular tubercle, and single, broken longitudinal brown line extending entire length of carapace medially; legs annulated; opisthosoma brown with black and cream blotches in posterior half; opisthosomal spike-like protuberances black on anterior faces and cream on posterior faces.

COLOUR IN VIVO (Fig. 9A–C). Carapace olive yellow, with black markings on lateral and posterior edges, two black blotches forming broken line medially behind ocular tubercle, and single, broken longitudinal black line extending entire length of carapace medially; legs annulated; opisthosoma olive yellow with black and white blotches in posterior half; opisthosomal spike-like protuberances black on anterior faces and white on posterior faces.

### Distribution

Known only from the Peaks National Park, Saint Helena (Fig. 9D–E).

### Remarks

*Ero lizae* sp. nov. has been found only in the cloud forest (Fig. 9D–E) and is presently only recorded from one specific plant, the endemic Black Scale Fern (*Diplazium filamentosum*). It is, however, likely to occur on other ferns, but this will require confirmation through future fieldwork. Diet, behaviour, and other aspects of its ecology are largely unknown presently and need to be studied in the future. Initial fieldwork efforts by DS have failed to find this species in non-cloud forest habitat, but future research is needed. Since we have multiple samples, we present additional plates of paratypes in RMCA, which show low intraspecific genitalic variation (Figs 5C–D, 6–8).

### *Ero natashae* sp. nov.

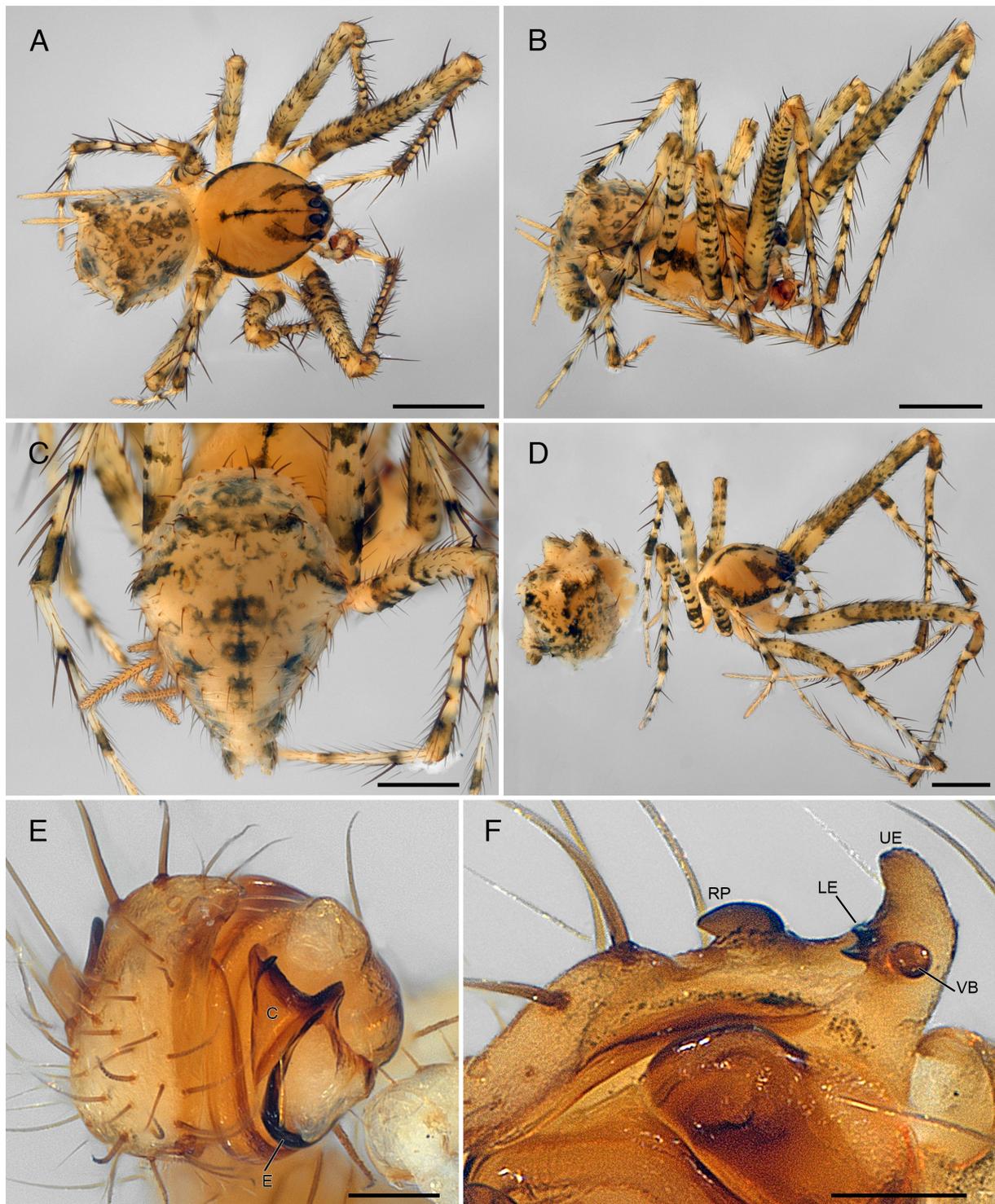
[urn:lsid:zoobank.org:act:DC475BB8-DEBF-41C2-AC9F-9476840FB9B5](https://doi.org/10.21203/rs.3.rs-1011111/v1)

Figs 10–13

*Ero aphana* – Unzicker 1977: 127–129 (misidentification).

### Diagnosis

*Ero natashae* sp. nov. can be distinguished from the male of *E. lizae* sp. nov. by smaller abdominal tubercles (Figs 10A–D) and by the shape of palpal structure, namely: 1) retrolateral cymbial process hooked, thinner and more developed conductor (retrolateral cymbial process non-hooked and conductor wider and less developed in *E. lizae*), and 2) lower dorsal triangular extension of paracymbium much smaller (lower dorsal triangular extension significantly larger in *E. lizae*). *Ero natashae* sp. nov. can be differentiated from *E. aphana* by the blunt hooked retrolateral cymbial process (retrolateral cymbial process more prominently hooked in *E. aphana*), and the different shape of the paracymbium and conductor (cf. Figs 10E–F, 11, 13A–B). Females can be distinguished from both species by its epigyne (Figs 12, 13C–D) with narrow copulatory openings and a strongly protruding anteromedian plate medially crossed by a thin septum (copulatory openings wider and anteromedian plate slightly protruding and not medially crossed by a thin septum in *E. aphana* and *E. lizae*). Both sexes appear to be smaller in body size and have comparatively longer legs than *E. lizae*, which serve as secondary taxonomic characteristics that may further separate them, also possibly indicating *E. natashae* inhabits darker habitats.



**Fig. 10.** *Ero natashae* sp. nov. **A–C, E–F.** Holotype, ♂ (BE\_RMCA\_ARA.Ara.129326). **D.** Paratype, ♀ (BE\_RMCA\_ARA.Ara.133379). **A.** Habitus, dorsal view. **B.** Habitus, lateral view. **C.** Habitus, posterior view. **D.** Habitus, lateral view. **E.** Palp, anterior view. **F.** Palp, showing the detail of cymbial basolateral extensions, retro-ventral view. Abbreviations: see Material and methods. Scale bars: A–B, D = 1 mm; C = 0.5 mm; E–F = 0.1 mm.

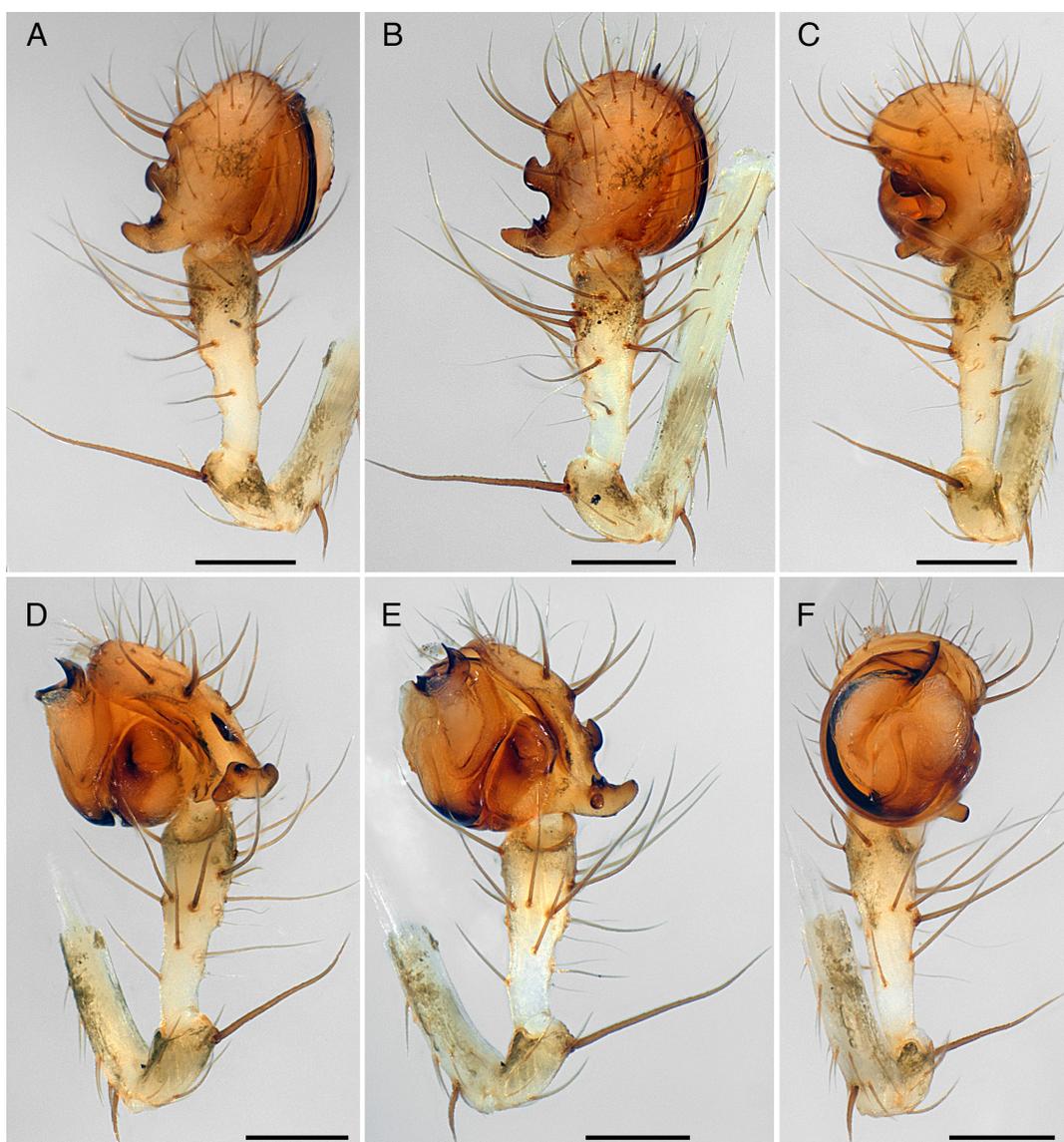
### Etymology

The specific epithet is a matronym honouring the Saint Helenian conservationist Natasha Stevens (Saint Helena National Trust) who has spent many years studying and conserving the invertebrates of Saint Helena, and who provided great help and kindness to the senior author during her expedition to the island.

### Material examined

#### Holotype

UNITED KINGDOM – Saint Helena, Ascencion and Tristan da Cunha • ♂; High Central Ridge, Mt Actaeon, Saint Helena; alt. 792–822 m; 11 Dec. 1965; P.L.G. Benoit, P. Basilewsky and N. Leleup leg.; BE\_RMCA\_ARA.Ara.129326.



**Fig. 11.** *Ero natashae* sp. nov., holotype, ♂ (BE\_RMCA\_ARA.Ara.129326), palp. **A.** Prolateral view. **B.** Idem, slightly dorsal. **C.** Dorsal view. **D.** Retrolateral view. **E.** Idem, slightly ventral. **F.** Ventral view. Scale bars = 0.2 mm.

### Paratypes

UNITED KINGDOM – **Saint Helena, Ascension and Tristan da Cunha** • 1 ♂, 1 ♀; High Central Ridge, Cabbage Tree Road, Saint Helena; alt. 701–822 m; 6 Feb. 1967; J. Decelle and N. Leleup leg.; in rotten trunk; BE\_RMCA\_ARA.Ara.133379 • 1 ♂, 1 imm.; same collection data as for preceding; Mar. 1967; BE\_RMCA\_ARA.Ara.133305 • 1 ♂; SW of Thompsons Wood, Saint Helena; alt. 518–548 m; 23 Nov. 1965; P.L.G. Benoit, P. Basilewsky and N. Leleup leg.; BE\_RMCA\_ARA.Ara.129109 • 1 ♂; High Peak, Saint Helena; 15°58' S, 5°42' W; alt. 731–792 m; Mar. 1967; J. Decelle and N. Leleup leg.; BE\_RMCA\_ARA.Ara.133333.

### Description

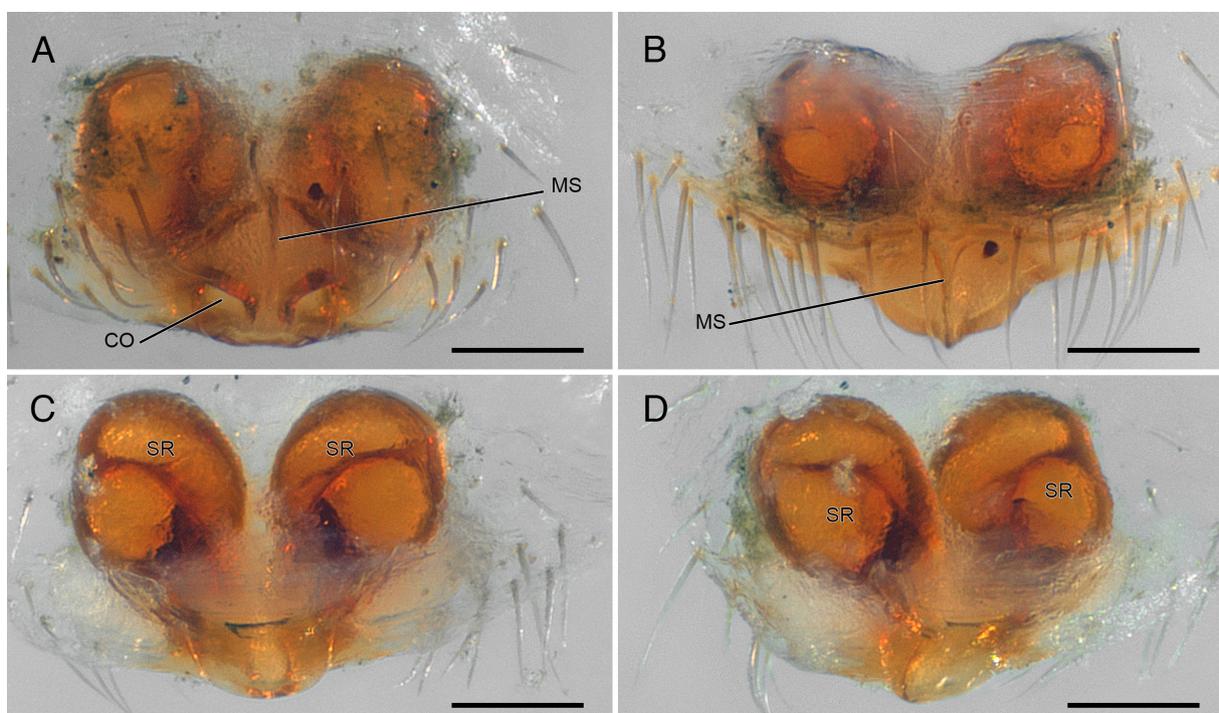
#### Male holotype

MEASUREMENTS. Total length including chelicerae: 2.92. Carapace: 1.48 long, 1.28 wide. Ocular tubercle: 0.26 long, 0.70 wide. PLE distinctly projecting over the outer edge of carapace, ALE distinctly projecting over the front of ocular tubercle. Chelicerae with 7 peg teeth. Stridulatory ridges absent. Opisthosoma: 1.45 long, 1.26 wide.

LEGS (femur + patella + tibia + metatarsus + tarsus). I 11.81 (3.59 + 0.80 + 3.56 + 2.56 + 1.30), II 7.49 (2.34 + 0.55 + 2.00 + 1.56 + 1.04), III 4.83 (1.66 + 0.50 + 1.15 + 0.85 + 0.67), IV 6.09 (2.21 + 0.53 + 1.56 + 1.13 + 0.66). Metatarsus I with 5 strong spines.

OPISTHOSOMA. With two pairs of tubercles, anterior pair smaller than posterior pair (Fig. 10 A–D).

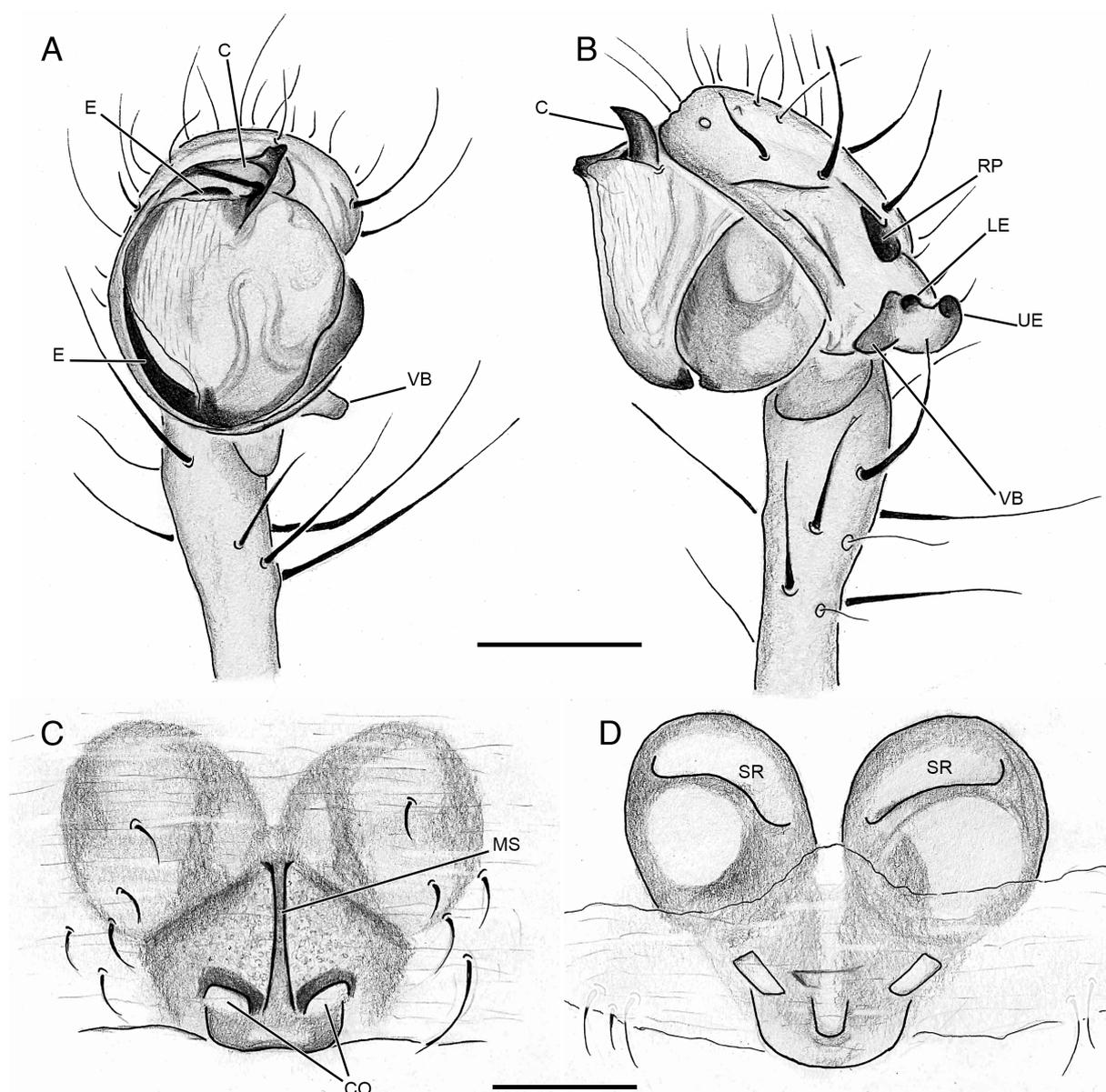
PALP. Embolus emergent proximally, twisted distally, conductor distally rounded, cymbium with retrolateral process (RP), paracymbium with two dorsal extensions and one ventral blade (VB), upper



**Fig. 12.** *Ero natashae* sp. nov., paratype, ♀ (BE\_RMCA\_ARA.Ara.133379), genitalia. **A.** Epigyne, ventral view. **B.** Idem, anterior view. **C.** Vulva, dorsal view. **D.** Idem, slightly lateral. Abbreviations: see Material and methods. Scale bars = 0.1 mm.

dorsal extension (UE) with apex somewhat triangular, lower dorsal extension (LE) rounded, ventral blade longer than lower dorsal extension and shorter than upper dorsal extension, apex somewhat triangular (Figs 10E–F, 11, 13A–B).

COLOUR (in alcohol; Figs 10A–D). Carapace brown, with brown markings on lateral and posterior edges, brown blotches forming broken line medially behind ocular tubercle, and single, broken longitudinal brown line extending entire length of carapace medially; legs annulated; opisthosoma brown with black and cream blotches in posterior half.



**Fig. 13.** *Ero natashae* sp. nov., genitalia illustrations. **A.** Holotype, ♂ (BE\_RMCA\_ARA.Ara.129326), palp, retrolateral view. **B.** Idem, ventral view. **C.** Paratype, ♀ (BE\_RMCA\_ARA.Ara.133379), epigyne, ventral view. **D.** Vulva, dorsal view. Abbreviations: see Material and methods. Scale bars: A–B = 0.2 mm; C–D = 0.1 mm.

**Female paratype** (BE\_RMCA\_ARA.Ara.133379)

MEASUREMENTS. Total length including chelicerae: 3.05. Carapace: 1.72 long, 1.35 wide. Ocular tubercle: 0.30 long, 0.73 wide. PLE distinctly projecting over the outer edge of carapace, ALE distinctly projecting over the ocular tubercle. Chelicerae with 7 peg teeth. Stridulatory ridges absent. Opisthosoma: 2.32 long, 1.87 wide.

LEGS (femur + patella + tibia + metatarsus + tarsus). I 10.17 (3.62 + 0.75 + 2.34 + 2.41 + 1.05), II 7.40 (2.14 + 0.65 + 2.03 + 1.56 + 1.02), III 3.59 (0.85 + 0.56 + 1.21 + 0.54 + 0.43), IV 4.75 (1.58 + 0.59 + 1.53 + 0.72 + 0.33). Metatarsus I with 5 strong spines.

OPISTHOSOMA. With two pairs of tubercles, anterior pair smaller than posterior pair (Fig. 10D).

EPIGYNE AND VULVA. Epigyne with very small septum, outer edges of septum weakly sclerotised, curved in anterior two thirds, copulatory openings circular, vulva with two globular spermathecal receptacles (Figs 12, 13C–D).

COLOUR (in alcohol; Fig. 10D). Carapace brown, with brown markings on lateral and posterior edges, brown blotches forming broken line medially behind ocular tubercle, and single, broken longitudinal brown line extending entire length of carapace medially; legs annulated; opisthosoma brown with black and cream blotches in posterior half.

### Distribution

Known only from the Peaks National Park, and southwest of Thompson's Wood, Saint Helena (see Fig. 9D–E).

### Remarks

*Ero natashae* sp. nov. is sympatric with *E. lizae* sp. nov. (see above) but is easily distinguished by the absence of large spike-like tubercles and by divergent genital organ morphology. Unlike the latter species, it has never been photographed, and no new specimens have been recorded since 1977. Further fieldwork is required to ascertain the population status of this species, and its habitat preference. Unfortunately, it was impossible to sequence this species molecularly within the timeframe and funding of the present work, especially as no fresh material was available.

### Discussion

This work clarifies the taxonomy of mimetid spiders (pirate spiders) on the remote island of Saint Helena, previously thought to be a single, common, and non-native species by Unzicker (1977). The discovery of two sympatric congeners in the internationally important cloud forest further demonstrates Saint Helena's remarkable diversity of invertebrates, and how the cloud forest in particular is a key biodiversity hotspot in the South Atlantic. Further studies are required to elucidate the ecology and life history of these species and to analyse the molecular data of *E. natashae* sp. nov. Nonetheless, this contribution is a significant step forwards in the taxonomy of spiders of this region.

### Acknowledgements

The Species Recovery Trust and St. Helena National Trust supported the development of this paper. This paper has been produced as part of the St. Helena Cloud Forest Project 'Restoring St Helena's Internationally Important Cloud Forest for Wildlife, Water Security and People', funded by the UK Foreign, Commonwealth and Development Office (FCDO). The senior author also wishes to thank Danilo Harms (Leibnitz Institute for the Analysis of Biodiversity Change, Hamburg) for discussions on potential cryptic speciation in the *Ero aphana* complex and for his patience. Joana Cristóvão (NHMUK)

is thanked for her assistance with tissue sampling. Jørgen Lissner (Natural History Museum, Aarhus, Denmark) and two anonymous reviewers are warmly thanked for their comments on the manuscript, which improved its contents. We are also grateful to Rudy Jocqué (Royal Museum for Central Africa) for his valuable editorial support.

## References

- Ashmole P. & Ashmole M. 2000. *St Helena and Ascension Island: a Natural History*. Anthony Nelson Ltd, Shropshire, UK.
- Benson G. 1999. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Research* 27 (2): 573–580.
- BOLD 2023. Barcode of Life Database, version 4.0. Available from <https://www.boldsystems.org> [accessed 23 Jan. 2024].
- Camacho C., Coulouris G., Avagyan V., Ma N., Papadopoulos J., Bealer K. & Madden T.L. 2009. BLAST+: Architecture and applications. *BMC Bioinformatics* 10: 1–9. <https://doi.org/10.1186/1471-2105-10-421>
- Challis R., Richards E., Rajan J., Cochrane G. & Blaxter M. 2020. BlobToolKit – Interactive quality assessment of genome assemblies. *G3: Genes, Genomes, Genetics* 10 (4): 1361–1374. <https://doi.org/10.1534/g3.119.400908>
- Chen S., Zhou Y., Chen Y. & Gu J. 2018. Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34 (17): i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>
- Dabney J., Knapp M., Glocke I., Gansauge M.-T., Weihmann A., Nickel B., Valdiosera C., García N., Pääbo S., Arsuaga J.-L. & Meyer M. 2013. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences* 110 (39): 15758–15763. <https://doi.org/10.1073/pnas.1314445110>
- Donath A., Jühling F., Al-Arab M., Bernhart S.H., Reinhardt F., Stadler P.F., Middendorf M. & Bernt M. 2019. Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. *Nucleic Acids Research* 47 (20): 10543–10552. <https://doi.org/10.1093/nar/gkz833>
- Emerit M. 1980. Une lacune de moins dans la faune malgache: la famille des Mimetidae (Araneae). *In: Comptes rendus Colloque d'Arachnologie d'Expression francaise* 5: 55–63. Barcelona.
- Emerit M. 1996. Contribution à l'étude des aranéides de Madagascar et des Comores: I. La famille des Mimetidae. *Revue Arachnologique* 11: 95–108.
- Hall A.C. & Price B.W. 2023. Museum DNA extraction protocol. Available from <https://www.protocols.io/view/museum-dna-extraction-cnufvetn> [accessed 24 Jan. 2024].
- Jin J.-J., Yu W.-B., Yang J.-B., Song Y., Depamphilis C.W., Yi T.-S. & Li D.-Z. 2020. GetOrganelle: A fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biology* 21 (1): 241. <https://doi.org/10.1186/s13059-020-02154-5>
- Kans J. 2013. Entrez Direct: E-utilities on the Unix Command Line. *In: Entrez Programming Utilities Help. National Center for Biotechnology Information*. Available from <https://www.ncbi.nlm.nih.gov/books/NBK179288/> [accessed 24 Jan. 2024].
- Key R., Fowler L. & Price D. 2021. *Terrestrial & Freshwater Invertebrates of St Helena*. NatureBureau, Newbury.
- Koch C.L. 1836. Arachniden. *In: Herrich-Schäffer G.A.W. (ed.) Deutschlands Insecten*: 137–141. Friedrich Pustet, Regensburg.

- Korlević P., Mcalister E., Mayho M., Makunin A., Flicek P. & Lawniczak M.K.N. 2021. A minimally morphologically destructive approach for DNA retrieval and whole genome shotgun sequencing of pinned historic dipteran vector species. *Genome Biology and Evolution* 13 (10): evab226. <https://doi.org/10.1093/gbe/evab226>
- Laetsch D.R. & Blaxter M.L. 2017. BlobTools: Interrogation of genome assemblies [version 1; peer review: 2 approved with reservations]. *F1000Research* 2017 6: 1287. <https://doi.org/10.12688/f1000research.12232.1>
- Li H. 2018. Minimap2: Pairwise alignment for nucleotide sequences. *Bioinformatics* 34 (18): 3094–3100. <https://doi.org/10.1093/bioinformatics/bty191>
- Nentwig W., Blick T., Bosmans R., Gloor D., Hänggi A. & Kropf C. 2023. Spiders of Europe. Version 05.2023. Available from <https://www.araneae.nmbe.ch> [accessed 4 Jun. 2023]. <https://doi.org/10.24436/1>
- Rohland N. & Reich D. 2012. Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Research* 22 (5): 939–946. <https://doi.org/10.1101/gr.128124.111>
- Rohland N., Gloeckle I., Aximu-Petri A. & Meyer M. 2018. Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. *Nature Protocols* 13: 1. <https://doi.org/10.1038/s41596-018-0050-5>
- Sherwood D. & Fowler L. 2023. Spider research on St Helena: past, present and future. *Wirebird* 52: 2–22.
- Simon E. 1895. *Histoire Naturelle des Araignées. Deuxième édition, tome premier*: 761–1084. Roret, Paris.
- Tamura K., Stecher G. & Kumar S. 2021. MEGA 11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* 38: 3022–3027.
- Thaler K., van Harten A. & Knoflach B. 2004. Pirate spiders of the genus *Ero* C.L. Koch from southern Europe, Yemen, and Ivory Coast, with two new species (Arachnida, Araneae, Mimetidae). *Denisia* 13: 359–368.
- Unzicker J.D. 1966. A review of the African Mimetidae (Arachnida: Araneae) with the description of a new species. *Journal of the Kansas Entomological Society* 39 (3): 506–513.
- Unzicker J.D. 1977. Fam. Mimetidae. In: La Faune Terrestre de l'Île de Sainte-Hélène IV. *Annales, Musée royal de l'Afrique centrale, Sciences zoologiques (Zool.-Ser. 8°)* 220: 127–129.
- Walckenaer C.A. 1802. *Faune Parisienne. Insectes. ou: Histoire Abrégée des Insectes de Environs de Paris*: 187–250. Dentu, Imprimeur Libraire, Palais du Tribunal, galeries de bois, Paris.
- World Spider Catalog 2023. *World Spider Catalog. Version 24.0*. Natural History Museum Bern. Available from <https://wsc.nmbe.ch> [accessed 4 Jun. 2023]. <https://doi.org/10.24436/2>

*Manuscript received: 8 June 2023*

*Manuscript accepted: 25 September 2023*

*Published on: 2 February 2024*

*Topic editor: Magalie Castelin*

*Section editor: Rudy Jocqué*

*Desk editor: Eva-Maria Levermann*

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