

# Molecular and taxonomic analyses in troglobiotic *Alpioniscus* (*Illyrionethes*) species from the Dinaric Karst (Isopoda: Trichoniscidae)

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Received 9 December 2018; revised 4 June 2019; accepted for publication 8 June 2019

Species richness of terrestrial isopods is high in caves of the Dinaric Karst, which hosts ~10% of the world's nominal oniscidean troglobionts. The most widespread taxon is the southern European genus *Alpioniscus*, which consists of two subgenera: *Alpioniscus s.s.* and *Illyrionethes*. Before this study, 14 nominal troglobiotic *Illyrionethes* taxa were recorded from the Dinaric Karst. Our molecular analyses using two mitochondrial DNA (16S rRNA and *COI*) fragments and a nuclear gene (*H3*) fragment on all known Dinaric taxa identified three distinct lineages: *strasseri*-, *heroldi*- and *magnus*-lineage. Our results confirmed the validity of most nominal species. The exceptions are *Alpioniscus balthasari*, which consists of two different species including *Alpioniscus iapodicus*, and *Alpioniscus heroldi*, which is paraphyletic with respect to *Alpioniscus bosniensis*. The *strasseri*-lineage was highly supported by all phylogenetic methods used; therefore, we performed a detailed morphological analysis to distinguish and characterize the species of this group. New morphological characters, such as body part ratios, are proposed for future species identification. In addition, we redescribe three known species (*Alpioniscus strasseri*, *Alpioniscus christiani* and *Alpioniscus balthasari*) and describe two new ones (*Alpioniscus hirci* sp. nov. and *Alpioniscus velebiticus* sp. nov.). As a result, 15 nominal species of *Illyrionethes* are currently known from the Dinaric Karst.

ADDITIONAL KEYWORDS: 16S – cave fauna – *COI* – histone *H3* – molecular clock – new species – Oniscidea – phylogeny – terrestrial isopods.

## INTRODUCTION

The Dinaric Karst is considered to be a hotspot of subterranean biodiversity (Culver & Sket, 2000; Sket *et al.*, 2004; Deharveng *et al.*, 2012; Sket, 2012; Simičević, 2017), with nearly 1000 aquatic and

terrestrial subterranean species recorded (Sket, 2012). Such high subterranean biodiversity in the Dinaric Mountains is related to their complex geological history and intensive karstification, enabling multiple colonizations of the subterranean realm (Bilandžija *et al.*, 2013). High levels of habitat fragmentation in subterranean ecosystems often lead to lineage isolations and, potentially, speciations (Gorički & Trontelj, 2006; Trontelj *et al.*, 2007, 2009; Zakšek *et al.*, 2007; Prevorčnik *et al.*, 2010). However,

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[Version of Record, published online 27 August 2019; <http://zoobank.org/urn:lsid:zoobank.org:pub:B90C1747-E672-404D-9464-343467765463>]

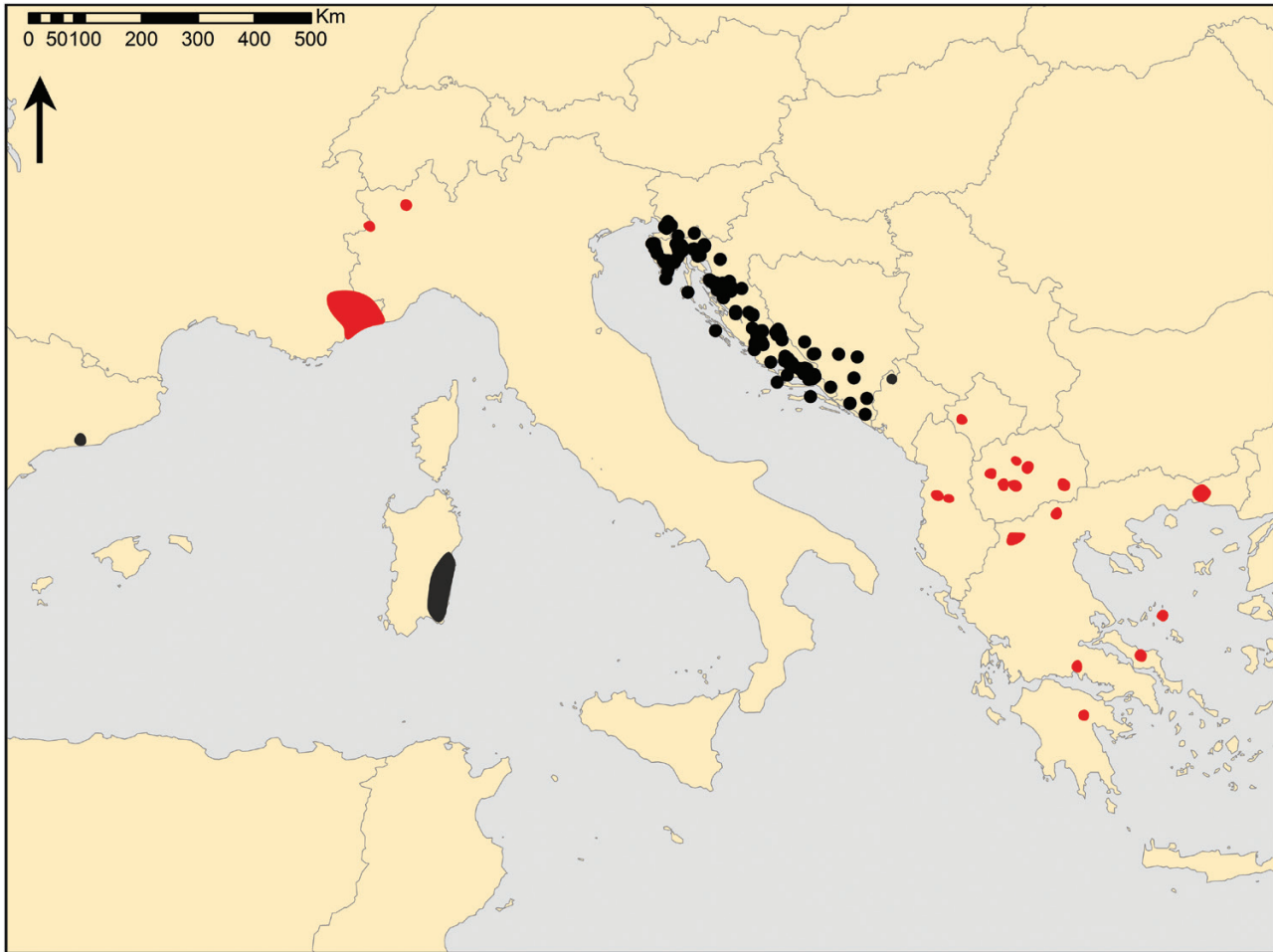
convergent adaptations, owing to high homogeneity of the cave environment, can result in cryptic species (Lefébure *et al.*, 2006; Finston *et al.*, 2007; Zakšek *et al.*, 2009; Baratti *et al.*, 2010; Bilandžija *et al.*, 2013; Delić *et al.*, 2017a, b). Therefore, molecular studies are often essential to clarify phylogenetic relationships and biogeography in troglotaxa. They are fundamental in the Dinaric Karst, where perplexed palaeogeography makes the interpretation of evolutionary history complex. Subterranean morphospecies can consist of deeply divergent lineages (Bilandžija *et al.*, 2013; Weigand *et al.*, 2013; Delić *et al.*, 2017b). Stygobiotic species can be widely distributed, with ranges  $\leq 400$  km (Baratti *et al.*, 2010; Jugović *et al.*, 2012), but more often they are stenoendemic (Zakšek *et al.*, 2009; Bilandžija *et al.*, 2013; Delić *et al.*, 2017a, b). In contrast, no widespread troglotaxa species has been phylogenetically analysed to date, and the widest ranges confirmed by molecular analyses in the Dinaric Karst are  $< 50$  km apart (Weigand *et al.*, 2013; Andersen *et al.*, 2016; Njunjić *et al.*, 2016, 2017, 2018; Polak *et al.*, 2016; Pavlek & Ribera, 2017; Lukić *et al.*, 2018).

Biodiversity of terrestrial isopods in caves of the Dinaric Karst is high, with  $\sim 10\%$  of the world's nominal oniscidean troglotaxa (i.e. 31 species out of  $\sim 300$ ) having been recorded from the area (Karaman, 1966; Bedek *et al.*, 2011, 2017; Campos-Filho *et al.*, 2017; Karaman & Horvatić, 2018). Altogether, 11 troglotaxa genera are currently known, six of which are endemic to the Dinaric Karst (Karaman, 1966; Schmalzfuss, 2003; Karaman & Horvatić, 2018). The most ubiquitous taxon is the genus *Alpioniscus* Racovitza, 1908. At present, it consists of two subgenera: *Alpioniscus s.s.*, with 14 species distributed in south-eastern France, the western Italian Alps and the southern Balkans (Schmalzfuss, 2003; Andreev, 2013a, b), and *Illyrionethes* Verhoeff, 1927, with 21 species known from north-eastern Spain, Sardinia and the Dinaric Karst (Schmalzfuss, 2003; Taiti & Argano, 2009; Bedek *et al.*, 2017; Taiti *et al.*, 2018) (Fig. 1). The subgenera are based solely on small morphological characters, i.e. the distal (second) article of the male pleopod 2 endopod longer or of subequal length than the proximal (first) article in *Alpioniscus* and distinctly shorter in *Illyrionethes*, and their monophyly have not been confirmed by molecular analyses. All *Alpioniscus* species are adapted to an underground environment, with the majority being terrestrial troglotaxa. The exceptions are Sardinian species: the endogean *Alpioniscus thanit* Taiti & Argano, 2009 and the stygobiotic *Alpioniscus stochi* Taiti & Argano, 2018, *Alpioniscus sideralis* Taiti & Argano, 2018 and *Alpioniscus kuenhi* (Schmalzfuss, 2005) (Taiti *et al.*, 2018). The north-eastern range of *Illyrionethes*

encompasses a  $\sim 600$ -km-long area of Dinaric Karst that stretches along the Adriatic coast, from north-eastern Italy to north-western Montenegro (Karaman, 1966; Schmalzfuss, 2003; Bedek *et al.*, 2011; Horvatić, 2014). To date, 14 nominal species, three subspecies and 11 putative undescribed species are recognized (Bedek & Taiti, 2011; Horvatić, 2014; Bedek *et al.*, 2017). Most species are stenoendemic, but there are a few widespread ones, e.g., *Alpioniscus balthasari* (Frankenberger, 1937) with a 180 km wide range (Bedek *et al.*, 2011). Morphological differences between species of *Illyrionethes* are small and based solely on male secondary sexual characters. Few attempts to understand relationships among *Illyrionethes* species have been made previously, but they relied on morphological analysis alone (Frankenberger & Strouhal, 1940; Buturović, 1957; Bedek & Taiti, 2011; Horvatić, 2014). In this study, we use an integrative approach to test the monophyly of different *Alpioniscus* (*Illyrionethes*) species blurred by small morphological differences. We needed to resolve the issue of whether it is justified to use such small morphological differences as species delimitation criteria.

Several phylogenetic studies on epigeal (e.g. Klossa-Kilia *et al.*, 2006; Montesanto *et al.*, 2007; Parmakelis *et al.*, 2008; Poulakakis & Sfenthourakis, 2008; Karasawa & Honda, 2012; Kamilari *et al.*, 2014; Lee *et al.*, 2014; Raupach *et al.*, 2014; Zimmermann *et al.*, 2015, 2018; Dimitriou *et al.*, 2018) and cave (Rivera *et al.*, 2002; Cooper *et al.*, 2008) Oniscidea have been performed thus far, but only one focused on Synocheta taxa, namely Sardinian *Illyrionethes* (Taiti *et al.*, 2018). They showed that traditional taxonomic characters should be revised in some cases (Klossa-Kilia *et al.*, 2006; Parmakelis *et al.*, 2008; Lee *et al.*, 2014; Zimmermann *et al.*, 2018; Dimitriou *et al.*, 2018), whereas in others they confirmed that current taxonomy based on rather small morphological differences is correct (Zimmermann *et al.*, 2015). In general, using multiple methods to solve taxonomic problems helps to overcome errors related to single-discipline approaches and increases rigor in species delimitation (Schlick-Steiner *et al.*, 2010).

The aim of this study is as follows: (1) to verify the validity of the current taxonomy and confirm the existence of new species before describing them; (2) to shed light on the evolutionary history; and (3) to develop a new taxonomical framework based on both traditional and new morphological characters, using well-supported and thoroughly sampled lineage as a model. In addition, we redescribe three known species, describe two new ones and provide additional information to a recent species description.



**Figure 1.** Distribution map of the genus *Alpioniscus*. *Alpioniscus s.s.* is marked in red, *Illyrionethes* in black.

## MATERIAL AND METHODS

### SAMPLING AND IDENTIFICATION

Altogether, 3038 specimens of *Illyrionethes* from the entire Dinaric Karst area (251 caves) were examined and deposited in the natural history collections in Zagreb (Croatia), Ljubljana (Slovenia), Novi Sad (Serbia), Florence (Italy) and Stuttgart (Germany). Specimens were usually collected by hand with tweezers, rarely by pitfall traps, fixed and stored in 70% ethanol, 75% ethanol with glycerol or 96% ethanol. A total of 193 specimens, mostly males, were dissected for species identification. The species were identified according to traditional morphological criteria based on original descriptions and, whenever possible, compared with topotypic specimens or with specimens collected from the closest possible site to the type locality. The locality coordinates are in the WGS84 coordinate system (Supporting Information, Tables S1 and S2).

### MOLECULAR ANALYSES

A total of 64 specimens from 47 different localities were included in the molecular analyses (Supporting Information, Table S2). Genomic DNA was isolated from pereopods (2–3) or entire individuals using the commercial kit QIAamp DNA Mini Kit (Qiagen Inc., USA), following the animal tissue protocol.

Parts of two mitochondrial loci, large ribosomal subunit 16S (16S) and cytochrome *c* oxidase I (*COI*), and of the nuclear histone *H3* (*H3*) were polymerase chain reaction (PCR) amplified. Unfortunately, we encountered several problems in *COI* amplification using Folmer region primers (LCO-HCO; Folmer *et al.*, 1994), due to the high variability at the HCO primer site and the presence of pseudogenes.

In our attempt to amplify *COI*, we used different primers in combination with LCO-1490 (5'-GGTCA ACAAATCATAAAGATATTG-3'): HCO-2198 (5'-TAA ACTTCAGGGTGACCAAAAAATCA-3') (Folmer

*et al.*, 1994), C1-J-2329 (alias K525; 5'-ACTGTAA ATATATGATGAGCTCA-3') (Simon *et al.*, 1994), H7005mod1 (5'-ARTGNGCANCANCRTARTANG TRTCRTG-3') (Donald *et al.*, 2005) and FLO-HCO (5'-GGRTGYCCAAARAATCA-3') (Baratti *et al.*, 2005). Despite multiple attempts *COI* was successfully amplified in only 37 specimens of 14 taxa (Supporting Information, Table S2). Therefore, we used *COI* sequences only to calculate genetic distances between successfully amplified taxa, not to infer phylogeny.

A region of ~550 bp of the 16S rDNA gene was amplified with the primers 16Sar (5'-CGCCTGTT TATCAAAAACAT-3') (Palumbi, 1994) and 16SH2 (5'-AGATAGAAACCAACCTGG-3') (Schubart *et al.*, 2000, equivalent to the primer 1472 described by Crandall & Fitzpatrick, 1996), and 302 bp of the nuclear histone *H3* were amplified using primers 5'-ATGGCTCGTACCAAGCAGACVGC-3' and 5'-ATA TCCTTRGGCATRATRGTGAC-3' (Colgan *et al.*, 1998). We followed PCR protocols as described by Baratti *et al.* (2005) for *COI* and as described by Baratti *et al.* (2004) for 16S. For histone *H3*, we followed the protocol of Colgan *et al.* (1998).

The PCR products were purified using a SureClean Plus kit (Bioline US Inc., Taunton, MA, USA) and sequenced using the ABI Big Dye Terminator Mix by the ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). All sequences are deposited in GenBank, with accession numbers listed in the Supporting Information (Table S2).

#### PHYLOGENETIC ANALYSIS

Chromatograms were visualized by CHROMAS software v.2.01 (Technelysium Pty Ltd, South Brisbane, Queensland, Australia). The sequences, manually corrected, were aligned and translated into amino acids using MEGA v.5.05 (Tamura *et al.*, 2011). For 16S, we assigned different gap penalty values (opening: 30, 60; extension: 5, 30), and the resulting alignments were used to construct tree topologies with the different combinations of gap values. Polymorphic sites, nucleotide statistics and genetic distances (K2P) were analysed with MEGA v.5.05 (Tamura *et al.*, 2011) and MEGA X (Kumar *et al.*, 2018). In order to determine whether sequences were nuclear (nuclear mitochondrial DNA segments; Song *et al.*, 2008; Buhay, 2009) or mitochondrial copies, the following steps were used. First, sequence chromatograms were checked for double signals. Second, coding sequences alignments were inspected for frameshift mutations and/or stop codons. Third, corrected sequences were compared with GenBank ones.

A  $\chi^2$  test of homogeneity of base frequencies across taxa and partition-homogeneity test of phylogenetic congruence among the gene portions were carried out

using PAUP\* v.4.0b10 (Swofford, 2002). The software ModelTest v.3.7 (Posada & Crandall, 1998) was used to determine the best model of evolution based on a likelihood ratio test. Different models of nucleotide substitutions were fitted to each dataset and for the combined dataset. ModelTest identified the TVM+I+G model as the best fit for 16S and 16S+*H3* datasets, whereas TrNef+G was the best model for histone *H3* (Tamura & Nei, 1993).

Phylogenetic trees were reconstructed separately for each marker and for the concatenated alignment, using both Bayesian inference (BI) and maximum likelihood (ML). The BI analysis was conducted in MrBayes v.3.12 (Huelsenbeck & Ronquist, 2001), using the best models selected by MrModeltest. Four chains of Markov chain Monte Carlo (MCMC) were run simultaneously and sampled every 1000 generations for a total of 4 million generations. The first 1000 sampled trees from each run were discarded as burn-in. The remaining trees were used to construct a 50% majority-rule consensus tree, and the proportion of trees that contained the clade was given as the posterior probability (PP) on the consensus tree to estimate the robustness of each clade.

We used the package BEAST v.2.1.2 (Bouckaert *et al.*, 2014) to estimate divergence times for the 16S sequence dataset. We carried out a clock-like evolution test using likelihood ratio tests in PAUP\* v.4.0b10. Given that no fossil or geological evidence was available for calibration, and the null hypothesis for constant evolution across all lineages was not rejected for the 16S, we used the strict clock model and a molecular rate of 0.14% base substitution per million years [considering transversions only, and as reported from previous studies for 16S of isopods (Held, 2001)]. Tree priors were set to calibrate a coalescent constant population, and two MCMC analyses were run for 100 million generations, with parameters sampled every 10 000 or 1000 generations. A convergence of the runs was assessed using TRACER v.1.6 (Rambaut *et al.*, 2014), which indicated that most parameter values had effective sample sizes well > 100. The two separate runs of a dataset were combined using LogCombiner v.2.1.2. A maximum clade credibility (MCC) consensus tree was obtained in TreeAnnotator v.2.1.2 that was used to calculate node ages and upper and lower bounds of the 95% highest posterior density interval for divergence times from a combined tree file of both runs. The chronogram was visualized using FigTree v.1.4.0 (<https://github.com/rambaut/figtree/releases>). The ML tree was constructed based on the TVM+I+G model with 1000 bootstrap replicates, using PhyML algorithms as implemented in the Seaview software (Gouy *et al.*, 2010). *Spelaeonethes mancinii* (Brian, 1912) from a Tuscan cave (Buca della Cava, Oliveto Terme, Monte Pisano)

and *Oritoniscus flavus* (Budde-Lund, 1906) [GenBank accession no. AJ388087.1 (Michel-Salzat & Bouchon, 2000)] were used as outgroups for 16S+H3 and 16S trees, respectively.

#### MORPHOLOGICAL ANALYSES

A subset of the largest males with fully developed secondary sexual characteristics were dissected: a total of 181 males collected in 102 different localities (details in Supporting Information, Table S1). The following appendages were usually dissected: antennulae, antennae, pereopods 1 and 7, genital papilla and pleopods 1 and 2. For illustrations and species descriptions, all appendages and entire bodies were used. The dissected body parts were mounted on micropreparations, in Hoyer's liquid (Anderson, 1954) or sometimes in glycerol. Specimens were examined under Wild M5, Wild M20, Zeiss Stemi 2000-C, Zeiss Primo Star and Nikon Labophot microscopes. Body parts in micropreparations were photographed using Zeiss Primo Star, Canon EOS 40D and EOS Utility software and measured from photographs using AxioVision LE Software.

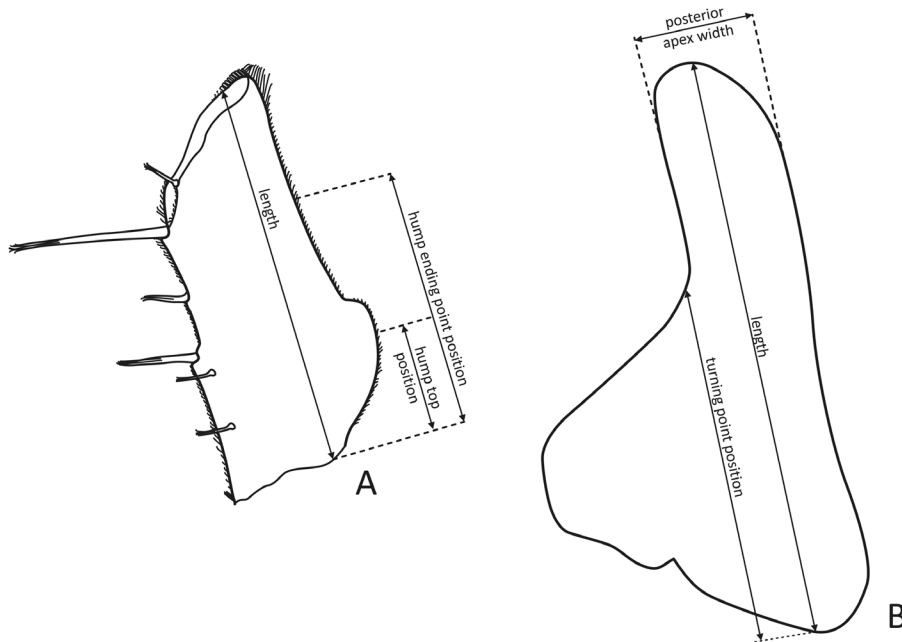
A detailed taxonomic analysis was performed on the three largest quartiles of dissected males (129 specimens), selected on the basis of the longest exopods of pleopod 1. The following appendages were measured: male pereopod 7 carpus length and hump (ending point position, top position; Fig. 2A), male pleopod 1 exopod (length, posterior point

width, position of concavity turning point; Fig. 2B) and body size. The following characters were counted: number of antennular aesthetascs, number of antennal flagellum articles, number of antennal flagellum articles bearing aesthetascs and number of setae on male pereopod 1 carpus. The analyses were performed and box-and-whiskers plots created using Orange (Demsar *et al.*, 2013). In species descriptions/redescriptions, the ratios are expressed as the arithmetic mean  $\pm$  SD.

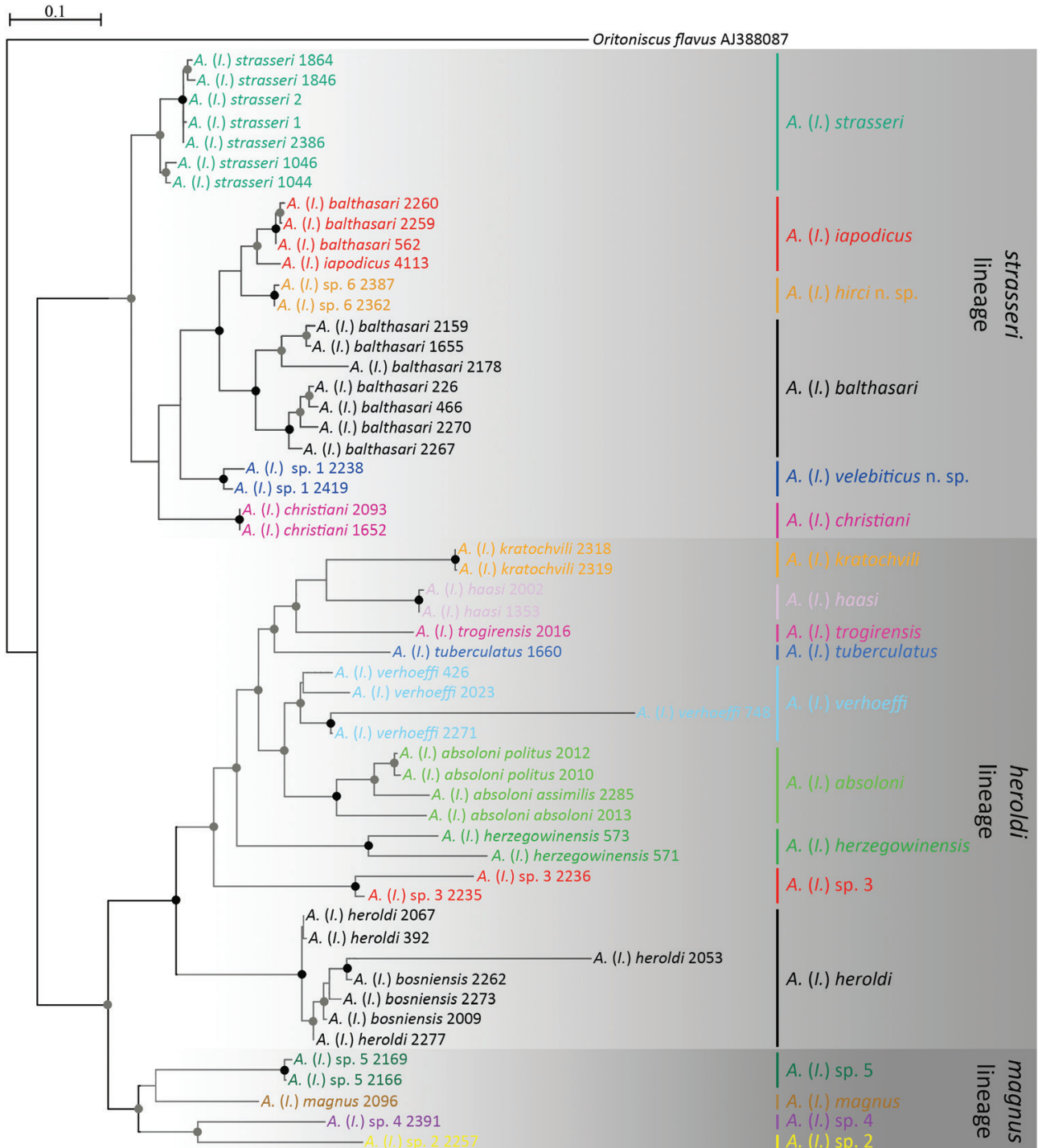
The drawings were made mostly from photographs or with the aid of a camera lucida. Species descriptions and redescriptions are based on examinations of the type material, when possible, or on topotypes and/or the closest possible population. The terminology used in species description is based mainly on Vandel (1960, 1962). Values given in the taxonomy section include data on all adult specimens of the given species. The number of specimens is indicated.

#### ABBREVIATIONS

- CBSSC Croatian Biospeleological Society Collection, Zagreb, Croatia  
 CCC Cave Cadaster of the Republic of Croatia, hosted by the Ministry of Environment and Energy, Zagreb, Croatia (Croatian Agency for the Environment and Nature, 2015)  
 CNHM Croatian Natural History Museum, Crustacea Collection, Zagreb, Croatia



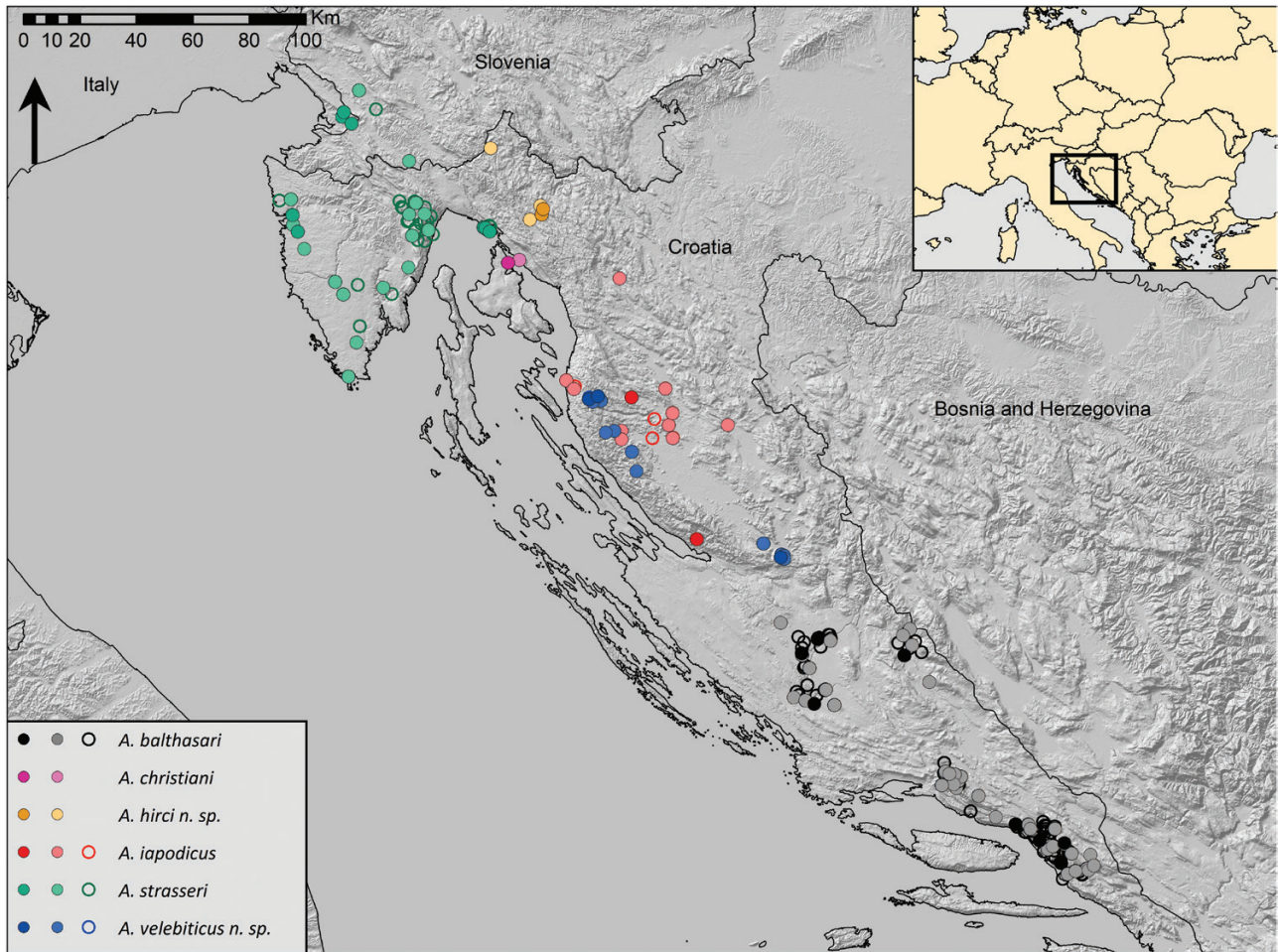
**Figure 2.** Measurements: A, the male carpus of pereopod 7; B, the male exopod of pleopod 1.



**Figure 3.** Maximum likelihood (ML) phylogenetic tree obtained by 16S dataset, with original taxa names in the tree and new taxa names in the right column. Black and grey dots indicate bootstrap values of  $\geq 0.95$  and  $> 0.75$ , respectively.

ECC eCadaster of caves database, Ljubljana, Slovenia ([Društvo za raziskovanje jam Ljubljana, 2018](#))

MZUF Museo di Storia Naturale dell'Università di Firenze, Sezione di Zoologia La Specola, Florence, Italy



**Figure 4.** Distribution map of the *strasseri*-lineage (dark circles, DNA and morphological analyses; pale circles, morphological analyses; open circles, sample sites).

RCCFVG	Regional Cadastre of the Caves of Friuli Venezia Giulia, Trieste, Italy
SMNS	Staatliches Museum für Naturkunde Stuttgart, Isopod Collection, Stuttgart, Germany
ZCDB	Zoological Collection at the Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
ZZDBE	Zoological Collection of Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

## RESULTS

### GENETIC ANALYSIS

We examined 605 aligned base pairs (bp) for *COI* (288 variable and 235 parsimony-informative sites), 515 bp for 16S (310 variable and 217 parsimony-informative

sites) and 292 bp for *H3* (66 variable sites and 35 parsimony-informative sites). The results of the  $\chi^2$  homogeneity test showed a homogeneous base composition within the group for all genes ( $P < 0.0001$ ). Congruence between 16S and *H3* sequences was not rejected according to the homogeneity partition test ( $P = 0.25$ ) for 55 specimens sequenced for both genes. The topology obtained by 16S alone was mostly consistent with the combined 16S+*H3* and 16S+*H3*+*COI* (not shown) analysis, whereas the *H3* tree (not shown) was mainly not resolved.

Three different lineages could be identified with ML and BI 16S analyses (Fig. 3; Supporting Information, Fig. S1): the largest, *heroldi*-lineage with nine species, the *strasseri*-lineage with six and the smallest, *magnus*-lineage with four. Species in the *strasseri*- and *heroldi*-lineage group together regardless which combination of methods, genes or outgroups were used in the analyses (e.g. Fig. 3; Supporting Information, Figs S1–S3). In contrast, the *magnus*-lineage is less

stable (Fig. 3; Supporting Information, Figs S1–S3); therefore, we consider it as tentative. Overall, the relationships we found here are presumptive, because not all analyses showed the same topology.

All species in the *heroldi*-lineage [*Alpioniscus haasi* (Verhoeff, 1931), *Alpioniscus kratochvili* (Frankenberger, 1938), *Alpioniscus absoloni* (Strouhal, 1939), *Alpioniscus trogirensis* Buturović, 1955, *Alpioniscus verhoeffi* (Strouhal, 1938), *Alpioniscus tuberculatus* (Frankenberger, 1939) and *Alpioniscus herzegowinensis* (Verhoeff, 1931)] are monophyletic, except for *Alpioniscus heroldi* (Verhoeff, 1931). The phylogenetic reconstruction showed that *A. heroldi* also includes *Alpioniscus bosniensis* (Frankenberger, 1939). The morphological analysis was coherent with this result; therefore, *A. bosniensis* should be treated as a junior synonym of *A. heroldi*, on the basis of priority (Verhoeff, 1931a; Frankenberger, 1939). The three subspecies of *A. absoloni*, *A. a. absoloni* (Strouhal, 1939), *A. a. politus* (Strouhal, 1939) and *A. a. assimilis* (Strouhal, 1939), form a clade, and each subspecies is clearly separated from the other two. The separate status of *A. sp. 3* is confirmed by our results.

The *strasseri*-lineage comprises four nominal species (the monophyletic *Alpioniscus christiani* Potočnik, 1983 and *Alpioniscus strasseri* (Verhoeff, 1927), the paraphyletic *Alpioniscus iapodicus* Bedek, Horvatović & Karaman, 2017 and the polyphyletic *A. balthasari*) and two new species described below (*Alpioniscus velebiticus* Bedek & Taiti sp. nov. and *Alpioniscus hirci* Bedek & Taiti sp. nov.). Our results show that the population from Paklenica (specimen numbers 562, 2259 and 2260) previously identified as *A. balthasari* (Bedek et al., 2011), clustered with *A. iapodicus*. Morphological analyses confirmed this, so Paklenica population is now included in *A. iapodicus*. *Alpioniscus balthasari* includes three subclades: *A. balthasari* s.s. with the Cetina–Krka populations (specimen numbers 1655 and 2159), the Stara jametina population (specimen number 2178) and the Biokovo–Mosor population (specimen numbers 226, 466, 2267 and 2270). The *A. balthasari* subclades are grouped together in all analyses performed, except in 16S BI (Supporting Information, Fig. S1). *Alpioniscus strasseri* consists of two subclades: *A. strasseri* s.s. with northern Istrian populations (specimen numbers 1, 2, 1846, 1864, 2386) and *A. strasseri* with southern Istrian populations (specimens numbers 1044, 1046) that correspond to *Alpioniscus enriquesi* (Arcangeli, 1932), currently considered as a junior synonym of *A. strasseri* (Strouhal 1939a).

The tentative *magnus*-lineage includes four species: the monophyletic *Alpioniscus magnus* (Frankenberger, 1938) and three undescribed species *A. sp. 2*, *A. sp. 4* and *A. sp. 5*. They form a clade with high support in 16S trees (Fig. 3; Supporting Information, Fig. S1), but

*A. magnus* and *A. sp. 5* are separated from rest of the lineage in the 16S+H3 (Supporting Information, Fig. S2) and 16S+H3+COI analyses (not shown).

COI and 16S distances (Supporting Information, Tables S3 and S4) between species from different lineages ranged from 17 to 26% and from 17 to 52%, respectively. Within the *strasseri*-lineage, interspecific COI and 16S distances varied from 12 to 18% and from 5 to 17%, respectively, whereas in the *heroldi*-lineage they varied from 15 to 23% and 16 to 47%, respectively. The interspecific COI distance in the *magnus*-lineage was 19%, and 16S varied from 17 to 24%. Interpopulation COI and 16S distances within the *strasseri*-lineage species varied from 2 to 9% and from 1 to 9%, respectively, and within the *heroldi*-lineage from 1 to 19% and from 1 to 31%, respectively.

The three major lineages of Dinaric *Illyrionethes* (Supporting Information, Fig. S3) split ~10–9 Mya, and speciation occurred from ~10 to 2 Mya.

The lineages identified in our study have overlapping distributions (Figs 4, 5) and follow a north-west to south-east direction, the so-called Dinaric direction (Zupan Hajna, 2012). The *strasseri*-lineage is the most widely distributed, from Italy in the north-west of the Dinaric Karst to the south of Biokovo Mt. (Fig. 4). The *heroldi*-lineage occurs from north Dalmatia in the north-west to south-eastern Herzegovina (Fig. 5), and the provisional *magnus*-lineage occupies the coast from the island of Lošinj to the island of Brač (Fig. 5). Although sympatry in Dinaric *Illyrionethes* is common, syntopy is relatively rare: only *A. heroldi* is syntopic with *A. absoloni* in the area of Bileća, with *A. verhoeffi* in the area of the Neretva river and Biokovo mountain and with *A. balthasari* on Biokovo mountain.

## TAXONOMY

FAMILY TRICHONISCIDAE SARS, 1899

SUBFAMILY TRICHONISCINAE SARS, 1899

GENUS *ALPIONISCUS* RACOVITZA, 1908

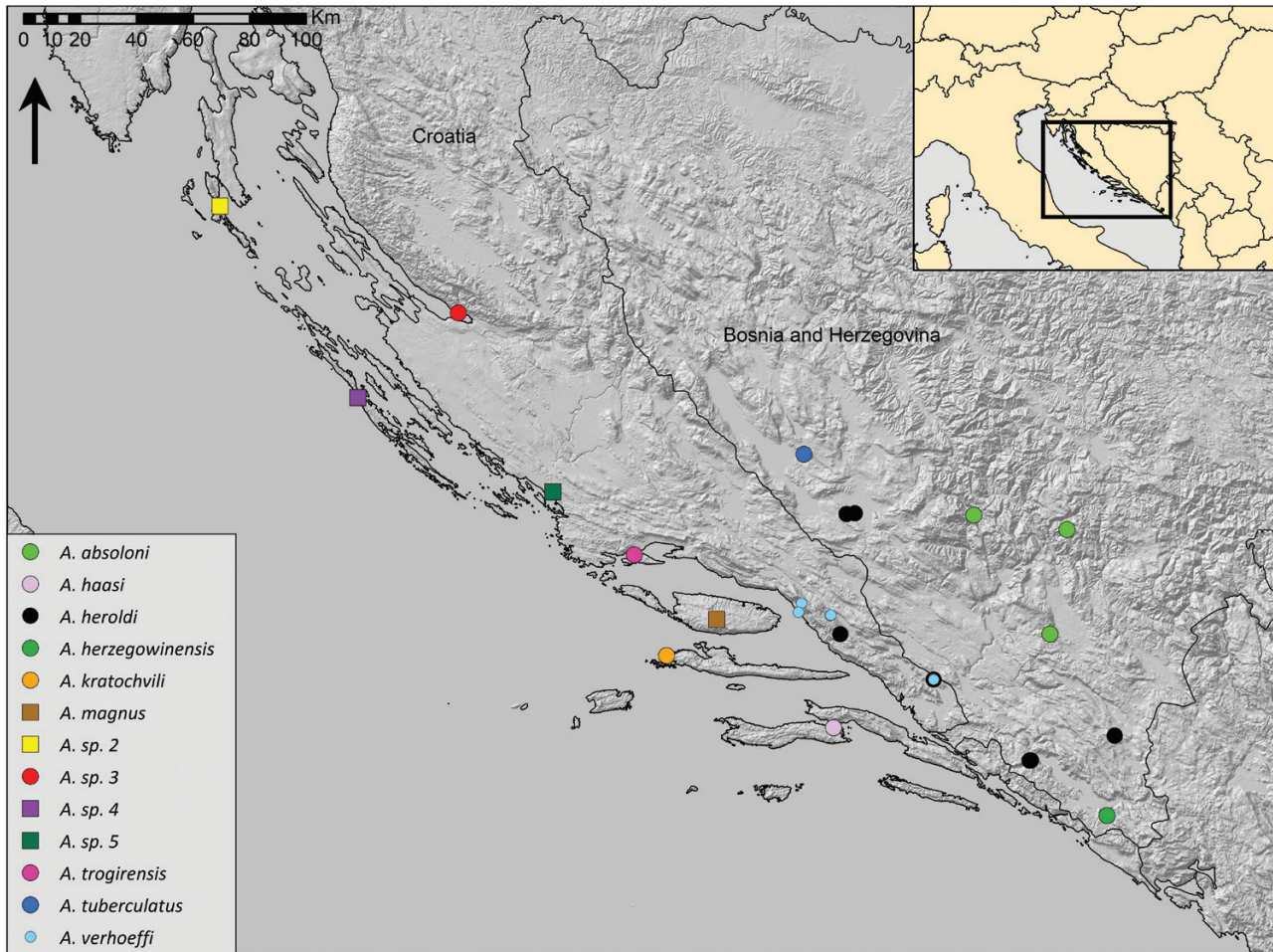
*Type species: Trichoniscus dispersus* Racovitza, 1907 [= *Alpioniscus feneriensis* (Parona, 1880)] by monotypy.

SUBGENUS *ILLYRIONETHES* VERHOEFF, 1927

*Type species: Illyrionethes strasseri* Verhoeff, 1927 by monotypy.

*Diagnosis:* Unpigmented body, eyes absent. Pleon narrower than pereon. Antennula of three articles, distal article bearing aesthetascs on subapical and/or apical margin. Antennal flagellum with five to 30 segments. Mandibles with one/two penicils in the right





**Figure 5.** Map of the populations included in the analyses of the *heroldi*- (circles) and *magnus*-lineage (squares).

and three penicils in the left; molar process with none/one penicil in the right and none in the left. Outer branch of maxillula with ten/11 teeth, apically entire and one/two slender stalks; inner branch with three penicils. Maxilla with setose apex. Pereopods with setose dactylar seta. Uropod endopod shorter than exopod. Male pereopod 7 ischium with straight sternal margin. Genital papilla with rounded apical part. Male pleopod 1 endopod elongated, bearing apical seta. Male pleopod 2 endopod longer than exopod; of two articles, with proximal article distinctly longer than distal article, and distal article bearing terminal seta.

*Remarks:* The above diagnosis is based on all species included in *Illyrionethes*, with the exception of *Alpioniscus escolai* Cruz & Dalens, 1989 from Catalonia, which needs a taxonomic revision owing to some morphological differences. The only character that distinguishes subgenus *Illyrionethes* from subgenus *Alpioniscus* is the male pleopod 2 endopod with proximal article distinctly longer than distal one.

*ALPIONISCUS (ILLYRIONETHES) STRASSERI*  
(VERHOEFF, 1927)

(FIGS 6–12; TABLE 1)

*Illyrionethes strasseri* Verhoeff, 1927: 270–274; figs 1–7. – 1929a: 28. – 1929b: 51. – 1931b: 20–21. – 1931c: 234. – 1932: 24. – Frankenberger, 1937: 173–175. – 1938: 34. – 1939: 98–99. – Brian, 1938: 176. – Strouhal, 1938: 272–273; figs 3–5. – 1939a: 116, 118–119 [partim: *nec* NW.-Kroatien; Lokve Špilja, Kroatien]. – 1939b: 6, 20. – 1940a: 94. – 1940b: 14. – Wolf, 1938: 78–79. – Frankenberger & Strouhal, 1940: 448–449 [partim: *nec* NW.-Kroatien]. – Creuwels, 2018. – Natural History Museum, 2018a. – 2018b. – 2018c. – Natural History Museum, 2018d.

*Alpioniscus strasseri* – Kesselyák, 1930: 247; figs 2a, 3b–e. – Vandell, 1947: 264, 267, 271. – Potočník, 1989: 64. – Schmalfuss, 2003: 14 [partim: *nec* island Cres]. – Lukić *et al.*, 2009: 16–17. – Lukić *et al.*, 2011: 19–22. – Bedek *et al.*, 2011: 275–278; fig. 16 [partim: *nec* Čampari; Ovčarica; Piskovica; Rabakova špilja; Vrtare

male; Lokvarka; Medvjeda špilja]. – [Bedeck et al., 2017](#): 213. – [Ozimec et al., 2011](#): 166 [partim: Jama kod Burići]. – [Polak et al., 2012](#): 10–16 [partim: *nec* Kubik, Piskovica].

*Illyrionethes enriquesi* [Arcangeli, 1932](#): 1042–1046; pls. 3–9; figs 7–26. – [Brian, 1938](#): 175–176. – [Wolf, 1938](#): 765.

*Alpioniscus (Illyrionethes) strasseri* – [Buturović, 1957](#): 6–49; fig. 24. – [Schmölzer, 1965](#): 59 [partim: NO-Italien]. – [Karaman, 1966](#): 376 [partim: *nec* Lokve špilja; o. Cres (pećina kod Petričevi)]. – [Paoletti, 1978](#): 4 [partim: *nec* Isola di Cherso]. – [Potočnik, 1979](#): 66. – 1993: 85–86, 163, 178. – [Potočnik & Novak, 1980](#): 4. – [Argano, Baldari & Manicastro, 1982](#): 132 [partim: *nec* Isola di Cherso]. – [Argano et al., 1995](#): 14. – [Tabacaru, 1996](#): 15, 40. – [Horvatović, 2014](#): 125, 172–174; figs 7.28.a, 7.33.

*Alpioniscus cf. strasseri* – [Polak et al., 2012](#): 10–16 [partim: Račiška pećina].

? *Illyrionethes strasseri* – [Verhoeff, 1938](#): 134. – 1940: 111.

? *Alpioniscus (Illyrionethes) strasseri* – [Karaman, 1966](#): 376 [partim: o. Cres (pećina kod Petričevi)]. – [Paoletti, 1978](#): 4 [partim: Isola di Cherso]. – [Argano et al., 1982](#) [partim: Isola di Cherso].

? *Alpioniscus strasseri* – [Schmalfuss, 2003](#): 14 [partim: including island Cres]. – [Bedeck et al., 2011](#): 275 [partim: Čampari; Ovčarica; Piskovica; Rabakova špilja]. – [Ozimec et al., 2011](#): 166 [partim: Ročka špilja]. – [Polak et al., 2012](#): 11, 16 [partim: Kubik, Piskovica].

? *Alpioniscus cf. strasseri* – [Polak et al., 2012](#): 10–11 [partim: Polina peć, Jama pod Krogom, Rabakova špilja].

*nec Alpioniscus strasseri* – [Casale et al., 2004](#): 310.

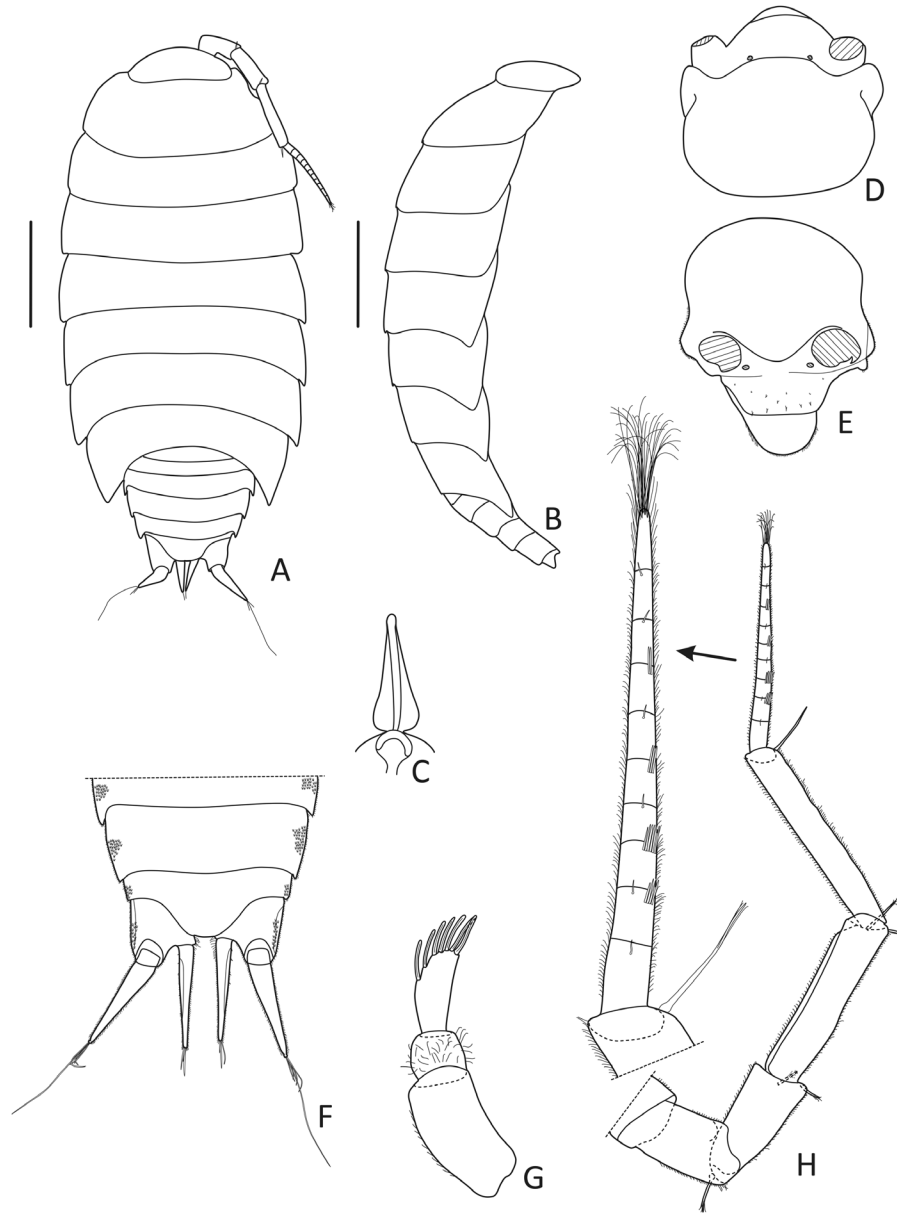
*nec Illyrionethes strasseri* – [Royal Belgian Institute of Natural Sciences, 2017](#).

#### *Material examined (collection details in Supporting Information, Table S1)*

**Northern Istria population:** ITALY. **Venezia Giulia, Trieste:** Basovizza, Caverna presso Basovizza (cave RCCFVG 689) (CBSSC IT2386; IT2473; IT3870); Longera, Grotta nel Bosco dei Pini (cave RCCFVG 16) (CBSSC IT2396; MZUF 1); San Dorlingo della Valle, Val Rosandra, Grotta del Guano (cave RCCFVG 527) (MZUF 2). SLOVENIA. Sežana, Štorje, Belinca jama (cave ECC 950) (CBSSC IT995, IT1648, IT1649, IT1650); Istria, Račice, Starod, Račiška pećina (cave ECC 942) (CBSSC IT2464); Divača, Škocjan, Škocjanske jame (cave ECC 735) (CBSSC IT2813). CROATIA. **Istria, Čičarija Mts.:** Lupoglav, Brgudac, Borušnjak, Borušnjak 1 (cave CCC HR01280) (CBSSC IT1735); Lupoglav, Brgudac, Borušnjak, Borušnjak 3 (cave CCC HR01291) (CBSSC IT1755); Lupoglav, Brest pod Učkom, Vojni poligon iznad Bresta, Crekvica (cave CCC

HR01531) (CBSSC IT2138, IT2139); Lupoglav, Brest pod Učkom, Vojni poligon iznad Bresta, Crekvica 1 (cave CCC HR01527) (CBSSC IT682, IT683, IT684, IT686, IT687); Lupoglav, Semići, Podbrus, Jama pod križ (cave CCC HR01761) (CBSSC IT1761, IT2105, IT2107, IT2108); Opatija, Veprinac, Mahenac, Jama SD 2 (cave CCC HR00925) (CBSSC IT1762); Lupoglav, Brgudac, Bončića kuća, Pećina kod planinarske kuće Pavlovac (cave) (CBSSC IT1732, IT1733); Opatija, Veprinac, Puhari, Pećina kod sela Puhari (cave CCC HR00437) (CBSSC IT1713, IT1717, IT1719); Opatija, Veprinac, Brložnik, Pećina pod Brložnikom (cave CCC HR00429) (CBSSC IT1759); Lupoglav, Brest pod Učkom, Vela peć (cave CCC HR00614) (CBSSC IT698, IT2127). **Istria, Učka Mt.:** Brest, Vojni poligon iznad Bresta, Barinova jama (cave CCC HR01262) (CBSSC IT1131); Lovran, Potoki, Mošćenička draga, Druška peć (cave) (CBSSC IT1744); Boljun, Pikulići, Vela draga, Jama iznad Vranjske drage (cave CCC HR01296) (CBSSC IT2117, IT2119); Veprinac, Menderi, Jama kod potoka Banine 3 (cave CCC HR00430) (CBSSC IT721, IT1132); Mala Učka, Jama mrtvih golubova (cave CCC HR00927) (CBSSC IT706, IT708, CNHM 3/1680); Poklon, Dobroč, Jama na Balaguštini (cave CCC HR01534) (CBSSC IT1140); Vela Učka, Molinarska draga, Mala peć (cave) (CBSSC IT727); Brest, Vojni poligon iznad Bresta, Oštirova jama (cave CCC HR01261) (CBSSC IT1125); Mala Učka, Podmaj, Petrc, Petrčeva jama (cave CCC HR00392) (CBSSC IT1135, IT1136); Lovran, Lovranska Dragica, Veliki Knezgrad, Proljetna jama (cave CCC HR00601) (CBSSC IT1747, IT1749); Boljun, Vranja, Vela draga, Pupičina pećina (cave CCC HR01318) (CBSSC IT2130, IT2131); Lovran, Medveja, Rudnik u Medveji (mine) (CBSSC IT1721, IT1722, IT1723); Mala Učka, Podmaj, Rupa II (cave) (CBSSC IT1142, IT1144); Poklon, Topol, Topolska peć (cave) (CBSSC IT718, IT1138). **Croatian Littoral, Kostrena:** Bakar, Rožmanići, Humčine, Bunetova jama (cave CCC HR00443) (CBSSC IT783, IT784, IT785, IT1851, IT1852, IT1853); Perilo, Mali štumbur (cave) (CBSSC IT1864, IT1865); Bakar, Paveki, Špilja u uvali Predpeć (cave CCC HR00442) (CBSSC IT779, IT781, IT782, IT1122, IT1123, IT1854, IT1855, IT1856, IT1857, CNHM 3/1679); Bakar, Paveki, Špilja u uvali Svežanj (cave CCC HR01336) (CBSSC IT1120, IT1121, IT1860, IT1863, IT1862); Rožmanići, Humčine, Tončakova jama (cave) (CBSSC IT1867); Urinj, Urinjska špilja (cave CCC HR01110) (CBSSC IT1841, IT1844, IT1846, IT1850, IT1849, IT1848).

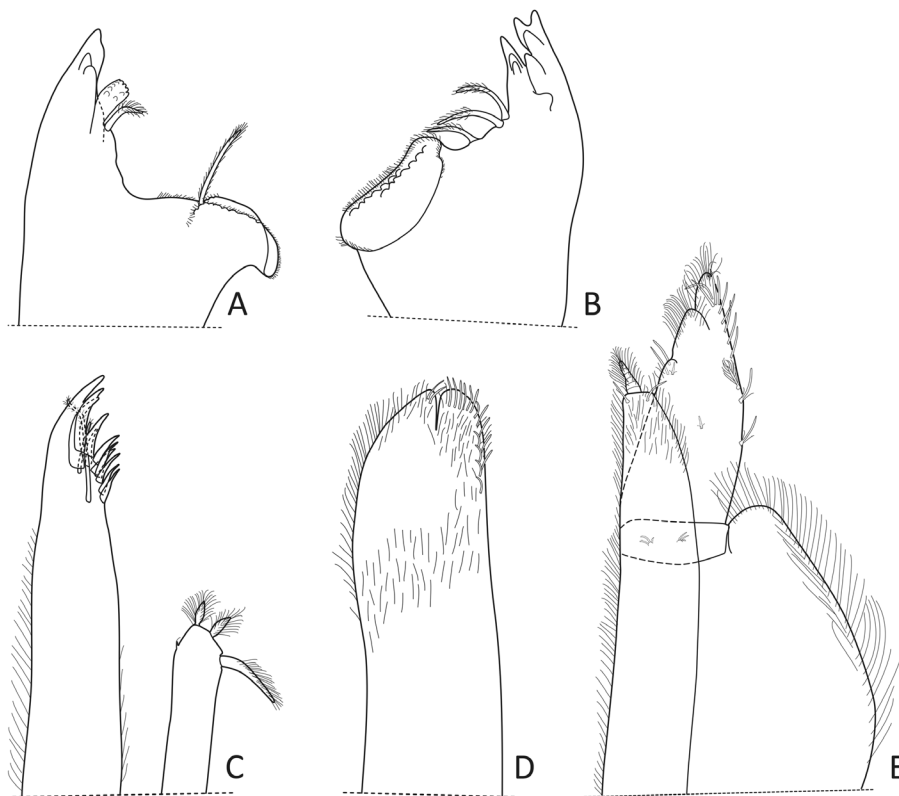
**Southern Istria population:** CROATIA. **Istria:** Poreč, Nova Vas, Baredine (cave CCC HR00312) (CBSSC IT1044, IT1046); Labin, Vozilići, Čepić tunel (Tunnel) (CBSSC IT3551); Žminj, Feštini, Festinsko kraljevstvo (cave) (CBSSC IT1045); Pula, Muntić, Ikinica (cave) (CNHM 10/1413, CBSSC IT3529); Poreč, Žbandaj, Ivanka (cave) (CBSSC IT3548); Kanfanar, Burići, Jama



**Figure 6.** *Alpioniscus (Illyrionethes) strasseri*. A, B, D, ♀ CBSSC IT2473, from Caverna presso Basovizza: A, specimen in dorsal view (scale bar: 1 mm); B, pereonites and pleonites in lateral view (scale bar: 1 mm); D, cephalon, dorsal. C, E–H, ♂ CBSSC IT 2386, from Caverna presso Basovizza: C, dorsal scale-seta; E, cephalon, dorsal; F, pleonites 4, 5, telson and uropods; G, antennula; H, antenna with enlargement (arrow) of flagellum.

kod Burići (cave CCC HR00754) (CBSSC IT3555, IT2229, IT2230, IT2440, IT2454, IT2455); Novigrad, Punta Karigador, Jama kod komune (cave) (CBSSC IT3547); Labin, Snašići, Jama na Snašići (cave) (CBSSC IT3549); Labin, Raša, Karlota (mine) (CBSSC IT3553); Pula, Marčana, Ljubićeva pećina (cave) (CBSSC IT3530); Poreč, Tar, Štancija Špin, Markova jama kod Tara (cave CCC HR00981) (CBSSC IT3540, IT3541, IT3542, IT3543, IT2231, IT2232, IT2233, IT2446, IT2447, IT2450); Umag, Brtonigla, Mramornica (cave) (SMNS 5326,

CBSSC IT3534, IT3531); Poreč, Tar, Vrh, Pincinova jama (cave CCC HR00313) (CBSSC IT3544, IT2434, IT2441); Svetvinčenat, Šikuti, Pliškovićeve jama (cave) (CBSSC IT3537, IT3538); Poreč, Nova Vas, Podšeš (cave) (CBSSC IT3546); Pula, Muntić, Staja (cave) (CBSSC IT3528); Pula, Premantura, Špilja na Gradini kod Premanture (cave) (CBSSC IT3512, IT3516, IT3517, IT3518, IT3520, IT3692, IT3775, IT3776, IT3778, IT3780, IT3782, IT3784, IT3786, IT3788, IT3790, IT3791, IT3793, IT3795, IT3798, IT3799, IT3801).

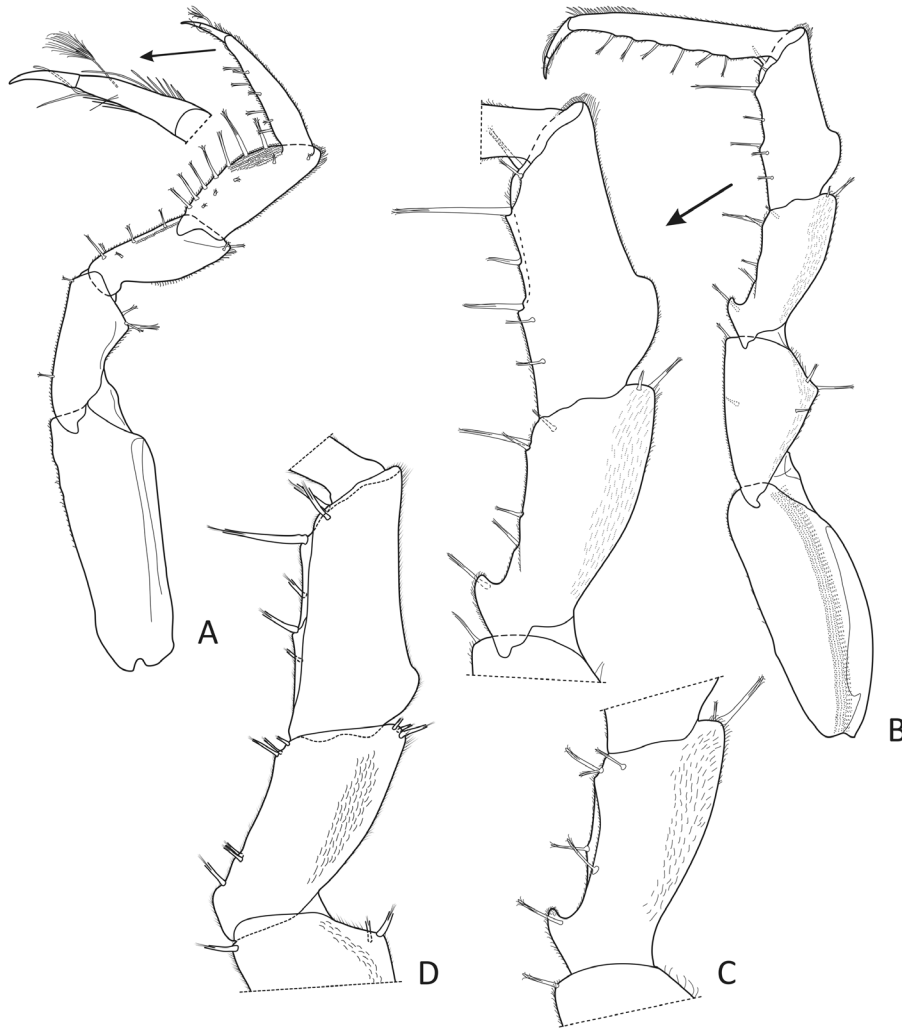


**Figure 7.** *Alpiniscus (Illyrionethes) strasseri*. ♂ CBSSC IT2386, from Caverna presso Basovizza: A, right mandible; B, left mandible; C, maxillula; D, maxilla; E, maxilliped.

**Redescription.** Maximum length: ♂, 5.8 mm; ♀, 8.0 mm. Pereon (Fig. 6A) with almost parallel sides. Back smooth, with some triangular scale-setae (Fig. 6C). Some gland pores on lateral margins of pleonites 4 and 5, telson and uropodal protopods (Fig. 6F). Cephalon (Fig. 6D, E) with suprantennal line bent downwards, rounded middle part; antennal lobes quadrangular. Posterior margin of pereonite 1 convex, of pereonites 2 and 3 straight, and of pereonites 4–7 progressively more concave (Fig. 6A, B). Pleonites 3–5 with small posterior points visible in dorsal view (Fig. 6A, F). Distal part of telson with concave sides and broadly rounded apex (Fig. 6F). Antennula (Fig. 6G) distal article flattened and bearing six to eight aesthetascs on apical margin. Antenna (Fig. 6H) smooth; flagellum of eight to 11 articles, with one row of aesthetascs on two to five different articles, always present on second and third. Mandibles (Fig. 7A, B) with one penicil in right; molar process with one penicil in right. Maxillula (Fig. 7C) outer branch with 5 + 6 teeth and two slender stalks; inner branch with outer and middle penicils subequal, inner distinctly longer. Maxilla (Fig. 7D) apex bilobate, lobes subequal in width. Maxilliped (Fig. 7E) endite narrow, with large segmented apical penicil; palp distally with three rounded lobes, basal article with two compound small setae; basis with rounded outer lobe protruding posteriorly and covered

with long setae on margin. Pereopods (Fig. 8A) similar in shape, with dactylar seta long and bifid. Uropod (Fig. 6F) with protopod slightly grooved on outer margin; endopod inserted proximally.

**Male:** Pereopod 1 (Fig. 8A) carpus bearing six to seven setae. Pereopod 1–4 with carpus and merus bearing numerous short scales on rostral surface. Pereopod 1–6 (Fig. 9) merus with sternal margin from straight (pereopod 1) progressively up to slightly concave (pereopod 6) and with sternal margin bearing no lobe (pereopod 1) progressively up to small lobe (pereopod 6). Pereopod 7 (Fig. 8B, C) merus with slightly concave sternal margin and large hook-shaped lobe in proximal part directed ventrally, bearing one seta; carpus with straight tergal margin and large rounded hump proximally, ratio of carpus length to top hump position  $3.73 \pm 0.53$  ( $N = 10$ ), and ratio of carpus length to ending hump position  $2.09 \pm 0.22$  ( $N = 10$ ). Pleopod 1 (Fig. 10A) exopod with posterior apex narrowly rounded, ratio of length to posterior apex width  $4.76 \pm 0.38$  ( $N = 10$ ), deeply concave outer margin, ratio of length to concavity turning point position  $1.73 \pm 0.07$  ( $N = 10$ ), slightly concave inner margin; endopod narrow with almost parallel sides, apical seta plumose. Pleopod 2 (Fig. 10C) exopod triangular with concave outer margin;



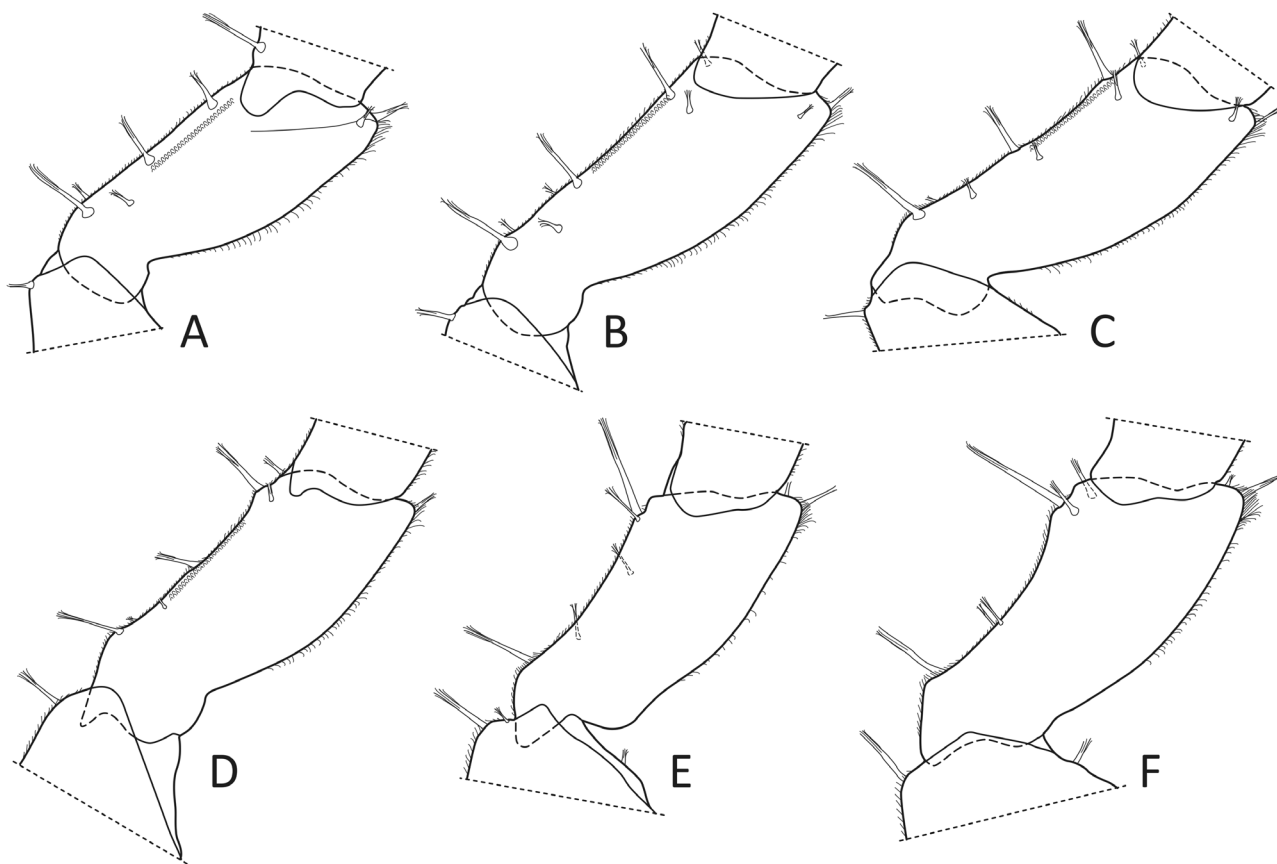
**Figure 8.** *Alpioniscus (Illyrionethes) strasseri*. A–C, ♂ CBSSC IT2386, from Caverna presso Basovizza: A, pereopod 1 with enlargement (arrow) of dactylus; B, pereopod 7 caudal view with enlargement (arrow) of merus and carpus; C, pereopod 7 merus rostral view. D, ♂ CBSSC IT3790 from Špilja na Gradini kod Premanture: pereopod 7 merus and carpus rostral view.

endopod distinctly longer than exopod, distal article narrower than proximal; terminal seta strong and bifid. Pleopod 3–5 exopods as in Fig. 10D–F.

*Type locality:* In the original description, Verhoeff (1927) clearly stated that the described specimens were collected in the cave ‘Höhle am Mt. Spaccato in Istrien, eine Stunde von Triest entfernt’ (presently known as Grotta ad W di Basovizza, Trieste, Italy; Friuli Venezia Giulia, RCCFVG 68) and that the specimens from the cave ‘Höhle Belinca bei Storje’ (presently known as Belinca jama, Štorje, Sežana, Slovenia; ECC. 950) were not included in the description. Therefore, the type locality encompasses only the cave Grotta ad W di Basovizza and not the cave Belinca jama, as previously reported by Bedek *et al.* (2011).

*Remarks:* The Grotta ad W di Basovizza was not available for collecting topotypic specimens owing to the Golf Club Trieste facilities. Therefore, in the species redescription we used specimens collected in two closest possible caves: the 2.5 km distant Caverna presso Basovizza, Basovizza, Trieste, Italy (RCCFVG 689) and the 1 km distant Grotta nel Bosco dei Pini, Basovizza, Trieste, Italy (RCCFVG 16).

The populations of *A. strasseri* from southern Istria show some small differences (Figs 8D, 10B, 11, 12; Table 1). Arcangeli (1932) described *I. enriquesi* from the Špilja na Gradini kod Premanture cave near Pula (caverna presso Promontore, dintorni di Pola) in Istria, which Strouhal (1939a) considered to be a junior synonym of *A. strasseri*. Taxonomic analyses confirm the differences in the males, i.e. the pereopod 7 merus with a small



**Figure 9.** *Alphoniscus (Illyrionethes) strasseri*. ♂ CBSSC IT2386, from Caverna presso Basovizza: A, pereopod 1 merus; B, pereopod 2 merus; C, pereopod 3 merus; D, pereopod 4 merus; E, pereopod 5 merus; F, pereopod 6 merus.

hump-shaped lobe on sternal margin in the proximal part; ratio of carpus length to carpus top hump position  $4.42 \pm 0.97$  ( $N = 7$ ); and ratio of pleopod 1 exopod length to concavity turning point position  $1.90 \pm 0.09$  ( $N = 7$ ). The population is geographically separated from *A. strasseri* s.s. and shows some molecular differences. More sampling should be conducted in regions between their distribution areas, followed by taxonomical and molecular analyses in order to validate the taxonomic status of the population.

*Alphoniscus strasseri* differs from the other species of the *strasseri*-lineage in the male characters (Fig. 12; Table 1). It differs from *A. christiani*, *A. balthasari*, *A. hirci* sp. nov. and *A. iapodicus* in the male pereopod 7 with a larger merus hook-shaped lobe. It is distinguishable in the ratio of the pereopod 7 carpus length to carpus top hump position from *A. hirci* sp. nov. ( $1.66 \pm 0.09$ ) and *A. velebiticus* sp. nov. ( $2.08 \pm 0.15$ ); in the ratio of the male pereopod 7 carpus length to ending tergal hump position from *A. velebiticus* sp. nov. ( $1.16 \pm 0.06$ ), *A. iapodicus* s.s. ( $1.26 \pm 0.03$ ) and *A. hirci* sp. nov. ( $1.27 \pm 0.03$ ); in the position of the concavity turning point of the outer margin of the male pleopod 1

exopod from *A. velebiticus* sp. nov. ( $2.21 \pm 0.14$ ); in the ratio of the male pleopod 1 exopod length to posterior apex width from *A. hirci* sp. nov. ( $3.57 \pm 0.19$ ).

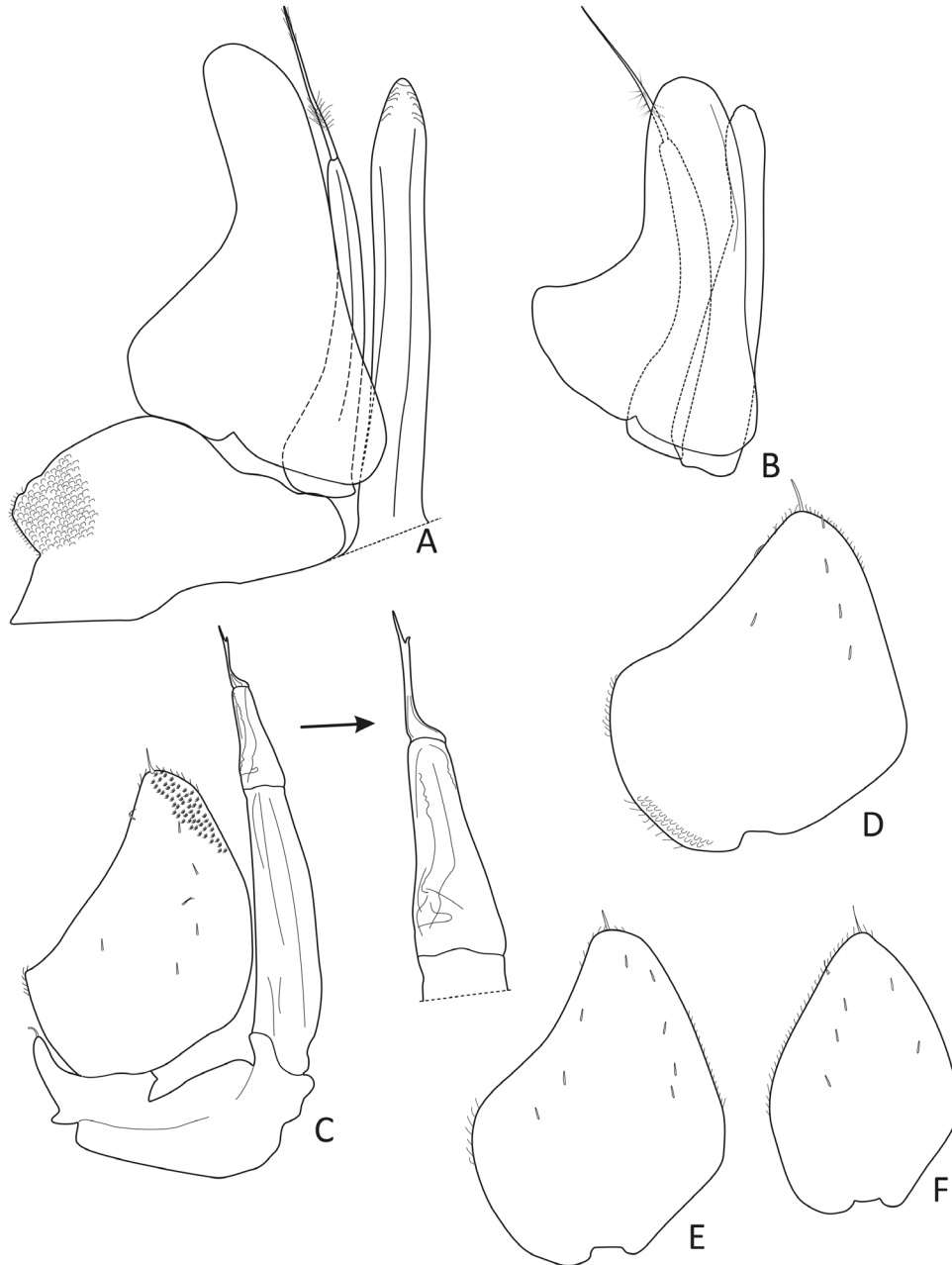
*Alphoniscus strasseri* is considered to be an amphibious species, because it was also observed in water (Verhoeff, 1927). The citations with ‘?’ are doubtful, and the material from those localities needs to be re-examined with both molecular and morphological analyses.

**Distribution:** Italian karst of Trieste, the southern part of the Slovenian Littoral, Croatian Istria and Kostrena surroundings (Figs 4, 11).

*ALPHONISCUS (ILLYRIONETHES) CHRISTIANI*  
POTOČNIK, 1983

(FIGS 12–14 TABLE 1)

*Alphoniscus (Illyrionethes) christiani* Potočnik, 1983: 389–395; figs 1–7. – 1993: 84, 178. – Tabacaru, 1996: 34–40. – Horvatović, 2014: 126, 173, 174, 178 [partim: island Krk]; figs 7.28.c, 7.33.



**Figure 10.** *Alpioniscus (Illyrionethes) strasseri*. A, C–F, ♂ CBSSC IT2386, from Caverna presso Basovizza: A, genital papilla and pleopod 1; C, pleopod 2 with enlargement (arrow) of endopod distal article; D, pleopod 3 exopod; E, pleopod 4 exopod; F, pleopod 5 exopod. B, ♂ CBSSC IT3790 from Špilja na Gradini kod Premanture: genital papilla and pleopod 1.

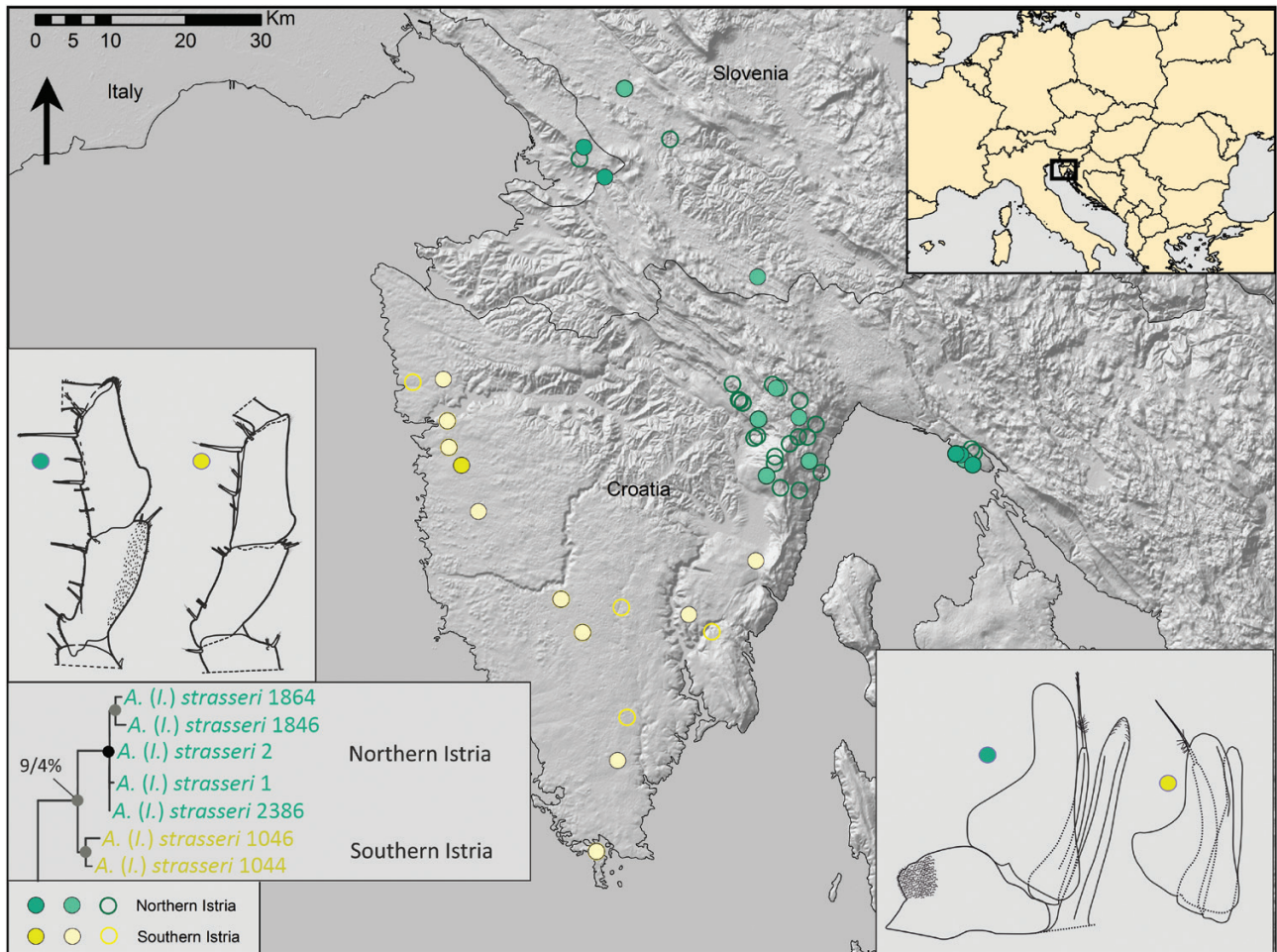
*Alpioniscus christiani* – Potočnik, 1989: 64. – Gottstein *et al.*, 2001: 2257. – Gottstein Matočec *et al.*, 2002: 48. – Schmalfuss, 2003: 13. – Tvrtković *et al.*, 2004: 76. – Bedek *et al.*, 2006: 15, 92. – Bedek *et al.*, 2011: 241, 268; fig. 11. – Jalžić *et al.*, 2010: 20. – Jalžić *et al.*, 2013: 13, 30, 43–45; fig. on p. 44.

*Alpioniscus strasseri* – Bedek *et al.*, 2011: 277 [partim: Vrtare male].

? *Alpioniscus christiani* – Horvatić, 2014: [partim: BiH: Hercegovina, Mosor, Bujanj].

*Material examined (collection details in Supporting Information, Table S1)*

CROATIA. **Croatian Littoral:** Krk Is., Omišalj, Čičići, Rudine, Biserujka (cave) (CBSSC IT2629, IT1657,



**Figure 11.** Distribution, maximum likelihood 16S tree, *COXI*/*16S* distances and male merus and carpus of pereopod 7, genital papilla and pleopod 1 of different populations of *Alpiomiscus strasseri* (dark circles, DNA and morphological analyses; pale circles, morphological analyses; open circles, sample sites; *COXI*/*16S* distances are indicated on the tree; black and grey dots indicate bootstrap values of  $\geq 0.95$  and  $> 0.75$ , respectively).

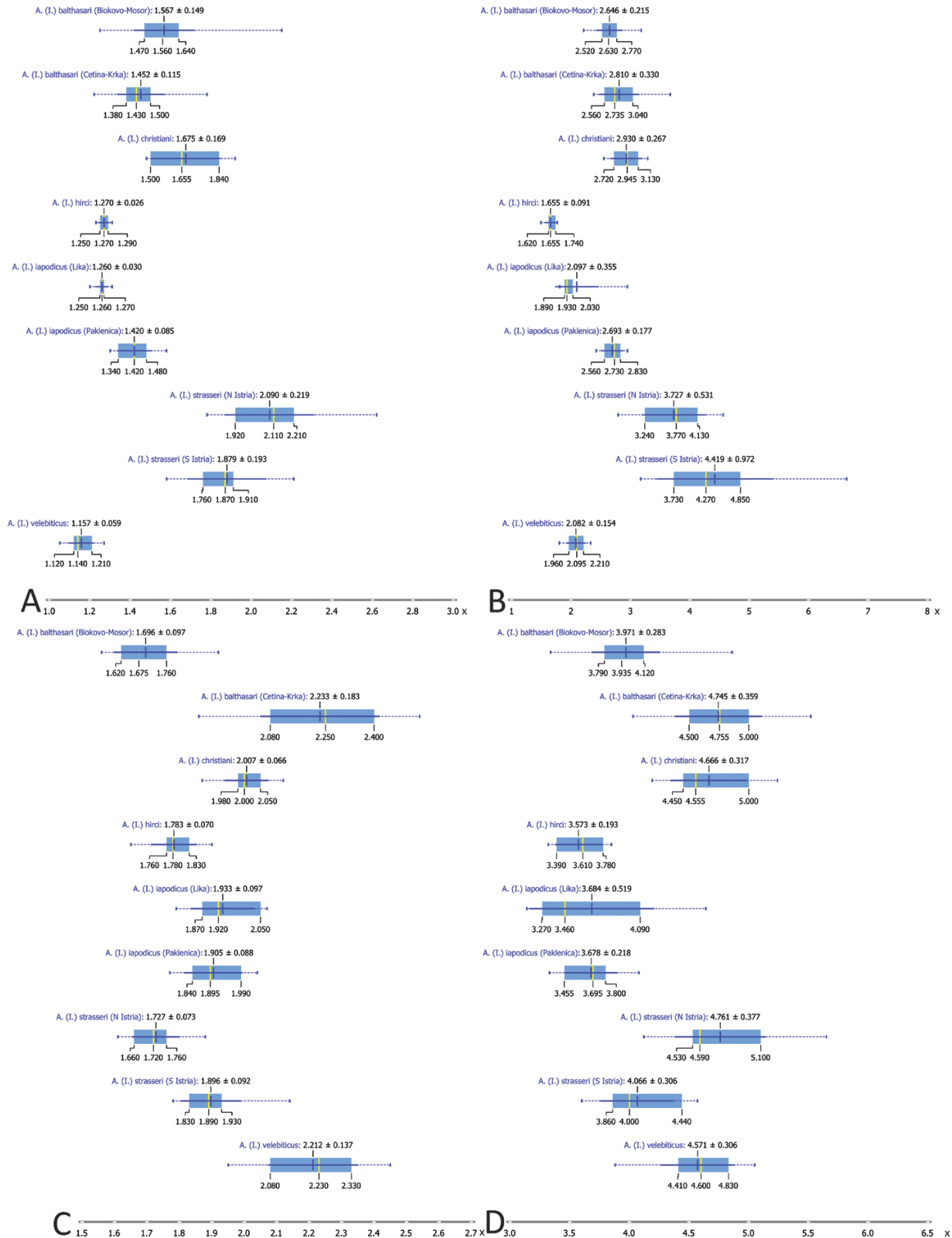
IT1652, IT1653, IT2092, IT2093, IT2409, IT2410, IT3654, IT2478); Crikvenica, Dramalj, Vrtare male (cave) (MZUF 9880, CBSSC IT325, IT3571, IT3573, IT3574, IT3577, IT3579, IT3580, IT3581).

**Redescription:** Maximum length: ♂, 5.3 mm; ♀, 8.0 mm. Pereon with almost parallel sides (Fig. 13A). Back smooth, with some triangular scale-setae. Some gland pores on lateral margins of pleonites 4 and 5 and uropodal protopods (Fig. 13C). Cephalon (Fig. 13B) with suprantennal line bent downwards, rounded middle part; antennal lobes broadly rounded. Posterior margin of pereonite 1 convex, of pereonites 2 and 3 straight, and of pereonites 4–7 progressively more concave. Pleonites 3–5 with small posterior points visible in dorsal view (Fig. 13C). Distal part of telson with concave sides and broadly rounded apex (Fig.

13C). Antennula (Fig. 13D) distal article flattened and bearing four to eight aesthetascs on apical margin. Antenna (Fig. 13E) smooth; flagellum of seven to nine articles, with one row of aesthetascs on two to four articles, always present on second and third. Mouthparts as in *A. strasseri*. Pereopods (Fig. 14A) similar in shape, with dactylar seta long and bifid. Uropod (Fig. 13C) with protopod slightly grooved on outer margin; endopod inserted proximally.

**Male:** Pereopod 1 (Fig. 14A) carpus bearing five to six setae. Pereopod 1–2 carpus and merus bearing numerous short scales on rostral surface. Pereopod 1–6 merus with sternal margin from straight (pereopod 1) progressively up to slightly concave (pereopod 6) and with a small lobe in proximal part increasing in size from pereopod 2 to pereopod 6. Pereopod 7 (Fig. 14B,





**Figure 12.** A, ratio of male pereopod 7 carpus length to hump ending point position. B, ratio of male pereopod 7 carpus length to hump top position. C, ratio of male pleopod 1 exopod length to turning point position. D, ratio of male pleopod 1

C) merus with slightly concave sternal margin and small hump-shaped lobe with three to five teeth in proximal part bearing one seta; carpus with straight sternal margin and large rounded dorsal hump in proximal part; ratio of carpus length to top hump position  $2.93 \pm 0.27$  ( $N = 5$ ), and ratio of carpus length to ending hump position  $1.68 \pm 0.17$  ( $N = 5$ ). Pleopod 1 (Fig. 14D) exopod with pointed or narrowly rounded posterior apex, ratio of length to posterior apex width  $4.67 \pm 0.32$  ( $N = 5$ ), deeply concave outer margin, ratio of length to concavity turning point position  $2.01 \pm 0.07$  ( $N = 5$ ), slightly concave inner margin; endopod narrow, slightly enlarged at base, apical seta plumose. Pleopod 2 (Fig. 14E) exopod triangular with concave outer margin; endopod distinctly longer than exopod, articles subequal in width, terminal seta strong.

*Type locality:* Höhle »Vitezičeva pećina« bei Rudine, Omišalj, Insel Krk (Potočnik, 1983) [presently known as Biserujka (cave), Rudine, Čižići, Island of Krk, Croatia].

*Remarks:* *Alpioniscus christiani* is the smallest species in the *strasseri*-lineage. It differs from all other species of the lineage (Fig. 12; Table 1) in having the lobe of the proximal part of the male pereopod 7 merus with teeth and the posterior apex of the male pleopod 1 exopod pointed instead of rounded. It differs in the ratio of the male pereopod 7 carpus length to carpus top hump position from *A. hirci* sp. nov. ( $1.66 \pm 0.09$ ) and *A. velebiticus* sp. nov. ( $2.08 \pm 0.15$ ); in the ratio of the male pereopod 7 carpus length to ending tergal hump from *A. velebiticus* sp. nov. ( $1.16 \pm 0.06$ ), *A. iapodicus* s.s. ( $1.26 \pm 0.03$ ) and *A. hirci* sp. nov. ( $1.27 \pm 0.03$ ); and in the ratio of the male pleopod 1 exopod length to posterior apex width from *A. hirci* sp. nov. ( $3.57 \pm 0.19$ ).

The citation with '?' is doubtful, and the material from this locality needs to be re-examined with both molecular and morphological analyses.

*Distribution:* Croatian island of Krk and Dramalj surroundings (Fig. 4).

*ALPIONISCUS (ILLYRIONETHES) BALTHASARI*  
(FRANKENBERGER, 1937)  
(FIGS 12, 15–19; TABLE 1)

*Titanethes albus* – Girometta, 1913: 13–16. – 1914: 3–8 [nec Špilja na Bardarovici (Vis Island); jama Propod (Hvar Island)]. – Langhoffer, 1915a: 19–20 [nec Ledenica kod Lokava; Mladenova pećina; Vražić-pećina;

Ledenica; Pustinja; Vrlovka; Vražić-pećina; pećina Jezero; Baračeva pećina II; Zobenica-pećina; Šupljara].

*Illyrionethes balthasari* Frankenberger, 1937: 175–176, figs 1–6. – 1938: 26. – 1939: 99. – Frankenberger & Strouhal, 1940: 449. – Strouhal, 1938: 273–274. – 1939b: 116; 119–122, figs 1, 2. – 1939c: 7, 22, 25, 28, 31. – 1939d: 17–18. – 1940a: 94. – Flasarová, 1967: 203–205, figs 12–14.

*Alpioniscus (Illyrionethes) balthasari* – Vandel, 1946: 155. – Buturović, 1955: 131–133. – 1957: 7–49; fig. 35. – Schmölzer, 1965: 59; fig. 221. – Karaman, 1966: 6–7. – Potočnik, 1993: 83, 178. – Tabacaru, 1996: 35. – Horvatović, 2014: 125, 173–175, 223; figs 7.28.b, 7.33.

*Alpioniscus balthasari* – Vandel, 1947: 271. – Potočnik, 1989: 64. – Erhard, 1997: 3. – Schmalfuss, 2003: 13. – Bedek et al., 2006: 34, 92, 127. – Bedek, 2007: 411–420. – Bedek et al., 2011: 237–240, 259–266; fig. 9 [nec Manita peć]. – 2017: 213 [nec eastern part of Mt. Velebit]. – Jalžić et al., 2007b: 498 [nec Velika Paklenica]. – 2010: 20, 104–105. – 2013: 17, 30. – Ozimec et al., 2012: 181, 217. – Stoev et al., 2013: 20. – Senckenberg, 2018.

*Alpioniscus* sp. – Hlaváč et al., 2019: 92, 97, 98.

? *Alpioniscus (Illyrionethes) balthasari* – Horvatović, 2014: 125 [partim: Milića špilja].

? *Alpioniscus balthasari* – Bedek et al., 2011: 260–266 [partim: Čude; Rastovača; Velika pećina; Kusa nad Manastirskim lukama; Kad jaganjci utihnu; Ivina jama; Pisanica; Bušljeta; Jama kod Jatara 2; Jatara; Vaganka; Gornja Cerovačka špilja].

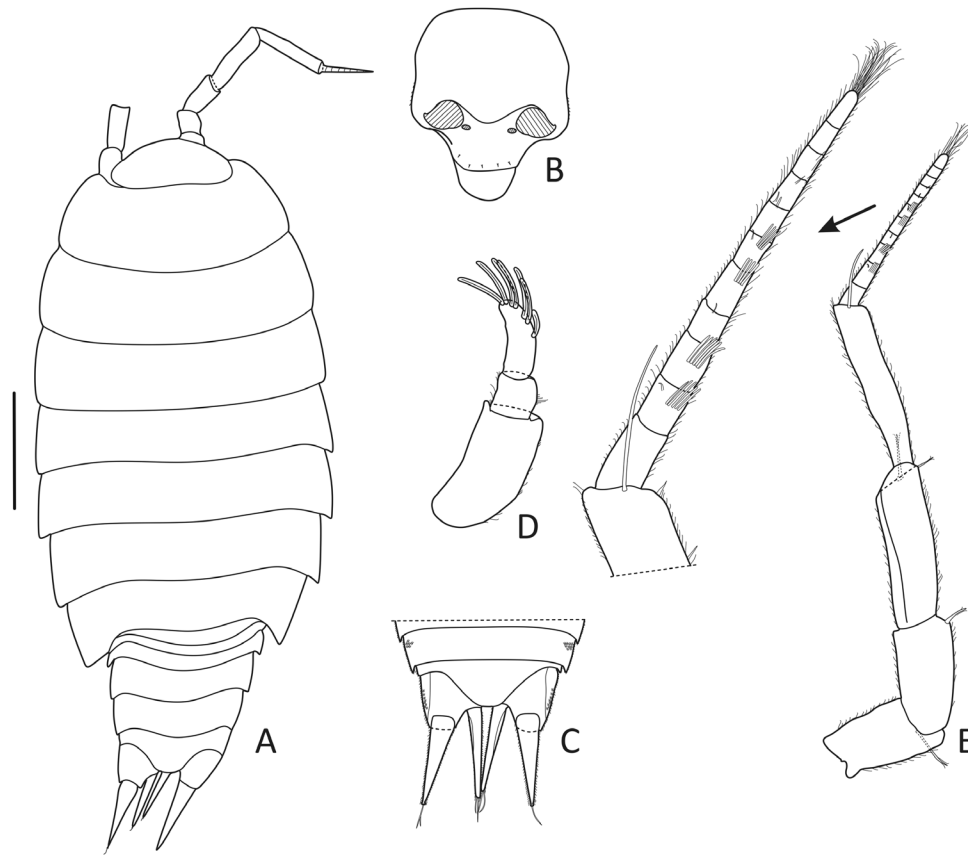
*Material examined (collection details in Supporting Information, Table S1)*

*Cetina–Krka population:* CROATIA. **Dalmatia, Vrljika:** Civljane, Kotluša, Kotluša (cave) (CBSSC IT1599, IT1602, IT1603, IT1601, IT1600, IT1654, IT1655, IT4028); Glavaš, Klačina draga, Babića pećina (cave) (CBSSC IT4041); Kijevo, Golubinjska kod Validžića (cave CCC HR00595) (CBSSC IT4040); Civljane, Tutići, Gornja Tutićeva špilja (cave) (CBSSC IT1616); Civljane, Četnici, Gospodska špilja (cave) (SMNS 5365, 5348; CBSSC IT1605, IT1607, IT1604, IT1606, IT1608, IT1609, IT4031, IT4032, IT4030, IT4029); Dinara Mt., Glavaš, Pekasova draga, Pećine (cave) (CBSSC IT1618); Vukovići, Civljane, Rudelića špilja (cave) (CBSSC IT1610, IT1611, IT1612); Koljane, Špilja ispod manastira Dragović (cave CCC HR00604) (CBSSC IT4038, IT4039); Kijevo, Velika Čulumova pećina (cave CCC HR01591) (SMNS 5356; CBSSC IT1656). **Dalmatia, Krka surroundings:** Kistanje, Lalići, Lalića stanovi, Drenovača (cave) (CBSSC IT2100, IT278, IT280, IT281, IT2099, IT2101, IT2102, IT2103, IT2873); Skradin, Golubinka (cave) (CBSSC

exopod length to apex width. The edge of each box marks the 25th and 75th percentiles; the whiskers show the largest and smallest values; yellow line indicates the median; and thick blue lines indicate the mean and SD.

**Table 1.** Differential characters of the *strasseri*-lineage species

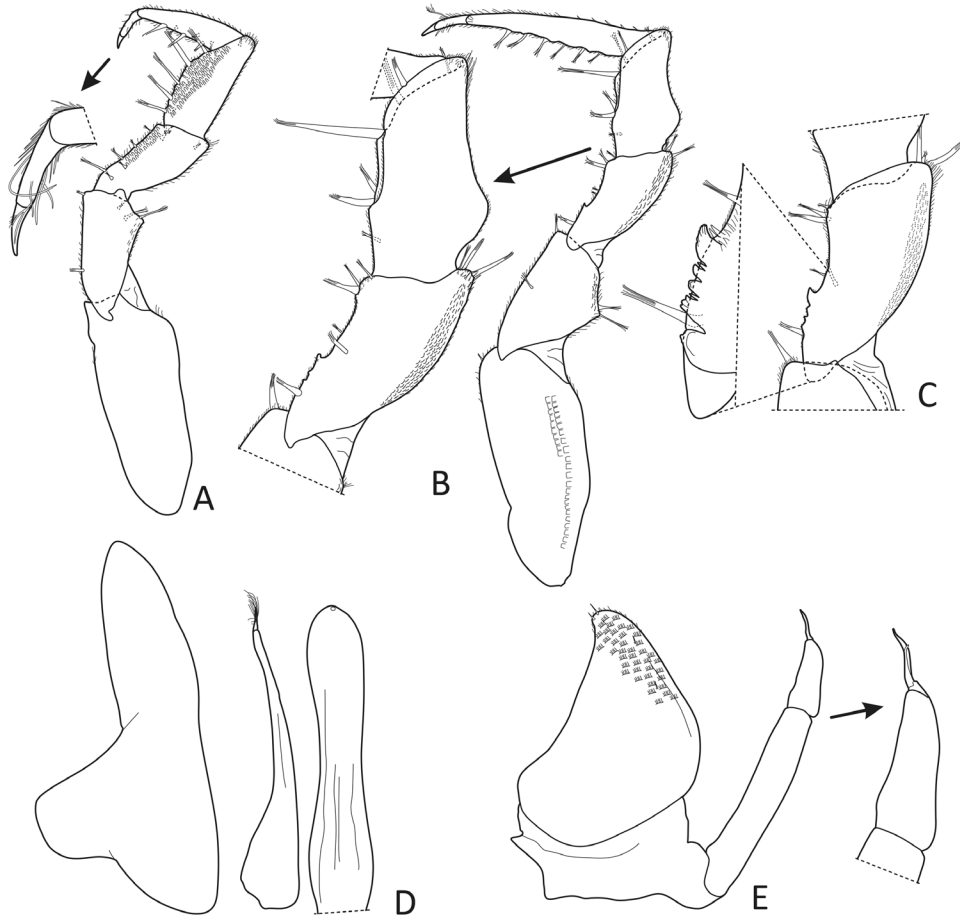
	<i>Alpioniscus strasseri</i> (N Istria)	<i>Alpioniscus strasseri</i> (S Istria)	<i>Alpioniscus christiani</i>	<i>Alpioniscus balthasari</i> (Cetina-Krka)	<i>Alpioniscus balthasari</i> (Biokovo-Mosor)	<i>Alpioniscus ipadicus</i> (Liika)	<i>Alpioniscus ipadicus</i> (Paklenica)	<i>Alpioniscus hirei</i> sp. nov.	<i>Alpioniscus vebititicus</i> sp. nov.
Ultimate body length [♂; ♀ (mm)]	5.8; 8.0	5.5; 7.5	5.3; 8.0	6.5; 7.8	7.5; 10.7	6.9; 8.5	6.5; 7.5	6.7; 7.4	6.5; 8.6
Male pereopod 7 merus	Hook	Hump	Hump with teeth	Hook	Hook	Hook	Hook	Hook	Hook
Male pereopod 7 carpus	Large Ventral Rounded	Small Ventral Rounded-pointed	Small Ventral Rounded	Medium Ventral Rounded	Medium Ventrolateral Straight-rounded	Small Lateral Straight-rounded	Medium Ventral Rounded	Medium Ventral Straight-rounded	Large Ventral Rounded
Hump	Tergal	Tergal	Tergal/tergolateral	Tergal	Tergal	Tergal/tergolateral	Tergal	Tergal	Tergal
Hump distally protuberant	-	-	-	-	-	-	-	+	-
Length/hump top (range; mean ± SD)	2.79-4.56 3.73 ± 0.53	3.17-6.64 4.42 ± 0.97	2.55-3.29 2.93 ± 0.27	2.38-3.67 2.81 ± 0.33	2.21-3.18 2.65 ± 0.22	1.81 ± 2.95 2.10 ± 0.36	2.42-2.95 2.69 ± 0.18	1.49-1.77 1.66 ± 0.09	1.80-2.33 2.08 ± 0.15
Length/hump ending (range; mean ± SD)	1.78-2.62 2.09 ± 0.22	1.58-2.21 1.88 ± 0.19	1.48-1.92 1.68 ± 0.17	1.22-1.78 1.45 ± 0.12	1.25-2.15 1.57 ± 0.15	1.20-1.31 1.23 ± 0.03	1.30-1.58 1.42 ± 0.09	1.23-1.31 1.27 ± 0.03	1.05-1.27 1.16 ± 0.06
Apex	Narrowly rounded	Narrowly rounded	Pointed/narrowly rounded	Narrowly rounded	Broadly rounded	Broadly rounded	Medium rounded	Broadly rounded	Narrowly rounded
Length/apex width (range; mean ± SD)	4.12-5.65 4.76 ± 0.38	3.60-4.57 4.07 ± 0.31	4.19-5.24 4.67 ± 0.32	4.03-5.52 4.75 ± 0.36	3.34-4.86 3.97 ± 0.28	3.14-4.64 3.68 ± 0.52	3.33-4.08 3.68 ± 0.22	3.32-3.85 3.57 ± 0.19	3.88-5.05 4.57 ± 0.31
Length/turning point (range; mean ± SD)	1.61-1.88 1.73 ± 0.07	1.78-2.14 1.90 ± 0.09	1.87-2.12 2.01 ± 0.07	1.86-2.54 2.23 ± 0.18	1.56-1.92 1.70 ± 0.10	1.79-2.07 1.93 ± 0.10	1.77-2.04 1.91 ± 0.09	1.65-1.90 1.78 ± 0.07	1.95-2.45 2.21 ± 0.14



**Figure 13.** *Alponiscus (Illyrionethes) christiani*. A, topotype ♀ CBSSC IT2629, from Biserujka: specimen in dorsal view (scale bar: 1 mm). B, C, topotype ♀ CBSSC IT1653, from Biserujka: B, cephalon, dorsal; C, pleonite 5, telson and uropods. D, E, topotype ♂ CBSSC IT1652, from Biserujka: D, antennula; E, antenna, with enlargement (arrow) of flagellum.

IT2221); Benkovac, Bjelina, Golubinka kod Bjeline (cave CCC HR01468) (CBSSC IT3955); Kistanje, Grulovići, Golubnjača u Grulovićima (cave CCC HR00250) (CBSSC IT2857, IT2858, IT2859); Oklaj, Ljubotić, Jagodnjak (cave) (CBSSC IT2871, IT2872); Drniš, Laškovića, Roški slap, Jama na Bibnovcu (cave CCC HR01364) (CBSSC IT2839, IT2833, IT2838); Drniš, Laškovića, Roški slap, Jama na Selinama (cave CCC HR01290) (CBSSC IT2875); Drniš, Ključ, kanjon Čikole, Torak, Jama nasuprot Torka (cave CCC HR01264) (CBSSC IT2222, IT3986); Oklaj, Puljanje, Jazinka (cave) (CBSSC IT2204, IT2205, IT2868, IT2869); Drniš, Ključ, Kaverna u kanjonu Čikole (cave) (CBSSC IT2206, IT2207); Dalmatia, Vrlika, Jare, Crvene grede, Kranjica (cave) (CBSSC IT1617); Oklaj, Ljubotić, Makeljina jama (cave) (CBSSC IT2876, IT3978); Skradin, Martina jama (cave CCC HR01367) (CBSSC IT305); Oklaj, HE Miljacka, Miljacka II (cave) (CBSSC IT3412, IT298, IT300, IT301, IT3065, IT2156, IT2157, IT2159, IT2162, IT2163, IT2820, IT3982); Oklaj, HE Miljacka, Miljacka I-V (cave) (CBSSC IT288,

IT289, IT2212, IT2213, IT2224); Oklaj, Bogatići, Roški slap, Oziđana pećina (cave CCC HR01356) (CBSSC IT2210, IT2211, IT3984, IT3985); Kistanje, Prčova pećina (cave CCC HR01306) (CBSSC IT284); Kistanje, Burze, Sedrena špilja iza mlina (cave CCC HR01269) (CBSSC IT3140, IT2192, IT2194, IT2195, IT2847); Kistanje, Burze, Sedrena špilja kod stola (cave CCC HR00244) (CBSSC IT2201, IT2202, IT2203, IT2853); Kistanje, Burze, Sedrena špilja s jamskim ulazom (cave CCC HR00245) (CBSSC IT2196, IT2197, IT2198, IT2199, IT2200); Konjevrate, Gornji Krnjići, Punčka draga, Stara jametina (cave CCC HR01349) (CBSSC IT303, IT304, IT2177, IT2178, IT2179, IT2180, IT2181, IT2182, IT2829, IT2830, IT3990, IT3991); Konjevrate, Škarići, Škarin Samograd (cave CCC HR00246) (CBSSC IT286, IT3010, IT302, IT287, IT2885, IT2886); Skradin, Gornje Grahovo, Visovac, Špilja ispod Vukovića poda (cave) (CBSSC IT2878, IT2884); Skradin, Špilja iznad Skradinskog mosta (cave CCC HR01550) (CBSSC IT2225); Oklaj, HE Miljacka, Špilja kod mlina na Miljacki (cave) (CBSSC IT293, IT3061,



**Figure 14.** *Alpioniscus (Illyrionethes) christiani*. Topotype ♂ CBSSC IT1653, from Biserujka: A, pereopod 1 with enlargement (arrow) of dactylus; B, pereopod 7 rostral view with enlargement (arrow) of merus and carpus; C, pereopod 7 merus caudal view; D, genital papilla and pleopod 1; E, pleopod 2 with enlargement (arrow) of endopod distal article.

IT294, IT2214, IT2216, IT2228); Skradin, HE Jaruga, Špilja u Skradinskom buku (cave CCC HR01232) (CBSSC IT2208, IT2209); Drniš, Ključ, kanjon Čikole, Štrikinica (cave CCC HR01191) (CBSSC IT2219, IT2220); Kistanje, Lalići, Lalića ograde, Tavnica (cave CCC HR01579) (CBSSC IT274, IT275, IT277); Drniš, Ključ, Kanjon Čikole, Topla pećina (cave CCC HR01217) (CBSSC IT290, IT291, IT292, IT2184, IT2186, IT2189, IT3987, IT3988); Kistanje, Lalići, Lalića ograde, Trišića jama (cave) (CBSSC IT282, IT283, IT315, IT2874, IT3981, IT3979, IT3980); Kistanje, Burze, Trogrla (cave CCC HR00248) (CBSSC IT2217, IT2218).

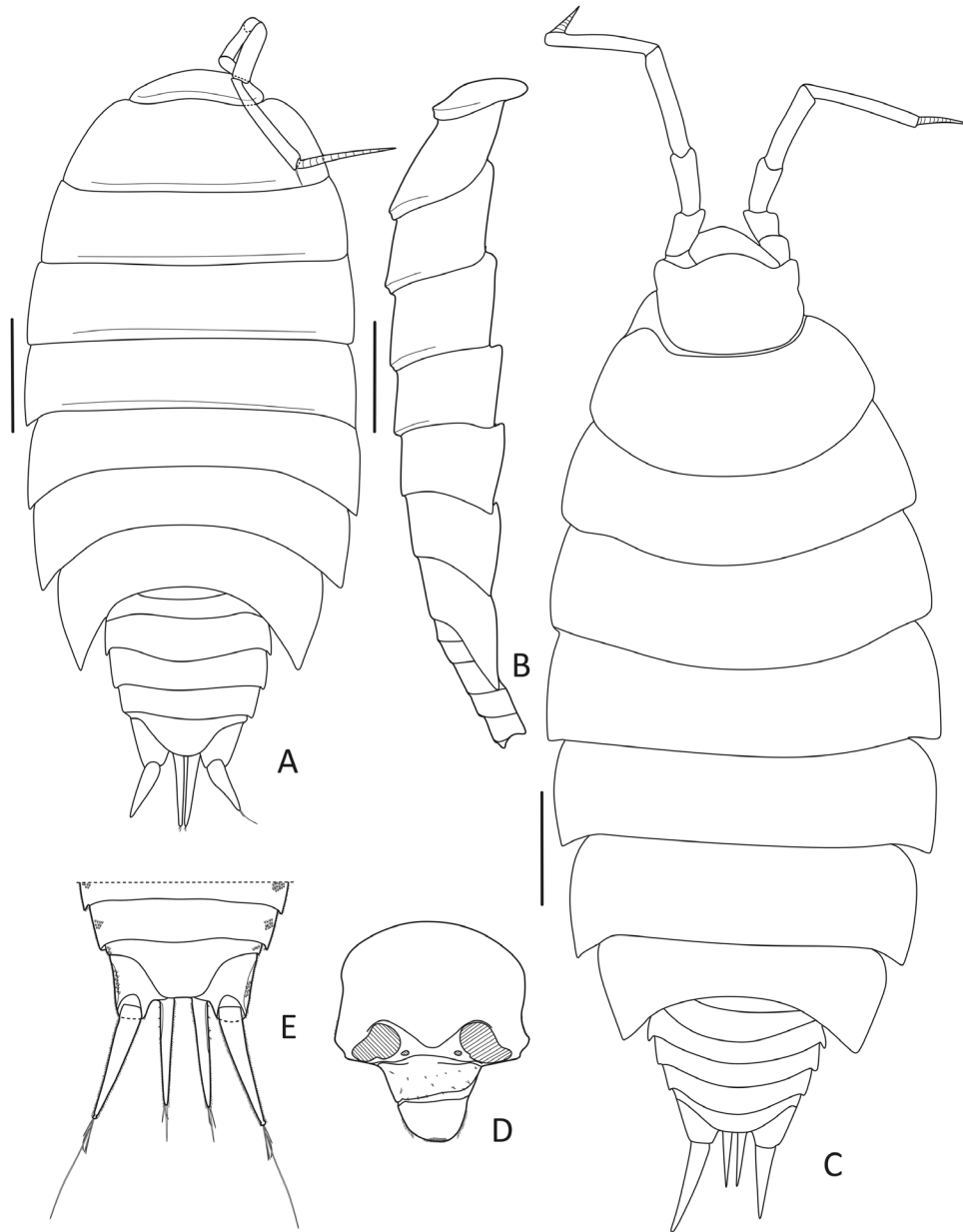
**Biokovo–Mosor population:** CROATIA. **Dalmatia, Biokovo Mt.:** Podgora, Šošići, Štedovac, Baba (cave) (CBSSC IT2540); Zagvozd, Gornje Raščane, Bebeci, Baba pećina (cave) (CBSSC IT3813); Zavodi, Ravna vlaška, Benova jama (cave) (CBSSC IT851); Župa, Lozovci, Bošak (cave) (CBSSC IT213, IT466); Makarska, Veliko Brdo, Bubnjavača (cave CCC HR00361) (CBSSC

IT495, IT3171, IT3173, IT2304, IT2480); Zagvozd, Svaguša, Čelar (a cave unknown at present) (SMNS 5273); Bartulovići, Grljak, Čulića jama (cave) (CBSSC IT85, IT86); Gornja Brela, Bartulovići, Drinova, Drinova II (cave) (CNHM 3/1417, CBSSC IT66, IT949, IT2264, IT3824); Zagvozd, Kaoci, Duboka trepetljika (cave) (CBSSC IT92); Zagvozd, Gačeše, Gaćina jama (cave) (CNHM 5/1677); Gornja Brela, Bartulovići, Grljak, Gornja jama na Grljaku (cave) (CBSSC IT215); Župa, Gradska spila (cave CCC HR00362) (CNHM 425; CBSSC IT2799); Sv. Jure, Lipi Dočić, Jama iznad Lipog dočića (cave) (CBSSC IT67); Gornja Brela, Nevistine stine, Jama iznad Prozorčića (cave) (CBSSC IT83, IT884, IT82, IT246, IT472); Kozica, Saranač, Jama iznad Saranača (cave CCC HR00798) (CBSSC IT99, IT3187, IT3149); Zagvozd, Svaguša, Jama kod Svaguše (a cave unknown at present) (SMNS 5353); Tučepi, Ravna vlaška, Jama kod Tučepške vilenjače (cave CCC HR00802) (CBSSC IT847); Prisika, Jama na grebenu (cave) (CBSSC IT69, IT70, IT868); Topići, Brezdan, Jama na Brezdanu

(cave) (CBSSC IT260, IT223); Kozica, Saranač, Jama na Docima (cave) (CBSSC IT89, IT90, IT918, IT895, IT3154); Zagvozd, Kačka glavica, Kaoci, Jama na Kačkoj glavici 3 (cave) (CBSSC IT94); Zagvozd, Dedići, Jama na Mrždolcu (cave CCC HR01853) (CBSSC IT443); Sv. Jure, Jama na putu za Staru školu (cave) (CNHM 3/1416); Sv. Jure, Jama pod Sv. Jurom (cave) (CBSSC IT849, IT850, IT3815); Makarska, Vošac, Jama pod Vošcem (cave) (CBSSC IT3144); Zagvozd, Čikeši, Jama u Čikešima (cave) (CBSSC IT490); Zagvozd, Kaoci, Jama u trepetljikama (cave) (CBSSC IT945); Podgora, Vrata Biokova, Supin, Jama za Supinom (cave CCC HR00959) (CBSSC IT867, IT241, IT459, IT2535); Nevistine stine, Jamina (cave) (CBSSC IT886, IT764); Kozica, Jujnovići, Jujnovića špilja (cave CCC HR00363) (CNHM 4/1418, (CBSSC IT865, IT483, IT484, IT3189, IT2544, IT3195); Makarska, Velo Brdo, Krjava 1 (cave) (CBSSC IT42); Makarska, Velo Brdo, Krjava 2 (cave) (CBSSC IT43, IT862); Baška Voda, Bast, Kukor (cave) (CBSSC IT11, IT41, IT830, IT832, IT229, IT225, IT228, IT508, IT501, IT503, IT750, IT751, IT3183, IT3191, IT3148, IT2270, IT2301, IT2539, IT3823); Čedor, Kuna (cave) (CBSSC IT856, IT858); Sv. Jure, Ledenica kod Stare škole (cave) (CNHM 1/1419); Župa, Mala jama (cave) (CBSSC IT97); Zagvozd, Spiljice, Kaoci, Mala jama u stijenama (cave) (CNHM 4/1676); Župa, Matijaševa peć (cave CCC HR01820) (CNHM 4/1600, CBSSC IT435, IT3184); Župa, Lozovci, Mravinjuša (cave) (CBSSC IT207); Zagvozd, Svaguša, Sridnja gora, Mužina špilja (cave) (CBSSC IT854, IT855); Topići, Pasji manastir (cave) (CBSSC IT245); Gornja Brela, Nevistine stine, Plošnica (cave CCC HR00055) (CNHM 5/1601); Podgora, Podrum (cave CCC HR00255) (CBSSC IT860, IT416, IT417); Dedići, Rastovac, Potkameničina jama (cave CCC HR00261) (CBSSC IT756); Gornja Brela, Ražanj, Pozjata (cave) (CBSSC IT88); Makarska, Lokve, Pretnerova jama (cave) (CBSSC IT3804; MZUF 9879); Zagvozd, Prodani, Pružina (cave) (CBSSC IT864, IT87, IT897, IT3806); Zagvozd, Kaoci, Pukotina kod doca Stražbenica (cave) (CNHM 7/1414; CBSSC IT899); Zagvozd, Rastovac, Raputinova špilja (cave) (CBSSC IT444, IT447, IT450); Gornja Brela, Drinova, Spasiteljica (cave) (CBSSC IT925, IT915); Kozica, Jujnovići, Spila, Spila 1 (cave) (CBSSC IT905, IT3817, IT3819); Kozica, Jujnovići, Spila, Spila 2 (cave) (CNHM 3/1678; CBSSC IT84, IT907, IT206, IT256, IT3186, IT2315, IT3816); Župa, Rogliči, Stonjska peć donja (cave CCC HR00264) (CBSSC IT96, IT3803); Župa, Rogliči, Stonjska peć gornja (cave) (CBSSC IT95, IT2803); Gornja Brela, Dubci, Svetica (cave) (CBSSC IT487); Zagvozd, Katušići, Špilja bei Katušići (a cave unknown at present) (SMNS 5346); Zagvozd, Kaoci, Špilja do Obilježene (cave) (CBSSC IT943); Zagvozd, Dedići, Sridnja gora, Špilja na Mrždolcu (cave) (CBSSC IT441, IT442); Zagvozd, Brnasi, Špilja u Gaju (cave) (CBSSC IT841, IT840); Tučepi, Srida sela, Ravna vlaška, Tučepska vilenjača (cave) (CBSSC IT757, IT758, IT759,

IT760, IT845, IT846, IT2633, IT2267, IT4086, IT4089, IT4096, IT4108; MZUF 9877, 9878); Zagvozd, Dedići, Bukovci, Velika špilja (cave) (CBSSC IT481, IT482); Zagvozd, Kaoci, Vodena trepetljika (cave) (CBSSC IT93). **Dalmatia, Mosor Mt.:** Dugopolje, Balešina jama (cave) (CBSSC IT4059, IT4060); Balići, Dugopolje, Balića špilja (cave CCC HR00478) (SMNS 5354, CBSSC IT2630, IT3046); Gornje Sitno, Jabukovac, Javor draga, Dana špilja (cave) (CBSSC IT4062); Kotelnice, Tukići, Bradarića staje, Drinovčuća (cave) (CBSSC IT2959, IT3072, IT3074, IT3117, IT3126); Dugopolje, Križani, Đuderina jama (cave) (CBSSC IT3085); Gornja Poljica, Trnbusi, Jama u Dočiću (cave) (CBSSC IT4067); Trilj, Ugljane, Jama u Krčevini (cave) (SMNS 5352); Kotelnice, Tukići, Bradarića staje, Kravska jama (cave) (CBSSC IT4045, IT4047, IT4048); Trilj, Ugljane, Majica pećina (a cave unknown at present) (SMNS 5332); Kute, Čevrljin klen, Dugopolje, Maklutača (cave) (CBSSC IT3124, IT3077, IT4050, IT4051); Žrnovnica, Miličevića pećina (cave) (CBSSC IT4056, IT4057); Kolić, Dugopolje, Peć u Čulinovim raljevinama (cave CCC HR01164) (CBSSC IT2991, IT3019, IT3137, IT3076); Dugopolje, Slipica (cave) (SMNS 5327); Ljubljana, Sniježnica pod Ljubljanom (cave) (CBSSC IT2973); Podgrade, Ščadin, Svinjarača (a cave unknown at present) (CBSSC IT4066); Gornje Poljice, Trnbusi, Špilja Jame (cave) (CBSSC IT3071); Gornji Dolac, Gornja Poljica, Trojama (cave CCC HR00875) (CBSSC IT3090, IT3100, IT3108, IT2966, IT3050, IT4064); Gornje Sitno, Velika Gajina (cave) (CBSSC IT4061); Kotlenice, Dugopolje, Vranjača (cave) (SMNS 5320, 5043; CBSSC IT2631, IT2986, IT3051, IT3045, IT3012); Palin umac, Žmirino gumno, Dugopolje, Žmirina jama (cave) (CBSSC IT4052, IT4054). **Dalmatia, Omiška Dinara Mt.:** Brela, Gornja Brela, Dovanj, Čolnjača (cave) (CBSSC IT98, IT226); Zadvarje, Slime, Jama na podu Slime (cave) (CBSSC IT3007); Zakučac, Naklice, Mandića špilja (cave) (CBSSC IT3040); Kučice, Nikolina špilja (cave) (CBSSC IT2996). **Dalmatia, Vrgorac:** Šibenik, Kozica, Antunovići, Mala špilja (cave) (CNHM 4/1415; CBSSC IT3152); Stilja, Martina jama (cave) (CBSSC IT306); Šibenik, Kozica, Antunovići, Velika špilja (cave) (CBSSC IT3192); Jurilji, Breskula u staroj paski (a cave unknown at present) (SMNS 5105).

*Redescription:* Maximum length: ♂, 6.5 mm; ♀, 7.8 mm. Pereon with almost parallel sides. Back smooth, with ridges near posterior margins of cephalon and pereonites 1–4 (Fig. 15A, B) (absent in populations from the cave Stara jametina) with some triangular scale-setae. Gland pores on lateral margins of pleonites 4 and 5, telson and uropodal protopods (Fig. 15E). Cephalon (Fig. 15D) with suprantennal line bent downwards, rounded middle part; antennal lobes quadrangular with central depression. Posterior margin of pereonite 1 convex, of pereonites 2 and 3

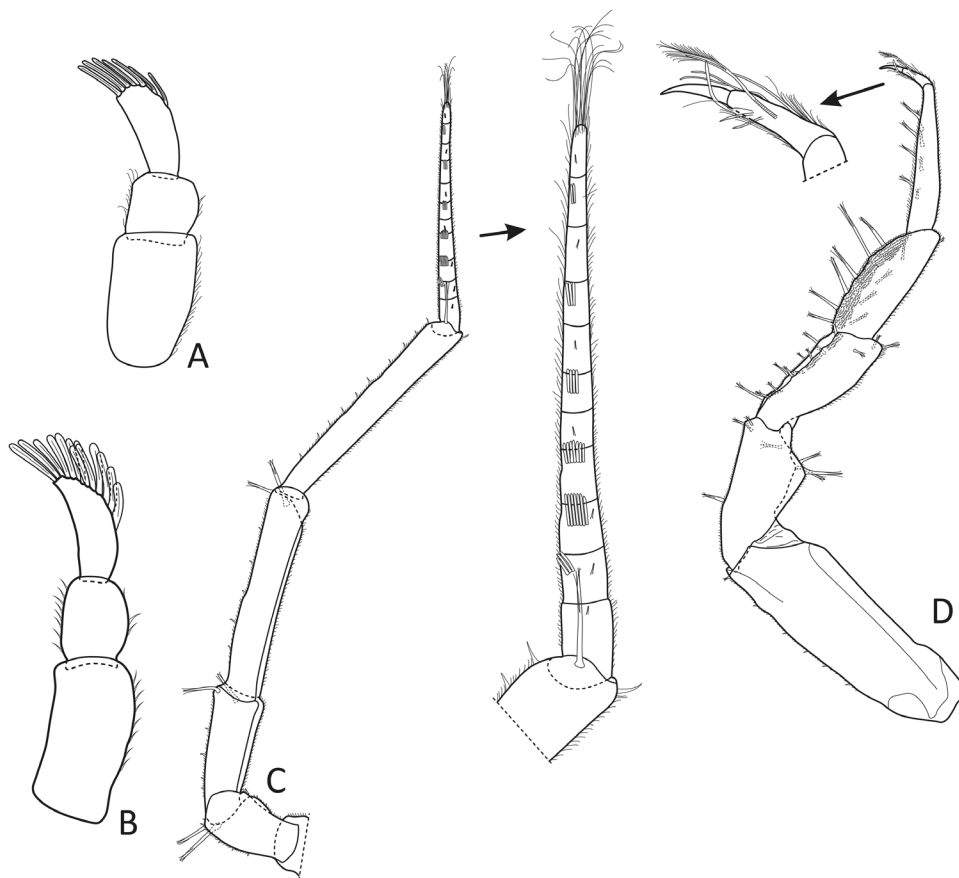


**Figure 15.** *Alponiscus (Illyrionethes) balthasari*. A, B, topotype ♀ CBSSC IT1601, from Kotluša: A, specimen in dorsal view (scale bar: 1 mm); B, pereonites and pleonites in lateral view (scale bar: 1 mm). C, ♂ CBSSC IT2629, from Tučepska vilenjača: specimen in dorsal view (scale bar: 1 mm). D, E, topotype ♂ CBSSC IT 1654/1, from Kotluša: D, cephalon, dorsal; E, pleonite 5, telson and uropods.

straight, and of pereonites 4–7 progressively more concave. Pleonites 3–5 with small posterior points visible in dorsal view (Fig. 15A, E). Distal part of telson with concave sides and broadly rounded apex (Fig. 15E). Antennula (Fig. 16A) distal article flattened and bearing four to seven aesthetascs on apical margin. Antenna (Fig. 16C) smooth; flagellum of eight to 13 articles, with one row of aesthetascs on two to seven articles, always present on second and third.

Mouthparts as in *A. strasseri*. Pereopods (Fig. 16D) similar in shape, with dactylar seta large and bifid. Uropod (Fig. 15E) with protopod slightly grooved on outer margin; endopod inserted proximally.

*Male:* Pereopod 1 (Fig. 16D) carpus bearing five to seven setae. Pereopod 1–3 with carpus and merus bearing numerous short scales on rostral surface. Pereopod 1–6 merus with sternal margin from straight



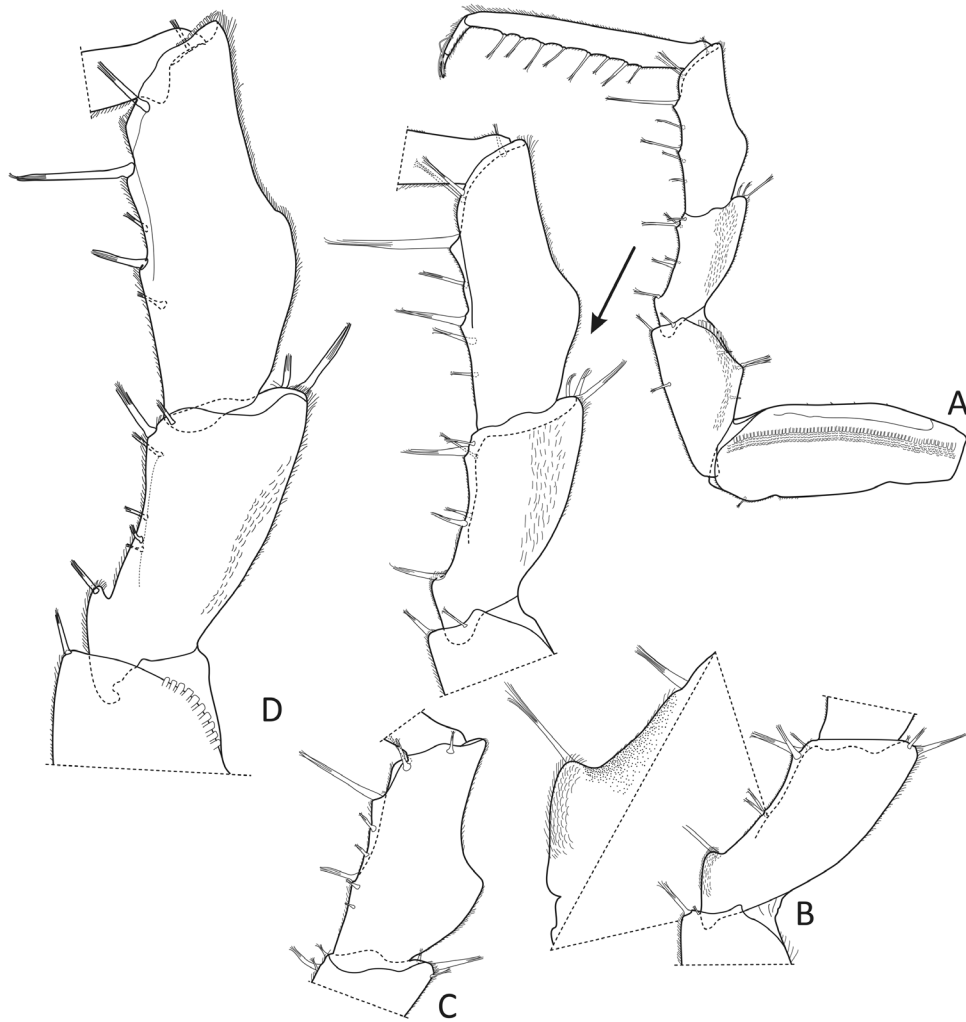
**Figure 16.** *Alphoniscus (Illyrionethes) balthasari*. A, C, D, topotype ♂ CBSSC IT1654/1, from Kotluša: A, antennula; C, antenna with enlargement (arrow) of flagellum; D, pereopod 1 with enlargement (arrow) of dactylus B, ♂ CBSSC IT4086, from Tučepška vilenjača: antennula.

(pereopod 1) progressively up to slightly concave (pereopod 6) and from none (pereopod 1) progressively up to small lobe (pereopod 6) in proximal part. Pereopod 7 (Fig. 17A–C) merus with slightly concave sternal margin and small hook-shaped lobe in proximal part bearing one seta; carpus with straight sternal margin and large more or less rounded dorsal hump in proximal part, ratio of carpus length to top hump position  $2.81 \pm 0.33$  ( $N = 25$ ), and ratio of carpus length to ending hump position  $1.45 \pm 0.12$  ( $N = 24$ ). Pleopod 1 (Fig. 18A) exopod with narrowly rounded posterior apex, ratio of length to posterior apex width  $4.75 \pm 0.36$  ( $N = 25$ ), deeply concave outer margin, ratio of length to concavity turning point position  $2.23 \pm 0.18$  ( $N = 25$ ), straight to slightly concave inner margin; endopod narrow with almost parallel sides, apical seta plumose. Pleopod 2 (Fig. 18C) exopod triangular with narrowly rounded posterior apex, concave outer margin; endopod articles subequal in width; terminal seta strong and bifid.

*Type locality:* ‘eine Höhle in der Umgebung der Stadt Knin; es ist dies jene Höhle, die in der Nähe des Dorfes Kotluša am westlichen Fusse des Dinara-Gebirges; »Höhle mit Wasser« (Frankenberger, 1937) [presently known as Kotluša (cave), Kotluša, Cviljane, Vrlika, Croatia].

*Remarks:* The Biokovo–Mosor population of *A. balthasari* shows some differences (Figs 12, 15C, 16B, 17D, 18B, D, 19; Table 1), as follows: the body is considerably larger (♂, 8.2 mm; ♀, 10.7 mm), without ridges near posterior margins of cephalon and pereonites; the distal article of the antennula bears seven to 14 aesthetascs on the apical margin; the male pleopod 1 exopod has a broadly rounded posterior apex, ratio of length to posterior apex width  $3.97 \pm 0.28$  ( $N = 33$ ), ratio of length to concavity turning point position  $1.70 \pm 0.10$  ( $N = 33$ ), slightly concave inner margin; endopod slightly enlarged at base; the male pleopod 2 exopod has a broadly rounded posterior apex and the endopod is distinctly longer





**Figure 17.** *Alpnioniscus (Illyrionethes) balthasari*. A, B, topotype ♂ CBSSC IT1654/1, from Kotluša: A, pereopod 7 rostral view with enlargement (arrow) of merus and carpus; B, pereopod 7 merus caudal view. C, topotype ♂ CBSSC IT1654/2, from Kotluša: pereopod 7 carpus. D, ♂ CBSSC IT4086, from Tučepska vilenjača: pereopod 7 merus and carpus rostral.

than the exopod, with the distal article narrower than the proximal. The population is geographically separated from *A. balthasari s.s.*, and also shows some molecular differences. More sampling should be conducted in regions between their distribution areas, with morphological and molecular analyses in order to validate the taxonomic status of that population.

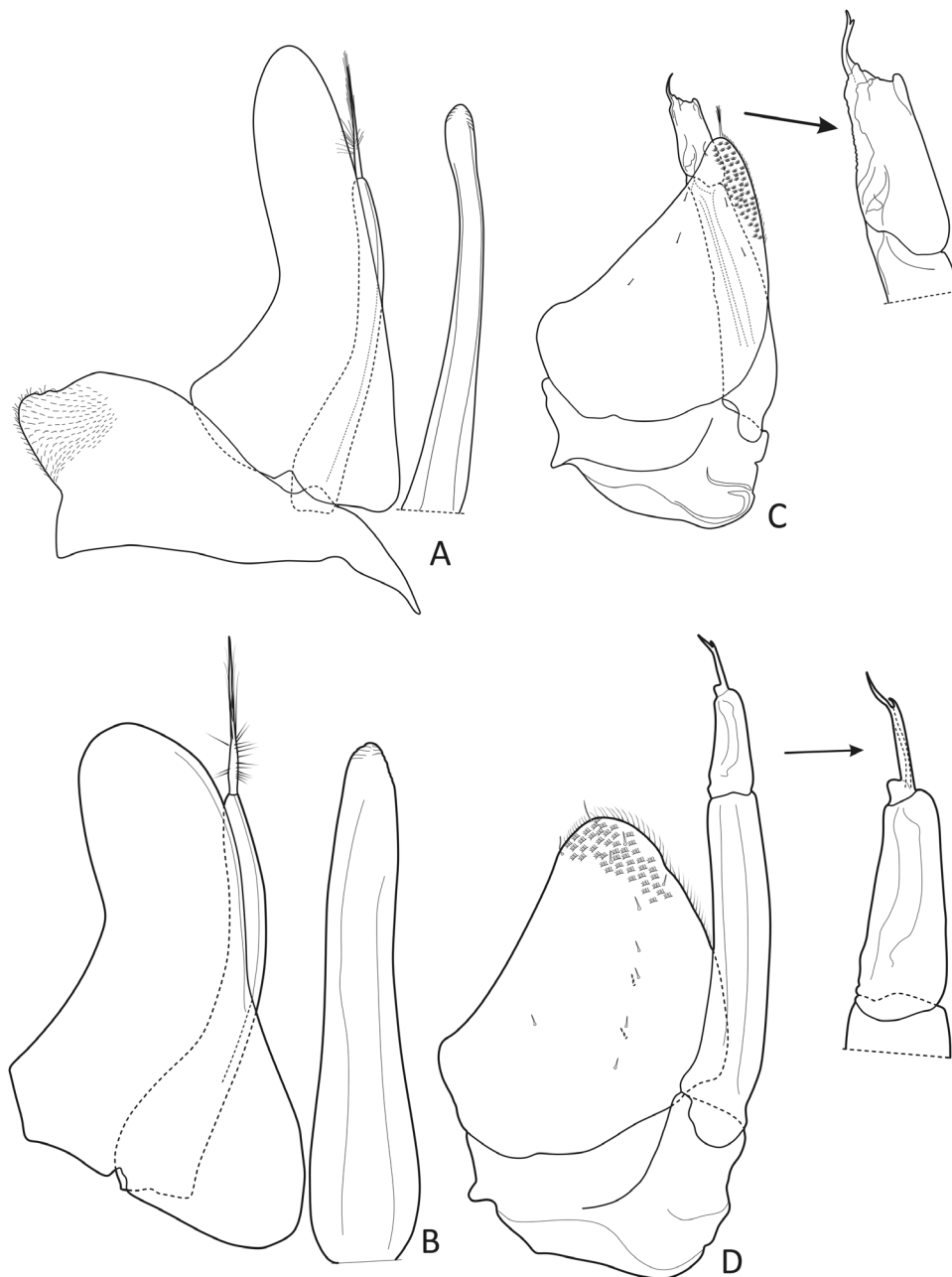
*Alpnioniscus balthasari* differs from other species of the *strasseri*-lineage (Fig. 12; Table 1) in the presence of ridges on the posterior part of cephalon and pereonites 1–4 (except the population from Stara jametina). Males differ in the ratio of the pereopod 7 carpus length to carpus top hump position from *A. hirci* sp. nov. ( $1.66 \pm 0.09$ ) and *A. velebiticus* sp. nov. ( $2.08 \pm 0.15$ ); in pereopod 7 carpus length to ending hump position from *A. velebiticus* sp.

nov. ( $1.16 \pm 0.06$ ); in the ratio of the pleopod 1 exopod length to turning point position from *A. strasseri s.s.* ( $1.73 \pm 0.07$ ); and in the ratio of the pleopod 1 length to apex width from *A. hirci* sp. nov. ( $3.57 \pm 0.19$ ) and *A. iapodicus* (Paklenica) ( $3.68 \pm 0.22$ ).

This species is amphibious, because it was also observed in water.

The citations with ‘?’ are doubtful, and the material from those localities needs to be re-examined with both molecular and morphological analyses.

**Distribution:** Croatian river Cetina source surroundings, river Krka surroundings, Biokovo Mt., Mosor Mt., Omiška Dinara Mt. and their surroundings (Figs 4, 19); Bosnia and Herzegovina, Tomislavgrad surroundings.



**Figure 18.** *Al pioniscus* (*Illyrionethes*) *balthasari*. A, C, topotype ♂ CBSSC IT1654/1, from Kotluša: A, genital papilla and pleopod 1; C, pleopod 2 with enlargement (arrow) of endopod distal article. B, D, ♂ CBSSC IT4086, from Tučepska vilenjača: B, genital papilla and pleopod 1; D, pleopod 2 with enlargement (arrow) of endopod distal article.

*ALPIONISCUS* (*ILLYRIONETHES*) *IAPODICUS* BEDEK,  
HORVATOVIĆ & KARAMAN, 2017

(FIGS 12, 20, 21; TABLE 1)

*Titanethes* (*albus*?) *dahli* – Božičević, 1965: 438  
[partim: Manita peč].

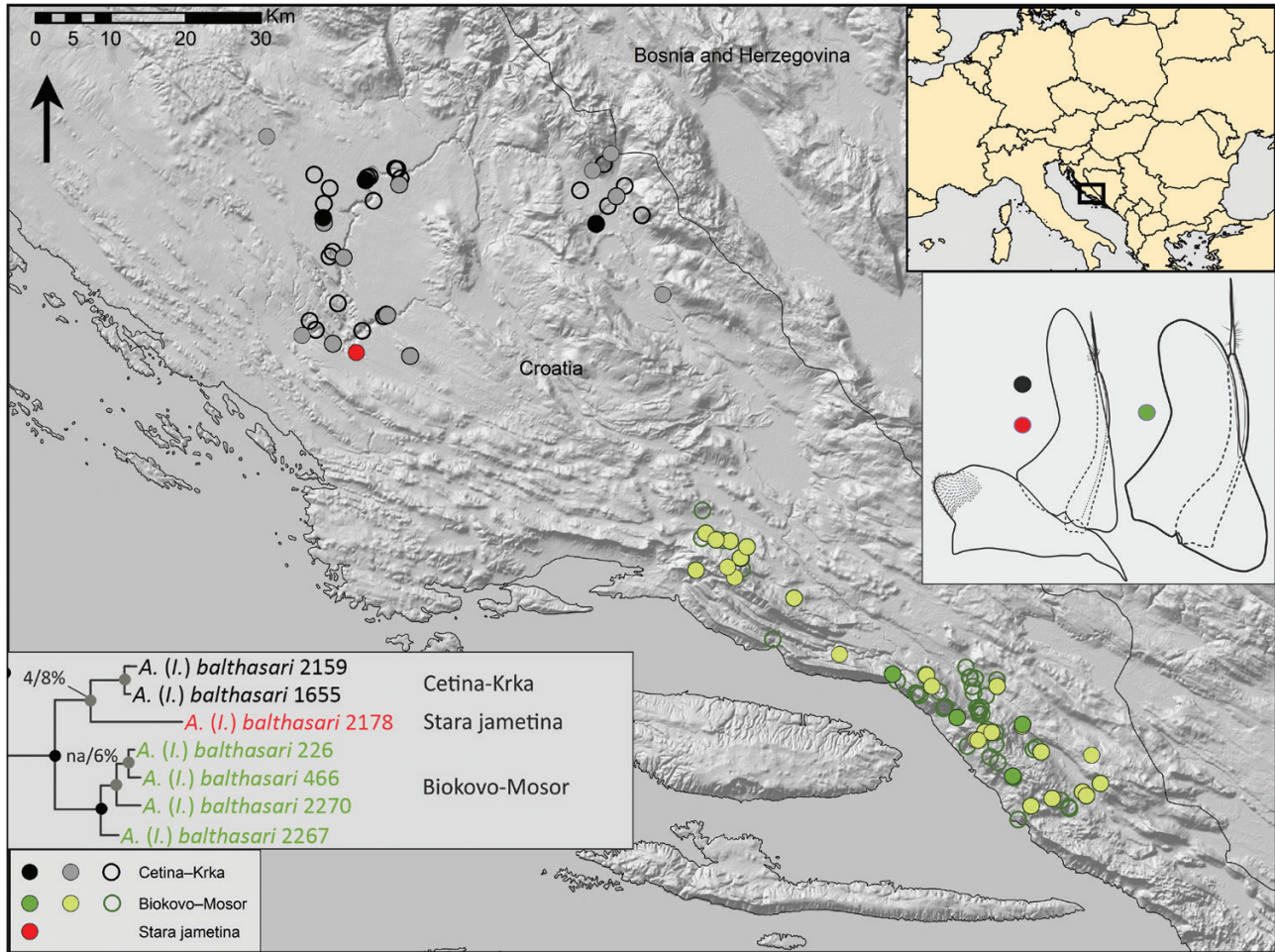
*Titanethes albus* – Langhoffer, 1912: 363 [partim:  
Medina pećina]. – 1915b: 68 [partim: Medina pećina,

Perusić környéke]. – Csiki, 1926: 73 [partim: Medina  
pećina] – Wolf, 1938: 77 [partim: Medina pećina].

*Titanthes dahli* – Strouhal, 1939b: 23 [partim:  
Medina pećina bei Perušić].

*Al pioniscus* sp. – Bregović *et al.*, 2008: 110 [partim:  
Manita Peć, Velika Paklenica].

*Al pioniscus balthasari* – Jalžić *et al.*, 2007b: 498  
[partim: Velika Paklenica]. – Bedek *et al.*, 2011: 261,



**Figure 19.** Distribution, maximum likelihood 16S tree, *COXI*/16S distances and male pleopod 1 of different populations of *Alpioniscus balthasari* (dark circles, DNA and morphological analyses; pale circles, morphological analyses; open circles, sample sites; *COXI*/16S distances are indicated on the tree; black and grey dots indicate bootstrap values of  $\geq 0.95$  and  $> 0.75$ , respectively).

fig. 9 [partim: Manita peč]. – [Bedek et al., 2017](#): 213 [partim: eastern part of Mt. Velebit].

*Alpioniscus* n. sp. 4 – [Horvatović, 2014](#): 126, 174, 223, 240, figs 7.29, 7.33 [partim: pećina pri Mušalučkom donjem selu; nekoliko pećina u Lici].

*Alpioniscus* n. sp. – [Cindrić et al., 2017](#): 51–52.

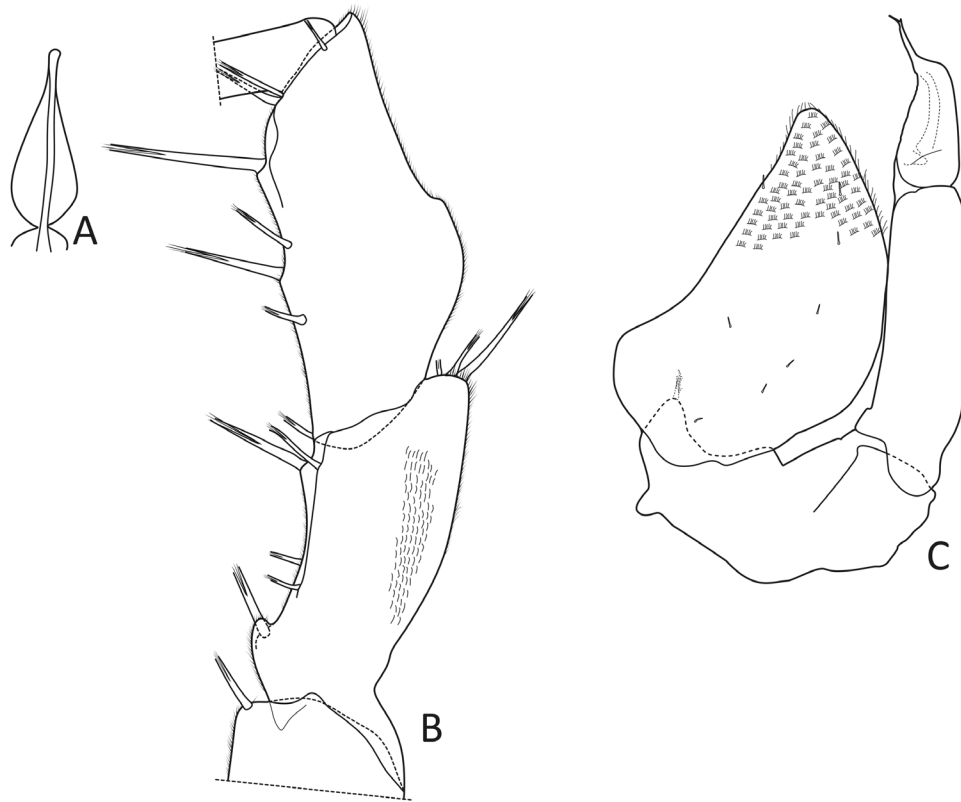
*Alpioniscus (Illyrionethes) iapodicus* [Bedek et al., 2017](#): 205–214, figs 1–7.

#### Type material re-examined (collection details in [Supporting Information, Table S1](#))

**Holotype:** ♂, Croatia, Lika, Donji Kosinj, Lipovo polje, Markov ponor (cave CCC HR01480) (CBSSC IT4109).

**Paratypes:** **Croatia, Lika:** ibid. (CBSSC IT2479; ZZDBE 1142; MZUF 9763); Ličko Lešće, Pećina (cave) (CBSSC IT3344, IT3347); Donji Kosinj, Lipovo polje, Petranović Draga, Mramorna špilja (cave CCC HR02057) (CBSSC

IT3367); Perušić, Studenci, Milkovići, Budina špilja (cave CCC HR01195) (CBSSC IT3373, IT3843, IT3848, IT3846, IT3374); Donji Kosinj, Mlakva, Javorinske drage, Pećina na Čakovcu (cave CCC HR01706) (CBSSC IT3765); Perušić, Sitvuki, Sitvukova pećina (cave CCC HR01962) (CBSSC IT3383); Perušić, Velika Plana, Rastovac, Prva poštena – Jama u Rastovcu (cave CCC HR00953) (CBSSC IT3921); Perušić, Grabovača, Medina pećina (cave) (CNHM 653; CBSSC IT2545, IT3381, IT3907, IT3382); Perušić, Grabovača, Amidžina pećina (cave) (CBSSC IT2546, IT3849, IT3850, IT3378); Pazarište, Vranovine, Vranovinski ponor (cave CCC HR00490) (SMNS 5276); Velebit, Pazarište, Japage, špilja Japa (cave CCC HR00344) (CBSSC IT544); Gospić, Mušaluk, Pećina pri mušalučkom donjem selu (present unknown cave) (ZZDBE 0899); Gospić, Klanac, Grčka pećina (present unknown cave) (SMNS 5344);



**Figure 20.** *Alponiscus (Illyrionethes) iapodicus*. A, C, paratype ♂ CBSSC IT2479, from Markov ponor: A, dorsal scale-seta; C, pleopod 2. B, ♂ CBSSC IT2958, from Manita peć: pereopod 7 merus and carpus rostral.

Pazarište, Mlakva, Mlakvena greda (present unknown cave) (SMNS 5295).

*Material examined (collection details in Supporting Information, Table S1)*

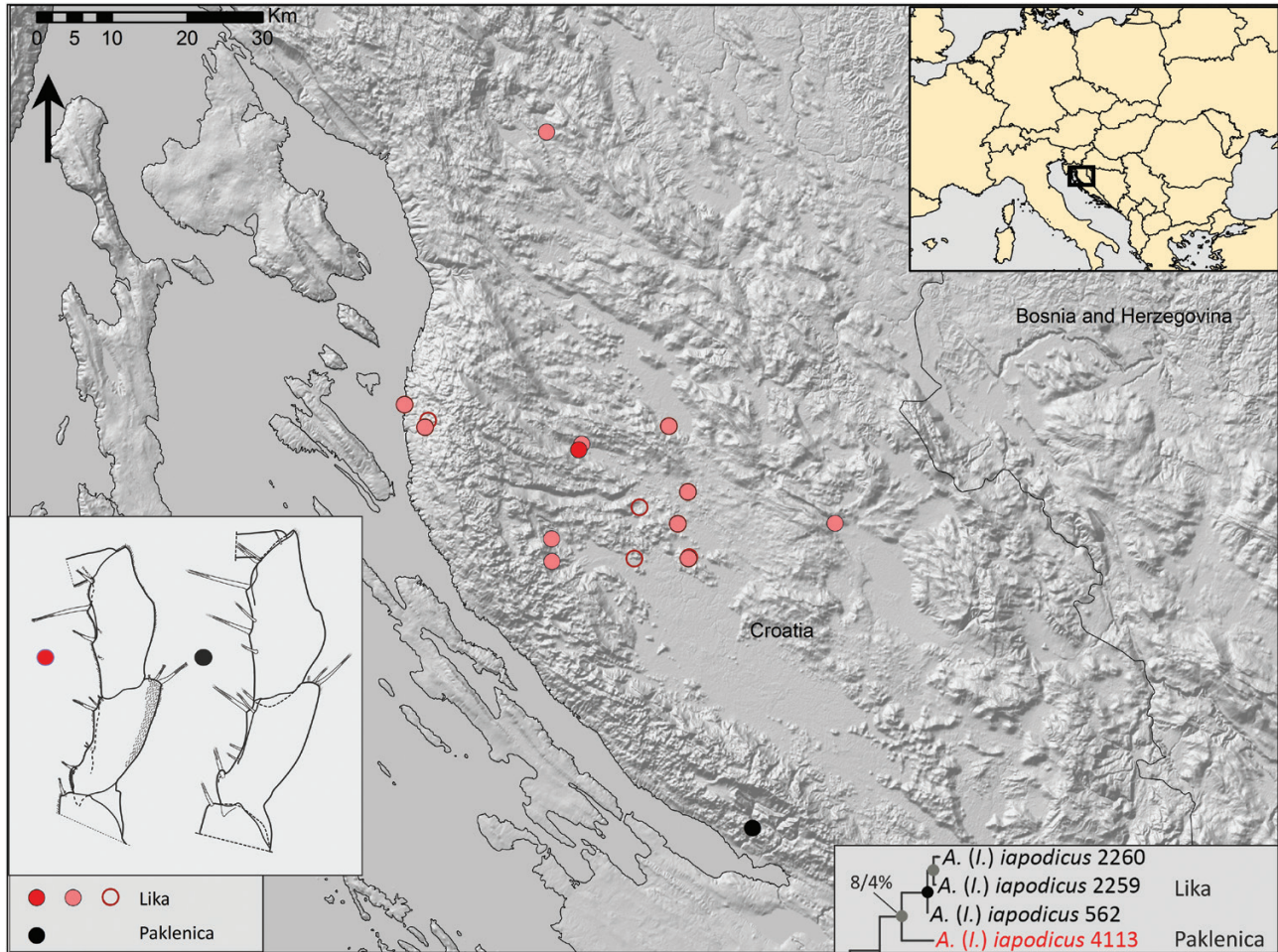
**Lika population: Croatia, Velebit Mt. (N):** Starigrad kod Senja, Velike Brisnice, Jama II kod Velikih Brisnica (cave) (CBSSC IT411, IT412, IT580, IT582, IT583, IT3112, IT3427, IT3428); Senj, Donja Klada, Jama na Krželjevcu (cave) (CBSSC IT542); Senj, Velike Brisnice, Jama na Vrančinici (cave) (CNHM 2/1669; CBSSC IT587); Donja Klada, Špilja u Vukotića dragi (cave) (CBSSC IT540, IT541, IT593). **Croatia, Lika:** Ogulin, Drežnica, Drežničko polje, Sopača (cave CCC HR01927) (CBSSC IT3252); Bunić, Zelena pećina (cave) (CBSSC IT3404).

**Paklenica population: Croatia, Velebit Mt. (S):** Starigrad Paklenica, Velika Paklenica, Manita peć (cave) (SMNS 5362, 5341; CBSSC IT14, IT15, IT1, IT562, IT563, IT2259, IT2260, IT2261, IT3211, IT2958, IT3208, IT3210, IT3212, IT3223, IT3225, IT3207, IT3221, IT3206, IT3202, IT3200, IT3218, IT3228, IT3229, IT3230, IT3231, IT3232, IT3204).

*Additional data to description:* Tergite with triangular scale-setae (Fig. 20A). Antennula distal article bearing four to nine aesthetascs on apical margin. Antennal flagellum of eight to ten articles, with one row of aesthetascs on three to five different articles, always on second and third. Male pereopod 1 carpus bearing five to seven setae. Male pereopod 7, ratio of carpus length to carpus top hump position  $2.10 \pm 0.36$  ( $N = 7$ ), and ratio of carpus length to ending hump position  $1.26 \pm 0.03$  ( $N = 7$ ). Male pleopod 1 ratio of exopod length to posterior apex width  $3.68 \pm 0.52$  ( $N = 7$ ), with deeply concave outer margin, ratio of length to concavity turning point position  $1.93 \pm 0.10$  ( $N = 7$ ), straight to slightly concave inner margin; endopod apical seta plumose. Male pleopod 2 endopod of two articles (Fig. 20C).

*Type locality:* Croatia, Lika, Donji Kosinj, Lipovo polje, Markov ponor (cave CCC HR01480) (Bedeck et al., 2017).

*Remarks:* The population from Manita peć, Paklenica (Figs 12, 20B, 21; Table 1) shows some differences from *A. iapodicus* s.s., i.e. the male pereopod 7 merus with hook-shaped lobe directed ventrally; pereopod 7 ratio of carpus length to carpus top hump position  $2.69 \pm 0.18$  ( $N = 6$ ); and pereopod 7 ratio of carpus length to ending hump position  $1.42 \pm 0.09$  ( $N = 6$ ). The population is geographically



**Figure 21.** Distribution, maximum likelihood 16S tree, *COXI*/*16S* distances and male merus and carpus of pereopod 7 of different populations of *Alpioniscus iapodicus* (dark circles, DNA and morphological analyses; pale circles, morphological analyses; open circles, sample sites; *COXI*/*16S* distances are indicated on the tree; black and grey dots indicate bootstrap values of  $\geq 0.95$  and  $> 0.75$ , respectively).

separated from *A. iapodicus* s.s., and it also shows some molecular differences. More sampling should be conducted between the type locality and Paklenica area, in order to validate the taxonomic status of this population.

*Alpioniscus iapodicus* differs from all the other species of the *strasseri*-lineage in the male characters (Fig. 12; Table 1). The hook-shaped lobe of the male pereopod 7 merus in the proximal part is directed laterally instead of ventrolaterally as in the *A. balthasari* Biokovo–Mosor population or ventrally as in all the other species. It differs in the ratio of the male pereopod 7 carpus length to ending tergal hump position from *A. strasseri* s.s. ( $2.09 \pm 0.22$ ) and *A. christiani* ( $1.68 \pm 0.17$ ).

**Distribution:** Croatian Lika and Velebit Mt. slopes (Figs 4, 21).

***ALPIONISCUS (ILLYRIONETHES) HIRCI* BEDEK & TAITI SP. NOV.**

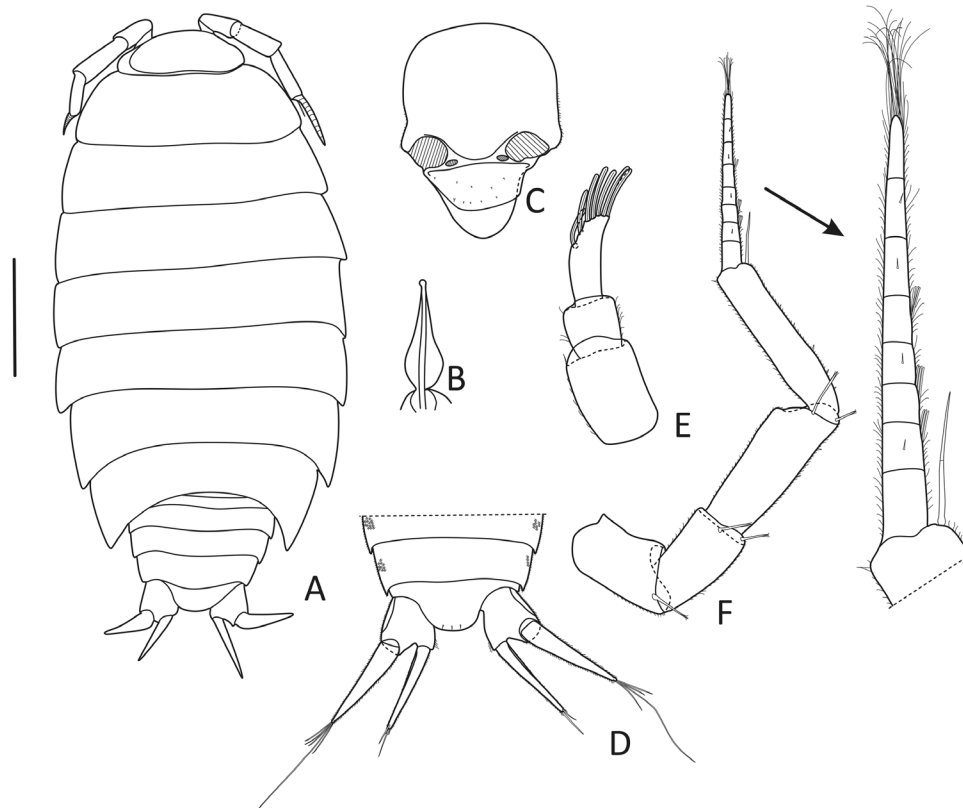
(FIGS 12, 22–25; TABLE 1)

LSID <http://zoobank.org/093F3A0E-D163-4CCF-9C82-1A302930E39D>

*Illyrionethes strasseri* – Strouhal, 1939b: 188 [partim: Lokve Špilja, Kroatien; NW.-Kroatien]. – Frankenberger & Strouhal, 1940: 449 [partim: NW.-Kroatien].

*Alpioniscus (Illyrionethes) strasseri* – Schmolzer, 1965: 59 [partim: NW-Kroatien]. – Karaman, 1966: 376 [partim: Lokve špilja].

*Alpioniscus strasseri* – Casale *et al.*, 2004: 310. – Bedek *et al.*, 2011: 277; fig. 16 [partim: Lokvarka (cave), Lokve, Delnice].



**Figure 22.** *Alpiniscus (Illyrionethes) hirci* Bedek & Taiti **sp. nov.** A, paratype ♀ CBSSC IT2389, from Bukovac: specimen in dorsal view (scale bar: 1 mm). B–F, paratype ♂ CBSSC IT2389, from Bukovac: B, dorsal scale-seta; C, cephalon, dorsal; D pleonite 5, telson and uropods; E, antennula; F, antenna with enlargement (arrow) of flagellum.

*Material examined (collection details in Supporting Information, Table S1)*

**Holotype:** ♂, CROATIA. **Gorski Kotar:** Fužine, Vrata, Donja Slavica, Bukovac (cave CCC HR00557) (CBSSC IT4254).

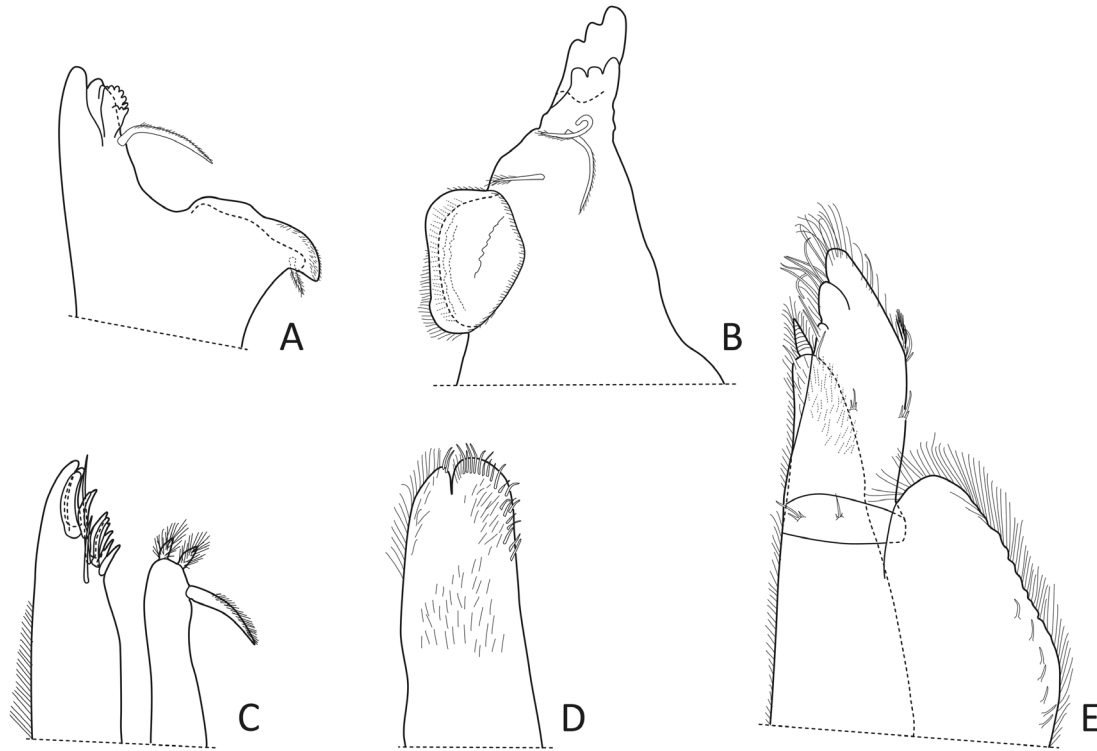
**Paratypes:** CROATIA. **Gorski Kotar:** ibid. (CBSSC IT2388, IT2389, IT3892; MZUF 9873); Lokve, Dimnjačareva špilja (cave CCC HR00559) (CBSSC IT3885, IT3887; MZUF 9874); Lokve, Lokvarka (cave) (SMNS 5284/6; CBSSC IT3439, IT2476); Lokve, Kameniti vrh, Medvjeda špilja (cave CCC HR01196) (CBSSC IT3449); Fužine, Vrelo (cave) (CBSSC IT2290, IT2296, IT3452).

**Additional material:** CROATIA. **Gorski Kotar:** Fužine, Vrata, Donja Slavica, Bukovac (cave CCC HR00557) (CBSSC IT2387, IT4097); Lokve, Lokvarka (cave) (CBSSC IT2362, IT2477); Čabar, Kapetanov dolac, Špilja u dolcu Kame Hame (cave CCC HR00032) (CBSSC IT3471); Fužine, Vrelo (cave) (CBSSC IT2403, IT4098).

**Description:** Maximum length: ♂, 6.7 mm; ♀, 7.4 mm. Pereon with almost parallel sides (Fig. 22A). Back smooth, with some triangular scale-setae (Fig. 22B). Some gland pores on lateral margins of pleonites 4 and 5 and telson

(Fig. 22D). Cephalon (Fig. 22C) with suprantennal line bent downwards, rounded middle part; antennal lobes rounded. Posterior margin of pereonite 1 convex, of pereonites 2 and 3 straight, and of pereonites 4–7 progressively more concave. Pleonites 3–5 with small posterior points visible in dorsal view (Fig. 22D). Distal part of telson with concave sides and broadly rounded apex (Fig. 22D). Antennula (Fig. 22E) distal article flattened and bearing five to eight aesthetascs on apical margin. Antenna (Fig. 22F) smooth; flagellum of nine to 11 articles with one row of aesthetascs on four to six different articles, always on second and third. Mouthparts (Fig. 23) as in *A. strasseri*. Pereopods (Fig. 24A) similar in shape, with dactylar seta long and bifid. Uropod (Fig. 22D) with protopod slightly grooved on outer margin; endopod inserted proximally.

**Male:** Pereopod 1 (Fig. 24A) carpus bearing six to seven setae. Pereopod 1 and 2 with carpus and merus bearing numerous short scales on rostral surface. Pereopods 1–6 merus with sternal margin from straight (pereopod 1) progressively up to slightly concave (pereopod 6), and small lobe in proximal part increasing in size from pereopod 2 to pereopod 6.



**Figure 23.** *Alpioniscus (Illyrionethes) hirci* Bedek & Taiti sp. nov. Paratype ♂ CBSSC IT2389, from Bukovac: A, right mandible; B, left mandible; C, maxillula; D, maxilla; E, maxilliped.

Pereopod 7 (Fig. 24B–D) merus with slightly concave sternal margin and medium-sized hook-shaped lobe in proximal part directed ventrally, bearing one seta; carpus with straight sternal margin and large straight to rounded and protuberant distally, tergal hump in proximal part, ratio of carpus length to top hump position  $1.66 \pm 0.09$  ( $N = 4$ ), ratio of carpus length to ending hump position  $1.27 \pm 0.03$  ( $N = 4$ ). Pleopod 1 (Fig. 25A) exopod with posterior apex broadly rounded, ratio of length to posterior apex width  $3.57 \pm 0.19$  ( $N = 4$ ), deeply concave outer margin, ratio of length to concavity turning point position  $1.78 \pm 0.07$  ( $N = 4$ ), straight to slightly concave inner margin; endopod narrow with almost parallel sides, apical seta not plumose. Pleopod 2 (Fig. 25B) exopod triangular with slightly concave outer margin; endopod distinctly longer than exopod, distal article narrower than proximal; terminal seta strong and bifid. Pleopod 3–5 exopods as in Figure 25C–E.

**Etymology:** The species is named after Dragutin Hirc (1853–1921), a natural science researcher and speleologist, focused on Gorski Kotar region.

**Remarks:** *Alpioniscus hirci* sp. nov. differs from the other species of the *strasseri*-lineage (Fig. 12; Table 1) in the shape of the male pereopod 7 carpus tergal hump

with protruding lateral edge, and the male pleopod 1 endopod with apical seta not plumose. It differs in the ratio of the male pereopod 7 carpus length to carpus top hump position from *A. strasseri* s.s. ( $3.73 \pm 0.53$ ) and *A. balthasari* s.s. ( $2.81 \pm 0.33$ ); in the ratio of the male pereopod 7 carpus length to ending tergal hump position from *A. strasseri* s.s. ( $2.09 \pm 0.22$ ) and *A. christiani* ( $1.68 \pm 0.17$ ); in the position of concavity turning point of outer margin of the male pleopod 1 exopod from *A. velebiticus* sp. nov. ( $2.21 \pm 0.14$ ); and in the ratio of the male pleopod 1 exopod length to posterior apex width from *A. strasseri* s.s. ( $4.76 \pm 0.38$ ), *A. balthasari* s.s. ( $4.76 \pm 0.36$ ) and *A. christiani* ( $4.67 \pm 0.32$ ).

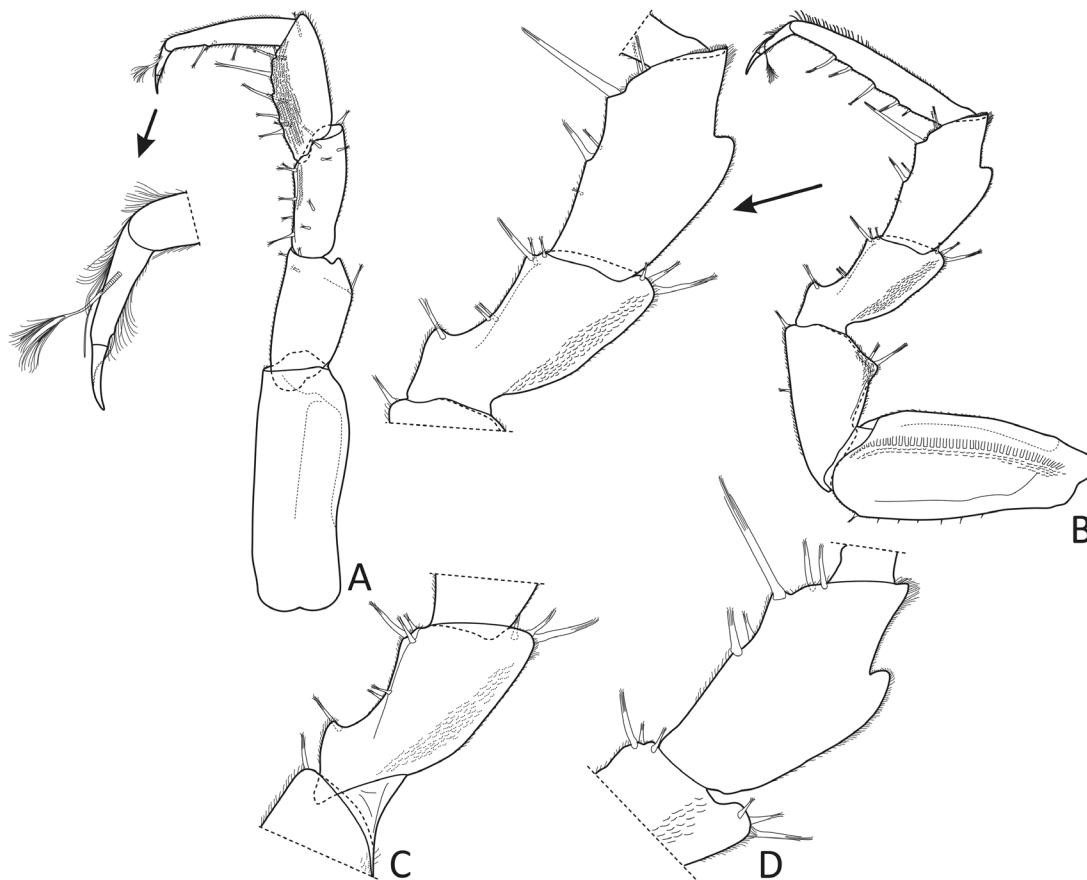
**Distribution:** Croatian Gorski Kotar area (Fig. 4).

***ALPIONISCUS (ILLYRIONETHES) VELEBITICUS*  
BEDEK & TAITI SP. NOV.**

(FIGS 12, 26–29; TABLE 1)

LSID <http://zoobank.org/153D609A-E8A6-4DB9-955F-B40A07617A93>

*Alpioniscus hercegowiniensis* – Kerovec *et al.*, 1999: 35.



**Figure 24.** *Al pioniscus (Illyrionethes) hirci* Bedek & Taiti **sp. nov.** A–C, paratype ♂ CBSSC IT2389, from Bukovac: A, pereopod 1 with enlargement (arrow) of dactylus; B, pereopod 7 rostral view with enlargement (arrow) of merus and carpus; C, pereopod 7 merus caudal view. D, paratype ♂ CBSSC IT 3892, from Bukovac: pereopod 7 carpus rostral.

*Al pioniscus* sp. – Casale *et al.*, 2004: 315–316. – Jalžić *et al.*, 2007a: 91. – Bregović *et al.*, 2013: 28 [partim: Burinka].

*Al pioniscus herzegowinensis* – Bedek *et al.*, 2011: 271.

*Al pioniscus* sp. n. – Stoev *et al.*, 2015: 110.

*Material examined (collection details in Supporting Information, Table S1)*

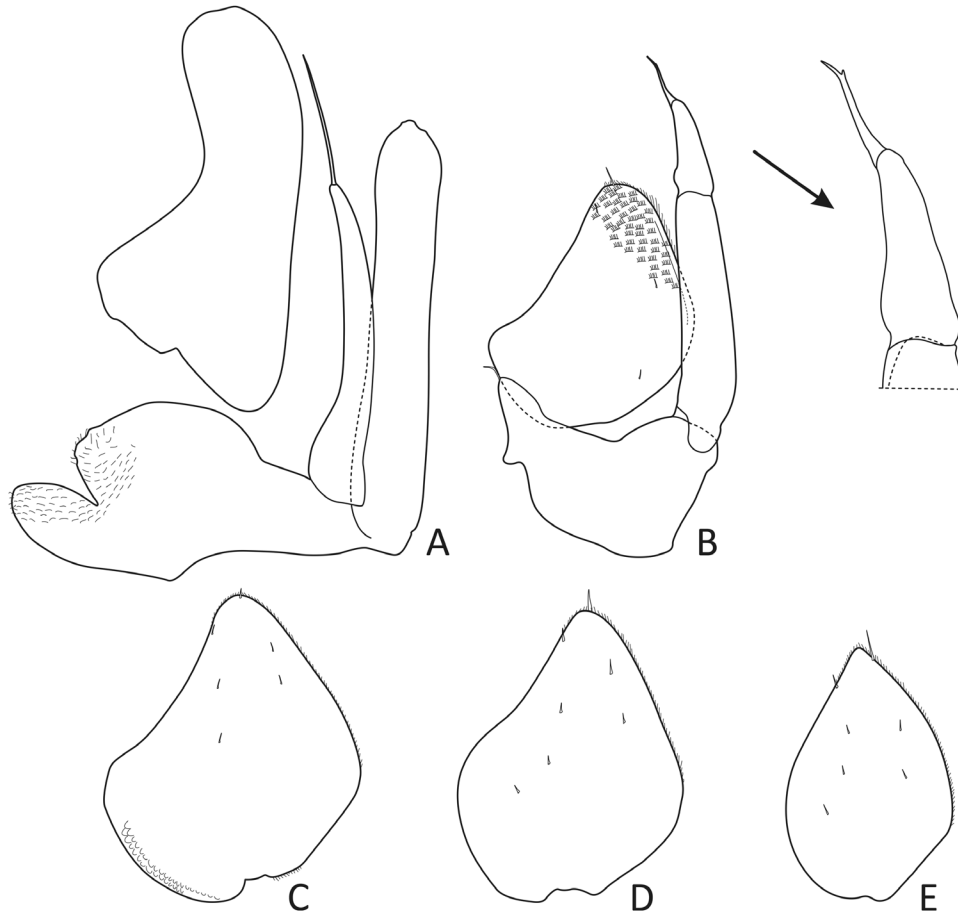
*Holotype*: ♂, CROATIA. **Velebit Mt. (N)**: Krasno, Crikvena, Velebita (cave) (CBSSC IT4110).

*Paratypes*: CROATIA. **Velebit Mt. (N)**: *ibid.* (CBSSC IT2420, IT2418, IT352, IT353, IT318; MZUF 9875); Krasno, Begovača, Jama Olimp (cave CCC HR00169) (CBSSC IT355, IT357, IT3916); Krasno, Hajdučki kukovi, Lukina jama-Trojama (cave CCC HR01481) (CBSSC IT2415, IT2416, IT2417, IT3237, IT3238, IT3239, IT3240, IT3243, IT3241); Krasno, Crikvena, Meduza (cave) (CBSSC IT365, IT3762); Krasno, Mali kuk, Slovačka jama (cave) (ZCDB, CBSSC IT25, IT24, IT4101; MZUF 9876).

*Additional material*: CROATIA. **Velebit Mt. (N)**: Krasno, Crikvena, Velebita (cave) (CBSSC IT317, IT2419); Krasno, Hajdučki kukovi, Lukina jama-Trojama (cave CCC HR01481) (CBSSC IT2258).

**Velebit Mt. (middle)**: Pazarište, Klementa, Klementina 1 (cave) (CBSSC IT3410); Pazarište, Bužim, Kalanjeva Ruja, Pepelarica (cave) (CBSSC IT3418, IT3933); Baške Oštarije, Crni vrh, Ponor Crnog vrela (cave CCC HR00171) (CBSSC IT383); Pazarište, Klepina duliba, Ponor u Klepinoj dulibi 2 (cave) (CBSSC IT3278). **Velebit Mt. (S)**: Gračac, Crnopac, Dragičevića stan, Duman, Burinka (cave) (CBSSC IT402, IT2914); Gračac, Crnopac, Kita Gačešina-Draženova puhaljka (cave) (CBSSC IT2594, IT2595, IT2596, IT3937); Gračac, Crnopac, Muda labudova (cave) (CBSSC IT2237, IT2238, IT2581, IT2582, IT2583, IT2584, IT2585, IT2586, IT2587, IT2588, IT4074, IT3936); Gračac, Crnopac, Munižaba (cave) (CBSSC IT625, IT401, IT2299, IT2589, IT2590, IT2592, IT2593). **Lika**: Gračac, Štikada, Jelar ponor (cave) (CBSSC IT617, IT3407, IT3406, IT3421, IT3422).

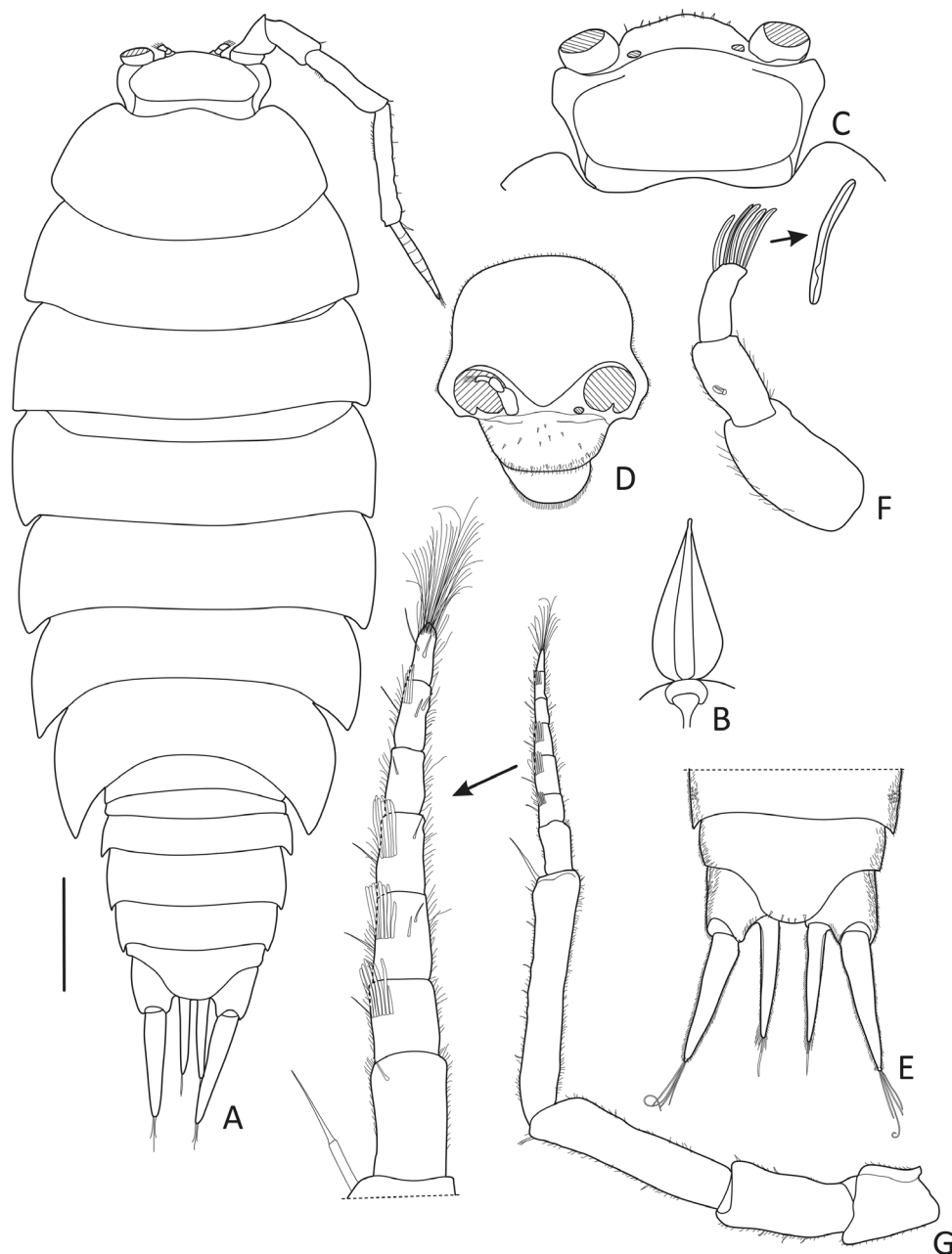




**Figure 25.** *Alpiniscus (Illyrionethes) hirci* Bedek & Taiti **sp. nov.** Paratype ♂ CBSSC IT2389, from Bukovac: A, genital papilla and pleopod 1; B, pleopod 2 with enlargement (arrow) of endopod distal article; C, pleopod 3 exopod; D, pleopod 4 exopod; E, pleopod 5 exopod.

**Description:** Maximum length: ♂, 6.5 mm; ♀, 8.6 mm. Pereon with almost parallel sides (Fig. 26A). Back smooth, with some triangular scale-setae (Fig. 26B). Gland pores on lateral margins of pleonites 4 and 5 (Fig. 26E). Cephalon (Fig. 26C, D) with suprantennal line bent downwards, rounded middle part; antennal lobes quadrangular. Posterior margin of pereonite 1 convex, of pereonite 2 straight and of pereonites 3–7 progressively more concave. Pleonites 3–5 with small posterior points visible in dorsal view (Fig. 26A, E). Distal part of telson with concave sides and broadly rounded apex (Fig. 26E). Antennula (Fig. 26F) distal article flattened and bearing three to seven aesthetascs on apical margin. Antenna (Fig. 26G) smooth; flagellum of six to nine articles with one row of aesthetascs on two to five different articles, always on second and third. Mouthparts (Fig. 27) as in *A. strasseri*. Pereopods (Fig. 28A) with dactylar seta large and bifid. Uropod (Fig. 26E) with protopod slightly grooved on outer margin; endopod inserted proximally.

**Male:** Pereopod 1 (Fig. 28A) carpus bearing four to five setae. Pereopod 1–3 with carpus and merus bearing numerous short scales on rostral surface. Pereopod 1–6 merus with sternal margin from straight (pereopod 1) progressively up to slightly concave (pereopod 6) and small lobe in proximal part increasing in size from pereopod 2 to pereopod 6. Pereopod 7 (Fig. 28B, C) merus with slightly concave sternal margin and large hook-shaped lobe in proximal part directed ventrally, bearing one seta; carpus with straight sternal margin and large rounded tergal hump in central part of tergal margin, ratio of carpus length to top hump position  $2.08 \pm 0.15$  ( $N = 16$ ), and ratio of carpus length to ending hump position  $1.16 \pm 0.06$  ( $N = 16$ ). Pleopod 1 (Fig. 29A) exopod with posterior apex narrowly rounded, ratio of length to posterior apex width  $4.57 \pm 0.31$  ( $N = 16$ ), deeply concave outer margin, ratio of length to concavity turning point position  $2.21 \pm 0.14$  ( $N = 16$ ), straight to slightly concave inner margin; endopod narrow with almost parallel



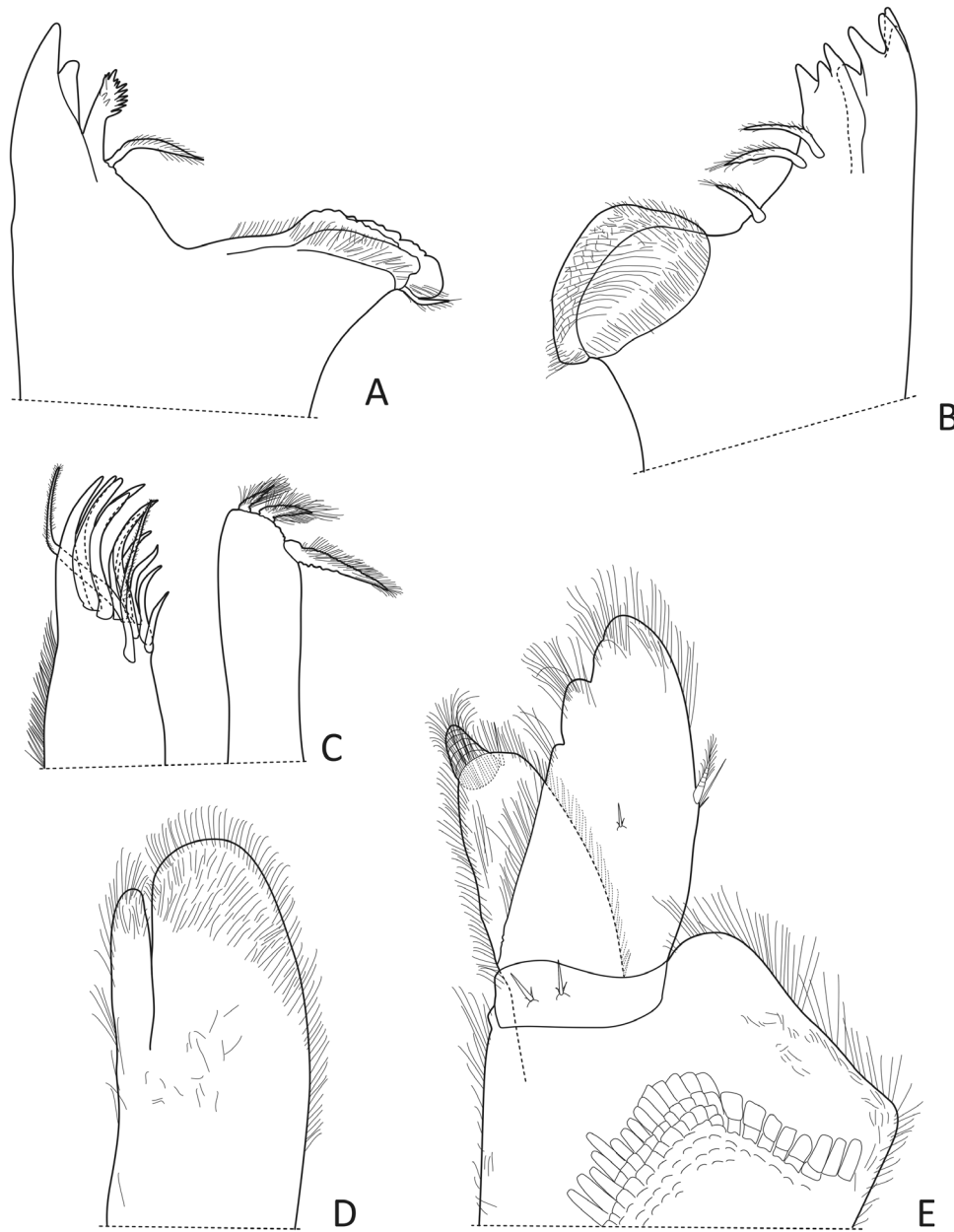
**Figure 26.** *Al pioniscus (Illyrionethes) velebiticus* Bedek & Taiti sp. nov. A, C, paratype ♀ CBSSC IT353, from Velebita: A, specimen in dorsal view (scale bar: 1 mm); C, cephalon, dorsal. B, D–G, paratype ♂ CBSSC IT353, from Velebita: B, dorsal scale-seta; D, cephalon, dorsal; E, pleonite 5, telson and uropods; F, antennula with enlargement (arrow) of aesthetasc; G, antenna with enlargement (arrow) of flagellum.

sides, apical seta plumose. Pleopod 2 (Fig. 29B) exopod triangular with concave outer margin; endopod distinctly longer than exopod, articles subequal in width, terminal seta strong. Pleopod 3–5 exopods as in Fig. 29C–E.

**Etymology:** The species is named after Velebit Mt., where the caves are located, and after the Speleological

Section of the University Mountaineering Society 'Velebit', Zagreb.

**Remarks:** *Al pioniscus velebiticus* sp. nov. differs from all the other species of the *strasseri*-lineage (Fig. 12; Table 1) in the smallest number of antennular aesthetascs (three to seven) and in the smallest



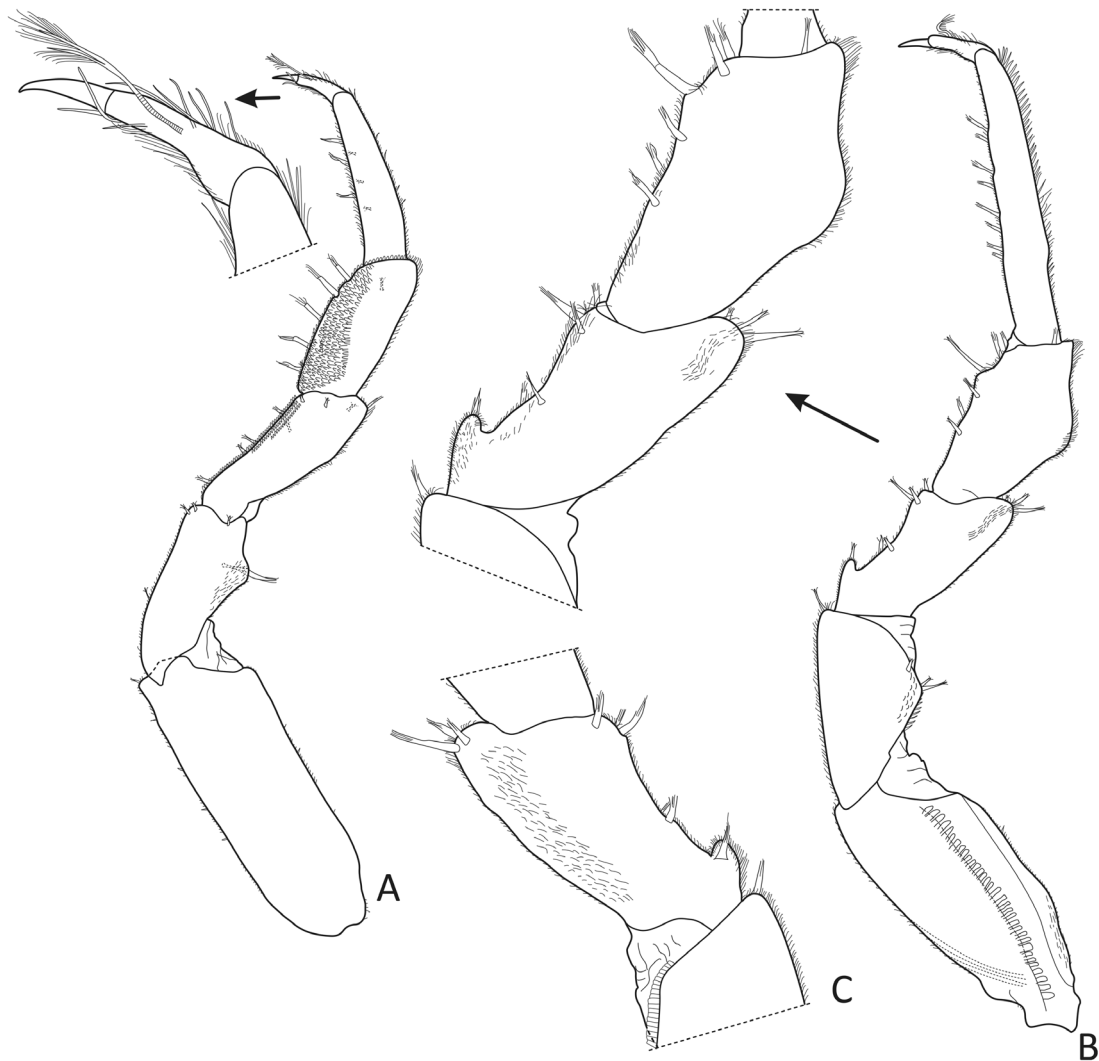
**Figure 27.** *Al pioniscus (Illyrionethes) velebiticus* Bedek & Taiti **sp. nov.** Paratype ♀ CBSSC IT353, from Velebita: A, right mandible; B, left mandible; C, maxillula; D, maxilla; E, maxilliped.

number of sternal setae on the male pereopod 1 carpus (four or five). It differs from *A. christiani*, *A. balthasari*, *A. hirci* sp. nov. and *A. iapodicus* in having a larger hook-shaped lobe directed ventrally in the proximal part of the male pereopod 7 merus. It also differs in the ratio of the male pereopod 7 carpus length to carpus top hump position from *A. strasseri* s.s. ( $3.73 \pm 0.53$ ) and *A. christiani* ( $2.93 \pm 0.27$ ); in the ratio of the male pereopod 7 carpus length to ending

tergal hump position from *A. strasseri* s.s. ( $2.09 \pm 0.22$ ) and *A. christiani* ( $1.68 \pm 0.17$ ); and in the position of the concavity turning point of the outer margin of the male pleopod 1 exopod from *A. hirci* ( $1.78 \pm 0.07$ ) and *A. strasseri* s.s. ( $1.73 \pm 0.07$ ).

The species occurs near and in the cave hygropeletic habitats (Sket, 2004).

Stoev *et al.* (2015) list it as *Al pioniscus* sp. n., without name and description.



**Figure 28.** *Alpioniscus (Illyrionethes) velebiticus* Bedek & Taiti sp. nov. Paratype ♂ CBSSC IT353, from Velebita: A, pereopod 1 with enlargement (arrow) of dactylus; B, pereopod 7 rostral view with enlargement (arrow) of merus and carpus; C, pereopod 7 merus caudal view.

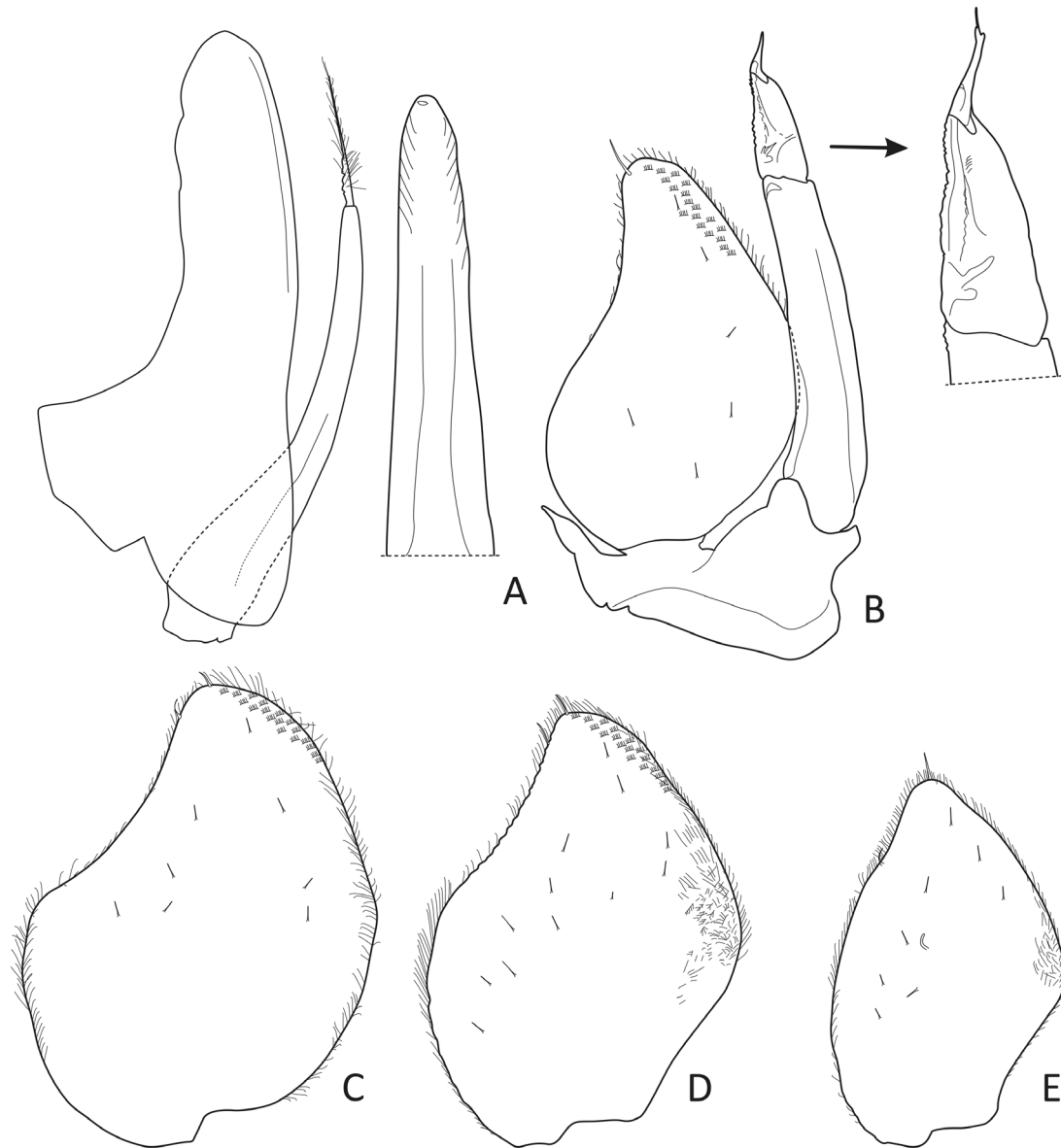
*Distribution:* Croatian Velebit Mt. (Fig. 4).

## DISCUSSION

Our phylogenetic analysis confirms the existence of six undescribed and 13 out of 14 nominal Dinaric *Alpioniscus (Illyrionethes)* species. With the newly described species (*A. hirci* sp. nov. and *A. velebiticus* sp. nov.), the Dinaric *Illyrionethes* now comprises 15 nominal and four still undescribed species. They were grouped in three lineages: *heroldi*-, *strasseri*- and tentative *magnus*-lineage.

The two morphogroups previously suggested by Bedek & Taiti (2011), *heroldi*- and *strasseri*-morphogroup, correspond in part to the lineages

identified by molecular analyses. The *heroldi*-morphogroup comprises the *heroldi*- and *magnus*-lineage, and the *strasseri*-morphogroup is consistent with the *strasseri*-lineage, with the only difference being that *A. absoloni* belongs to the *heroldi*-instead of to the *strasseri*-lineage. From a morphological point of view, the *strasseri*-lineage can be distinguished from the *heroldi*- and *magnus*-lineage by the deeply concave outer margin of the male pleopod 1 exopod with regard to a non-concave or slightly concave outer margin, as proposed by Bedek & Taiti (2011). The other distinguishing character proposed by Bedek & Taiti (2011), the prominent hump on the male pereopod 7 carpus, is a common feature in the *strasseri*-lineage, but is present also in *A. absoloni* and *A. magnus*.



**Figure 29.** *Alphoniscus (Illyrionethes) velebiticus* Bedek & Taiti **sp. nov.** Paratype ♂ CBSSC IT353, from Velebita: A, genital papilla and pleopod 1; B, pleopod 2 with enlargement (arrow) of endopod distal article; C, pleopod 3 exopod; D, pleopod 4 exopod; E, pleopod 5 exopod.

The molecular analyses of the *heroldi*-lineage reveals higher *COI* and 16S interspecific distances and earlier divergence times compared with the *strasseri*-lineage and, to some extent, with the tentative *magnus*-lineage. Our analyses confirms that *A. absoloni* is monophyletic and consists of three clades, which correspond to the nominal subspecies. The taxonomical distinction among the subspecies is further confirmed by the sympatry of *A. a. absoloni* and *A. a. politus* and their distant placement in the phylogenetic tree (Strouhal, 1939d; Vandell, 1946). The status of *A. verhoeffi* needs to be re-examined, because

the high interpopulation *COI*/16S distances ( $\leq 19/31\%$ ) (Supporting Information, Tables S3 and S4), early divergence time (specimen number 748 vs. the rest; Supporting Information, Fig. S3) and the geographical separation within the clade (Fig. 5) suggest that it could consist of more than one species. More extensive molecular analyses and detailed morphological investigation are essential to clarify the relationships in the *heroldi*-lineage.

The *magnus*-lineage is closely related to the *heroldi*-lineage according to 16S tree, which was supported by previous morphological analyses by Bedek & Taiti (2011).

However, the inclusion of the *H3* molecular marker (Supporting Information, Fig. S2) causes dispersal of the *magnus*-lineage species throughout the tree, and most of their inter-relationships are unresolved.

Our morphological study focuses on the *strasseri*-lineage because of its robust and highly supported phylogeny as well as the availability of sufficient material due to intensive sampling in the lineage distribution area. Here we establish a new taxonomical framework and use both current and new diagnostic characters to enable morphological recognition of species identified by molecular analyses. Our results show that species diagnostic characters with the highest specific discriminant values are as follows: (1) the ratio of the length and concavity turning point position in the male pleopod 1 exopod (Table 1; Fig. 12); (2) the ratio of the male pereopod 7 carpus length and hump ending position (Table 1; Fig. 12); and (3) the shape and position of the male pereopod 7 merus sternal lobe (Table 1). Additional differential characters, informative only for a subset of species, are as follows: (1) the ratio of the male pereopod 7 carpus length and hump top position (Fig. 12); (2) the shape of posterior apex of the male pleopod 1 exopod (Table 1); and (3) the ratio of length and posterior apex width of the male pleopod 1 exopod (Fig. 12). Overall, our quantitative analysis enables a far more precise definition of the most important diagnostic characters.

Three species in the *strasseri*-lineage show intraspecific molecular divergence and small but consistent morphological differences between different populations. *Alpioniscus strasseri* (Fig. 11) and *A. iapodicus* (Fig. 21) include two subclades each. The *COI/16S* distances (Supporting Information, Tables S3 and S4) between subclades (8–9/3–5% and 8/4%, respectively) are higher than other interpopulation distances of the *strasseri*-lineage, but also smaller than interspecific distances in our study (from 12 to 25% for *COI* and from 5 to 17% for 16S) or other Oniscidea (from 13 to 30% for *COI* and from 7.6 to 24.4% for 16S) (e.g. Klossa-Kilia *et al.*, 2006; Sicard *et al.*, 2014; Zimmermann *et al.*, 2015). Morphological differences are present in the male pereopod 7 merus sternal lobe, one of the main characters used for species recognition (Figs 11, 21). Overall, these results suggest an ongoing speciation process, perhaps as a consequence of recent geographical isolation. The two different populations of *A. iapodicus* occurring in Lika and Paklenica are separated by the Velebit Mountain, whereas the distinct subclades of *A. strasseri* correspond to different geological regions of the Istrian peninsula (northern and southern Istria). *Alpioniscus balthasari* comprises three subclades (Fig. 19), clearly seen in the 16S BI tree (Supporting Information, Fig. S1) and supported by morphological analyses. The 16S distances between

*A. balthasari* s.s. (specimens 1655 and 2159) and the Biokovo–Mosor population (specimens 226, 466, 2267 and 2270) are 6% on average (Supporting Information, Table S4). Morphological differences, already observed in part by Buturović (1955), are present in both male and general characters (Figs 15C, 16B, 17D, 18B, D, 19). Although the morphological differences and 16S distance borderline species level distinction, the population in between needs to be analysed in more detail to solve their relationships. The *COI/16S* distances between *A. balthasari* s.s. and the Stara jametina population (specimen number 2178) are 4/8%. Molecular differences were supported by the absence of ridges on the posterior part of the cephalon and pereonites 1–4 (Fig. 15A) in the Stara jametina population, whereas no male morphological differences are present.

The three *Illyrionethes* lineages have overlapping ranges even though syntopic species are relatively rare. The rareness of syntopy is probably attributable to ecological (e.g. competition) rather than geographical reasons. Species within the *strasseri*-lineage are not syntopic and always occur in geographically or elevationally separated areas (e.g. *A. velebiticus* sp. nov. at higher elevation on Velebit Mt. compared with *A. iapodicus*). Unlike other troglobionts in the Dinaric Karst, which are usually restricted to smaller geographical units, several *Alpioniscus* species show large ranges. The amphibious species show larger ranges according to our study (*A. balthasari* ~130 × 50 km, *A. strasseri* ~120 × 80 km, and *A. velebiticus* sp. nov. ~110 × 10 km), probably because they can use both aquatic and terrestrial dispersal pathways. A very peculiar distribution is present in the tentative *magnus*-lineage. Its distribution shows a paralittoral pattern (Fig. 5): three species endemic to islands, *A. magnus* (island of Brač), *A. sp. 4* (island of Dugi otok) and *A. sp. 2* (island of Lošinj), and one coastal: *A. sp. 5* (Šibenik area). Such a distribution has never been reported previously for troglobionts and is usually exhibited by anchialine species, such as *Hadzia fragilis* S. Karaman, 1932, *Niphargus hebereri* Schellenberg, 1933 (Amphipoda), *Tethysbaena halophila* (S. Karaman, 1953) (Thermosbaenacea) and by the stygobiont *Sphaeromides virei virei* (Brian, 1923) (Isopoda: Cirolanidae) (Sket, 2012).

Vicariant effects of Alpine and Dinaric orogeny during the Middle and Late Miocene are coherent with an important phylogeographical split (north-west vs. south-east) that has been observed for freshwater fauna in this area (Verovnik *et al.*, 2005; Franjević *et al.*, 2010; Perea *et al.*, 2010). According to the results of BEAST analysis (Supporting Information, Fig. S3), the first split within Dinaric *Illyrionethes* occurred around the Middle Miocene, ~10 Mya, and separated

the *heroldi*-lineage in the south-eastern Dinarides from the *strasseri*- + *magnus*-lineage in the north-west (Figs 4, 5; Supporting Information, Fig. S3), when the Dinaric Mountains were already uplifted to some extent (Popov *et al.*, 2004; Zupan Hajna, 2012). It was followed shortly thereafter by a split between *A. heroldi* and other *heroldi*-lineage species, whereas the *strasseri*-lineage separated from the *magnus*-lineage ~9 Mya. Species from the *strasseri*-lineage have the youngest divergence times compared with those from the *heroldi*- and *magnus*-lineage. The separation of *Illyrionethes* coastal species coincides with the Messinian salinity crisis (6.0–5.3 Mya), when changes in sea level might have caused speciation, as was previously suggested for other Dinaric stygobionts (Sbordoni *et al.*, 1980; Zakšek *et al.*, 2007; Delić *et al.*, 2017a) and other Mediterranean subterranean fauna (e.g. Faille *et al.*, 2018). Several mainland clades of the *heroldi*-lineage have also split during the same turbulent period, as already observed for different Dinaric cave species (Bilandžija *et al.*, 2013; Njunjić *et al.*, 2018). The Pleistocene species origin hypothesis (e.g. Vandel, 1964; Barr & Holsinger, 1985) was confirmed by speciation in the *strasseri*-lineage, namely between *A. christiani* and *A. strasseri* and between *A. hirci* sp. nov. and *A. iapodicus*. The intraspecific diversity seems also to be affected by Pleistocene events, specifically the splits between two morphologically different populations of *A. strasseri* (northern vs. southern Istria), *A. iapodicus* (Lika vs. Paklenica population) and *A. balthasari* (Cetina–Krka vs. Biokovo–Mosor population). Similar timings were found previously for *Niphargus* Schiödte, 1849 species (Delić *et al.*, 2017a).

Lineage diversifications might already have occurred in a surface ancestor, which subsequently colonized subterranean habitats multiple times independently, as proposed for other Dinaric stygobionts (Zakšek *et al.*, 2007). The geographical overlapping of different lineages suggests that the reproductive isolation was followed by secondary contact, which partly superimposed ranges of different lineages. Speciation in each lineage could have proceeded when subsequent vicariance events (formation of mountains, which in some cases became islands) isolated different populations (Holsinger, 2012). Less likely, a single invasion with subsequent diversification and dispersal through the underground environment followed by further speciations within lineages and, in some cases, secondary contact could have produced the geographical pattern we see today, similar to the scenario proposed for the Dinaric troglobiotic genus *Anthroherpon* Reitter, 1889 (Coleoptera: Leiodidae) by Njunjić *et al.* (2018). Future detailed phylogeographical analyses might

confirm which of the two scenarios played a role in shaping the evolutionary history of *Illyrionethes* in the Dinaric Karst.

The disjunct distribution of the two *Alpioniscus* subgenera (Fig. 1) questions their monophyly. This disjunct distribution is not a unique feature among European troglobionts (e.g. Sendra *et al.*, 2004; Lukić *et al.*, 2015), but is rarely tested and supported by molecular analyses (e.g. Weigand *et al.*, 2013; Pavlek & Ribera, 2017; Inäbnit *et al.*, 2019). To test the monophyly of the Dinaric *Illyrionethes*, future studies should include the most similar Sardinian species of *Illyrionethes* and the more different but geographically closer southern Balkan species of *Alpioniscus* s.s. With the inclusion of those specimens, we expect to have a clearer idea of the monophyly of the two subgenera and of the relationships between the lineages of Dinaric *Illyrionethes*, although we do not expect any effect on the phylogeny at the species level.

In conclusion, the integrative taxonomical approach in our study was essential to enlighten the Dinaric *Illyrionethes* taxonomy. It showed that small morphological differences among different species are reliable diagnostic characters. In order to distinguish the species better, we supplemented the traditional descriptive differential characters with quantitative analysis, using the *strasseri*-lineage as a test group. In subsequent studies, all the other species of the *heroldi*- and *magnus*-lineage will be redescribed or newly described, special attention being paid to resolving the possible species complexes inferred in this study.

#### ACKNOWLEDGEMENTS

The authors are grateful to all collectors and (bio) speleologists from the Croatian Biospeleological Society (Zagreb, Croatia), the Speleological Society Zelena Brda (Trebinje, Bosnia and Herzegovina), Speleo Dodo Speleological Society (Sarajevo, Bosnia and Herzegovina), the Biological Students Society BIUS (Zagreb, Croatia), the Speleological Club Željezničar (Zagreb, Croatia), the Speleological Society Velebit (Zagreb, Croatia), the Speleological Society Estavela (Kastav, Croatia) and the Mountaineering Society Dubovac (Karlovac, Croatia) for collecting the material and help with the fieldwork. We wish to thank Marko Lukić for his help at all stages of the study. We are also grateful to the following colleagues for enabling us to examine their isopod collections: Dr Helmut Schmalzfuss of the Staatliches Museum für Naturkunde, Stuttgart; Dr Irena Grbac of Croatian Natural History Museum, Zagreb; Dr Ivo M. Karaman and Dr Mladen Horvatović of the Department

of Biology and Ecology, Faculty of Sciences, University of Novi Sad; and Dr Maja Zagmajster of the Department of Biology, Biotechnical Faculty, University of Ljubljana. We greatly appreciate the anonymous reviewers for their suggestions and comments that helped us to improve the manuscript to a great extent. Dr Josip Balabanić is particularly acknowledged for reviewing the new Latin names. The work was partly supported by the National Park Krka (Croatia). The majority of examined specimens were collected through different projects conducted by the Croatian Biospeleological Society and partly supported by the State Institute for Nature Protection (Croatia); The Environmental Protection and Energy Efficiency Fund (Croatia); Nature park Biokovo (Croatia); Nature park Učka (Croatia); Nature park Velebit (Croatia); National park Sjeverni Velebit (Croatia); National park Paklenica (Croatia); Municipality Kostrena (Croatia); Public Institute for the Management of Protected Natural Areas and Other Protected Natural Areas in Šibenik-Knin County (Croatia); The town of Crikvenica (Croatia); Public Institution Kamenjak (Croatia); Cave park Grabovača (Croatia); European Union Instrument for Pre-Accession Assistance (IPA); and Whitley Fund for Nature (UK).

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### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** List of samples used in analyses, with specimens, locality and collectors' details.

**Table S2.** List of taxa included in the DNA analysis, with specimen numbers and GenBank accession numbers.

**Table S3.** *COI* p-distances between 29 specimens. All positions containing gaps and missing data were eliminated, resulting in a total of 579 bp in the final dataset. Codon positions included were 1st + 2nd + 3rd + non-coding. Statistical analyses were conducted in MEGA v.5.

**Table S4.** 16S distances between 54 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Statistical analyses were conducted in MEGA X.

**Figure S1.** Phylogenetic tree obtained using Bayesian inference (MrBayes) for 16S dataset, with original taxa names in the tree and new taxa names in the right column. Posterior probabilities for nodes are indicated.

**Figure S2.** Phylogenetic tree obtained using Bayesian inference (MrBayes) for 16S+*H3* dataset, with original taxa names in the tree and new taxa names in the right column. Posterior probabilities for nodes are indicated.

**Figure S3.** Chronogram obtained by Bayesian inference (BEAST) using 16S dataset. Nodal scale bars indicate the 95% posterior probability intervals for time divergence dates. Posterior probabilities for nodes are indicated.