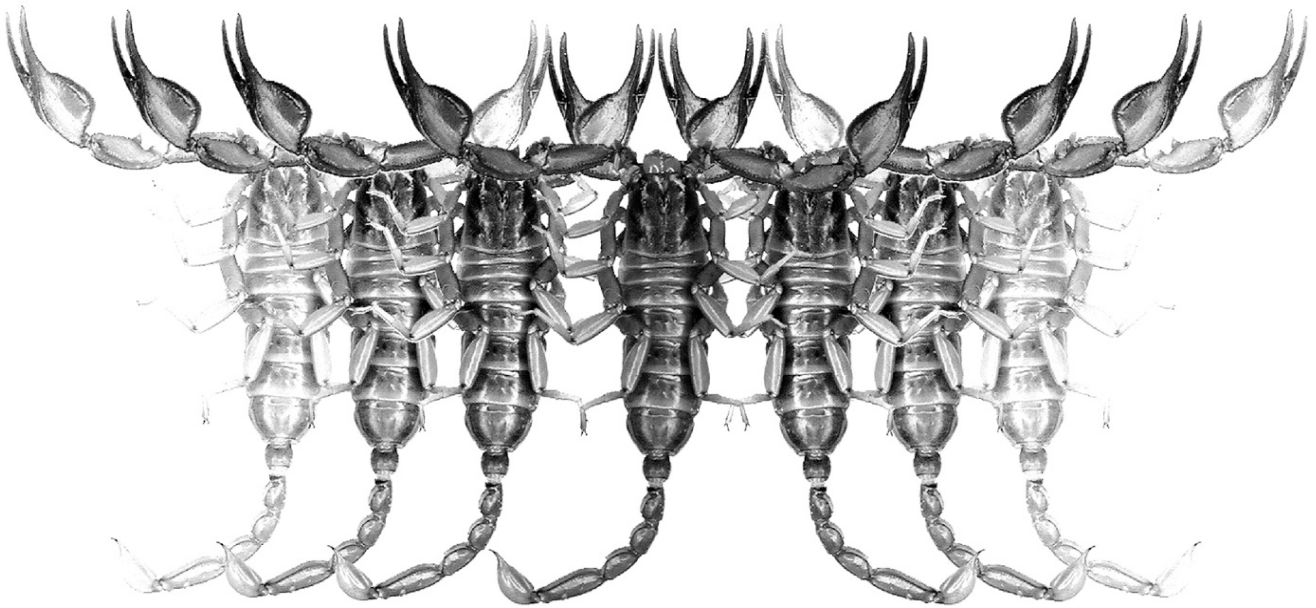


Euscorpius

Occasional Publications in Scorpiology



Euscorpius thracicus sp. n. (Scorpiones: Euscorpiidae)
from Bulgaria

František Kovařík, Graeme Lowe, Markéta Byronová & František Šťáhlavský

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Euscorpius

Occasional Publications in Scorpiology

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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/>.

Publication date: 30 November 2020

<http://zoobank.org/urn:lsid:zoobank.org:pub:3E4FA71B-1C3A-4BBD-A928-EF5A8344B419>

Euscorpius thracicus sp. n. (Scorpiones: Euscorpiidae) from Bulgaria

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<http://zoobank.org/urn:lsid:zoobank.org:pub:3E4FA71B-1C3A-4BBD-A928-EF5A8344B419>

Summary

Euscorpius thracicus sp. n. from eastern Bulgaria is described, fully complemented with color photographs of both live and preserved specimens, as well as their habitats. This species is described based on morphology and the cytochrome oxidase I (COI) DNA barcoding marker. In addition to the analyses of external morphology and hemispermatophore, we also describe the karyotype of *E. thracicus* sp. n. (2n=92).

Introduction

Our understanding of the scorpion genus *Euscorpius* Thorell, 1876, species of which are widespread in southern Europe and Anatolia, underwent a dramatic change in the last two decades. These changes were triggered by the first application of molecular phylogenetic data in scorpions combined with modern reassessments of morphology based on detailed analysis of trichobothrial patterns (Gantenbein et al., 1999, 2000, 2001, 2002; Fet & Soleglad, 2002; Fet et al., 2003, Kovařík & Štáhlavský, 2020). As a result, the discovery of numerous new cryptic species followed. Here we describe another new species of *Euscorpius* from eastern Bulgaria. Results of our continued molecular analysis show that, especially in Greece and Bulgaria, there are other cryptic species of *Euscorpius* awaiting description.

Methods, Material & Abbreviations

Nomenclature and measurements follow Stahnke (1971), Soleglad & Sissom (2001), Kovařík (2009), Kovařík & Ojanguren Affilastro (2013), and Kovařík & Štáhlavský (2020) except for trichobothriotaxy (Vachon, 1974), and hemispermatophores (Kovařík et al., 2020; Jacob et al., 2004a, 2004b; Molteni et al., 1983; Monod et al., 2017; Vachon, 1948).

Nomenclature of hemispermatophore capsule structures in genera *Euscorpius*, *Alpiscorpius* and *Tetratrachobothrius* varies widely between different authors. Here we use the following terminology (presumed equivalent terms in parentheses): **distal carina** (Monod et al., 2017) (= capsular lobe complex = median projection = basal lobe of primary acuminate process (in part)); **basal carina** (Monod et al.,

2017) (= ental channel = internal projection = internal lobe = lateral lobe); **distal external lobe**, *lde* (Vachon, 1948) (= outer distal lobe = auxillary lobe of primary acuminate process (in part)); **distal internal lobe**, *ldi* (Vachon, 1948) (= inner distal lobe = auxillary lobe of primary acuminate process (in part) = laterodistal lobe = median lobe); **basal lobe**, *bl* (Vachon, 1948) (= acuminate process = secondary acuminate process); and **crown-like structure**, *cls* (Jacob et al., 2004a, 2004b) (= spinose distal ental channel = internal projection crown = crown-like process = couronne dentaire).

We used fragments (~610 bp) of the COI gene for the comparison of the genetic distances among the species known from Bulgaria. Our fragments were amplified by PCR for two paratypes (Nos. 1865, 1866) using the protocol already described by Kovařík et al. (2009). The obtained DNA sequences were edited and aligned using SeqMan 5.05 program. These two new DNA sequences were deposited in GenBank (<http://www.ncbi.nlm.nih.gov/>), with the following accession numbers: MW291129, MW291114. Additionally, we used 13 sequences of several species included in previous DNA analyses from this region (Parmakelis et al., 2013; Fet et al. 2014; Tropea et al., 2015a, 2015b) (specimen code/accession number in GenBank): *Euscorpius deltshevi* (VF-0746-1/KM111244) and (VF-0821-1/KM111243), *E. solegladi* (VF-0801-1/KM111247) and (VF-0802-1/KM111246), *E. popovi* (FESP13/KC215733) and (FESP21/KC215737), *E. drenskii* (117F/KT602916), and *E. sp.* (113F/KC215662), (FESP12/KC215732), (FESP11/KC215731), (FESP09/KC215729), (FESP10/KC215730) and (120F/KC215663). Sequence distances (p-distance and Kimura 2-parameter distance) (Table 2) were conducted using MEGA Version X (Kumar et al., 2018).

For chromosome preparations we used the “plate spreading” method already used for scorpions (e. g. Plíšková



Figures 1–2. *E. thracicus* sp. n., male (1) and female (2) paratypes in vivo habitus.

et al., 2016). The chromosomes were stained by 5% Giemsa solution in Sörensen phosphate buffer for 30 min. The relative length of the chromosomes of the diploid set was measured using the software Image J 1.45r (<http://rsbweb.nih.gov/ij>) with the plugin Levan (Sakamoto & Zacaro, 2009) based on 15 postpachytene or metaphase I.

Specimen Depository: FKCP (František Kovařík, private collection; will in future be merged with the collections of the National Museum of Natural History, Prague, Czech Republic).

Morphometrics: D, depth; L, length; W, width. *Pedipalp finger dentition*: MD, median denticles; ID, inner denticles; IAD, inner accessory denticles; OD, outer denticles. *Hemispermaphore*: *lde*, distal external lobe; *ldi*, distal internal lobe; *bl*, basal lobe; *cls*, crown-like structure.

Systematics

Family Euscorpiidae Laurie, 1896

Euscorpius Thorell, 1876

(Figures 1–61, Tables 1–2)

<http://zoobank.org/urn:lsid:zoobank.org:act:CEC71123-0F84-45DD-857E-2D4702C2FD80>

Euscorpius Thorell, 1876: 15; Fet & Sissom, 2000: 357–377 (in part, complete reference list until 1998); Kovařík & Štáhlavský, 2020: 1–37, figs. 1–186, tabs. 1–4.

TYPE SPECIES. *Scorpio carpathicus* Linnaeus, 1767

DIAGNOSIS. Total length 20–50 mm. Movable fingers of pedipalp chela with MD aligned in a single straight row along entire length of chela fingers; ID, IAD (5 in number) and OD present. OD are displaced to outer aspect of fingers. Pedipalp chela flat in appearance. Trichobothrial pattern type C. Two subdistal denticles present on cheliceral movable finger dorsal edge. Ventral edge of cheliceral movable finger smooth; serrula absent. Two pedal spurs present on legs. Tarsal spurs on legs absent. Sternum pentagonal in shape. Hemispermaphore lamelliform in shape with broad trunk and distal lamina. Telson without subaculear tubercle. Metasoma I–IV with single median carina usually absent.

Euscorpius thracicus sp. n.

(Figures 1–49, 58–61, Tables 1–2)

<http://zoobank.org/urn:lsid:zoobank.org:act:1BDEC12C-018B-4123-9A1A-397BA3266047>

TYPE LOCALITY AND TYPE REPOSITORY. **Bulgaria**, Kardzhali Province, Krumovgrad Municipality, Bryagovets Village, 41.6485862°N 25.8132185°E; FKCP.

TYPE MATERIAL. **Bulgaria**, Kardzhali Province, Krumovgrad Municipality, Bryagovets Village, 41.6485862°N 25.8132185°E (Figs. 59–60, 1♂ (holotype) 6♂8♀ (paratypes, Nos. 1864, 1865, 1866, 1878, 1879, 1882, 1883), 25 September 2020, leg. M. Byronová et al., FKCP.

ETYMOLOGY. Named after Thracia (Thrace), the ancient name of the region where the type locality is situated.

DIAGNOSIS (♂♀). Total length 28–32 mm. Color reddish yellow to brown, telson yellow. Pedipalp patella external trichobothria numbers: *4eb*, *4eb_a*, *2esb*, *4em*, *4est*, *6et*, ventral aspect of patella with 7 trichobothria. Pectinal teeth number 8–10 in males, 6–9 in females. Chelicerae yellow, very slightly reticulated. Male with pedipalp finger marginal profile type C (Kovařík & Štáhlavský, 2020: 2), female with fingers very weakly undulate, almost linear. Dorsal metasomal carinae on segments III–IV irregularly granulated, mainly in male. Dorsolateral carinae on metasomal segments II–IV absent. Ventrolateral carinae on metasomal segments II–IV present or indicated and smooth. Metasoma V ventrally with median carina indicated. Metasoma finely granulated dorsally in male and smooth in female. Chela length/width ratio, 2.35–2.48 in male, 2.50–2.55 in female. Metasoma IV length/width ratio, 1.8–1.9 in both sexes. Telson length/depth ratio, 2.5–2.6 in male, 3.1–3.2 in female.

DESCRIPTION (♀♂). Total length 28–32 mm in both sexes. The habitus is shown in Figs. 1–6. For position and distribution of trichobothria on pedipalps, see Figs. 32–38. For sexual dimorphism, see below in the description. For measurements, see Table 1.

Coloration (Figs. 1–6). Base color uniformly reddish yellow to brown including sternites, telson yellow, pedipalps reddish brown. Chelicerae yellow and very slightly reticulate.

Carapace and mesosoma (Figs. 7–10). Carapace finely granulated with several smooth areas; carinae absent. Anterior margin of carapace straight. Carapace with two lateral eyes. Tergites finely granulated, more so in male, without carinae developed. Tergite VII lacking median and paired lateral carinae. Sternites III–VII smooth and lustrous; VII lacking median and paired lateral carinae. Stigmata small, narrow elliptical. Pectinal teeth number 8–10 (8 x 8, 4 x 9, 2 x 10) in males and 6–9 (1 x 6, 12 x 7, 2 x 8, 1 x 9) in females, fulcra present. Pectines with 3 marginal lamellae and 4–6 middle lamellae.

Metasoma and telson (Figs. 15–22). Metasoma very sparsely hirsute and smooth. Metasoma I–V finely granulated in male, several fine granules also present on lateral surfaces of metasoma I and V in both sexes. Dorsal carinae on metasomal segments I–V irregularly granulated in male, reduced in female; dorsolateral carinae absent; ventrolateral carinae present or indicated and smooth on segments II–IV, granulated on segment V. Metasoma V ventrally granulated with median carina indicated, metasoma I–IV with ventral median carinae absent. Anal arch with small pigmented granules. Telson rather smooth, elongate in female and swollen in male, with annular ring indicated in female and developed in male. Aculeus short, more curved in male.

Pedipalps (Figs. 23–39). Pedipalps very sparsely hirsute. Patella with 24 (*4eb*, *4eb_a*, *2esb*, *4em*, *4est*, *6et*) external and 7 ventral trichobothria. Chela with 4 trichobothria in ventral series, of which *V₄* is located external to the

Dimensions (mm)		<i>Euscorpius thracicus</i> sp. n.	<i>Euscorpius thracicus</i> sp. n.
		♂ holotype	♀ paratype
Carapace	L / W	4.13 / 3.97	4.00 / 4.00
Mesosoma	L	9.92	13.58
Tergite VII	L / W	1.73 / 3.31	2.23 / 3.73
Metasoma + telson	L	15.26	13.00
Segment I	L / W / D	1.61 / 1.51 / 1.23	1.32 / 1.42 / 1.27
Segment II	L / W / D	1.76 / 1.33 / 1.21	1.54 / 1.20 / 1.13
Segment III	L / W / D	1.90 / 1.25 / 1.18	1.66 / 1.10 / 1.13
Segment IV	L / W / D	2.23 / 1.17 / 1.18	1.97 / 1.06 / 1.08
Segment V	L / W / D	3.54 / 1.15 / 1.23	3.09 / 1.00 / 1.01
Telson	L / W / D	4.22 / 1.64 / 1.63	3.42 / 1.07 / 1.06
Pedipalp	L	14.37	13.85
Femur	L / W	3.52 / 1.34	3.38 / 1.32
Patella	L / W	3.49 / 1.48	3.44 / 1.44
Chela	L	7.36	7.03
Manus	W / D	2.98 / 2.15	2.78 / 1.77
Movable finger	L	4.18	3.86
Total	L	29.31	30.58

Table 1. Comparative measurements of adults of *Euscorpius thracicus* sp. n. Abbreviations: length (L), width (W, in carapace it corresponds to posterior width), depth (D).

ventroexternal carina, on the external surface near *Eb*₁. Entire femur finely granulated and patella smooth with fine granulated dorsointernal and ventrointernal margins. Femur with granulated developed carinae; ventroexternal carina incomplete. Patella with 5 complete carinae including irregular wide externomedian carina. Dorsal patellar spur well developed in male. Manus dorsally with fine, rounded granules, which do not form a median carina (mainly in male); only five chelal carinae developed. Male with pedipalp finger undulation profile type C, female with fingers very weakly undulate, almost linear.

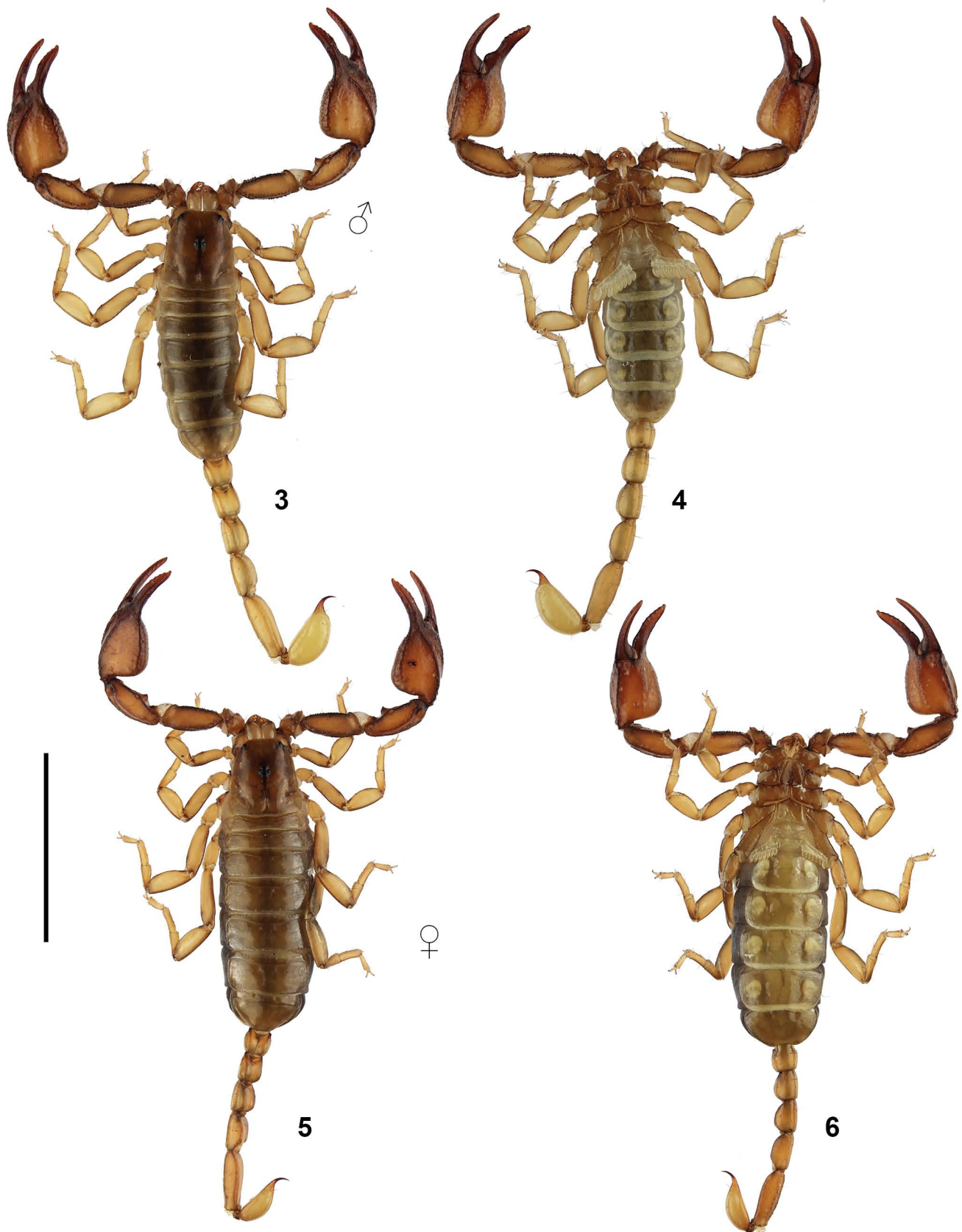
Legs (Figs. 11–14). Both pedal spurs present on all legs, lacking spinelets; tibial spurs absent. Tarsus with single row of spinules on ventral surface, terminating distally with two essentially adjacent spinules.

Hemispermatothore (Figs. 44–58). Lamelliform. Shape and proportions typical of the genus (Fet & Soleglad, 2002). Distal lamina proximally broad, tapered distally, with strong basal constriction to half maximal width at base. Apex of lamina narrow but blunt, bent posteriorly. Capsule broad, with transverse trough on convex surface. Distal margin of trough strengthened with transverse rib that curves and connects to posterior axial rib of distal lamina. Truncal flexure well developed, separating trunk from capsule. Trunk relatively short, ca. 2/3 of length of distal lamina, gradually tapered towards base. Pedicel almost as long as trunk. Basal carina of capsule weakly sclerotized, terminating in typical crown-like structure (*cls*) with 4–7 hook-like tines. Distal carina of capsule strongly sclerotized, distal external lobe (*lde*) apparently divided into two cristate ‘sub-lobes’, one broader

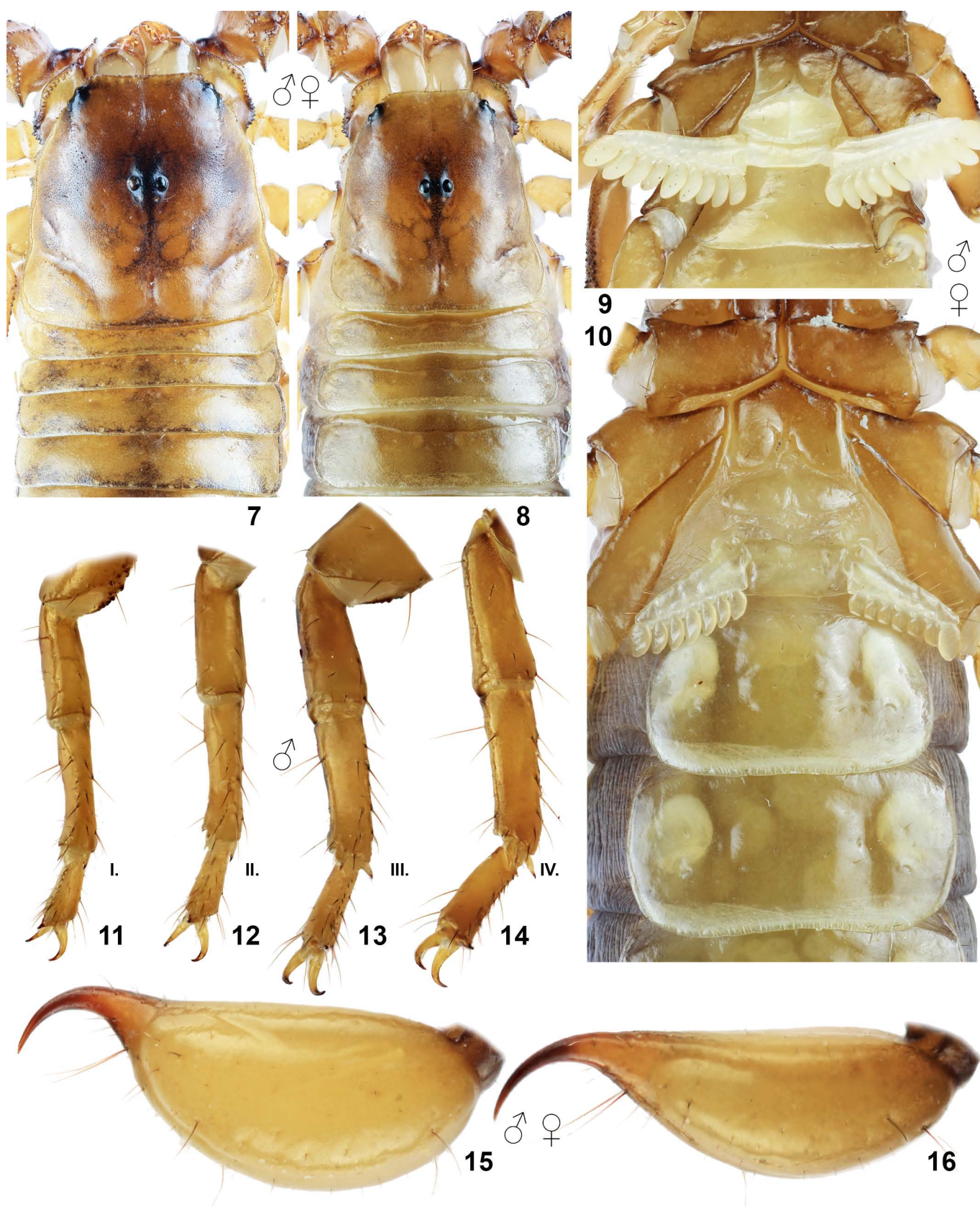
and one narrower, separated by a deep cleft. Distal internal lobe (*ldi*) apparently absent. Basal lobe (*bl*) absent. Terminal membrane of sperm duct with numerous fine spicules. Both left and right hemispermatothores from 3 individuals (paratypes No. 1864, 1865 and 1866) displayed similar morphology. Measurements of left hemispermatothore No. 1864 (mm): distal lamina length from base of basal constriction to apex 2.50, maximal width 0.78; trunk length from truncal flexure to pedicel 1.34; pedicel length 1.06. Variation: *cls* tine counts (with bifid tines counting as 2, trifid as 3, etc.) varied among 3 examined paratypes: No. 1864, right 4, left 7; No. 1865, right 4, left 5; No. 1866, right 6, left 4.

Karyotype (Figs. 40–43). We analyzed the chromosomes of four paratype males (Nos. 1864, 1965, 1866, 1878). The diploid complement of these samples is composed of 92 chromosomes (Figs. 40, 41). In all observed postpachytene and metaphase I nuclei, we observed 46 bivalents without visible chiasmata (Fig. 42). The relative length of the chromosomes of the diploid set decreased gradually from 2.23% to 0.37%. The chromosomes exhibited monocentric organization and the morphology of all chromosome pairs was telocentric with only one exception. The chromosomes of the pair number 18 were metacentric. The arm ratio of chromosomes belonging to this pair was 1.17 (the standard deviation is 0.12). These two biarmed chromosomes were clearly visible during postpachytene (Fig. 42) as well as during metaphase II (Fig. 43).

The number of chromosomes in *E. thracicus* sp. n. (2n=92) fully corresponds to the known 2n values of two *Euscorpius* species from the Balkan Peninsula (*E. janstai* from North



Figures 3–6: *E. thracicus* sp. n. **Figures 3–4.** Male holotype in dorsal (3) and ventral (4) views. **Figures 5–6.** Female paratype in dorsal (5) and ventral (6) views. Scale bar: 10 mm.



Figures 7–16: *E. thracicus* sp. n. **Figures 7, 9, 11–15.** Male holotype, carapace and tergites I–IV (7), coxosternal area (9), left legs I–IV, retro-lateral aspect (11–14 respectively), and telson lateral (15). **Figures 8, 10, 16.** Female paratype, carapace and tergites I–III (8), coxosternal area and sternites III–IV (10), and telson lateral (16).



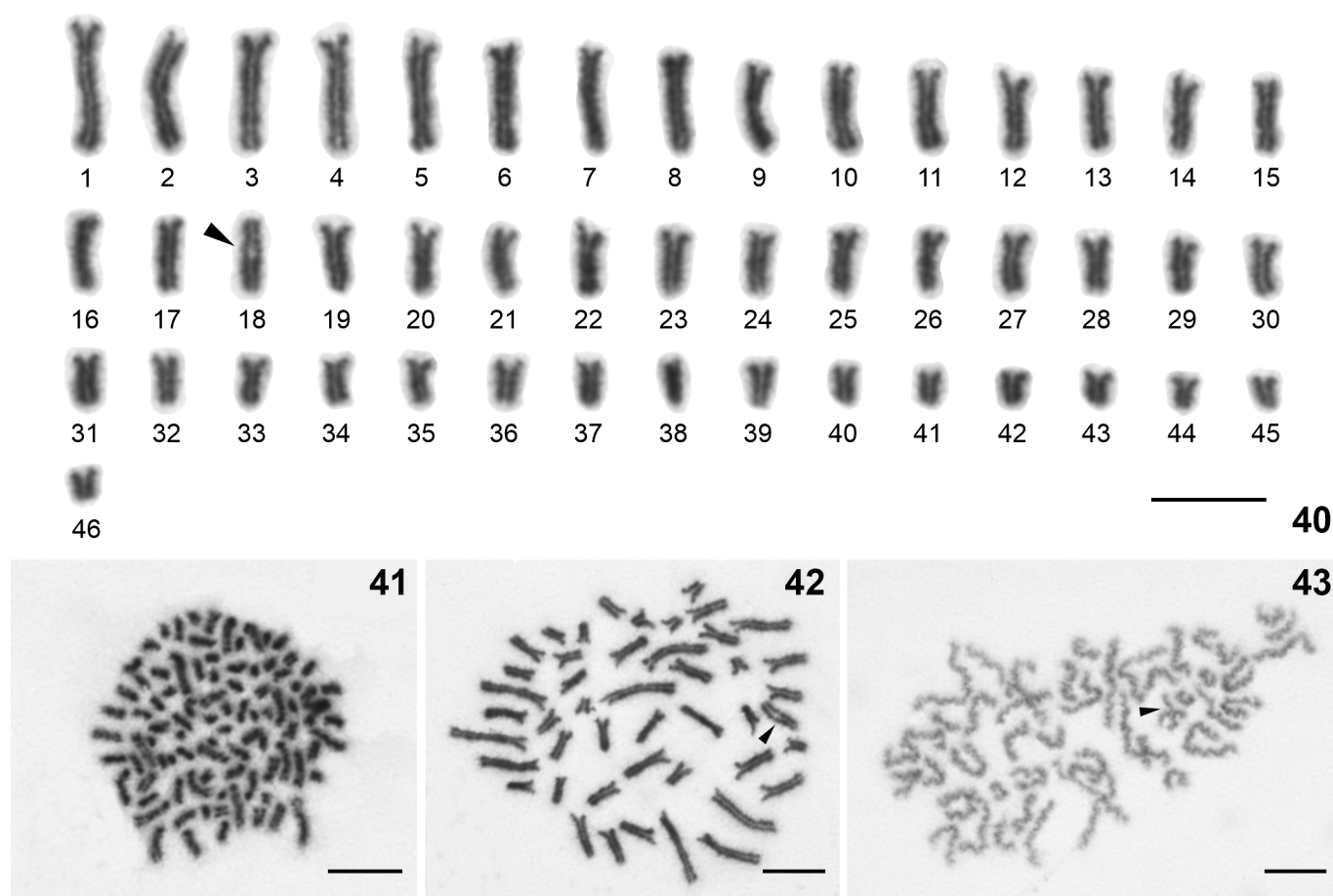
Figures 17–22: *E. thracicus* sp. n. **Figures 17–19.** Female paratype, metasoma and telson lateral (17), ventral (18), and dorsal (19) views. **Figures 20–22.** Male holotype, metasoma and telson lateral (20), ventral (21), and dorsal (22) views. Scale bars: 10 mm (17–19, 20–22).



Figures 23–31. *E. thracicus* sp. n., male holotype, pedipalp segments. Chela dorsal (23), external (24) and ventral (25) views. Patella dorsal (26), external (27) and ventral (28) views. Trochanter and femur dorsal (29) and ventral (30) views. Movable finger dentition (31).



Figures 32–39. *E. thracicus* sp. n., female paratype, pedipalp segments. Chela dorsal (32), external (33) and ventral (34) views. Patella dorsal (35), external (36) and ventral (37) views. Trochanter and femur dorsal (38) and ventral (39) views. Trichobothrial pattern is indicated by white circles (32–38).



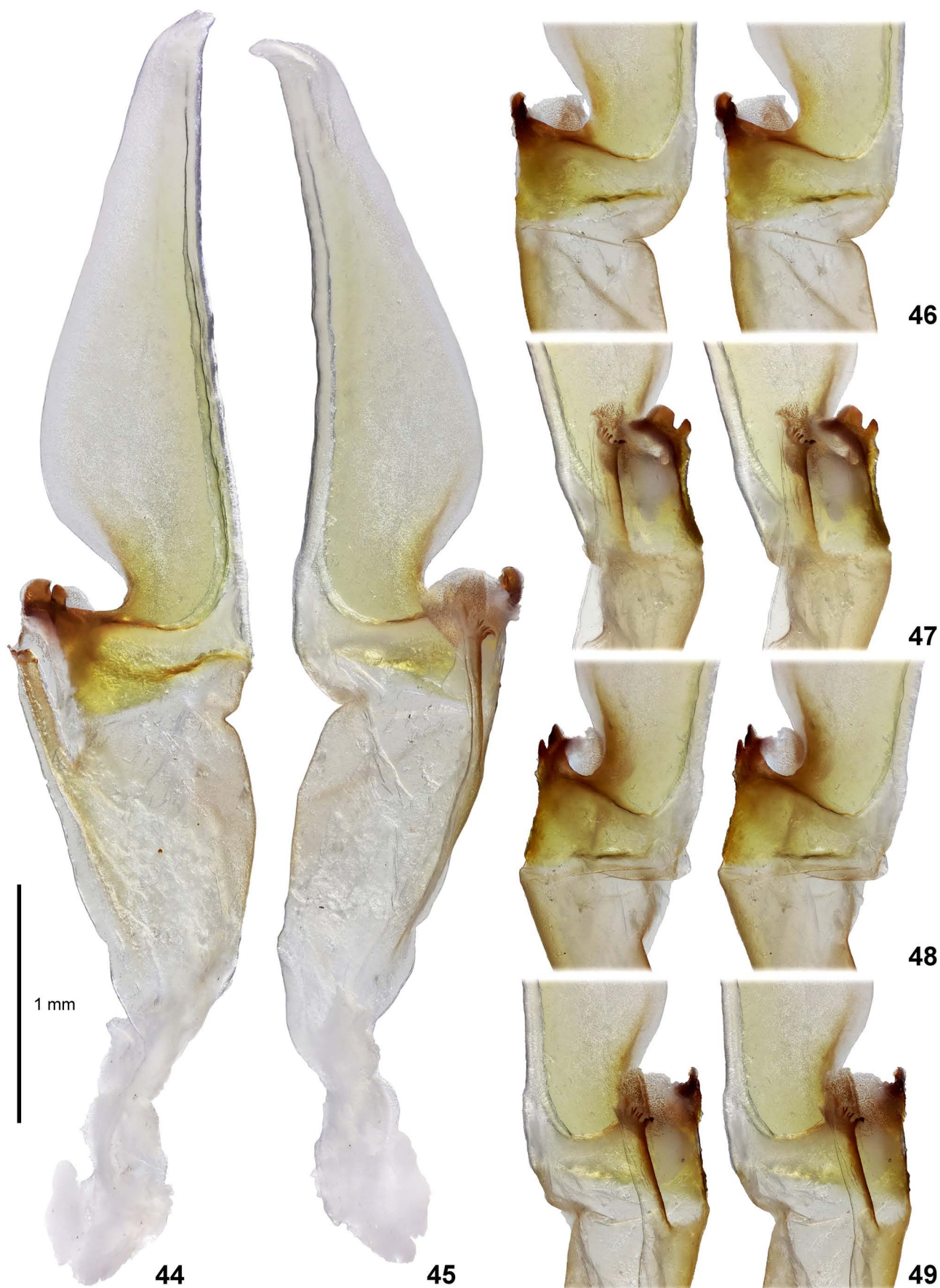
Figures 40–43. Chromosomes of *E. thracicus* sp. n., karyotype based on postpachytene ($2n=92$) (40), mitotic metaphase (41), postpachytene (42), one sister metaphase II ($n=46$) (43). Scale bars: 10 μ m.

Macedonia: $2n=112$ and *E. sadileki* from Serbia: $2n=90$) (Kovařík & Štáhlavský, 2020). However, the karyotypes of these species differ conspicuously from that of *E. thracicus* sp. n. by the presence of a higher amount of biarmed chromosomes in their karyotypes. Our cytogenetic results from the Balkan Peninsula document a high karyotype variability in the genus *Euscorpius* in this region. We support the application of this marker in taxonomy of the family Euscorpiidae, as already implemented in the genus *Alpiscorpius* (Euscorpiidae) from the Alps (Štundlová et al., 2019).

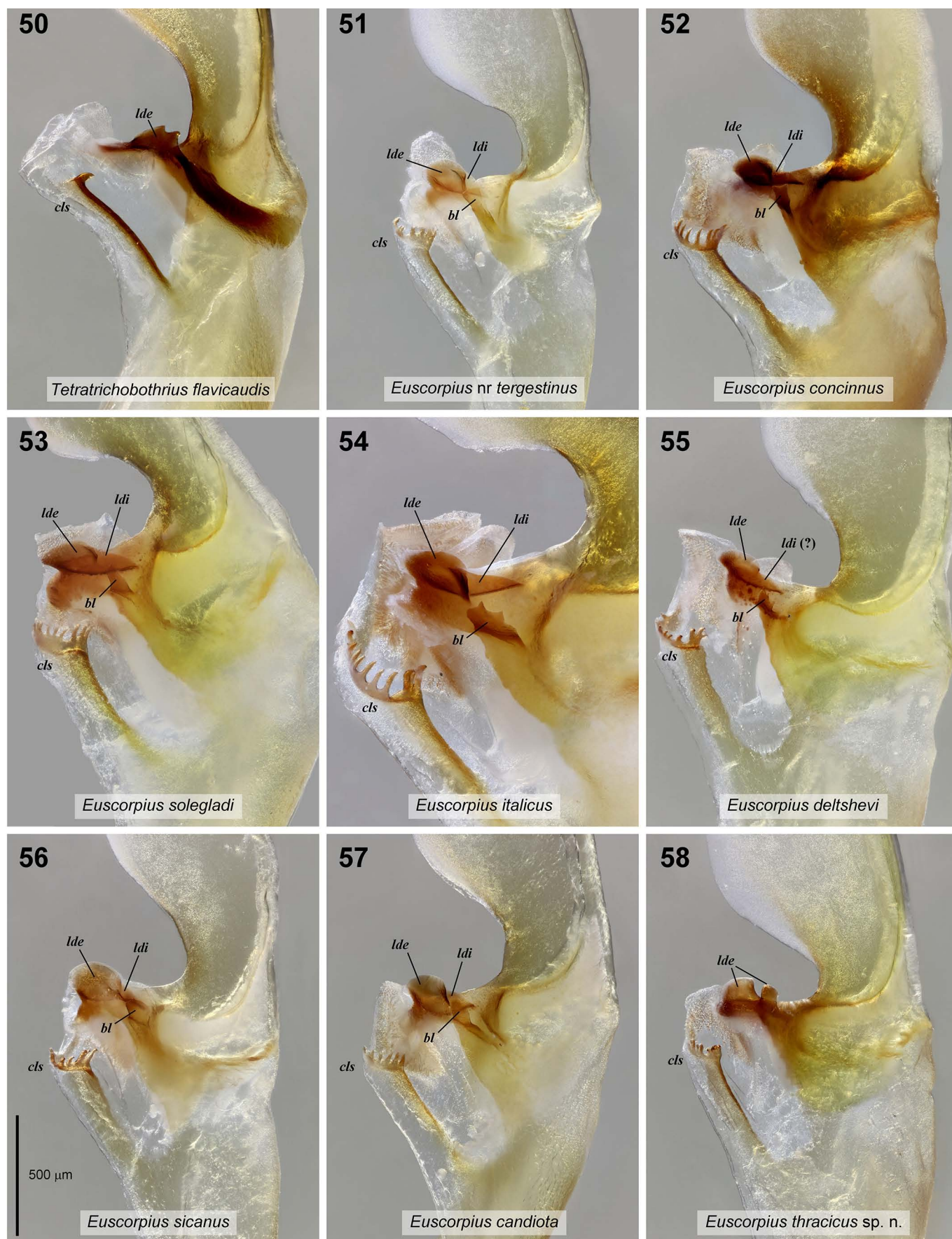
DNA analysis (Table 2). The comparison of the differences among the sequences of the mitochondrial 16S rRNA or/and COI genes within the genus *Euscorpius* represents one of the primary important tools for species delimitation in this morphologically uniform group (e.g. Gantenbein et al., 1999; Parmakelis et al., 2013; Štundlová et al., 2019). Our comparison of the genetic distances (p-distances and Kimura 2-parameter distances) (Table 2) among the species known from the studied region (Bulgaria, Eastern Serbia, and Northern Greece) corresponds to the previous analyses of the species from this area (Parmakelis et al., 2013; Fet et al., 2014; Tropea et al., 2015a, 2015b) and retrieved values of distances always exceeded 0.046 among all analyzed species. This fact supports a separate position and species status of *E. thracicus* sp. n.

AFFINITIES. DNA analysis separates *E. thracicus* sp. n. from all other species of the genus. The morphologically most similar species is *E. popovi* Tropea et al., 2015, from southwestern Bulgaria and northeastern Greece. These two species are differentiated according to the granulation of the dorsal surface of the pedipalp patella, which is well developed in *E. popovi* and reduced to absent in *E. thracicus* sp. n. (Fig. 26 versus fig. 9 in Tropea et al., 2015a: 5). The male of *E. thracicus* sp. n. has pedipalp finger margin profile type C versus type A in *E. popovi*. Females have fingers very weakly undulate, almost linear in *E. thracicus* sp. n. and undulate in *E. popovi* (Figs. 24, 33 vs. Figs. 7, 11 in Tropea et al., 2015a: 5; see Kovařík & Štáhlavský, 2020: 2).

The hemispermatophore distal carina of *E. thracicus* sp. n. differed from those of other examined species of related euscorpiids (cf. Fig. 58 vs. Figs. 50–57) in: (i) lacking a basal lobe (*bl*), (ii) having the distal external lobe (*lde*) apparently divided into two separate sub-lobes, and (iii) lacking the distal internal lobe (*ldi*). An alternative interpretation of the anatomy is that the broader distal sub-lobe corresponds to the *lde*, and the narrower distal sub-lobe to the *ldi*. However, in other species the *ldi* is always directly joined to the *lde* by an oblique non-terminal suture (which may be partial or limited to a short notch), and its axis is distinctly slanted relative



Figures 44–49. *Euscorpius thracicus* sp. n., left hemispermatophore. **Figures 44–45.** Whole hemispermatophore in convex (44) and concave (45) views. **Figures 46–49.** Capsule of left hemispermatophore, 3-dimensional form displayed in cross stereoscopic pairs, in posterior-convex (46), anterior (47), convex (48) and concave (49) views. Paratype No. 1864. Scale bar: 1 mm (Figs. 44–49).



Figures 50–58. Comparison of the capsule lobes of the hemispermatophores of nine euscorpiids (anterior-convex views). 50. *Tetratrachobothrius flavicaudis*. 51. *Euscorpius* sp., nr *tergestinus*. 52. *E. concinnus*. 53. *E. solegladi*. 54. *E. italicus*. 55. *E. deltshevi*. 56. *E. sicanus*. 57. *E. candiota*. 58. *E. thracicus* sp. n., paratype No. 1864 Abbreviations: lde: distal external lobe; ldi: distal internal lobe; bl: basal lobe; cls: crown-like structure. Capsules of left hemispermatophores shown except for Fig. 57, which shows the right capsule (in mirror image for comparison). Scale bar: 500 µm (in Fig. 56) applies to Figs. 50–58.



Figures 59–60. *E. thracicus* sp. n., type locality, Bulgaria, Bryagovets, Krumovgrad, 41.6485862°N 25.8132185°E.

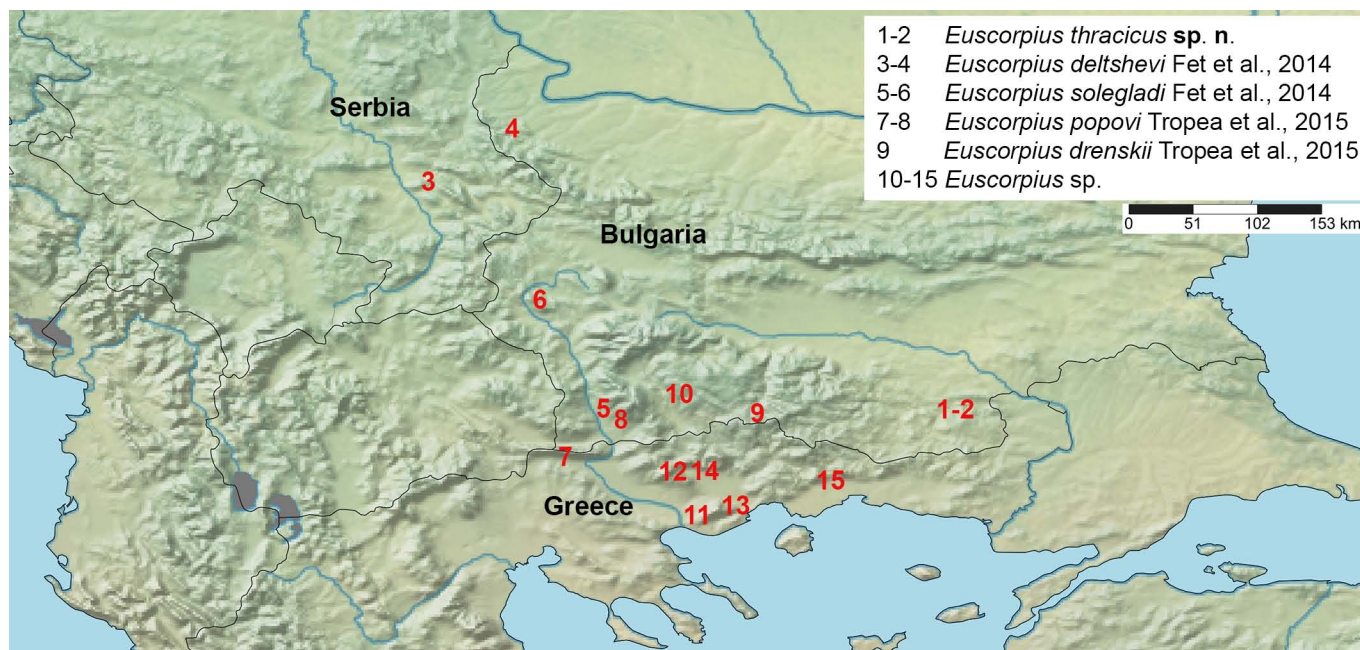


Figure 61. Map of distribution of *Euscorpius* populations analysed by DNA (see Table 2).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 <i>E. thracicus</i> sp. n. (S1865/MW291129)	-	0.002	0.094	0.102	0.099	0.099	0.064	0.066	0.078	0.060	0.050	0.049	0.052	0.048	0.067
2 <i>E. thracicus</i> sp. n. (S1866/MW291114)	0.002	-	0.096	0.104	0.101	0.101	0.067	0.068	0.080	0.062	0.052	0.052	0.054	0.050	0.069
3 <i>E. deltshevi</i> (VF-0746-1/KM111244)	0.087	0.089	-	0.047	0.079	0.081	0.086	0.087	0.101	0.089	0.075	0.079	0.081	0.076	0.084
4 <i>E. deltshevi</i> (VF-0821-1/KM111243)	0.094	0.096	0.045	-	0.089	0.089	0.097	0.093	0.106	0.083	0.092	0.097	0.099	0.093	0.086
5 <i>E. solegladi</i> (VF-0801-1/KM111247)	0.093	0.094	0.074	0.083	-	0.003	0.088	0.089	0.112	0.085	0.081	0.083	0.085	0.082	0.092
6 <i>E. solegladi</i> (VF-0802-1/KM111246)	0.093	0.094	0.076	0.083	0.004	-	0.090	0.091	0.115	0.087	0.083	0.085	0.087	0.084	0.090
7 <i>E. popovi</i> (FESP13/KC215733)	0.061	0.063	0.081	0.090	0.083	0.084	-	0.012	0.091	0.064	0.059	0.056	0.058	0.055	0.082
8 <i>E. popovi</i> (FESP21/KC215737)	0.063	0.065	0.082	0.087	0.083	0.085	0.012	-	0.086	0.064	0.056	0.056	0.058	0.054	0.078
9 <i>E. drenskii</i> (117F/KT602916)	0.073	0.075	0.094	0.098	0.104	0.106	0.084	0.081	-	0.067	0.062	0.063	0.063	0.064	0.072
10 <i>E. sp.</i> (113F/KC215662)	0.057	0.059	0.084	0.078	0.080	0.082	0.061	0.061	0.063	-	0.040	0.047	0.050	0.046	0.058
11 <i>E. sp.</i> (FESP12/KC215732)	0.048	0.050	0.071	0.086	0.076	0.078	0.057	0.054	0.058	0.039	-	0.010	0.010	0.008	0.041
12 <i>E. sp.</i> (FESP11/KC215731)	0.048	0.050	0.074	0.090	0.078	0.080	0.054	0.053	0.060	0.046	0.010	-	0.000	0.000	0.048
13 <i>E. sp.</i> (FESP09/KC215729)	0.050	0.052	0.076	0.092	0.080	0.082	0.056	0.055	0.060	0.048	0.010	0.000	-	0.000	0.048
14 <i>E. sp.</i> (FESP10/KC215730)	0.046	0.048	0.071	0.087	0.077	0.079	0.052	0.052	0.060	0.044	0.008	0.000	0.000	-	0.047
15 <i>E. sp.</i> (120F/KC215663)	0.063	0.065	0.079	0.081	0.086	0.084	0.077	0.073	0.067	0.056	0.039	0.046	0.046	0.045	-

Table 2. A matrix of genetic distances among the COI genes of the *Euscorpius* species from Bulgaria, eastern Serbia, and northern Greece. p-distance (below the diagonal); Kimura 2-parameter distance (above the diagonal). In the brackets are mentioned specimen codes/accession number from GenBank.

to the *lde* axis. In contrast, the two sub-lobes observed here were strongly separated from each other with their axes nearly parallel. We also reject the hypothesis that the narrower distal sub-lobe is homologous to the basal lobe (*bl*), since the *bl* invariably arises from a more basal position on the distal carina in other species.

Of 33 other species of related euscorpiids with described hemispermatophores, the *bl* was absent in only one species, *Tetratrachobothrius flavicaudis* (De Geer, 1778) (Fig. 50; Fet & Soleglad, 2002; Jacob et al., 2004b; Vachon, 1948). It has been reported to be present in 6 species of *Alpiscorpius* Gantenbein et al., 1999 (*A. alpha* (Di Caporiacco, 1950); *A. gamma* (Di Caporiacco, 1950); *A. germanus* (C.L. Koch, 1837); *A. mingrelicus* (Kessler, 1874); *A. phrygius* (Bonacina, 1980); *A. uludagensis* (Lacroix, 1995)) (Jacob et al. 2004b; Molteni et al., 1983; Scherabon, 1987; Tropea et al., 2015c),

and in 26 species of *Euscorpius* (*E. avcii* Tropea et al., 2012; *E. balearicus* Di Caporiacco, 1950; *E. birulai* Fet et al., 2014; *E. candiota* Birula, 1903; *E. carpathicus* (Linnaeus, 1767); *E. ciliciensis* Birula, 1898; *E. concinnus* (C. L. Koch, 1837); *E. deltshevi* Fet et al., 2014; *E. drenskii* Tropea et al., 2015; *E. feti* Tropea, 2013; *E. giachinoi* Tropea & Fet, 2015; *E. gocmeni* Tropea et al., 2014; *E. hadzii* Di Caporiacco, 1950; *E. italicus* (Herbst, 1800); *E. kinzelbachi* Tropea et al., 2014; *E. kritscheri* Fet et al., 2013; *E. lycius* Yağmur et al., 2013; *E. mylonasi* Fet et al., 2014; *E. oglasae* Di Caporiacco, 1950; *E. sicanus* (C. L. Koch, 1837); *E. solegladi* Fet et al., 2014; *E. stahlavskiyi* Tropea et al., 2014; *E. tauricus* (C. L. Koch, 1837); *E. tergestinus* (C.L. Koch, 1837); *E. sp. cf. tergestinus*; *E. vailatii* Tropea & Fet, 2015) (Figs. 51–57; Fet & Soleglad, 2002; Fet et al., 2013; Fet et al., 2014; Gantenbein et al., 2002; Jacob et al., 2004a, 2004b; Scherabon, 1987; Tropea et al., 2014a, 2014b, 2015b, 2015c;

Tropea & Fet, 2015; Tropea & Ozimec, 2019; Vignoli et al., 2007; Yağmur et al., 2013; Yağmur & Tropea, 2013). The *bl* was not directly reported in two other species. Gantenbein et al. (2002) described and illustrated the hemispermatophore of *E. naupliensis* (C. L. Koch, 1837), which seemed to lack the *bl*, but their text and figure were inconclusive. Tropea et al. (2013) omitted mention of the *bl* in *E. erymanthus*, but did not exclude it, and its presence is unclear.

Absence of the *ldi* is another character shared between *E. thracicus* sp. n. and *T. flavicaudis*. When this lobe is weakly developed, it can be intraspecifically variable, being absent among some individuals of a species (e.g., *A. alpha*, *A. gamma* and *A. germanus*; Jacob et al., 2004b). It has been reported to be present in 15 species of *Euscorpius* (i.e., *E. balearicus* Di Caporiacco, 1950; *E. birulai* Fet et al., 2014; *E. candiota* Birula, 1903; *E. carpathicus* (Linnaeus, 1767); *E. concinnus* (C. L. Koch, 1837); *E. deltshevi* Fet et al., 2014; *E. hadzii* Di Caporiacco, 1950; *E. italicus* (Herbst, 1800); *E. kritscheri* Fet et al., 2013; *E. mylonasi* Fet et al., 2014; *E. oglasae* Di Caporiacco, 1950; *E. sicanus* (C. L. Koch, 1837); *E. solegladi* Fet et al., 2014; *E. tergestinus* (C. L. Koch, 1837); *E. sp. cf. tergestinus*) (Figs. 51–57; Fet & Soleglad, 2002; Fet et al., 2013; Fet et al., 2014; Gantenbein et al., 2002; Jacob et al., 2004a, 2004b; Scherabon, 1987; Vignoli et al., 2007).

The *lde* appears to be almost universally present in hemispermatophores of these genera that have been described to date. However, none have been reported to exhibit apparent division into two sub-lobes. Perhaps the most proximate condition is in *T. flavicaudis*, whose *lde* is bilobate or bidentate, but undivided (Fig. 50). In molecular studies, *T. flavicaudis* resolved as a basal lineage and outgroup that was not closely related to *E. popovi* (Tropea et al., 2015a: 11, fig. 20), which is the species most similar morphologically to *E. thracicus* sp. n. Thus, in *E. thracicus* sp. n., *ldi* and *bl* may have been independently lost much later in the evolutionary history of the group. Their absence is probably the primitive state in *T. flavicaudis*.

The unusual morphology of the distal carina of *E. thracicus* sp. n., was consistent across 6 hemispermatophores from 3 males, indicating a taxonomically stable character. This underlines the importance of accurately characterizing and illustrating these structures for comparative analysis. Hemispermatophore anatomy has yet to be reported in the majority of other related euscorpiids (9 *Alpiscorpius* spp., 36 *Euscorpius* spp.).

DISTRIBUTION. Bulgaria (eastern Rodopi Mts.) (Fig. 61).

COMPARATIVE HEMISPHERMATOPHORE MATERIAL (♂, Figs. 50–57). *Euscorpius concinnus* (C. L. Koch, 1837), **France**, Var Department, Le Thoronet, 43.459761°N 6.281816°E, No. 1642, leg. M. Stockmann.

Euscorpius candiota Birula, 1903, **Greece**, Crete, Lassithi Plateau, 35.149605°N 25.504660°E, No. 1728, leg. M. Stockmann.

Euscorpius deltshevi Fet et al., 2014, **Bulgaria**, Sofia Province, Dragoman Municipality, Petrovski Krast Mtn., 42.9403326°N 22.9667976°E, 2 October 2020, No. 1867, leg. O. Vaněk.

Euscorpius italicus (Herbst, 1800), **Croatia**, Istria, 2 km SE Presika, 45.066421°N 14.138763°E, No. 1646.

Euscorpius sp., cf. *tergestinus*, **Croatia**, Krk Island, No. 1481.

Euscorpius sicanus (C. L. Koch, 1837), **Italy**, Sicily, Syracuse Province, Noto Antica, August 2014, No. 881, leg. C. Turiel.

Euscorpius solegladi Fet et al., 2014, **Bulgaria**, Blagoevgrad Province, Petrich Municipality, Rupite, Kozhuch Mt., 41.646179°N 23.361132°E, October 2020, No. 1861, leg. T. Ryšan.

Tetratrichobothrius flavicaudis (De Geer, 1778), **France**, Var Department, Gonfaron, 43.341578°N 6.294433°E, No. 1350, leg. M. Stockmann.

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References

- FET, V., M. R. GRAHAM, M. M. WEBBER & G. BLAGOEV. 2014. Two new species of *Euscorpius* (Scorpiones: Euscorpiidae) from Bulgaria, Serbia, and Greece. *Zootaxa*, 3894: 83–105.
- FET, V. & W. D. SISSOM. 2000. Family Euscorpiidae. Pp. 355–381. In: Fet, V., W. D. Sissom, G. Lowe & M. E. Braunwalder (eds.). *Catalog of the Scorpions of the World (1758-1998)*. New York: New York Entomological Society, 690 pp.
- FET, V. & M. E. SOLEGLAD. 2002. Morphology analysis supports presence of more than one species in the “*Euscorpius carpathicus*” complex (Scorpiones: Euscorpiidae). *Euscorpius*, 3: 1–51.
- FET, V., M. E. SOLEGLAD, B. GANTENBEIN, V. VIGNOLI, N. SALOMONE, E. V. FET & P. J. SCHEMBRI. 2003. New molecular and morphological data on the “*Euscorpius carpathicus*” species complex (Scorpiones: Euscorpiidae) from Italy, Malta, and Greece justify the elevation of *E. c. sicanus* (C. L. Koch, 1837) to the species level. *Revue suisse de Zoologie*, 110(2): 355–379.
- FET, V., M. E. SOLEGLAD, A. PARMAKELIS, P. KOTSAKIOZI & I. STATHI. 2013. A new species of *Euscorpius* from Tinos Island, Greece (Scorpiones: Euscorpiidae). *Revista Ibérica de Aracnología*, 23: 3–10.
- FET, V., M. E. SOLEGLAD, A. PARMAKELIS, P. KOTSAKIOZI & I. STATHI. 2014. Two new species of *Euscorpius* from Euboea Island, Greece (Scorpiones: Euscorpiidae). *Arthropoda Selecta*, 23 (2): 111–126.

- GANTENBEIN, B., V. FET, M. BARKER & A. SCHOLL. 2000. Nuclear and mitochondrial markers reveal the existence of two parapatric scorpion species in the Alps: *Euscorpius germanus* (C. L. Koch, 1837) and *E. alpha* Caporiacco, 1950, stat. nov. (Scorpiones, Euscorpiidae). *Revue suisse de Zoologie*, 107(4): 843–869.
- GANTENBEIN, B., V. FET, C.R. LARGIADÈR & A. SCHOLL. 1999. First DNA phylogeny of *Euscorpius* Thorell, 1876 (Scorpiones: Euscorpiidae) and its bearing on taxonomy and biogeography of this genus. *Biogeographica* (Paris), 75(2): 49–65.
- GANTENBEIN, B., M. E. SOLEGLAD & V. FET. 2001. *Euscorpius balearicus* Caporiacco, 1950, stat. nov. (Scorpiones: Euscorpiidae): molecular (allozymes and mtDNA) and morphological evidence for an endemic Balearic Islands species. *Organisms Diversity & Evolution*, 1(4): 301–320.
- GANTENBEIN, B., M. E. SOLEGLAD, V. FET, P. CRUCITTI & E. V. FET. 2002. *Euscorpius naupliensis* (C. L. Koch, 1837) (Scorpiones: Euscorpiidae) from Greece: elevation to the species level justified by molecular and morphological data. *Revista Ibérica de Aracnología*, 6: 13–43.
- JACOB, A., B. GANTENBEIN, M. E. BRAUNWALDER, W. NENTWIG & C. KROPF. 2004a. Morphology and function of male genitalia (spermatophores) in *Euscorpius italicus* (Euscorpiidae, Scorpiones): complex spermatophore structures enable safe sperm transfer. *Journal of Morphology*, 260: 72–84.
- JACOB, A., B. GANTENBEIN, M. E. BRAUNWALDER, W. NENTWIG & C. KROPF. 2004b. Complex male genitalia (hemispermaphores) are not diagnostic for cryptic species in the genus *Euscorpius* (Scorpiones: Euscorpiidae). *Organisms Diversity & Evolution*, 4: 59–72.
- KOVAŘÍK, F. 2009. *Illustrated catalog of scorpions. Part I. Introductory remarks; keys to families and genera; subfamily Scorpioninae with keys to Heterometrus and Pandinus species*. Prague: Clairon Production, 170 pp.
- KOVAŘÍK, F., G. LOWE, M. STOCKMANN & F. ŠTÁHLAVSKÝ. 2020. Revision of genus-group taxa in the family Scorpionidae Kraepelin, 1905, with description of 15 new species (Arachnida: Scorpiones). *Euscorpius*, 325: 1–140.
- KOVAŘÍK, F. & A. A. OJANGUREN AFFILASTRO. 2013. *Illustrated catalog of scorpions. Part II. Bothriuridae; Chaerilidae; Buthidae I. Genera Compsobuthus, Hottentotta, Isometrus, Lychas, and Sassanidotus*. Prague: Clairon Production, 400 pp.
- KOVAŘÍK, F. & F. ŠTÁHLAVSKÝ. 2020. Five new species of *Euscorpius* Thorell, 1876 (Scorpiones: Euscorpiidae) from Albania, Greece, North Macedonia, and Serbia. *Euscorpius*, 315: 1–37.
- KUMAR, S., G. STECHER, M. LI, C. KNYAZ & K. TAMURA. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35: 1547–1549.
- MOLTENI, M. G., A. BONACINA, R. CHIODERO & G. VAILATI. 1984. Aspetti anatomici ed istologici dell'organo parassiale di *Euscorpius germanus* (C.L. Koch) (Scorpiones, Chactidae). *Rivista del Museo Civico di Scienze Naturali "Enrico Caffi" Bergamo*, 6: 125–154.
- MONOD, L., L. CAUWET, E. GONZÁLEZ-SANTILLÁN & S. HUBER. 2017. The male sexual apparatus in the order Scorpiones (Arachnida): a comparative study of functional morphology as a tool to define hypotheses of homology. *Frontiers in Zoology*, 14: 51: 1–48.
- PARMAKELIS, A., P. KOTSAKIOZI, I. STATHI, S. POULIKARAKOU & V. FET. 2013. Hidden diversity of *Euscorpius* (Scorpiones: Euscorpiidae) in Greece revealed by multilocus species-delimitation approaches. *Biological Journal of the Linnean Society, London*, 110: 728–748.
- PLÍŠKOVÁ, J., F. KOVAŘÍK, O. KOŠULIČ & F. ŠTÁHLAVSKÝ. 2016. Description of a new species of *Heterometrus* Ehrenberg, 1828 (Scorpiones: Scorpionidae) from Thailand with remarks about the utilization of cytogenetic data in taxonomy of the genus. *Annales Zoologici (Warszawa)*, 66(3): 467–476.
- SAKAMOTO, Y. & A. A. ZACARO. 2009. LEVAN, an ImageJ plugin for morphological cytogenetic analysis of mitotic and meiotic chromosomes. Available at: <http://rsbweb.nih.gov/ij/plugins/levan/levan.html>. Accessed 3rd June 2016.
- SCHERABON, B. 1987. Die Skorpione Österreichs in vergleichender Sicht unter besonderer Berücksichtigung Kärntens. *Carinthia II*, 45: 77–154.
- SOLEGLAD, M. E. & W. D. SISSOM. 2001. Phylogeny of the family Euscorpiidae Laurie, 1896: a major revision. Pp. 25–112 in: Fet, V. & P. A. Selden (eds.). *Scorpions 2001. In Memoriam Gary A. Polis*. Burnham Beeches, Bucks: British Arachnological Society.
- STAHNKE, H. L. 1971. Scorpion nomenclature and mensuration. *Entomological News*, 81: 297–316.

- ŠTUNDLOVÁ, J., J. ŠMÍD, P. NGUYEN & F. ŠTÁHLAVSKÝ. 2019. Cryptic diversity and dynamic chromosome evolution in Alpine scorpions (Euscorpiidae: Euscorpius). *Molecular Phylogenetics and Evolution*, 134: 152–163.
- TROPEA, G. & V. FET. 2015. Two new *Euscorpius* species from central-western Greece (Scorpiones: Euscorpiidae). *Euscorpius*, 199: 1–16.
- THORELL, T. 1876. On the classification of scorpions. *Annals and Magazine of Natural History*, 4(17): 1–15.
- TROPEA, G., V. FET, A. PARMAKELIS, P. KOTSAKIOZI & I. STATHI. 2013. A new species of *Euscorpius* Thorell, 1876 from Peloponnese, Greece (Scorpiones: Euscorpiidae). *Euscorpius*, 169: 1–11.
- TROPEA, G., V. FET, A. PARMAKELIS, P. KOTSAKIOZI & I. STATHI. 2014a. Three new species of *Euscorpius* (Scorpiones: Euscorpiidae) from Greece. *Euscorpius*, 190: 1–22.
- TROPEA, G., V. FET, A. PARMAKELIS, P. KOTSAKIOZI & I. STATHI. 2015a. A new species of *Euscorpius* from Bulgaria and Greece (Scorpiones: Euscorpiidae). *Euscorpius*, 207: 1–15.
- TROPEA, G., V. FET, A. PARMAKELIS, P. KOTSAKIOZI & I. STATHI. 2015b. A new species of *Euscorpius* (Scorpiones: Euscorpiidae) from southern Bulgaria. *Arachnologische Mitteilungen*, 49: 10–20.
- TROPEA, G. & R. OZIMEC. 2019. Description of the adult male of *Euscorpius feti* Tropea, 2013 (Scorpiones: Euscorpiidae), with notes on cave ecology of this species. *Euscorpius*, 291: 1–10.
- TROPEA, G., E. A. YAĞMUR & V. FET. 2015c. A revision of the Anatolian-Caucasian “*Euscorpius mingrelicus* Complex” (Scorpiones: Euscorpiidae). *Euscorpius*, 203: 1–32.
- TROPEA, G., E. A. YAĞMUR, H. KOÇ, F. YEŞİLYURT & A. ROSSI. 2012. A new species of *Euscorpius* Thorell, 1876 (Scorpiones, Euscorpiidae) from Turkey. *ZooKeys*, 219: 63–80.
- TROPEA, G., E. A. YAĞMUR & F. YEŞİLYURT. 2014b. A new species of *Euscorpius* Thorell, 1876 from the Antalya Province, southern Turkey (Scorpiones: Euscorpiidae). *Euscorpius*, 184: 1–13.
- VACHON, M. 1948. Scorpions récoltés dans l’île de Crète par Mr le docteur Otto von Wettstein. *Annalen des Naturhistorischen Museums in Wien*, 56: 61–69.
- VACHON, M. 1974. Études des caractères utilisés pour classer les familles et les genres des scorpions (Arachnides). 1. La trichobothriotaxie en arachnologie. Sigles trichobothriaux et types de trichobothriotaxie chez les Scorpions. *Bulletin du Muséum national d’Histoire naturelle*, 3e série, 140 (Zoologie, 104): 857–958.
- VIGNOLI, V., N. SALOMONE, F. CICONARDI & F. BERNINI. 2007. The scorpion of Montecristo, *Euscorpius oglasae* Di Caporiacco, 1950, stat.nov. (Scorpiones, Euscorpiidae): a paleoendemism of the Tuscan Archipelago (northern Tyrrhenian, Italy). *Comptes Rendus Biologies*, 330 (2): 113–125.
- YAĞMUR, E. A. & G. TROPEA. 2013. A new species of *Euscorpius* Thorell, 1876 (Scorpiones, Euscorpiidae) from Marmara Region of Turkey. *ZooKeys*, 281: 91–105.
- YAĞMUR, E. A., G. TROPEA & F. YEŞİLYURT. 2013. A new species of *Euscorpius* Thorell, 1876 (Scorpiones, Euscorpiidae) from south western Turkey. *ZooKeys*, 348: 29–45.