UNIVERSITY OF LJUBLJANA
BIOTECHNICAL FACULTY
MSc ECOLOGY AND BIODIVERSITY

VID ŠVARA

INTEGRATIVE TAXONOMY OF *Niphargus arbiter* - *Niphargus salonitanus* COMPLEX

M.SC. Thesis
Master Study Programm

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INTEGRATIVNA TAKSONOMIJA SLEPIH POSTRANIC IZ KOMPLEKSA *Niphargus arbiter - Niphargus salonitanus*

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AB With over 350 described species, *Niphargus* is the most species rich genus of freshwater amphipods. The genus shows a high ecological and morphological diversity. *Niphargus* amphipods are important as top invertebrate predators in the subterranean environment of the Dinaric Karst. The most fascinating species belong to the cave-lake ecomorphs with large body size and raptorial appendages. Nevertheless the species inventory and distribution of cave-lake *Niphargus* ecomorphs remains incompletely studied. One of the understudied groups is the *Niphargus arbiter/Niphargus salonitanus* species complex, which consists of several cryptic species. In this research, 109 individuals from 34 localities were assigned to species using molecular uni- and multilocus species delimitation. Based on the suggested phylogeny morphological characteristics of the given species were analyzed. Additionally, species ecological niche models were compared. The combination of different molecular delimitation methods revealed that the complex consists of 9 species. Additionally, morphological diagnosis yielded significant differences between most of the species except for two pairs. Ecological models proved to be applicable in data sets that were acquired from five or more locations. The new species of the complex *Niphargus arbiter/Niphargus salonitanus* are provided with diagnosis and discussed within a broader biodiversity and nature conservation context.

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AI Rod jamskih postranic *Niphargus* je, z več kot 350 opisanimi vrstami, vrstno najbogatejši rod sladkovodnih postranic. Za rod je značilna visoka ekološka in morfološka raznolikost, v jamskih sistemih Dinarskega krasa pa so te postranice pomembni plenilci nevretenčarjev. Najimpresivnejše vrste najdemo med takoimenovanimi jamsko-jezerskimi ekomorfi, za katere sta značilna veliko telo in izrazito plenilski gnatopodi. Kljub ekoloüki pomembnosti teh organizmov, sta taksonomija in filogenija skupin nepopolno proučeni. Primer takšne skupine je tudi kompleks vrst *Niphargus arbiter/Niphargus salonitanus*, ki sestoji iz kriptičnih vrst. V tej raziskavi smo proučili 109 osebkov kompleksa, najdenih na 34 različnih lokacijah v Dinaridih. Opravili smo molekulsko delimitacijo na osnovi uni- in multilokusne vrstne delimitacije. Glede na dobljeno filogenetsko drevo, smo analizirali morfologijo vrst ter zanje izdelali modele ekoloških niš. Kombinacija štirih različnih molekulskih delimitacijskih metod podpira obstoj devetih vrst v tem kompleksu. Poleg tega smo našli zanesljive diagnostične morfološke znake pri večini vrst. Ekološki modeli so uporabni le v primeru večjega nabora podatkov iz vsaj petih lokalitet. Za nove vrste kompleksa *Niphargus arbiter*/*Niphargus salonitanus* smo podali diagnoze in jih obravnavali v širšem naravovarstvenem ter biodiverzitetnem kontekstu.

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1 INTRODUCTION

Amphipods are among the most important and diverse freshwater invertebrates. They are a key group in aquatic ecosystems and commonly used in biodiversity monitoring or ecotoxicology tests. The most species rich freshwater amphipod genus in the Western Palearctics is *Niphargus* Schiödte, 1849 (Väinölä et al., 2008). With over 350 described species (Horton et al., 2016) it constitutes an important part of freshwater biodiversity. It is distributed across Europe, with the bulk of the species found south of the Pleistocene ice sheet boundary (Karaman & Ruffo, 1986; Proudlove et al., 2003). Several species were described from the Arabian peninsula, Turkey and Iran (Karaman, 1986; Fišer et al., 2009a; b; Esmaeili-Rineh et al., 2015a; b).

Niphargus species are limited almost exclusively to subterranean waters, where they inhabit all the available ecological niches including cave streams, lakes, and water filled crevices (Sket, 1999). Ecological diversity could be the reason for the high morphological diversity of the genus. This diversity can be illustrated by variation in body size of different species spanning between 2 mm and 40 mm. Beside that no less than five ecomorphs were recognized (Trontelj et al., 2012 Delić et al., 2016). The most attractive and charismatic members of *Niphargus* are cave-lake ecomorphs with body size exceeding 20 mm, elegant long appendages, often attractively ornamented pleon segments with spines and huge raptorial gnathopods (Fišer et al., 2006; Trontelj et al., 2012; Petković et al., 2015). Lake ecomorphs have independently evolved several times (Trontelj et al., 2012; unpublished data) at mid-latitudes of the genus range, in France (Lefébure et al., 2006a), Italy (Iannilli & Taglianti, 2004), Central-West Balkan Peninsula (Fišer et al., 2006) and the Crimean Peninsula (Birstein, 1964). Species of cave-lake ecomorphs are an intriguing research object in evolutionary ecology for two reasons. First, cave-lake *Niphargus* amphipods are opportunistic predators and large-bodied species that may be top invertebrate predators in Dinaric Mountains (Ginet, 1960; Fišer et al., 2010). As such, they are important for the maintenance of high regional species diversity (Boulton et al., 2008). Second, large-bodied species represent an evolutionary phenomenon deviating from the global rule, stating that amphipod body sizes increase with geographic latitude and availability of dissolved oxygen (Chapelle & Peck, 1999, 2004); a comparison to the published information indicates that body sizes of the lake ecomorphs that reach over 20 mm between latitudes 42 to 47 °N (WGS 1984) are unexpectedly large.

Although cave-lake ecomorphs are attractive research objects for ecologists and evolutionary biologists, the species inventory and distribution of cave-lake *Niphargus* ecomorphs remains incompletely studied. *Niphargus* taxonomy below morphologically distinct ecomorphs is notoriously difficult. The main problem of *Niphargus* taxonomy is high intra-specific variation and small inter-specific differences, in addition to general

problems of taxonomy like small sample sizes due to species rarity (Lim et al., 2012). Indeed, molecular taxonomy unveiled that nominal *Niphargus* species often comprise several morphologically hardly distinguishable species (Lefébure et al., 2006b; Fišer et al., 2008, 2009b; Trontelj et al., 2009; Zagmajster & Fišer, 2009; Švara et al., 2015), so called morphologically cryptic species (Bickford et al., 2007). Such species commonly remain undescribed and neglected (Pante et al., 2015) although clarification of their taxonomic status could open new venues of eco-evolutionary research and conservation practices. Recent conceptual and technical progress in taxonomy permit diagnosing and description of cryptic species. This practice should be applied at least to charismatic and ecologically important species complexes such as cave-lake ecomorphs of genus *Niphargus.*

The acknowledgment that speciation is not a uniform process and that divergence within each speciation event may affect different sets of biological traits has ultimately classified taxonomy as an interdisciplinary science (Carstens et al., 2013). The evidence for species hypotheses may be based upon traits as diverse as DNA sequences, morphology, ecological or behavioral characteristics (Padial et al., 2010; Schlick-Steiner et al., 2010) and hence diagnostic combinations need to be appropriately adjusted. Additional diagnostic characters can even be more informative than morphology by itself (Jörger & Schrödl, 2013).

In this study, we explore the taxonomy of the *Niphargus* species complex of cave-lake ecomorphs, endemic to the Dinaric Mountains. Originally, the complex was composed of two species: *Niphargus arbiter* G. Karaman, 1984 and *Niphargus salonitanus* S. Karaman, 1950, described from the northern and southern part of the region, respectively (Karaman, 1984). While the individuals collected from the locus typicus show obvious morphological differences between the two species, several populations with transitional morphology have been found in this study (Karaman & Sket, 1989). Indeed, early molecular analyses (Fišer et al., 2008; Trontelj et al., 2009) indicate that the populations are genetically strongly structured and that the complex *Niphargus arbiter/Niphargus salonitanus* may contain other, morphologically non-differentiated species. The interdisciplinary approach was used to show how morphologically cryptic species can be included in broader biodiversity research. The morphological analyses were combined with multilocus species delimitation methods and ecological modeling, and it was shown that the complex contains seven additional species. The species are diagnosed, and discussed within a broader biodiversity context.

1.1 THESIS GOALS

The main focus of this Master's thesis is to provide a better insight into the taxonomy and phylogeny of the species complex *Niphargus arbiter/Niphargus salonitanus*. Using molecular taxonomy as the backbone and morphological and supplementary ecological data, we aim to diagnose the species.

Research goals:

- Provide a phylogenetic position of the *Niphargus arbiter/Niphargus salonitanus* species complex within the genus *Niphargus* using multilocus phylogeny.

- Delineate species using unilocus and multilocus species delineation with addition of morphological analysis and ecological niche modeling.

- Diagnosis of new species and nominal species.

2 LITERATURE REVIEW

2.1 SUBTERRANEAN AMPHIPODS OF THE GENUS *Niphargus*

The amphipod genus *Niphargus* Schiödte, 1849 (Crustacea: Amphipoda) consists of over 350 described species, which represents about 1/6 of all freshwater amphipods in the world (Väinölä et al., 2008). Most of these species can be found in European the ground waters and therefore constitute a significant proportion of freshwater fauna in the region (Väinölä et al., 2008; Zagmajster et al., 2014). With its number of species and morphological and ecological diversity (Sket, 1958; Ginet, 1960; Fišer et al., 2010, 2016), *Niphargus* is one of the most important invertebrate model organisms for evolutionary and ecological studies (Fišer, 2012).

The first record of *Niphargus* species dates back into 1836 when *Gammarus puteanus* Koch, 1836 was described. The genus *Niphargus* was erected in 1849, based on the description of *Gammarus stygius* Schiödte, 1847 collected in the cave Postonjska jama in Slovenia. The diagnostic characteristics of *Niphargus* are complete reduction of the eyes, lack of integumental pigmentation, distinctive shape of gnathopods, pedicellate gills, separated segments of the urosome, reduced inner ramus of uropod III and the absence of facial spine on basis of uropod I (Lowry & Myers, 2013). The family Niphargidae was introduced and distinguished from the family Gammaridae in 1978 (Bousfield, 1982). Beside *Niphargus* the family consists of several additional genera, among which some members may not be phylogenetically justified (Englisch et al., 2003; Trontelj et al., 2012; Esmaeili-Rineh et al., 2015b; Fišer et al., 2015). The family Niphargidae is extremely heterogeneous and difficult to provide with comprehensive diagnosis (Fišer et al., 2008). Morphological traits are inappropriate for inference of phylogenetic relationships which depend strongly on molecular data instead (Fišer et al., 2008). Morphology is highly sensitive to the local selective regime. On the one hand, specialization to microniches yield morphologically extremely different ecomorphs among closely related species (Trontelj et al., 2012), and substantial divergence within single species (Delić et al., 2016). On the other hand, strong convergence (Trontelj et al., 2009) or morphological stasis (Meleg et al., 2013) yield morphologically cryptic species. The latter are rather common phenomena (Meleg et al., 2013).

The distribution of the genus is strongly determined by Pleistocene glaciations with possible extinctions in glaciated and arid areas of the North and East (Fišer et al., 2009a; Karaman & Ruffo, 1986). On the other hand speciation processes mediated by habitat heterogeneity and high productivity enhanced species richness in the territories of North Italy and West Balkans (Eme et al., 2014; McInerney et al., 2014). *Niphargus* is notably absent in the Iberian peninsula where the related genus *Haploginglymus* inhabits

subterranean aquatic habitats instead. The distribution of *Niphargus* extends to the Middle East across Turkey to Iran (Karaman, 1986; Fišer et al., 2009a; Esmaeili-Rineh et al., 2015a). Species range sizes vary in space; the degree of endemism is remarkably higher in southern latitudes whereas large-ranged species are more common in the north (Eme et al., submitted). However, ranges larger than 200 km between the two distal-most points are rare and hard to explain. As such those might be the cases of taxonomically unresolved cryptic species. Still, there are some species with distributional ranges well over 200 km in southern latitudes, including across the Dinaric ridge. The 650 km long Dinaric limestone massif ranges from western Slovenia in the north along the Adriatic sea to Montenegro in the south (Mihevc et al., 2010). Cave fauna of the area have been studied for more than a century and the region itself can be considered as one of the most thoroughly explored areas for subterranean fauna in the world. The region is extremely rich with crustaceans from the genus *Niphargus* (Zagmajster et al., 2014). The Dinaric species show exceptional morphological and also ecological diversity. So far, close to 200 species have been described from this area and apparently this is not the end of the line (Švara et al., 2015; Karaman, 2016).

The largest subterranean amphipod species from the Dinaric karst belong to *Niphargus orcinus* group (Fig. 1), which consists of more than two dozen species that share the similar characteristics of long bulky body and long appendages mostly equipped with long spines. Species of the group are the top arthropod predator in cave waters (Fišer et al., 2010). The main importance of predators in the ecosystem is in their contribution to high biodiversity and ecological balance of the ecosystem (Boulton et al., 2008). All of the species from the *Niphargus orcinus* group are endemic to Dinaric Karst but they are not protected. The understanding of ecology and phylogeny of each of those species is crucial for future conservation and preservation of biodiversity in the area (Sket, 1999; Baker et al., 2003; Bickford et al., 2007; Ferreira et al., 2007).

Figure 1: Amphipod from the species complex *Niphargus arbiter/Niphargus salonitanus* (Photo taken by Teo Delić).

Slika 1: Jamska postranica iz kompleksa vrst *Niphargus arbiter/Niphargus salonitanus* (Fotografija Tea Delića).

2.2 SPECIES DELINEATION AND INTEGRATIVE TAXONOMY

Taxonomy, the essential discipline for identification and description of species, is facing crisis due to a gap in the taxonomic knowledge of less attractive organisms, limited taxonomic infrastructure (bad databases and specimens accessibility) and a decline of experts (Godfray, 2002; Coleman, 2015). The term species is theoretically defined with more than 24 species concepts (Mayden, 1997; De Queiroz, 2005). The results of different species delimitation approaches can sometimes dissagree. The inappropriate species delineation, due to the choice of species concept can have an important impact on the outcome of studies that include species traits such as ecology, evolution and behavior.

In searching for a solution to the problem of a lack of consensus over what defines a species de Queiroz (De Queiroz, 2005) prposed a general species concept. The concept defines a species as a metapopulation lineage that evolves separately of others metapopulations by divergence which can manifest itself in different ways. The divergence can be indirectly observed through genetic comparison, interbreeding, phylogenetic relationship, the same ecological role or morphological distinctness. The general species concept defines a common base that unifies different approaches for describing a species.

Traditional taxonomists have been using methods based on morphology and often rely on them to distinguish between species. The more novel and reliable approach to distinguish

and also delimit species is with molecular delimitation. It usually defines species based on their specific genetic sequence. On the other hand it does not provide additional data which can be useful in the field (Sites & Marshall, 2003) such as species ecological preferences or morphological characteristics. Alone, a morphological or phylogenetic approach to taxonomy fails in part of its essential role: either delimitation, classification and naming species or in providing tools for species identification (Dayrat, 2005). To provide all of those services an integrative approach to taxonomy is suggested to rigorously and robustly delineate species (Dayrat, 2005; Padial et al., 2010; Schlick-Steiner et al., 2010). In integrative taxonomy each criterion, e.g. molecular sequence, morphological character, ecological models, is equally important for species delimitation. As such every species is defined by a set of parameters. The definition of each species can be tested and supplemented with additional experiments or more novel methods. Combining methods from different biological disciplines such as molecular data, biogeography, morphology and ecology seem to be the most effective in robust species delineation especially concerning cryptic species (Jörger & Schrödl, 2013).

The robustness of species hypothesis increases if several methods agree on the same species delimitation (Schlick-Steiner et al., 2010). Schick-Steiner et al. (2010) suggest that at least three disciplines are required for robust taxonomy, and recommend that morphological and genetic divergence should be ideally supplemented by ecological or behavioral data.

The first step of integrative taxonomy is deciding which group of species is going to be tested and to try to support it with additional data. This can usually be done based on experience or with already published research. One of the possible next step is to follow the general species concept in species delimitation using genetic information (de Quiroz, 2007). Currently, molecular taxonomy uses uni- and multilocus species delimitation methods. Combining at least one nuclear (e.g. ITS) and one mitochondrial (e.g. COI, 16S) genetic marker makes delimitation more robust (Lefébure et al., 2006a). Quite often different delimitation methods yield different species composition; Generalized Mixed Yule Coalescence (GMYC) often identifies higher number of species than Poisson Tree Process (PTP) or even Automatic Barcode Gap Discovery (ABGD) (Fontaneto et al., 2015). However, the result of molecular analyses can be a series of alternative species hypotheses which can then be critically evaluated.

Morphology is the most traditional and often the least expensive and the least time consuming, straight forward method. The use of powerful light microscopes and visualizing instruments such as electronic microscopy and micro computer tomography can provide data that was not available in the past (Pilz et al., 2008). As many morphological characters as possible must be taken into account to allow thorough

statistical analysis. Knowledge of variable but informative characters of the studied group can significantly improve the speed and quality of acquiring large datasets (Fišer et al., 2009b). Even though morphological variation does not yield diagnostic traits in cryptic species, morphological analysis may identify clusters of species, which may be in turn identified with the help of another data source.

Finally, in some cases ecological data successfully delimit species and complement morphological and molecular analyses (Raxworthy et al., 2007). Ecological niche modeling combines bioclimatic information with species' distribution data to visualize important biogeographic species' traits (Barry & Elith, 2006). Ecological modeling is conducted in four steps. The first step is selection of an appropriate grid for analysis. In the second step species distribution (dependent variable) is applied to a map, which is overlaid by environmental variables in the third step. In the final step, the ecological niche (Fig. 2) is modeled from ecological properties of those cells where species were found (Raxworthy et al., 2007). Part of the data is used for model training whereas a small part of the data is used for model validation. Jackknife validation approach (Pearson et al., 2007) permits ecological niche modeling in MAXENT software (Phillips et al., 2004) as long as there are at least five presence data points available. Such models of ecological niches can be applied in taxonomy, under assumptions that niches of distinct species do not overlap or overlap only in part. Niche overlap estimate various indices and the significance of the overlap can be estimated from randomization procedure using Schoener's D index and Hellinger distance I (Warren et al., 2008).

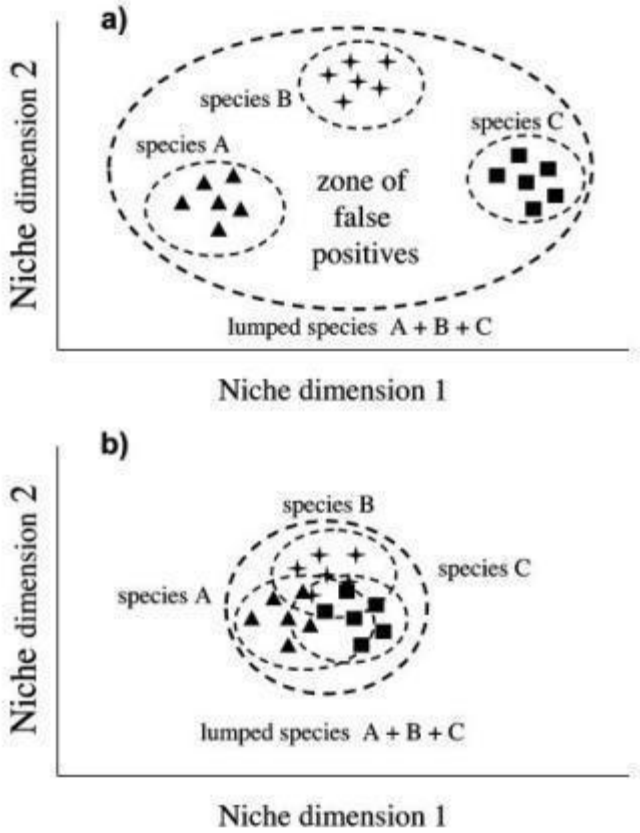


Figure 2: Niche examples of three cryptic species (after Raxworthy et al., 2007).

Slika 2: Primer ekoloških niš treh kriptičnih vrst (po Raxworthy in sod., 2007).

3 MATERIAL AND METHODS

3.1 DATA

The specimencs were collected from 34 localities (in total 109 individuals) from the entire Dinaric Karst. The sampling area covers the entire 500 km long range of the complex *Niphargus arbiter*/*Niphargus salonitanus* (Fig. 3) The samples were collected between September 2000 and July 2013 by hand nets or baited traps and are stored in 96% ethanol at the Department of Biology at the Biotechnical faculty, University of Ljubljana. Details on localities, vouchers and accession numbers are accessible in the Appendix A.

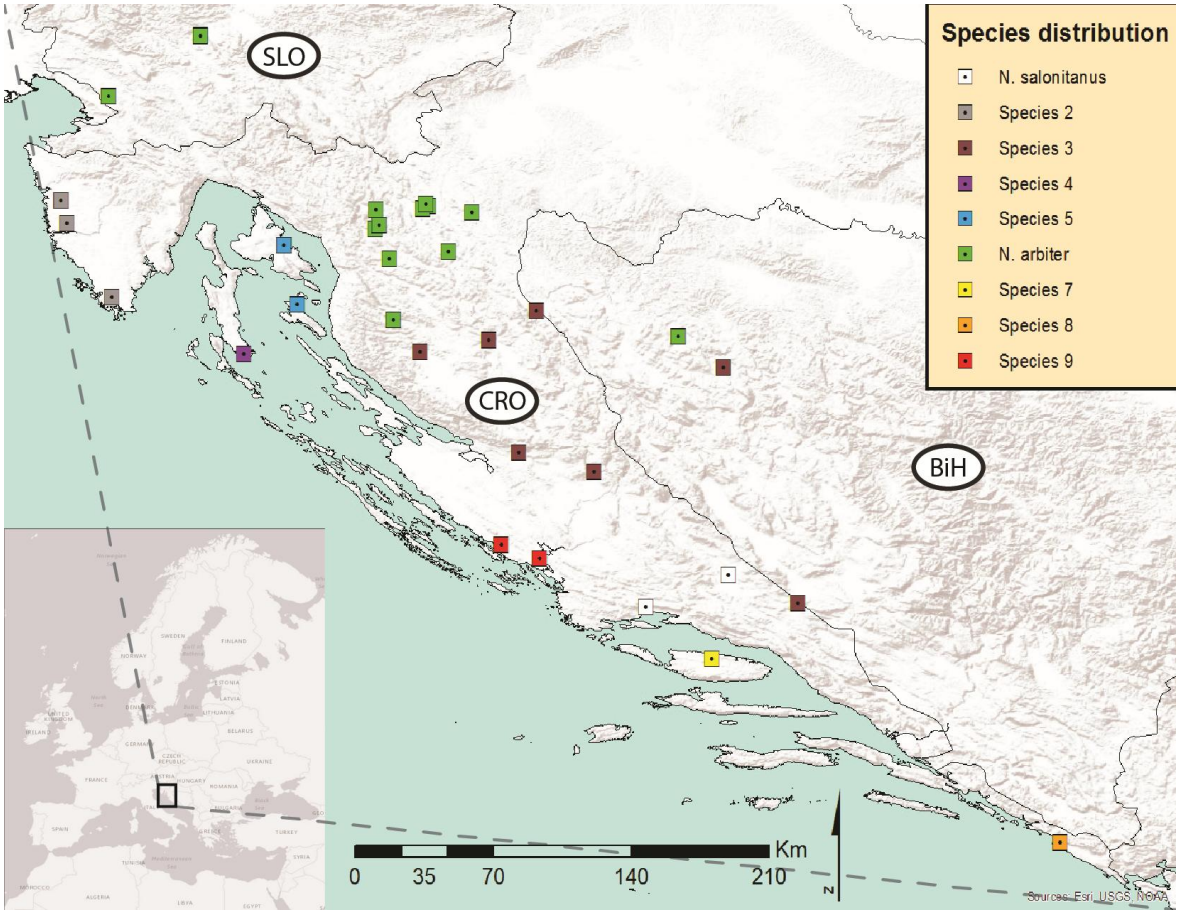


Figure 3: Distribution map of the species complex *Niphargus arbiter*/*Niphargus salonitanus*.

Slika 3: Mapa razširjenosti kompleksa vrst *Niphargus arbiter/Nipahrgus salonitanus*.

3.2 MOLECULAR ANALYSIS

One of the pereopods was removed for DNA extraction, while the rest of the specimen was stored for morphological analyses. Genomic DNA was extracted using GeneElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich) according to the Mammalian Tissue Preparation protocol. The nuclear DNA (nDNA) loci including two parts of 28S ribosomal subunit (28S rRNA I and 28S rRNA II), internal transcribed spacer (ITS), histone 3 subunit A (H3) and two fragments of mitochondrial (mtDNA) cytochrome oxidase I (COI I and COI II) were amplified. Also partial 28S rRNA fragments were amplified using primers 28S lev2 (Verovnik et al., 2005) and 28S des2 (Zakšek et al., 2007) for 28S rRNA I and primers 28S lev3 and 28S des5 (Fišer et al., 2013) for 28S rRNA II. ITS region was amplified using primers ITS f1 and ITS r1 (Flot et al., 2010), H3 was amplified using H3aF2 and H3aR2 primers (Colgan et al., 1998). The first COI (COI I) fragment was amplified using primers Jerry and Maggie (Simon et al., 1994) and the second part (COI II) with LCO (Folmer et al., 1994) and COIspr1 (Fišer et al., 2015). The

ITS region was additionally sequenced with four extra internal primers (ITS sf1, ITS sr1, ITS sf2, ITS sr2).

The polymerase chain reaction (PCR) cycling setting was identical to protocols from Fišer et al. (2013) and an additional program counting 30 cycles of 94 °C for 30 sec, 54 °C for 45 sec, 72 °C for 2 min, following by a final extension at 72°C for 10 min was used for ITS.

Successfully amplified PCR products were purified using Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific Inc., US), and sequenced using the same amplification primers in the forward and backward direction by Microsynth AG (Balgach, Switzerland). Resulting chromatograms were assembled and edited in Geneious 6.0.5. (Biomatters Ltd, New Zealand), with gaps coded as (–) and missing data as (?). Edited sequence were then aligned in MAFFT v7 (Katoh & Standley, 2013).

3.3 PHYLOGENETIC ANALYSIS

To position the studied species complex within the *Niphargus* evolutionary tree, a concatenated alignment of ITS, 28S rRNA I, 28S rRNA II, COI I and COI II was assembled. A dataset of 83 specimens of the studiesd species complex was assembled, 29 specimens of other *Niphargus* species and two outgroup species (*Synurella ambulans* and *Gammarus fossarum*) were included. The included species within the genus *Niphargus* covered all major lineages identified hitherto (Lefébure et al., 2006a, 2007; Fišer et al., 2008; Esmaeili-Rineh et al., 2015a) The best fitted evolutionary model of for each partition was selected using PartitionFinder (Lanfear et al., 2012). Phylogenetic relationships were reconstructed with Bayesian inference (BA) in MrBayes v3.2 (Ronquist & Huelsenbeck, 2003) and BEAST v1.8.1 (Drummond et al., 2012). Two parallel Markov chain Monte Carlo (MCMC) algorithms with four cold chains each, were run for 10 million generations sampling every 200th generation in MrBayes. The first 25% of sampled trees were discarded as a burn-in while the remaining trees were used to assemble the majority-rule consensus tree (Fig. 4).

Alternatively, a multilocus gene phylogeny was run in BEAST v. 1.8.1 using different clock (strict, relaxed and exponential) and speciation (Yule process, Birth-death) settings. MCMC run was set to 80 million generations, sampling every 5000th generation. Resulting data was checked for parameter convergence in Tracer v1.6 (Rambaut et al., 2014) and the maximum credibility tree was assembled using Tree Annotator version 1.8.1. (Drummond et al., 2012) after discarding the first 2000 trees as a burn-in. Outcomes of different runs were compared according to AICM values and the analysis run under strict clock with pure birth-death speciation model was selected as the most appropriate. Evolutionary

diversification of niphargids was estimated using 45 million years old amber remains (Jażdżewski & Kupryjanowicz, 2010).

Molecular species delineation analysis include three unilocus delimitation methods and one multilocus method. The deatails about delimitation procedures, which were performed by Teo Delić are documented in the Appendix B.

3.4 MORPHOLOGICAL ANALYSIS

Selected specimens were treated in a 10% hot solution of KOH, briefly rinsed with diluted HCl and washed with distilled water. Cleared exoskeletons were stained with either chlorazol black or lignin pink, partly dissected in glycerol and mounted on slides in a glycerol-gelatine medium. Morphology was studied under a stereomicroscope Olympus SZX9 (magnifications 3.14–114×) and a Zeiss microscope (magnifications 100–400x). Landmarks that were used are described in Fišer et al. (2009). Digital drawings (digital inking) were created in Adobe Illustrator CS3, using photographs of the appendages, a Bamboo digital drawing board and a digital pen (Coleman, 2003, 2006, 2009).

In the morphological analysis, 63 specimens were analyzed. We tested the hypothesis that molecularly determined species are also morphologically different. For that purpose 26 morphometric characters and 99 other characters (counts, categorical, list of selected characters is available in Appendix C) were analyzed. In order to remove the impact of body length, all measures were plotted against body length and residuals calculated. All subsequent tests were based on residual values. Differences among species were tested for each trait using either ANOVA with applied post-hoc Scheffe, Bonferroni and Hochberg corrections for normally distributed data or Kolmogorov-Smirnov (MannWhitney U tests with adjusted alpha level for pairwise comparisons) for non-normally distributed data. Damaged specimens (e.g. with broken appendages) were excluded from analyses. Taxonomically important characters (Tab. 1, Appendix C) that showed differences in a smaller sample of specimencs were checked for every specimen. Differences in proportions of appendages and number of spines between the species that may be important diagnostically were visualized on plots using IBM SPSS Statistics v20. Non-quantitative characters and frequencies (e.g. number of spines) were analyzed using population aggregation analysis (PAA) (Davis & Nixon, 1992).

Terminological note: true spines, i.e. extrusions of cuticle, are not known in *Niphargus*. Species from this genus have appendages armed with flexible thin setae, flexible plumose setae and stout spiniform setae. To simplify descriptions, we refer to the thin flexible setae as ‘setae’ and stout spiniform setae as ‘spines’.

3.5 ECOLOGICAL MODELING USING MAXENT

The occurrence data of species were obtained from the molecularly identified species. Altogether species spatial data from 34 localities that had been molecularly delimited was included in the analysis (Tab. 3).

Many species within the focal species complex turned out to be narrowly endemic, which strongly hampered species' ecological niche modeling and pairwise comparisons of ecological differentiation at species level; only one species pair could have been tested for ecological niche overlap at the species level. Instead, the niche differentiation was explored at the clade level. The data was pooled along the phylogenetic hierarchy such that minimally five occurrence data per taxon were obtained (see below, (Pearson et al., 2007)). For each taxon (species, clade, group of clades) the hypothetical bioclimatic niche was reconstructed and in the second step tested whether or not taxon pair differs with respect to available ecological data.

The ecological niche was modeled using data from BioClim (Hijmans et al., 2005). Short-term climatic oscillations are buffered in subterranean ecosystems (Culver & White, 2005), however, annual precipitation regime and long term temperature oscillations affect productivity on the surface. It has been shown that productivity determines species richness of subterranean crustaceans and may at least indirectly affect ecological needs of closely related species (Eme et al., 2014). The Bioclim dataset includes 19 layers of various climatic parameters at resolution 1km x 1km. These layers were applied to a grid with cell size 10 km x 10 km, and edited in ArcGIS to fit their size to the area of Dinaric Karst. To account for the non-independence among climatic parameters, first we calculated pairwise correlations among parameters and removed strongly correlated parameters. For the needs of the analysis three alternative datasets were prepared, in which parameters correlate to different degrees (coefficient of correlation, spearman's rho > 0.6; 0.7; 0.8). All analyses of correlation and calculations of spearman r were calculated using package agricolae (Mendiburu, 2015) in R (R Development Core Team, 2016). Ecological niches were modeled in program Maxent using presence only data (Barry & Elith, 2006; Ortega-Huerta & Townsend Peterson, 2008). It has been shown that the method effectively constructs ecological niches even when sample sizes are small (Pearson et al., 2007; Kumar & Stohlgren, 2009).

Using the given data we created 4 sets of models: Species 3 model, Species 6 model; Species [2, 7, 9] (clade A) model and species [1, 3, 4, 5, 6, 8] (clade B) model. All models were trained using 80 % of the occurrence data and tested with remaining 20 % of the data. (Kumar & Stohlgren, 2009). Other settings were set to default (Phillips et al., 2006). The quality of the model was assessed using jackknife procedure. Each taxon model is an

average of ten replicates. In the second step we tested whether estimated ecological niches of target taxa (species / clades) are equivalent to each other (Warren et al., 2008) using the R packages phyloclim (Heibl & Calenge, 2013) and dismo (Hijmans et al., 2016). These packages calculate and estimate significance for indices of similarity (niche equivalency) and overlap (niche overlap), respectively. Schoener's D index and Hellinger distance were calculated (Warren et al., 2008). Usually the D index is used to interpret the results (Aguirre-Gutierrez et al., 2015). The indexes range from 0 (no overlap) to 1 (identical potential distributions).

4 RESULTS

4.1 PHYLOGENETIC ANALYSIS AND MOLECULAR SPECIES DELIMITATION

The *Niphargus arbiter/Niphargus salonitanus* species group is a monophyletic complex nested within a clade of 'cave lake' (*Niphargus ictus*, *Niphargus longiflagellum*, *Niphargus steueri*) and 'lake giant' (*Niphargus rejici*, *Niphargus stenopus*, *Niphargus pachytelson*) ecomorphs (Fig. 4). The phylogenetic analysis hence suggests that the complex derives from pre-adapted ancestor distributed within the broader Dinaric area and Italy (*Niphargus ictus*) (Fig. 4).

The complex itself is comprised of four major lineages. The first lineage comprises mainly coastal populations along the eastern Adriatic coast, including the Istrian Peninsula, the Zadar region and the Island of Brač (Fig. 3, 4). This lineage diverged into three species. The species from Brač is supported by all three unilocus and multilocus delimitation methods. Less clear is the species structure of Istra-Zadar populations. While a 0.16 threshold distance indicates this should be treated as a single species, the GMYC and PTP method support two species. The two groups are separated by a distance of approximately 200 km of sea, and as multilocus BPP supports a two-species structure (under all settings tested), we suggest the two groups to be treated as the as two separated species in the future.

The second lineage (Fig. 3) includes populations from the islands of Cres and Krk from the Gulf of Kvarner. Again, the more conservative 0.16 threshold distance indicates that all populations from Kvarner islands should be treated as a single species, whereas GMYC, PTP and BPP (under all settings tested) suggest that the population from each island should be treated as a separate species (Fig. 4). Given that the two island populations are already monophyletic, genetically differentiated and physically separated, we treat them as separate species.

The third lineage (Fig. 3) is distributed along the southern part of the Adriatic Coast, and consists of two species according to all four species delimitation methods (Fig. 4). Populations the from the vicinity of the city of Split (from a well near Church of Stomorija) likely belong to the population of *Niphargus salonitanus*, from which also the type specimen was collected by S. Karaman. The second population from the anchihialine cave Šipun deserves a separate species status based on strong support in all for molecular delimitation approaches.

Finally, the fourth lineage is distributed across inland montane areas between Slovenia, Croatia and Bosnia and Herzegowina (Fig. 3). All four species delimitation methods indicate that this lineage consists of two species. The northern species includes specimens from the population of *Niphargus arbiter*, while the southern can be treated as a new species.

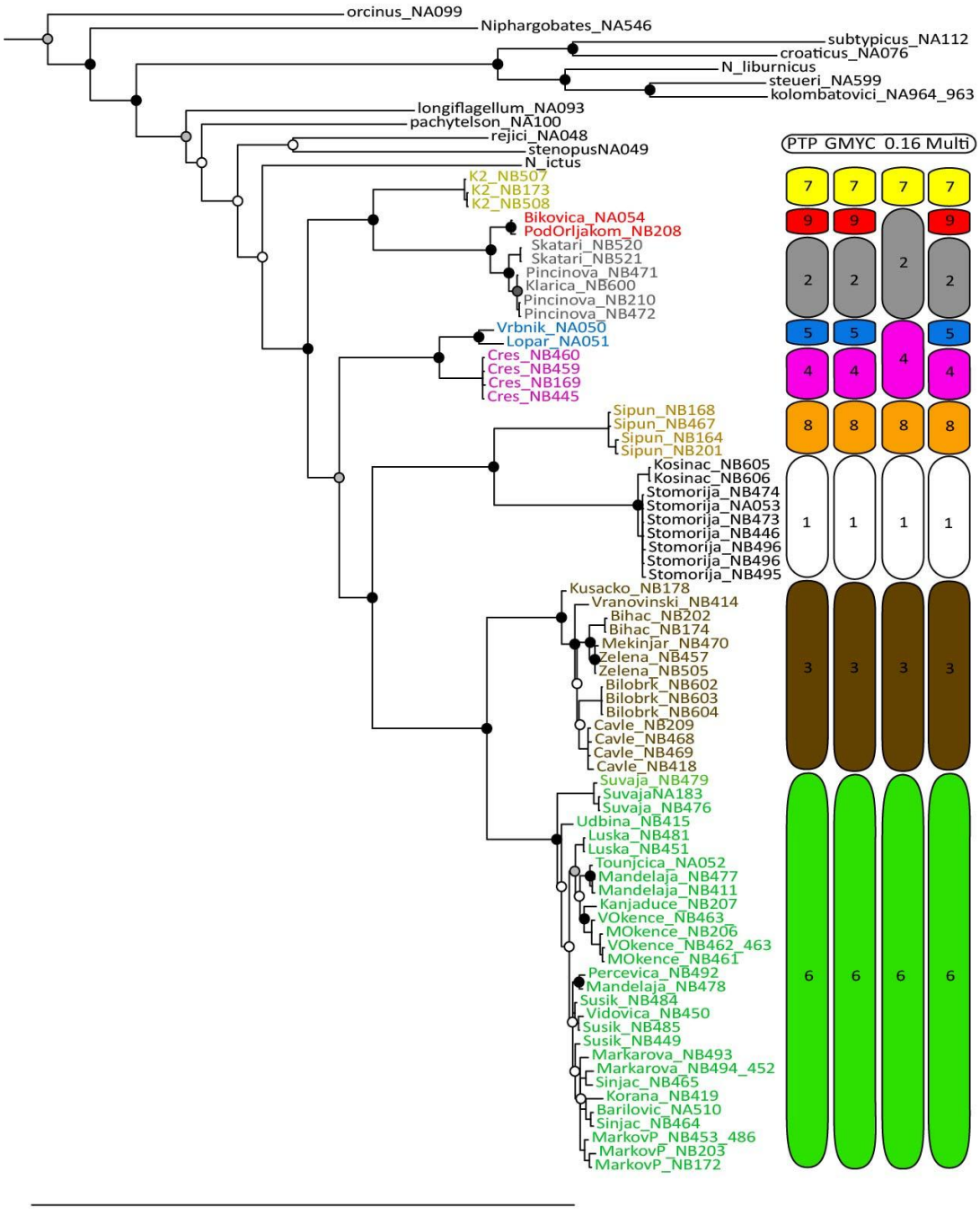


Figure 4: Phylogenetic tree of the *Niphargus arbiter/Niphargus salonitanus* species complex based on multilocus analysis. The colors of the nodes represent different support to the appropriate clade, where black ≥ 0.99; grey ≥ 0.95 and < 0.99; white < 0.95. Nodes without any circle present individuals from the same locality and support of 1. Columns on the right show different delimitations and the species identificafication number proposed by each approach. The bar below shows 0.1 nucleotide substitute per base pair.

Slika 4: Filogenetsko drevo kompleksa vrst *Niphargus arbiter/Niphargus salonitanus* osnovano na multilokusni primerjavi. Barve na mestih cepitev predstavljajo podporne vrednosti pripadajočim kladom: Črna ≥ 0.99, siva ≥ 0.95 in < 0.99, bela < 0.95. Cepitve brez krogov predstavljajo osebke iz iste lokacije in podporo 1. Stolpci na desni predstavljajo različne delimitacije in pripadnost osebkov posamezni vrsti. Merilo prikazuje 0.1 nukleotidno zamenjavo na bazno mesto.

4.2 MORPHOLOGICAL RESULTS

All nine species showed high morphological variation within and outstanding morphological similarity between them. Among 99 studied qualitative and numerical counted characters and 27 morphometric characters, only 34 numerical counted (Tab. 1) and 8 morphometric characters turned to be potentially useful in species discrimination¸, as they are significantly different between some species pairs (Tab. 2). Nine species morphologically differ from each other to various extent. The most differentiated species pairs (species pair 4–7, species pair 4–9) differ in 17 numerical counted traits. Two pairs (species pair 3–6 and species pair 2–3) cannot be discriminated from each other based on morphology. Traits that diagnose species are listed in Tables 2 (qualitative, counts) and 3 (morphometric).

Table 1: An analysis of numeric counted taxonomic characters. All characters are expressed as intervals and presented in absolute values (left column) and as corrected by the body length (right column). Species character that differ in at least one other species character in the complex are in bold.

Preglednica 1: Analiza ütevilskih ütetih taksonomskih znakov. Vsi znaki so izraženi kot intervali absolutnih vrednosti (levi stolpec) ter kot razmerja s telesno dolžino (desni stolpec). Znaki, v katerih se vrsta razlikuje vsaj od ene druge vrste so označeni s krepkim tiskom.

Species	n set pereonite VII		n set pleosome I		n sp pleosome I		n set pleosome II		n sp pleosome II		n set pleosome III	
1	0-4	0-0.194	**12-18**	**0.492-0.875**	**0**	**0**	**12-17**	**0.523-0.778**	0-1	0-0.047	13-16	**0.369-0.778**
2	0-2	0-0.067	**5-14**	**0.167-0.849**	**3-12**	**0.181-0.504**	**3-9**	**0.100-0.546**	1-15	**0.060-0.560**	**2-8**	**0.067-0.485**
3	0-1	**0-0.042**	5-18	0.210-0.451	**0-9**	0-0.534	2-15	0.101-0.843	0-14	**0-0.652**	2-13	**0.84-0.376**
4	**1-3**	**0.063-0.367**	**14-20**	**1.258-1.712**	**0**	**0**	6-21	**0.617-1.957**	0	0	**12-25**	**1.234-1.835**
5	**1**	**0.054**	**7-15**	0.485-2.311	**0**	**0**	7-17	**0.843-2.619**	0	0	5-20	0.345-3.082
6	0-2	0-0.128	2-16	**0.126-0.938**	**0-15**	0-0.701	4-23	0.112-0.877	0-16	0-1.022	2-21	**0.080-0.866**
7	0	0	14	0.483	1	0.034	7	0.241	18	0.621	6	0.207
8	**1-2**	**0.056-0.140**	**6-19**	0.068-1.328	**0**	**0**	3-14	0.204-0.978	0-6	0-0.419	**1-8**	**0.068-0.619**
9	0	0	4	0.121	11	0.332	7	0.211	14	0.423	4	0.121

Species	n sp pleosome III		n sp urosomite I		n sp urosomite II		n sp epim plate II		n sp epim plate III		n apical sp	
1	**0-5**	0-0.236	3	**0.092-0.147**	**3-4**	**0.123-0.194**	**2-3**	0.092-0.146	3-4	**0.123-0.197**	**5-6**	**0.184-0.292**
2	**4-13**	**0.242-0.616**	**2-3**	0.100-0.287	**2-4**	0.112-0.383	1-3	0.056-0.191	1-5	0.056-0.287	5-16	**0.303-0.897**
3	**7-15**	**0.225-1.570**	2-4	0.087-0.314	3-5	0.125-0.419	1-5	0.042-0.258	2-5	0.058-0.258	**6-18**	0.200-1.256
4	**0**	0	**3**	**0.189-0.367**	**3-4**	**0.252-0.489**	**2-3**	0.126-0.367	2-4	**0.206-0.489**	**6-8**	**0.674-0.978**
5	**0**	**0**	**3**	**0.207-0.462**	**4**	**0.276-0.616**	**2-3**	0.138-0.462	3-4	**0.207-0.616**	**7-9**	**0.483-1.387**
6	**0-16**	0-0.958	3-5	0.097-0.300	3-6	0.126-0.318	1-4	0.076-0.217	2-4	0.122-0.300	**6-16**	0.198-0.866
7	15	0.517	4	0.138	5	0.172	2	0.069	3	0.103	5	0.172
8	**2-16**	**0.136-1.118**	3-4	**0.223-0.280**	3-5	**0.223-0.349**	1-2	0.068-0.155	3-4	**0.210-0.310**	**6-8**	**0.408-0.527**
9	12	0.362	4	0.121	4	0.121	1	0.030	3	0.90	5	0.151

Species	n set cx 1		n set gI/3		n gr set gI6/post		n gr set gI6/ant		n set palm sp gI		n gr seta gI/7	
1	**9-11**	**0.307-0.486**	7-17	0.461-0.707	**14-20**	0.615-0.807	4-6	**0.184-0.292**	**3-5**	0.123-0.236	6-17	**0.523-0.807**
2	5-12	**0.364-0.485**	8-18	0.543-0.874	9-17	0.569-0.861	**3-5**	0.167-0.388	2-7	0.121-0.287	**9-14**	0.435-0.861
3	7-13	**0.294-0.733**	**9-28**	0.260-1.256	9-18	0.426-0.942	3-6	0.029-0.419	2-4	0.087-0.419	7-27	0.202-1.361
4	**6-10**	**0.629-0.899**	**4-11**	0.449-0.692	**7-10**	0.629-0.856	**4-5**	**0.314-0.514**	2-4	0.189-0.411	**8-13**	**0.818-1.061**
5	**12**	**1849**	**4-8**	0.276-1.233	**7-12**	0.483-1.849	2-5	0.138-0.770	2-3	0.138-0.462	**3-7**	0.207-1.079
6	7-14	**0.265-0.636**	6-31	0.477-0.977	9-20	0.496-0.901	4-7	0.165-0.432	1-5	0.100-0.318	4-20	0.289-1.034
7	11	0.379	14	0.483	21	0.724	6	0.207	2	0.069	21	0.724
8	**4-7**	**0.280-0.476**	**6-10**	0.408-0.619	**11-15**	0.748-0.856	**3-5**	0.198-0.387	**2**	0.112-0.155	**8-15**	0.544-1.048
9	14	0.423	25	0.755	23	0.694	5	0.151	2	0.060	19	0.573

continued

continuation of Table 1. An analysis of numerical counted taxonomic characters**.**

Species	n seta cxII		n seta gII3		n gr set gII/6 post		n gr set gIIa		n set gII/6 antdist		n gr set gII/7	
1	10-14	0.430-0.632	2-4	0.092-0.147	**12-15**	**0.461-0.660**	2-4	**0.092-0.097**	6-14	0.184-0.681	6-16	**0.553-0.760**
2	5-13	**0.342-0.546**	1-4	**0.056-0.134**	8-16	0.485-0.861	3-4	**0.224-1.052**	0-6	**0-0.478**	**8-15**	**0.469-0.845**
3	6-14	0.252-0.904	2-14	0.119-0.589	**10-16**	0.347-0.861	2-6	**0.101-0.228**	2-14	0.084-0.702	**8-29**	**0.231-1.012**
4	7-11	**0.692-0.899**	2-5	**0.063-0.122**	**7-9**	0.566-0.899	2-4	**0.206-0.354**	5-11	**0.514-0.787**	**9-14**	**0.943-1.236**
5	8-11	**0.552-1.695**	2-3	**0.138-0.462**	7-11	0.483-1.695	1-3	0.069-0.462	3-7	0.207-1.079	**3-6**	**0.207-0.924**
6	6-17	**0.348-0.658**	1-14	0.093-0.556	8-18	0.463-0.954	2-6	**0.120-0.371**	2-15	0.132-0.572	4-18	**0.289-0.954**
7	12	0.414	6	0.207	21	0.724	4	0.138	7	0.241	15	0.517
8	**8-10**	0.502-0.699	2-5	**0.132-0.309**	**10-13**	**0.680-0.774**	1-3	**0.068-0.167**	5-8	0.279-0.544	**7-16**	0.476-1.006
9	11	0.332	7	0.211	18	0.543	4	0.121	6	0.181	20	0.604

Species	n set gII7		n set cxIII		n set cx IV		n gr sp pIV/4 post		n gr sp pIV4ant		n gr sp pVII2 ant	
1	6-16	**0.553-0.760**	**8-14**	**0.338-0.583**	**11-14**	0.430-0.681	3-5	**0.092-0.197**	3-4	**0.123-0.197**	**6-9**	**0.184-0.380**
2	8-15	0.469-0.845	**8-11**	0.368-0.765	**10-13**	0.603-0.957	3-5	0.134-0.302	3-4	0.134-0.287	**7-9**	**0.268-0.546**
3	8-30	0.231-1.012	**7-13**	0.276-1.047	11-16	0.387-1.361	3-5	0.075-0.344	3-4	**0.100-0.258**	6-10	**0.225-0.534**
4	9-15	**0.943-1.236**	6-12	**0.708-1.011**	**6-11**	0.674-0.944	**2-3**	0.189-0.337	3-4	**0.252-0.411**	**6-8**	**0.708-0.978**
5	3-6	0.207-0.924	**6**	0.414-0.924	**6-8**	0.414-1.233	**4**	**0.276-0.616**	3-4	0.207-0.616	**8-9**	**0.552-1.387**
6	4-19	0.289-0.954	4-15	0.378-0.716	5-19	0.430-0.875	2-4	0.088-0.279	2-5	0.076-0.398	7-11	0.231-0.795
7	15	0.517	11	0.379	15	0.517	5	0.172	4	0.138	10	0.345
8	7-16	0.476-1.006	**8-10**	0.446-0.696	**7-13**	0.489-0.884	3-4	0.210-0.309	4-5	**0.272-0.329**	**10**	**0.680**
9	20	0.604	13	0.392	13	0.392	4	0.121	4	0.121	9	0.272

Species	n set pVII2p		n sp lat uI1		n gr sp uI endo		n sp uIeks		n apical set uIII/3		n inner gr sp uIII2	
1	11-14	**0.369-0.570**	7-8	**0.246-0.380**	8-15	**0.389-0.688**	19-34	**1.045-1.321**	2-4	**0.123-0.194**	7-14	0.369-0.660
2	11-12	**0.402-0.874**	6-8	0.268-0.670	9-12	0.402-0.861	14-36	0.785-1.722	**3-4**	0.134-0.287	8-11	0.368-0.957
3	12-17	**0.376-0.771**	5-9	0.125-0.516	4-15	0.100-0.861	15-39	0.376-1.897	**3-8**	0.152-0.200	**9-12**	0.251-0.711
4	11-12	**1.345-1.415**	6-7	**0.440-0.856**	**7-9**	0.566-0.944	14-23	**1.446-2.201**	**2**	**0.206**	4	0.411
5	6-19	**0.621-2.465**	6-7	0.414-1.079	5-9	0.345-1.387	9-22	0.621-3.390	**3**	**0.207**	**5-8**	0.345-1.233
6	7-20	**0.355-0.875**	5-10	0.165-0.600	7-14	**0.298-0.722**	13-44	0.529-1.621	2-5	0.099-0.398	6-21	0.221-0.901
7	14	0.483	7	0.241	x	x	15	0.517	x	x	x	x
8	15	**1020**	7	**0.461-0.541**	**11-12**	**0.724-0.928**	18-25	1.186-1.747	2-4	0.136-0.263	7-11	0.476-0.724
9	13	0.392	7	0.211	12	0.362	30	0.905	x	x	19	0.573

Species	n D set md		n gr set md palp2.		n sp out segm mxp		n set ap mxp in lobe	
1	**31-49**	1.507-2.162	**6-8**	**0.246-0.377**	9-13	0.338-0.617	**6-12**	**0.154-0.285**
2	16-50	1.165-2.966	5-7	0.234-0.478	9-15	0.502-957	**4-7**	**0.134-0.383**
3	28-60	1.177-3.663	5-8	0.200-0.523	**10-17**	0.376-1.047	5-8	0.150-0.628
4	18-32	2.005-2.201	4-6	**0.377-0.590**	**7-9**	0.566-978	4-7	**0.308-0.674**
5	**14-29**	x	**4-5**	0.276-0.770	**4-9**	0.276-1.387	5-8	**0.414-1.079**
6	22-70	0.860-2.943	4-8	0.194-0.600	8-17	0.397-0.801	4-10	0.161-0.500
7	38	1310	7	0.241	14	0.483	9	0.103
8	28-40	1.844-2.230	5-7	0.279-0.489	9-13	0.502-0.884	5-6	**0.279-0.387**
9	50	1509	6	0.181	17	0.513	9	0.151

*the list of abbreviations: n - number; set - setae; sp - spine like setae; epim - epimera; cx - coxa; g - gnathopod; palm - palmar; p - pereopod; u - uropod; md - mandible; palp - palpus; mxp - maxilliped; eks - exopodite; endo - endopodite; gr - groups; out - outer; ant- anterior; post - posterior; lat - lateral; dist- distal; Roman numerals indicate the number of an appendage; Arabic numerals represent the number of the segment.

Table 2: Results for Kruskal-Wallis and ANOVA test. Only traits that significantly differ in at least one pair of species are presented. On the right side of the table are pairs, that show significant result between two groups after preforming tests. p values are corrected for multiple comparisons (here the Bonferroni correction was performed).

Preglednica 2: Signifikantni rezultati testov Kruskal-Wallis in ANOVA. Na desni strani tabele so pari, ki kažejo signifikantno razliko med dvema vrstama po izvedbi testov. V analizi smo uporabili reziduale regresij na telesno velikost (z izjemo telesne velikosti same). Vrednosti p (v oklepajih) so korigirane za multiplo primerjavo (Bonferroni).

Character	**Kruskal-Wallis / ANOVA* p-value**	**Speices pairs (p-value after correction)**					
a13	0.029	1–7 (0.030)	2–8 (0.011)	6–8 (0.025)	7–8 (0.025)		
a23	0.023	1–2 (0.028)	1–7 (0.030)	2–6 (0.029)	2–8 (0.009)	6–7 (0.033)	7–8 (0.025)
g16/3	0.022	1–7 (0.014)	2–7 (0.014)	3–7 (0.037)	6–7 (0.003)	7–8 (0.014)	
g25	0.026	1–2 (0.028)	2–6 (0.009)	2–8 (0.009)	6–7 (0.029)	7–8 (0.027)	
cx3h	0.009	3–4 (0.009)					
cx3v	0.002	3–4 (0.001)	4–6 (0.023)				
a11	0.005	3–4 (0.012)					
a12	0.002	2–4 (0.002)	3–4 (0.001)	4–6 (0.012)			

*italic letters indicate normally distributed characters tested with anova, regular letter indicate non-normal distributions tested with Kruskal-Wallis test.

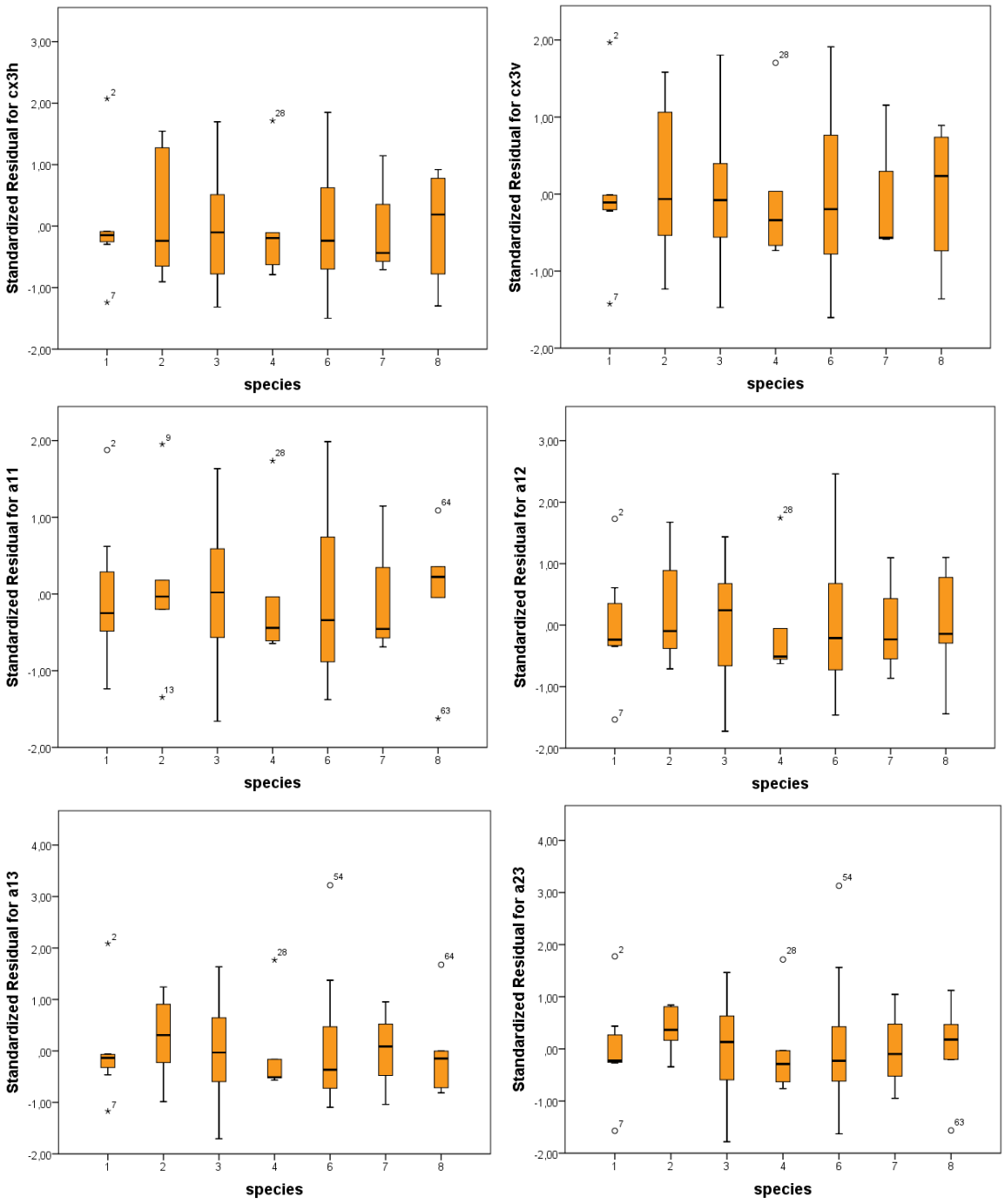


Figure 5**:** The graphs of selected residuals represent morphometric characters that distinguish between species (see Tab. 2). Continued.

Slika 5: Diagrami predstavljajo reziduale izbranih merjenih znakov, po katerih lahko razlikujemo posamezne vrste.

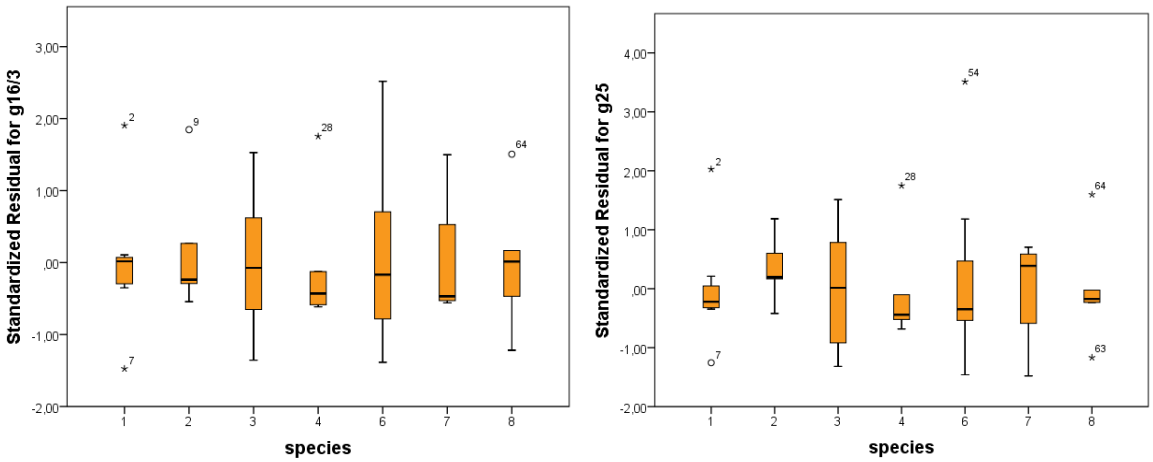


Figure 5: The graphs of selected residuals represent merphometric characters that distinguish between species (see Tab. 2). Continuation of figure 5.

Slika 5: Diagrami predstavljajo reziduale izbranih merjenih znakov, po katerih lahko razlikujemo posamezne vrste.

4.3 ECOLOGICAL NICHE COMPARISON

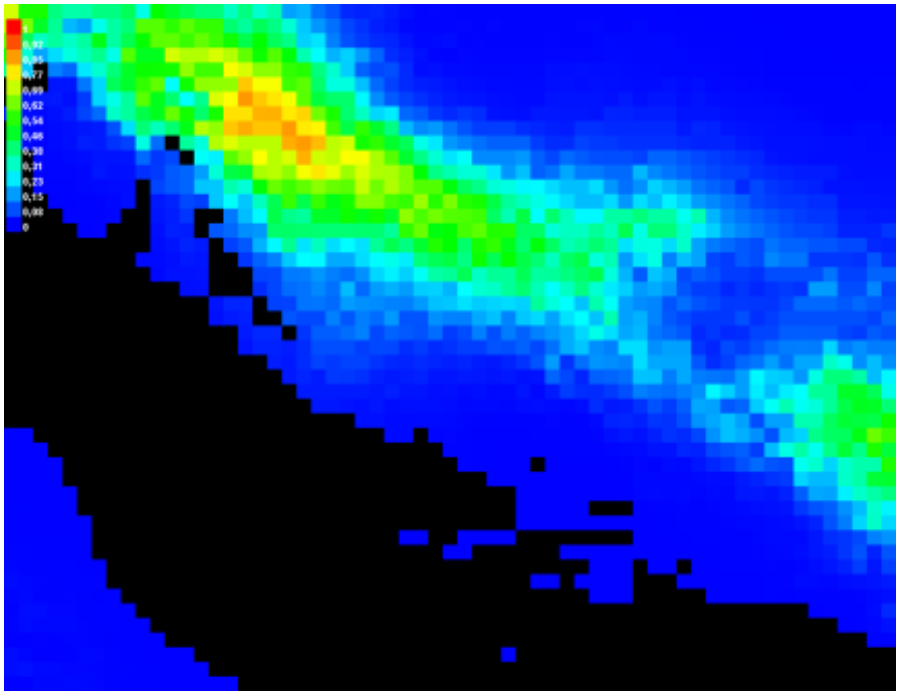
Here we present results of ecological niche models of the following taxa: species 3, *Niphargus arbiter*, clade A and clade B. The models can be considered as acceptably predictive as area under the curve (AUC) in these cases always exceeded 0.7 (Hosmer & Lemeshow, 2000) in all of the models. Pairwise comparisons indicate differentiation in most of the cases. Changes in the parameter selection did not affect the results. The comparison of the clades shows stronger differentiation than the species comparison. In the species comparison the D value is always close to 0.6, which indicates some equivalency and some overlap. However high p value does not support the significance of the result. The result shows that the species actually do not possess the same ecological niche. The I value is higher and indicates an overlap and equivalency of the niches, again without significant p value. In the clade comparison, the D and I value show low equivalency and overlap even though the p value is high again. The suggested difference (D value) can be observed in the visual presentation of the model (Fig. 6, 7), where the B clade exhibits a suitable area more centrally than clade A, for which suitable area is located near the coastal area. We cannot pinpoint such a specific area for the species comparison, as there are no clear suitable areas for species 3.

Table 3: Parameters used in ecological niche modeling based on different threshold levels of correlation (X indicates included parameter at a corresponding correlation value). Parameters were selected in a way that allow the smallest number of selected parameters.

Preglednica 3: Parametri uporabljeni v modelih ekološke niše, na osnovi različnih stopenj korelacije (X označuje parameter, ki je bil uporabljen pri dani korelacijski vrednosti). Parametri so bili izbrani na podlagi najmanjšega skupnega števila v danem modelu.

Layer	**rho = 0.6**	**rho = 0.7**	**rho = 0.8**	**Parameter description**
bio1		**X**	**X**	Annual Mean Temperature
bio2	**X**	**X**	**X**	Mean Diurnal Range (Mean of monthly (max temp - min temp))
bio9			**X**	Mean Temperature of Driest Quarter
bio11	**X**			Mean Temperature of Coldest Quarter
bio12			**X**	Annual Precipitation
bio15	**X**	**X**		Precipitation Seasonality (Coefficient of Variation)
bio16	**X**	**X**		Precipitation of Wettest Quarter
bio19			**X**	Precipitation of Coldest Quarter

A)



B)

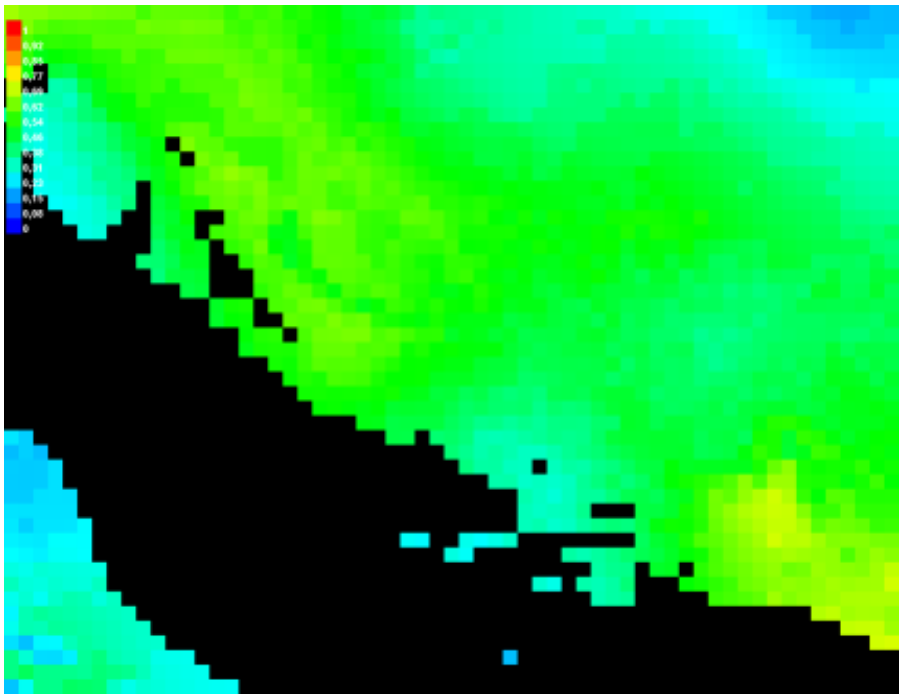
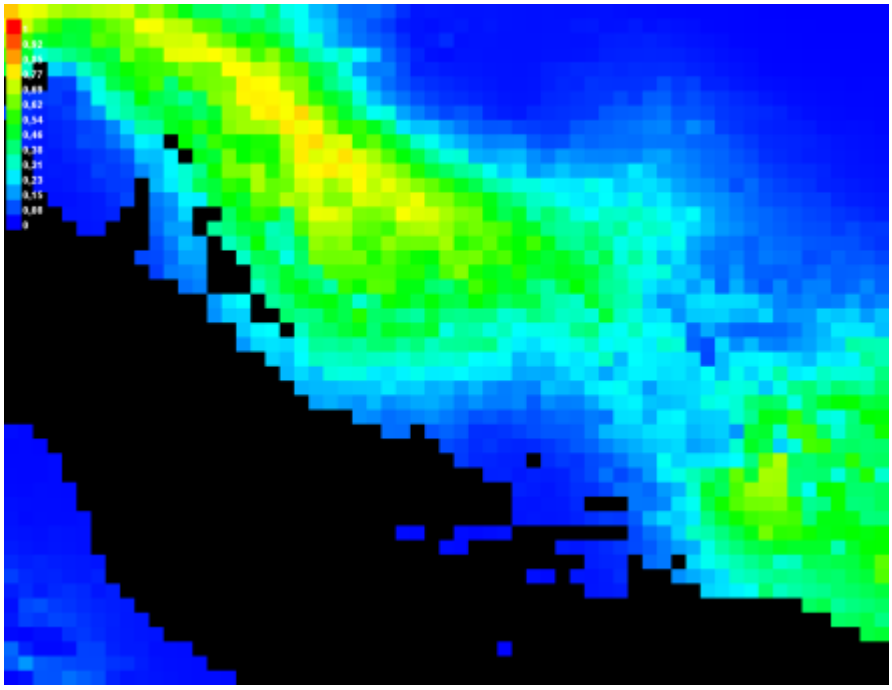


Figure 6: Visual presentation of ecological niche models of species 3 (B) and *Niphargus arbiter* (A) at layer selection of rho = 0.7. Red color represents more suitable areas and blue less suitable areas. The probability of species presence is decreasing from red to blue color.

Slika 6: Vizualna predstavitev modelov ekoloških niš vrst 3 (B) in *Niphargus arbiter* (A) pri izbiri slojev za vrednost rho = 0.7. Rdeča barva prikazuje ugodnejše območje in modra manj ugodne. Verjetnost rezüirjenosti pada od rdeče proti modri.

A)



B)

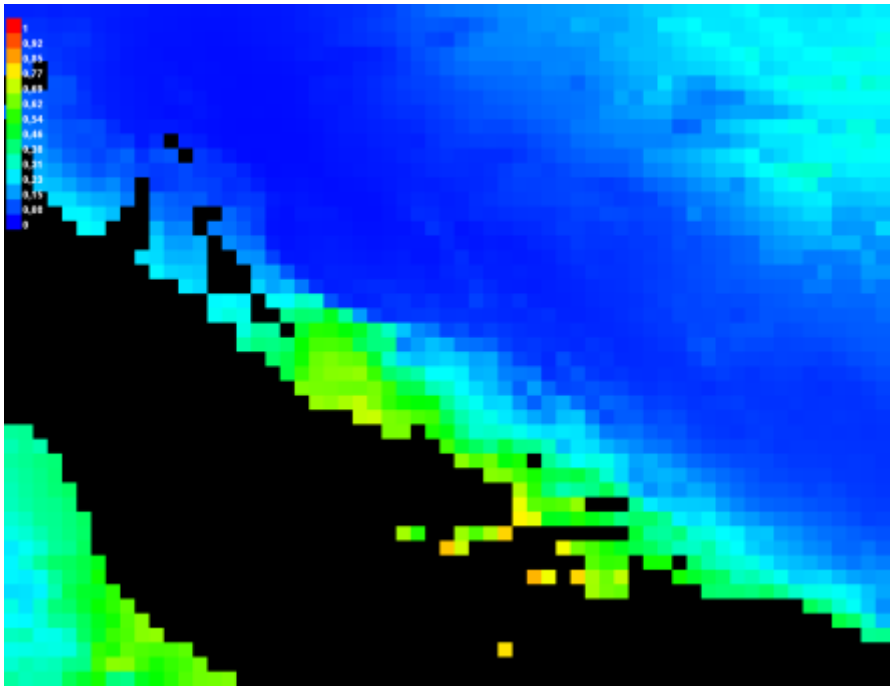


Figure 7: Visual presentation of ecological niche models of group of clade A (A) and of clade B (B) species at layer selection of p = 0.7. Red color represents more suitable areas and blue less suitable areas. The probability of species presence is decreasing from red to blue color.

Slika 7: Vizualna predstavitev modelov ekoloških niš klada A (A) in klada B (B) pri izbiri slojev za vrednost rho = 0.7. Rdeča barva prikazuje ugodnejše območje in modra manj ugodne. Verjetnost rezüirjenosti pada od rdeče proti modri.

Table 4: Niche equivalency (top part) and niche overlap values (bottom part). p values are given in brackets.

Preglednica 4: Vrednosti za analizo niche equivalency (zgoraj) in niche overlap (spodaj). Vrednosti p so podane v oklepajih.

Correlation	**Niche model**	**D**	**I**
rho > 0.6	species 3 : species 6	0.674 (0.964)	0.902 (0,979)
rho > 0.7		0.519 (0.624)	0.8 (0.526)
rho > 0.8		0.668 (0.510)	0.902 (0.412)
rho > 0.6	clade A: clade B	0.434 (1)	0.759 (1)
rho > 0.7		0.531 (1)	0.808 (1)
rho > 0.8		0.356 (1)	0.647 (1
rho > 0.6	species 3 : species 6	0.685 (0.847)	0.908 (0.839)
rho > 0.7		0.535 (0.548)	0.818 (0.572)
rho > 0.8		0.668 (0.411)	0.901 (0.403)
rho > 0.6	clade A : clade B	0.312 (0.914)	0.625 (0.898)
rho > 0.7		0.342 (0.986)	0.651 (1)
rho > 0.8		0.274 (1)	0.549 (1)

4.4 CLADE VARIABILITY AND DIAGNOSIS

Variability of the studied clade comprising of all 9 studied species:

Here we present the complete variability of the *Niphargus arbiter/Niphargus salonitanus* clade to show the range of studied characters. For each species its characters can be compared to the total clade variability.

Body (Fig. 8). Body stout, 8.1–39.9 mm long. Head from 0.4–2.3 mm long, without rostrum. Pereonite VII with 0–4 postero–ventral setae, pleon segments I–III with 1–25 dorso–posterior setae and 0–19 dorso–posterior spines. Epimeral plates II–III rectangular, with concave–straight posterior and convex distal margin. Epimeral plates II–III with 4–20 setae accompanied by 0–6 spines posteriorly; sub–ventrally with 1–5 spines respectively. Urosomites I–III with 2–6; 2–6; 0 dorsolateral spines and up to 2 seta on each side of the body.

Telson (Fig. 13) with 2–8 apical spines (per lobe), 0–1 lateral spines (per lobe), 0–1 mesial (per lobe) and 0–2 dorsal spines (per lobe). Laterally 2 plumose setae on each lobe.

Antennae I–II (Fig. 9)**.** Antenna I 0.29–0.73 of the body length. Peduncle segments 1–3 in ratio 1 : (0.67–1.13) : (0.92–2.48), flagellum of 15–48 articles, each bearing seta and 1 aestethasc. Accessory flagellum bi-articulated, distal article about 1/3 of the length of basal article.

Antenna II (Fig. 9) 0.17–0.30 of body length. Lengths of peduncle articles 4 : 5 as 1 : (0.76–1.40). Flagellum II with 7–25 articles, each bearing short seta.

Mouthparts (Fig. 10). Mandibular palpus three-articulated, basal article without setae, middle article with 4–10 long setae along inner margin, distal article with 1 group of 3–15 A setae, 2–8 groups of B setae, 15–70 D setae and 3–9 E setae.

Maxilla I (Fig. 10) with 1–9 setae on its inner lobe, outer lobe with 7 spines. Palpus bi-articulated, with 2–16 distal and subdistal seta. Maxilla II (Fig. 5e) with sub–equal lobes, each with a group of long apical and subapical seta. Labium with inner lobes.

Maxilliped (Fig. 10) inner lobe with 3–6 strong flattened spines and 3–8 strong hairy seta apically and subapically. Outer lobe with 4–17 strong medial flattened spines and 4–12 hairy apical seta. Dactylus with setae at the base of nail.

Gnathopods. Gnathopod I (Fig. 11) 2.0–13.4 mm in length (from the top of the coxa to tip of the dactylus). Coxa of rhomboid shape with 4–16 setae ventro–distally. Article 3 with 1–2 rows of 4–31 posteroventral setae. Length of article 5 is 0.4–2.3 mm. Article 5 with proximal bulbus; 1 group of setae disto–anteriorly; setae also on bulbus and along postero-mesial margin.

Article 6 distaly rounded–rectangular in shape. Anterior margin with 1–7 groups of setae and antero–distal group of 3–17 setae. Posterior margin with 7–23 rows of setae. Palmar corner with 1 long palmar spine, 1 small smooth inner spine and 1–8 outer denticulated

spines. On outer surface proximally to palmar spine a group of 1–8 long setae; inner surface with several groups of small setae. Dactylus with 3–29 mostly single setae along outer margin, inner margin with small setae.

Gnathopod II (Fig. 11) 2.4–18.8 mm in length. Coxa with 5–18 setae ventro–distally. Article 3 with 1 row of 1–15 postero–ventral setae. Length of article 5 (0.4–3.0 mm). Article 5 with proximal bulbus; 1 group of setae disto–anteriorly; setae also on bulbus and along postero mesial margin.

Article 6 of gnathopod II distally rounded–rectangular in shape, larger than article 6 of gnathopod I. Anterior margin with 1–6 groups of setae (in total 2–29 setae) and antero–distal group of 0–16 setae. Posterior margin with 7–22 rows of setae. Palmar corner with one long palmar spine, 1 small smooth inner spine and 0–4 outer denticulated spines. On outer surface proximally to palmar spine a group of 0–7 long setae; inner surface with several groups of small setae. Dactylus with 3–30 mostly single setae along outer margin, inner margin with small setae.

Pereopods III–IV (Fig. 12) coxa with 4–17 and 6–19 setae on ventral margins acompanied by 1–2 spine like seta. Each dactylus with 0–1 spine/setae at the base of nail.

Pereopods V–VII (Fig. 15) up to 19.4/24.8/18.4 in length. Coxae V–VI with anterior lobe, posterior margin with seta, distal seta may be spine–like. Coxa VII semicircular, seta posteriorly. Articles 2 with small disto–posterior lobe; bases (articles 2) of pereopods V–VII with 8–25, 9–20, 7–20 posterior setae and 5–13, 6–12, 6–11 anterior groups of setae and spines, respectively. Each dactylus with 0–1 tiny seta at the base of nail; 1 plumose seta dorsally.

Pleopods (Fig. 13) with inner ramus is longer than outer, each ramus of 10–33 articles, 2 retinacles on each pleopod.

Uropods I–III. Uropod I (Fig. 13) peduncle with 5–10 lateral and 3–6 mesial spines. Inner ramus with 4–15 groups of totally 6–31 spines and setae, outer ramus with 5–17 groups of totally 9–44 spines.

Uropod III (Fig. 1) peduncle with 4–21 lateral spines and 4–18 apical spines. Outer ramus proximal article with 4–21 groups of spines, setae and plumose setae along outer margin respectively. Apical article of expopodite with 1–5 setae laterally and 2–8 setae apically. Inner ramus with 0–2 lateral spines and 1–5 apical spines and setae.

Species diagnosis (in bold is the selected voucher of the type specimen for the diagnosis. In case of *Niphargus arbiter/Niphargus salonitanus* is the reference speciemen for the given diagnosis). The following species names, *Niphargus* sp. n. 2, *Niphargus* sp. n. 3, *Niphargus* sp. n. 4, *Niphargus* sp. n. 5, *Niphargus* sp. n. 7, *Niphargus* sp. n. 8 and *Niphargus* sp. n. 9, and their diagnoses are only used for the purpose of this thesis. This agrees with the article 8 (8.2. and 8.3.) of the International Code of Zoological Nomenclature (ICZN, 1999).

Species 1 – *Niphargus salonitanus* S. Karaman 1950

Analyzed material. 7 individuals from one locality (voucher No. **NB179**, NB446, NB473, NB474, NB496, NB522, NB529), coll. Cene Fišer, 2002. The series is stored in the collection of Department of Biology, Biotechnical Faculty, University of Ljubljana.

Locality of the reference specimen. Sveta Stomorija, Split; Croatia

Distribution. Sveta Stomorija, Split; Kosinac, Han, Sinj; Croatia. (Fig. 3)

Diagnosis. Pleonite I with 18 (12–18) setae and without spines, pleonite II with 16 (12–17) setae and 0 (0–1) spines, mesosoma III with 16 (13–16) setae and 3 spines (0–5). Urosomite I with 3 (3) spines, urosomite II with 4 (3–4) spines. Epimeral plate II with 3 (2–3) spines, epimeral plate III with 4 (3–4) spines. Telson with 6 (5–6) apical spines total. Coxa of gnathopod I with 10 (9–11) setae. Gnathopod I propodus with 15 (14–20) posterior group of setae and 6 (4–6) anterior groups of setae, 3 (3–6) setae under gnathopod spine. Dactylus with 14 (6–17) groups of setae. Gnathopod II propodus with 12 (12–15) posterior groups of setae and 2 (2–4) anterior groups setae, dactylus with 13 (6–16) groups of setae. Coxa of pereopod III with 12 (8–14) setae. Coxa of pereopod IV with 14 (11–14) setae, article 4 with 4 (3–5) posterior group of spines and 3 (3–4) anterior groups. Pereopod 7 with x (6–9) anterior and x (11–14) posterior groups of spines on 2nd article. Uropod I with 7 (7–8) lateral spines on the basal article, with 8 (7–8) groups of spines on endopodid and 27 (19–36) on exopodid. Uropod III with 4 (2–4) setae on 3rd article. Mandible with 35 (31–49) D setae, on second article of palp 6 (6–8) setae. Maxilliped with 8 (6–12) strong spines on outer lobe.

***Niphargus* sp. n. 2**

Type material. 4 individuals from type locality (voucher No. NB210, **NB471**, NB472, NB513), coll. Branko Jalžić, 2010. The type series is stored in the collection of Department of Biology, Biotechnical Faculty, University of Ljubljana.

Type locality. Pincinova cave; Tar; Poreč; Croatia; Coordinates: WGS–84; 13,658716; 45,263453

Distribution. Pincinova cave, Poreč; Klarićeva cave, Vrsar; Škatari bunar, Pula; Croatia. (Fig. 3)

Diagnosis. Pleonite I with 5 (5–14) setae and 9 (3–12) spines, pleonite II with 4 (3–9) setae and 10 (1–15) spines, pleonite III with 4 (2–8) setae and 11 (4–13) spines. Urosomite I with 2 (2–3) spines, urosomite II with 2 (2–4) spines. Epimeral plate II with 1 (1–3) spines, epimeral plate III with 1 (1–5) spines. Telson with 16 (5–16) apical spines total. Coxa of gnathopod I with 8 (5–12) setae. Gnathopod I propodus with 5 (3–5) anterior groups of setae, dactylus with 14 (9–14) groups of setae. Gnathopod II coxa with 8 (5–13) setae, 3rd article with 1 (1–4) setae, propodus with 3 (2–4) anterior groups setae, 4 (0–6) antero–distal setae, dactylus with 15 (8–15) groups of setae. Coxa of pereopod III with 8

(8–11) setae. Coxa of pereopod IV with 13 (10–13) setae. Pereopod VII with (7–9) anterior and (11–12) posterior groups of spines on 2nd article. Uropod III with (3–4) setae on 3rd article. Maxilliped with 6 (4–7) strong spines on outer lobe.

***Niphargus* sp. n. 3**

Type material. 6 individuals from type locality (voucher No. NB209, NB418, NB447 **NB468**, NB469, NB512), coll. P. Bregović, A. Čukušić, 2010. The type series is stored in the collection of Department of Biology, Biotechnical Faculty, University of Ljubljana.

Type locality. Čavle špilja, Muškovci, Zrmanja, Croatia; Coordinates: WGS–84; 15,746365 N; 44,212411 E.

Distribution. Čavle špilja, Zrmanja; Kusačko jezero, Zrmanja; Suvaja pećina, Udbina; Vranovinski ponor, Gospić; Zelena špilja, Korenica; Croatia. Bilobrkova pećina, Trilj; Izvor pećina, Bihač; BiH (Fig. 3)

Diagnosis. Diagnosis. Pereonit VII with 0 (0–1) setae. Pleonite I with 0 (0–9) spines, pleonite II with 11 (0–14) spines, pleonite III with 4 (2–13) setae and 11 (8–15) spines. Telson with 12 (6–18) apical spines total. Coxa of pereopod I with 8 (7–13) setae. Gnathopod I article 3 with 11 (9–28) setae. Gnathopod II propodus with 12 (10–16) posterior groups of setae, 2 (2–6) anterior groups of setae, dactylus with 16 (8–29) groups of setae. Coxa of pereopod III with 10 (7–13) setae. Pereopod IV with 4 (3–4) anterior groups of spines on 4th article. Pereopod VII with 9 (6–10) anterior groups of spines and 13 (12–17) posterior setae on 2nd article. Uropod III with 3 (3–8) setae on 3rd article, 12 (9–12) groups of spines on inner side of 2nd article. Maxilliped with 14 (10–17) strong spines on outer lobe.

***Niphargus* sp. n. 4**

Type material. 5 individuals from type locality (voucher No. NB169, NB445, NB459, NB460, **NB514**), coll. Tom Turk, 2007. The type series is stored in the collection of Department of Biology, Biotechnical Faculty, University of Ljubljana.

Type locality. Jašek p. mostu, bay Jadriščica, Punta Križa, Cres, Croatia; Coordinates: WGS–84; 14,494554 N; 44,624289 E.

Distribution. Bay Jadriščica, Cres, Croatia. (Fig. 3)

Diagnosis. Pereonit VII with 1 (1–3) spine. Pleonite I with 14 (14–20) setae and without spines, pleonite II with 6 (6–21) setae and without spines, pleonite III with 12 (12–25) setae and without spines. Urosomite I with 3 (3) spines, urosomite II with 4 (3–4) spines. Epimeral plate II with 2 (2–3) spines, epimeral plate III with 3 (2–4) spines. Telson with 8 (6–8) apical spines total. Coxa of pereopod I with 7 (6–10) setae. Gnathopod I article 3 with 6 (4–11) setae, propodus with 8 (7–10) posterior groups of setae and 5 (4–5) anterior groups of setae, dactylus with 8 (8–13) groups of setae. Gnathopod II coxa with 8 (7–11) setae, third article with 5 (2–5) setae, propodus with 8 (7–9) posterior groups of setae and 2 (2–4) anterior groups of setae, 5 (5–11) anterodistal setae, dactylus with 10 (9–14) groups

of setae and 10 (9–14) setae. Coxa of pereopod III with 7 (6–12) setae. Coxa of pereopod IV with 9 (6–11) setae, article 4 with 3 (2–3) posterior group of spines and 4 (3–4) anterior groups. Pereopod VII with (6–8) anterior and (11–12) posterior groups of spines on 2nd article. Uropod I with 7 (6–7) lateral spines on the basal article, with 8 (7–9) groups of spines on endopodid and 18 (14–23) spines on exopodid. Uropod III with 2 (2) setae on 3rd article. Mandible with 5 (4–6) setae on second article of palp. Maxilliped with 9 (7–9) strong spines and 7 (4–7) setae on outer lobe.

***Niphargus* sp. n. 5**

Type material. 1 individual from type locality (voucher No. **NA050**), coll. Boris Sket, 2004. The type series is stored in the collection of Department of Biology, Biotechnical Faculty, University of Ljubljana.

Type locality. Vrbnik, Krk, Croatia; Coordinates: WGS–84; 14,678183 N; 45,078399 E. Izvirček v luki Vrbnik, Vrbnik, Krk; Croatia; 14.678183 N; 45.078399 E; 4/29/2004 Boris Sket.

Distribution. Vrbnik, Krk; Lopar, Rab; Croatia. (Fig. 3)

Diagnosis. Pereonit VII with 1 (1) spines. Pleonite I with 14 (7–15) setae and without spines, pleonite II with 6 (7–17) setae and without spines, pleonite III with 12 (5–20) setae and without spines. Urosomite I with 3 (3) spines, urosomite II with 4 (4) spines. Epimeral plate II with 2 (2–3) spines, epimeral plate III with 3 (3–4) spines. Telson with 8 (7–9) apical spines total. Coxa of pereopod I with 12 (12) setae. Gnathopod I article 3 with 14 (4–8) setae, propodus with 8 (7–12) posterior groups of setae, dactylus with 8 (3–7) groups of setae. Gnathopod II coxa with 8 (8–11) setae, third article with 5 (2–3) setae, dactylus with 10 (3–6). Coxa of pereopod III with 6 (6) setae. Coxa of pereopod IV with 9 (6–8) setae, article 4 with 4 (4) posterior group of spines. Pereopod VII with x (8–9) anterior and x (6–19) posterior groups of spines on 2nd article. Uropod III with (5–8) groups of spines on inner side and 3 (3) setae on 3rd article. Mandible with (14–29) D setae and 5 (4–5) setae on second article of palp. Maxilliped with 9 (4–9) strong spines and 7 (5–8) setae on outer lobe.

Species 6 – *Niphargus arbiter* G. Karaman 1984

Studied material. 4 individuals from type locality (voucher No. **NB172**, NB203, NB453, NB519), coll. Branko Jalžić, 2000. The series is stored in the collection of Department of Biology, Biotechnical Faculty, University of Ljubljana.

Locality of the reference specimen. Markov ponor; Lipovo polje; Otočac; Lika; Croatia; Coordinates: WGS–84; 15, 176874 N; 44, 765318 E.

Distribution. Izvor Sinjac, Plavča draga, Plaški; Markov ponor, Lipovo polje, Otočac; Sinjac izvor, Plavča draga, Plaški; Mandelaja, Oštarije, Ogulin; Suvaja pećina, Mekinjar, Udbina; Ponor Sušik, Drežnica, Ogulin; Vidovića špilja, Drežnica, Ogulin; Pećina uz Koranu, Blagaj, Slunj; Croatia; Veliko okence; Retovje; Vrhnika; Malo okence, Retovje,

Vrhnika; Jama v Kanjeducah, Sežana; Slovenia; Hrustovača; Hrustovo; Sanski Most; BiH (Fig. 3)

Diagnosis. Pleonite I with 14 (2–16) setae and 0 (0–15) spines, pleonite II with 6 (4–23) setae and 0 (0–16) spines, pleonite III with 12 (2–21) setae and 0 (0–16) spines. Telson with 8 (6–16) apical spines total. Coxa of pereopod I with 12 (7–14) setae. Gnathopod II coxa with 8 (6–17) setae, propodus with (2–6) anterior groups of setae, dactylus with 10 (4–18). Pereopod VII with (7–20) posterior groups of spines on 2nd article. Uropod I with (7–14) groups of spines on endopodid.

Remarks: Original *Niphargus arbiter* Karaman 1984

***Niphargus* sp. n. 7**

Type material. 1 individual from type locality (voucher No. **NB163**), coll. Branko Jalžić, 2010. The type series is stored in the collection of Department of Biology, Biotechnical Faculty, University of Ljubljana.

Type locality. Kaptaža K–2, Dunaj, Postira, Brač island; Croatia; Coordinates: WGS–84; 16,624100 N; 43.351808 E.

Distribution. Kaptaža K–2, Postira, Brač island; Croatia. (Fig. 3)

Diagnosis. Due to limited samples size the data is given only in the table (Tab. 1).

***Niphargus* sp. n. 8**

Type material. 5 individuals from type locality (voucher No. NB164, NB168, NB201, **NB467**, NB510), coll. Boris Sket, 2007. The type series is stored in the collection of Department of Biology, Biotechnical Faculty, University of Ljubljana.

Type locality. Šipun špilja, Konavle donje, Cavtat, Croatia; Coordinates: WGS–84; 18,213091 N; 42,586420 E.

Distribution. Šipun špilja, Cavtat, Croatia. (Fig. 3)

Diagnosis. Pereonit VII with 2 (1–2) spines. Pleonite I with 19 (6–19) setae and without spines, pleonite II with 14 (3–14) setae and 6 (0–6) spines, pleonite III with 5 (1–8) setae and 16 (2–16) spines. Urosomite I with 4 (3–4) spines, urosomite II with 5 (3–5) spines. Epimeral plate III with 3 (3–4) spines. Telson with 8 (6–8) apical spines total. Coxa of pereopod I with 4 (4–7) setae. Gnathopod I article 3 with 8 (6–10) setae, propodus with 12 (11–15) posterior and 5 (3–5) anterior groups of setae, 2 (2) setae under propodus spine, dactylus with 15 (8–15) groups of setae. Gnathopod II coxa with 10 (8–10) setae, third article with 2 (2–5) setae, propodus with 10 (10–13) posterior and 2 (1–3) anterior grups of setae, dactylus with 14 (7–16). Coxa of pereopod III with 8 (8–10) setae. Coxa of pereopod IV with 7 (7–13) setae. Pereopod VII with 10 (10) anterior and 15 (15) posterior groups of spines on 2nd article. Uropod I peduncle with 7 (7) lateral spines, endopodid with 11 (11–12) groups of spines. Maxilliped with 5 (5–6) setae on inner lobe.

***Niphargus* sp. n. 9**

Type material. 1 individual from type locality (voucher No. **NB208**), coll. Branko Jalžić, 2010. The type series is stored in the collection of Department of Biology, Biotechnical Faculty, University of Ljubljana.

Type locality. Jama pod Orljakom; Zaton; Šibenik; Croatia; Coordinates: WGS–84; 15.841372 N; 43.770483 E.

Distribution. Jama pod Orljakom; Zaton; Šibenik; Croatia. (Fig. 3)

Diagnosis. Due to limited sample size the data is given only in the table (Tab. 1).

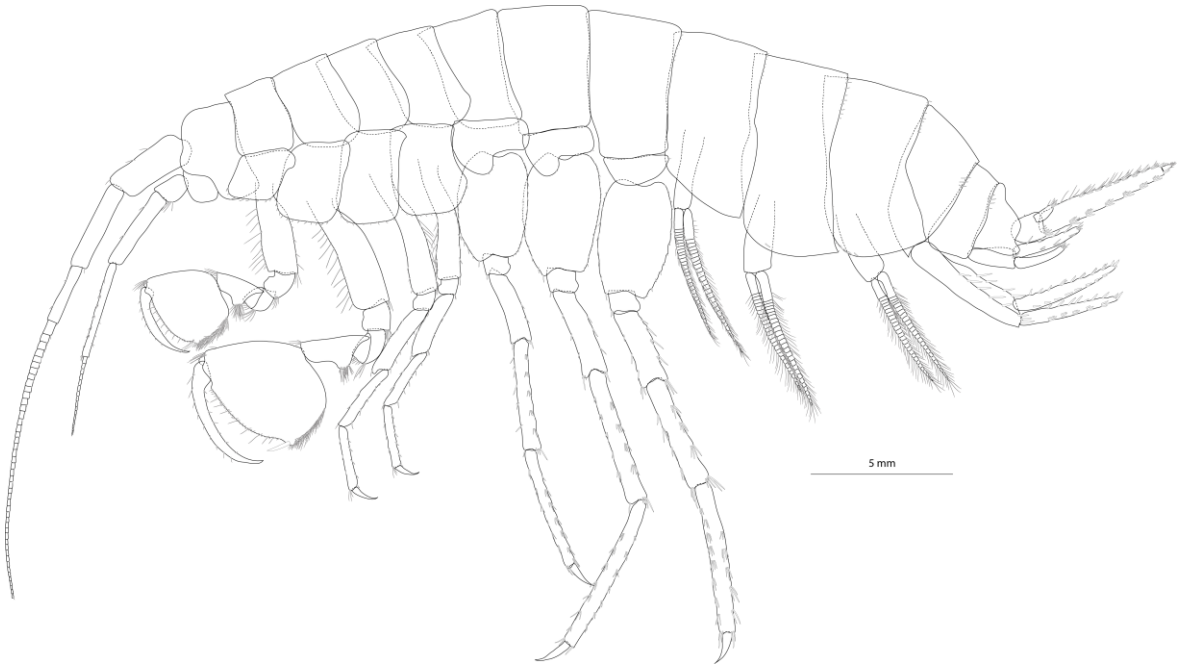


Figure 8: Habitus of *Niphargus* sp. n. 3.

Slika 8: Habitus osebka vrste 3 kompleksa *Niphargus arbiter/Niphargus salonitanus*.

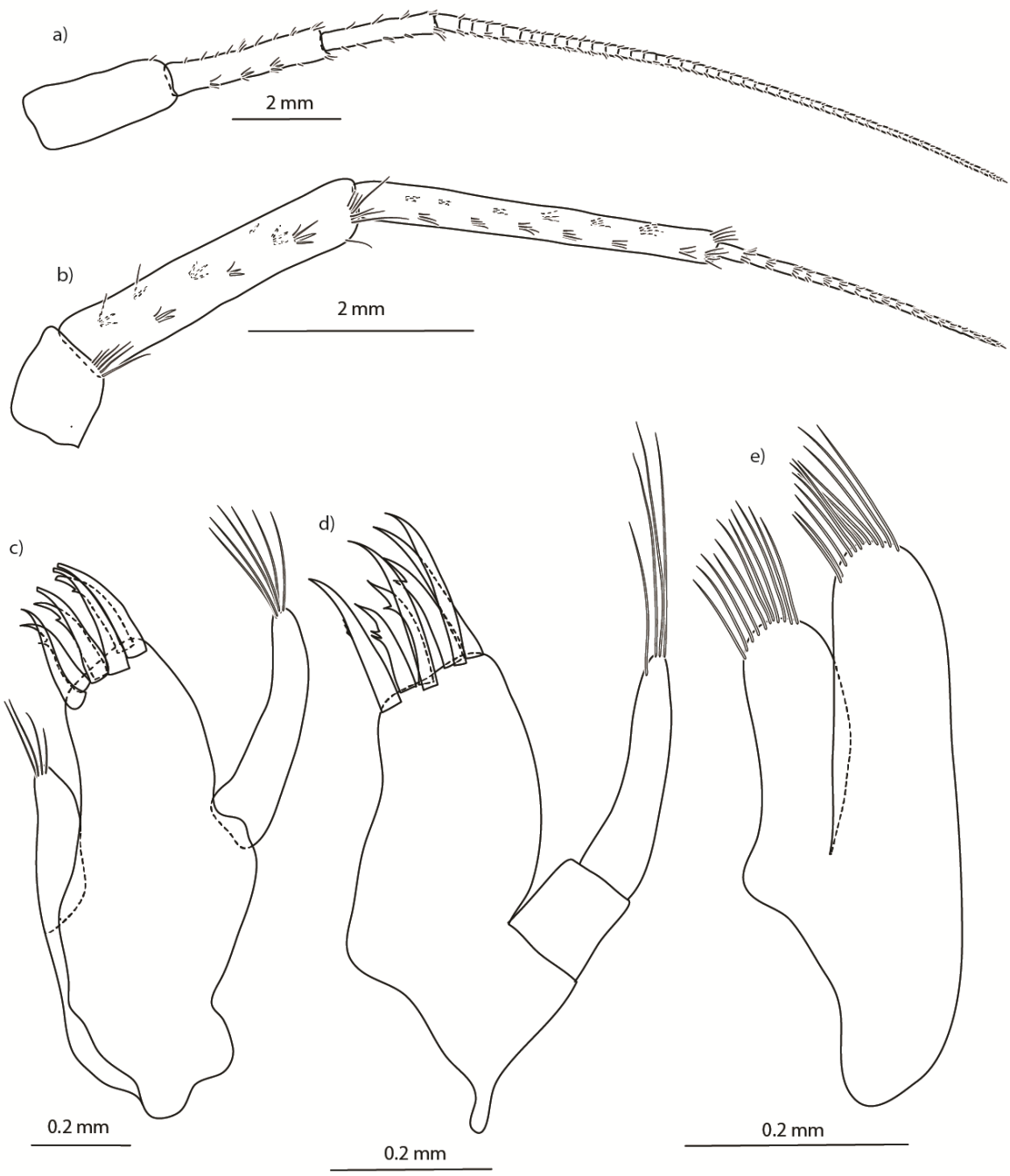


Figure 9: Digital drawings of a) antenna 1 and b) antenna 2, c) maxilla 1 of species 3, d) maxilla 1 and e) maxilla 2 of species 2.
Slika 9: Digitalna risba a) antene 1, b) antene 2, c) maxile 1 vrste 3, d) maksila 1 in e) maksila 2 vrste 2.

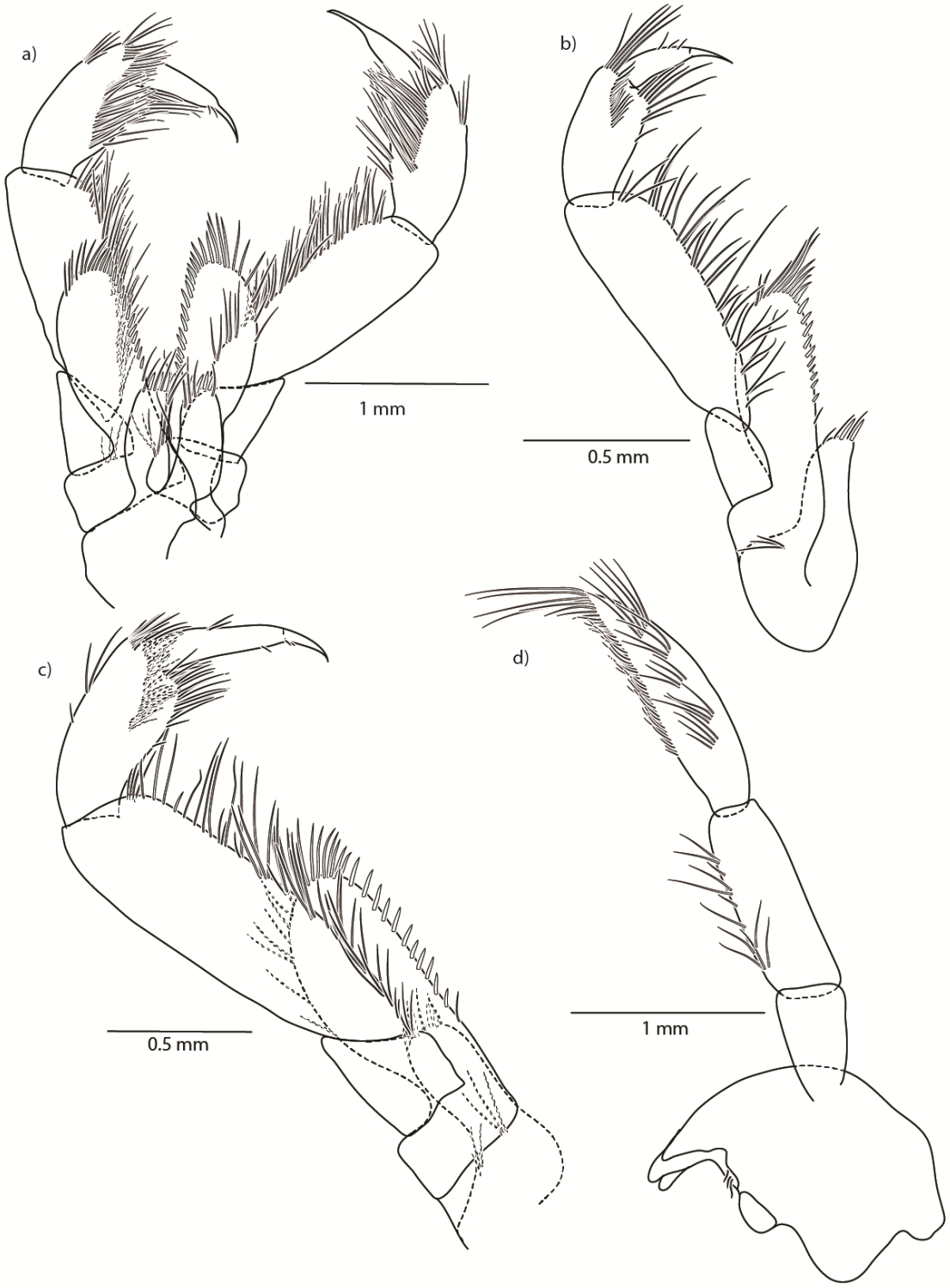


Figure 10: Digital drawings of a) maxilliped and d) mandible of species 3, b) maxilliped of species 2 and c) maxilliped of *Niphargus arbiter.*

Slika 10: Digitana risba a) maksilipeda in d) mandibule vrste 3, b) maksiliped vrste 2 in c) maksiliped vrste *Niphargus arbiter.*

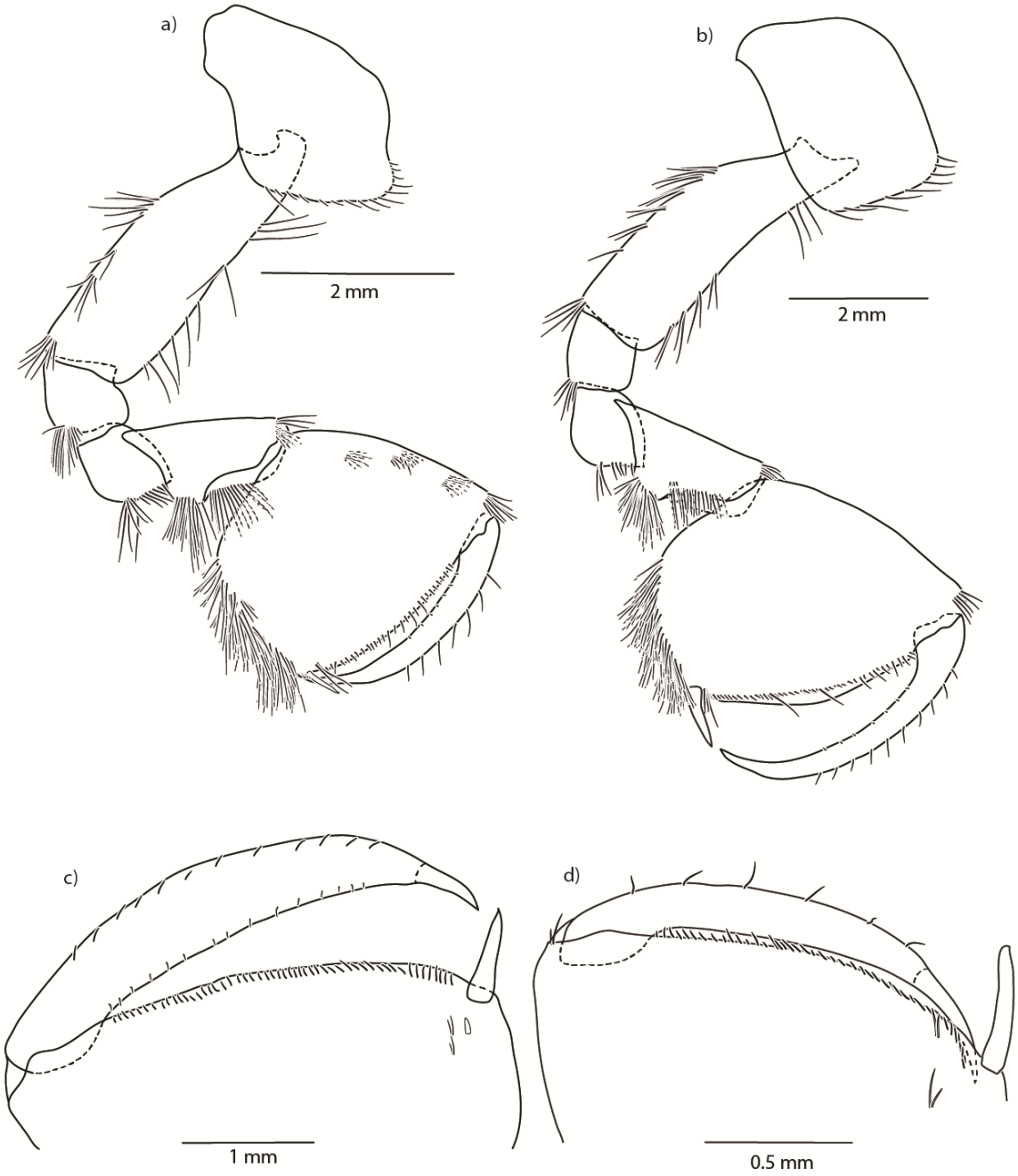


Figure 11: Digital drawing of a) gnathopods 1, b) gnathopod 2, c) dactylus of gnathopod 2 of species 3, d) dactyl of gnathopod 2 of species 5.

Slika 11: Digitalna risba a) gnatopoda 1, b) gnatopoda 2 in c) daktil gnatopoda 2 vrste 3, d) daktil gnatopoda 2 vrste 5.

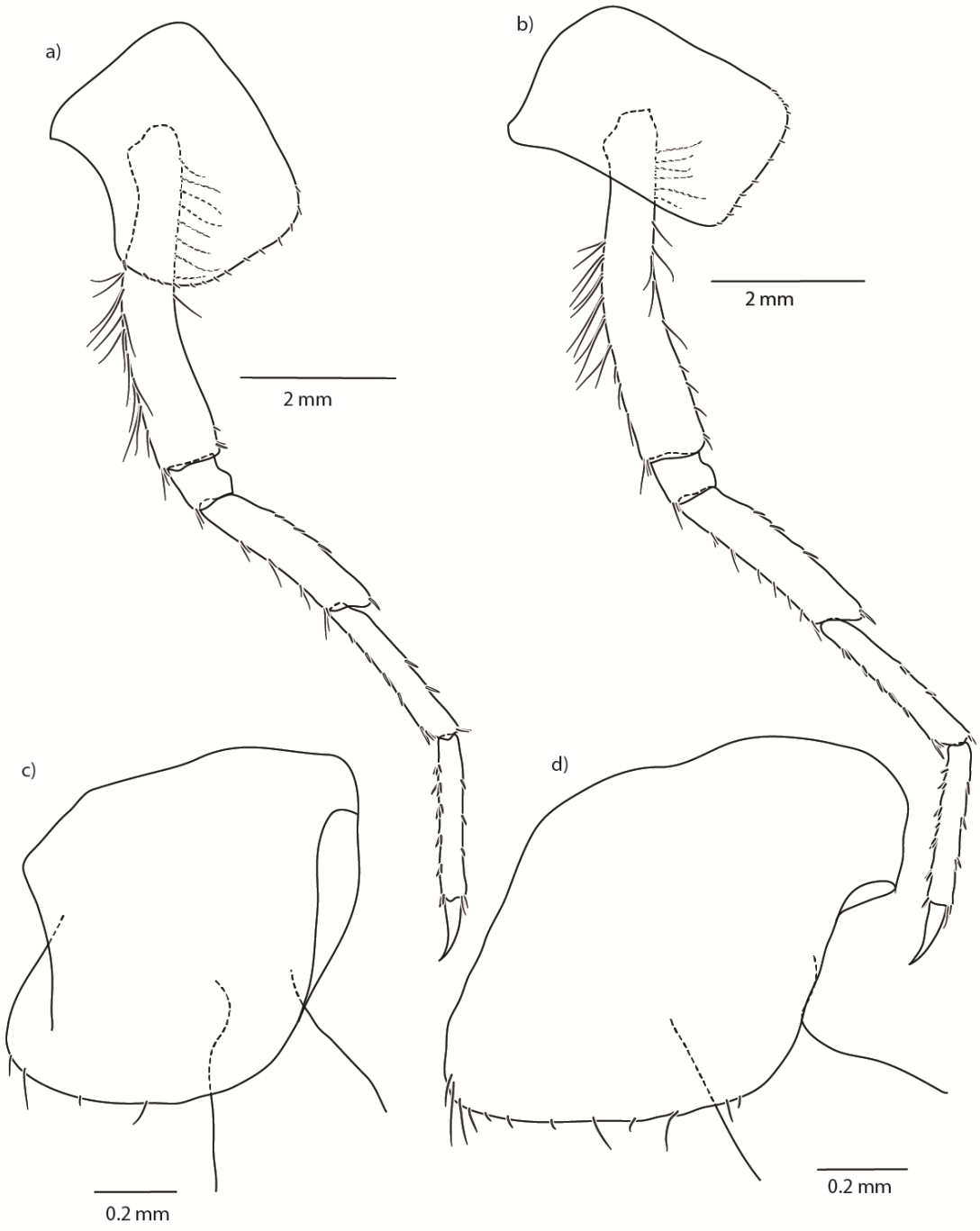


Figure 12: Digital drawings of a) pereopod 3 and b) pereopod 4 of species 3, c) coxa 1 of species 8, and d) coxa 1 of species 5.

Slika 12: Digitalna risba a) pereopoda 3 in b) pereopoda 4 vrste 3, in c) koksa 1 vrste 8 ter d) koksa 1 vrste 5.

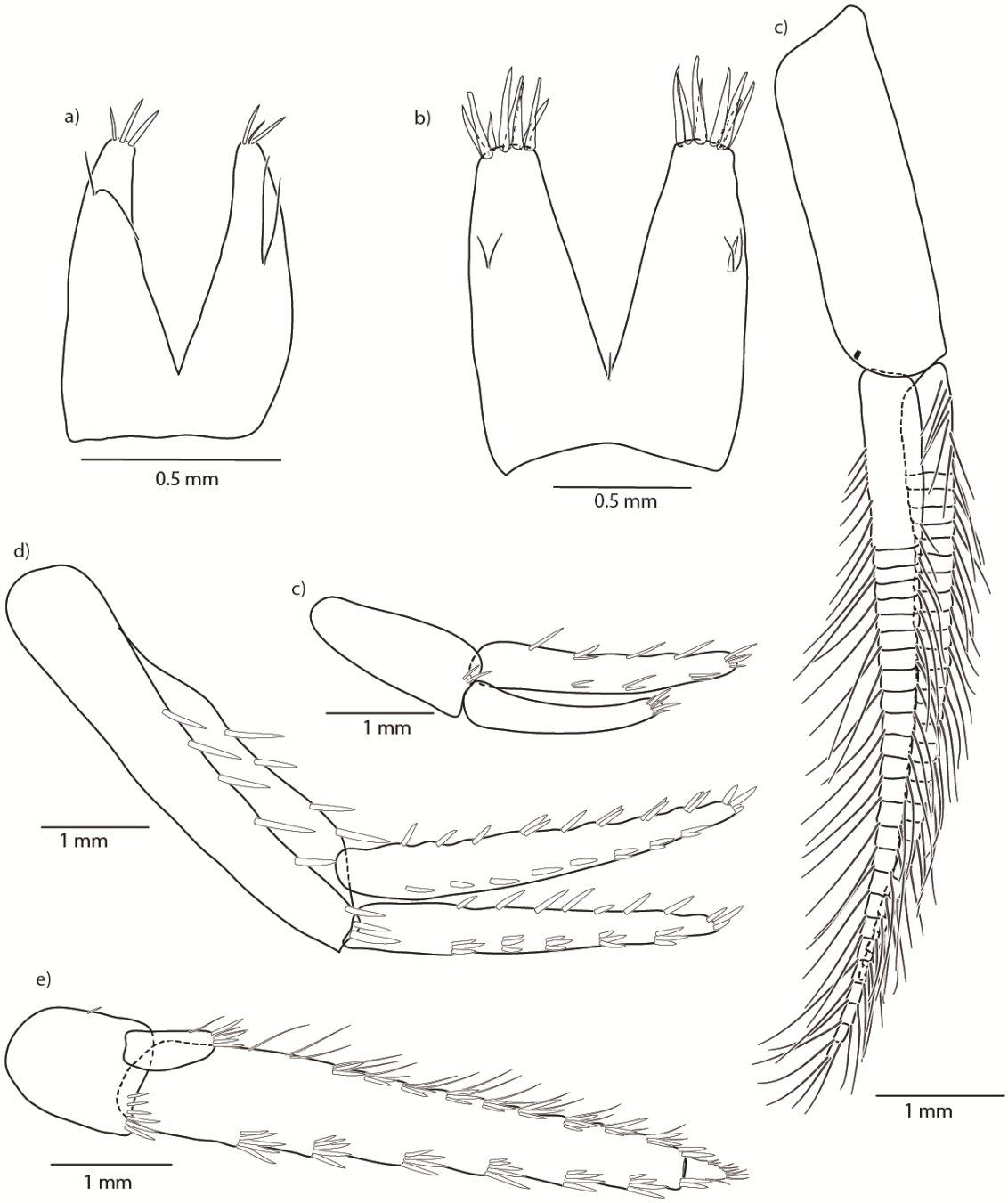


Figure 13: Digital drawings of a) telson of *Niphargus arbiter,* b) telson of species 2, c) uropod 1, d) uropod 2, e) uropod 3 and f) pleopod of species 3.

Slika 13: Digitalna risba a) telzona vrste *Niphargus arbiter,* b) telzona vrste 2, c) uropoda 1, d) uropoda 2, e) uropoda 3 in f) pleopoda vrste 3.

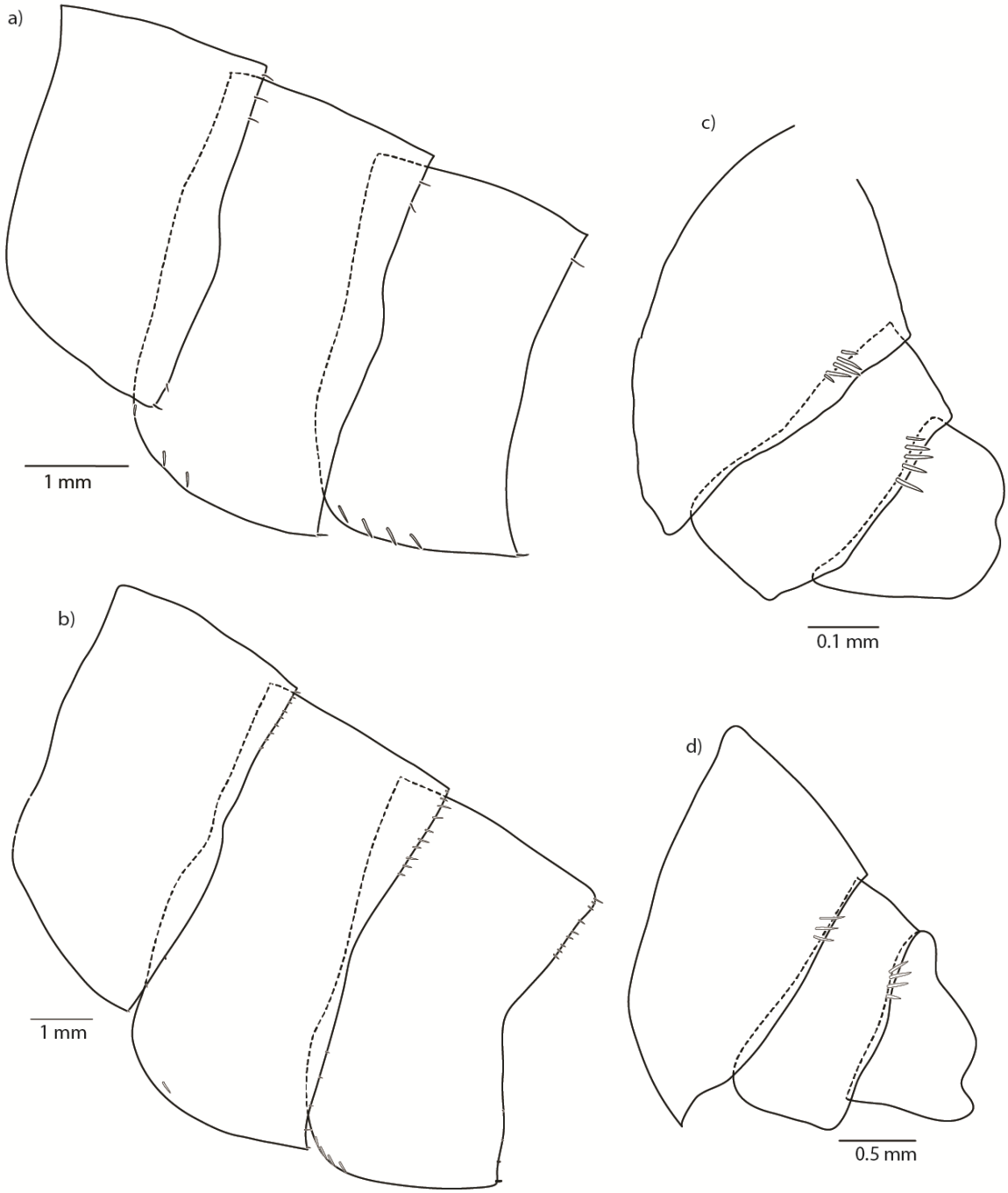


Figure 14: Digital drawings of a) pleosoma and c) urosoma of species 3 and b) pleosoma of *Niphargus salonitanus* and d) urosoma of *Niphargus salonitanus*.

Slika 14: Digitalna risba a) pleosome in c) urosome vrste 3 ter b) pleosome in d) urosome vrste *Niphargus salonitanus.*

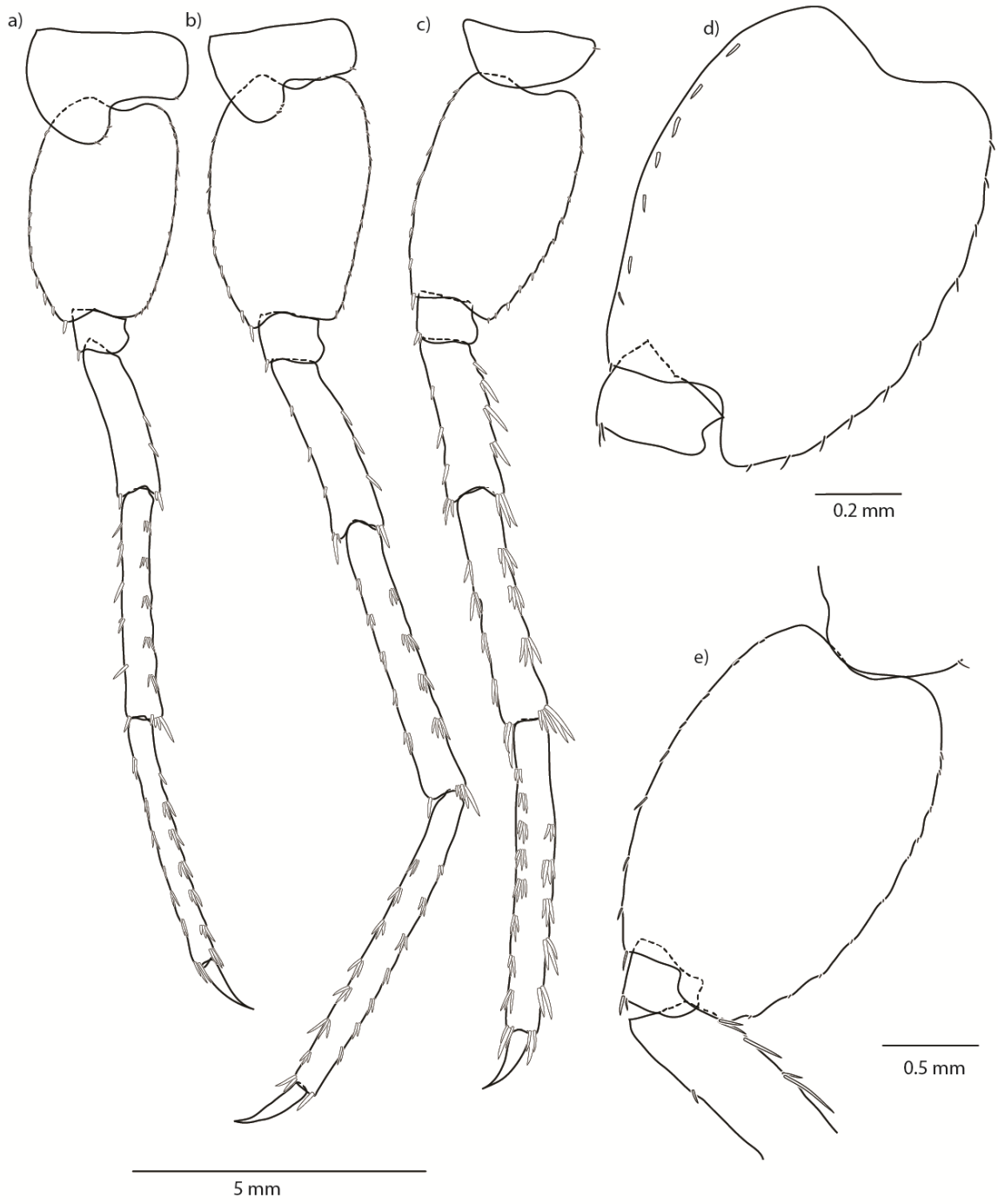


Figure 15: Digital drawing of a) peropod 5, b) pereopod 6, c) pereopod 7 of species 3 and d) basis of pereopod 7 of species 4 and e) basis of pereopod 7 of species 8.

Slika 15: Digitalna risba a) pereopod 5, b) pereopoda 6, c) pereopoda 7 vrste 3 in d) basis pereopoda 7 vrste 4 in e) basis pereopoda 7 vrste 8.

5 DISCUSSION

5.1 EVOLUTIONARY DIVERGENCE OF THE *Niphargus arbiter/Niphargus salonitanus* COMPLEX

The results of the phylogenetic analysis based on three genetic markers (28S rRNA, COI, ITS) suggest monophyly of the species complex *Niphargus arbiter/Niphargus salonitanus*. Within the clade between six and nine independent lineages can be recognized based on different delimitation approaches. Two nominal species from the northern (*Niphargus arbiter* - species 6) and southern part (*Niphargus salonitanus* - species 1) of distribution range can be recognized. New species were discovered in 2) the Istria Peninsula, 3) the Central Dinarides, 4) the island of Cres, 5) the islands of Krk and Rab, 7) the island of Brač, 8) Southern Dalmacija and 9) the Zadar Region. This means that the whole monophylum has a large area, which includes most of the Dinaric Karst except the southern-most part. Small species ranges agree with the observation (Trontelj et al., 2009) that species ranges are smaller than 200 km. Similar allopatric distribution patterns have recently been recognized in studies of the subterranean amphibian *Proteus* (Gorički & Trontelj, 2006) and the cave shrimp *Troglocaris* (Zakšek et al., 2007). In both cases the larger species complex is presented and devided into species with smaller distribution ranges. In my thesis we are facing a similar pattern including some specifics. Those include the northern species where only the western clade is present. Beside that two separate species are found in Istria and in Lika (*Niphargus arbiter*).

Most of the species are morphologically similar to each other, yet there are some minor differences between them in morphology as well as ecological niche. Such a state can be the result of two scenarios; either we are witnessing relatively young species or the described species have been facing identical selection pressure for a longer period of time.
The latter explanation seems more probable because the species that settled subterranean habitats have been under the same selection pressure and retained the same morphological characterstics (Smith et al., 2011). As mentioned, some morphological characters exhibit distinctness although even these differences are hard to locate and identify. It cannot be pointed out if such tiny differences are the consequence of constrained evolution or nonadaptive evolution (Smith et al., 2011; Bravo et al., 2014).

Bioclimate data as an equivalent of surface influence on conditions in caves suggest that at least two sister species might differ in their ecological niche. Beside that differences between Clade 1 and Clade 2 (Tab. 4) are present but hard to distinguish as the data does not show significant differences. Different paths can lead to the observed state and all of them seem possible: 1) Species are actually adapted to different climate (Schluter, 2009); 2) Species accumulated neutral mutations that result in difference as a consequence of

allopatry and 3) Species can tolerate different environmental condition with the same efficiency, so they actually share the same characteristic (Seehausen et al., 2008; Smith et al., 2011).

5.2 TAXONOMIC REVISION OF THE *Niphargus arbiter/Niphargus salonitanus* SPECIES COMPLEX

The most obvious character to distinguish between species is the presence of spines on top of the pleonite segments (Fig. 14). This distinction can be made because 4 species have no spines present on pleosoma 1 while the other 5 species posses spines on pleosoma (see table 3). On the other hand some characters that have been thought to be useful actually proved to be extremely variable even within the newly described species. Some characters that seemed distinct between *Niphargus arbiter* and *Niphargus salonitanus* after the work of Karaman (1984) in *Niphargus orcinus* group revision proved not so in this study. For example an additional spine on the dactyls of pereopods 5-7 (fig. 15, 16). A detailed analysis of the character showed that the spine can be present or absent in new species which makes the trait useless in distinguishing between species. The same trend can be seen in the apical spines of the telson. Even though many morphological differences between species 7 and species 9 in comparison to other species have been found we cannot conclude that any of these are informative. The number of analyzed specimens was with a single or two specimens which is too small to provide a reliable a dataset.

The use of morphometry in species delimitation is useful in 12 species pairs. Several statistical approaches fail to show differences due to a large standard deviation of the data which can also be a consequence of small sample sizes. The morphology of all species seems extremely variable in most of the characters examined. In fact recent research of subterranean amphipods suggests that the morphological characteristics of the individual are often strongly affected by the environment in which the individual lives (Delić et al., 2016). Therefore a large variability in the specimens from the same population can be present. In this case only large datasets from many sampling sites proves to be reliable for morphological characterization of the species.

Even though additional aspects in species delimitation show differences between species they can often be extremely limited. They add robustness to the method yet they cannot be applied to all the species because of a lack of universality and repetability. That makes integrative taxonomy less attractive but still valuable in the case of cryptic and species that lack other clear taxonomic characters.

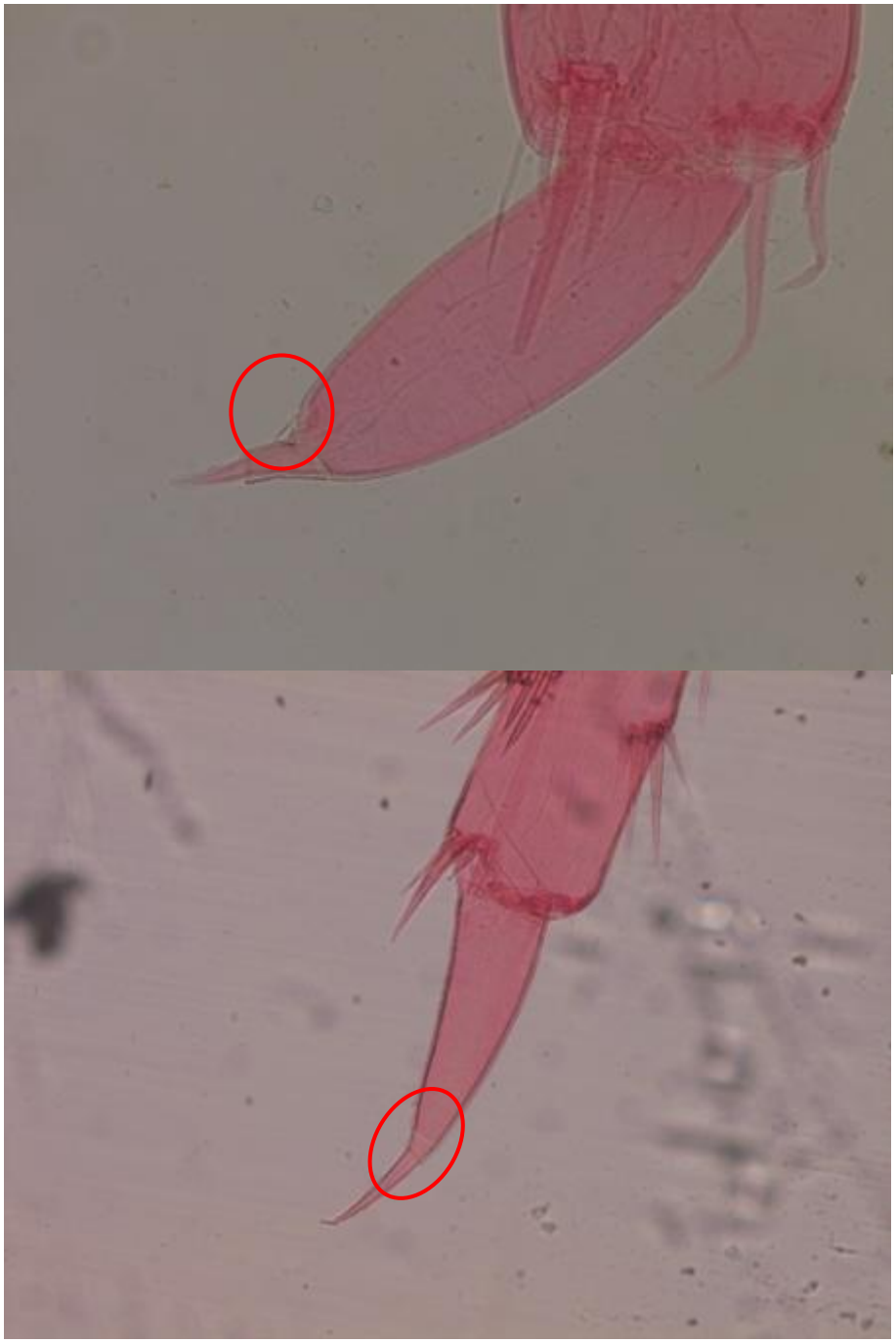


Figure 16: Additional spines of dactyls of pereopods 5, 6, 7 were described as one of the characters based on which *Niphargus arbiter* and *Niphargus salonitanus* can be distinguished. On the photos above we can see that the character is intraspecifically variable in *N. arbiter*.

Slika 16: Dodatni trni na daktilih pereopodov 5, 6 in 7 so veljali za znak, po katerem se potancialno ločita vrsti *Niphargus arbiter* in *Niphargus salonitanus*. Na sliki je vidno da je znak znotraj vrste *Niphargus arbiter* variabilen.

Figure 17: Additional spines of dactyls of pereopods 5, 6, 7 were described as one of the characters based on which *Niphargus arbiter* and *Niphargus salonitanus* can be distinguished. On the photos above we can see that the character can be present in species 3 (which was included in the type series) and also specimens found in Istria region.

Slika 17: Dodatni trni na daktilih pereopodov 5, 6 in 7 so veljali za znak, po katerem se potancialno ločita vrsti *Niphargus arbiter* in *Niphargus salonitanus*. Na sliki je vidno, da je znak lahko prisoten tako pri vrsti 3 (v preteklosti *Niphargus salonitanus*), kot pri primerkih najdenih v Istri.

5.3 CRYPTIC SPECIES AND THEIR CONSERVATION

The analysis unveiled that two nominal species, each with unexpectedly large range (>200 km, Trontelj *et al.*, 2009), are in fact part of a complex of nine, mostly narrowly endemic species. With exception of *Niphargus arbiter*, which covers a large range (200 km in diameter), all other species have much narrower distributions, with three single site species, two double site and one species that occurs in three known sites. Higher species richness and a higher degree of endemism are the most common and the most expected consequences when cryptic species are detected; such cases are not limited only to amphipods but were reported for several other groups of animals (Hebert et al., 2004; Stuart et al., 2006; Smith et al., 2011; Weis et al., 2014; Scherz et al., 2016).

The consequences for nature conservation are far reaching. Different species composition does affect regional species richness patterns as well as patterns of endemism (Bickford et al., 2007). According to IUCN categorization, 3 species of the complex were found in the area of < 5.000 km², and 2 species in the area of < 20.000 km² which make them candidates for Endangered (EN) or Vulnerable (VU) category, respectively. The species that previously seemed widely distributed and by no means endangered are in fact more vulnerable to many distractions, like various pollutions on the surface, habitat destruction and increasing pressure of tourism (Reboleira et al., 2011; Silva & Ferreira, 2015).

Understanding the role of these raptorial species may be important for understanding patterns of species richness and endemism in the Dinaric Karst. Additional data on species conservation status, e.g., population dynamics, more detailed distributional data, are urgently needed.

6 CONCLUSIONS

- Multilocus phylogenetic analyses show that *Niphargus arbiter/Niphargus salonitanus* species complex is monophyletic with nine clades that form separate species.

- Unilocus species delineations support from 6 (0.16) to 9 (PTP, GMYC) independently evolving lineages, while multilocus approach provides high support for 9 separate lineages which can be interpreted as separate species.

- All of the lineages are allopatric.

- Besides the two nominal species *Niphargus arbiter* and *Niphargus salonitanus*, new species are recognized in Istria region, Island Cres, Islands Krk and Rab, Northern Dalmacija region, Middle Dalmacija region, Island of Hvar and the Konavle region.

- Most of the species can be morphologically distinguished. No morphological differences have been found between species *Niphargus arbiter* and species 3 and *Niphargus arbiter* and species 2 (Istria region). Species 7 and 9 were not diagnosed as there were not enough specimens available for reliable analysis.

- Ecological models showed the differences of ecological niches based on Bioclim ecological factors between *Niphargus arbiter* and species n. 3. Also the niche differences between clades A and B, was shown at different correlation values.

- Additional sampling and data are needed for a more reliable comparison of morphology and ecological niches of the species.

- Due to small ranges of all of the newly described species (less than 200 km), all of them should be considered for future protection as the risk of extinction is considerably high.

7 SUMMARY

7.1 SUMMARY

The amphipod genus *Niphargus* constitutes an important part of freshwater biodiversity with many narrowly endemic species. High genetic and morphological diversity is due to efficient diversification. The most attractive members of *Niphargus* are species that belong to cave-lake ecomorphs with a large body size and predatory appendages. They hold an important role as top invertebrate predator in subterranean waters. Regardless of their importance many species are lacking basic taxonomical and phylogenetic characterization and therefore neglected in biodiversity and nature conservation assessments. The traditional taxonomical methods have often proved to be insufficient for species delimitation and therefore an integrative approach is being used more often. In this approach we rely on the general species concept and try to collect the data from different biological disciplines (molecular studies, morphology, ecology) that may support the hypothesis of species.

Cave-lake amphipod species *Niphargus arbiter* and *Niphargus salonitanus* were described in the middle of 20th century based on morphology. Recent analysis showed that the complex consists of more than two species. Therefore the purpose of this work is to delineate species from this complex and provide additional data for species determination. 109 individuals from 34 localities were included in the study to cover most of the species complex distribution range. Gene regions 28S rRNA, COI and ITS were sequenced and analyzed to provide a phylogenetic tree. Based on COI barcoding gene region sequences unilocus PTP, GMYC and 0.16 delimitation of selected specimens were made. On top of that bayesian multilocus delineation was carried out. Morphological characters were checked, measured and compared for species taxonomic diagnosis. Finally, ecological models were produced in MAXENT software using a Bioclim dataset to compare ecological niches of species *Niphargus arbiter* and species 3 and two monophyletic clades within the complex.

The results of the phylogenetic analysis and species delineation show that the species complex forms a monophyletic group which consists of nine species. This number of species has the support of three different delineation approaches. The most conservative 0.16 patristic distances approach supports six species, however we chose to adhere to the consensus of nine species. New species can be found in the Istria region, the Cres island, Islands Krk and Rab, the Upper Dalmacija region, the Middle Dalmacija region, the Island of Hvar and the Konavle region. Morphological analysis of the species showed differences between most of the species while two pairs cannot be distinguished morphologically. In two species a small number of specimens limits reliable morphological diagnosis. Some

characters such as spines on metasomal segments distinguish between two groups, but not between the originally described species. We were able to provide 4 ecological models as the distribution data for most of the species was limited. We tested those at different values of Pearson's correlation rho where a selection of Bioclim parameters were included in the model. The tests of niche equivalency and niche overlap showed that the species *Niphargus arbiter* and species 3 have different ecological niches. This data complements the molecular delimitation methods as we could not find any morphological differences between this pair. The results at different selection of Bioclim parameters for modeling show similar result. The comparison of the monophyletic species clade A (species 2, 7, 9) to the clade B, consisting of the rest of the species, showed differences at any rate of correlation. The p value of the tests is higher than 0.05, which means that the niches are not equivalent.

The analysis unveiled that two nominal species are in fact part of a complex of nine, mostly narrowly endemic species. Large intraspecific morphological variability and interspecific similarity on top of clear molecular distinctness suggest allopatric speciation of amphipods, where they were facing similar selective pressure. With exception of *Niphargus arbiter*, which covers a range of over 200 km, all other species have much narrower distributional ranges, with 3 single site species, 2 double site and 1 species which was found in 3 known sites. Due to the narrow distribution and a potential pressure on habitats through pollution, habitat destruction and tourism, the species should be placed on the IUCN red list and protected. Additional sampling and further morphological and ecological analysis are needed for stronger support of some of the given species. In any case a Dinaric Karst and the amphipod genus *Niphargus* once more proved to be an important and exciting combination for the evolutionary and ecological labyrinth.

7.2 POVZETEK

Postranice (Amphipoda), spadajo med najpomembnejše in najbolj raznolike sladkovodne nevretenčarje. So ena izmed ključnih skupin v vodnih ekosistemih, saj jih pogosto uporabljamo pri vrednotenju ekološkega stanja voda, ali v ekotoksikoloških testih. Najbolj vrstno bogata skupina sladkovodnih postranic v zahodni Palearktiki je rod slepih postranic *Niphargus* Schiödte, 1849. Z več kot 350 opisanimi vrstami, rod pomembno prispeva k biodiverziteti celinskih voda. Slepe postranice so razširjene po celotni Evropi, z izrazito večino vrst južno od meje nekdanjega pleistocenskega ledenega pokrova. Poleg tega, rod poseljuje tudi vode Arabskega polotoka, Turčije in Irana. Slepih postranic na območju Iberskega polotoka, kjer podzemlje poseljujejo postranice iz rodu *Haploginglymus*. Velikost območje razširjenosti posameznih vrst iz rodu *Niphargus* variira, je pa večini vrst skupna ozka endemnost, saj območje razširjenosti večinoma ne presega 200 km med najbolj oddaljenima točkama. Kar nekaj takšnih vrst se nahaja na območju Dinarskega gorstva, ki se razteza od zahodne Slovenije, preko 650 km ob Jadranu, vse do Črne Gore. Jamsko favno tega območja raziskovalci proučujejo že več kot stoletje, samo območje pa velja za eno najbolj dobro prouenih v svetu jamske favne. Regija je izjemno bogata tudi z raki iz rodu *Niphargus*, saj je bilo do sedaj od tu opisanih več kot 200 vrst.

Vrste iz rodu *Niphargus* so skoraj izključno vezane na podzemne vode, kjer zasedajo praktično vse razpoložljive ekološke niše. Njihova izjemna ekološka raznolikost je verjetno glavni razlog za morfološko raznolikost rodu. To dobro oriše razpon telesnih velikosti različnih vrst postranic, ki segajo od 2 mm do 40 mm, poleg tega pa poznamo pet različnih ekomorfov postranic. Med njimi so najbolj atraktivne in karizmatične živali jamsko-jezerskega ekomorfa, katerih telesne dolžine običajno presegajo 20 mm, poleg tega pa jih krasijo elegantne in dolge okončine, pleon oborožen s trni ter ogromni plenilski gnatopodi. Jezerski tip postranic se je tekom evolucije razvil večkrat, vsakič na srednjih zemljepisnih širinah razširjenosti rodu. Ta tip se je razvil večkrat neodvisno v Franciji, Italiji, centralno-zahodnem Balkanu ter na polotoku Krim. Vrste tega ekomorfa so zanimiv objekt za študij evolucijske ekologije, saj predstavljajo glavne predatorje nevretenčarjev v podzemlju Dinarskega pogorja. S svojo vlogo pripomorejo k visoki vrstni pestrosti v regiji. Poleg tega predstavljajo svojevrsten fenomen, saj so presenetljivo velike za postranice z območja zemljepisne širine med 42° in 47° severne poloble.

Navkljub privlačnosti postranic jamsko-jezerskega ekomorfa, te še vedno ostajajo brez kompletnega inventarja vrst ter študije njihove razširjenosti. Taksonomija rodu *Niphargus* je na nivoju vrste pogosto izjemno zahtevna, zaradi velike znotrajvrstne raznolikosti ter medvrstne podobnosti. Seveda pa identifikacije ne olajšajo niti ostale okoliščine znane taksonomom, kot so majhni vzorci v primeru redkih vrst. Z uporabo molekulskih tehnik in razkritjem takoimenovanih kriptičnih vrst, so pomankljivosti morfoloških diagnoz vrst iz

rodu *Niphargus* postale več kot očitne. Pogosto te vrste ostanejo neopisane in prezrte, kljub temu, da imajo velik potencial za ekološko-evolucijske študije ter naravovarstvo. Sodobni taksonomski koncepti in tehnološki napredek omogočajo razlikovanje in opisovanje kriptičnih vrst, kar bi bilo vredno izkoristiti vsaj pri karizmatičnih in ekološko pomembnih vrstnih kompleksih, kakršne najdemo med jamsko-jezerskimi postranicami iz rodu *Niphargus.*

Taksonomija, osnovna biološka disciplina opisovanja in identifikacije vrst, se je v zadnjem desetletju znašla v krizi. Kriza je predvsem posledica primankljaja v taksonomskem znanju nekaterih taksonomov, omejene taksonomske infrastrukture (nedostopnost baz podatkov, zbirk) ter upada števila taksonomov za posamezne skupine organizmov. Disciplina je predvsem zaradi spoznanj, da speciacija ni enosmeren proces ter, da se vrste ne spreminjajo zgolj morfološko, prerasla v interdisciplinarno vedo. Značilnosti vrste izhajajo iz dednega zapisa, morfologije, ekologije ali pa vedenjskih lastnosti. Posledično moramo pri diagnozi vrst upoštevati raznolike dejavnike. Zdi se, da je uvedba "general species concept", ameriškega biologa Kevina de Queiroza, odpravila številne dvome nasprotujočih si koncepcij vrste. Koncepcija definira vrsto kot metapopulacijo, ki evoluira ločeno od drugih metapopulacij ter se od njih poljubno loči. Tako lahko najdemo razlike med vrstami v njihovem genskem zapisu, ekološki vlogi, morfoloških ali vedenjskih značilnostih.

Integrativni pristop k taksonomiji omogoča robustnejše diagnoze, ki pripomorejo k delimitaciji, klasifikaciji, poimenovanju in bodoči identifikaciji organizmov. Vsak izmed obravnavanih kriterijev v integrativni taksonomiji enakovredeno prispeva k delimitaciji. Tako je vsaka vrsta določena z različnimi parametri, ki jih lahko testiramo in dopolnimo z novimi podatki, pridobljenimi z novejšimi metodami. Za zadostno robustnost taksonomskega pristopa avtorji predlagajo, da se za opis uporabi vsaj tri discipline; tj. genetske podatke dopolnimo z morfološkimi, ekološkimi ali vedenjskimi podatki. Ključnega pomena je tako molekulska delimitacija. Ta vključuje kombinacijo sekvenc več genskih regij. Sekvence različnih vrst primerjamo z delimitacijskimi modeli, ki lahko podajo različne rezultate. Med konzervativnejše spada PTP, medtem ko GMYC pogosto izračuna več potencialnih vrst. Analiza morfologije lahko z modernimi pristopi, kot sta računalniška tomografija ali elektronska mikroskopija doda podatke, ki jih v preteklosti ni bilo mogoče analizirati. Obsežne baze podatkov in bogato predznanje, lahko proces identificiranja vrst močno pospešijo. Dodatno težo delimitaciji vrste dajo ekološki podatki, ki jih analiziramo kot modele ekološke niše. To omogočajo dostopni in uporabniku prijazni programi kot je Maxent. Modele lahko testiramo že pri nizkem število podatkov o prisotnosti vrste (najmanj 5). Za delimitacijo vrst lahko uporabimo pakete v R-ju, ki primerjajo ekvivalentnost in prekrivanje izračunanih niš.

V tej nalogi obravnavamo kompleks postranic jamsko-jezerskega ekotipa iz rodu *Niphargus*, endemita Dinarskega gorovja. Primarno je bil kompleks opisan kot dve prostorsko ločeni vrsti, in sicer severna vrsta *Niphargus arbiter* Karaman, 1984 in južna vrsta *Niphargus salonitanus* (Karaman, 1950). Kljub temu, da sta holotipa opisanih vrst jasno morfološko ločena, pa so bile najdene populacije z vmesnimi morfološkimi stanji. Večje število genetskih linij pa predvidijo tudi nekatere molekularne analize. Molekularno delimitacijo vrst smo združili z morfološkimi podatki in ekološkim modeliranjem ter ugotovili, da kompleks sestavlja devet vrst, ki smo jim pripisali diagnoze in jih umestil v širši biodiverzitetni in naravovarstveni kontekst.

V nalogi poskušamo razjasniti vrstno strukturo kompleksa vrst *Niphargus arbiter/Niphargus salonitanus.* Glavni cilji raziskave so: testiranje filogenetske umestitve kompleksa med ostale vrste rodu *Niphargus* z uporabo multilokusne delimitacije, analiza morfologije vrst in izdelava ekoloških modelov kot podpore molekulskim podatkom, diagnoza novih vrst ter nominalnih vrst. Pridobljeni podatki bodo pripomogli predvsem k poznavanju kriptičnih vrst, speciacijskih procesov med jamskimi organizmi ter načinu opisovanja taksonomsko zahtevnih vrst. Poleg tega, bo možna aplikacija pridobljenega znanja v varstveno biologijo ter zaščito jamskih organizmov. Postavili smo štiri hipoteze. Prvič, pričakujemo, da je vrstni kompleks monofiletski. Drugič, klad sestoji iz več kot dveh vrst. Tretjič, vrste se razlikujejo morfološko, vendar pa so neočitni in niso uporabni za hitro in učinkovito prepoznavo vrst. Četrtič, ekološke niše analiziranih vrst se jasno ločijo, prav tako niše kladov.

Skupaj je bilo v analizo vključenih 109 osebkov iz 34 lokalitet, večinoma iz Dinarskega krasa. Povzorčenih je bilo vseh 500 km vzdolž razširjenosti kompleksa vrst. Osebki so bili ujeti z vzorčenjem z vodno mrežo in vodnimi pastmi in shranjeni v 96% EtOH na Oddelku za biologijo Biotehniške fakultete na Univerzi v Ljubljani. Podatki o vavčerskih kodah in lokaliteah so v prilogi A.

Za pridobitev DNA smo vsakemu osebku odstranili po en pereopod. DNA genoma smo izolirali z uporabo GeneElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich). Pomnožili smo jedrno DNA, dva dela ribosomalne podregije 28S, ITS regijo, histon 3 podenoto A ter dva fragmenta mitohondrijske DNA in sicer, COI I in COI II regijo. Začetniki 28S lev2, 28S des2, 28S lev3, 28S des5, ITS f1, ITS r1, H3aF2, H3aR2, Jerry, Maggie, LCO, COI, spr1. ITS regija je bila dodatno sekvencirana z uporabo štirih začetnikov (ITS sf1, ITS sr1, ITS sf2, ITS sr2). PCR program za markerje COI, 28S in ITS genetske markerje je bil identičen kot v Fišer et *al.* (2013). Izpeljan je bil dodaten program za ITS regijo s 30 cikli po 94 °C za 30 sekund, 54 °C za 45 sekund, 72 °C za 2 minuti ter s končnim podaljškom na 72 °C za 10 minut. Uspešno pomnoženi PCR produkti so bili očiščeni z Eksonukleazo I in FastAP termosenzitivno alkalno fosfatazo (Thermo Fisher

Scientific Inc., US). Sekvenciranje je bilo izvedenso s strani Microsynth AG (Balgach, Switzerland). Kromatogrami so bili analizirani v programu Geneious 6.0.5. Sekvence so bile poravnane v programu MAFFT v7.

Za pozicioniranje skupine znotraj evolucijskega drevesa rodu *Niphargus* je bilo v niz podatkov združenih 83 osebkov iz kompleksa *Niphargus arbiter/Niphargus salonitanus* in 29 osebkov drugih vrst iz rodu *Niphargus* ter zunanjika *Synurella ambulans* in *Gammarus fossarum.* Najbolje prilegajoči se model je bil izbran s programom PartitionFinder. Filogenetski odnosi so bili rekonstruirani z Bayesian inference v programu MrBayes v3.2 in BEAST v1.8.1, v dveh neodvisnih MCMC algoritmih. Prvih 25% dreves smo zavrgli, medtem ko smo preostale prikazali na filogenetskem drevesu. Alternativno smo izpeljali multilokusno filogenijo v programu BEAST, ter sestavili drevo v program Tree Annotator v1.8.1.. Diverzifikacija je bila ocenjena s pomočjo 45 mio. let starega fosila v jantarju. Molekularna delimitacija je vključevala tri unilokusne metode (0.16, PTP in GMYC) ter eno multilokusno metodo. Podrobnosti so predstavljene v prilogi (Appendix B).

Za odstranitev mehkih tkiv smo izbrane osebke dali v 10% vrelo raztopino KOH, nevtralizirali z razredčeno klorovodikovo kislino ter sprali z destilirano vodo. Prosojne eksoskelete smo pobarvali s klorazol črnim ali pa lignin roza barviloma, deloma smo jim odstranili okončine v glicerolu in jih namestili na objektna stekla, v mešanico glicerola in želatine. S stereomikroskopom Olympus SZX0 in Zeisovim mikroskopom, smo proučili morfološke znake. Znake smo analizirali po predlogu Fišer in sod. (2009). Digitalne risbe izbranih znakov smo zrisali v Adobe Illustrator CS3, z uporabo digitalne risalne mize Bamboo.

Vrste so bile molekulsko delimitirane. Glede na vrste, ki jih podpirajo različni molekulski delimitacijski pristopi (PTP, GMYC; 0.16, MULTI), smo poskušali prepoznati za diagnozo posamičnih vrst pomembne morfološke znake. Analiziranih je bilo 26 merjenih znakov ter 99 številskih štetih in pa kategorialnih znakov. Da smo odstranili vpliv telesne dolžine, smo izmerjene vrednosti delili s telesno dolžino. Nato smo izračunali reziduale. Vsi naslednji testi so temeljili na vrednostih ostankov. Normalno porazdeljeni podatki so bili testirani z ANOVA testom in posthoc testi, s Scheffejevimi, Bonferronijevimi in Hochbergovimi korekcijami. Podatke, ki niso porazdeljeni normalno, smo analizirali s Kolmogorov-Smirnovim testom. Poškodovanih delov osebkov nismo vključili v raziskavo. Razlike med podatki smo pregledali na grafih ustvarjenih v SPSS Statistics v20. Merjene znake in frekvence štetih številskih znakov smo analizirali s populacijsko agregatno analizo.

Tudi za ekološko modeliraje smo podatke lokalitet vrst povzeli glede na molekulske delimitacije. Večina vrst se je izkazala za ozko endemne, število znanih nahajališč pa

omejeno. Posledično smo zaradi primanjkljaja znanih lokacij lahko primerjali zgolj en par vrst. Poleg tega smo primerjali še dva monofiletska klada z različno razširjenostjo, enega celinskega in drugega obalnega. Za vsak takson smo najprej rekonstruirali potencialno bioklimatsko nišo, čemur je sledil test, ki primerja razliko v ekološki niši dveh kladov/vrst. Modele smo pripravili z BioClim podatki, ki simulirajo bioklimatske razmere na površju, ki preko produkcije organskih snovi, padavin in temperature vplivajo na organizme v podzemlju. Sklop 19-ih slojev je bil prilagojen na celico velikosti 10 x 10 km in v ArcGIS-u umerjen na velikost Dinaridov. Izračunali smo vrednosti korelacije med danimi parametri in izločili odvečne sloje pri Spearmanovih korelacijskih vrednostih (0.6, 0.7, 0.8), izračunanih v paketu agricolae v statističnem programu R. Ekološke niše smo izračunali v programu Maxent s pomočjo podatkov o prisotnosti vrst, saj lahko program učinkovito ustvari modele z majhnim številom podatkov o prisotnosti vrste. Pripravili smo štiri sete podatkov: vrsta 3, vrsta 6, klad A (monofilum vrst 2, 7, 9) in klad B (monofilum vrst 1, 3, 4, 5, 6, 8). Model smo pripravili z 80 % podatkov in ga testirali s preostalimi 20%. V primerjavi dveh parov modelov smo uporabili paketa Phyloclim in dismo, v katerih smo izračunali podobnost niš ter njihovo prekrivanje, izraženo z Schoenerjevim indeksom D in Hellingerjevimi razdaljami. Vrednost indeksov se giblje med 0 in 1, pri čemer vrednost 0 pomeni različna modela brez prekrivanja, vrednost 1 pa enaka modela s popolnim prekrivanjem.

Rezultati filogenetskih analiz so pokazali, da je kompleks vrst *Niphargus arbiter/Niphargus salonitanus* monofiletski in vgnezden znotraj vrst jamsko-jezerskih ekomorforfov (*Niphargus ictus, Niphargus longiflagelum, Niphargus steueri*). Kompleks sestavljajo štiri glavne linije. Prva je večinoma razporejena ob obali vzhodnega Jadrana, vključno z Istrskim polotokom, Zadrom in Bračem. V tej skupini najdemo tri vrste, ki so podprte z vsemi delimitacijskimi metodami, razen konzervativnejšo metodo z 0.16 substitucije na nukleotidno mesto. Slednja podpira eno vrsto iz več filetskih linij. Vrsti 7 in 9 sta oddaljeni okoli 200 km zračne linije, kar podpira idejo od dveh ločenih vrstah. Druga linija vsebuje populacije iz Kvarnerskih otokov. Metoda z 0.16 substitucije podpira populacije kot eno vrsto, medtem ko preostali trije testi podpirajo dve ločeni vrsti. Ker sta populaciji 4 in 5 prostorsko ločeni, genetsko diferencirani in monofiletski, predvidevamo, da gre za ločeni vrsti. Tretja linija je razširjena na povsem južnem delu Dalmacije. Molekularne metode podpirajo dve vrsti (*Niphargus salonitanus* in vrsto 8), od katerih se ena nahaja ob tipski lokaliteti *Niphargus salonitanus*. Četrta linija je razširjena po goratem Dinarskem krasu Slovenije, Hrvaške ter Bosne in Hercegovine. Vse delimitacijske metode v četrti liniji potrjujejo obstoj dveh vrst, severne *Niphargus arbiter* in nove vrste na jugu (vrsta 3).

Morfološki rezultati kažejo predvsem na visoko variabilnost morfoloških znakov znotraj vrst ter veliko podobnost med nekaterimi predlaganimi vrstami. Med 123 merjenimi in

analiziranimi znaki, smo našli 33 znakov, ki razlikujejo vsaj med enim parom vrst in so potencialno uporabni za njihovo identifikacijo. Med najbolj različnimi sta si par vrst oziroma filetskih linij 4-7 in par 4-9, ki se razlikujeta v 17 znakih, med parom vrst 3-*Niphargus arbiter* in parom 2-3 pa morfoloških razlik nismo našli.

Bioklimatski modeli za vrste 3 in *N. arbiter* ter monofiletska klada A in B, se zdita sprejemljivo predvidljiva z vrednostjo AUC, večjo od 0.7. Primerjava parov modelov kaže na diferenciacijo med analiziranimi vrstami/kladi. Sprememba korelacijske vrednosti med vključenimi parametri ni bistveno vplivala na rezultat. Primerjava kladov A in B kaže manjšo ekvivalentnost in prekrivanje niš, kot primerjava vrst 3 in *Niphargus arbiter*. Visoka vrednost p pri obeh testih kaže na neznačilno razliko med primerjavo enakosti ekoloških niš. Rezultate smo grafično prikazali in lahko vidimo, da je razlika očitna predvsem med kladoma A in B. Klad A ima povsem drugačnom bolj kontinentalno nišo kot obalni klad, ki mu očitno bolj ustreza območje bližje Jadranskemu morju (slika 7). Razlika med vrstama 3 in *Niphargus arbiter* je manj očitna, saj le *Niphargus arbiter* kaže izrazito preferenčno bioklimatsko območje globlje na celini.

V nalogi smo podali predloge diagnoz vrst, med katerimi so znaki za vrsti 7 in 9 zgolj informativni, saj je število analiziranih osebkov prenizko za zanesljivo diagnozo. Poleg tega smo podali tudi splošen opis variabilnosti analiziranih taksonomskih znakov kompleksa novo-odkritih vrst. Pomembne taksonomske znake smo prikazali z digitalnimi risbami.

Rezultati kažejo na jasno genetsko diferenciacijo znotraj kompleksa, katerega vrste imajo parapatrično distribucijo. Nasprotno pa so morfološki in bioklimatski znaki za divergenco manj izraziti in ne podpirajo vseh predlaganih vrst. Poleg dveh nominalnih vrst na severu in jugu območja razširjenosti kompleksa vrst *Niphargus arbiter/Niphargus salonitanus*, najdemo ločene vrste še v zahodni Istri (vrsta 2), osrednji Dalmaciji (vrsta 3), na otoku Cresu (vrsta 4), Krku in Rabu (vrsta 5), Braču (vrsta 7), južni Dalmaciji (vrsta 8) in v okolica Zadra (vrsta 9). Velik areal celotnega klada je tako razdeljen na vrste z manjšimi areali, kar potrjuje dognanja, da areali jamskih vrst ne presegajo 200 km. Do opisa več kriptičnih in ozko endemnih vrst v Dinaridih, je v zadnjem desetletju prišlo tudi pri drugih vrstah, kot na primer pri človeški ribici ali pa jamskih rakih iz rodu *Troglocaris*.

Morfološka in ekološka diagnoza s slabo definiranimi razlikami sta lahko posledici enega izmed dveh možnih scenarijev. Ena možnost je, da so vrste relativno mlade in še ni prišlo do jasne diferenciacije. Na to morda nakazuje razlika v ekološki diferenciaciji na nivoju kladov in vrst, kjer so starejši kladi bolje diferencirani kot pa mlajši. Možna razlaga je tudi, da so vrste starejše in jasno diferencirane, vendar pa ne kažejo izrazith morfoloških razlik, saj so bile v preteklosti izpostavljene enakim selekcijskim pritiskom. Posledično so vse

vrste pridobile podobno morfološko strukturo oziroma se le-ta ni spremenila, saj je že predniška vrsta pridobila vse sedanje morfološke značilnosti. Na podlagi danih rezultatov za nekatere vrste ni mogoče podati uporabne morfološke diagnoze, zato se moramo zanašati na filogenetske analize.

Podobna je razlaga ekoloških podobnosti vrst, kjer so možni trije scenariji. Vrste so dejansko prilagojene na specifične bioklimatske razmere, kar se odraža v ekoloških lastnostih vrst. Druga možnost je akumulacija nevtralnih genetskih sprememb zaradi alopatrije. Tretja možnost je, da so vrste ekološko enake in zgolj dobro tolerirajo raznolike okoljske razmere.

Zaključimo lahko, da dodatni podatki pomagajo razlikovati med kriptičnimi vrstami, vendar pa v primeru majhnega števila analiziranih osebkov, ne dosežejo namena. Naši podatki so predvsem pokazali, da je na obravnavanem območju devet fenotipsko podobnih vrst z izjemno omejenim območjem razširjenosti. *Niphargus arbiter* je ohranil za jamske organizme velik areal (200 km v premeru). Poleg tega lahko sedaj prepoznamo kar tri vrste zgolj iz ene lokalitete, dve vrsti iz dveh in eno iz treh lokalitet. Prispevek kompleksa k regionalni diverziteti jamskih postranic je velik, visoka pa je tudi stopnja endemnosti. Najmanj šest vrst bi morali vključiti na rdeči seznam ogroženih vrst, saj je njihov areal manjši od 20.000 km² oziroma celo manjši od 5.000 km². To jih uvršča med ranljive (VU) ali pa ogrožene (EN). Posledično tem vrstam grozi izumrtje v primeru zanje neustreznih življenjskih razmer. Za ustrezno oceno stopnje ogroženosti posamezne vrste, bi bila potrebna pridobitev dodatnih podatkov o razširjenosti vrst ter njihovi populacijski dinamiki.

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APPENDIX A

List of the analyzed specimens (x and y coordinates in WGS1984 coordinate system)

Sample number	Collecting locality	x	y	date	Collected by
JFF_Saktari		13,892528	44,861469	24.01.2010	B. Jalžić
NA048	Podpeško jezero; Podpeč; Ljubljana				
NA050	Izvirček v luki Vrbnik, Vrbnik, Krk; HRV	14,678183	45,078399	29.04.2004	Boris Sket
NA051	Lopar, Rab; otok Rab; HRV	14,736155	44,830481	06.08.2005	Boris Sket
NA052	Tounjčica jama, Tounj, Ogulin, HRV	15,322633	45,248823	01.08.2002	Boris Sket
NA053	Sveta Stomorija; Kaštel Stari; Split; HRV	16,324805	43,569863	27.03.2002	C. Fišer
NA054	Bikovica; Pirovac; Zadar; HRV	15,665955	43,829961	02.04.2005	T. Rađa
NA183	Suvaja; Lušci Palanka; Sanski Most; BIH	16,472892	44,698076	01.09.2003	S. Polak, P. Trontelj
NA510	Izvir pod Cesto; Barilović; Karlovac; HRV	15,568470	45,385008	27.05.2007	B. Sket
NA587	Jama nad kobilo, kš.547; Idrija, SLO	14,010913	45,980880	22.04.2009	S. Prevorčnik, P. Trontelj
NB161	Jama nad Kobilo (547); Zagoda; Idrija; SLO	14,010913	45,980880	24.02.2007	B. Sket
NB162	Vogršček(3903);G. Log; Most na Soči; SLO	13,712331	46,125339	03.03.2007	V.Zakšek, J. Jugovič
NB163	Kaptaža K-2; Dunaj; Postira; o. Brač; HRV	16,624100	43,351808	28.02.2010	B. Jalžič, H. Bilandžija
NB164	Šipun špilja; Konavle donje; Cavtat; HRV	18,213091	42,586420	07.04.2007	B. Sket
NB168	Šipun špilja; Konavle donje; Cavtat; HRV	18,213091	42,586420	07.04.2007	B. Sket
NB169	Jašek p. mostu, zaliv Jadriščica, Punta Križa, Cres, HRV	14,494554	44,624289	01.05.2007	T. Turk
NB170	Rupa na Brodu; Novo mesto, SLO	15,144295	45,788852	02.03.2008	V. Zakšek
NB171	Jelovička jama, Žaga, Kostel, SLO	14,909154	45,520446	08.05.2009	B. Sket
NB172	Markov ponor, v sifonu, Kosinj, Lipovo polje, Lika, HRV	15,176874	44,765318	01.09.2000	B. Jalžić
NB173	Kaptaža K-2; Dunaj, Postira; Brač; HRV	16,624100	43,351808	17.04.2010	B. Jalžić
NB174	Izvor pećina, Vedro polje, Bihač, BIH	15,827389	44,805101	17.04.2007	S. Polak
NB175	Jama Velika Betina, Kokorići, Vrgorac	17,322505	43,194454	25.08.2005	P. Rade
NB176	Pećina poli vrtića, Brest pod Učkom, Ćićarija	14,154617	45,330332	06.11.2006	H. Cvitanović
NB177	Špilja pod Mačkovim dragom, Vrelo, HOC Bjelolasica	15,006647	45,256428	14.06.2009	P. Baković
NB178	Kusačko jezero, Palanka, Zrmanja; HRV	16,088643	44,132514	01.05.2010	B. Jalžić
NB179	Sveta Stomorija; Kaštel Stari; Split; HRV	16,324805	43,569863	03.02.2013	T. Delić
NB201	Šipun špilja; Konavle donje; Cavtat; HRV	18,213091	42,586420	07.04.2007	B. Sket
NB202	Izvor pećina, Vedro polje, Bihač, BIH	15,827389	44,805101	17.04.2007	S. Polak
NB203	Markov ponor, v sifonu, Kosinj, Lipovo polje, Lika, HRV	15,176874	44,765318	15.08.2009	M. Lukić
NB204	Pećina poli vrtića, Brest pod Učkom, Ćićarija	14,154617	45,330332	06.11.2006	M. Pavlek
NB205	Jelovička jama, Žaga, Kostel, SLO	14,909154	45,520446	08.05.2009	B. Sket
NB206	Malo okence, Retovje, Vrhnika, SLO	14,295418	45,951446	21.11.2001	A. Hodalič
NB207	Jama v Kanjeducah, Sežana, SLO	13,876198	45,696775	11.12.2005	P. Trontelj
NB208	Jama pod Orljakom, Zaton, Šibenik, HRV	15,841372	43,770483	24.04.2010	B. Jalžić
NB209	Čavle špilja, Muškovci, Zrmanja, HRV	15,746365	44,212411	06.05.2010	M. Pavlek
NB210	Pincinova jama; Tar; Poreč; HRV	13,658716	45,263453	29.05.2010	P. Bregović, A. Čukušić
NB211	Čude špilja, Kanjon Zrmanje, Obrovac	15,703476	44,208232	04.08.2006	M. Pavlek
NB411	Mandelaja, Oštarije; Ogulin; HRV	15,310024	45,231269	10.09.2009	M. Lukić
NB412	Jablan izvor; Grab; Gračac; HRV	15,898603	44,279056	25.03.2005	J. Bedek
NB413	Jama kod Špikovca; Zadar; HRV	15,320172	44,310351	11.03.2007	T. Dražina

NB414	Vranovinski ponor, Vranovine, Gospić; HRV	15,297667	44,633635	25.07.2011	B. Jalžić, H. Bilandžija
NB415	Suvaja pećina; Mekinjar; Udbina; HRV	16,679796	44,566551	10.04.2010	B. Jalžić
NB416	Tounjčica; Tounj; Ogulin; HRV	15,322633	45,248823	23.06.2001	C. Fišer
NB417	Pećina špilja; Ličko Lešće; Otočac; HRV	15,331365	44,796383	24.06.2006	P. Rade
NB418	Čavle špilja, Muškovci, Zrmanja, HRV	15,746365	44,212411	06.05.2010	P. Bregović, A. Čukušić
NB419	Pećina uz Koranu; Blagaj; Slunj; HRV	15,534040	45,216276	08.10.2009	B. Jalžić
NB420	Jelovička jama, Žaga, Kostel, SLO	14,909154	45,520446	08.05.2009	B. Sket
NB424	Vodna jama v Lozi; Slavina; Pivka; SLO	14,118600	45,716700	21.04.2013	T. Delić
NB425	Vranovinski ponor, Vranovine, Gospić; HRV	15,297667	44,633635	25.07.2011	B. Jalžić, H. Bilandžija
NB445	Jašek p. mostu, zaliv Jadriščica, Punta Križa, Cres, HRV	14,494554	44,624289	01.05.2007	T. Turk
NB446	Sveta Stomorija; Kaštel Stari; Split; HRV	16,324805	43,569863	28.12.2013	T. Delić
NB447	Čavle špilja, Muškovci, Zrmanja, HRV	15,746365	44,212411	22.09.2005	P. Rade
NB448	Jazbina; Katići; Generalski stol; HRV	15,414654	45,348586	21.03.2010	B. Jalžić
NB449	Ponor Sušik, Drežnica; Ogulin; HRV	15,089984	45,145582	08.09.2009	J. Bedek
NB450	Vidovića špilja, Drežnica; Ogulin; HRV	15,110512	45,161451	08.09.2009	J. Bedek
NB451	Luška Špilja; Debeli Lug: Jasenak; HRV	15,093663	45,224745	09.09.2009	B. Jalžič
NB452	Markarova špilja, Stajnica, Jezerane; HRV	15,154641	45,019871	31.01.2008	M. Pavlek
NB453	Markov ponor, Kosinj, Lipovo polje, HRV	15,176874	44,765318	15.08.2009	J. Bedek
NB454	Tamnica, Potok Tounjski, Tounj; HRV	15,360043	45,272377	30.01.2008	H. Cvitanović
NB455	Hrustovača; Hrustovo; Sanski Most; BIH	16,696950	44,673751	27.03.2012	J. Bedek
NB456	Dabarska pećina; Dabar; Sanski Most; BIH	16,635845	44,710690	14.05.2011	B. Jalžić
NB457	Zelena špilja; Bunić; Korenica; HRV	15,608562	44,681883	21.06.2011	D. W. Fong, M. L. Porter
NB458	Špilja za Gromačkom vlakom; Gromača; Dubrovnik; HRV	18,012716	42,727815	05.10.2011	L. Đud
NB459	Jašek p. mostu, zaliv Jadriščica, Punta Križa, Cres, HRV	14,494554	44,624289	01.05.2007	T. Turk
NB460	Jašek p. mostu, zaliv Jadriščica, Punta Križa, Cres, HRV	14,494554	44,624289	01.05.2007	T. Turk
NB461	Malo okence, Retovje, Vrhnika, SLO	14,295418	45,951446	21.11.2001	A. Hodalič
NB462	Veliko okence; Retovje; Vrhnika; SLO	14,295828	45,949694	01.06.2005	M. Simonič
NB463	Veliko okence; Retovje; Vrhnika; SLO	14,295828	45,949694	01.06.2005	M. Simonič
NB464	Sinjac izvor; Plavča draga; Plaški; HRV	15,427142	45,050062	10.09.2009	B. Jalžić
NB465	Sinjac izvor; Plavča draga; Plaški; HRV	15,427142	45,050062	10.09.2009	B. Jalžić
NB466	Šipun špilja; Konavle donje; Cavtat; HRV	18,213091	42,586420	07.04.2007	B. Sket
NB467	Šipun špilja; Konavle donje; Cavtat; HRV	18,213091	42,586420	07.04.2007	B. Sket
NB468	Čavle špilja, Muškovci, Zrmanja, HRV	15,746365	44,212411	06.05.2010	P. Bregović, A. Čukušić
NB469	Čavle špilja, Muškovci, Zrmanja, HRV	15,746365	44,212411	06.05.2010	P. Bregović, A. Čukušić
NB470	Suvaja pećina; Mekinjar; Udbina; HRV	16,679796	44,566551	10.04.2010	B. Jalžić
NB471	Pincinova jama; Tar; Poreč; HRV	13,658716	45,263453	29.05.2010	B. Jalžić
NB472	Pincinova jama; Tar; Poreč; HRV	13,658716	45,263453	29.05.2010	B. Jalžić
NB473	Sveta Stomorija; Kaštel Stari; Split; HRV	16,324805	43,569863	28.12.2011	T. Delić
NB474	Sveta Stomorija; Kaštel Stari; Split; HRV	16,324805	43,569863	28.12.2011	T. Delić
NB475	Suvaja pećina; Mekinjar; Udbina; HRV	16,679796	44,566551	10.04.2010	B. Jalžić
NB476	Suvaja; Lušci Palanka; Sanski Most; BIH	16,472892	44,698076	08.11.2006	S. Polak, P. Trontelj, A. Kapla
NB477	Mandelaja, Oštarije; Ogulin; HRV	15,310024	45,231269	10.09.2009	M. Lukić
NB478	Mandelaja, Oštarije; Ogulin; HRV	15,310024	45,231269	10.09.2009	M. Lukić
NB479	Suvaja; Lušci Palanka; Sanski Most; BIH	16,472892	44,698076	18.07.2004	P. Trontelj
NB480	Pećina špilja; Ličko Lešće; Otočac; HRV	15,331365	44,796383	22.09.2005	B. Jalžić, H. Bilandžija
NB481	Luška Špilja; Debeli Lug: Jasenak; HRV	15,093663	45,224745	09.09.2009	B. Jalžić

NB482	Luška Špilja; Debeli Lug: Jasenak; HRV	15,093663	45,224745	09.09.2009	R. Ozimec
NB483	Špilja pod Mačkovom dragom, Vrelo, HOC Bjelolasica	15,006647	45,256428	14.06.2009	P. Baković
NB484	Ponor Sušik, Drežnica; Ogulin; HRV	15,089984	45,145582	08.09.2009	J. Bedek
NB485	Ponor Sušik, Drežnica; Ogulin; HRV	15,089984	45,145582	08.09.2009	J. Bedek
NB486	Markov ponor, Kosinj, Lipovo polje, HRV	15,176874	44,765318	15.08.2009	M. Lukić
NB487	Vidovića špilja, Drežnica; Ogulin; HRV	15,110512	45,161451	08.09.2009	J. Bedek
NB488	Vidovića špilja, Drežnica; Ogulin; HRV	15,110512	45,161451	08.09.2009	J. Bedek
NB489	Zagorska peć; Zagorje; Ogulin; HRV	15,21978	45,196991	26.01.2009	V. Jalžić
NB490	Špilja pod Mačkovom dragom, Vrelo, HOC Bjelolasica	15,006647	45,256428	20.06.2009	P. Baković
NB491	Pećina uz Koranu; Blagaj; Slunj; HRV	15,534040	45,216276	08.10.2009	B. Jalžić
NB492	Perčevića špilja; Tounj; Ogulin; HRV	15,336124	45,242623	29.01.2008	H. Cvitanović
NB493	Markarova špilja, Stajnica, Jezerane; HRV	15,154641	45,019871	31.01.2008	M. Pavlek
NB494	Markarova špilja, Stajnica, Jezerane; HRV	15,154641	45,019871	31.01.2008	M. Pavlek
NB495	Sveta Stomorija; Kaštel Stari; Split; HRV	16,324805	43,569863	03.02.2013	T. Delić
NB496	Sveta Stomorija; Kaštel Stari; Split; HRV	16,324805	43,569863	03.02.2013	T. Delić
NB497	Izvor Graba; Grab; Trilj; HRV	16,770135	43,641461	17.08.2011	P. Kovač-Konrad
NB498	Izvor Graba; Grab; Trilj; HRV	16,770135	43,641461	17.08.2011	P. Kovač-Konrad
NB499	Hrustovača; Hrustovo; Sanski Most; BIH	16,696950	44,673751	27.03.2012	I. Napotnik
NB500	Hrustovača; Hrustovo; Sanski Most; BIH	16,696950	44,673751	27.03.2012	J. Bedek
NB501	Šipun špilja; Konavle donje; Cavtat; HRV	18,213091	42,586420	05.06.2011	J. Bedek
NB502	Šipun špilja; Konavle donje; Cavtat; HRV	18,213091	42,586420	05.06.2011	J. Bedek
NB503	Špilja za Gromačkom vlakom; Gromača; Dubrovnik; HRV	18,012716	42,727815	05.10.2011	L. Đud
NB504	Špilja za Gromačkom vlakom; Gromača; Dubrovnik; HRV	18,012716	42,727815	05.10.2011	L. Đud
NB505	Zelena špilja; Bunić; Korenica; HRV	15,608562	44,681883	21.06.2011	D. W. Fong, M. L. Porter
NB506	Zelena špilja; Bunić; Korenica; HRV	15,608562	44,681883	21.06.2011	D. W. Fong, M. L. Porter
NB507	Kaptaža K-2; Dunaj; Postira; o. Brač; HRV	16,624100	43,351808	28.02.2010	B. Jalžič, H. Bilandžija
NB508	Kaptaža K-2; Dunaj; Postira; o. Brač; HRV	16,624100	43,351808	28.02.2010	B. Jalžič, H. Bilandžija
NB509	Kaptaža K-2; Dunaj; Postira; o. Brač; HRV	16,624100	43,351808	28.02.2010	B. Jalžič, H. Bilandžija
NB510	Šipun špilja; Konavle donje; Cavtat; HRV	18,213091	42,586420	07.04.2007	B. Sket
NB511	Suvaja; Lušci Palanka; Sanski Most; BIH	16,472892	44,698076	18.07.2004	P. Trontelj
NB512	Čavle špilja, Muškovci, Zrmanja, HRV	15,746365	44,212411	06.05.2010	P. Bregović, A. Čukušić
NB513	Pincinova jama; Tar; Poreč; HRV	13,658716	45,263453	29.05.2010	B. Jalžić
NB514	Jašek p. mostu, zaliv Jadriščica, Punta Križa, Cres, HRV	14,494554	44,624289	01.05.2007	T. Turk
NB515	Veliko okence; Retovje; Vrhnika; SLO	14,295828	45,949694	01.06.2005	M. Simonič
NB516	Mandelaja, Oštarije; Ogulin; HRV	15,310024	45,231269	10.09.2009	M. Lukić
NB517	Ponor Sušik, Drežnica; Ogulin; HRV	15,089984	45,145582	08.09.2009	J. Bedek
NB518	Sinjac izvor; Plavča draga; Plaški; HRV	15,427142	45,050062	10.09.2009	B. Jalžić
NB519	Markov ponor, Kosinj, Lipovo polje, HRV	15,176874	44,765318	15.08.2009	M. Lukić
NB520	Škatari bunar; Šaktari; Pula; HRV	13,892528	44,861469	24.01.2010	B. Jalžić
NB521	Škatari bunar; Šaktari; Pula; HRV	13,892528	44,861469	24.01.2010	B. Jalžić
NB522	Sveta Stomorija; Kaštel Stari; Split; HRV	16,324805	43,569863	28.12.2011	T. Delić
NB523	Markarova špilja, Stajnica, Jezerane; HRV	15,154641	45,019871	31.01.2008	M. Pavlek
NB524	Mandelaja, Oštarije; Ogulin; HRV	15,310024	45,231269	10.09.2009	M. Lukić
NB525	Sinjac izvor; Plavča draga; Plaški; HRV	15,427142	45,050062	10.09.2009	B. Jalžić
NB526	Suvaja; Lušci Palanka; Sanski Most; BIH	16,472892	44,698076	08.11.2006	P. Trontelj
NB527	Ponor Sušik, Drežnica; Ogulin; HRV	15,089984	45,145582	08.09.2009	J. Bedek
NB528	Markarova špilja, Stajnica, Jezerane; HRV	15,154641	45,019871	31.01.2008	M. Pavlek

NB529	Sveta Stomorija; Kaštel Stari; Split; HRV	16,324805	43,569863	28.12.2011	T. Delić
NB530	Suvaja; Lušci Palanka; Sanski Most; BIH	16,472892	44,698076	18.07.2004	P. Trontelj
NB531	Čavle špilja, Muškovci, Zrmanja, HRV	15,746365	44,212411	06.05.2010	P. Bregović, A. Čukušić
NB532	Sinjac izvor; Plavča draga; Plaški; HRV	15,427142	45,050062	10.09.2009	B. Jalžić
NB533	Mandelaja, Oštarije; Ogulin; HRV	15,310024	45,231269	10.09.2009	M. Lukić
NB600	Klarićeva jama; Vrsar; HRV	13,68651	45,169184		
NB602	Bilobrkova pećina; Bilobrci; Trilj; BIH	17,016441	43,583185	27.07.2013	T. Delić, T. Rađa
NB603	Bilobrkova pećina; Bilobrci; Trilj; BIH	17,016441	43,583185	28.07.2013	T. Delić, T. Rađa
NB604	Bilobrkova pećina; Bilobrci; Trilj; BIH	17,016441	43,583185	29.07.2013	T. Delić, T. Rađa
NB605	Kosinac; Han; Sinj; HRV	16,701257	43,7030368	22.07.2013	T. Delić, B. Radej
NB606	Kosinac; Han; Sinj; HRV	16,701257	43,7030368	22.07.2013	T. Delić, B. Radej

APPENDIX B

Methods for species delimitation written by Teo Delić

Unilocus species delimitation

Dataset containing 150 niphargid COI II sequences was used to build a Maximum likelihood tree in PhyML v3.0 (Guindon et al., 2010). Evolutionary substitution model was set to GTR+I+G, proportion of invariable sites was estimated from the dataset and the tree was searched under Nearest Neighbor Interchanges (NNI) and Subtree Pruning and Regrafting (SPR) topology search with substitution rate categories set to 6. R package ape (Paradis et al., 2004) was used to compute patristic distances and cluster to delimit putative species by a 0.16 molecular divergence threshold proposed by Lefébure et al., 2006. Lineages exceeding the 0.16 threshold are highly possible belonging to independently evolving species.

Same COI dataset was used to delimit putative species via Poisson Tree Process (PTP) and Generalized Mixed Yule Coalescence (GMYC). Differently from previously mentioned, distance based method (previous paragraph), PTP and GMYC are tree-based methods, implying that substitution patterns differ at allele coalescence and cladogenesis level and use these intra-interspecific differences to delimit evolutionary independent species.

PTP analysis was done on a phylogenetic tree inferred in MrBayes after two MCMC runs with four cold chains for 2 million generations, sampling every 100th generation. First 25 % of the resulting trees were discarded as a burn in, while the rest was used to build a majority-rule consensus tree. The resulting tree was submitted to a web server (http://species.h-its.org/ptp/) for species delimitation. Bayesian posterior probabilities for putative species were acquired after running 500 000 generations, sampling every 100 generation and discarding first 20 % of the samples as a burn-in.

Third alternative species delimitation procedure was applied to the same COI dataset using GMYC method (Pons et al., 2006). Ultrametric tree required for the analysis was inferred via BEAST 1.8.1. (Drummond et al., 2012). Again, the GTR+G+I evolutionary model was used. The tree prior was set to Yule process and the molecular clock was set to an uncorrelated lognormal relaxed clock prior. Root-node of the genus *Niphargus*, estimated to be 45 Mya (Jażdżewski & Kupryjanowicz, 2010), served as a single calibration point for inference of evolutionary diversification. The MCMC run for 100 million generations sampling every 5000 generations. Convergence and effective sample sizes were checked using Tracer 1.6 (Rambaut et al., 2014). A subset of the first 3000 resulting trees was discarded as a burn-in and the MCC tree was summarized from the remaining 17001 trees in TreeAnnotator 1.8.1 (Drummond et al., 2012). The resulting ultrametric tree was used for species delimitation using single threshold GMYC method implemented in R-package splits v1.0–19 (Ezard et al., 2009) in the statistical environment R (R Development Core Team, 2016).

Multilocus species delimitation

Evolutionary independence of the a priori determined species was estimated in Bayesian Phylogenetics & Phylogeography 3.1 (BPP) (Yang & Rannala, 2010). Based on the molecular data from multiple loci, guiding species tree and a priori determined species, coalescent-based Bayesian posterior probabilities for putative species are returned. Putative species were taken from morphological analyses and unilocus species delimitations (0.16 molecular divergence treshold, PTP, GMYC) and tested within BPP multilocus species delimitation framework.
The guiding trees were acquired by editing BEAST multilocus phylogenetic tree in R package ape (Paradis et al., 2004). Evolutionary independence of alternative putative species was estimated using a concatenated multilocus dataset and guiding tree in BPP. The reversal jump MCMC (rjMCMC) algorithm was run on clean dataset, with missing data and ambiguities excluded, for 100 000 generations. Every fifth generation was sampled and the first 20 000 generations were discarded as a burn-in. All five molecular fragments were used in the analysis, with species delimitation prior set to 1, treating a priori species tree as a guide tree, algorithm set to 0, and fine tune parameter automatically adjusted. Regarding the small population sizes and recent splits θ and τ values were set to 2 and 2000, respectively. Each run was repeated twice to confirm consistency of the resulting output. Species receiving more than 0.95 posterior probabilities are considered evolutionary independent.

APPENDIX C

Residuals table of morphometric analysis. The folowing characters were analyzed (in brackets are the abraviations used in the table): body length (l), length and height of coxa 3 (cx), horizontal (hr), vertical (vr), total length of antenna (a) 1 and 2 and peduncular articles (x) of antenna 1 and 2 (5 measures in total), whole gnathopod (g) 1 and 2 length and the lengths of distal parts of gnathopods (carpus 1 measurement and propodus 3 measurements), lengths of peropods (p) 5, 6 and 7, and the length of uropod (u) 3 and its basal and distant segment. The names of appendages in the table are as follows: first letter represents the type of the appendage, numbers represent the number of a appendage, number of a segment. R at the end represent the residual measurment.

species	lave	cx3hr	cx3vr	a11r	a12r	a13r	a1xr	a1lr	a22r	a23r	a2xr	a2lr	g1lr	g2lr	g15r	g161r	g162r	g163r	g25r	g261r	g262r	g263r	p5r	p6r	p7r	u31r	u3xr
1	20,573	-0,09485	-0,01547	-0,36018	-0,31946	-0,13586	0,313388	-0,11334	-0,24734	-0,22724	0,094921	-0,14869	-0,0576	-0,21966	-0,0038	-0,07609	-0,10426	0,107064	-0,12148	-0,15665	-0,18777	-0,12719	-0,33824			-0,40825	-0,14257
1	32,524	2,071615	1,968147	1,877078	1,72854	2,086346	1,493051	1,868317	1,910715	1,77402	1,744864	1,777671	1,934702	2,003208	1,9349	1,946507	1,873651	1,905738	2,026838	2,041761	2,041694	2,110967	1,46571	0,707107	0,707107	2,041241	1,568231
1	20,355	-0,20966	-0,18694	-0,60711	-0,34441	-0,06127	0,315936	-0,14083	-0,33268	-0,26947	0,205894	-0,15017	-0,27641	-0,23298	-0,22428	-0,29541	-0,22043	-0,35217	-0,34527	-0,35991	-0,2994	-0,31353		-0,70711	-0,70711	-0,40825	-0,99797
1	21,202	-0,29702	-0,21943	0,620166	0,608148	-0,46531	-0,4062	-0,55145	0,376946	0,436718	-0,952	-0,10283	0,203765	0,169578	0,163459	0,244184	0,467668	0,033714	0,212701	0,185684	0,219443	-0,11062	-0,78923			-0,40825	0,712832
1	20,327	-0,14726	-0,10933	-0,24905	-0,23555	-0,0737	-1,79334		-0,28776	-0,24132			-0,30208	-0,27036	-0,24329	-0,36851	-0,36043	-0,23736	-0,29753	-0,42409	-0,26167	-0,30317					
1	21,064	-0,08237	-0,01186	-0,0441	0,097848	-0,17938	0,345782	0,019987	-0,02278	0,098873	-0,09981	-0,04069	-0,07448	-0,11999	-0,18247	-0,06565	-0,23235	0,017768	-0,21995	-0,07998	-0,28368	-0,11062	-0,33824			-0,40825	-0,14257
1	13,047	-1,24047	-1,42512	-1,2368	-1,53511	-1,17082	-0,26862	-1,08269	-1,39711	-1,57158	-0,99388	-1,33529	-1,4279	-1,32981	-1,44452	-1,38503	-1,42386	-1,47475	-1,25532	-1,20682	-1,22861	-1,14584				-0,40825	-0,99797
2	29,89814	1,544523	1,582414	1,951578	0,888855	1,242555	1,094483	1,384332	1,548458	0,839684	1,511448	1,460098	1,524802	1,485201	1,081573	1,894102	1,551297	1,846877	1,185771	1,841317	1,688468	1,769939				0,545517	0,779138
2	17,77075	-0,148	-0,06351	0,179526	0,119145	0,907525	0,791512	0,732859	0,744857	0,808865	-0,39709	0,481038	0,618807	0,621239	0,692583	0,160941	0,542554	0,265684	0,601403	0,332013	0,41422	0,33584					
2	16,412	1,275058	1,061309	-0,16287	-0,37942	0,33135			0,069988	0,164868	0,235433	0,092665	-0,09898	-0,21022	0,266634	-0,25701	-0,08617	-0,29117	0,167248	-0,41325	-0,13734	-0,30392					
2	16,45956	-0,33198	-0,06713	0,096669	-0,3104	0,284464	0,36765	0,262416	0,221776	0,364739	-0,13109	0,133288	-0,02347	0,108746	0,270061	-0,33938	-0,02322	-0,23775	0,194113	-0,2255	-0,09792	-0,04368				0,608609	0,93298
2	10,31568	-0,90385	-1,23239	-1,34759	1,675135	-0,98649	-1,64991	-1,28797	-1,40826																	-1,15413	-0,68751
2	13,72434	-0,65106	-0,53504	-0,20057	-0,71144	-0,22433	-0,04109	-0,21192	-0,18983	-0,34468	-1,2187	-0,72145	-0,66478	-0,58879	-0,63751	-0,46343	-0,59091	-0,54364	-0,41909	-0,54345	-0,61324	-0,63968					
3	11,612	-1,31514	-1,47167	-1,31773	-1,30349	-1,28371	-1,09254	-1,23462	-1,33352	-1,30803	-1,17469	-1,33121	-1,39804	-1,59729	-1,3052	-1,2822	-1,27207	-1,3126	-1,31774	-1,436	-1,47705	-1,61289					
3	17,743	-0,77125	-0,48067	-0,42102	-0,61352	-0,55395	-0,89163	-0,78844	-0,57772	-0,62133	-0,31067	-0,53039	-0,66821	-0,93089	-0,72691	-0,51846	-0,65193	-0,58908	-0,72739	-0,88297	-0,97408	-0,88612					
3	9,679	-1,18284	-1,1678	-1,66003	-1,7252	-1,70564	-1,45871	-1,63054	-1,75451	-1,78261	-1,54167	-1,77303	-1,35261		-1,24717	-1,2687	-1,16283	-1,35716	-1,18439								
3	23,736	-0,10056	-0,42858	0,019281	0,242001	1,034147	-0,27773	-0,0258	0,525366	1,124781	-0,61327	0,382734	0,543938	0,553961	0,617762	0,063996	0,516102	0,187487	0,970257	0,065664	0,422346	0,525292					
3	26,344	0,63811	0,356529	0,50925	0,243209	0,373195	0,331039	0,363931	0,299252	0,132481	0,357615	0,272605	-0,09176	-0,2475	0,121515	-0,33331	-0,64016	-0,39812	0,015801	-0,45508	-0,55603	-0,5265		-0,86549	-0,83783	-0,10559	-0,09549
3	19,999	-0,29534	-0,07882	-0,20221	-0,08063	-0,03243	-0,1886	-0,16615	-0,15548	-0,09043	-0,0686	-0,10981	-0,19898	-0,30573	-0,40275	-0,30052	-0,21161	-0,07349	-0,22993	-0,34877	-0,19093	-0,03295				-0,58588	-0,46244
3	39,358	1,696505	1,803955	1,634163	1,159143	-0,11754	2,139821	1,777671	1,418578	0,386548	1,458554	1,123292	1,641959	1,322265	1,964433	1,805569	1,653879	1,490253	1,511165	1,035827	0,907673	1,076014	0,707107	1,441529		1,50201	1,476077
3	16,92	-0,78227	-0,63943	-0,71366	-0,70898	-0,63725	-0,24389	-0,44028	-0,7039	-0,56501	-0,81801	-0,7242	-0,70129	-0,96701	-0,7189	-0,59946	-0,50067	-0,71639	-1,10947	-0,80223	-0,75127	-0,90418				-1,13365	-1,22613
3	28,228	0,298175	0,254825	0,352191	0,712051	0,681036	0,657629	0,658163	0,709005	0,750871	0,736183	0,765153	0,494507	0,483331	0,39165	0,590518	0,726179	0,777339	0,597165	0,704814	0,871274	0,726923	-0,70711	-0,34723	-0,21691		
3	29,474	0,384537	0,435908	0,668993	0,63955	0,606792	0,331341	0,475283	0,492885	0,508788	0,568789	0,546628	0,367295	0,221029	0,283596	0,370652	0,003514	0,463317	0,301988	0,232516	-0,03982	0,036263			-0,39316	0,323107	0,307972
3	34,648	1,430069	1,415742	1,130771	1,435856	1,635342	0,693279	1,010776	1,080032	1,463937	1,40576	1,378238	1,363183	1,467838	1,021964	1,471912	1,539597	1,528445	1,172536	1,886233	1,787882	1,598147		-0,22881	1,447901		
4	15,89	1,714011	1,703331	1,735941	1,743197	1,765078	1,488172	1,485206	1,758923	1,714011	1,706256	1,732326	1,717411	1,719135	1,729204	1,786047	1,726903	1,754781	1,748065	1,762651	1,737621	1,756437	-0,44721	-0,44721	1,488172		
4	8,96	-0,19414	-0,33871	-0,61152	-0,62682	-0,56719	-0,67218	-0,66939	-0,63226	-0,63051	-0,75827	-0,68148	-0,31853	-0,40039	-0,41345	-0,50195	-0,31561	-0,42995	-0,68259	-0,23786	-0,36495	-0,45405	-0,44721	-0,44721	-0,67218		
4	8,349	-0,78836	-0,73329	-0,64463	-0,55253	-0,52339	-0,39072	-0,48947	-0,48808	-0,76187	-0,59397	-0,61017	-0,78313	-0,78367	-0,60195	-0,48179	-0,55839	-0,58493	-0,52221	-0,5017	-0,52616	-0,57353	-0,44721	-0,44721	-0,39072		
4	8,149	-0,62596	-0,66705	-0,44226	-0,51008	-0,51244			-0,49863	-0,28819	-0,39866	-0,40094	-0,57822	-0,5195	-0,68992	-0,44651	-0,77478	-0,61492	-0,44203	-0,68679	-0,7491	-0,6034	-0,44721	-0,44721			
4	9,664	-0,10556	0,035714	-0,03753	-0,05377	-0,16205	-0,42528	-0,32635	-0,13995	-0,03344	0,04464	-0,03974	-0,03753	-0,01558	-0,02388	-0,3558	-0,07811	-0,12498	-0,10124	-0,33631	-0,09741	-0,12546	1,788854	1,788854	-0,42528		

continued

continuation of Appendix C table. Residuals table of morphometric analysis .

6	31,837	1,849873	1,91091	1,561693	1,294029	1,166405	0,958309	1,198011	0,935703	1,107925	1,028735	1,170129	1,469014	1,544517	1,315118	1,543659	1,314613	1,609587	1,181636	1,66042	1,558751	1,647341		1,380097	0,762503	1,304142	0,753151
6	24,82	0,630766	0,955656	0,66693	0,52632	0,497928	0,560397	0,588767	0,283594	0,498976	0,240991	0,397154	0,553298	0,22305	0,282606	0,437306	0,43905	0,763218	0,167026	0,281457	0,296839	0,660148					
6	17,19	-0,41896	-0,52434	-0,46188	-0,4813	-0,41853	-0,106	-0,29423	-0,50713	-0,37286	-0,77897	-0,61657	-0,53219	-0,47009	-0,65787	-0,51185	-0,41783	-0,53618	-0,41801	-0,51845	-0,44884	-0,57561				-0,52384	-0,55081
6	21,319	0,084434	0,155607	-0,40389	0,039647	-0,02823	0,042684	-0,04505	-0,14169	0,043007	0,4089	0,102018	0,187402	0,229496	0,112917	0,149594	0,011776	-0,16725	0,122024	-0,01735	0,254574	0,051966				-0,2476	-0,06472
6	21,216	0,387422	0,521449	-0,00829	0,277843	0,021368	-0,30549	-0,13861	-0,02198	0,118755	-0,10733	0,00364	0,221553	0,348581	0,141678	0,140696	0,291956	0,353597	0,122024	0,281457	0,259103	0,312867	0,178547			-0,17939	-0,16075
6	25,253	0,587823	0,959351	0,658645	0,67712	0,689845	0,637413	0,688628	0,534042	0,659382	0,391037	0,612243	0,78552	0,63832	0,58747	0,956372	0,705221	0,692687	0,610918	0,548713	0,823642	0,593159	1,548281			0,362868	0,441895
6	26,227	0,692795	-0,09753	0,805701	0,404652	-0,34953	2,33442	1,508933	0,430083	0,319262	2,582788	1,181739	0,520124	0,380472	0,458047	0,796203	0,200897	0,041634	0,492274	0,416941	-0,06694	0,069594		0,64945	0,27418	0,785759	0,420703
6	22,608	0,959996	0,447542	0,32518	0,26242	0,206816	0,28924	0,286666	2,540774	-0,68328	0,178471	0,765616	0,218138	-0,0063	0,245217	0,348323	0,182218	0,239663	0,222257	0,209076	0,138346	0,076645	0,457455				
6	17,89	-0,2782	-0,29892	-0,33761	-0,46074	-0,43579	-0,3456	-0,41519	-0,47247	-0,22879	0,089158	-0,24689	-0,4195	-0,42123	-0,32712	-0,44659	-0,58828	-0,20251	-0,34846	-0,44793	-0,58167	-0,37993	-0,96791			-0,5443	-0,44618
6	23,156	0,618837	0,733933	0,741494	0,690829	0,450487	0,695174	0,703118	0,167034	0,351938	-0,61106	-2,7E-05	0,407428	0,643069	0,483931	0,698322	0,64685	0,717102	0,643648	0,62295	0,662129	0,612551		0,379901	-0,13758	0,448128	0,076335
6	30,923	1,659015	1,79081	1,683895	1,631616	1,373417	1,350872	1,540434	1,047539	1,562409	2,221962	1,809284	1,35827	1,255796	1,079279	1,31527	1,471046	1,330176	0,486138	1,634437	1,288557	1,335318					
6	15,741	-0,6623	-0,75161						-0,50083	-0,19612	-0,38063	-0,40576	-0,5639	-0,55965	-0,45079	-0,7521	-0,70969	-0,84814	-0,38733	-0,66693	-0,55601	-0,55798					
6		-1,22772	-1,28005	-1,19509	-0,87886	-1,03957	-1,11361	-1,16273	-0,87886	-0,92389	-0,84685	-1,01009	-1,06299	-1,08722	-1,07203	-1,14956	-0,91282	-1,07058	-1,45921	-1,00285	-0,9696	-0,9934					
6	9,96	-1,4973	-1,60339	-1,37736	-1,4615	-0,37972	-2,08272	-1,77229	-1,25847	-1,62937	-1,37022	-1,62663	-1,26447	-1,22768	-1,22734	-1,30379	-1,30507	-1,38526	-1,001	-1,25526	-1,21715	-1,19613				-0,95696	-1,00247
6	18,433	-0,23764	-0,19545	-0,34589	-0,55156	-0,63417	-0,56328	-0,59034	-0,43152	-0,38326	0,053433	-0,30677	-0,54341	-0,55253	-0,43641	-0,79362	-0,85912	-0,79388	-0,40778	-0,71889	-0,6979	-0,55798	-1,19344				
6	17,35	-0,27104	-0,2398	-0,41424	-0,66466	-0,44657			-0,61739	-0,35652	-0,23058	-0,46442	-0,5239	-0,47144	-0,44217	-0,48515	-0,23105	-0,11299	-0,4221	-0,43679	-0,54242	-0,43634	-0,02293	-0,7965			
6	13,907	-1,22295	-1,01398	-0,90926	-0,72635	-0,72474	-0,71356	-0,80991	-0,7812	-0,47534	-0,93795	-0,81944	-0,67806	-0,62445	-0,65212	-0,79066	-0,56259	-0,76133	-0,54279	-0,66507	-0,46544	-0,64788		-1,35146		-0,84442	-0,62101
6	10,728	-1,33746	-1,39091	-1,24066	-1,13077	-0,86059	-0,94407	-1,09028	-1,13403	-0,98627	-1,19874	-1,25573	-1,2796	-1,14863	-1,15831	-1,38091	-1,11828	-1,29302	-1,06851	-1,10122	-1,09036	-1,13619					
6	24,048	0,509094	0,793059	0,816057	0,802216	0,469895	1,011257	0,923631	0,500964	0,592547	1,071606	0,804112	0,604524	0,484968	0,570214	1,095779	0,562796	0,97481	0,453408	0,769569	0,767792	0,767681		-0,26149	0,005347	0,328764	0,291565
6	12,532	-1,12513	-0,82921	-1,21166	-1,12905	-1,09564	-0,97884	-1,14005	-0,57958	-0,90903	-0,79683	-0,87138	-0,88736	-0,92573	-0,84194	-0,96862	-0,99454	-0,88883	-0,84758	-0,98615	-1,12659	-0,90702			-1,29847	-0,82737	-0,87465
6	30,207	1,675715	1,332584	1,986291	2,459302	3,219278	0,500497	1,507042	2,38641	3,127853	0,316014	2,320731	2,853564	3,044103	3,273726	2,279251	2,993357	2,518348	3,511557	2,690467	2,912739	2,851365			1,531326	2,6001	3,063068
6	15,903	-0,64321	-0,57053	-0,45567	-0,6955	-0,80453	-0,52851	-0,6376	-0,66937	-0,58971	-0,50031	-0,67279	-0,60245	-0,57933	-0,55146	-0,48218	-0,44352	-0,41139	-0,53256	-0,67621	-0,47752	-0,74308			-1,13731	-0,69777	-0,5634
6	14,098	-0,73387	-0,80519	-0,88441	-0,88572	-0,87784	-0,69859	-0,84897	-0,83161	-0,64763	-0,82363	-0,87016	-0,82101	-0,71809	-0,73265	-0,69574	-0,677	-0,76947	-0,57757	-0,62239	-0,72205	-0,84709				-1,00812	-0,76273
7	29,41	1,144098	1,154629	1,146829	1,095319	0,953515	1,080451	1,08975	1,061931	1,045185	1,137943	1,106495	0,992702	0,676084	0,682841	1,230236	0,87657	-0,44062	0,70439	1,149222	1,05726	0,761667			0,707107		
7													-1,36254	-1,47479	-1,46472	-1,05752	-1,42702	1,498214	-1,47971	-1,19559	-1,32167	-1,40469					
7	20,51445	-0,70725	-0,58842	-0,68998	-0,86422	-1,04077	-0,89303	-0,87553	-0,92368	-0,94767	-0,73872	-0,83917	-0,02679	0,242902	0,187661	-0,50664	0,12734	-0,56006	0,304839	-0,3295	-0,09189	-0,02488			-0,70711	-0,70711	-0,70711
7	22,98314	-0,43685	-0,56621	-0,45685	-0,2311	0,087253	-0,18742	-0,21422	-0,13825	-0,09752	-0,39923	-0,26733	0,396623	0,5558	0,594221	0,333917	0,423111	-0,49754	0,470485	0,375872	0,356296	0,667904				0,707107	0,707107
8	12,928	-1,2985	-0,11422	-1,62292	-1,44257	-0,71373	-1,11086	-0,1733	-1,47941	-1,56515	-0,23349	0,922321	-0,91961	-1,04979	-1,14268	-1,56733	-1,01708	-1,218	-1,16744	-1,24865	-1,14063	-1,28015					
8	17,936			1,08942	1,100858	1,675573	1,283083	1,021564	1,34117	1,121482	-1,63115	-0,19454	1,641541	1,621242	1,495561	1,068003	1,593816	1,506307	1,597546	1,466057	1,613528	1,442842					
8	15,183	0,920496	0,584161	-0,04633	-0,14173	-0,00153		-1,61999	-0,0078	-0,20335	0,374902	0,963963	-0,66705	-0,49643	0,394895	-0,26353	-0,33513	-0,46866	-0,02353	-0,29253	-0,27542	-0,41795				-1,15404	-1,13403
8	14,31	-0,2555	0,890927	0,222666	0,775649	-0,81328	-0,31508	0,33986	0,042364	0,468311	0,613326	-0,23298	0,05379	0,06314	-0,26887	0,527067	0,255894	0,16638	-0,17387	-0,28115	-0,107	0,317601				0,543075	0,755377
8	14,708	0,633497	-1,36087	0,357162	-0,2922	-0,14703	0,142858	0,431865	0,103681	0,178698	0,876415	-1,45876	-0,10867	-0,13816	-0,47892	0,235793	-0,4975	0,013971	-0,2327	0,35627	-0,09048	-0,06235				0,61096	0,378654