

## MOLECULAR AND MORPHOLOGICAL EVIDENCE SUPPORTS THE ELEVATION OF *EUSCORPIUS GERMANUS CROATICUS* DI CAPORIAMO, 1950 (SCORPIONES: EUSCORPIIDAE) TO *E. CROATICUS* STAT. NOV., A RARE SPECIES FROM CROATIA

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**Abstract:** The taxonomic identity of *Euscorpium germanus croaticus* Di Caporiacco, 1950, described from Croatia and Bosnia, has remained unclear ever since its discovery. We studied the lectotype from the Velebit Mountains as well as new material from Biserujka Cave on Krk Island, Croatia. We reassessed *E. g. croaticus* using both morphology and DNA barcodes (*cox1* sequences) from one of the Biserujka Cave specimens and 15 congeneric species. The resulting DNA phylogeny suggests that *E. g. croaticus* is not a subspecies of *E. germanus*. The taxon appears to be a separate lineage, which groups close to subgenus *Alpiscorpius* but differs from all its members in several morphological characters. We elevate *E. g. croaticus* to species rank as *Euscorpium croaticus* Di Caporiacco, 1950, **stat. nov.**, and provide a detailed redescription of both sexes.

**Key words:** Scorpiones, Euscorpidae, *Euscorpium*, barcode, *cox1*, mitochondrial DNA, Croatia, Krk, Biserujka Cave, Velebit.

**Los datos moleculares y morfológicos apoyan la elevación de *Euscorpium germanus croaticus* Di Caporiacco, 1950 (Scorpiones: Euscorpidae) a *E. croaticus* stat. nov., especie rara de Croacia**

**Resumen:** La identidad taxonómica de *Euscorpium germanus croaticus* Di Caporiacco, 1950, descrito de Croacia y Bosnia, ha estado poco clara desde su descubrimiento. Hemos estudiado el lectotipo, de las montañas de Velebit, así como nuevo material de la cueva de Biserujka, en la isla de Krk, Croacia. Hemos reevaluado *E. g. croaticus* sobre la base de la morfología y códigos de barras de ADN (secuencias *cox1*) de uno de los especímenes de la cueva de Biserujka y 15 especies del mismo género. La filogenia de ADN resultante sugiere que *E. g. croaticus* no es una subespecie de *E. germanus*. El taxón parece ser un linaje aparte y queda más próximo al subgénero *Alpiscorpius*, aunque se diferencia de todos sus miembros por varios caracteres morfológicos. Elevamos *E. g. croaticus* al rango de especie, como *Euscorpium croaticus* Di Caporiacco, 1950, **stat. nov.**, y damos una redesccripción detallada de ambos sexos.

**Palabras clave:** Scorpiones, Euscorpidae, *Euscorpium*, código de barras, *cox1*, ADN mitocondrial, Croacia, Krk, cueva de Biserujka, Velebit.

**Taxonomy / Taxonomía:** *Euscorpium croaticus* Di Caporiacco, 1950, **stat. nov.**

### Introduction

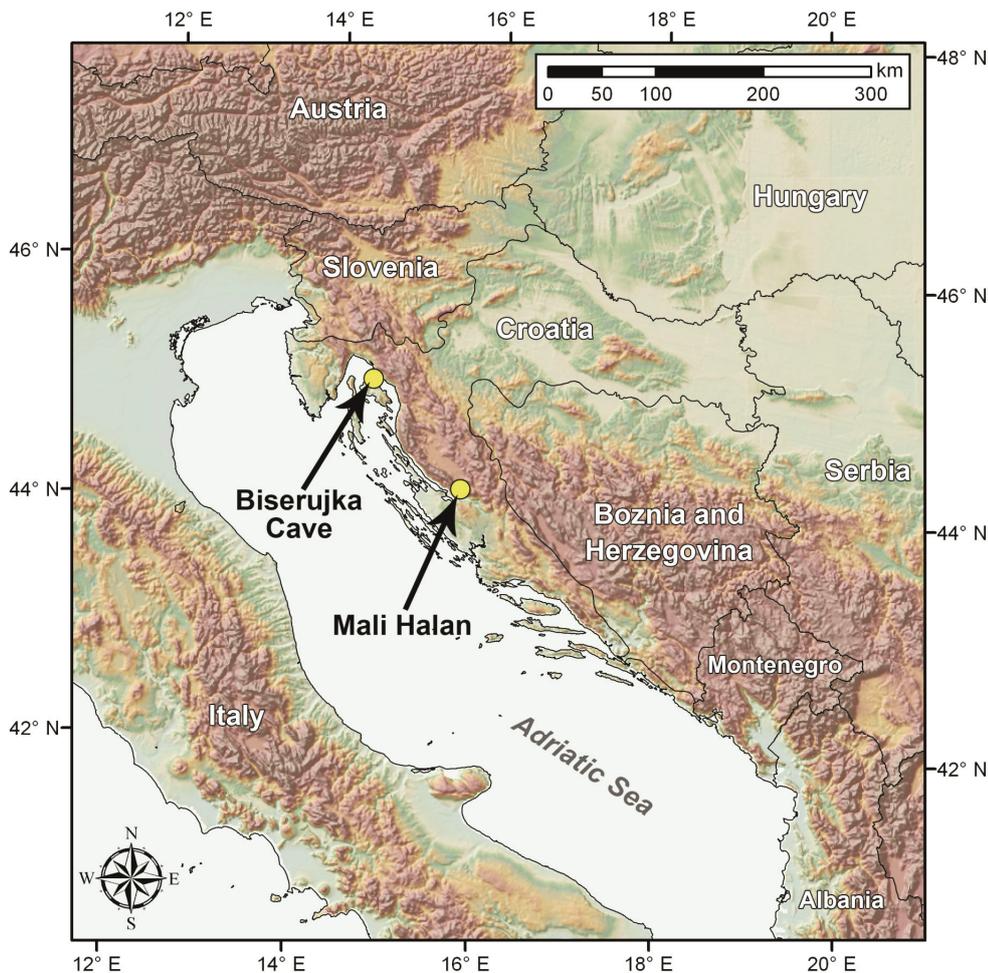
An appropriate taxonomic assignment for the enigmatic subspecies *E. germanus croaticus* Di Caporiacco, 1950 has remained ambiguous ever since its description. Di Caporiacco (1950) listed several specimens (syntypes) from Croatia and Bosnia, but only a single male specimen from the Velebit Mountains of Croatia was found to exist at Museo Zoologico “La Specola” (MZUF); this specimen was designated as a lectotype by Gantenbein *et al.* (2000). Using evidence from mitochondrial DNA and allozymes, Gantenbein *et al.* (1999) established the subgenus *Alpiscorpius* with *E. germanus* as the type species. Diagnostic characters for subgenus *Alpiscorpius* included 5–6 trichobothria on the ventral aspect of the pedipalp patella, reduced to obsolete metasomal carinae, and elongated pectinal sensilla. More species have since been assigned to *Alpiscorpius* (Scherabon *et al.*, 2000; Fet, 2000), but the scope of this subgenus, which is distributed from the Alps to the Caucasus, remains unclear (see Discussion).

Gantenbein *et al.* (2000) discussed all described subspecies of *E. germanus*. Based on the number of *et* trichobothria on the external surface of the pedipalp patella (*et* = 5), presence of carination on the metasoma, shape of pedipalps, and leg spination, they concluded that *E. g. croaticus* was not a subspecies of *E. germanus*, or even a closely related species. As such, Gantenbein *et al.* (2000) did not consider *E. g. croaticus* a member of subgenus *Alpiscorpius* and treated it as a

form belonging to the (loosely defined at that time) “*E. carpathicus* complex” (=subgenus *Euscorpium*), leaving the species designation undecided.

Recently, we obtained several female *Euscorpium* collected from Biserujka Cave (formerly known formerly known as “Vitezičeva pečina”) on Krk Island, Croatia. Earlier authors identified forms from this cave as *E. germanus* (Potočnik, 1983; Christian & Potočnik, 1985), but the population was never carefully studied. Since unique troglomorphic scorpions continue to be discovered (Fet *et al.*, 2011), we began examining the new material from Biserujka Cave to assess whether they could represent the first cave-adapted *Euscorpium*. However, other than being slightly paler in color than most *Euscorpium* spp., our investigation did not uncover any morphological features indicating that the specimens may be troglomorphic. Instead, the specimens appear to represent the first known females of the enigmatic taxon described as *E. germanus croaticus*, the lectotype locality of which lies nearby at Mali Halan Pass in the Velebit Mountains (Fig. 1; note: the Mali Halan locality name was misspelled as “Mali Halam” by Di Caporiacco (1950: 215) and most subsequent authors).

To assess the phylogenetic position of these rare scorpions, we sequenced DNA barcodes (mitochondrial *cox1* sequences) from one of the new specimens from Biserujka



**Fig. 1.** Localities for the male lectotype (Mali Halan) and recently collected females (Biserujka Cave) of *Euscorpium croaticus* Di Caporiacco, 1950, *stat. nov.*

Cave and 15 congeners. Phylogenetic results of this analysis indicated that the specimen from Biserujka Cave appears to be closely related to subgenus *Alpiscorpius*, not subgenus *Euscorpium* s.str. However, the specimens markedly differ from other *Alpiscorpius* spp. in several morphological characters. Accordingly, we elevate *E. germanus croaticus* to species rank as *E. croaticus* Di Caporiacco, 1950, *stat. nov.*, and provide a thorough redescription of both sexes (with the first available description of females). Currently, its subgeneric affiliation remains undetermined. Our descriptions and morphological comparisons follow standards set by Fet & Soleglad (2002), who redescribed and defined two species of subgenus *Euscorpium* s.str. found in Croatia, including its Adriatic islands. We used both of these species, *E. tergestinus* and *E. hadzii*, as outgroups in our phylogenetic study.

## Material and Methods

**Specimens used for DNA barcoding.** Of 16 sequences used for our phylogeny, 12 are reported here for the first time: *Euscorpium croaticus* Di Caporiacco, 1950, *stat. nov.* CROATIA: Krk Island, Dobrinj District, Rudine, Biserujka Cave, 45°10'59"N, 14°36'47"E, 1997, VF-0824, AMSC O082-10. *Euscorpium (Alpiscorpius) alpha* Di Caporiacco, 1950. ITALY, Trentino-Alto Adige, Aprica, 46°09'N, 10°08'E, 1001 m, leg. F. Štáhl'avský, VF-0731, AMSCO013-10. *Euscorpium (Alpiscorpius) germanus* (C. L. Koch, 1837). ITALY: Trentino-Alto Adige, Egna (Neumarkt), 46°19'1.2"N, 11°16'1.2"E, 214 m, leg. F. Štáhl'avský, VF-0726, AMSC O011-10 (HM418272); SLOVENIA: Soča Valley, Trnovo ob

Soči, 46°16'47"N, 13°33'11"E, 12 June 1999, leg. V. Fet & D. Huber, VF-0733, AMSCO015-10. *Euscorpium (Alpiscorpius) gamma* Di Caporiacco, 1950. AUSTRIA, Carinthia, Trögerner-Klamm, 46°27'28"N, 14°30'02"E, 13 June 1999, leg. V. Fet & B. Scherabon, VF-0732, AMSCO014-10 (HM418273). *Euscorpium (Alpiscorpius) mingrelicus* (Kessler, 1874). BOSNIA & HERZEGOVINA: Herzegovina, Lebršnik, 43°12'07"N, 18°38'16"E, 1700 m, 9 September 2006, leg. I. Karaman, VF-0737, AMSCO018-10 (HM418276); SERBIA: Tara River, Kaluderske Bare, 43°54'23"N, 19°31'37"E, 14 July 2007, leg. D. Pavicevic, VF-0744, AMSCO024-10 (HM418278); GEORGIA: Abkhazia, Ritsa Lake, 43°28'N, 40°30'E, 14 October 2004, leg. Yu. Marusik, VF-0747, AMSCO027-10 (HM418279). *Euscorpium (Polytrichobothrius) italicus* (Herbst, 1800). SLOVENIA: Koper District, Marezige, 45°30'35"N, 13°48'11"E, 11 August 2002, leg. T. Brstilo, VF-0706, AMSCO005-10 (HM418270). *Euscorpium (Euscorpium) tergestinus* (C. L. Koch, 1837), CROATIA: 10 km E of Split, 0.5 km S of Dubrava Village, 43°29'N, 16°39'E, 11 October 2009, leg. H. Bringsøe, VF-0772, AMSCO046-10 (HM418286). *Euscorpium (Euscorpium) balearicus* Di Caporiacco, 1950, SPAIN: Balearic Islands, Mallorca, Deia, 39°44'48"N, 02°38'46"E, March 1998, leg. M. Vences, VF-0770, AMSC O045-10 (HM418285). *Euscorpium (Euscorpium) sp.*, BULGARIA: West Rodopi Mts., Kovatchevitsa, 41°41'13"N, 23°50'33"E, 1353 m, 1 June 1999, leg. V. Fet & V. Sakalian, VF-0819, AMSCO077-10 (HM418303).

The remaining four barcode sequences used as outgroups were published in our recent study (Graham *et al.*, 2012): *Euscorpium (Euscorpium) sicani* (C.L. Koch, 1837):

MALTA, Buskett Gardens, 35°51'41"N, 14°23'56"E, 17 September 2001, leg. P. Schembri, VF-0792, AMSCO053-10 (HM418288). *Euscorpius (Euscorpius) carpathicus* (L., 1767): ROMANIA, Caraş-Severin County, Băile Herculane, 44°52'43"N, 22°24'51"E, 4 June 2008, leg. F. Štáhlavský, VF-0768, AMSCO044-10 (HM418284). *Euscorpius (Euscorpius) hadzii* Di Caporiacco, 1950: MONTENEGRO, Budva District, Visnjevo, 42°17'52"N, 18°46'37"E, sea level, 29 October 2005, leg. F. Franeta, VF-0807, AMSCO066-10 (HM418296). *Euscorpius (Tetratrichobothrius) flavicaudis* (DeGeer, 1787): FRANCE, Vaucluse, Pernes-les-Fontaines, 43°59'55"N, 05°03'35"E, 230 June 2007, leg. V. Fet, VF-0700, AMSCO001-10 (HM418267).

**Specimens studied for morphological comparison:** *E. germanus*: SLOVENIA, Soča Valley, Trnovo ob Soči, 46°16'47"N, 13°33'11"E, 12 June 1999, leg. V. Fet & D. Huber, VF-0733 (see label above). *E. gamma*: SLOVENIA, Bohinj, 46°16'N, 13° 57'E, 7-26 June 2008, leg. F. Stahlavsky; AUSTRIA, Carinthia, VF-0732 (see label above); *E. mingrelicus*: BOSNIA & HERZEGOVINA: Lebršnik, VF-0737 (see label above); SERBIA: Tara River, VF-0744 (see label above); GEORGIA: Abkhazia, Ritsa Lake, VF-0747 (see label above); TURKEY: Konya Prov., Doğanhisar Distr., Başköy Village, Kirazlıdere vicinity, 38°08'N, 31°47'E, ca. 1300 m, 21 August 2001, leg. A. Ersöz, VF-0762; Denizli Prov., Honaz Distr., Honaz Dağı National Park, 40°09'N, 29°05'E, ca. 1500 m, 25 July 2001, leg. E. Şivkm, VF-0759.

**List of depositories:** MZUF, Museo Zoologico "La Specola", University of Florence, Florence, Italy; NHMW, Naturhistorisches Museum Wien, Vienna, Austria; VF, Personal collection of Victor Fet, Huntington, West Virginia, USA.

#### **Molecular Techniques and Phylogenetic Analysis:**

Mitochondrial barcodes (*cox1* sequences) were generated for 16 *Euscorpius* specimens at the Canadian Centre for DNA Barcoding, University of Guelph using standard protocols as outlined in DeWaard *et al.* (2008). In brief, tissue from a single scorpion leg was used for extraction of genomic DNA using a 96 AcroPrep™ 1 ml filter plate (PALL) with 3.0 µm Glass fiber over 0.2µm Bioinert membrane as described in Ivanova *et al.* (2006). DNA was eluted in 40 µl of dH<sub>2</sub>O. Full-length *cox1* barcodes (649 bp) were amplified using newly designed primer sets (designed by N. Ivanova, see also Graham *et al.*, 2012): ScorpF1\_t1 (5' – TGTAACACGACGG CCAGTTTTCTACTAATCAYAAAGAYATTGG – 3') and ScorpR1\_t1 (5' – CAGG AACAGCTATGACGGRTGTCC AAAAAAYCAAAAYAAATG – 3'). Specimens which failed to produce full-length barcodes due to DNA degradation were amplified using two overlapping fragments with primers: ScorpF1\_t1/MScorpR1 (5' – CCHGTTCCCHGCHC CDCTYTC – 3', this study) and MScorpF1 (5' – GCTTTTC CRCGDTTRAATAATA – 3', this study)/ScorpR1\_t1.

All PCR products were sequenced bi-directionally with BigDye 3.1 (Applied Biosystems) using corresponding primers [M13F(-21) and M13R(-27) (Messing, 1983) were used for M13-tailed ScorpF1\_t1 and ScorpR1\_t1]. Cycle sequencing reactions were analyzed on an ABI3730XL (Applied Biosystems) following manufacturer's instructions. The forward and reverse sequences were used to generate a single

consensus sequence using CodonCode Aligner v. 3.0.2 (CodonCode Corporation). *Cox1* was chosen because it is commonly used in barcoding and has been demonstrated as highly effective in discriminating among insect (Zhang & Hewitt, 1997; Foottit *et al.*, 2009; Zhou *et al.*, 2009) and arachnid species (e.g. Barrett & Hebert, 2005; Thomas & Hedin, 2008; Robinson *et al.*, 2009; Graham *et al.*, 2012; Sousa *et al.*, 2012). All new barcodes were submitted to GenBank and can be accessed through BOLD (<http://www.boldsystems.org>, Ratnasingham & Hebert, 2007) under project "Scorpions of the Ancient Mediterranean 2b (AMSCB)". Voucher specimens are in a private collection of V. Fet.

We imported the barcodes into SEQUENCHER v. 4.9 (Gene Codes Corp., Inc., Ann Arbor, MI, USA) and aligned them by eye. The best-fit model of nucleotide substitution was determined by MEGA v. 5 (Tamura *et al.*, 2011) with the Akaike Information Criterion (Posada, 2008). Using the appropriate model (GTR+I+G model), we assessed phylogenetic patterns using Bayesian inference (BI) implemented in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003) ran through the Cyberinfrastructure for Phylogenetic Research cluster (CIPRES Gateway v 3.1) at the San Diego Supercomputer Center. We based our interpretations on final runs of 10 million generations with trees sampled every 1,000 generations. We set the temperature to 0.01 and discarded the first 2.5 million generations as burn-in.

#### **Results**

Phylogenetic analysis of *cox1* barcodes under the criterion of Bayesian inference rendered *E. croaticus* **stat. nov.** as a unique lineage which formed a strongly supported (PP = 0.96) monophyletic group (Fig. 2) together with subgenus *Alpiscorpius*. The recovered lineage was positioned as sister to *Euscorpius tergestinus* (C. L. Koch, 1837) (PP = 0.94). Two monophyletic groups were recovered within *Alpiscorpius* with strong support (PP = 1.00); one comprised of *E. gamma* and *E. mingrelicus* from Serbia and Bosnia & Herzegovina, and another containing *E. germanus* from Italy and Slovenia.

#### **SYSTEMATICS:**

**Order SCORPIONES C. L. Koch, 1850**

**Parvorder Iurida Soleglad et Fet, 2003**

**Superfamily Chactoidea Pocock, 1893**

**Family Euscorpiidae Laurie, 1896**

**Subfamily Euscorpiinae Laurie, 1896**

**Genus *Euscorpius* Thorell, 1876**

***Euscorpius croaticus* Di Caporiacco, 1950, **stat. nov.****

Fig. 3–17, Table I.

*Euscorpius germanus croaticus* Di Caporiacco, 1950: 215.

**MATERIAL STUDIED:** *Lectotype* (designated by Gantenbein *et al.*, 2000: 858): 1 ♂ (MZUF 5580), Mali Halan, Velebit Mts., Croatia, 44°17'30"N, 15°39'20"E, 1045 m. No other syntypes exist in MZUF (see Note below on the syntype series).

*Other material studied:* Biserujka Cave, Rudine, Krk Island, Dobrinj District, Croatia, 45°10'59" N, 14°36'47" E, 3 ♀ (NHMW, 21.950/1-3), 11 August 1990, leg. C. Komposch & P. Schnitter; 1 ♀ (VF-0824).

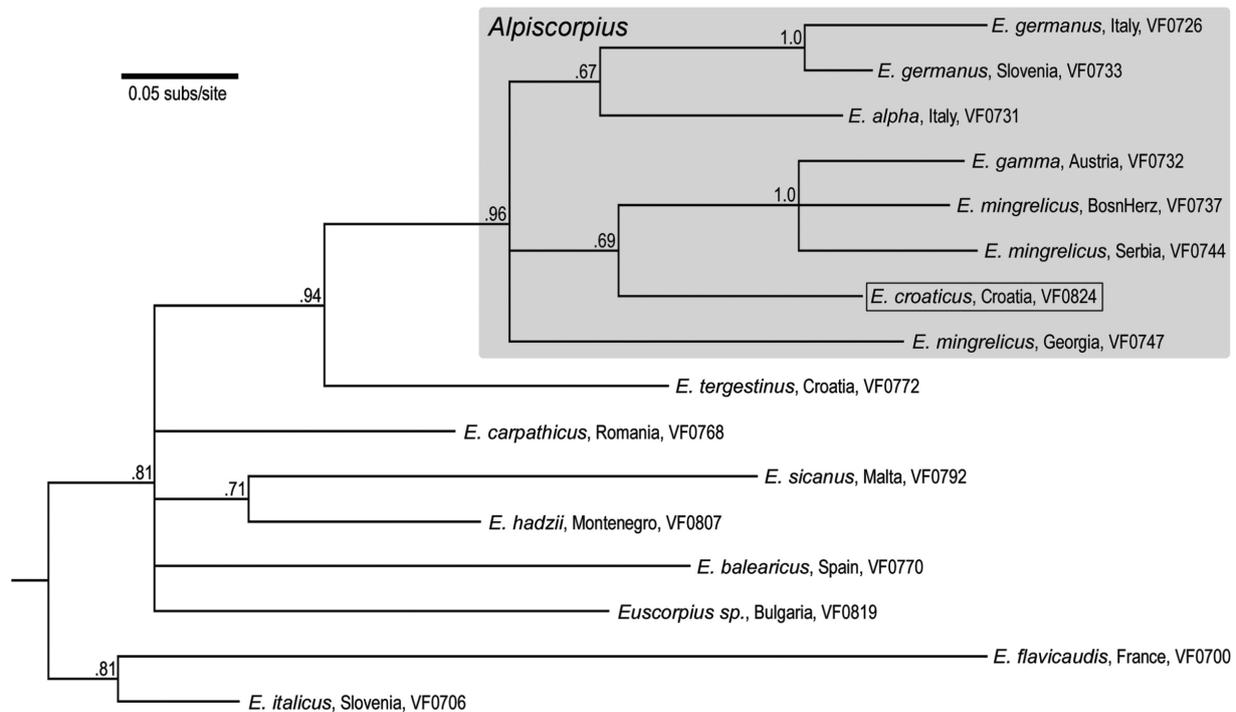


Fig. 2. Phylogenetic position of *Euscorpium croaticus* Di Caporiacco, 1950, *stat. nov.* (outlined) within subgenus *Alpiscorpius* (grey shading) based on DNA barcode data. Posterior probabilities greater than 0.5 are indicated above each node.

Table I. Measurements (in mm) of the male lectotype and two female *E. croaticus* *stat. nov.*

Specimen	Lectotype	NHMW	NHMW
	♂	21.950/1	21.950/2
Sex	♂	♀	♀
Total Length	32.74	34.40	31.43
Carapace Length	4.64	4.76	4.76
Mesosoma Length	11.07	10.36	13.10
Metasoma Length	17.02	19.29	13.57
Met I Length	1.79	2.14	2.14
Met I Width	1.90	1.67	1.67
Met I Depth	1.67	1.55	1.31
Met II Length	2.02	2.38	2.26
Met II Width	1.79	1.43	1.43
Met II Depth	1.43	1.31	1.19
Met III Length	2.26	2.50	2.38
Met III Width	1.67	1.31	1.43
Met III Depth	1.55	1.43	1.43
Met IV Length	2.38	2.62	2.62
Met IV Width	1.55	1.31	1.31
Met IV Depth	1.31	1.43	1.31
Met V Length	4.40	4.17	4.17
Met V Width	1.67	1.19	1.43
Met V Depth	1.43	1.43	1.31
Telson Length	4.17	4.29	4.40
Vesicle Length	2.98	3.10	3.21
Vesicle Width	2.02	1.67	1.67
Vesicle Depth	1.90	1.43	1.55
Aculeus Length	1.19	1.19	1.19
Pedipalp Length	14.40	15.83	15.83
Femur Length	3.21	4.05	3.93
Femur Width	1.43	1.43	1.43
Patella Length	3.57	3.93	4.17
Patella Width	1.19	1.43	1.31
Chela Length	7.62	8.33	7.74
Palm Length	4.05	4.52	4.05
Palm Width	2.62	2.62	2.74
Palm Depth	2.02	2.02	2.02
Movable Finger Length	4.05	4.52	4.76
Fixed Finger Length	3.57	3.81	3.69
Pectine Teeth	7/7	5/5	5/5
Middle Lamellae	3/4	2/2	2/2

#### REFERENCES:

- Euscorpium germanus mesotrichus*: Hadži, 1930: 37 (in part: Velebit Mts).  
*Euscorpium germanus croaticus*: Valle *et al.*, 1971: 96; Bonacina, 1980: 57–58, 70–71, 75; Bartolozzi *et al.*, 1987: 296; Fet, 1993: 5; Fet & Braunwalder, 2000: 19–20; Fet & Sissom, 2000: 368; Fet *et al.*, 2003: 1520.  
*Euscorpium germanus*: Potočnik, 1983: 394; Christian & Potočnik, 1985: 13.  
*Euscorpium carpathicus croaticus*: Gantenbein *et al.*, 2000: 856–858; Fet & Soleglad, 2007: 415–416.  
*Euscorpium "croaticus"*: Fet & Soleglad, 2007: 415, fig. 14.

**DIAGNOSIS:** Small to medium in size, ranging from 31.4 to 34.4 mm in length among the specimens examined (Table I). Light brown in coloration with darker carapace and pedipalps and no obvious patterning (Fig. 3–6). Anterior edge of the carapace is straight. The metasoma is elongated and covered with moderate to weak granulation. The vesicle is swollen, especially in the male, and possesses two dark brown bands on the lateral and ventral surfaces. The pectine tooth count is 6–7 in males and 5–6 in females. The genital operculum is separated most of its length, and genital papillae in the male protrudes well beyond the opercular plates. Metasomal carinae are present but highly reduced. The DPS is sharp and well-developed, and the VPS is present as a small granule. Number of trichobothria on ventral surface of patella is 5 to 6 (Fig. 13); number of external median (*em*) trichobothria on external surface of patella is always 3 (Fig. 12). Metasomal carinae are reduced.

**DESCRIPTION OF MALE LECTOTYPE:** *Coloration* (Figs. 3, 4): Basic color light to dark brown, pedipalps and carapace darkest; mesosoma, metasoma and telson slightly lighter; legs and chelicerae orange-brown; sternum, genital operculum, pectines and sternites I–IV dark yellow. No discernable patterns present.



**Fig. 3–6.** Habitus of *Euscorpium croaticus* Di Caporiacco, 1950, **stat. nov.** **3.** Male lectotype, dorsal. **4.** Male lectotype, ventral. **5.** Female NHMW 21.950/1, dorsal. **6.** Female NHMW 21.950/1, ventral.

*Carapace:* Overall surface slightly granular in appearance at 10x. Anterior edge straight. Two pairs of lateral eyes, anterior eye slightly larger; median eyes and tubercle medium to small, situated anterior of middle with an AE/PE ratio = 0.62.

*Mesosoma:* Tergites slightly rough and granular in appearance at 10x; carinae of tergite VII essentially obsolete. Sternites lustrous, carinae of segment V obsolete. Spiracles small, short, oval like in shape.

*Metasoma:* Generally elongate in overall proportions. Carinae: Segments I–IV: dorsally rounded with scattered granulation; dorsal lateral carinae moderate, and slightly rounded on I–III, weak on IV, weak to obsolete on V; lateral carinae weak to obsolete on I and II, and obsolete on III and IV; inferior (ventral) lateral weak to obsolete on segments I–III, moderate on IV; inferior (ventral) median obsolete on segments I–III, slight trace on IV. Carinae: Segment V: dorsal

lateral carinae small, rounded and slightly rough; lateral obsolete; inferior lateral moderate and granular; inferior median irregularly granulate. Intercarinal spaces are finely granular on I–IV, with small scattered granules on V.

*Telson*: Vesicle swollen both laterally and dorsally (Fig. 16). Two dark brown bands present on lateral and ventral surface of vesicle. Aculeus forming short conspicuous curve; 4–5 pairs of setae at vesicle/aculeus juncture. Pair of setae present at base of aculeus.

*Pectines*: Well developed, teeth elongated, tooth count 7/7; middle lamellae 4–4. Fulcra developed for entire pecten; delicate yellow setae found on anterior lamellae; sensorial areas of teeth, approximately  $\frac{1}{3}$  to  $\frac{1}{2}$  of surface. Basal piece with slight rounded anterior indentation.

*Genital operculum*: Separated most of length, genital papillae extend well below plates.

*Sternum* (Fig. 14): Pentagonal in shape, length/width ratio = 0.82.

*Chelicerae*: Movable finger: dorsal distal denticle considerably shorter than ventral counterpart; dorsal edge with two subdistal denticles; ventral edge smooth, no serrula, covered with thick brush-like setae. Fixed finger: four denticles configured normally.

*Pedipalps*: Prominent scalloping at chelal finger bases. Femur: dorsal internal and external, and ventral internal granular to crenulate; ventral external crenulate; dorsal and ventral surfaces covered with minute granules; external surface smooth; internal face with 8 or more large granules. Patella: dorsal and ventral carinae denticulate, exteromedian rounded, scattered granulation; dorsal surface with minute granules; ventral face smooth; DPS well developed and sharp, VPS present as weak small granule. Chela carinae: digital strong and generally smooth, slight trace of granulation; sub-digital as one rounded granule; dorsal secondary obsolete; dorsal tertiary very weakly developed; dorsal marginal are round, and irregularly covered with larger granules; dorsal internal rounded and rough; ventroexternal strong and smooth, extending to external condyle, external to trichobothrium *Et*<sub>2</sub>; ventral median weak, highly rounded; ventral internal medium, smooth and rounded; external secondary are irregularly granulate. Chelal finger dentition: median denticle row straight; 6/7 internal denticles, 5/6 outer denticles, 4/5 internal accessory denticles for fixed and movable fingers respectively. Trichobothria patterns (Fig. 7–13): Type C, neobothriotaxic (major additive on patella). Femur: trichobothrium *d* and *i* equally proximal, *e* distal, situated on dorsoexternal carina. Patella: ventral series number 6/6 and external series number *eb* = 4/4, *eba* = 4/4, *esb* = 2/2, *em* = 3/3, *est* = 4/4, *et* = 5/5. Chela: Ventral series trichobothria number 4/4, *V*<sub>4</sub> on external surface removed from ventroexternal carina. Ratio of distances between trichobothria *est-dst/esb-est* on fixed finger 1.17/1.02.

*Legs*: Two smooth pedal spurs. Ungues stout, unguicular spine well developed. Tarsus III: 6 stout median ventral spinules visible (tarsi are damaged) terminating in one pair of stout spinules.

**DESCRIPTION OF FEMALE (based on specimen NHMW 21.950/1):** *Coloration* (Fig. 5–6): Overall color dark orange-brown, carapace and chelae darkest; chelicerae and telson orange-brown, genital operculum, pectines and sternites yellow-orange; internal carinae of pedipalp femur brown-black;

chelal digital and ventroexternal carinae red-black; dorsal carinae of metasoma dark brown. Sternum, genital operculum orange. No discernable patterns present.

*Carapace*: Overall surface smooth with very fine granulation, interocular area shiny in appearance. Anterior and posterior margins straight. Two pairs of lateral eyes, anterior eye much larger. Median eyes medium sized, AE/PE ratio = 0.62. Slight median furrow on posterior 1/4.

*Mesosoma*: Tergites smooth with very fine granulation. Carinae of tergite VII obsolete. Sternites smooth with no carinae on sternite V. Spiracles are ovoid.

*Metasoma*: Carinae on segments I–IV: dorsal rounded with scattered granulation; dorsal lateral weak on I–IV; lateral weak on I–IV; inferior lateral weak on I–III and slight trace on segments IV; inferior median nearly obsolete on I and weak on segments II–IV. Carinae: Segment V: dorsal lateral weak, rounded and slightly rough; lateral obsolete; inferior lateral rough and granulate; inferior median rounded and irregularly granulate. Dorsal groove on metasomal segments is deeper on anterior  $\frac{1}{2}$  of segments I–III, anterior  $\frac{1}{3}$  of segment IV, and one-half of segment V. Dorsal lateral surface is rounded with scattered granulation; lateral granulation weak to obsolete; intercarinal spaces smooth on I–IV, scattered small granules on V.

*Telson*: Vesicle moderately swollen both laterally and dorsally (Fig. 17), smooth, and with stripes of dark brown flank both sides of the vesicle, with two rows of dark brown on dorsal surface. Aculeus forming short conspicuous curve; 5 pairs of setae at vesicle/aculeus juncture.

*Pectines*: Well developed, teeth are elongate, tooth count 5/5; middle lamellae 3/4. Fulcra are weak; 1–2 minute yellow setae scattered along anterior lamellae; sensorial areas elongated along edge of pectinal teeth, approximately  $\frac{1}{3}$  of tooth surface. Basal piece with slight rounded anterior indentation.

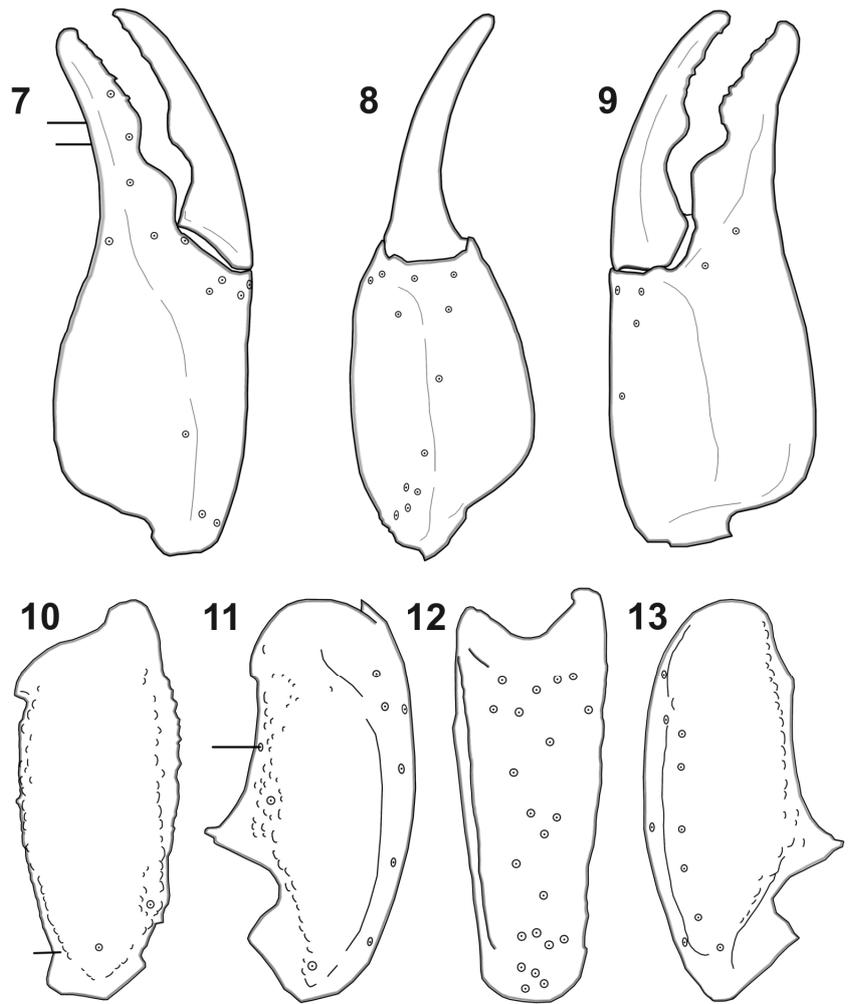
*Genital operculum*: Separated most of length with no genital papillae visible on male lectotype.

*Sternum* (Fig. 15): Pentagonal, wider than long, length/width ratio = 0.82.

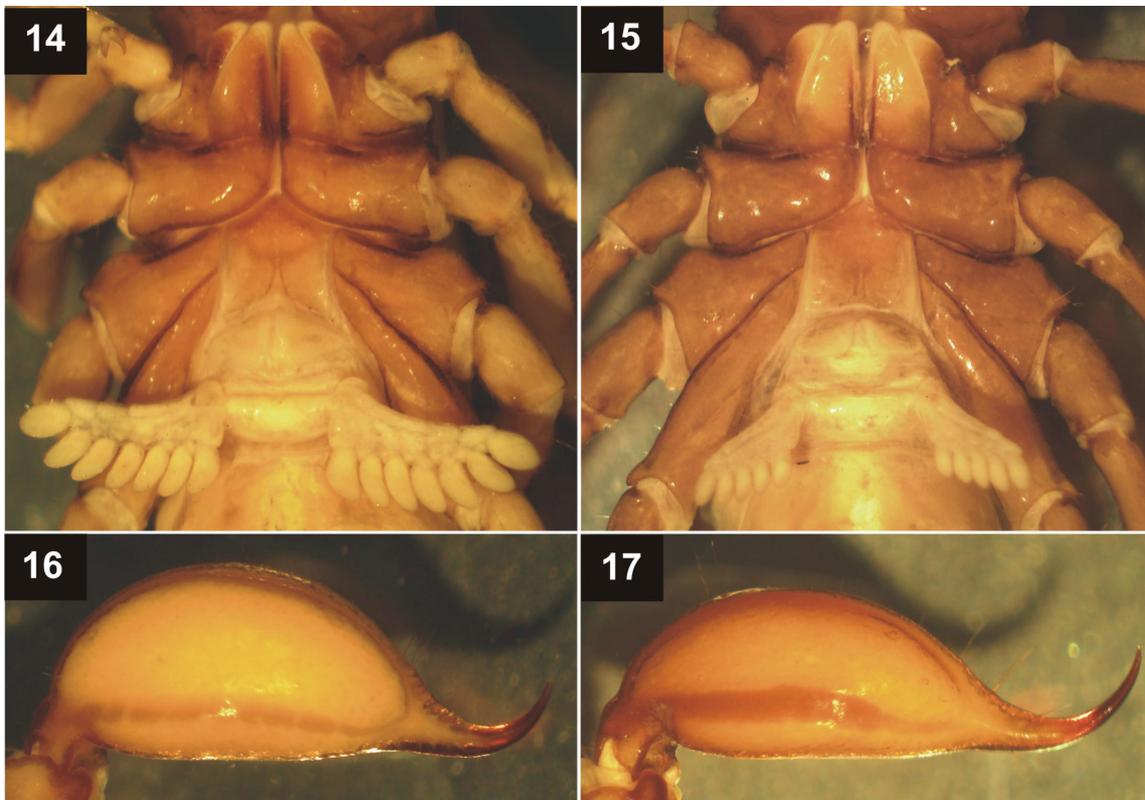
*Chelicerae*: Movable finger: ventral distal denticle extends beyond dorsal counterpart; dorsal edge with two subdistal denticles; ventral edge smooth, no serrulae, covered with heavy brush-like setae. Fixed finger: four denticles configured normally.

*Pedipalps*: Prominent scalloping at chelal finger bases. Femur: dorsal internal are black and crenulate, ventral internal are crenulate to dentate, and external granular and crenulate; dorsal external crenate; dorsal and ventral surfaces covered with small granules; external surface granular; internal face with 8 or more large granules. Patella: dorsal and ventral carinae crenulate; exteromedian rounded, scattered granulation; dorsal and ventral surface with minute granules; DPS acute and well-developed, VPS present as small granule. Chela carinae: digital rounded and generally smooth, slight trace of granulation; sub-digital as two rounded granules; dorsal secondary obsolete; dorsal tertiary very weakly developed; dorsal marginal very rounded, irregularly covered with larger granules; dorsal internal weak, rounded and rough; ventroexternal strong, smooth, extending to external condyle, external to trichobothrium *Et*<sub>2</sub>; ventral median rounded and nearly obsolete; ventral internal moderate, smooth and rounded; external secondary rounded and irregularly granu-

► **Fig. 7–13.** Trichobothrial patterns for *Euscorpius croaticus* Di Caporiacco, 1950, *stat. nov.*, male lectotype.



▼ **Fig. 14–17.** Sternum and telson of *Euscorpius croaticus* Di Caporiacco, 1950, *stat. nov.* **14.** Male lectotype, sternum. **15.** Female NHMW 21.950/1, sternum. **16.** Male lectotype, telson, lateral view. **17.** Female NHMW 21.950/1, telson, lateral view.



late. Chelal finger dentition: median denticle row straight; 5/5 internal denticles, 5/5 outer denticles, 4/4 internal accessory denticles for fixed and movable fingers respectively. Trichobothria pattern: Type C, neobothriotaxic (major additive) on patella. Femur: trichobothrium *d* and *i* equally proximal, and *e*, distal to both on dorsoexternal carina. Patella: ventral series number 6/6 and external series number  $eb = 4/4$ ,  $eb_a = 4/4$ ,  $esb = 2/2$ ,  $em = 3/3$ ,  $est = 4/4$ ,  $et = 5/5$ . Chela: Ventral series number 4/4,  $V_4$  on external surface removed from ventroexternal carina. Ratio of distances between trichobothria  $est-dst$  and  $esb-est$  on fixed finger 1.16/1.23.

**Legs:** Two smooth pedal spurs. Ungues slightly elongate, unguicular spine well developed. Tarsus III: 10 stout ventral spinules terminating in one pair of stout spinules.

**COMPARISON TO AN ADDITIONAL FEMALE:** Another female specimen (NHMW 21.950/2) from Biserujka Cave, Krk Island, Croatia was used for comparison. This female is larger in overall body length, and has a longer metasoma than the female 21.950/1, but the mesosoma is shorter. NHMW 21.950/2 is also lighter in overall coloration but retains dark carinae. Chelae of 21.950/2 are longer and thinner than those of the 21.950/1. Ratio of distances between trichobothria  $est-dst$  and  $esb-est$  on one of the fixed fingers, 1.07. Patella of 21.950/2 are slightly shorter but more robust than 21.950/1. Granulation is similar in both females. The dorsal groove on metasomal segments are deeper on 21.950/1. The pectines are slightly more prominent on the 21.950/1, even though both exhibit identical pectine tooth counts (5/5) and middle lamellae counts (2/2). Two additional juvenile females from Biserujka Cave (NHMW 21.950/3 and VF-0824) both have 6/6 pectinal teeth.

**NOTE ON SYNTYPE SERIES:** Nine syntypes are listed in the original description but eight of those are missing from MZUF (Bartolozzi *et al.*, 1988; S. Whitman, pers. comm.) where most of Di Caporiacco's *Euscorpius* types are deposited. Di Caporiacco (1950: 215) listed five specimens from Mali Halan ("Mali Halam") (four males 35–39 mm long and a female of 38 mm), two specimens from Vrbas, Bosnia (a 29 mm male and 28 mm female), and two specimens from Trebevic, Bosnia (immediately south of Sarajevo) (a 33 mm male and 30 mm female). Di Caporiacco (1950) recorded the number of pectinal teeth as 7/7 for one female from Vrbas and three males from Mali Halan, 6/6 for a male and female from Mali Halan, 8/9 in males from Vrbas and Trebevic, and 6/7 for a female from Trebevic. All syntypes had 22 trichobothria on the external surface of pedipalp patella (i.e.  $et=5$ ,  $em=3$ ), and the number of trichobothria on ventral surface of pedipalp patella was usually 6/6; except 5/6 for a female from Trebevic, 6/7 for a male from Trebevic, and 6/7 for a male from Mali Halan. Since the trichobothria scores and pectine tooth counts recorded by Di Caporiacco (1950) are similar to those of the *E. croaticus* **stat. nov.** lectotype and the females from Biserujka Cave, we find it reasonable to assign the four missing syntypes from Mali Halan (which are, technically, paralectotypes) to *E. croaticus* **stat. nov.** The missing Bosnian specimens, however, have slightly higher pectine tooth counts, so we do not consider them to be *E. croaticus* **stat. nov.**

**VARIATION:** Of the five studied specimens and all four additional syntypes (paralectotypes) from Mali Halan (Di Caporiacco, 1950), all have 22 trichobothria on external surface of pedipalp patella (i.e.,  $et=5$ ,  $em=3$ ) and 5 to 6 trichobothria on

the ventral surface of pedipalp patella (except one lost paralectotype male with 6/7). Pectine tooth counts are as follows: males 7/7 (3), 6/6 (1); females 6/6 (2), 5/5 (3). In addition to the syntype series, Di Caporiacco (1950) also mentioned five specimens from Velebit Mts. listed by Hadži (1930) as "*E. germanus mesotrichus*" (an unavailable name): two males with 7/7 pectinal teeth, two females with 6/6, and one female with 6/5; number of ventral trichobothria was 5 to 6.

**GEOGRAPHICAL DISTRIBUTION** (Fig. 1): CROATIA: Velebit Mountains and Biserujka Cave, Island of Krk.

## Discussion

In this study, we used DNA barcodes to assess the phylogenetic position of *Euscorpius germanus croaticus* Di Caporiacco, 1950. Based on our results (Fig. 2), this taxon does not appear to be related to *E. germanus*, and forms a separate, species-level clade. This clade, based on DNA marker data alone, appears to fall inside subgenus *Alpiscorpius*. Indeed, *Euscorpius croaticus* **stat. nov.** resembles species of subgenus *Alpiscorpius* (Gantenbein *et al.*, 1999) in having 5 to 6 trichobothria on the ventral surface of the patella,  $em=3$  trichobothria on the external surface of the patella, and reduced metasomal carination. At the same time, *E. croaticus* **stat. nov.** has a number of unique morphological characters (Table II), as already indicated by Gantenbein *et al.* (2000).

Di Caporiacco's placement of *E. croaticus* in *E. germanus* was based solely on the presence of  $em = 3$  trichobothria. We know today, however, that this character has independently evolved at least two more times in genus *Euscorpius* (Fet, 2000; Fet *et al.*, 2003; Fet & Soleglad, 2007). Other *Euscorpius* lineages also possess  $em=3$ , which, according to molecular data for *Euscorpius carpathicus* (L., 1767) from Romania (Fet *et al.*, 2003) and a Bulgarian population from Trigrad, Rhodope Mountains (Fet & Soleglad, 2007), could be a result of convergence (Fig. 2). The population from the Rhodope Mts. was erroneously referred to as *E. germanus croaticus* (Fet, 1993), but recent multilocus data (Parmakelis *et al.*, unpublished data) indicate that it actually represents an independent new species. This species is represented in our current barcoding study by a nearby population (VF-0819) from Kovatchevitsa, Bulgaria, which, however, has  $em=4$ . Cases of independent reduction from  $em=4$  to  $em=3$ , have been observed in other *Euscorpius* populations as well (Fet, 2000; Fet *et al.*, unpublished data). The same trend was observed in reduced metasomal carination.

Subgenus *Alpiscorpius* Gantenbein *et al.*, 1999 (type species *E. germanus*) is morphologically quite distinct from the other subgenera currently recognized in Euscorpiinae and can be defined by the following major diagnostic characters: (1) number of external median trichobothria on pedipalp patella ( $em$ ) is 3 (rather than 4 in most *Euscorpius* s.str.); (2) Dorsal Patellar Spur (DPS) is not well developed, but instead very reduced (Soleglad & Sissom, 2001: Fig. 149, 150); (3) the telson vesicle of sexually mature males is not symmetrically swollen from a lateral aspect, but swollen more at the base, tapering towards the elongated aculeus which lacks any trace of a subaculear tubercle (as defined by the subaculear setal pair) (Soleglad & Sissom, 2001: Figs. 179, 180); (4) ventral median spinule row of leg tarsus does not terminate in a pair of well developed spinules, but instead terminates in an offset single spinule (Soleglad & Sissom, 2001: Table 6). Of

**Table II. Morphometric ratios of *Euscorpium croaticus* stat. nov. compared to other *Euscorpium* spp.** Single asterisks (\*) indicate ratios that distinguish *E. croaticus* from subgenera *Euscorpium* s.str and *Alpiscorpium*, ratios with two asterisks (\*\*) distinguish *Alpiscorpium*, and the ratio with three asterisks (\*\*\*) distinguishes all three groups. Abbreviations: Vele, *E. croaticus* lectotype male, Velebit Mts., MZUF; Bis-A, Biserujka, female NHMW 21.950/1; Bis-B, Biserujka, female NHMW 21.950/2; *E. gamma*: EgBO, Bohinj; EgSO, Soca Valley; *E. mingrelicus*: EmBJ, Mt. Bijela Gora, Montenegro; EmLE, Lebrsnik, Bosnia & Herzegovina; EmTA, Tara River, Serbia; EmDE, Denizli, Turkey; EmKO, Konya, Turkey; Ehad, *E. hadzii*; Eter, *E. tergestinus*; DPS, dorsal patellar spur; D2DE, distance between trichobothrium  $d_2$  and dorsoexternal carina. NOTE: Two specimens were missing parts, hence the missing values.

Ratios	<i>Euscorpium croaticus</i>			Other spp. in subgenus <i>Alpiscorpium</i>							Subgenus <i>Euscorpium</i>	
	Vele	Bis-A	Bis-B	EgBO	EgSO	EmBJ	EmLE	EmTA	EmDE	EmKO	Ehad	Eter
DPS/pat L*	0.131	0.177	0.141	0.111	0.086	0.059	0.056	0.026	0.045	–	0.096	0.108
DPS/D2DE*	0.444	0.611	0.500	0.316	0.278	0.176	0.176	0.067	0.125	–	0.313	0.333
MF L/Met V L	0.985	1.091	1.044	1.360	1.196	1.094	1.082	1.125	–	0.878	1.172	1.030
Pedipalp L/Met L	1.068	1.224	1.074	1.382	1.451	1.224	1.131	1.266	–	1.023	1.189	1.164
Chela L/W*	2.786	3.341	2.907	2.625	2.500	2.487	2.55	2.548	2.606	2.321	2.523	2.404
Chela L/D	3.774	4.893	4.167	3.621	3.704	3.593	3.517	3.435	3.739	3.095	3.469	3.906
Chela W/D	1.355	1.464	1.433	1.379	1.481	1.444	1.379	1.348	1.435	1.333	1.375	1.625
Met I L/W**	0.969	1.217	1.357	0.87	0.864	0.731	0.828	0.696	–	0.818	1.043	1.143
Met II L/W***	1.462	1.545	1.440	1.100	1.105	1.000	1.160	0.905	1.250	0.952	1.400	1.333
Met III L/W	1.429	1.905	1.783	1.381	1.200	1.174	1.231	1.048	1.444	1.100	1.684	1.952
Met IV L/W**	1.875	2.100	2.286	1.550	1.526	1.500	1.423	1.350	1.722	1.421	2.111	1.909
Met V L/W	2.393	3.300	2.957	2.381	2.429	2.409	2.346	1.905	2.778	2.158	3.222	3.300
Met I W/MF L	0.485	0.319	0.394	0.338	0.361	0.448	0.439	0.511	–	0.611	0.338	0.412
Met II W/MF L	0.394	0.306	0.352	0.294	0.311	0.414	0.379	0.467	–	0.583	0.294	0.353
Met III W/MF L	0.424	0.292	0.324	0.309	0.328	0.397	0.394	0.467	–	0.556	0.279	0.309
Met IV W/MF L	0.364	0.278	0.296	0.294	0.311	0.379	0.394	0.444	–	0.528	0.265	0.324
Met V W/MF L	0.424	0.278	0.324	0.309	0.344	0.379	0.394	0.467	–	0.528	0.265	0.294

these four characters, only (1) is present in *E. croaticus*, while (2) to (4) in this species do not conform with all other *Alpiscorpium* taxa. In addition, *Euscorpium croaticus* stat. nov. differs from *Alpiscorpium* species by possessing more elongate chelae and longer metasomal segments, especially segments I and IV. Useful diagnostic ratios for these features include: DPS/patella L, chela L/W, metasoma segment I L/W, met II L/W, and Met IV L/W (Table II). *Euscorpium croaticus* also differs from sympatric Balkan forms of *Alpiscorpium* (the “*Euscorpium mingrelicus* complex”) by having the ratio of distances between trichobothria *est-dst* and *esb-est* on fixed finger of chela ranging from 1.02 to 1.23. In the “*mingrelicus* complex”, this ratio ranges 1.5 to 3.0 (Bonacina, 1980; Fet, 1993).

Therefore, the morphology of *E. croaticus* does not conform with that of subgenus *Alpiscorpium* as currently defined. At this moment, we choose not to assign *E. croaticus* to any existing subgenus, as its phylogenetic position warrants further investigation.

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