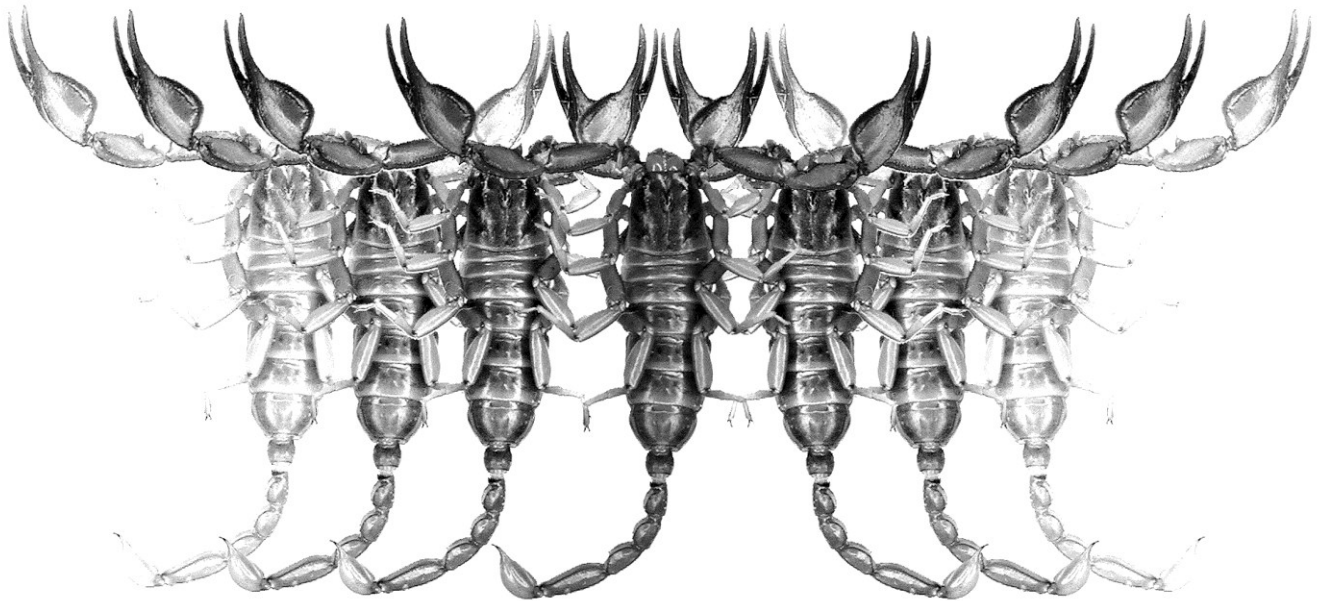


Euscorpilus

Occasional Publications in Scorpiology



**A New Species of *Euscorpilus* from Bulgaria and Greece
(Scorpiones: Euscorpiidae)**

**Gioele Tropea, Victor Fet, Aristeidis Parmakelis, Panayiota Kotsakiozi
& Iasmi Stathi**

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Euscorpius

Occasional Publications in Scorpiology

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Derivatio Nominis

The name *Euscorpius* Thorell, 1876 refers to the most common genus of scorpions in the Mediterranean region and southern Europe (family Euscorpiidae).

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In September 2012, ICZN Article 8. *What constitutes published work*, has been amended and allowed for electronic publications, disallowing publication on optical discs. From January 2013, *Euscorpius* discontinued CD-ROM production; only online electronic version (*ISSN 1536-9307*) is published. For further details on the new ICZN amendment, see <http://www.pensoft.net/journals/zookeys/article/3944/>.

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<http://zoobank.org/urn:lsid:zoobank.org:pub:39CA1923-AD1C-431F-B83C-C0BBF8085B46>

A new species of *Euscorpius* from Bulgaria and Greece (Scorpiones: Euscorpiidae)

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<http://zoobank.org/urn:lsid:zoobank.org:pub:39CA1923-AD1C-431F-B83C-C0BBF8085B46>

Summary

A new scorpion species, *Euscorpius popovi* **sp. nov.**, is described from southwestern Bulgaria and northeastern Greece based on morphological and molecular evidence analyses.

Introduction

The genus *Euscorpius* Thorell, 1876, widespread in southern Europe and Anatolia, is one of the most studied scorpion taxa. Despite this, its taxonomy is still far from being resolved. This is also true for Bulgaria, where this genus has been insufficiently studied in the past. Taxonomic studies of *Euscorpius* are further hindered by the existence of cryptic species complexes, difficult to resolve even with phylogenetic analyses (Parmakelis et al. 2013a, Tropea et al., 2014a). Several relatively recent studies have provided information on different *Euscorpius* populations from Bulgaria, assuming the possibility of new species (Valle, 1975; Fet, 2000; Teruel et al., 2004; Fet & Soleglad, 2007). However, they did not focus on resolving the systematic position of these forms, but rather grouped different populations based on a few morphological characteristics, and placed them in the following species complexes: “*E. carpathicus* complex”, “*E. hadzii* complex” and “*E. mingrelicus* complex”. Most recently, three new species have been described from Bulgaria: *E. deltshevi* Fet et al., 2014, *E. solegladi* Fet et al., 2014, and *E. drenskii* Tropea et al., 2015 (Fet et al., 2014; Tropea et al., 2014b).

Here, we describe a new species from southwestern Bulgaria and northeastern Greece, *Euscorpius popovi* **sp. nov.**, based on morphological and molecular evidence analyses.

Methods and Material

The trichobothrial notation follows Vachon (1974). Morphological measurements are given in millimeters (mm) following Tropea et al. (2014b). Morphological nomenclature follows Stahnke (1970), Hjelle (1990) and Sissom (1990); the chela carinae and denticle configuration follows Soleglad & Sissom (2001); and sternum terminology follows Soleglad & Fet (2003). The map was generated by Earth Explorer 6.1, with positional and altitude data compiled through Google Maps.

All DNA work was performed in the University of Athens by PK and AP; for details on DNA extraction, amplification and sequencing, see Parmakelis et al. (2013a). Phylogenetic analysis was conducted by AP as specified below. Nomenclature for reporting DNA sequences from non-type (“geneseq-3”) specimens follows Chakrabarty et al. (2013).

Abbreviations

V: trichobothrial series on pedipalp chela manus ventral surface (not including *Et*₁); *Pv*: trichobothria on the ventral aspect of pedipalp patella; *Pe*: trichobothria on the external surface of pedipalp patella; *et*: external terminal; *est*: external subterminal; *em*: external median; *esb*: external suprabasal; *eba*: external basal-*a*; *eb*: external basal; *db*: dorsal basal trichobothrium on fixed fin-

ger; *Dp*: pectinal teeth number; *L*: length; *H*: height; *Lchel*: chela length; *Wchel*: chela width (= *Wchel-A* of Tropea et al. 2014a); *Lcar*: carapace length; *Wcar*: carapace width; *Lfem*: femur length; *Lpat*: patella length; *Lmet*: sum of the length of all metasomal segments; *Wmet*: sum of the width of all metasomal segments; *met.seg*: metasomal segment; *CarA/CarP* %: average ratio of distances from center of median eyes to anterior and posterior margins of the carapace; *DPS*: dorsal patellar spur; *DD*: distal denticle; *MD*: median denticles; *OD*: outer denticles; *ID*: inner denticles; *IAD*: inner accessory denticles; *imm.*: immature specimen (in any stage of development).

Depositories: MZUR, Museo di Zoologia dell'Università di Roma "Sapienza", Rome, Italy; NHMW, Naturhistorisches Museum Wien, Vienna, Austria; NHMC, Natural History Museum of Crete, Heraklion, Crete, Greece; NMNHS, National Museum of Natural History, Sofia, Bulgaria; VFPC: personal collection of Victor Fet, Huntington, West Virginia, USA.

Material Studied

A detailed list of material with label data is provided below.

Phylogenetic Analysis

Fourteen published *16S rDNA* sequences together with 16 published and one unpublished *COI* sequences were used in our phylogenetic analyses (Table 1). The phylogenetic analyses was performed on the concatenated dataset (1122 bp) of the *16S rDNA* (439 bp) and *COI* (683 bp) sequences. In those individuals where one of the two sequences was not available (four *16S rDNA* sequences, one *COI* sequence, see Table 1), the missing data were replaced with N. The sequences were aligned using CodonCode Aligner and the Clustal X algorithm. Phylogeny was performed within a Bayesian framework (BI) using the software BEAST v.1.8.2 (Drummond et al., 2012). The species *E. flavicaudis* was used to root the tree. The strict clock option was implemented and the rate was fixed to 1. In the tree prior the speciation model of Yule was enforced. The Akaike Information Criterion (Akaike, 1974) as implemented in Modeltest 3.7 (Posada & Crandall, 1998) was used to choose the best-fit model of *DNA* substitution for each gene fragment. The model selected was the HKY+I+G for *16S rDNA* and the TN93 for *COI*. Four independent runs with the settings described above were run simultaneously. The chain length was set to 200·10⁶, parameters were sampled every 20000 steps and a random starting tree was used. Each run was evaluated for convergence using Tracer v.1.6.0 (Rambaut et al., 2014). Parameters receiving effective sample sizes

(ESS) values above 200 were considered as having been sufficiently sampled (Kuhner, 2009). The results of the four converged and independent runs were combined (after removing of 10% burn-in) using LogCombiner v.1.8.2 of the BEAST package. The sampled posterior trees from all four analyses combined, were summarized using TreeAnnotator v.1.8.2 to generate a maximum clade credibility tree (Fig. 20: maximum posterior probabilities). The genetic distances (Tables 3 and 4) were computed using the Kimura 2-parameter method of Kimura (1980) and are expressed as the number of base substitutions per site.

Systematics

Genus *Euscorpius* Thorell, 1876 Subgenus *Incertus*

Euscorpius popovi Tropea, Fet, Parmakelis, Kotsakiozi et Stathi, sp. n.

(Figures 1–20, Tables 1–4)

<http://zoobank.org/urn:lsid:zoobank.org:act:87870C36-3317-450C-B812-3ECE87E83641>

References:

- Euscorpius carpathicus*, sensu lato (= "*Euscorpius carpathicus* complex"), "Subgroup A1": Fet, 2000: 52 (in part).
Euscorpius sp. 1 ("*carpathicus* complex"): Teruel et al., 2004: 5–7, figs. 16–17.
Euscorpius sp. ("*carpathicus* complex"): Fet & Soleglad, 2007: 413, figs. 13–15 (in part: "southwestern group").
Euscorpius sp., clade E4: Parmakelis et al., 2013a: 10 (in part).
Euscorpius sp. "FESP21 Bulgaria": Tropea et al., 2015: fig. 21.

Type material (28 specimens: 10 ♂, 18 ♀):

Holotype: ♂, BULGARIA. *Blagoevgrad Province*: Sandanski District, Melnik, 29 April 1983, leg. P. Beron, S. Andreev & V. Pomakov (NMHNS 88).

Paratypes: BULGARIA. *Blagoevgrad Province*: Petrich District, Belasitsa Mts., 41.4°N, 23.22°E, 22 July 1930, leg. J. Zonkov, 1 ♂ (NMHNS 315); Sandanski District, Slavyanka (=Alibotush) Mts., Summer Post No. 10, 41.38°N, 23.62°E, 28 June 1937, leg. J. Zonkov, 3 ♀ (NMHNS 501); same data, 3 ♀ (NMHNS 506); Slavyanka Mts., Post No. 16, 1020 m, 41.38°N, 23.62°E, 6 June 1935, leg. P. Drenski, 1 ♂, 2 ♀ (NMHNS 508); same data, 1 ♂, 1 ♀ (MSNB); Slavyanka Mts., Post No. 17, 1400 m, 7 June 1935, leg. J. Zonkov, 2 ♀ (NMHNS 519); same data, 1 ♀ (NHMC); Slavyanka Mts., 20 June 1929, leg. K. Tuleschkov, 1 ♂ (NMHNS 522); same data, 1 ♂, 1 ♀ (MZUR); same data, 1 ♂ (NHMC); Sandanski District, Pirin Mts., above Popina Laka, 1350 m, 9 June 1988, leg. P. Beron, 1 ♀ (NMHNS); same data, 1 ♂, 1 ♀ (NHMW).



Figures 1–2: Dorsal and ventral views of *Euscorpium popovi*, sp. nov., male.

GREECE. *Central Macedonia*: Serres, Kerkini Mts., 800 m, 19 July 2003, leg. G. Ramel, 1 ♂, 2 ♀ (VFPC; used for DNA as FESP13; geneseq-2).

Other *E. popovi* sp. n. examined (not included in type series) (75 specimens: 32 ♂, 43 ♀):

BULGARIA. *Blagoevgrad Province*: Gotse Delchev District, Musomishta, Grebenaro, no date, leg. P. Beron & S. Andreev, 2 ♂ (NMHNS 31); same locality, 19 May 2004, leg. P. Beron & B. Petrov, 1 ♂, 2 ♀ (NMHNS); Hadzhidimovo District, Ilinden, Pazlaka, 6 May 1981, leg. P. Beron, S. Andreev & V. Pomakov, 6 ♂, 4 ♀ (NMHNS 7, NMHNS 13); Hadzhidimovo District, Paril, Hambardere, 8 May 1981, leg. P. Beron & S. Andreev, 3 ♀ (NMHNS 11); Hadzhidimovo District, Paril, near Rupata Cave, 9 May 1981, leg. P. Beron & S. Andreev, 2 ♂ (NMHNS 2); Hadzhidimovo District, Stargach Mts., below Laki, close to Parilska Reka, 500 m, 18 May 2004, leg. P. Beron & B. Petrov, 1 ♀ (NMHNS); Kresna District, Struma Valley, Stara Kresna, 6–7 April 1988, leg. M. Langourov, 2 ♀ (NMHNS); Petrich District, Belasitsa Mts., 22 July 1930, leg. J. Zonkov, 1 ♂

(NMHNS 315); Petrich District, Belasitsa Mts., 4 May 1979, leg. Z. Hubenov, 1 ♀ (NMHNS); Petrich District, Belasitsa Mts., 8 June 1989, leg. P. Mitov, 2 ♂ (NMHNS); Petrich District, Belasitsa Mts., near Petrich, 14 May 1995, leg. P. Beron, 1 ♂ (NMHNS 268); Sandanski District, Goleshovo, near karstic source, 10 May 1984, leg. P. Beron & S. Andreev, 3 ♀ (NMHNS); Sandanski District, Melnik, 31 May 2000, leg. B. Petrov, 2 ♂ imm. (NMHNS 245); Sandanski District, Melnik, 31 May 1999, leg. V. Fet & V. Sakalian, 1 ♂ (VFPC; used for DNA as FESP 21; geneseq-3); Sandanski District, Pirin Mts., between Lilyanovo and Popina Laka, 800–1000 m, 8 June 1988, leg. P. Beron, 1 ♀ (NMHNS); Sandanski District, Pirin Mts., above Popina Laka, 1350 m, 9 June 1988, leg. P. Beron, 1 ♂ imm. (NMHNS); Sandanski District, Pirin Mts., southern part, 900 m, 14 April 1996, leg. B. Petrov, 1 ♀ (NMHNS 196). Sandanski District, Slavyanka (=Alibotush) Mts., 22 May 1925, leg. P. Drenski, 2 ♀ (NMHNS 537); Slavyanka Mts., 20 June 1929, leg. K. Tuleschkov, 1 ♀ (NMHNS 511); Slavyanka Mts., 6 June 1935, leg. J. Zonkov, 1 ♂, 1 ♀ (NMHNS 286); Slav-



Figures 3–4: Dorsal and ventral views of *Euscornius popovi*, **sp. nov.**, female.

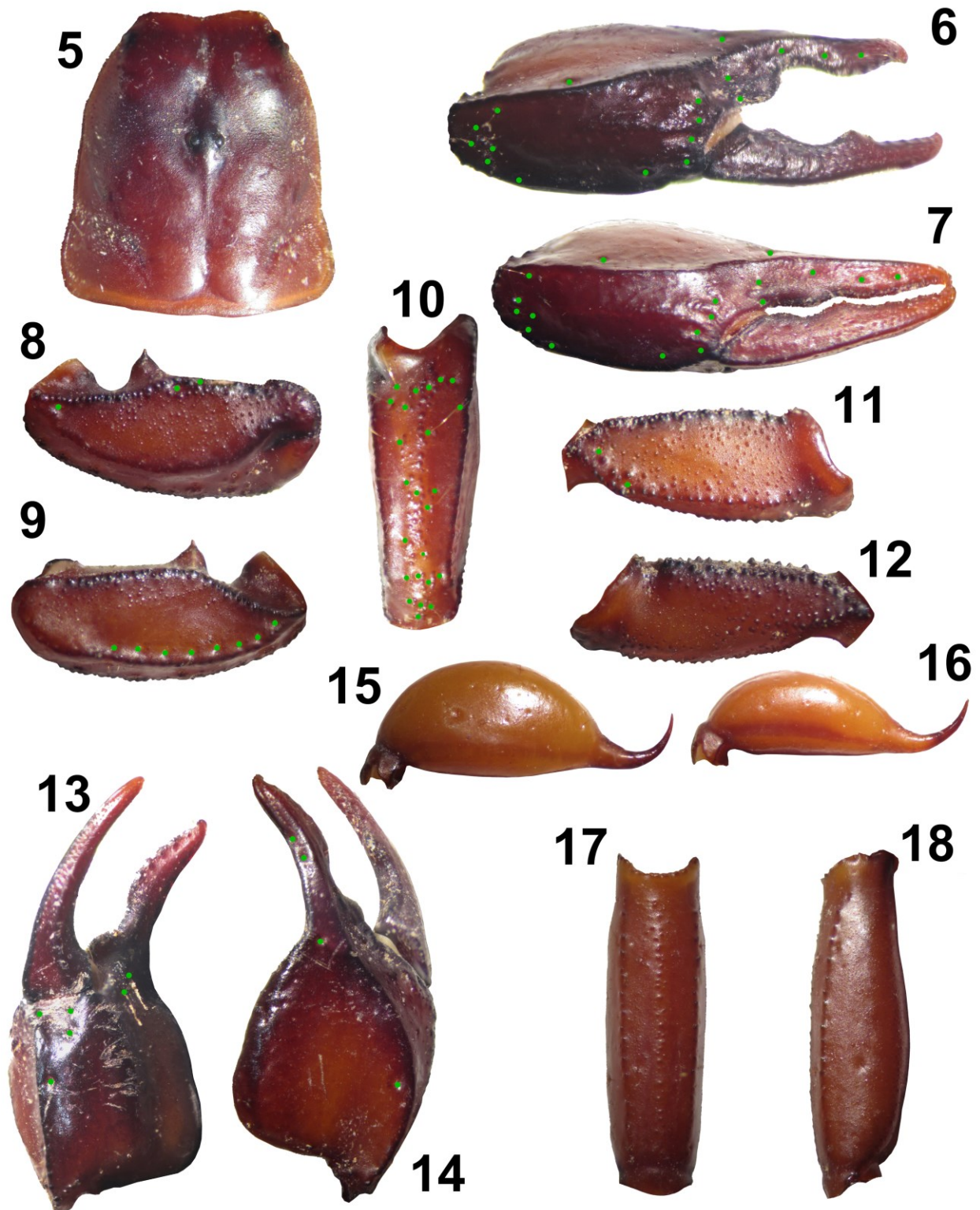
yanka Mts., Post No. 16, 1020 m, 6 June 1935, leg. P. Drenski, 1 ♂, 2 ♀ (NMHNS 287); Slavyanka Mts., 6 June 1935, leg. P. Drenski, 2 ♀ (NMHNS 298); Slavyanka Mts., Post No. 17, 1400 m, 7 June 1935, leg. J. Zonkov, 1 ♂ imm. (NMHNS 519); Slavyanka Mts., 8 June 1935, leg. J. Zonkov, 3 ♀ (NMHNS 515); Slavyanka Mts., Hambardere, 7 June 1936, leg. J. Zonkov, 2 ♀ (NMHNS 292); Slavyanka Mts., Tsarev Vrah summit, 7 June 1936, leg. J. Zonkov, 1 ♀ (NMHNS 531); Slavyanka Mts., Summer Post No. 1, 1450 m, below Tsarev Vrah summit, 7 June 1936, leg. J. Zonkov, 1 ♂, 1 ♀ (NMHNS 545); Slavyanka Mts., 1850 m, below Tsarev Vrah summit, 8 June 1936, leg. J. Zonkov, 3 ♂, 3 ♀ (NMHNS 520); Slavyanka Mts., Post No. 18, 1650 m, 9 June 1936, leg. N. Atanasov, 1 ♂, 4 ♀ (NMHNS 544); Slavyanka Mts., 2100 m, Tsarev Vrah summit, 9 July 1936, leg. J. Zonkov, 1 ♀ (NMHNS 548); Slavyanka Mts., Summer Post 10, 41.38°N,

23.62°E, 28 June 1937, leg. J. Zonkov, 1 ♀ imm. (NMHNS 506); Slavyanka Mts., below Gotsev Vrah summit, 2000 m, 26 July 2004, leg. B. Petrov, 2 ♂ (NMHNS); Slavyanka Mts., Livade, MSS trap, 9 September–17 October 2006, 1780 m, leg. N. Simov, 1 ♂, 1 ♀ (NMHNS).

GREECE. *Central Macedonia*: Serres, Kerkini Mts., 800 m, 19 July 2003, leg. G. Ramel, 2 ♀ (VFPC).

Etymology: We are pleased to name this new species after the great Bulgarian entomologist, Dr. Alexi Popov (National Museum of Natural History, Sofia, Bulgaria), whose contributions over many decades greatly increased the knowledge of the entomofauna and zoogeography of Bulgaria and the Balkan Peninsula.

Distribution: Bulgaria (southwest), Greece (northeast, near southwestern Bulgarian border) (Fig. 19).



Figures 5–18: *Euscorpius popovi*, sp. nov. 5. Carapace. 6. External view of the chela of adult male. 7. External view of the chela of adult female. 8. Dorsal view of pedipalp patella. 9. Ventral view of pedipalp patella. 10. External view of pedipalp femur. 11. Dorsal view of pedipalp femur. 12. Ventral view of pedipalp femur. 13. Dorsal view of the chela of adult male. 14. Dorsal view of the chela of adult female. 15. Telson of adult male. 16. Telson of adult female. 17. Ventral view of the metasomal segment V. 18. Lateral view of the metasomal segment V.

Diagnosis. A medium-sized *Euscorpius* species, total length 31–39 mm (mean 34.5). Color of adults light to medium brown/reddish, carapace darker. Reticulation or marbling varies from absent to marked on chelicerae, carapace, mesosoma and metasoma. The number of trichobothria on the pedipalp manus ventral surface is 4 ($V_{1-3}+Et_1$). The number of trichobothria on the pedipalp patella ventral surface usually from 7 to 8. The number of trichobothria on pedipalp patella external surface is: $eb = 4$, $eb_a = 4$, $esb = 2$, $em = 4$, $est = 4$, $et = 5$ to 6. The pectinal teeth number in males is 8 to 10, mostly 9; in females, usually 7 to 8. Dorsal patellar spur well-developed. Femur usually more or less as long as wide; $Lfem/Lpat$ ratio is 0.99. Carapace more or less as long as wide; mean ratio $Lcar/Wcar$ 1.041 in males and 0.982 in females; mean distance from center of median eyes to anterior margin of the carapace is 41.62% of the carapace length. Mean ratio of $Lmet/Lcar$ is 2.82 in males and 2.55 in females.

Trichobothrial and pectinal teeth count variation

The variation observed in 103 studied specimens (42 ♂, 61 ♀) is given below.

Pectinal teeth in males (n=42): 7/7 (2), 7/8 (2), 8/? (1), 8/8 (2), 8/9 (3), 9/8 (5), 9/9 (18), 9/10 (4), 10/9 (4), 11/10 (1); in total, 7 in 7.23 % (6), 8 in 18.07 % (15), 9 in 62.65 % (52) and 10 in 10.84 % (9); mean = 8.81, SD = 0.77.

Pectinal teeth in females (n=60): 6/7 (1), 7/6 (1), 7/7 (13), 7/8 (4), 8/7 (10), 8/8 (28), 9/8 (3); in total, 6 in 1.67 % (1), 7 in 35.00 % (42), 8 in 60.83 % (73) and 9 in 2.50 % (3); mean = 7.64, SD = 0.56.

Pedipalp patella trichobothria Pv (n=102): 6/6 (2), 6/7 (1), 6/8 (1), 7/? (1), 7/7 (27), 7/8 (13), 8/? (1), 8/7 (9), 8/8 (45), 8/9 (1), and 9/9 (1); in total, 6 in 0.99 % (2), 7 in 34.16 % (69), 8 in 55.88 % (38), and 9 in 1.49 % (3); mean = 7.57, SD = 0.58.

Pedipalp patella trichobothria Pe (n=102): $et = 4/5(1)$, $5/4(1)$, $4/6(2)$, $5/5(35)$, $5/6(3)$, $6/?(2)$, $6/5(7)$, $6/6(48)$, $6/7(1)$, $7/6(1)$, $8/5(1)$; in total, 4 in 1.98 % (4), 5 in 41.09 % (83) and 6 in 55.45 % (112); mean = 5.57, SD = 0.58; $est, eb_a, eb = 4/?(2)$, $4/4(33)$; $esb = 2/?(2)$, $2/2(100)$; $em = 3/3(1)$, $4/3(1)$, $4/4(100)$.

Description of the male holotype

Coloration: Whole color medium brownish with carapace and pedipalps darker reddish; sternites and pectines and genital operculum very light brownish/ivory; chelicerae orange, without marbling; telson yellowish/orange, with a longitudinal lighter line and dark reddish aculeus tip; all pedipalps carinae darker, dark brown to blackish colored; no marbling is present.

Carapace: Granulation of variable size is present on entire surface, except in the anterior area between the

anterior edge, the lateral eyes and median eyes, which is almost smooth, very finely punctated, and the lateral area behind the lateral eyes, which has larger granules; anterior edge slightly granulated and concave; deep posterior lateral and anterior median furrows; two pairs of lateral eyes (with a larger anterior eye), and a pair of median eyes, situated distally of the middle; distance from center of median eyes to anterior margin is 40.91 % of carapace length.

Mesosoma: Tergites very finely granulated; sternites glossy and punctated. Small spiracles inclined about 45° downward toward outside.

Metasoma: All the metasomal segments are longer than wide. Dorsal carinae on segments I–IV granulated; ventrolateral carinae absent on segment I, obsolete or smooth on segments II–IV, serrulated on segment V; ventromedian carina absent on segments I–IV, the V with serrulated granules; the dorsal and lateral intercarinal spaces have very fine granulation, while the ventral intercarinal spaces is smooth or punctated.

Telson: Vesicle with a few very small granules, and ventral setae of different size, especially near the vesicle/aculeus juncture.

Pectines: Teeth number 9/9; middle lamellae number 6/6; several microsetae on proximal area of teeth, marginal lamellae, middle lamellae and fulcra.

Genital operculum: The genital operculum is formed by two longitudinally separated subtriangular sclerites; genital papillae protruding; a few microsetae are present.

Sternum: Pentagonal shape, type 2; more or less as long as wide, with a deep posterior emargination.

Pedipalps: Coxa and trochanter with tuberculated carinae. Femur: dorsal and ventral internal carinae tuberculated; dorsal external carinae formed by slightly spaced tubercles; external median carinae serrulated; ventral external carinae formed by spaced tubercles, well-formed only in the proximal one-third; anterior median formed by 10/12 spaced conical tubercles, varying in size; dorsal and ventral intercarinal spaces with granules of variable size. Patella: dorsal and ventral internal carinae tuberculated to granulated; dorsal and ventral external carinae crenulated, with low tubercles; dorsal intercarinal surface with scattered granules; ventral intercarinal surface with a few scattered granules, especially near ventral internal carinae. Dorsal patellar spur well-developed. Chelal carina $D1$ distinct, strong, dark, smooth to rough; $D4$ dark, rounded, smooth to rough; $V1$ distinct, strong, dark, and rough with a few tubercles proximally; $V3$ rounded, dark, and smooth; external carina granulated; intercarinal tegument finely granulated. Typical *Euscorpius* chela finger dentition.

Trichobothria: Chela: trichobothria on the pedipalp manus ventral surface $4/4$ ($V_{1-3}+Et_1$). Patella ventral (Pv): $8/8$. Patella external (Pe): $et = 6/6$, $est = 4/4$, $em = 4/4$, $esb = 2/2$, $eb_a = 4/4$, $eb = 4/4$. Femur: trichoboth-

Species	Locality/Specimen Code	Accession number, <i>16S rDNA</i>	Accession number, <i>COI</i>
<i>Euscorpius popovi</i> sp. n.	Greece, Central Macedonia, Serres, Kerkini Mts. (geneseq-2), FESP13	KC215647	KC215733
<i>Euscorpius popovi</i> sp. n.	Bulgaria, Blagoevgrad Province, Sandanski District, Melnik (geneseq-3), FESP21	KC215651	KC215737
<i>E. avcii</i> Tropea et al., 2012	Turkey: Aydin Province: Guzelcamli, Gzm 2	KF030937	KF030935
<i>E. carpathicus</i> (Linnaeus, 1767)	Romania: Băile Herculane	n/a	HM418284
<i>E. concinnus</i> (C.L. Koch, 1837)	Italy: Elba Island, Procchio, 134F	KC215581	KC215664
<i>E. deltshevi</i> Fet et al., 2014	Bulgaria: Vidin Province, Belogradchik, VF0821	n/a	KM111243
<i>E. drenskii</i> Tropea et al., 2015	Bulgaria: Trigrad, 117F	KP123428	KT602916
<i>E. flavicaudis</i> (De Geer, 1778)	Italy: Sardinia, Chiamonti, FEF1	KC215632	KC215716
<i>E. germanus</i> (C.L. Koch, 1837)	Austria: Oberdrauburg, Ege_OB	AJ249553	n/a
<i>E. italicus</i> (Herbst, 1800)	Greece: Epirus, Chionades, EI201	KC215622	KC215706
<i>E. solegladi</i> Fet et al., 2014	Bulgaria: Sandanska Bistritsa, VF801	n/a	KM111247
<i>E. stahlavskyi</i> Tropea et al., 2014	Greece, Epiros, Mt. Smolikas, FESP33	KC215653	KC215739
<i>E. tergestinus</i> (C.L. Koch, 1837)	Croatia: Rab Island, FETE1	KC215656	KC215827
<i>E. sp.</i> (Rhodope2)	Bulgaria: Kovachevitsa, 113F	KC215579	KC215662
<i>E. sp.</i> (Rhodope4)	Greece: Central Macedonia, Serres, Mt. Menikio, FESP11	n/a	KC215731
<i>E. sp.</i> (Rhodope5)	Greece: Kavala, Elefteroupoli [incorrectly reported in Parmakelis et al. (2013a) as “Serres, on the road Dramas-Strymonas, Mikro Souli”], FESP9	KC215644	KC215729
<i>E. sp.</i> (Rhodope6)	Greece: Central Macedonia, Drama, Prosotsani, Angkitis, FESP10	KC215645	KC215730
<i>E. sp.</i> (Rhodope7)	Greece: Thrace, road Drama to Xanthi, 120F	KC215580	KC215663

Table 1 : DNA sequences used in phylogenetic analysis. GenBank accession numbers of the sequences used are given after Gantenbein et al., 2000 (AJ249553); Fet et al., 2014 (HM418284); Parmakelis et al., 2013a, 2013b; Tropea et al., 2015 (KP123428). “Genseq” designations according to Chakrabarty et al. (2013). Unpublished COI sequence (*E. drenskii*) was deposited in GenBank under number KT602916.

rium *d* is slightly proximal to *i*, while trichobothrium *e* is located well distally to both *d* and *i*, and situated on dorsal external carina.

Legs: With two pedal spurs; no tarsal spur; ventral row of tarsus III with a total of 11/11 spinules, of increasing size from proximal to distal, ending with a decentralized spinule. 5–10 prolateral spinules on ventral part of the basitarsus of the legs I and II, with none or two small spinules near the pedal spurs of the legs III and IV. Granulation well present on dorsal and ventral surface of leg femora, it is mostly marked and dark ventrally.

Chelicerae: Movable finger: the dorsal distal denticle is much smaller than the ventral distal denticle; ventral edge is smooth with brushlike setae on the inner part; dorsal edge has five denticles: one large distal, two small subdistal, one large median, and a small basal. Fixed finger has four denticles: one distal, one subdistal, one median, and one basal, the last two in a fork arrangement; the internal surface has brushlike setae.

Discussion

The species of the genus *Euscorpius* in Bulgaria have been insufficiently studied until recent time, and were commonly addressed as “*Euscorpius carpathicus*”. A limited information on the Bulgarian populations was given mostly in relatively recent papers (Valle, 1975; Fet, 2000; Teruel et al., 2004; Fet & Soleglad, 2007). These authors assumed the possibility of new species present in Bulgaria, however, they did not focus on resolving the systematic position of these forms, but rather grouped several populations based on their morphology, and addressed them as belonging to species groups or complexes: “*E. carpathicus* complex”, “*E. hadzii* complex” and “*E. mingrelicus* complex”. Teruel et al. (2004) reported a population from Pirin Mts. as “*Euscorpius* sp. 1 *carpathicus* complex”, which belongs to the new species described here, *E. popovi* sp. n.

Recently, the study of genus *Euscorpius* was intensified, including also the populations of Bulgaria, based on extensive collections of NMNHS as well as new field studies by one of the authors (V.F., 1999 and 2005). This led to the description of three new Bulgarian species: *E. deltshevi* Fet et al., 2014, from the Stara Planina (= Balkan) Mts. in central Bulgaria; *E. solegladi* Fet et al. 2014, from southwestern Bulgaria (both belonging to the subgenus *Euscorpius*), and *E. drenskii* Tropea et al., 2015, from the Western Rhodope Mts. in southern Bulgaria (subgenus incertus).

Parmakelis et al. (2013a), in a large phylogenetic study of *Euscorpius* from Greece, also included two populations from southwestern Bulgaria (Melnik, FESP 21) and from northeastern Greece (Kerkini, FESP13), which belong to the new species described here, *E. popovi* sp. n. The latter clustered with several pop-

ulations from northeastern Greece and southern Bulgaria in the phylogenetic tree of Parmakelis et al. (2013a) (Clade E4), as well as with *E. drenskii* in the phylogenetic tree of Tropea et al. (2015), and the phylogenetic tree proposed here (Fig. 20).

Several methods of species delimitation and a species validation method were used in Parmakelis et al. (2013a), based on the phylogeny inferred using sequence data from one nuclear and three mtDNA loci. Except from one species delimitation method (see Fig. 3 in Parmakelis et al., 2013a), *E. popovi* sp. n. was always strongly supported as corresponding to an independent evolutionary unit.

According to our pilot phylogeny based on *16S rDNA* and *COI* sequence data (Fig. 20), and to Parmakelis et al. (2013a), *E. popovi* sp. n., together with *E. drenskii* and other populations from southwestern Bulgaria and northeastern Greece, form a clade that is well-separated from the existing subgenera: *Polytrichobothrius* Birula, 1917 (represented here by the type species *E. italicus*); *Tetratrachobothrius* Birula, 1917 (represented here by the type species *E. flavicaudis*); *Euscorpius* Thorell, 1876 (represented here by the type species *E. carpathicus*, and also by *E. tergestinus* and *E. concinnus*); and *Alpiscorpius* Gantenbein et al., 1999 (represented here by the type species *E. germanus*).

Euscorpius popovi sp. n. exhibits genetic distance in the *16S rDNA* sequences of 3.4 % from *E. drenskii*, the closest related described species, and from 3.4 % to 5 % from other populations of its clade (Rhodope populations in our phylogeny, Fig. 20), which are a subject of our further studies (Tropea et al., in progress). This genetic distance is similar or higher than among other closely related species (e.g. in our analysis the distance between *E. concinnus* and *E. tergestinus* is 2.4 %). A much higher genetic distance is shown among the remaining species, 8.3 % to 13.2 % (Table 3).

The genetic distance between the *COI* mtDNA sequences is usually higher than in *16S rDNA* but is suitable for diagnosing the species level (see Table 4). *E. popovi* sp. n. exhibits genetic distance between the *COI* sequences of 9.1 to 9.3 % from *E. drenskii*, and 6.3 % to 8.4 % from other populations of its clade. With the other two Bulgarian species, *E. solegladi* and *E. deltshevi*, this divergence is 9.0 % to 9.3 % and 10.0 % to 10.2 %, respectively. With the remaining species of our phylogenetic tree the divergence ranges from 8.6 % to 13.4 % (Table 4).

Though the Bulgarian species, *E. solegladi* and *E. deltshevi*, were not included in the phylogenetic analysis of Parmakelis et al. (2013a), in our phylogeny we include these two species based on their published *COI* sequences (*16S rDNA* data is not available for these species) (Fet et al., 2014). It is clear that these two Bulgarian species are not closely related to the clade that includes *E. popovi* sp. n. and *E. drenskii*, and that the

<i>Euscorpius popovi</i> sp. n.		Holotype ♂	Paratype ♀
Total	Length	38.22	34.17
Carapace	Length	5.52	5.10
	Post. width	5.05	5.16
Metasoma	Length	15.72	12.93
Segment I	Length	2.10	1.65
	Width	1.92	1.80
Segment II	Length	2.46	1.98
	Width	1.66	1.56
Segment III	Length	2.76	2.22
	Width	1.62	1.50
Segment IV	Length	3.24	2.64
	Width	1.56	1.35
Segment V	Length	5.16	4.44
	Width	1.59	1.38
Telson	Length	5.22	4.14
Vesicle	Length	3.84	2.85
	Width	1.88	1.51
	Height	2.10	1.38
Aculeus	Length	1.38	1.29
Femur	Length	4.49	4.08
	Width	1.68	1.56
Patella	Length	4.50	4.14
	Width	1.80	1.86
Chela	Length	9.36	8.40
	Width	3.96	3.36
Movable finger	Length	5.54	4.86
Ratios	<i>CarA (%)</i>	41.30	42.35
	<i>Lcar/Lfer</i>	1.230	1.250
	<i>Lcar/Ltel</i>	1.057	1.232
	<i>Lchel/Wchel</i>	2.364	2.500
	<i>L/W met.seg I</i>	1.094	0.916
	<i>L/W met.seg II</i>	1.485	1.269
	<i>L/W met.seg III</i>	1.704	1.480
	<i>L/W met.seg IV</i>	2.077	1.955
	<i>L/W met.seg V</i>	1.883	1.703
	<i>Lmet/met.seg V</i>	3.046	2.912
	<i>Lmet/Lcar</i>	2.850	2.535
	<i>Lfem/Lpat</i>	1.227	1.232

Table 2: Measurements (mm) and morphometric ratios of *Euscorpius popovi* sp. n.

two latter species do not belong to the subgenus *Euscorpius* s. str. We confirm the conclusion of Fet et al. (2014) that *E. deltshevi* is the closest known species to the Romanian *E. carpathicus*, while *E. solegladi* (which is related to *E. hadzii* Di Caporiacco, 1950) groups close to the clade that includes *E. carpathicus*. In addition, *E. popovi* sp. n. shows a high genetic divergence from *E. tergestinus* and *E. concinnus* (two species belonging to the subgenus *Euscorpius*), which is 9.7 % to 10.3 % in *16S rDNA* (Table 3).

Comparison with other Bulgarian species

E. popovi sp. n. can be easily differentiated from the allopatric *E. drenskii* (Western Rhodope) since (1) the latter has the reduced external trichobothrial series $em = 3$, versus “standard” $em = 4$ in *E. popovi* sp. n.; (2) *E. drenskii* is smaller than *E. popovi* sp. n. (mean 29 mm, with a range of 26.5–31.7 in *E. drenskii*, and mean 34.5, with a range of 31.2–39.0 in *E. popovi* sp. n.).

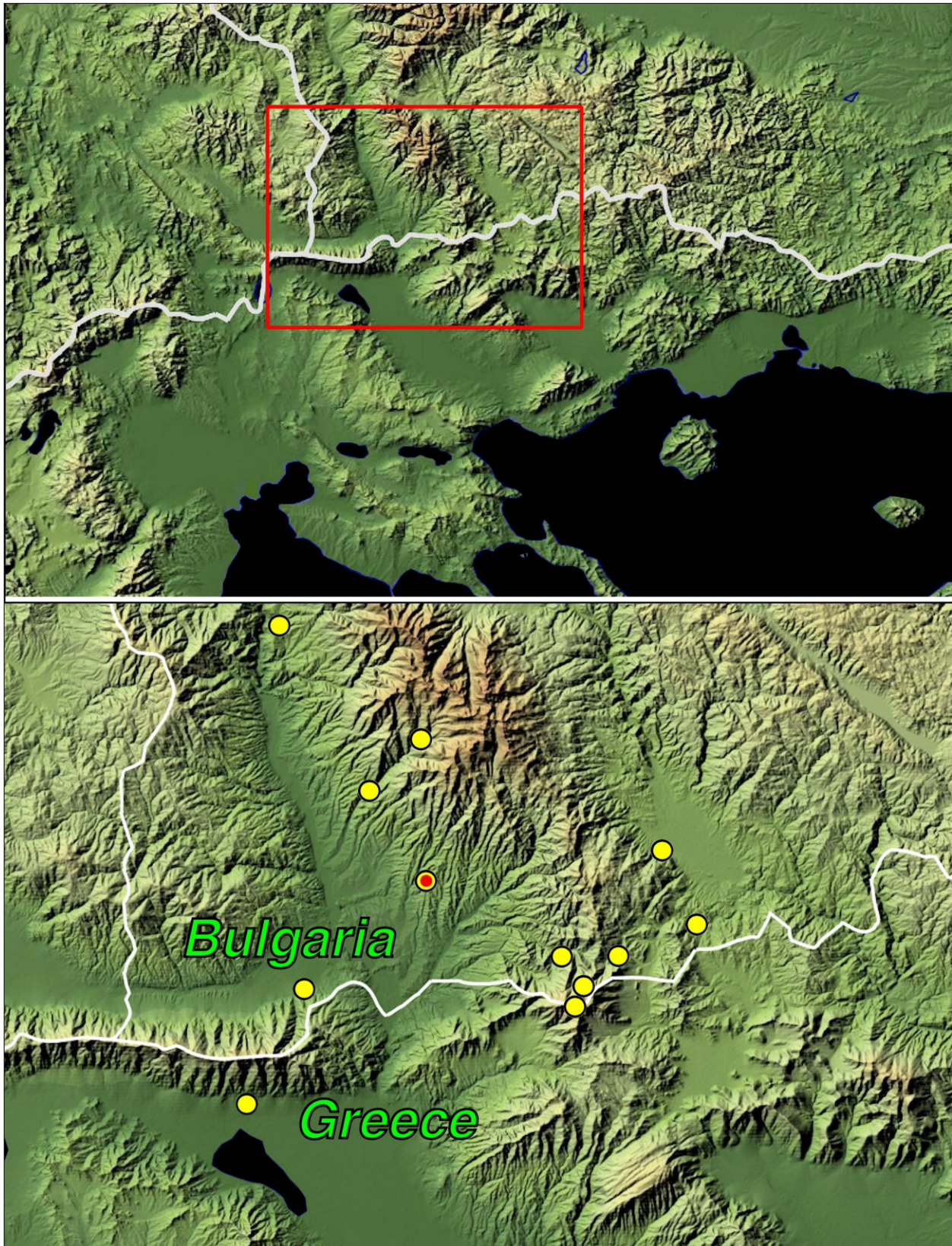


Figure 19: Distribution of *Euscorpius popovi*, sp. nov., red icon indicating type locality.

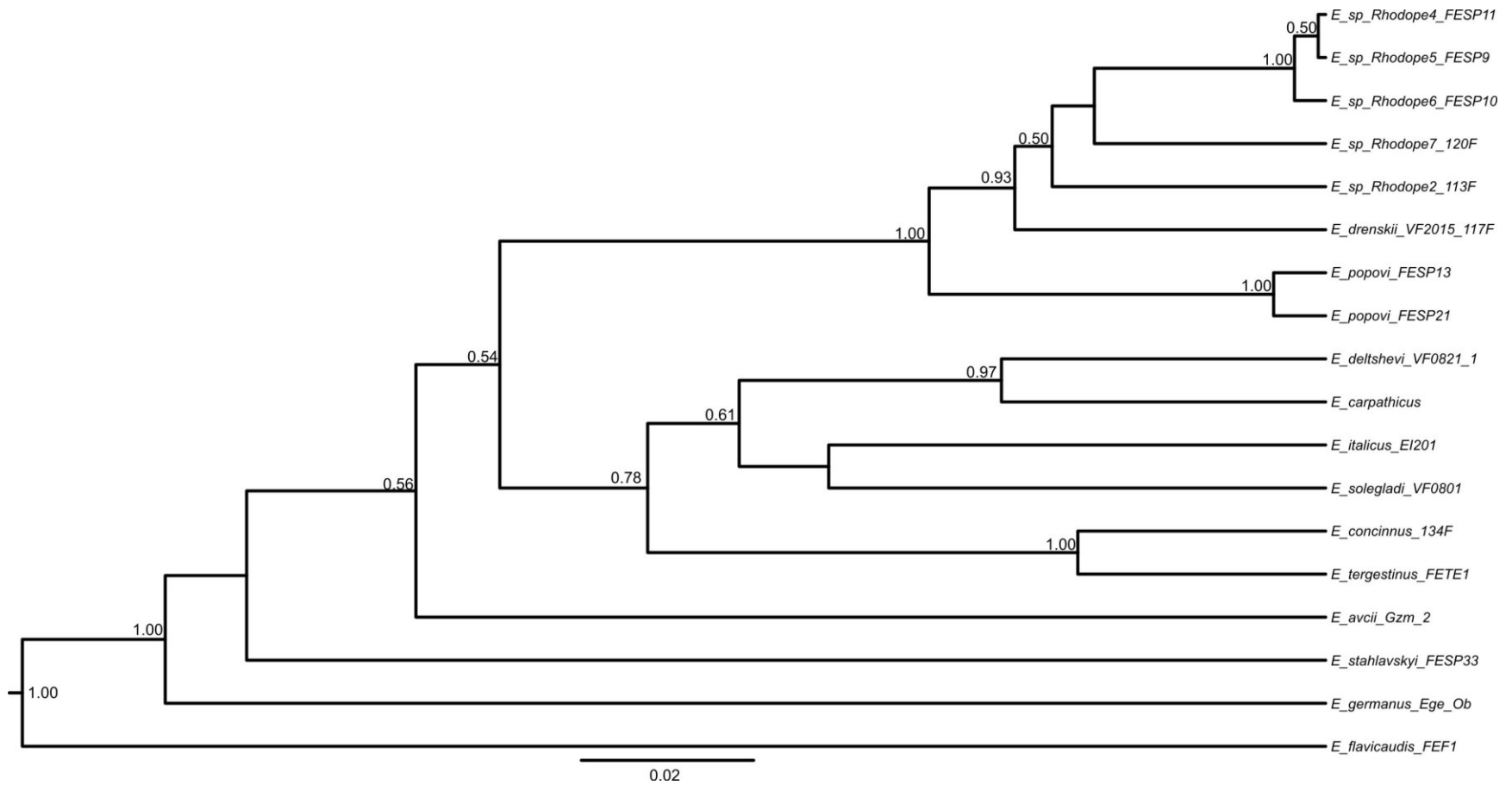


Figure 20: Maximum credibility tree inferred with BEAST v.1.8.2. Numbers above nodes indicate posterior probabilities (only values above 0.5 are reported).

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	<i>E. flavicaudis</i> FEF1	–	0.017	0.018	0.019	0.018	0.019	0.019	0.019	0.019	0.018	0.019	0.020	0.021
2	<i>E. germanus</i> Ege Ob	0.103	–	0.017	0.020	0.015	0.017	0.015	0.018	0.018	0.018	0.018	0.019	0.019
3	<i>E. stahlavskiyi</i> FESP33	0.107	0.093	–	0.018	0.015	0.017	0.016	0.016	0.016	0.016	0.015	0.017	0.017
4	<i>E. avcii</i> Gzm 2	0.120	0.121	0.113	–	0.017	0.017	0.019	0.018	0.018	0.017	0.018	0.018	0.019
5	<i>E. italicus</i> EI201	0.103	0.082	0.076	0.089	–	0.012	0.012	0.015	0.016	0.014	0.017	0.016	0.016
6	<i>E. tergestinus</i> FETE1	0.121	0.096	0.097	0.099	0.050	–	0.008	0.015	0.016	0.015	0.017	0.018	0.017
7	<i>E. concinnus</i> 134F	0.124	0.082	0.097	0.113	0.050	0.024	–	0.016	0.016	0.016	0.017	0.017	0.017
8	<i>E. sp.</i> 113F	0.121	0.104	0.083	0.099	0.069	0.079	0.083	–	0.009	0.008	0.010	0.011	0.010
9	<i>E. sp.</i> FESP9	0.121	0.104	0.090	0.106	0.076	0.086	0.090	0.025	–	0.010	0.011	0.011	0.011
10	<i>E. sp.</i> 120F	0.114	0.107	0.087	0.096	0.066	0.076	0.083	0.021	0.034	–	0.013	0.013	0.012
11	<i>E. drenskii</i> 117F	0.128	0.107	0.083	0.109	0.086	0.097	0.100	0.034	0.037	0.050	–	0.010	0.010
12	<i>E. popovi</i> FESP13	0.128	0.114	0.093	0.113	0.086	0.103	0.100	0.040	0.040	0.057	0.034	–	0.005
13	<i>E. popovi</i> FESP21	0.132	0.111	0.090	0.117	0.083	0.100	0.097	0.034	0.041	0.050	0.034	0.009	–

Table 3: Genetic distances between *16S rDNA* sequences. The number of base substitutions per site between 13 sequences are shown. Standard error estimates are above the diagonal. See Methods and Material for explanations.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	<i>E. flavicaudis</i> FEF1	–	0.018	0.017	0.016	0.016	0.016	0.016	0.018	0.018	0.017	0.018	0.018	0.018	0.017	0.018	0.017	0.017
2	<i>E. stahlavskiyi</i> FESP33	0.137	–	0.016	0.016	0.016	0.017	0.017	0.016	0.016	0.016	0.017	0.017	0.017	0.017	0.017	0.015	0.015
3	<i>E. avcii</i> Gzm 2	0.111	0.114	–	0.014	0.013	0.014	0.015	0.015	0.015	0.014	0.014	0.014	0.014	0.014	0.017	0.015	0.014
4	<i>E. italicus</i> EI201	0.114	0.122	0.095	–	0.013	0.014	0.013	0.014	0.013	0.014	0.016	0.016	0.016	0.014	0.016	0.014	0.014
5	<i>E. carpathicus</i>	0.112	0.114	0.079	0.076	–	0.013	0.013	0.011	0.012	0.013	0.015	0.015	0.015	0.014	0.015	0.014	0.014
6	<i>E. tergestinus</i> FETE1	0.117	0.127	0.090	0.095	0.072	–	0.011	0.013	0.014	0.013	0.013	0.013	0.013	0.013	0.015	0.013	0.013
7	<i>E. concinnus</i> 134F	0.119	0.127	0.093	0.088	0.079	0.052	–	0.014	0.014	0.013	0.013	0.013	0.013	0.013	0.015	0.014	0.014
8	<i>E. deltshevi</i> VF0821	0.142	0.114	0.098	0.093	0.054	0.084	0.091	–	0.013	0.013	0.015	0.015	0.015	0.014	0.016	0.015	0.015
9	<i>E. solegladi</i> VF801	0.134	0.112	0.098	0.076	0.072	0.100	0.090	0.081	–	0.014	0.014	0.014	0.014	0.014	0.016	0.014	0.014
10	<i>E. sp.</i> 113F	0.126	0.122	0.092	0.100	0.076	0.076	0.083	0.085	0.090	–	0.011	0.011	0.011	0.012	0.012	0.012	0.013
11	<i>E. sp.</i> FESP9	0.140	0.128	0.095	0.100	0.090	0.081	0.076	0.103	0.090	0.054	–	0.000	0.000	0.010	0.012	0.011	0.012
12	<i>E. sp.</i> FESP10	0.140	0.128	0.095	0.100	0.090	0.081	0.076	0.103	0.090	0.054	0.000	–	0.000	0.010	0.012	0.011	0.012
13	<i>E. sp.</i> FESP11	0.140	0.128	0.095	0.100	0.090	0.081	0.076	0.103	0.090	0.054	0.000	0.000	–	0.010	0.012	0.011	0.012
14	<i>E. sp.</i> 120F	0.134	0.123	0.102	0.088	0.086	0.086	0.079	0.088	0.097	0.063	0.052	0.052	0.052	–	0.013	0.014	0.014
15	<i>E. drenskii</i> 117F	0.139	0.128	0.117	0.112	0.100	0.097	0.107	0.108	0.117	0.070	0.066	0.066	0.066	0.075	–	0.014	0.014
16	<i>E. popovi</i> FESP13	0.131	0.113	0.095	0.093	0.088	0.088	0.090	0.102	0.090	0.067	0.061	0.061	0.061	0.087	0.093	–	0.004
17	<i>E. popovi</i> FESP21	0.134	0.110	0.093	0.090	0.086	0.086	0.093	0.100	0.093	0.069	0.063	0.063	0.063	0.084	0.091	0.010	–

Table 4: Genetic distances between *COI* sequences. The number of base substitutions per site between 17 sequences are shown. Standard error estimates are above the diagonal. See Methods and Material for explanations.

E. popovi sp. n. can be easily differentiated from the sympatric *E. solegladi* since (1) the latter has the external trichobothrial series $eb = 5$ and $eb_a = 5-6$, versus “standard” $eb = 4$ and $eb_a = 4$ in *E. popovi* sp. n.; (2) *E. solegladi* has the ventral trichobothrial series $Pv = 9-10$, while *E. popovi* sp. n. has $Pv = 7-8$; (3) *E. solegladi* has dark brown coloration, while *E. popovi* sp. n. is light to medium brown/reddish. (The data for *E. solegladi* were taken from Fet et al., 2014).

E. popovi sp. n. can be easily differentiated from the allopatric *E. deltshevi* (Central Bulgaria) by coloration: the latter is dark brown while *E. popovi* sp. n. is light to medium brown/reddish colored.

Biogeographic Notes

The new species follows a pattern of localized distribution common to a number of other terrestrial invertebrates from the highly fragmented mountains of southwestern Bulgaria (see e.g. Fet & Popov, 2007, for a review of Bulgarian zoogeography, and Hubenov, 2012, for an update). Other endemics have been recently described among arthropods of this area, e.g. in the millipede genus *Balkanopetalum*, which has one endemic species in Stara Planina, one in Slavyanka and adjacent Greek mountains, three in the West Rhodopes, and one in East Rhodopes (Stoev & Enghoff, 2003). The cave spider genus *Troglohyphantes* also shows similar patterns; an endemic species has been recently identified from caves in Slavyanka Mts. (Deltshev et al., 2014). Another spider, *Inermocoelotes brevispinus* (Deltshev et Dimitrov, 1996) (Aranei: Agelenidae) has been described from Slavyanka.

Euscorpius popovi sp. n. is found to the north to the high altitudes of Pirin Mts. (first report by Teruel et al., 2004). On the isolated Slavyanka (Alibotush) massif, which separates Bulgaria from Greece. *E. popovi* sp. n. is found up to 2100 m on its highest peak, Gotsev Vrah (summit 2212 m). For a list of rare spiders of Slavyanka, see Naumova (2009).

The new species appears to be allopatric to *E. deltshevi*, which is known from Stara Planina Mts., Bulgaria, and adjacent areas of Bulgaria and Serbia (Fet et al., 2014), while *E. popovi* sp. n. is only known from southwestern Bulgaria and northeastern Greece (Serres, near southwestern Bulgarian border.)

E. popovi sp. n. is allopatric also to localized Bulgarian endemic *E. drenskii*, known so far from western Rhodope Mts. in southern Bulgaria.

Additional populations of *Euscorpius* exist in Bulgaria for which taxonomic status has not been yet determined, especially in the southeast (Central and Eastern Rhodope) and the Black Sea coast (see map in Fet & Soleglad, 2007).

Conclusions

In the past, the genus *Euscorpius* has been intensively studied; over 40 species and subspecies were described. Most of these taxa were later downgraded to subspecies status or moved to synonymy. However, since 1999, when this genus had only four recognized species, the number steadily increased and has gradually reached 17 in 2007. Thanks to further detailed studies, based both on morphological and molecular data, from 2012 to the present, the species number now increased to 48 (including *E. popovi* sp. n.), and several other species are in press or in description. This large increase in species diversity, and in the studies that led to establishing these taxa, reflects a great degree of speciation and endemism in *Euscorpius*. As many other Mediterranean terrestrial invertebrates, these scorpion taxa are often restricted to very limited areas such as a mountain range or an island, or a small group of mountains or islands, as the species, which is the subject of this paper.

Another interesting point that was understood during these studies, and noted for the first time by Tropea (2013), is that the existing subgeneric division of the genus *Euscorpius* was not consistent with the taxonomic situation. Parmakelis et al. (2013a), in a much larger and detailed molecular phylogenetic study, arrived at the same result. Currently, there are a number of forms without a clear subgeneric placement. These include also the new, localized species described herein, *E. popovi* sp. n. According to the traditional diagnostic characters, it would be classified in the subgenus *Euscorpius*; however, genetically it is completely separate, and clearly belongs to a different subgenus (or even genus). Therefore we addressed it here as “subgenus incertus”. Further phylogenetic studies are needed to clarify systematics of this growing and complicated scorpion group.

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