

ALPISCORPIUS LIBURNICUS SP. N.
WITH A NOTE ON THE "ALPISCORPIUS
CROATICUS GROUP" (SCORPIONES:
EUSCORPIIDAE) IN CROATIA

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Alpiscorpius liburnicus sp. n. is described from Krk Island, Croatia, as the first cave dwelling scorpion species in the genus. Clear differences from the genetically closest epigeal species were found in metasomal morphometry. Its delineation is supported phylogenetically based on mitochondrial (*COI* and *16S rRNA*) and nuclear (*ITS1*) markers. In the barcode fragment of the mitochondrial cytochrome oxidase subunit 1 gene (*COI*), the uncorrected pairwise genetic distances to other known taxa in "*Alpiscorpius croaticus* group" range between 4.2 and 4.8 %. The first molecular evidence of the species' presence on the coastal part of Mt. Velebit implies its potential distribution in the whole of the eastern Adriatic coastal area, roughly from Rijeka City area to the source of the Zrmanja River. The new species is sympatric with common epigeal taxa of the "*Euscorpius tergestinus* group" and with the synanthropic *E. italicus* in Mediterranean habitats. The phylogenetic relationships within the "*A. croaticus* group" are estimated. Localities of epigeal *A. croaticus* are restricted to the continental side of southern and southeastern parts of Mt. Velebit. There it is sympatric and syntopic with the smaller *A. omega* in karst beech forests, with an extrazonal enclave in the Velika Paklenica canyon area on the southern slopes of the mountain. The third taxon in the group is known at the moment from a single locality in the middle part of Mt. Velebit, where it is also syntopic with *A. omega* in a beech forest near limestone cliffs.

Key words: taxonomy, phylogeny, mitochondrial and nuclear molecular markers, cave dwelling species, distribution, habitats

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Alpiscorpius liburnicus sp. n. je opisan s otoka Krka na temelju razlika u morfometrijskim značajkama metasome u odnosu na genetički srodnu vrstu *A. croaticus* (Di Caporiacco, 1950). Status ove prve špiljske vrste roda *Alpiscorpius* je podržan filogenetskim analizama temeljenim na zasebnim mitohondrijskim (*COI* i *16S rRNA*) i jezgrinim (*ITS1*) biljezima, kao i opsegom nekorigiranih udvojenih genetičkih udaljenosti u DNA barkod regiji mitohondrijskog gena za podjedinicu 1 citokrom oksidaze (*COI*) koji, prema ostalim svojstama "*Alpiscorpius croaticus* skupine", iznosi 4,2 do 4,8%. S prvom molekularnom

potvrdom ove vrste u jednoj špilji u obalnom dijelu Velebita njena rasprostranjenost se očekuje i u drugim špiljama istočne obale Jadrana od šireg područja Rijeke do izvorišta Zrmanje. Nova vrsta je simpatrička s nadzemnim vrstama iz "*Euscorpius tergestinus* skupine" i sinantropnom vrstom *E. italicus* u mediteranskim staništima. Utvrđeni su filogenetski odnosi unutar "*A. croaticus* skupine". Nalazi vrste *A. croaticus* su ograničeni na krške bukove šume kontinentalne strane južnog i jugoistočnog Velebita, gdje je utvrđena na istoj lokaciji s manjom vrstom *A. omega*, uz izdvojenu populaciju na južnim padinama Velebita u kršu uz kanjon Velike Paklenice. Treća svojta iz iste skupine je trenutno poznata samo s jednog lokaliteta na srednjem Velebitu, gdje je također nađena u bukovoj šumi ispod okomitih vapnenačkih stijena uz vrstu *A. omega*.

Ključne riječi: taksonomija, filogenija, mitohondrijski i jezgrični molekularni biljezi, špiljske vrste, rasprostranjenost, staništa

INTRODUCTION

The story about the identification of a scorpion first found by Austrian zoologist Erhard Christian in Vitezovića Cave (today Biserujka Cave) on Krk Island (Croatia) in 1978, and published later as a finding of *Euscorpius germanus* (C. L. Koch) (POTOČNIK, 1983), began 40 years ago. However, those cave specimens are morphologically similar to another taxon, the Croatian endemic described as *Euscorpius germanus croaticus* by DI CAPORIAMCO (1950) from Mali Halan, Mt. Velebit (Croatia), Mt. Trebević near Sarajevo and the surroundings of the Vrbas River (Bosnia and Herzegovina). GANTENBEIN et al. (2000), examining syntypes in museum collections, found only the specimen from Mali Halan and designated it as a lectotype, and suggested a restriction of its range to Croatia alone. GRAHAM et al. (2012) elevated it to species level as *Euscorpius croaticus*, **comb. n.** concluding that it does not belong to the subgenus *Alpiscorpius* Gantenbein, Fet, Largiadè & Scholl, 1999, as it possesses three distinct morphological traits known at that time only in *Euscorpius* s.l.. Two specimens from the Biserujka Cave deposited in the Arachnoidea Collection at the Natural History Museum Vienna (NHMW), Austria, were described by GRAHAM et al. (2012) as females belonging to the same species as the male lectotype of *E. croaticus* from Mt. Velebit, and thus the DNA barcode of the VF-0824 specimen from Biserujka was temporarily assigned to *E. croaticus*. FET et al. (2016) suggested that the phylogenetic position of this taxon should lie within *Alpiscorpius*. KOVAŘÍK et al. (2019) elevated *Alpiscorpius* to the genus level, but did not include the enigmatic *E. croaticus* into the genus. PODNAR et al. (2021) referred to the lectotype from Mt. Velebit as *Alpiscorpius croaticus* (Di Caporiamco, 1950) **comb. n.**, and found molecular differences between the Velebit sample and the Krk Island sample (Acr1 = *A. cf. croaticus*) within the range of interspecific divergence for *Alpiscorpius* species (ŠTUNDOVÁ et al., 2019; KOVAŘÍK et al., 2019). After the review of morphologically cryptic *Alpiscorpius* taxa in the Alps and the westernmost Dinarides, it seems that identification without genetic markers is possible only by using a combination of different traits including karyotype "races" (Kovařík et al., 2019), as findings of morphological differences, necessary for an accurate species description, did not seem likely. However, data from GRAHAM et al. (2012), as well as the morphological examination of the first male from Biserujka Cave, have opened the door to the morphological classification of species in the "*A. croaticus* group". With additional new findings from Mt. Velebit, together with data obtained from the old material deposited in the Collection of Scorpiones and Pseudoscorpiones at the Croatian Natural History Museum (CNHM), Zagreb, Croatia and fresh samples in the Scorpion Collection of the Croatian Biospeleological Society (CBSS) in Zagreb, Croatia, we present new data on the taxonomy, diversity, variability and distribution of the "*A. croaticus* group".

MATERIAL AND METHODS

A total of 15 specimens of the “*A. croaticus* group” are examined (five *A. liburnicus* **sp. n.**, eight *A. croaticus*, two *Alpiscorpius* sp.: 13 morphologically, 9 morphometrically, 8 molecularly) from seven locations in Croatia (Tab. 1, Fig. 11:1-7), seven of which are new findings, made from 2018 to spring 2022. Apart from the finding in Biserujka Cave (1) near Rudine on Krk Island, all findings are from the Mt. Velebit area, including the finding of a third instar nymph in Markova špilja Cave (2), Seline, near the coast below the Velika Paklenica canyon. In support of the published data on the lectotype of *A. croaticus* described in GRAHAM *et al.* (2012), we examined two more specimens from the *locus typicus* - Mali Halan pass (3), four specimens from Paklenica National Park: Velika Paklenica and Bojin kuk (4, 5), and one found under Crnopac peak (6), south-eastern part of Mt. Velebit. In this study, together with the diagnosis and morphological overview of *Alpiscorpius liburnicus* **sp. n.**, we took only some morphological data (Tab. 2) of *A. croaticus* into consideration, necessary for the distinguishing of a new species and discussion about the differential traits of the genus *Alpiscorpius* (GANTENBEIN *et al.*, 2000; GRAHAM *et al.*, 2012; KOVAŘÍK *et al.*, 2019; TROPEA, 2021); other data will be published with the description of a third, yet unnamed taxon from the group, found below Filipov kuk (7) Baške Oštarije, in the middle part of Mt. Velebit (wrongly attributed to *A. croaticus* in PODNAR *et al.*, 2021: Fig 9., Tab. S1). Sequences of two *A. omega* Kovařík, Štundlová, Fet & Šfáhlavský, 2019 and one *Euscorpius tergestinus* (C.L. Koch, 1837) were used as outgroups.

Trichobothrial notation and designations follow VACHON (1974), SOLEGLAD & SISSOM (2001) and TROPEA (2021), while terminology and measurements follow STAHNKE (1971), HJELLE (1990), SISSOM (1990) and TROPEA *et al.* (2014). New specimens studied herein were first preserved in 96% ethanol and those from the old collection (V. Paklenica, from 1911) were dry in the past, but all specimens were finally conserved in 75% ethanol. Measurements (in mm) were taken with micrometric ocular lens applied to an XTL-3400 D stereomicroscope with a digital camera, and photographed using DinoLite, Capture 2.0. Only adult specimens were measured. Granulation of metasoma and other morphological traits were observed using the same stereomicroscope.

In the genetic part of the study, total genomic DNA was extracted from single legs using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma) according to the manufacturer's protocols and eluted in 100 µl of Elution Solution. A partial region of the mitochondrial (mt) *cytochrome c oxidase subunit 1* gene (*COI*, the DNA barcode region, HEBERT *et al.*, 2003) was amplified in all specimens, and a fragment of the mitochondrial *16S rRNA* gene (*16S*) as well as the internal transcribed spacer 1 region (*ITS1*) of the nuclear ribosomal rRNA gene cluster were amplified in four specimens (Tab. 1) using PCR primers and conditions described by PODNAR *et al.* 2021. Sequencing of PCR products using amplification primers was conducted at MacroGen Europe sequencing services (Amsterdam, The Netherlands), *COI* sequences were deposited in BOLD, and *16S* and *ITS1* sequences were submitted to GenBank (BOLD accession codes and GenBank accession numbers are given in Tab. 1).

Sequences were aligned using MAFFT version 7 (KATOH & STANDLEY, 2013) online service. Phylogenetic relationships within the “*A. croaticus* group” were reconstructed using phylogenetic tree-building as well as the phylogenetic network approach. The Bayesian phylogenetic analysis was conducted using MrBayes (v.3.2.7a, HUELSENBECK & RONQUIST, 2001; RONQUIST & HUELSENBECK, 2003) on concatenated mitochondrial and nuclear sequences listed in Tab. 1, whereby gap positions in *ITS1* sequences were coded

as matrix of binary characters. Data set was partitioned by gene and by *COI* codon position and the optimal model of sequence evolution for each partition was selected under the Bayesian information criterion (BIC) using jModelTest2 (DARRIBA *et al.*, 2012). The run settings were the same as described by PODNAR *et al.* (2021), while *Euscorpius tergestinus* was used as an outgroup. Median-joining (BANDELT *et al.* 1999) and the 95% statistical parsimony network (TEMPLETON *et al.*, 1992) of the “*A. croaticus* group” *COI* haplotypes were constructed by using PopART v. 1.7 (LEIGH & BRYANT, 2015) and TCS v1.21 (CLEMENT *et al.*, 2000) software, respectively.

Species delimitation based on a DNA barcode fragment of the mt *COI* gene was carried out by using two species delineation approaches: Automated Barcode Gap Discovery (ABGD) analysis (PUILLANDRE *et al.*, 2012) and Assemble Species by Automatic Partitioning (ASAP, PUILLANDRE *et al.*, 2021). Five newly obtained *COI* sequences (Tab. 1) were added to the ABGD data set used by PODNAR *et al.* (2021) and the ABGD analysis of the resulting data set was conducted using the web server (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>) under the settings described by ŠTUNDLOVA *et al.* (2019). The ASAP analysis of the same data set was also run on the web server (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html#>) under default settings and by using a simple distance substitution model. The ranges of intra-and inter-clade uncorrected pairwise distances (p-distances) for *COI* sequences within the “*A. croaticus* group” were calculated with MEGA version 11 (TAMURA *et al.*, 2021), treating missing data with pairwise deletion options.

Abbreviations: n – the number of samples, Dp – pectinal teeth; DPS – dorsal patellar spur; Pv – patella ventral trichobothria; Pe – patella external trichobothria; *et* – external terminal; *est* – external subterminal; *em* – external median; *esb* – external suprabasal; *eb_a* – external basal; *eb* – external subbasal; V₁₋₅ – trichobothria series of the chela (pedipalp manus) ventral surface, last often proximal external; Et₁, Et₂ – two trichobothria of Et series on the chela external surface close to V₁ carina, first distal on the ventral surface; chela fixed finger trichobothria: *et* – proximal, *est* – in the middle, *dsb* – distal; V₁ – ventroexternal carina of the chela (between the external and ventral surfaces); m – male; f – female; L – length; W – width; HR = Croatia, SLO = Slovenia, BiH = Bosnia and Herzegovina.

Museum and collection acronyms: MZUF – Museo Zoologico “La Specola”, Sezione del Museo di Storia Naturale, University of Florence, Italy; NHMW – Naturhistorisches Museum Wien, Austria; CNHM – Hrvatski prirodoslovni muzej (Croatian Natural History Museum), Collection of Scorpiones and Pseudoscorpiones, Zagreb, Croatia; CBSS SC – Hrvatsko biospeleološko društvo (Croatian Biospeleological Society, Scorpion collection), Zagreb, Croatia; VF – private collection of V. Fet, USA;

RESULTS

In detailed descriptions of the Biserujka Cave specimens and after the comparison with the male lectotype in GRAHAM *et al.* (2012) we found morphometric differences between *E. croaticus* from Mali Halan, Mt. Velebit and the two females from Biserujka Cave (Tab. 2). These differences were also visible in the first male specimen collected in Biserujka Cave and deposited in the Croatian Natural History Museum (CNHM) collection, therefore we gained morphological support (Fig. 1 and Fig. 2) for the description of a new, genetically separated species. In comparison to the sample of *A.*

Tab. 1. List of specimens used in the study, with species assignment (according to the results of this study): Collection inventory numbers (ID); Localities; Sample codes (ID); BOLD (COI, DNA barcode region), and GenBank (16S and ITS1) sequence accession numbers. Abbreviations of references (Ref). A = present study, B = PODNAR *et al.*, 2021, C = GRAHAM *et al.*, 2012, D = PARMAKELIS *et al.*, 2013. For localities 1 - 7, see map in Fig. 11.

| Taxon | Collection ID | Locality | Sample ID | Accession number | | | Ref |
|---|--|---|-----------|-----------------------|----------|----------|-----|
| | | | | COI | 16S | ITS1 | |
| <i>Alpiscorpius croaticus</i> | CNHM 837 | (3) HR: M. Halan, Velebit Mt. | CROB1006 | CROSS001-22 | OP538654 | OP538658 | A |
| | CNHM 838 MZUF 5580 | HR: M. Halan, Velebit Mt. | CROB1008 | CROSS002-22 | - | - | A |
| | CNHM 19 | HR: M. Halan, Velebit Mt. | - | - | - | - | C |
| | CNHM 69_1 | (4) HR: V. Paklenica, Velebit Mt. | - | - | - | - | A |
| | CNHM 69_2 | HR: V. Paklenica, Velebit Mt. | - | - | - | - | A |
| | CNHM 777 | HR: V. Paklenica, Velebit Mt. | - | - | - | - | A |
| <i>Alpiscorpius liburnicus</i> sp.n. | CBS5 SC37 | (5) HR: Bojin kuk, Veliko Rujno, Velebit Mt. | CROB704 | CROSS003-22 | OP538655 | OP538659 | A |
| | CNHM 257 NHMW21.950/1 NHMW21.950/2 VF0824 | (6) HR: Crnopac, Velebit Mt. | CROB1278 | CROSS004-22 | OP538656 | OP538660 | A |
| | CBS5 SC26 | (1) HR: Biserujka Cave, Krk isl. | CROB220 | CROSC038-21 | OK491402 | OK491455 | B |
| | | HR: Biserujka Cave, Krk isl. | - | - | - | - | C |
| | | HR: Biserujka Cave, Krk isl. | - | - | - | - | C |
| | | HR: Biserujka Cave, Krk isl. | VF0824 | AMSCO082-10/HIM418306 | - | - | C |
| <i>Alpiscorpius sp.</i> (cf. <i>croaticus</i>) | CNHM 250 CNHM 778 | (2) HR: Markova špilja Cave, Selina, Starigrad Paklenica, Velebit Mt. | CROB1281 | CROSS005-22 | OP538657 | OP538661 | A |
| | CNHM 351 | (7) HR: Filipov kuk, Baške Oštarije, Velebit Mt. | CROB 227 | CROSC037-21 | OK491406 | OK491459 | B |
| | - | HR: Kalnik Mt., Hrvatsko Zagorje | CROB580 | CROSC019-21 | - | - | A |
| <i>Euscorpius tergestinus</i> | - | SLO: Sovitce, Haloze | FEG1 | GACO2280-19/KC215717 | KC215633 | KC215888 | D |
| | CNHM 232 | HR: Trstenik, Čičarija Mt. | CROB178 | CROSC079-21 | OK491399 | OK491452 | B |

croaticus, *A. liburnicus* sp. n. has longer than wide metasomal segment I (Fig. 1) and mainly thinner metasomal segment V (Fig. 2).

Tab. 2. Selected traits, measurements and ratios that highlight the differences and variability of the studied *Alpiscorpius* species. All measurements are in mm, only adult specimens are measured. Data of NHMW 21.950/1, NHMW 21.950/2 and MZUF 5580 taken from GRAHAM et al. (2012).

| Taxon | Collection ID | Gender | Carapax length | Chela mov. finger length | Tarsus terminal spinules | Metasoma segment V | | | Metasoma segment I L/W ratio |
|-----------------------------|---------------|-------------|----------------|--------------------------|--------------------------|--------------------|-------|-----------|------------------------------|
| | | | | | | length | width | L/W ratio | |
| <i>A. liburnicus</i> sp. n. | CNHM 257 | male | 4.53 | 4.05 | 1 | 4.28 | 1.32 | 3.25 | 1.15 |
| | NHMW 21.950/1 | female | 4.76 | 4.52 | 2 | 4.17 | 1.19 | 3.5 | 1.28 |
| | NHMW 21.950/2 | female | 4.76 | 4.76 | 2 | 4.17 | 1.43 | 2.92 | 1.28 |
| <i>A. croaticus</i> | MZUF 5580 | male | 4.64 | 4.05 | 2 | 4.4 | 1.69 | 2.6 | 0.94 |
| | CNHM 837 | female | 4.96 | 4.34 | 1 | 4.14 | 1.57 | 2.64 | 0.95 |
| | CNHM 838 | sub. male | | | 1 | | | | |
| | CNHM 19 | female | 4.35 | 3.92 | 2 | 3.59 | 1.28 | 2.35 | 0.65 |
| | CNHM 69.1 | female | 4.15 | 3.94 | 1 | 3.64 | 1.27 | 2.82 | 0.88 |
| | CNHM 69.2 | female | 4.24 | 3.65 | 1 | 3.69 | 1.33 | 2.77 | 0.85 |
| | CNHM 777 | female | 4.36 | 3.92 | 1 | 3.97 | 1.34 | 2.95 | 0.93 |
| | CBS SC37 | male | 4.46 | 4.25 | 1 | 3.44 | 1.16 | 2.96 | 0.96 |
| <i>A. sp. Filipov kuk</i> | CNHM 250 | sub. female | | | 1 | | | | |
| | CNHM 778 | female | | | 2 | | | | |

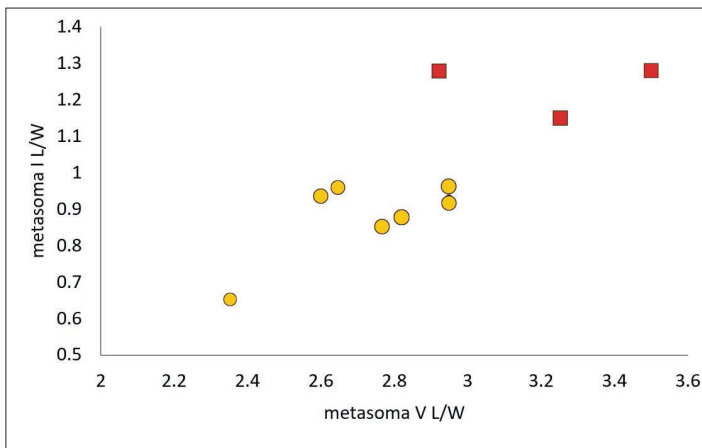


Fig. 1. Bivariate plot showing metasomal segment V length/width ratio (x) against metasomal segment I length/width ratio (y) in *A. liburnicus* sp. n. = ■ and *A. croaticus* = ●.

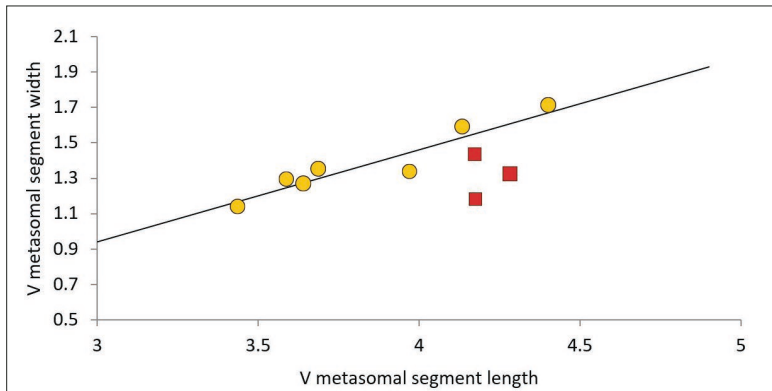


Fig. 2. Bivariate plot showing metasomal segment V length (x) against metasomal segment V width (y) in *A. liburnicus* sp. n. = ■, *A. croaticus* = ●. All measurements are in mm. Regression line for *A. croaticus* sample ($y=0.5214x - 0.6245$; $R^2=0.9278$).

Alpiscorpius liburnicus Tvrtković et Rebrina, sp. n.

<http://zoobank.org/urn:lsid:zoobank.org:act:872B02C8-169D-434F-B4E4-2B3E-739A1AD9>

Euscorpius germanus (C. L. Koch), POTOČNIK, 1983: p. 394; CHRISTIAN & POTOČNIK, 1985: p.13;

Euscorpius croaticus Di Caporiacco, 1950 (partim), GRAHAM *et al.*, 2012: pp. 41-49, description of two females from Biserujka Cave as additional material for the re-description of *E. croaticus* lectotype;

Euscorpius croaticus Di Caporiacco, 1950, FET *et al.*, 2016: VF-0824, 3-6, Fig.2, p.3, Tab. 1, p.6.; KOVAŘÍK *et al.*, 2019; TROPEA, 2021;

Alpiscorpius cf. *croaticus* from the "*A. croaticus* group": candidate species (Acr1), PODNAR *et al.*, 2021: pp.14 – 17, Fig. 7, Fig. 9;

TYPE LOCALITY AND TYPE REPOSITORY: Croatia, Krk Island, Rudine, **Biserujka Cave**, 45.1311°N 14.631°E; 40 m a.s.l., CNHM 257 (holotype), male, conserved in ethanol with left legs 1-3 removed, sample ID CROB 220, 21.9.2018. leg. Irena Grbac and Martina Podnar, Croatian Natural History Museum (Hrvatski prirodoslovni muzej), Zagreb, Croatia; Croatia, Krk Island, **Biserujka Cave**, NHMW 21.950/1, NHMW 21.950/2 (paratypes), two females, 11.8.1990, leg. C. Komposch & P. Schnitter, Natural History Museum Vienna (Naturhistorisches Museum Wien), Vienna, Austria.

OTHER MATERIAL EXAMINED: Croatia, Seline, **Markova špilja Cave**, at the foot of the Velebit Mountain slopes, 10 m a.s.l., 44°16.79'N 15°28.63'E, CBSS SC 26, sample ID CROB 1281, nymph 3rd instar, 20.2.2018, leg. Petra Bregović.

ETYMOLOGY

The species epithet of the newly described, oldest known taxon from the "*A. croaticus* group" refers to the Liburni (Liburnians), an ancient ethnic group that inhabited the coastal region of the northeastern Adriatic with adjacent islands, called Liburnia in pre-Roman and Roman times, before the Croats arrived.

DIAGNOSIS

The diagnosis is based on the Biserujka Cave (Krk Island) population sample, i.e. male holotype described here and two females (paratypes in our study) described in GRAHAM *et al.* (2012).

Medium in size, ranging approximately from 31.4 to 34.4 mm in length, carapace length 4.55 to 4.76 mm, chela movable finger length 4.25 in the male, 4.5 and 4.8 mm in females among the specimens examined. Carapace coloration light brown, tergites and pedipalps with dark brown-black carinae, chela fingers orange-brown, sternites yellowish, lamellae and pectines yellowish-white, chelicerae in freshly caught specimens light yellow, when preserved in ethanol become darker to light orange. Carapace slightly longer than wide, anterior edge of the carapace mostly straight. Chelicerae light yellow, with the dorsal distal denticle of movable finger much smaller than the ventral distal denticle. Metasoma is markedly narrow distally and elongated, metasomal lateral carinae are present. On metasomal segment V both lateral carinae are strongly granulated, ventral median carina is present, with irregular smaller granules, in some cases reduced in distal and proximal parts. Metasomal segment I is longer than wide, metasomal segment V with length/width ratio mostly > 3 or near 3. In the mature male, vesicle (telson) is symmetrically swollen on the proximal and distal side, and the dorsal side is slightly swollen as well. On each side of the telson, dorsolateral and ventrolateral surfaces bear two connected light brown lines in the male, dark brown bands in females. Pectinal tooth count (Pd) is 5/5 and 6/6 in females. Pectinal teeth are larger in the male, anterior lamellae bear numerous long white microsetae. DPS on the internal surface of patella is sharp and well-developed. Trichobothrium *est* on the external surface of the chela fixed finger is distal to the notch, ratio of distances between trichobothria *et-est/est-dsb* ratio close to 1 (about 1.06 – 1.19 in the specimens examined). Trichobothrial pattern on the chela ventral surface is generally 4/4 ($Et_1 + V_{1-3}$). The number of trichobothria on the ventral surface of the patella (Pv) is 6/6; the numbers of trichobothria *et* and *est* on the external surface distally are 5/5 and 4/4, respectively, median *em* trichobothria series is always 3/3. Tarsi bear a single row of elongated spinules on the ventral median surface, with one or two terminal spinules.

DESCRIPTION OF MALE HOLOTYPE

Total length about 33 mm (Fig. 3), carapace length 4.55 mm, chela movable finger length 4.26 mm (Tab. 2). Coloration of the carapace and tergites light brown, and chela pedipalp fingers orange-brown; metasoma light brown-reddish with darker mottling, telson yellow to light brown, legs and sternites yellowish. Carapace slightly longer than wide, anterior edge straight with sporadic very small granules, mostly below the lateral eyes. There are two equal-sized lateral pairs of eyes on each side of the carapace. Median pair is larger than lateral, but still relatively small in size. Chelicerae light yellow, only the apical parts of movable finger denticles reddish. Dorsal distal denticle of the movable finger is much smaller than the ventral distal denticle. Tergites I–VII essentially smooth; tergite VII with shorter carina of small granules in the external distal part on both sides, median carinae lacking. Sternites III–VII smooth and lustrous, lacking lateral and median carinae. Stigmata are small, narrow elliptical. Metasomal segments III, IV and V with lateral dorsal and ventral carinae. Segment V with ventral median carinae, but with smaller and sometimes irregular granulae, reduced in the proximal and distal parts of the segment. Metasomal segment I is longer than

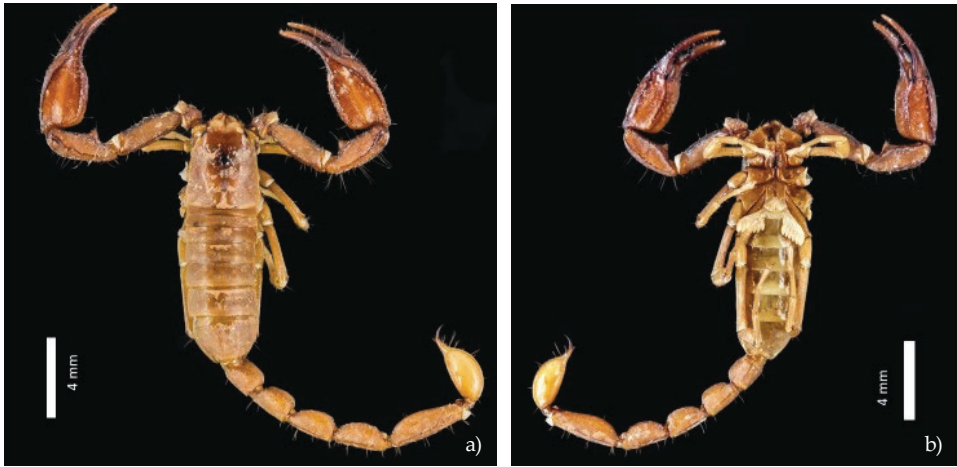


Fig. 3. Dorsal (a) and ventral (b) view of *Alpiscorpius liburnicus* sp. n. male holotype. Photo: Nives Borčić.

wide, metasomal segment V with length/width ratio > 3. Mature male telson vesicle swollen, laterally from all sides including the distal side (Fig. 4) and ellipsoidally elongated, yellow, with the curved aculeus black at the tip. At the ventral base of the aculeus juncture there are three pairs of setae, the middle pair longer than the other two. Pectinal teeth number Pd = 6/7, with large and ellipsoid teeth, middle lamellae 3/4, anterior lamellae with numerous long white microsetae (Fig. 5). Pedipalp carinae blackish-brown and well-developed, along the middle part of the patella external surface there are additional irregular carinae from the distal to the proximal part, traversing the space between and around individual trichobothria in series. Chela movable fingers bear seven rows of denticles with both external and internal denticles. Ratio of distances between the fixed finger trichobothria *et-est/est-dsb* equals 1.15, trichobothrium *est* distal to finger notch. Trichobothrial patterns on the chela ventral surface = 4/5 (Fig. 6a, b): on the right chela $Et_1 + V_{1-4}$ (irregular dislocated trichobothrium between V_1 and V_3),



Fig. 4. Adult male telson lateral view of *Alpiscorpius liburnicus* sp. n. male holotype.



Fig. 5. Left pectines of *Alpiscorpius liburnicus* sp. n. male holotype.

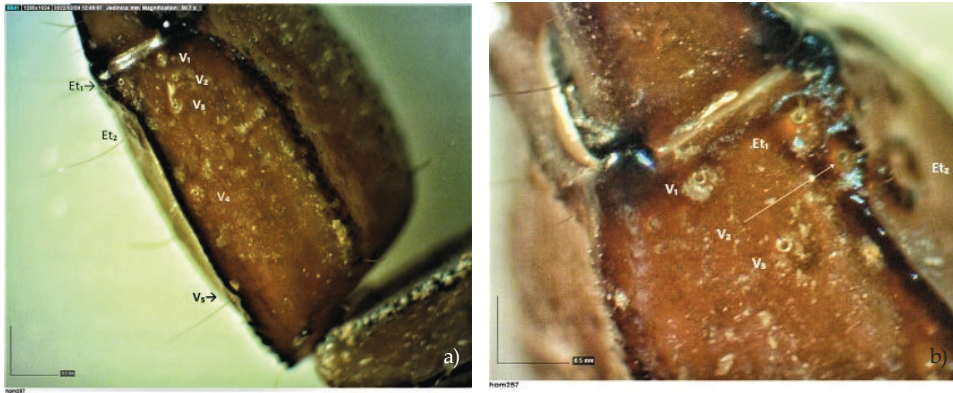


Fig. 6. Ventral chela trichobothria of *Alpiscorpius liburnicus* sp. n. male holotype: (a) Left chela and (b) right chela distal part.

V_5 captured in carina $V1$ from the external side; on the left chela ventral $Et_1 + V_{1-3'}$ (V_2 in carina $V1$ distal between Et_1 and external Et_2 , V_4 lost, V_5 captured in carina $V1$ external). Patellar external trichobothria $eb = 4/4$, $eba = 4/4$, $esb = 2/2$, $em = 3/3$, $est = 4/4$, $et = 5/5$. Patellar ventral $Pv = 6/6$. Legs bear two relatively large acute pedal spurs with black tips. Tarsi (including those of the leg III) bear a single row of six elongated spinules with one terminal spinule on the ventral median surface (Fig. 7a).

GENETIC ANALYSIS

Bayesian phylogenetic analysis revealed three deeply separated lineages within the "A. croaticus group": the well-supported (BPP = 1) monophyletic *A. croaticus* and *A. liburnicus* sp. n. clades, and *A. cf. croaticus* lineage represented by a single specimen (Fig. 8a). The same phylogenetic structure was revealed also in phylogenetic networks.

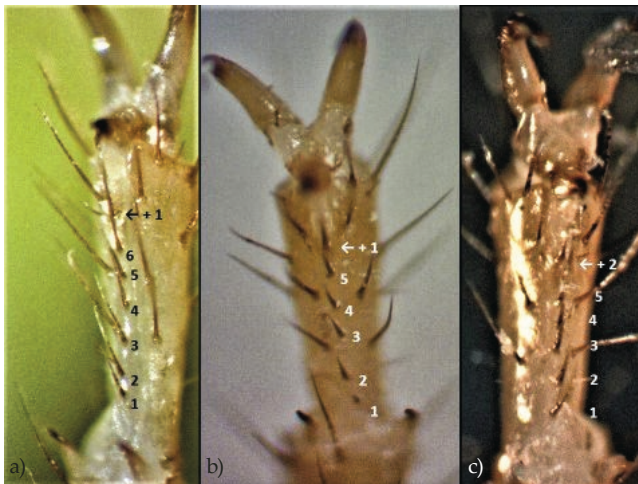


Fig. 7. Tarsus III ventral median spinules in (a) *Alpiscorpius liburnicus* sp. n. male holotype, (b) *Alpiscorpius croaticus* CNHM69.2 from Velika Paklenica, Velebit Mt., (c) *Alpiscorpius* sp. female CNHM 778 from Filipov kuk, Velebit Mt.

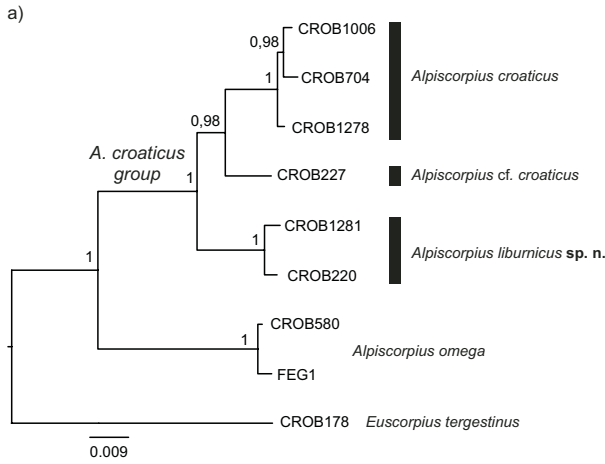


Fig. 8. a) Bayesian phylogram based on the concatenated mitochondrial (mt) (*COI* and *16S*) and nuclear *ITS1* sequences. Numbers at nodes are Bayesian posterior probabilities. The candidate species within “*A. croaticus* group” as delimited by ABGD and ASAP species delimitation methods are depicted by bars on the right side of the phylogram.

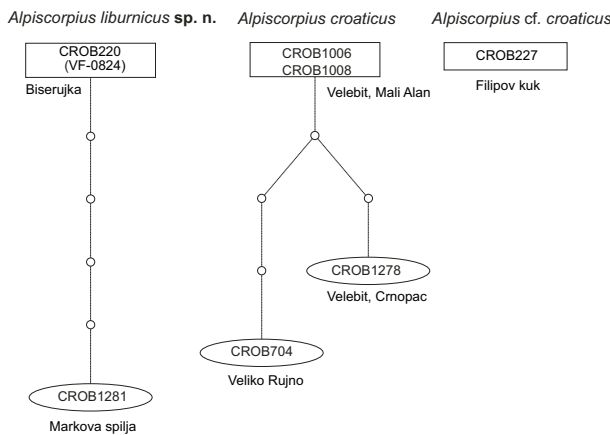


Fig. 8. b) Statistical 95% parsimony network (TCS) based on DNA barcode sequences of “*Alpiscorpium croaticus* group”. Small white circles represent missing intermediates and the size of oval corresponds to haplotypes frequencies.

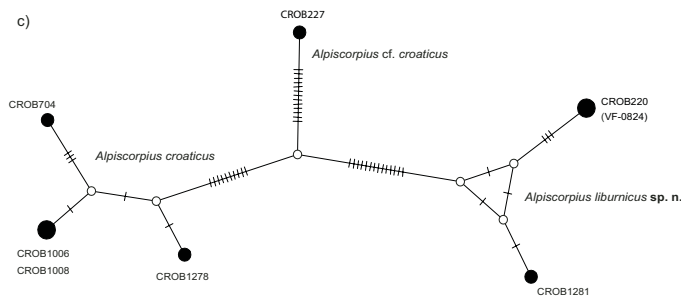


Fig. 8. c) Median-joining network (MJ) based on DNA barcode sequences of “*Alpiscorpium croaticus* group”. Small white circles represent median vectors. Mutational steps are denoted by vertical bars on the branches. The size of a circle corresponds to haplotype frequencies.

In TCS analysis those three groups could not be parsimoniously connected under the 95% confidence level and appear as separate networks (Fig. 8b), while in the MJ network they are well defined and separated by a large number of mutational steps (Fig. 8c).

The members of the “*A. croaticus* group” were delineated into three putative species across all obtained ABGD partitions (Fig. 8a), as well as in the best partition found by ASAP (ASAP-score =4, threshold distance = 0.036). In the second-best ASAP partition (ASAP-score =5.5, threshold distance = 0.038), however, *A. croaticus* and *A. cf. croaticus* samples comprised the same group. The inter- and intraspecific p-distance intervals within the “*A. croaticus* group” are shown in Tab. 3.

Tab. 3. The ranges of uncorrected pairwise genetic distances (p-distances; in percentages) based on DNA barcode fragment of the *COI* gene within and among three tentative species of „*Alpiscorpius croaticus* group” *sensu* PODNAR et al. (2021). Abbreviations: n.d. = no data.

| | <i>A. croaticus</i> | <i>A. liburnicus</i> sp.n. | <i>A. sp.</i> Filipov kuk |
|---------------------------------------|---------------------|----------------------------|---------------------------|
| <i>Alpiscorpius croaticus</i> | 0.6 - 0.8 | | |
| <i>Alpiscorpius liburnicus</i> sp. n. | 4.2 - 4.8 | 0.8 | |
| <i>Alpiscorpius</i> sp. Filipov kuk | 3.7 - 4.0 | 4.3 | n.d. |

RELATIONSHIPS TO MORPHOLOGICALLY SIMILAR SPECIES

Alpiscorpius liburnicus sp. n. is morphologically separated from *A. croaticus* by a combination of a longer than wide metasomal segment I and a narrower metasoma (Tab. 2, Fig. 2) and metasomal segment V with length/width ratio mostly > 3 or near 3 (in *A. croaticus* the ratio is < 3, mean = 2.75). The observed difference in lateral profile of the swollen adult male telson must be proved in a larger sample. From *Alpiscorpius* (*Hadzius*) *karamani* Tropea, 2021, distributed in the eastern Dinarides (TROPEA, 2021), *A. liburnicus* sp. n. is separated on the basis of a longer than wide metasomal segment I, the presence of a carina on the ventral surface of the metasomal segment V, a lower number of trichobothria on the Pv (6 versus 7), a lower number of trichobothria of Pet, and a variable number of terminal spinules in the ventral median row of tarsi.

From the geographically closest but smaller species from the “*Alpiscorpius gamma* group”, *A. omega* Kovařík, Štundlová, Fet & Šťáhlavský 2019, *A. liburnicus* sp. n. differs in the distal position of *est* trichobothrium on the fixed finger, *et-est/est-dsb* ratio of the fixed finger smaller than 1.4, the presence of a median carina on the ventral side of metasomal segment V, longer than wide metasomal segment I, thinner metasomal segment V, well developed DPS, light-coloured chelicera without ornamentation in adults, and Pv = 6 versus 5 (6) in *A. omega*.

KNOWN DISTRIBUTION:

Croatia: Krk Island, Biserujka Cave (Fig. 9) and Seline, Markova špilja Cave (Fig. 10) at the foot of the coastal slopes of Mt. Velebit (Fig. 11: 1 and 2).

HABITATS AND SYMPATRIC/SYNTOPIC SCORPION SPECIES:

The new species is found only in caves within the Mediterranean vegetation belt of pubescent oak (*Quercus pubescens*). Biserujka Cave (JALŽIĆ & PAVLEK, 2013: p. 43-45) is a 110 m long cave visited by tourists, rich in cave invertebrates, for example the endemic cave pseudoscorpion *Neobisium insulare* Beier, 1938. Markova špilja Cave (JALŽIĆ et al., 2022: p. 188-189) is a simple anchialine cave with the dry part only 18 m long and

9 m deep covered with stone blocks and rubble, known as the rare habitat of an endemic cave terrestrial isopod. In epigeal natural habitats around the cave on Krk Island there is only *Euscorpius* cf. *tergestinus* (PODČAR *et al.*, 2021) from the “*E. tergestinus* group”, and in Seline the typical *E. tergestinus* was found near Markova špilja Cave, a common species on the coastal slopes of Mt. Velebit (PODČAR *et al.*, 2021).

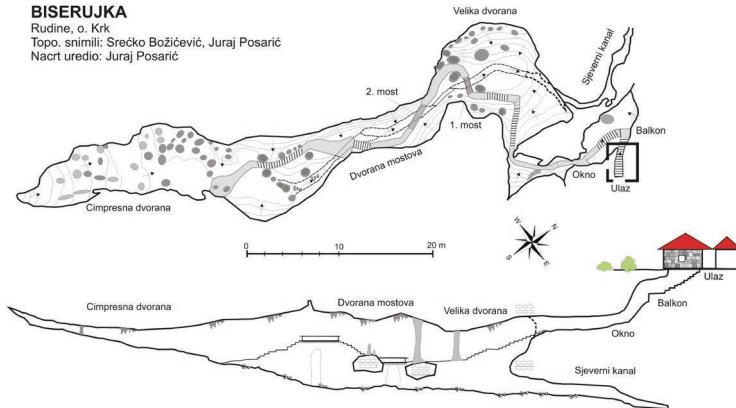


Fig. 9. Biserujka Cave, Rudine, Krk Island. Ground plan and longitudinal section. Taken from JALŽIĆ & PAVLEK (eds.) (2013).

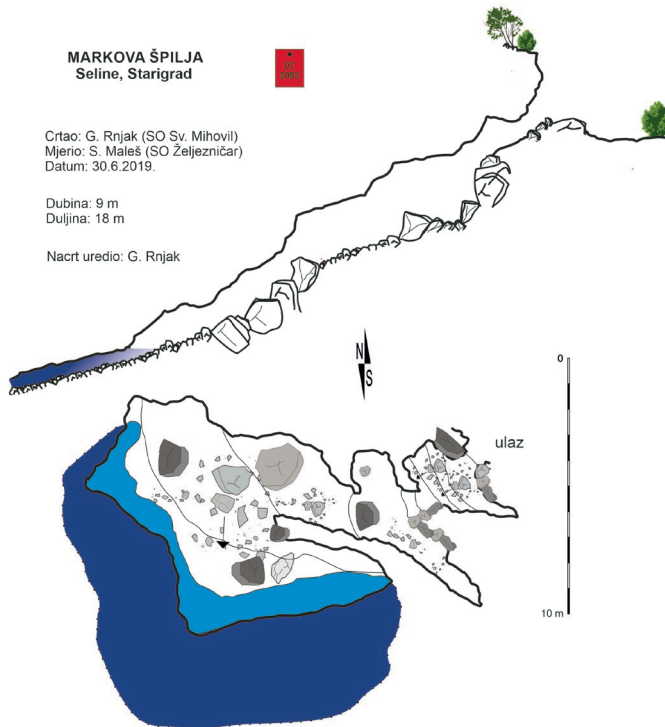


Fig. 10. Markova špilja Cave, Seline, Starigrad/Paklenica. Ground plan and longitudinal section. Taken from JALŽIĆ *et al.* (eds.) (2022).



Fig. 11. Known distribution of “*Alpiscorpius croaticus* group”: *A. liburnicus* sp. n. = ■, *A. croaticus* = ●, *A. sp.* (Filipov kuk) = ▲. Velebit Mountain division after POLJAK (1974): NV = northern part, MV = middle part, SV = southern part, SEV = southeastern part.

DISCUSSION

After the molecular analysis of specimens from the topotype locality of *A. croaticus* designated in GANTENBEIN *et al.* (2000), we depict clear phylogenetic positions of *A. croaticus* and *A. liburnicus* sp. n. within the “*A. croaticus* group” (Fig. 8 a). Interspecific p-distance values within the “*A. croaticus* group” (Tab. 3), with the relation of *A. liburnicus* sp. n. : *A. croaticus* 4.2 - 4.8 %, are larger than or comparable to those obtained for the same gene fragment in the pairwise comparisons of several *Alpiscorpius* species (*A. ypsilon* : *A. omega* 1.9 - 2.8 %, *A. ypsilon* : *A. omikron* 4.7 - 4.9 %, *A. sigma* : *A. omikron* 4.4 %, *A. kappa* : *A. lambda* 2.3 - 3.6 %, *A. germanus* : *A. lambda* 3.7 - 6.0 %, PODNAR *et al.*, 2021).

The first data on scorpions of the “*A. croaticus* group” for Croatia were published in a report on the geographic distribution of scorpions in the Kingdom of Serbs, Croats and Slovenes (HADŽI, 1931: noted in the text, not on the map) under the homonym *Euscorpium germanus polytrichus* Hadži, 1929 (GANTENBEIN *et al.*, 2000), after the identification of scorpions from the Collection of the National Museum in Zagreb by Hadži (today the Croatian Natural History Museum). We found four specimens in bad condition in old museum collection vials including labels with handwritten determinations “*Euscorpium germanus polytrichus* J. Hadži”. We also found the mention of this taxon in an old documentation list of scorpions included in the collection (handwritten list from 21 October 1929). Three polytrichus females (CNHM19, CNHM69a, CNHM69b) from Velika Paklenica (Mt. Velebit) collected around the year 1911 have large DPS, trichobothrium *est* on the chela fixed finger distal of the notch, have the carina with small

granules on metasomal segment V median surface, L/W ratio of metasomal segment V < 3, L/W ratio of metasomal segment I < 0.9: all clear morphological traits of *A. croaticus*. However, we identified one female from Senj (Mt. Velebit) designated polytrichus by Hadži and collected in 1904 as *Alpiscorpius omega*: it has $P_v=6/6$ (common in *A. croaticus*, rare in *A. omega*), but chela fixed finger trichobothrium *est* is in proximal position, the ventral surface of metasomal segment V is without traces of the granules or carina, and it has small to medium DPS.

KOVAŘÍK *et al.* (2019) confirmed that the ventral surface of metasomal segment V is smooth in most of the members of the *Alpiscorpius* “*germanus* complex”, e.g. in *A. omega* and *A. ypsilon* Kovařík, Štundlová, Fet & Štáhlavský, 2019, with granulation found only in *A. sigma* Kovařík, Štundlová, Fet & Štáhlavský, 2019. TROPEA (2021) noted a median carina with few granules in *A. caporiaccoi* Tropea, 2021 and only little granules without delimitation of ventral carinae in *A. karamani* Tropea, 2021. Both the presence of a median carina or the granulation of the ventral surface of metasomal segment V are not distinguishing traits between *Alpiscorpius* and *Euscorpius* Thorell, 1876.

In the description of the lectotype of *E. croaticus*, GRAHAM *et al.* (2012: 46) wrote “Tarsus III: median ventral spinules visible (tarsi are damaged) terminating in one pair of stout spinules” and in the description of a female from the Biserujka Cave, in the additional material (p. 48) it is written: “Tarsus III: ...spinules terminating in one pair of stout spinules.”. In a sample of 13 accessible specimens belonging to this group, only five specimens (38% in our sample: two *A. liburnicus* **sp. n.**, two *A. croaticus* and one *A. cf. croaticus*) possessing one pair of terminal spinules (Fig. 7. c) were found. Male *A. liburnicus* **sp. n.** (Fig. 7. a), one *A. cf. croaticus* and six *A. croaticus* specimens have only one terminal spinule in the row, like in SCHERABON (1987: 119, Abb. 19) and SOLEGLAD & SISSOM (2001: 61, Tab. 6). In some cases, this terminal spinule is displaced from the tentative row, as is sometimes the case with other individual spinules in the row as well. We suppose that the appearance of two terminal spinules is restricted to individuals with damaged tarsi (common in cave-dwelling invertebrates like cave crickets, personal observation), but for the moment this is only speculation. Among the specimens examined, all species in the “*A. croaticus* group” seem to have a variable number of terminal spinules, mostly one, in the row or dislocated, but in some cases two (one pair). DPS is well developed in all taxa of the “*A. croaticus* group”, but very much reduced in other *Alpiscorpius* species (SOLEGLAD & SISSOM, 2001, figs. 149, 150; KOVAŘÍK *et al.*, 2019); however, TROPEA (2021) found well-developed DPS also in *A. (Hadzius) karamani*. Regarding the differences in the lateral shape of the telson between *A. liburnicus* **sp. n.** (Fig. 4) and *A. croaticus* (GRAHAM *et al.*, 2012: 47, Fig. 16), we initially thought that the examined adult males of both species have a different telson shape from the diagnosis of *Alpiscorpius* (KOVAŘÍK *et al.*, 2019). In the diagnosis it is stated: “(telson) ... not symmetrical swollen from lateral aspect, but swollen more at base” – the maximal telson height is prolonged to the basal third. Both *A. liburnicus* **sp. n.** and *A. croaticus* males have telson more symmetrically swollen in the lateral aspect (maximal telson height near the middle). In the diagnosis of the genus *Alpiscorpius* with type species *A. germanus* C. L. Koch, 1837, KOVAŘÍK *et al.* (2019) cited SOLEGLAD & SISSOM (2001: fig. 88, 89, p. 109) in the description of telson, but on Fig. 89 only *E. mingrelicus* s.l., with telson swollen more at the base, was shown as representative of *Alpiscorpius*. Formally, from the diagnosis of the genus *Alpiscorpius* (KOVAŘÍK *et al.*, 2019), the following must be removed: (1) all text about the lateral shape of the telson, (2) “DPS is not well developed”, and (3) “the ventral median spinule row of leg tarsus does not terminate in a pair of spinules”.

We find the unusual variability of trichobothria on the chela ventral aspect in the holotype (Fig. 6 a, b) particularly interesting. Four V trichobothria on the one chela ventral aspect are mentioned by TROPEA *et al.* (2015: Fig. 49) only for *A. mingrelicus* (Kessler, 1874): the holotype of *A. mingrelicus* has five V trichobothria, four on the ventral surface ($Et_1 + V_{1-4}$, V_4 in carina V1!) and V_5 external, instead of 4/4 in the diagnosis and description of this species. Another interesting finding in the current case is a "broken" tentative straight line between V_{1-3} , as for example in *Belisarius* Simon, 1879 (SOLEGLAD & FET, 2003, Fig. 66). At the moment, we can explain this case of variability as the effect of the inbreeding, probably because of the long-term isolation of small populations in distant caves or karst underground units connected only by a local network of fissures and caves. Indication of a presumably higher variability in some traits of *A. liburnicus* sp. n. in comparison to the epigean *A. croaticus* are different ratios of mesosoma and metasoma lengths in two females from Biserujka Cave (GRAHAM *et al.*, 2012: p. 48), but at this moment our sample is too small for any conclusions to be drawn.

According to the current data, *Alpiscorpius liburnicus* sp. n. is a relict scorpion taxon (PODNAR *et al.* 2021) known from only two caves in Croatia, one on Krk Island and the second on the foothills of Southern Mt. Velebit, near the coast in Seline below Velika Paklenica National Park. Unfortunately, the cave fauna of the coastal part of the country between the Rijeka area and the source of the Zrmanja River (Fig. 11) is largely unexplored, and our knowledge about the possible rarity of this species remains undocumented. *Alpiscorpius croaticus*, an epigean species, is restricted to the area south and east of the highest peaks of the southern part of Mt. Velebit (Fig. 11). Another epigean *Alpiscorpius* candidate species (*A. cf. croaticus*) is known from a single location near Filipov kuk, Baške Oštarije, but on the opposite (NW) side of the highest mountain peaks area. *Alpiscorpius liburnicus* sp. n. is the first known cave-dwelling species from the genus *Alpiscorpius* (TROPEA *et al.*, 2014; FET *et al.*, 2016; KOVAŘÍK *et al.*, 2019; TROPEA, 2021). This is not surprising, as at the moment four other relict cave-dwelling species are known within the heterogeneous genus *Euscorpius* (as currently defined) in the Dinaric karst, namely *E. feti* Tropea, 2013, *E. biokovensis* Tropea et Ozimec 2020 (TROPEA & OZIMEC, 2019, 2020), enigmatic *E. studentium* Karaman, 2020 (KARAMAN, 2020) and *E. aquilejensis* (C. L. Koch, 1837) (PODNAR *et al.*, 2021).

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