Ekologija i biogeografija odabranih endemskih epigejskih vrsta trčaka (Coleoptera: Carabidae) dinarskog krša

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PRIRODOSLOVNO-MATEMATIČKI FAKULTET
BIOLOŠKI ODSJEK

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FACULTY OF SCIENCE
DIVISION OF BIOLOGY

Željka Jambrošić Vladić

ECOLOGY AND BIOGEOGRAPHY OF SEVERAL ENDEMIC EPIGEIC GROUND BEETLES (COLEOPTERA: CARABIDAE) FROM DINARIC KARST

DOCTORAL THESIS

Zagreb, 2020.

Ovaj je doktorski rad izrađen na Zoologijskom zavodu Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu, pod vodstvom dr. sc. Lucije Šerić Jelaska, u sklopu Sveučilišnog poslijediplomskog doktorskog studija Biologije pri Biološkom odsjeku Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu.

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Ekologija i biogeografija odabranih endemskih epigejskih vrsta trčaka (Coleoptera: Carabidae) dinarskog krša

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Ciljevi ove disertacije bili su doprinijeti boljem poznavanju faune trčaka naše zemlje s posebnim naglaskom na biologiju i ekologiju endemskih vrsta, kombinacijom morfoloških i molekularnih metoda doprinijeti razrješavanju taksonomskih odnosa između odabranih endemskih vrsta roda *Carabus* te otkriti eventualne promjene u sastavu zajednica trčaka na području Nacionalnoga parka Risnjak nakon 25 godina. U ovom istraživanju po prvi puta smo koristili mitohondrijske sekvence dviju endemskih vrsta *Carabus (Platycarabus) creutzeri*, *Carabus (Eucarabus) parreyssi* te vrste *Carabus (Eucarabus) ulrichii* u rekonstrukciji filogenetskog stablo roda *Carabus*. Kombinacijom linijske morfometrije, geometrijske morfometrije i molekularnih analiza pokušali smo utvrditi filogenetske odnose unutar dviju sestrinskih endemskih vrsta roda *Carabus* – *C. croaticus* i *C. caelatus* te utvrditi stvaran broj podvrsta i fenotipskih varijanti. Predložen je značajno manji broj podvrsta, a metode geometrijske morfometrije i u ovom su se primjeru pokazale korisnima za intraspecijsko razdvajanje vrsta nadilazeći probleme linijske morfometrije. Analiza sastava zajednica trčaka u razmaku od 25 godina ukazala je na smanjenje broja planinskih specijalista i širenje generalista unutar Nacionalnoga parka Risnjak. Izostanak endemskih vrsta *Molops alpestris, Pterostichus unctulatus* i *Trechus croaticus* u ulovu naglašava važnost daljnjeg kontinuiranog praćenja stanja zajednica kornjaša s ciljem poduzimanja ispravnih i pravovremenih konzervacijskih mjera.

(108 stranica, 17 slika, 129 literaturnih navoda, jezik izvornika hrvatski)

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Ecology and biogeography of several endemic epigeic ground beetles (Coleoptera: Carabidae) from Dinaric karst

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Aims of this dissertation were to contribute to a better knowledge of the entomofauna of our country with special emphasis on the biology and ecology of endemic species, a combination of morphological and molecular methods to contribute to the resolution of taxonomic relationships between selected endemic species of the genus *Carabus* and to recognise possible changes in the composition of the carabids' assemblages in the Risnjak National Park after period of 25 years. For the first time, in this study, we used the mitochondrial sequences of two endemic species *Carabus (Platycarabus) creutzeri*, *Carabus (Eucarabus) parreyssi* and another *Eucarabus* species *Carabus ulrichii* in the reconstruction of the *Carabus* phylogenetic tree. Combining traditional morphometry, geometric morphometry and molecular analyses, we attempted to determine the phylogenetic relationships within two sister endemic species of the genus *Carabus* - *C. croaticus* and *C. caelatus* and to determine the true number of subspecies and phenotypic variants. A significantly smaller number of subspecies have been suggested, and geometric morphometric methods have also proved to be useful for intraspecific delimitation, overcoming traditional linear morphometry problems. An analysis of the carabids' assemblages after 25-years indicated a decrease in the number of mountain specialists and the spread of generalists within the Risnjak National Park. The absence of endemic species *Molops alpestris*, *Pterostichus unctulatus* and *Trechus croaticus* in the catch highlight the importance of further continuous monitoring of carabids' assemblages to take accurate and well-timed conservation measures.

(108 pages, 17 figures, 129 references, original in Croatian)

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Keywords: Carabidae, taxonomy, endemic species, geometric morphometric, molecular philogeny, carabids' assemblages.

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1. UVOD

Trčci (Carabidae) se smatraju jednom od evolucijski najuspješnijih skupina organizama. Njihova brojnost i raznolikost, široki areal rasprostranjenosti, osjetljivost na promjene u okolišu te poznate dobrobiti za poljoprivrednu proizvodnju čine ih pogodnim organizmima za proučavanje i rješavanje mnogih ekoloških pitanja (Kotze i sur. 2011).

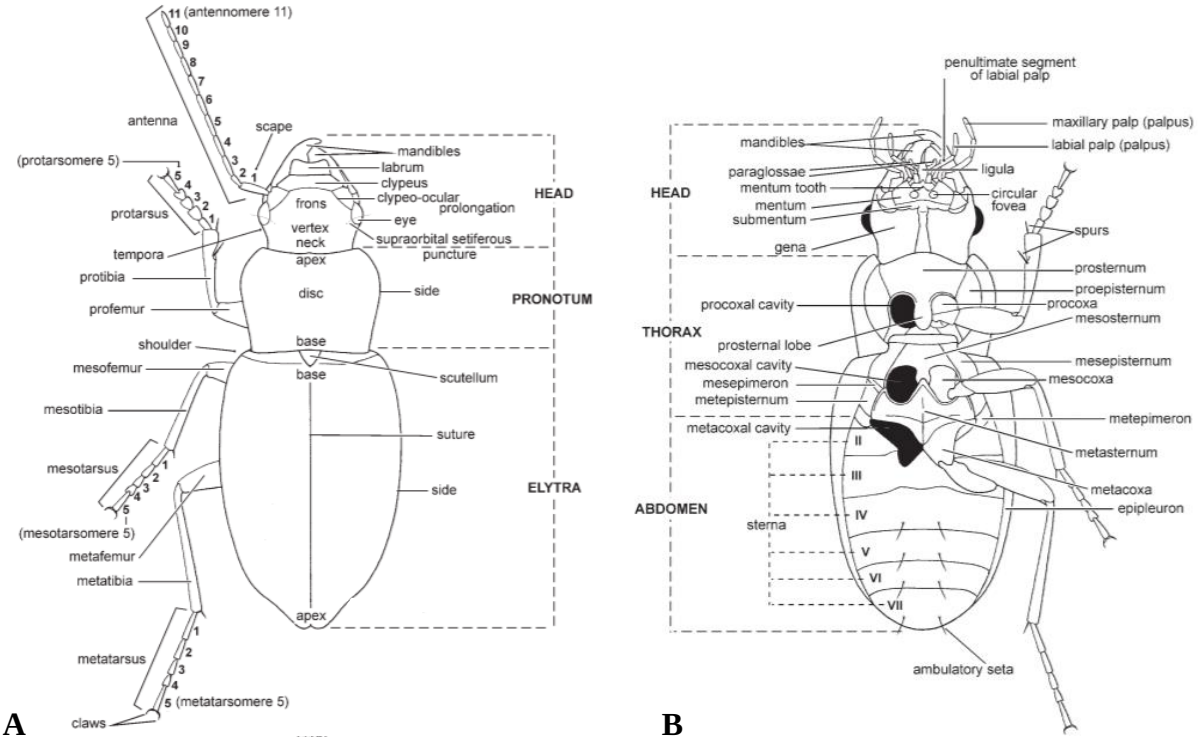
1.1. Biologija, ekologija i biogeografija trčaka

Trčci (Carabidae) su porodica unutar razreda kukaca (Insecta), reda kornjaši (Coleoptera) i podreda Adephaga. S više od 40 000 opisanih vrsta klasificiranih u 86 tribusa (Lorenz 2005; 2018) jedna je od najbrojnijih porodica unutar reda kornjaša koji pak predstavlja jednu od vrstama najbogatijih skupina na Zemlji (Lövei i Sunderland 1996). Nastanjuju većinu kopnenih staništa od tla, sloja listinca i prizemne vegetacije do sloja stabala i krošnji (Arndt 2005). Pojavili su se u ranom tercijaru u tropima kao predatori generalisti vlažnih staništa i odavde su se proširili prema većim geografskim širinama i visinama. Fosilni zapisi pokazuju da je na prijelazu perma u trijas nekoliko linija razvilo kozmopolitski obrazac rasprostranjenja (Erwin i Adis 1982). Thiele (1977) navodi temperaturu, vlažnost, svjetlost i karakteristike supstrata kao najvažnije abiotičke čimbenike. Vlažnost se izdvaja kao tzv. ograničavajući faktor za trčke (Lövei i Sunderland 1996) zbog čega su trčci danas rasprostranjeni na svim glavnim staništima izuzev pustinja gdje su ograničeni na oaze i potoke (Erwin 1985).

Radi se o morfološki homogenoj skupini, monofiletičkog porijekla (Thiele 1977). Glavne morfološke značajke uključuju glavu prognatnoga položaja s nitastim ticalima građenim od 11 članaka; prsa građena od tri kolutića od kojih je prvi prekriven vratnim štitom (*pronotum*); čvrsta prednja krila - pokrilja (*elitrae*) različite rebraste strukture; drugi par krila koji može biti dobro razvijen – makropterni oblici, reduciran – brahipterni oblici ili potpuno nedostajati – apterni oblici; tri para nogu prilagođenih hodanju i trčanju te stopalo uvijek građeno od pet članaka (Slika 1.). Uglavnom su tamne boje, poneki metalnoga sjaja, a veličinom variraju od 1mm do 8 cm.

U Europi i ostalim umjerenim područjima trčci su univoltni organizmi (jedna generacija u godini), (Thiele 1971). Životni vijek odrasle jedinke može trajati i duže od jedne sezone. Hibernirati mogu kao odrasle jedinke i kao ličinke, a u rijetkim slučajevima u stadiju jajašaca. Uglavnom prezimljuju pod korom drveta, u trulim panjevima, u tlu i pod mahovinom (Turin i sur. 2003). Larsson (1939) razlikuje jesenske vrste (razmnožavaju se u

jesen i prezimljuju kao ličinke); proljetne vrste s jesenskom aktivnošću (hiberniraju kao odrasli, razmnožavaju se u proljeće, ali ne i u jesen) i proljetne vrste bez jesenske aktivnosti (hiberniraju kao odrasli, razmnožavaju se u proljeće, ali nova generacija nije aktivna do sljedeće godine).



Slika 1. A: Dorzalna shema građe trčaka (antenna-ticalo; antennomere-članci ticala; apex-vršni dio pronotuma odnosno krajnji dio elitre; base-donji dio pronotuma; claws-kandžice; clypeo-ocular prolongation-čeono-štitni očni šav; clypeus-čeoni štitić; elytra-pokrilje, elitra; eye-oko; femur-bedro (pro-, meso-, meta-), frons-čelo; head-glava; labrum-usna; mandibles-donja čeljust; neck-vrat; pronotum-istaknuta pločasta struktura koja prekriva prvi prsni kolutić; scape-prvi članak ticala; scutellum-skutelum ili trokutasti štitić; shoulder-vanjski anteriorni kut elitre, supraorbital setiferous puncture-udubina supraorbitalne sete; tarsomere-članci stopala (pro-, meso-, meta-); tempora-posteriorni dio obraza; tibia-goljenica ili gnjat (pro-, meso-, meta-); vertex-tjeme); (Slika preuzeta iz: Lariviere i Larochelle 2013).

B: Ventralna shema građe trčaka (abdomen-zadak; circular fovea-kružni utor; coxa-kuk (pro-, meso-, meta-); coxal cavity-šupljina kuka (pro-, meso-, meta-); epipleuron-bočni sklerit; episternum-bočne pločice ili sklerite između šupljina kuka (pro-, meso-, meta-); gena-obraz; head-glava; labial palp-usni palpi; ligula-jezičac; mandibles-donja čeljust; maxillary palp -maksilarni palpi; mentum tooth-zubić brade; mesepimeron-pločica između humeralnog i prvog bočnog šava; metepimeron-pločica između drugog bočnog šava i sternuma; paraglossae-pajezik; penultimate segment of labial palp-pretposljednji dio usnog palpa; prosternal lobe-režanj između prednjih nogu; spurs-bodljice; sterna-sterniti ili zadčani kolutići; thorax-prsa); (Slika preuzeta iz: Lariviere i Larochelle 2013).

Lindroth, 1949. godine, predlaže podjelu na vrste koje prezimljuju u ličinačkom stadiju (*larval hibernators*) i vrste koje prezimljuju kao odrasle jedinke (*adult hibernators*), a den Boer i den Boer-Daanje (1990) predlažu jednostavnu podjelu na zimske i ljetne ličinke.

Period razmnožavanja ovisi o zemljopisnoj širini i nadmorskoj visini zbog čega se gotovo sve vrste u sjevernijim područjima, s kratkim vegetacijskim periodom, razmnožavaju ljeti kako bi dostigle potreban stupanj razvoja prije prezimljavanja.

Razvoj trčaka je holometabolan (potpuna preobrazba). Ličinka je kampodeiformna (dobro razvijene noge i ticala te spljošteno tijelo), a obično prolazi tri stadija prije kukuljena (Crowson 1981). Zbog slabe hitiniziranosti i pokretljivosti osjetljiva je na promjene vanjskih uvjeta, a prema Lövei i Sunderland (1996) predstavlja najosjetljiviji stadiji u životnom ciklusu trčaka. Kompletan razvoj trčaka od jajašca do odrasle jedinke traje manje od godinu dana premda u uvjetima nedostatka hrane ili nepovoljnih klimatskih čimbenika može trajati i nekoliko godina.

Većina trčaka su noćne životinje. Dnevni i godišnji ritam (entomofenologija) vrsta ove porodice ovisi o nizu čimbenika kao što su: promjene u temperaturi (Jones 1979), vlaga, intenzitet svjetla (Thiele 1977), period aktivnosti plijena (Alderweireld i Dessender 1990) i dr. Razdobljima mirovanja, bilo zbog visokih temperatura (npr. *Rhytidognathus sp*.), (Castro i sur. 2014) ili niskih temperatura (Desender 1982), omogućava se preživljavanje, usklađuje se životni vijek sa zalihama hrane, izbjegava kompeticija i dr. (Danks 1987, Delinger 2002, Kotze i sur. 2011). Po načinu ishrane, trčci se ubrajaju u polifage, dok je svega nekoliko vrsta fitofagno (Thiele 1977, Luff 1987). Najčešći plijen su im gujavice, puževi, skokuni i ličinke drugih kukaca (Turin i sur. 2003), a dnevno konzumiraju količinu hrane približno jednaku vlastitoj tjelesnoj masi (Thiele 1977). Veliko bogatstvo vrsta te raznolikost u obliku tijela i staništima koja nastanjuju imali su za posljedicu razvitak čitavog niza trofičkih specijalizacija (Hengeveld 1980, Zetto Brandmayr i sur. 1998b).

1.2. Sistematika trčaka

Početkom karabidologije, u užem smislu, smatra se 1810. godina kada je Latreille utemeljio porodicu Carabidae. Povijest taksonomije i sistematike ove porodice bazirala se isključivo na analizama vanjskih obilježja ličinki i odraslih jedinki pri čemu je ključnu ulogu igrala subjektivna moć zapažanja i razlikovanja. Takav način opisivanja i sistematizacije doveo je do postojanja brojnih oprečnih mišljenja i klasifikacija. Početkom 20. stoljeća morfološke se analize proširuju i na genitalne privjeske, prvenstveno na *edeagus* (Holdhaus 1912), čime je razvijen cijeli novi sustav klasifikacije, a omogućeno je i lakše razlikovanje kriptičnih vrsta.

Prema Bousquet i sur. (2017) porodica Carabidae Latreille, 1802 unutar reda Coleoptera Linné, 1758 i podreda Adephaga Schellenberg, 1806 sadrži sljedeće podporodice: Nebriinae Laporte, 1834; Cicindinae Csiki, 1927; Carabinae Latreille, 1802; Cicindelinae Latreille, 1802; Loricerinae Bonelli, 1810; Elaphrinae Latreille, 1802; Omophroninae Bonelli, 1810; Scaritinae Bonelli, 1810; Broscinae Hope, 1838; Apotominae LeConte, 1853; Melaeninae Csiki, 1933, Gehringiinae Darlington, 1933; Trechinae Bonelli, 1810; Patrobinae Kirby, 1837; Psydrinae LeConte, 1853; Paussinae Latreille, 1806; Brachininae Bonelli, 1810 i Harpalinae Bonelli, 1810.

Carabinae predstavlja veliku podporodicu unutar porodice trčaka (Carabidae) i taksonomski je klasificirana u 2 tribusa: Carabini Latreille, 1802 i Cychrini Perty, 1830. Tribus Carabini dalje se dijeli na dva podtribusa Carabina Latreille, 1802 i Calosomatina Jeannel, 1940 (Imura i sur. 2018). Unutar podtribusa Carabina nalazi se rod *Carabus* Linné, 1758 i vrste roda *Calosoma* Weber, 1802 (Osawa i sur. 2003).

Podtribus Carabina ima holarktičko rasprostranjenje dok je podtribus Calosomina kozmopolitski. Smatra se da je diferencijacija tribusa Carabini započela prije 52 milijuna godina (Osawa i sur. 2003).

1.2.1. Sistematika roda *Carabus*

Autor prve značajnije klasifikacije roda *Carabus* je Reitter koji je 1896. godine podijelio rod *Carabus* u grupe prema slijedećim morfološkim karakteristikama: prisutnost ili odsutnost dlake na prvom prsnom kolutiću (*pronotae setae*), veličina gornje usne, dvije ili više dlaka (*seatae*) na donjeusnenom pipalu (*labial palpae*), prisutnost dlačnih pora na zatku, značajke pokrilja (*elitrae*) i drugo (Deuve 2004).

Početkom 20. stoljeća dvojica karabidologa, Bengtsson i Lapouge, neovisno utvrđuju 3 grupe ličinaka roda *Carabus* prema obliku prednjeg ruba čeone pločice (*clipeo frons*); (Bengtsson: Archeocarabi («nemoralis tip») – nos ima 5 zubića, Metacarabi («hortensis tip») – nos ima 4 zubića, Neocarabi («violaceus tip») – nos ima pojedinačan jednostruki ili dvostruki zub; Lapouge: Serrilabres (=Archeocarabi Bengtsson), Quadricuspides (=Metacarabi Bengtsson) i Rostrilabres (=Neocarabi Bengtsson)).

Nakon što je Nakana (1962) opisao dijelove endofalusa (*endophallus*) za japanske vrste, Meurques i Ledoux (1966) te Sturani (1967) su ukazali na tehniku izvrtanja ovog organa prema van te na njegovu varijabilnost unutar roda *Carabus*.

Tablica 1. Klasifikacija do roda *Carabus* prema Bousquet i sur. (2017):

CARSTVO:	Animalia
PODCARSTVO:	Eumetazoa
KOLJENO:	Arthropoda
POTKOLJENO:	Hexapoda
RAZRED:	Insecta
RED:	Coleoptera Linné, 1758
PODRED:	Adephaga Schellenberg, 1806
PORODICA:	Carabidae Latreille, 1802
POTPORODICA:	Carabinae Latreille, 1802
TRIBUS:	Carabini Latreille, 1802
PODTRIBUS	Carabina Latreille, 1802
ROD:	*Carabus* Linné, 1758

Zbog toga su, navedeni autori, predložili da se pri izradi sistematike roda *Carabus* koriste karakteristike endofalusa (Deuve 2004). Prema morfološkim karakteristikama endofalusa Deuve razlikuje 5 osnovnih grupa: Spinulati (endofalus sa spinulom, pojedinačan, zubičast, asimetrična pločica, možda homologna s jezičcem ostalih vrsta roda *Carabus*, nema režnjića kod otvora (*ostium lobe*) na distalnom membranskom području penisa); Digitulati (endofalus sa prstićem (*digitulus*) udaljenim od jezičca (*ligulum*), središnji režanj aedeagusa (*penis*) obično manji i pri bazi manje svinut); Lipastrimorphi (veliki endofalus s ravnom hitiniziranom zonom («lipastradian plate») na mjestu gdje se unutar grupe Digitulati nalazi prstić (*digitulus*); nema režnja kod otvora (*ostial lobe*); Archicarabomorphi (endofalus sa dojezičnim naborima, prekriven pločama blizu jezičca (*ligula*), nema prstića (*digitalus*), ni režnja kod otvora (*ostial lobe*); Lobifera (blizu baze endofalusa jednostruki ili dvostruki režnjić kod otvora (*ostium lobe*), nema prstića (*digitulus*), ali često postoji vrećica (*sacculus*) na stražnjoj površini endofalusa, jezičac (*ligulum*) je ili rudimentaran ili vrlo dobro razvijen).

Navedene grupe (Spinulati, Digitulati, Lobifera, Archicarabomorphi i Lipastrimorphi) uglavnom zadovoljavaju opisivanje 5 glavnih tipova endofalusa unutar roda *Carabus* međutim primjena novih metoda dovodi do novih spoznaja i promjena u taksonomskim odnosima unutar ovog roda.

1.3. Odabrane endemske epigejske vrste trčaka dinarskog krša

Središnje mjesto u ovoj doktorskoj disertaciji pripalo je rodu *Carabus* odnosno vrstama: *C. caelatus, C. croaticus, C. catenulatus, C. parreyssi, C. creutzeri, C. intricatus* i *C. ulrichii*. Ovaj, drugi najbrojniji rod unutar porodice trčaka (vrstama je brojniji samo rod *Bembidion*), obuhvaća više od 940 vrsta grupiranih u 91 podrod. Na području Republike Hrvatske zabilježeno je tridesetak vrsta (Šerić Jelaska i sur. 2004).

Razlozi odabira navedenih vrsta leže u njihovom značaju za istraživano područje (alpsko-dinarski endemi), slabom poznavanju biologije i ekologije, ugroženosti kao i nepostojanju molekularnih sekvenci u relevantnim genetskim bazama.

Rod *Carabus* je vjerojatno nastao tijekom oligocena na euroazijskom kontinentu (Andujar i sur. 2012), pretežno je palearktičkog rasprostranjenja sa samo 11 vrsta zabilježenih na nearktičkom području (Bousquet i Larochelle 1993). Iako su njegova staništa i klimatske sklonosti uglavnom umjereni, rod pokriva većinu krajobraznih zona i visinske pojaseve holarktičkog područja, osim najsuših pustinja. Ekstenzivan areal čini rod *Carabus* izrazito pogodnim za analizu biogeografskih obrazaca i procesa u holarktičkom području. Sa stajališta konzervacijske biologije zanimljiv je zbog velike raznolikosti (oko 800 vrsta), veličine (12-50 mm) i atraktivne obojenosti.

Carabus (Megodonthus) caelatus Fabricius, 1801

Carabus caelatus (smežurani trčak) je vrsta koja je raširena u krškom području duž obale Jadranskog mora od Albanije do Soče (Durbešić 1967, Šerić Jelaska 1999, Šerić Jelaska i sur. 2004). Endem je Alpa, Dinarskoga gorja i zapadnog Balkana, a pojavljuje se od razine mora do 2200 m nadmorske visine (Turin i sur. 2003). Raširena je na području zapadnog Balkana. Odrasli se pojavljuju od svibnja do kolovoza. Vrsta je navedena na Crvenom popisu trčaka Hrvatske uključujući sljedeće podvrste: *Carabus (Megodontus) caelatus caelatus* Fabricius, 1801; *Carabus (Megodontus) caelatus dalmatinus* Duftschmid, 1812 i *Carabus (Megodontus) caelatus schreiberi* Kraatz, 1877). Navedene podvrste svrstane su unutar kategorije ugroženosti NT – niskorizične.

Carabus (Megodonthus) croaticus Dejean, 1826

Carabus croaticus (saboriti trčak, Slika 2.) je tipična krška vrsta koja dolazi na brdskim terenima u šumama od Albanije do Snježnika (Durbešić 1967, Šerić Jelaska 1999, Šerić Jelaska i sur. 2004). Planinski je specijalist i endemična vrsta Dinarskoga gorja

rasprostranjena na zapadnom dijelu Balkanskog poluotoka duž Dinarskoga gorja (Turin i sur. 2003). Pronađena je na nadmorskim visinama od 900 do 2400 m većinom u šumama jele, bora i bukve.



Slika 2. *Carabus croaticus* Dejean, 1826; Park prirode Blidinje, Bosna i Hercegovina. (Fotografija: Ž. J. Vladić).

Odrasli se pojavljuju od svibnja do rujna, a najbrojniji su u razdoblju od lipnja do srpnja (Turin i sur. 2003, Šerić Jelaska i sur. 2004). *Carabus (Megodontus) croaticus croaticus* Dejean, 1826 nalazi se na Crvenom popisu trčaka Hrvatske u kategoriji NT – niskorizična vrsta.

C. caelatus i *C. croaticus* su sestrinske vrste koje obiluju fenotipskim varijetetima što je rezultiralo opisom brojnih podvrsta i varijeteta te neslaganjima u taksonomiji.

Carabus (Eucarabus) catenulatus Scopoli, 1763

Carabus catenulatus (verižasti trčak) pojavljuje se u šumama, od nizina do subalpske zone, posebno na staništima s vapnenastim ili pješčanim tlom (Casale i sur. 1982, Marggi 1992) na području jugoistočne Europe (SI Italija, JI Austrija, SZ dio Balkanskog poluotoka).

U Hrvatskoj se javlja uglavnom u šumama, izloženih jugozapadu, na kaljužnom tlu (Rukavina i sur. 2010). Većinom je noćna vrsta premda postoje i dnevno aktivne populacije (Risnjak, osobno opažanje). Odrasli se pojavljuju u razdoblju od travnja do studenoga, a najbrojniji su u periodu lipanj-srpanj. Podvrsta *Carabus (Eucarabus) catenulatus catenulatus* Scopoli, 1763 nalazi se na Crvenom popisu trčaka Hrvatske u kategoriji LC – najmanje zabrinjavajuća vrsta.

Carabus (Eucarabus) parreyssii Palliardi, 1825

Carabus parreyssii (Pareyssiev trčak) je endemska vrsta sjeverozapadnoga dijela Balkana. Obično se pojavljuje u vrlo velikim šumskim ekosustavima (Casale i sur. 1982), a rjeđe u subalpskim staništima – otvorena staništa u gorju i planinama (Pavičević i sur. 1997) Podvrsta *Carabus (Eucarabus) parreyssi parreyssi* Palliardi, 1825 nalazi se na Crvenom popisu trčaka Hrvatske u kategoriji LC – najmanje zabrinjavajuća vrsta.

Carabus catenulatus i *Carabus parreyssi* smatraju se geografskim i ekološkim vikarijantima. Odrasle jedinke su morfološki slične, ali mužjaci mogu biti odvojeni na temelju oblika kopulatornog organa (*aedeagus*). Prema genetskim podacima, dobivenih istraživanjima u sklopu ove doktorske disertacije, te vrste još uvijek mogu hibridizirati na granicama svojih areala.

Carabus (Platycarabus) creutzeri Fabricius, 1801

Carabus creutzeri (zrnato-prugavi trčak) endemska je vrsta središnjih i istočnih Predalpa i Alpa na južnim padinama. Tipična je planinska vrsta koja se pojavljuje u šikarama i šumama, većinom u zoni od 2000 do 2300 m nadmorske visine (Hurka 1973), ali povremeno i na nižim nadmorskim visinama (200 do 300 m). Na području sjeverozapadnog Balkana pojavljuje se u dolinama šuma (200 m) pa sve do otvorenih staništa na većim visinama (2500 m). Podvrsta *Carabus creutzeri humilis* prema Winkleru (1932) te Droveniku i Peksu (1994) smatra se hrvatskim endemom. Podvrsta *Carabus (Platycarabus) creutzeri creutzeri* Fabricius, 1801 nalazi se na Crvenom popisu trčaka Hrvatske u kategoriji LC – najmanje zabrinjavajuća vrsta.

Carabus (Chaetocarabus) intricatus Linné, 1761

Carabus intricatus (prepleteni trčak) je ugrožena vrsta koja se nalazi na europskom popisu ugroženih vrsta (IUCN) kao i na Crvenom popisu trčaka Hrvatske u kategoriji niskorizična vrsta. Pojavljuje se do 1700 m nadmorske visine i dobar je pokazatelj gospodarenja starim šuma i tla bogatog organskom tvari (Mesaroš 1997; Turin i sur. 1993).

Carabus (Eucarabus) ulrichii Germar, 1824

Carabus ulrichii nastanjuje listopadne šume i otvorena staništa, od nizina do brežuljaka i planina visine do 500 m na toplijoj ekspoziciji u središnjoj i jugoistočnoj Europi (Turin i sur. 2003). U Hrvatskoj dolazi na sjevernom i sjeverozapadnom dijelu zemlje (Šerić Jelaska i sur. 2010). Pokazuje dnevnu i noćnu aktivnost. Odrasli se pojavljuju od ožujka

do rujna. U sjevernoj Europi nalazi se na Crvenim listama kao ugrožena i vrsta u opadanju dok na području južne Europe vjerojatno nije ugrožena (Turin i sur. 2003).

1.4. Područje i metode istraživanja

1.4.1. Područje istraživanja

Europska fauna rezultat je procesa koji su se odvijali tijekom i nakon posljednjeg ledenog doba. Područja Pirinejskog, Apeninskog i Balkanskog poluotoka odigrala su važnu ulogu u preživljavanju i ponovnom raseljavanju vrsta. Smatra se da je većina danas široko rasprostranjenih vrsta preživjela posljednje ledeno doba na jednom od 3 spomenuta poluotoka (Hewitt 2000). Od navedenih refugijalnih područja, Balkanski se poluotok izdvaja kao vrstama najbogatije područje, a filogeografska istraživanja upućuju na puno zamršenije procese koji su se odvijali na ovom području kao i na postojanje mikrorefugija (Previšić i sur. 2009).

Balkanski poluotok smješten je na jugoistoku Europe između Crnoga, Mramornoga, Egejskoga, Jonskoga i Jadranskoga mora. Poluotočna svojstva slabo su izražena, a zbog nepostojanje jedinstvene planinske pregrade sjeverna i zapadna granica nisu točno određene. Geografski uključuje: dio Republike Hrvatske, Bosnu i Hercegovinu, veći dio Srbije, Kosovo, Crnu Goru, Bugarsku, Sjevernu Makedoniju, Grčku, Albaniju, mali dio Rumunjske i europski dio Turske. Gotovo 70% poluotoka čine planinski lanci (Dinaridi, Šarsko-Pindsko gorje, Rodopi, Stara planina). Najviši vrh je Rila (2926 m) na sjeveru planinskog lanca Rodopi (Bugarska), (Reed i sur. 2004).

Balkanski poluotok je područje s vrlo bogatom biološkom raznolikošću, bogatijom od bilo kojeg drugog područja u Europi (Polunin 1980). Najbrojnija kopnena skupina su kornjaši (Coleoptera), a prema Guéorguiev (2007) ovo područje ima najraznovrsniju entomofaunu kornjaša u Europi. Visoka stopa endemizma flore i faune posljedica je vrlo varijabilnih klimatskih uvjeta tijekom povijesti.

Dinaridi ili Dinarsko gorje (Slika 3.) je gorski sustav mlađih ulančanih planina nastalih alpskom orogenezom u jugoistočnoj Europi. Triangularnog je oblika, a pruža se u dužinu od 645 km, od rijeke Soče i Trnovskog Gozda na sjeverozapadu pa do rijeke Drim i Prokletija u sjevernoj Albaniji, između jadranske obale i rijeke Save. Najviši vrh Dinarida je Maja Jezerces (2694 m, Prokletije, Albanija).

Planinski sustav obuhvaća više od 200 planina na kojima se nalazi 20 nacionalnih parkova, 19 lokaliteta svjetske baštine pod zaštitom UNESCO-a, 2200 km riječnih tokova, 200 prirodnih jezera te više od 38 000 biljnih i životinjskih vrsta (www.viadinarica.com). Ovo najveće i najpoznatije krško područje na svijetu uključuje i nekoliko prašuma (npr. Perućica i Biogradska gora).

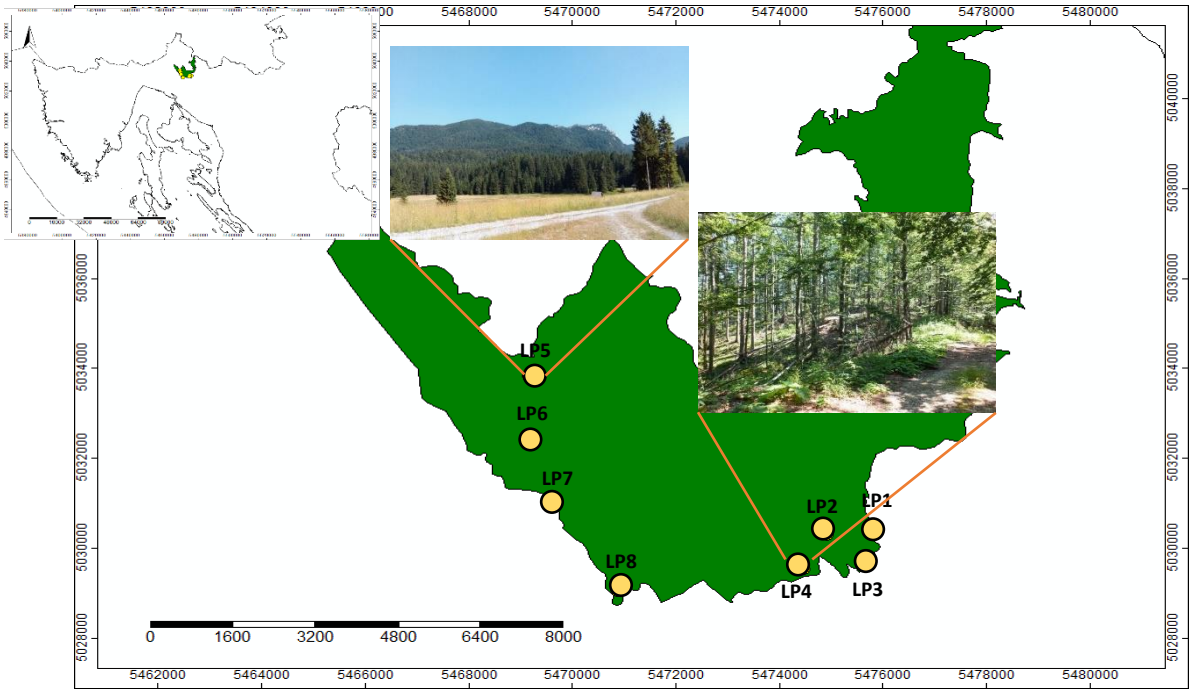


Slika 3. Dinarsko gorje, (slika preuzeta i prilagođena s www.viadinarica.com).

Istraživanjem u sklopu ove disertacije obuhvaćeno je gotovo 100 lokaliteta duž Dinarskoga gorja, jadranske obale i jadranskih otoka. Najopsežnije istraživanje provedeno je na sjevernom dijelu Dinarskoga gorja u kojem je smješten Nacionalni park Risnjak.

Nacionalni park Risnjak (Slika 4.), smješten na zapadu Hrvatske, obuhvaća planinu Risnjak (1528 m), planinu Snježnik (1506 m), gornji dio doline rijeke Kupe i najveći dio sliva potoka Krševica. Predstavlja prirodnu vezu Alpa i Balkanskog poluotoka. Na površini od samo 64 m^2 nalaze se brojni geografski, geološki, vegetacijski, klimatološki i hidrološki fenomeni karakteristični za ovaj dio hrvatskog dinarskog krškog područja (np-risnjak.hr).
Klima je perhumidna i umjereno hladna. Srednja godišnja temperatura je 5,4°C, a prosjek padalina iznosi 3770 mm godišnje (Buzjak i Fiedler 1999). Zbog specifičnog položaja (udaljenost od mora iznosi samo 15 km) i izloženosti maritimnim i kontinentalnim utjecajima Risnjak obiluje raznolikom florom i faunom. Vegetacija Nacionalnoga parka Risnjak sastoji se od oko 30 biljnih zajednica, među kojima je 14 šumskih, a najveći dio područja prekriva mješovita šuma bukve i jele (*Omphalodo-Fagetum*), (Trinajstić 1995).

Posljednje opsežnije istraživanje entomofaune na području Parka provedeno je prije tridesetak godina (Vujčić-Karlo 1999).



Slika 4. Nacionalni park Risnjak; raspored lovnih ploha i fotografije ploha Lazac (LP5) i Risnik (LP4). (Fotografija: Ž. J. Vladić.)

1.4.2. Metode uzorkovanja

Metode istraživanja uključivale su uzorkovanje kornjaša pomoću lovnih posuda i rukom (aktivno traženje jedinki kukaca ispod panjeva, trupaca i mahovine prilikom svakog izlaska na teren za potrebe DNA analiza).

Metoda lovnih posuda ili *"pitfall trapping"* najčešće je korištena metoda za proučavanje kornjaša. Uključuje upotrebu lovnih posuda (tzv. Barberove lovne posude) koje se do ruba otvora posude ukapaju u zemlju (Slika 5.). Lovni napor uključuje broj lovnih posuda i vrijeme izloženosti posuda. Ulov ovisi o aktivnosti tražene vrste pa lovne posude predstavljaju pasivno sredstvo lova. Osim o aktivnosti organizama ulov ovisi i o nizu drugih čimbenika kao što su: veličina i oblik lovne posude, materijal od kojeg je izrađena, raspored posuda na istraživanoj plohi, vremenu izlaganja posuda, korištenom atraktantu itd. Dodatni problem predstavlja različita efikasnost ulova na područjima s različitom vegetacijom (Sunderland i sur. 1995) zbog čega se rezultati uzorkovanja ovom metodom na staništima s različitim vegetacijskim pokrovom ne mogu uspoređivati. Osim u navedenom slučaju primjena lovnih posuda nije primjerena ni za istraživanje utjecaja pesticida (toksičnosti) na

kornjaše s obzirom da se ne zna njihov utjecaj na aktivnost kornjaša (Luff 1987). Nadalje, upitan je i izbor atraktanta i njegova primamljivost za različite predstavnike ove porodice. Kao moguće rješenje koriste se suhe zamke, međutim u ovom slučaju postoji opasnost od predacije unutar zamke ili izvan zamke (mali sisavci), (Stapp 1997). Usprkos navedenim ograničenjima za sada ne postoji ni jedna pouzdanija metoda uzorkovanja.



Slika 5. Modificirane Barberove lovne posude. (Fotografija: Ž. J. Vladić)

Određeni napori ulažu se u njezino standardiziranje (upotreba istog atraktanta, posude istog volumena i promjera otvora te izrađene od istog materijala, izjednačavanje rasporeda lovnih posuda na uzorkovanoj plohi i dr.) kako bi dobiveni rezultati istraživanja iz različitih dijelova svijeta bili usporedivi (Brown i Matthews 2016).

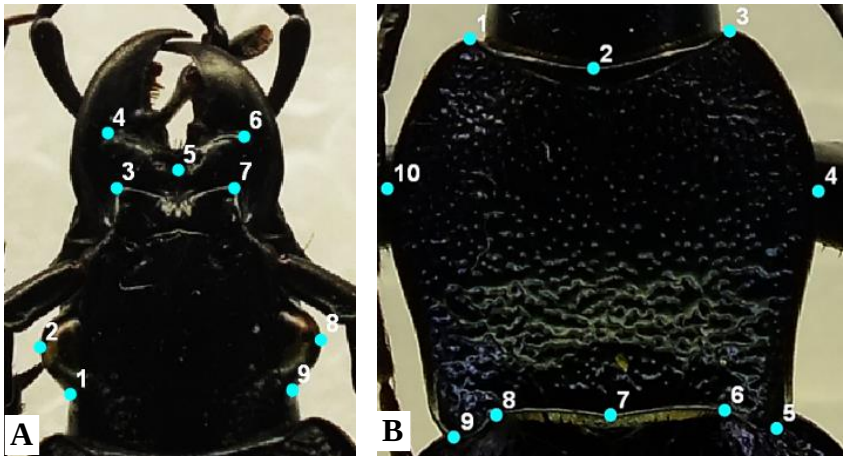
1.5. Morfološke analize – linearna (LM) i geometrijska morfometrija (GMM)

Tradicionalne morfometrijske analize su prvi i neizostavni korak u proučavanju biologije, taksonomije i filogenije vrste. Glavne prednosti tradicionalne ili linearne morfometrije (LM) su jednostavnost postupka i mogućnost proučavanja velikog broja vrsta bez ograničenja vezanih uz skupljeni materijal. Nedostaci leže u korelaciji LM s veličinom, a problem predstavlja i pojava paralelne evolucije kada isključiva primjena rezultata linijske morfometrije može dovesti do pogrešnih zaključaka o taksonomiji vrsta (Mossakowski 2003). Upravo je nemogućnost otkrivanja taksonomskih odnosa, pogotovo na intraspecijskoj razini, dovela do razvoja novih morfoloških metoda.

Geometrijska morfometrija (GMM) kao oblik morfometrijske analize za kvantifikaciju i vizualizaciju morfoloških varijacija omogućava bolju interpretaciju podataka dobivenih proučavanjem morfologije (Alibert i sur. 2001). Temelji se na analizi kartezijanskih geometrijskih koordinata, a ne linearnih, prostornih ili volumetrijskih varijabli. Za razliku od tradicionalne morfometrije, koja se temelji na linearnoj udaljenosti, metodama geometrijske morfometrije, koristeći koordinate točaka orijentira (*landmarks*), predstavlja se oblik proučavane strukture i omogućuje vizualni pregled njegovih varijacija (Mitteroecker i sur. 2013).

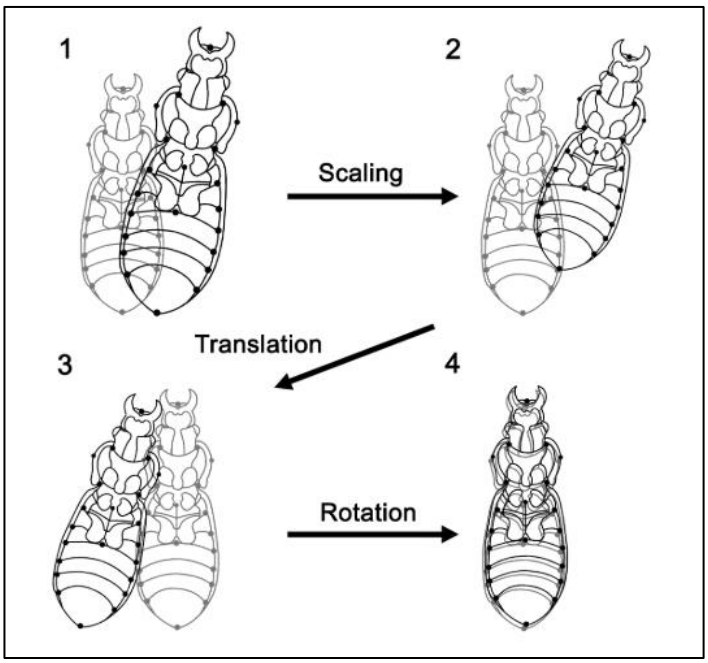
Među nekoliko geometrijskih pristupa morfometriji, *Procrustes* metoda je najraširenija i u matematičkim i statističkim svojstvima najrazumljivija (Bookstein 1996, Small 1996, Dryden i Mardia 1998).

Prvi korak u analizi geometrijskom morfometrijom je određivanje orijentira (*landmarks*), (Slika 6.). To su točke podudarnosti na svakom uzorku koji se podudaraju između i unutar populacija odnosno biološki homologni anatomski lokusi prepoznatljivi na svim uzorcima u istraživanju (Bookstein 1991; Dryden i Mardia 1998).



Slika 6. Odabrani orijentiri (*landmarks*) na glavi (A) i pronotumu (B) vrste *Carabus caelatus schreiberi* Kraatz ,1877. (Fotografija: Ž. J. Vladić.)

Nakon odabira orijentira primjenjuje se metoda za uklanjanje informacija o veličini, položaju i rotaciji (Slika 7.). Uklanjanjem tih informacija daljnje se analize temelje na obliku i koordinatama orijentira.



Slika 7. Shematski prikaz tri koraka metode *Procrustes superimposition*: skaliranje na jednaku veličinu (*scaling*), prevođenje na istu poziciju centroida (*translation*) i rotacija za minimiziranje sume kvadrata euklidskih udaljenosti među homolognim orijentirima (*rotation*). (Preuzeto iz Benítez 2013).

Proučavanjem oblika, a ne veličine nadilaze se problemi tradicionalne morfometrije te se podaci dobiveni geometrijskom morfometrijom uspješno koriste u rješavanju filogenije, ontogeneze i sistematike vrsta na inter- i intraspecijskoj razini (Loy i sur. 1993, Auffray i sur. 1996, David i Laurin 1996, Naylor 1996, Klingenberg i McIntre 1998, Adams i sur. 2013, Zúñiga-Reinoso i Benitez 2015).

1.6. Molekularne analize

Razvoj metoda molekularne biologije omogućio je mnoga saznanja o taksonomskim odnosima među kornjašima. DNA sekvence omogućuju brojne analize na svim taksonomskim nivoima što znatno unapređuje znanje o evoluciji i sistematici trčaka, a kombinacijom podataka dobivenih sekvenciranjem s filogeografijom i sistematikom bliže smo razumijevanju povijesti vrste.

Kao osnovni genetički biljezi, za tumačenje evolucijskih promjena i događaja koji su se odvijali u životinjskim vrstama i populacijama, najčešće se koriste geni na mitohondrijskoj DNA (mtDNA) te nuklearni geni. Mitohondrijski biljezi pokazali su se boljima u proučavanju filogenetskih odnosa srodnijih taksona dok su za udaljenije taksone prikladniji nuklearni biljezi zbog manje varijabilnosti.

U istraživanjima vezanima uz ovu disertaciju odabrani su sljedeći filogenetski biljezi za utvrđivanje filogenetskih odnosa između različitih vrsta i podvrsta roda *Carabus*: citokrom c oksidaza 1 – *COI* (mitohondrijski biljeg) te gen za *ITS-2* (nuklearni biljeg).

Kao početnice za lančanu reakciju polimerazom korištene su:

LCO-1490: 5' – GGTCAACAAATCATAAAGATATTGG – 3'
HCO-2198: 3' – TAAACTTCAGGGTGACCAAAAAATCA – 5'
(Hebert i sur. 2003)

5.8sF: 5' – GTGAATTCTGTGAACTGCAGGACACATGAAC – 3'
28sR: 3' – ATGCTTAAATTTAGGGGGTA – 5'
(Porter i Collins 1991)

Gen za citokrom C oksidazu 1 (*COI*) nalazi se na mitohondrijskoj DNA. Često se koristi pri proučavanju filogenetskih odnosa unutar porodice Carabidae (Galián i sur. 1999, Martínez-Navarro i sur. 2005, Deuve i sur. 2012). Općenito se smatra dobrim genetskim biljegom za razlikovanje vrsta zbog njegove brzine evolucijskih promjena i posljedične prikladnosti za razlikovanje srodnih vrsta i filogeografskih skupina unutar vrste (Cox i Hebert 2001, Wares i Cunningham 2001, Hebert i sur. 2003b). Dokazano se smatra najučinkovitijim jedinstvenim genetskim biljegom čemu u korist ide i postojanje univerzalnih početnica koje umnažaju segmente gotovo svih životinjskih vrsta (Folmer i sur. 1994; Zhang i Hewitt 1997).

Nuklearni biljeg *ITS-2* (*second internal transcribed spacer*) smješten je između male i velike podjedinice ribosomske DNA (rDNA) i koristan je za utvrđivanje povezanosti bliskih svojti koje su se nedavno razišle (<50 milijuna godina), a pokazao se izvrsnim i za razlikovanje vrsta te eksperimente hibridizacije (Marcilla i sur. 2001). Ubraja se u brzo evoluirajuće gene. Nadalje, *ITS-2* predstavljaju tandemno ponavljajuće sekvence ili mikrosatelite (Almeyda-Artigas i sur. 2000) čije su jedinice ponavljanja između 1 – 5 pb i koji su vrlo dobri

molekularni biljezi za diferencijaciju populacija unutar određene vrste (Jarne i Lagoda 1996).

1.7. Uloga trčaka u konzervacijskoj biologiji

Porodica trčaka zbog svoje je brojnosti i uloge u ekosustavu nezaobilazna u testiranju i proučavanju raznih ekoloških i evolucijskih hipoteza, a u novije vrijeme zauzima i sve važnije mjesto u konzervacijskoj biologiji. Brojna istraživanja (Eyre i Luff 1990; Kromp 1999; De Vries 1994; Spence i sur. 1996; Lövei i Sunderland 1996, Davies i Margules 1998; Duelli i Obrist 1998; Irmler 2003; Rainio i Niemelä 2003; Šerić Jelaska i sur. 2010) potvrđuju trčke kao dobre bioindikatore u procjeni stanja ekosustava zbog njihovog brzog reagiranja na promjene abiotskih i biotskih čimbenika. Sastav zajednica trčaka odražava stanje ekosustava koji nastanjuju (Gilgado 2012) zbog čega je od presudne važnosti imati informacije o njihovoj biologiji i ekologiji.

Ubrzane promjene u načinima korištenja zemljišta posljednjih desetljeća ostavile su dalekosežne posljedice na živi svijet. Uništavanje staništa, fragmentacija, korištenje insekticida i umjetnih gnojiva doveli su do smanjenja populacija što je u konačnici rezultiralo efektom uskog grla i pojačanim utjecajem genetičkog drifta. Zbog nemogućnosti prilagodbe na nagle promjene ekoloških čimbenika često nestaju cijele populacije. Dodatni problem predstavlja velika mobilnost ljudske populacije koja doprinosi slučajnom prenošenju vrsta u staništa u koja prirodno ne bi dospjele. Na prvi pogled takve slučajne migracije povećavaju lokalnu raznolikost entomofaune, ali će u konačnici rezultirati velikim gubitcima globalne bioraznolikosti uslijed homogenizacije. Upravo je ubrzana homogenizacija faune razlog povećanja globalne stope izumiranja za približno dva reda veličine (Lövei i Sunderland 1996).

Unutar porodice trčaka posebno mjesto i značaj u konzervacijskoj biologiji pripada rodu *Carabus* Linné, 1758. Zbog pojačanog intenziteta i promjena u načinu korištenja zemljišta te posljedičnim klimatskim promjenama svjedočimo znatnom smanjenju brojnosti populacija roda *Carabus* (Turin i den Boer 1988, Desender i Turin 1989 Balletto i Casale 1991, Brandmayer i Pizzoloto 2016). Dodatni problem predstavlja beskrilnost koja je česta kod vrsta ovog roda i s njime povezane slabije disperzijske mogućnosti većine vrsta čime se znatno povećava osjetljivost na izolaciju i fragmentaciju staništa. Posebno su pogođene stenovalentne planinske endemske vrste. Kao planinski specijalisti takve se vrste suočavaju

i sa smanjenim kapacitetom nosivosti okoliša, uslijed pomicanja prema većim nadmorskim visinama, zbog porasta prosječne temperature i promjene drugih abiotičkih čimbenika uzrokovanim naglim promjenama u klimi. Očekuje se da će odgovor trčaka na klimatske promjene biti sličan onome u prošlosti s iznimkom što se očekuje brže izumiranje zbog antropogenog utjecaja na stanište.

1.8. Hipoteze i ciljevi istraživanja

Pregledom dostupnih literaturnih izvora i proučavanjem stanja na terenu definirane su slijedeće hipoteze:

1. Analize mitohondrijskih i nuklearnih biljega otkrivaju jasniju sliku o evolucijskim procesima i filogenetskom položaju odabranih endemskih epigejskih vrsta trčaka dinarskog krša.

2. Detaljne morfološke analize upotpunjene s geometrijskom morfometrijom* utvrđuju postojanje manjeg broja podvrsta sestrinskih vrsta roda *Carabus* – *C. croaticus* i *C. caelatus*, od trenutno opisanih.

3. Sastav zajednica trčaka i njihove značajke (krilatost, afinitet prema staništu te afinitet za temperaturu i vlagu), na istim plohama unutar Nacionalnoga parka Risnjak, u razmaku od 25 godina pokazuju razlike koje su u trendu s globalnim klimatskim promjenama i mogu se objasniti promjenama u klimatskim čimbenicima istraživanog područja.

4. Analizom visinskoga gradijenta rasprostranjenosti planinskih specijalista utvrđuje se pomicanje njihovog areala prema višim nadmorskim visina.

i ciljevi ove doktorske disertacije:

1. Doprinijeti boljem poznavanju faune trčaka naše zemlje s posebnim naglaskom na biologiju i ekologiju endemskih vrsta.

2. Dopuniti filogenetske odnose u sistematici trčaka unutar roda *Carabus* s endemskim vrstama dinarskog krša koje do sada nisu bile uključene u molekularna istraživanja.

3. Ispitati stupanj usklađenosti molekularnih podataka s postojećim morfološkim klasifikacijama na temelju strukture frontalnih štitova ličinki i strukture endofalusa u odraslih primjeraka.

4. Upotrebom metoda geometrijske morfometrije pridonijeti razrješavanju taksonomskih odnosa na intraspecijskoj i interspecijskoj razini dviju endemskih sestrinskih vrsta roda *Carabus* (*C. caelatus* i *C. croaticus*).

5. Morfološki usporediti populacije dviju endemskih sestrinskih vrsta roda *Carabus* kroz njihov areal na Dinaridima i istražiti geografske uzorke fenotipskih varijantiti te poboljšati razumijevanje filogenetskih odnosa između ovih vrsta primjenom molekularnih analiza mitohondrijske i nuklearne DNA.

6. Usporediti rezultate geometrijske morfometrije s taksonomskim klasifikacijama temeljenim na ne-metričkim i subjektivnim vizualnim usporedbama morfoloških značajki prisutnih u literaturi.

7. Utvrditi promjene u sastavu entomofaune na području Nacionalnoga parka Risnjak uzorkovane 2015. i 2016. u odnosu na podatke iz 1990. i 1991. te ih povezati s mogućim promjena u klimatskim parametrima i drugim uočenim promjenama unutar razdoblja od 25 godina.

8. Provjeriti razmještanje vrsta duž visinskoga gradijenta unutar Nacionalnoga parka Risnjak.

9. Unaprijediti mogućnosti za razvoj primjerenih konzervacijskih mjera upoznavanjem evolucijskih procesa koji se pojavljuju duž Dinarida.

* Zbog poteškoća na koje se naišlo prilikom molekularnih analiza, a koje su detaljno opisane u odjeljku Rasprava (3.3.1. Primjena molekularnih analiza u interspecijskom i intraspecijskom razdvajanju sestrinskih vrsta *C. croaticus* i *C. caelatus*), kao potencijalnu alternativu molekularnim analizama u istraživanje su uključene metode geometrijske morfometrije kako bi se ustanovila varijabilnost populacija vrsta *C. croaticus* i *C. caelatus* duž njihovog areala.

2. ZNANSTVENI RADOVI

2.1. Popis znanstvenih radova

Šerić Jelaska L, Jambrošić Vladić Ž, Radovanović H, Franjević D (2014) Comparison of molecular and morphological systematics of *Carabus* species (Coleoptera: Carabidae) with special emphasis on species from Dinaric karst. Periodicum biologorum, 116, 249 – 257.

Jambrošić Vladić Ž, Benítez AH, Pirnat A, Hristovski S, Šerić Jelaska L (2018) Variations in body shape of mountain habitat specialist *Carabus croaticus* and its sister species *Carabus caelatus* (Coleoptera: Carabidae) populations across Dinaric Alps. Zoomorphology, 138 (1) 85 – 96.

Jambrošić Vladić Ž, Šerić Jelaska L (2020) Long term changes (1990-2016) in carabid beetle assemblages (Coleoptera: Carabidae) in protected forests on Dinaric Karst on Mountain Risnjak, Croatia. European Journal of Entomology 117, 56 – 67.

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Comparison of molecular and morphological systematics of *Carabus* species (Coleoptera: Carabidae) with special emphasis on species from Dinaric karst

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Abstract

***Background and Purpose**: Despite morphological and molecular data analysed so far, phylogenetic relationships of many lineages of genus* Carabus *Linnaeus 1758 (Carabini, Carabinae, Carabidae) present in Europe, have not been yet fully understood and molecular data have not been fully integrated with the morphological classifications. The aim of this research was to: (i) complement the phylogenetic relationships in the systematics of carabids within the genus* Carabus *with endemic species from Dinaric karst not included so far in molecular systematics research, and to (ii) examine the degree of matching of the molecular data with the existing morphological classifications based on the structure of frontal shields of larvae and the structure of the endophallus in adult specimens.*

***Materials and Methods**: In this research, phylogenetic relationships between 31 species from genus* Carabus *were analysed. Analyses were based on the DNA sequences of mitochondrial gene for cytochrome c oxidase subunit I. For phylogenetic inference maximum likelihood and Bayesian analysis methods were used.*

***Results:** The obtained results showed a greater concordance of molecular data with the classifications based on the structure of endophallus than with the classification based on structure of frontal shields of larvae. The results mainly corresponded to the phylograms in the previous studies confirming the taxonomic status of some species. For three species,* Carabus creutzeri *and* C. parreyssi *alpine-dinaric endemic species and* C. ulrichi*, all included for the first time in molecular analysis, results indicated taxonomic congruence of molecular data with the classification based on the structure of endophallus.*

***Conclusions:** Systematic categories within the genus* Carabus *cannot be based only on the structure of the endophallus, but the results of molecular analysis should also be included. As the topology of several groups still remains uncertain, molecular phylogeny requires further investigations with a larger data set and additional molecular markers.*

Carabus *species from the western part of Balkan Peninsula, mainly endemics, have been under-represented in hitherto molecular systematic analyses. Further studies, including their distribution and ecology as well as studies including other Balkan's endemic species are required to explain speciation events. This study contributes to the Barcode of Life Initiative.*

INTRODUCTION

The genus *Carabus* Linnaeus 1758 belongs to the subfamily Carabinae, family Carabidae, suborder Adephaga and order Coleoptera. It is the most numerous genus within Carabidae. Genus *Carabus* comprises about 940 valid species classified into 91 subgenera *(1).* 132 species occur in Europe while thirty species have been recorded in Croatia *(2),* including 53 subspecies and 81 forms and aberrations *(2).* Almost all species occur in the Palaearctic region with a dozen species occurring in the Nearctic *(3).*

Carabus species represent the largest Carabids, with body size from around 12 to 50 mm long. They are mostly wingless, nocturnal predators of snails, earthworms and caterpillars in forest and open habitats *(4, 5, 6). Carabus* species as distinctly terricolous and mostly non-flying insects are very sensitive to habitat fragmentation and changing environmental variables *(7),* and are therefore, considered as good bioindicators *(8).*

Within genus *Carabus* several species are endangered and listed in the European list of endangered species (IUCN). Three of them: *Carabus (Platycarabus) creutzeri* Fabricius 1801, *Carabus (Chaetocarabus) intricatus* Linnaeus 1761 and *Carabus (Eucarabus) parreyssi* Palliardi 1825, included in this research, have been placed in a number of national Red lists (i.e. Croatian Red List).

Carabus (Platycarabus) creutzeri Fabricius 1801 is an endemic species of the Central and Eastern Prealps and Alps on the southern side. This is a typical mountain species, occurring in bushes and forests, mostly in the alpine zone (2000-2300 m a.s.l.: 9), but occasionally also at lower altitudes (200-300 m a.s.l. in few places in Veneto: 10). In North - Western Balkans *C. creutzeri* occurs from the low valley forests (200 m a.s.l.) to open habitats at high altitudes (2500 m a.s.l.: 11). Pavičević and Mesaroš *(11)* mention the form *humilis* Berneau as possibly endangered. According to Winkler *(12)* and Drovenik and Peks *(13) Carabus creutzeri humilis* is Croatia's endemic subspecies. *Carabus (Chaetocarabus) intricatus* Linnaeus 1761 is an endangered species listed in the European Red List of Threatened Species (IUCN). It occurs up to 1700 m a.s.l. and is a good indicator of maintenance of old-growth forests and soil rich in organic matter *(14, 15). Carabus (Eucarabus) parreyssi* Palliardi 1825 is an endemic to North-Western part of Balkan Peninsula. According to Pavičević and Mesaroš *(11)* all forms are endangered. Generally it occurs in very large, undisturbed forests *(10),* however according to Pavičević and Mesaroš *(11),* it is regarded as a species of subalpine habitats which occurs in open habitats in hills and mountains as well.

Despite numerous studies, phylogenetic relationships within the genus *Carabus* are still unresolved. The two most well-known classifications based on morphological characteristics are the classification based on the structure of frontal shields of larvae and the classification based on the structure of endophallus in adults. The first one divided genus *Carabus* into three large groups - Archeocarabi, Metacarabi and Neocarabi *(16).* The classification based on the structure of endophallus, first suggested by Ishikawa (1978), divided the genus into eight groups: Spinulati, Digitulati, Lipastrimorphi, Archicarabomorphi, Tachypogenici, Arcifera and Neocarabi *(17).* The classification based on the structure of endophallus is more widely used nowadays, than classification based on the structure of larval shields *(1, 17).*

Phylogeny based on ND5 gene by Sota and Ishikava *(18)* gave the first picture of the relationships among *Carabus* subgenera but failed to resolve the fundamental relationships within the genus. These results did not corroborate classifications based on morphological characters (19 – 23). Deuve *et al. (24)* produced the first phylogeny of the genus *Carabus* based on both mitochondrial and nuclear markers in research which included *Carabus* species from the Iberian and Balkan Peninsula while Andujar *et al. (3)* conducted calibration analyses for the genus *Carabus* combining five mitochondrial and four nuclear DNA fragments to find out the rates of molecular evolution for this genus.

Genus *Carabus* is known as a hyper-diverse genus *(1).* Diversity is specially noticed in areas labelled as refuge. Of the different refuge areas, the Balkan Peninsula is considered as the most taxon-rich *(25, 26),* with Dinaric karst, referred as the greatest natural treasure of the Balkan Peninsula. Sket *(27)* pointed out a rich geological history of Dinaric karst and high diversity of flora and fauna of that specific area. Therefore, Balkan Peninsula is placed among 25 World's biodiversity hotspots *(28).*

Here, in this research, we used for the first time mitochondrial sequences from two endemic species, *Carabus (Platycarabus) creutzeri and Carabus (Eucarabus) parreyssi,* and another *Eucarabus* species *Carabus ulrichi* in reconstruction of the *Carabus* phylogenetic tree.

The aim of this research was to complement the phylogenetic relationships in the systematics of Carabids within the genus *Carabus* Linnaeus 1758 (Carabini, Carabinae, Carabidae) with some endemic species from Dinaric karst and to check the matching of phylogenetic trees with existing morphological classifications based on the structure of frontal shields of larvae and the structure of endophallus in adult specimens.

MATERIALS AND METHODS

Taxonomic sampling

Our phylogenetic analysis included 31 species of genus *Carabus*, belonging to 18 different subgenera. The specimens used in this analysis are listed in Table 1. All the main subgroups (Archicarabomorphi, Arcifera, Digitulati, Li-

TABLE 1

List of *Carabus* species included in this study, with division according to endophallus (17) and larval morphology (10, 41 – 43), and outgroup species included in this study with accession numbers from the GenBank and localities of individual species.

No.	Species/Sequence	*Carabus* division (endophallus morphology)	*Carabus* division (larval morphology)	Locality/ GenBank	GenBank Accession number
1	*Archicarabus nemoralis*1	ARCHICARABOMORPHI	ARCHEOCARABI	Croatia: Medvednica	KP067550
2	*Archicarabus nemoralis*2	ARCHICARABOMORPHI	ARCHEOCARABI	Croatia: Medvednica	KP067554
3	*Archicarabus nemoralis*3	ARCHICARABOMORPHI	ARCHEOCARABI	Croatia: Medvednica	KP067548
4	*Archicarabus nemoralis*4	ARCHICARABOMORPHI	ARCHEOCARABI	Croatia: Medvednica	KP067547
5	*Archicarabus nemoralis*5	ARCHICARABOMORPHI	ARCHEOCARABI	Croatia: Medvednica	KP067547
6	*Archicarabus nemoralis*6	ARCHICARABOMORPHI	ARCHEOCARABI	Croatia: Medvednica	KP067549
7	*Chaetocarabus intricatus*1	ARCIFERA	NEOCARABI	Croatia: Medvednica	KP067569
8	*Chaetocarabus intricatus*2	ARCIFERA	NEOCARABI	Croatia: Medvednica	KP067569
9	*Chaetocarabus intricatus*3	ARCIFERA	NEOCARABI	Croatia: Medvednica	KP067570
10	*Chaetocarabus intricatus*4	ARCIFERA	NEOCARABI	Croatia: Medvednica	KP067572
11	*Chaetocarabus intricatus*5	ARCIFERA	NEOCARABI	Montenegro: Durmitor	KP067571
12	*Platycarabus creutzeri*1	ARCIFERA	NEOCARABI	Croatia: Velebit	KP067564
13	*Platycarabus creutzeri*2	ARCIFERA	NEOCARABI	Croatia: Velebit	KP067563
14	*Platycarabus irregularis*	ARCIFERA	NEOCARABI	GenBank	JQ689887
15	Carabus arvensis	DIGITULATI	ARCHEOCARABI	GenBank	JQ646568
16	Carabus deyrollei	DIGITULATI	no data	GenBank	JQ646588
17	*Eucarabus parreyssi*1	DIGITULATI	no data	Croatia: Velebeit	KP067568
18	*Eucarabus parreyssi*2	DIGITULATI	no data	Croatia: Poštak	KP067567
19	*Eucarabus catenulatus*1	DIGITULATI	no data	Croatia: Gorski Kotar	KP067558
20	*Eucarabus catenulatus*2	DIGITULATI	no data	Croatia: Gorski Kotar	KP067557
21	*Eucarabus catenulatu*3s	DIGITULATI	no data	Croatia: Gorski Kotar	KP067559
22	*Eucarabus sternbergi*	DIGITULATI	no data	GenBank	HM180573
23	*Eucarabus ulrichi*1	DIGITULATI	ARCHEOCARABI	Croatia: Medvednica	KP067556
24	*Eucarabus ulrichi*2	DIGITULATI	ARCHEOCARABI	Croatia: Medvednica	KP067555
25	*Morphocarabus monilis*1	LIPASTRIMORPHI	ARCHEOCARABI	GenBank	GU347147
26	*Morphocarabus monilis*2	LIPASTRIMORPHI	ARCHEOCARABI	GenBank	GU347148
27	*Eurycarabus famini*1	METACARABI	METACARABI	GenBank	JQ689884
28	*Eurycarabus famini*2	METACARABI	METACARABI	GenBank	JQ689878
29	*Mesocarabus lusitanicus*	METACARABI	no data	GenBank	JQ689901
30	Mesocarabus macrocephalus	METACARABI	no data	GenBank	JQ689879
31	*Nesaeocarabus abbreviatus*1	METACARABI	METACARABI	GenBank	JQ689894
32	*Nesaeocarabus abbreviatus*2	METACARABI	METACARABI	GenBank	JQ689874
33	*Oreocarabus hortensis*1	METACARABI	METACARABI	Croatia: Dalmatia	KP067566
34	*Oreocarabus hortensis*2	METACARABI	METACARABI	Croatia: Gorski Kotar	KP067565
35	Orinocarabus baudii	METACARABI	no data	GenBank	JQ646601
36	Orinocarabus fairmairei	METACARABI	no data	GenBank	JQ646595
37	*Tomocarabus convexus*1	METACARABI	METACARABI	Croatia: Medvednica	KP067546
38	*Tomocarabus convexus*2	METACARABI	METACARABI	Croatia: Medvednica	KP067552
39	*Tomocarabus convexus*3	METACARABI	METACARABI	Croatia: Medvednica	KP067553
40	Chrysocarabus auronitens	NEOCARABI	NEOCARABI	GenBank	GU347140
41	*Chrysocarabus rutilans*	NEOCARABI	NEOCARABI	GenBank	JQ689891
42	Macrothorax morbillosus	NEOCARABI	NEOCARABI	GenBank	JQ689883
43	Macrothorax rugosus	NEOCARABI	NEOCARABI	GenBank	JQ689882
44	Megodontus caelatus	NEOCARABI	NEOCARABI	Montenegro: Durmitor	KP067573
45	*Megodontus violaceus*1	NEOCARABI	NEOCARABI	Croatia: Medvednica	KP067561
46	*Megodontus violaceus*2	NEOCARABI	NEOCARABI	Croatia: Medvednica	KP067562
47	*Procrustes coriaceus*1	NEOCARABI	NEOCARABI	Croatia: Medvednica	KP067574
48	*Procrustes coriaceus*2	NEOCARABI	NEOCARABI	Croatia: Medvednica	KP067574
49	*Apotomopterus kouanping*	SPINULATI	no data	GenBank	JQ646606
50	*Apotomopterus skyaphilus*	SPINULATI	no data	GenBank	JQ646604
51	Tachypus auratus	TACHYPOGENICI	ARCHEOCARABI	GenBank	JQ646600
52	Tachypus cancellatus	TACHYPOGENICI	ARCHEOCARABI	Croatia: Dalmatia	KP067551
53	Tachypus cristofori	TACHYPOGENICI	no data	GenBank	JQ646597
54	Cychrus caraboides	outgroup		GenBank	AB109838
55	Cychrus attenuatus	outgroup		Croatia: Medvednica	KP067560

pastrimorphi, Metacarabi, Neocarabi, Spinulati and Tachypogenici) as defined by the endophallic characters of male genitalia were represented *(17).* Also, two species of *Cychrus,* which served as an outgroup to root the phylogenetic trees were included. 32 DNA sequences from 12 species of *Carabus* and one *Cychrus* species were obtained by DNA extraction and sequencing from specimens collected in the field, while the rest 23 sequences were retrieved from the NCBI GenBank database (Table 1).

Ground beetles were sampled in the area of Mt. Medvednica (Croatia), from May to October 2007, Mts. Velebit (Croatia) and Durmitor (Monte Negro) in 2009, Mt. Poštak and Krka riverside in Dalmatia (Croatia) in 2010 and Gorski Kotar area (Croatia) in 2010 and 2013, (Table 1). Immediately after collecting, samples were stored in 96% EtOH until the beginning of laboratory processing.

DNA extraction, amplification and sequencing

DNA was extracted using the Qiagen „DNeasy Tissue Kit", following standard protocols. The reaction mixture for PCR was prepared according to the "HotMasterMix (2.5X)" (Eppendorf) reagent kit manufacturer's instructions. Amplification of the Cytochrome Oxidase Subunit I (COI) gene fragments was accomplished by using LCO-1490 (5' – GGTCAACAAATCATAAAGATATTGG – 3') and HCO-2198 (5' – TAAACTTCAGGGTGACCAAAAAATCA – 3') primers (29). Polymerase chain reaction began with a two minute initialization step at 94 °C, followed by 35 cycles of denaturation for 2 minutes at 94 °C, primer annealing for 45 seconds at 45 °C and fragment elongation for 1 minute at 65 °C. Each program ended with the final reaction of DNA synthesis lasting 7 minutes at 65 °C. PCR products were purified using Roche „High Pure PCR Product Purification Kit", following the manufacturer's instructions, and sequenced in one direction in Macrogen Inc. company (South Korea) using the LCO-1490 primer (29). Sequences are deposited in GenBank under accession numbers listed in Table 1.

Phylogenetic analyses

Our sample consisted of 55 COI sequences (53 *Carabus* + 2 outgroups), 560 bp long. The sequences were aligned using default settings in ClustalW (implemented in MEGA 5 *(29)*). The most appropriate evolutionary model for our data set was identified as TPM1uf+I+G, using the corrected Akaike information criterion implemented in jModelTest 2.1.1 *(30).*

Phylogenetic trees were reconstructed using maximum likelihood and Bayesian methods. Maximum likelihood (ML) analysis was performed using PhyML 3.0 *(31).* We used a custom TPM1uf model with fixed gamma shape parameter (0.531) and proportion of invariable sites (0.352), both chosen according to the results of the jModelTest analysis. The number of substitution rate categories was set to 4. Tree topology search operations were set to best of NNIs and SPRs, starting from 5 random trees. To estimate the node reliability, we used approximate likelihood ratio test with SH-like supports.

Bayesian analysis (BA) was conducted using MrBayes 3.1.2 *(32).* We used the same model parameters (nst = 6, shapepr = 0.531, pinvarpr = 0.352) as in ML analysis. To improve mixing of the cold chain and prevent it from becoming trapped in local optima, we used Metropolis-coupled Markov chain Monte Carlo (MCMC) method, with each run including a cold chain and three incrementally heated chains (8 chains in total for two parallel runs). The heating parameter was set to 0.2. We ran the analysis for 1.000.000 generations with all the parameters and trees sampled every 100 generations. Convergence between the two runs was monitored in Mr. Bayes through the standard deviation of split frequencies, and runs were continued until this value dropped to less than 0.01. Then, the convergence of each run towards stationarity was monitored with Tracer v. 1.4 *(33)* using likelihood values as well as all other parameters estimated. Stationarity was reached after 250000 of generations. Hence, 2500 trees were discarded as burn-in.

After the summarizing consensus tree was assigned, the posterior probabilities were obtained accordingly.

RESULTS

The phylogenetic analyses based on two different methods of phylogenetic reconstruction (Maximum likelihood and Bayesian analysis) resulted in similar topologies (Figures 1 and 2).

Half of the eight subgroups (Lipastrimorphi, Spinulati, Archicarabomorphi and Digitulati) based on endophallic characters of the male genitalia were recovered as monophyletic in both trees. Tachypogenici were monophyletic only in the ML tree, with moderate support. The monophyly of Arcifera, Metacarabi and Neocarabi was not supported by our data. The clade comprising Digitulati + Archicarabomorphi + Neocarabi was strongly supported in the ML tree (0.92 SH), while it was not supported in the BA tree. Both trees suggested a close relationship between Spinulati and Tachypogenici (0.92 SH and 0.96 PP). Lipastrimorphi were not clustered within Carabogenici (Digitulati + Archicarabomorphi + Lipastrimorphi, 19) as was recorded in previous studies *(18, 24).* In the ML tree Lipastrimorphi appeared as a sister group to the clade comprising subgenera *Oreocarabus*, *Nesaeocarabus* and *Eurycarabus* (Metacarabi), while in BA tree their relationship to other crown group members was unresolved.

All represented subgenera were monophyletic in both trees except for *Megodontus, Machrothorax* (only in BA tree) and *Tachypus* (moderate support in ML tree). Mono-

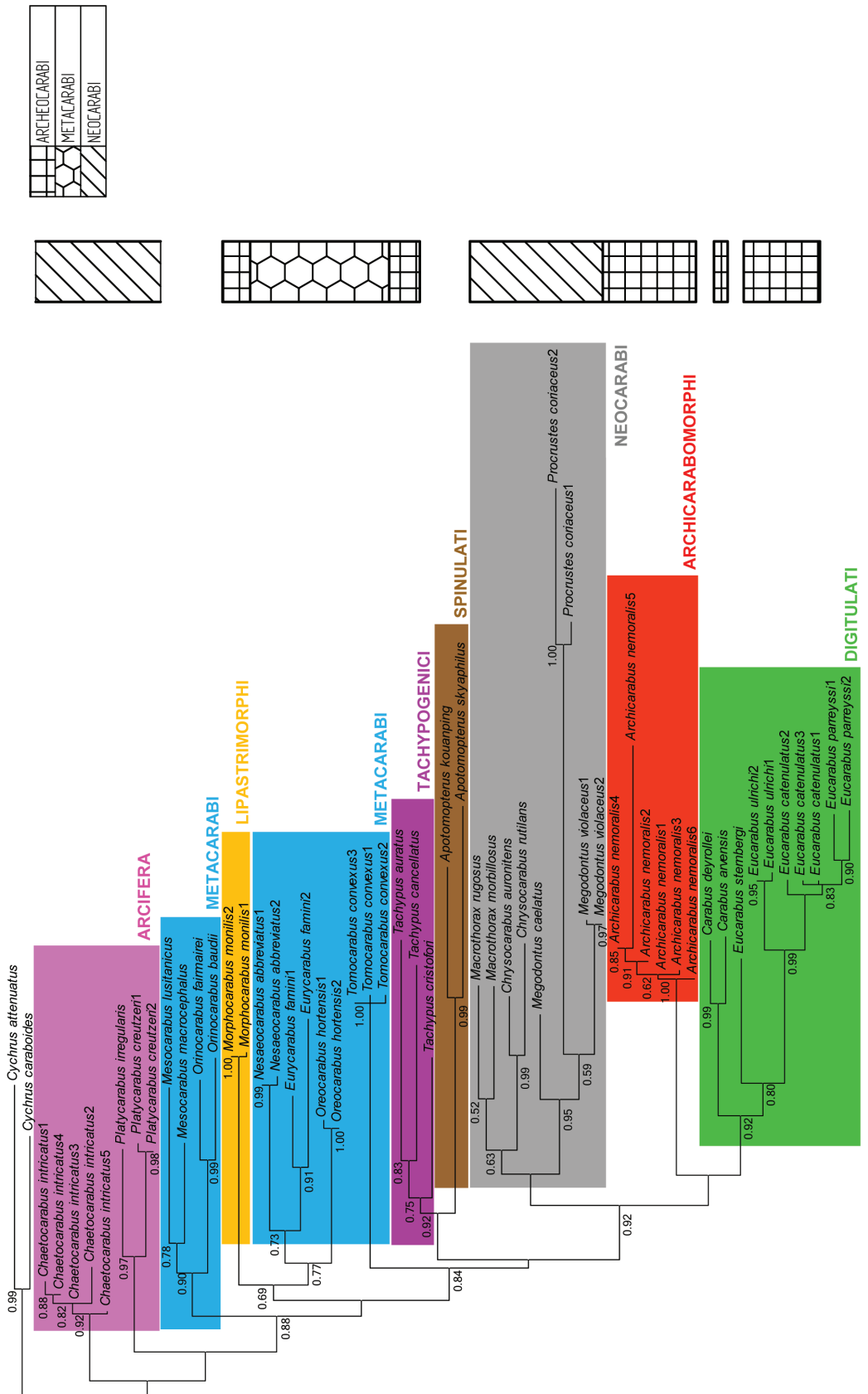


Figure 1. *The rooted Maximum Likelihood phylogram of 31 Carabus species and two outgroup Cychrus species under TPM1uf+I+G model of nucleotide substitution. Colours denote grouping based on the structure of endophallus. Different subgroups (Spinulati, Digitulati, Lipastrimorphi, Archicarabomorphi, Tachypogenici, Arcifera and Neocarabi) are covered by coloured rectangles, and the name of each subgroup is written using the same colour beside each rectangle. Groupings according to larval morphology (Archeocarabi, Metacarabi and Neocarabi) are shown in rectangles filled with lines and shown in the column beside the tree together with the explanatory legend. Numbers at nodes represent aLRT SH-like supports (only values ≥ 0.5 are shown).*

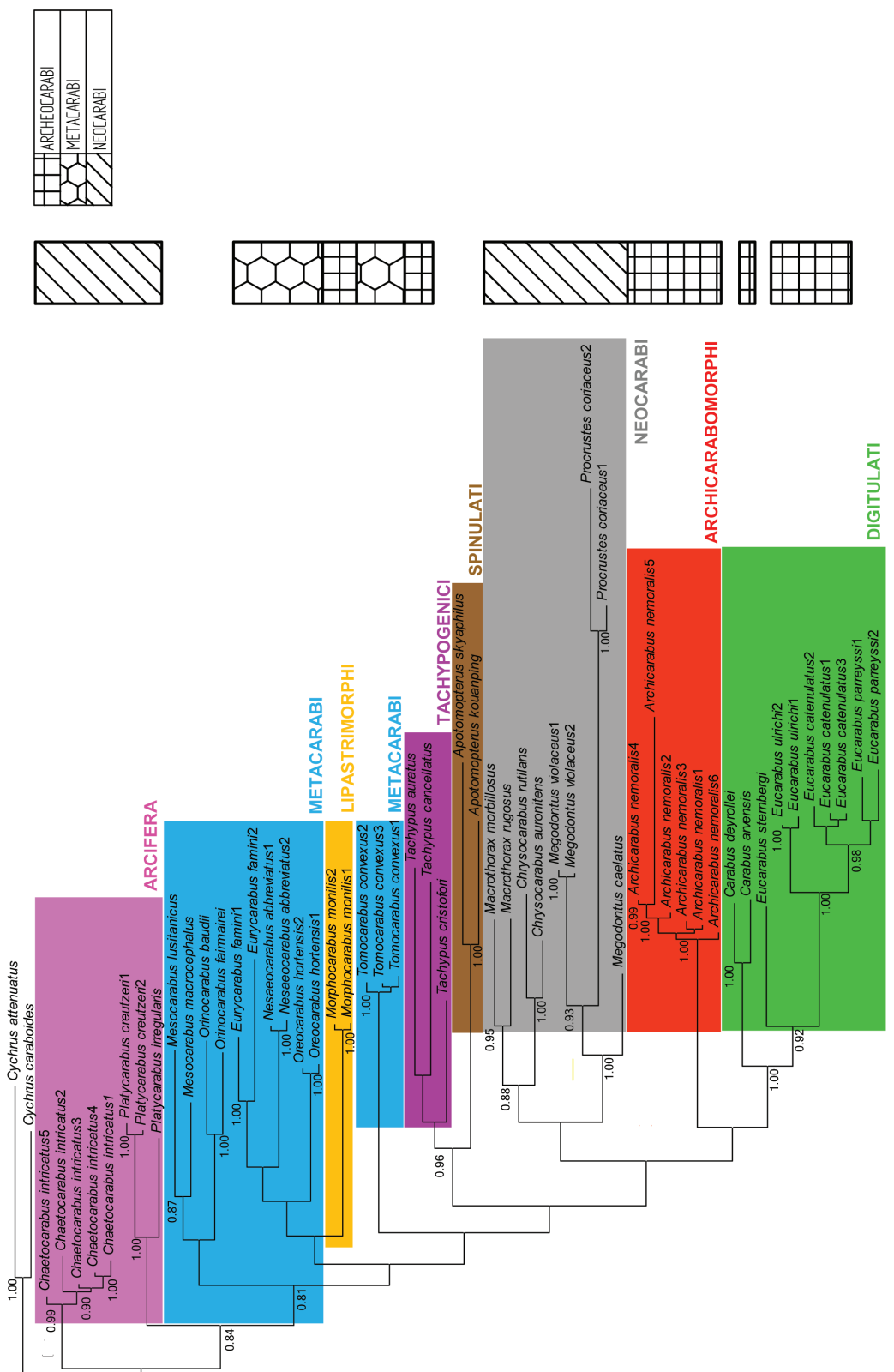


Figure 2. *The rooted Bayesian phylogram of 31 Carabus species and two outgroup Cychrus species using Metropolis-coupled Markov chain Monte Carlo (MCMC) method under TPM1uf+I+G model of nucleotide substitution. Different subgroups (Spinulati, Digitulati, Lipastrimorphi, Archicarabomorphi, Tachypogenici, Arcifera and Neocarabi) are covered by coloured rectangles, and the name of each subgroup is written using the same colour beside each rectangle. Groupings according to larval morphology (Archeocarabi, Metacarabi and Neocarabi) are shown in rectangles filled with lines and shown in the column beside the tree together with the explanatory legend. Numbers at nodes represent Bayesian posterior probabilities (only values ≥ 0.7 are shown).*

phyly of *Mesocarabus* was only moderately supported. On the ML tree subgenus *Orinocarabus* was sister to subgenus *Mesocarabus*, while subgenus *Eurycarabus* was sister to subgenus *Nesaeocarabus*. The same results were corroborated in previous research *(24).* Subgenus *Megodontus* was paraphyletic in both trees. *Megodontus caelatus* was sister to the clade comprising *Megodontus violaceus* + *Procrustes coriaceus*. Both trees supported the monophyly of the crown group excluding the two clades (*Chaetocarabus* and *Platycarabus*) traditionaly classified as Arcifera. The BA tree moderatly supported the sister status of *Platycarabus* relative to the crown group species, but this relationship was not supported in the ML tree.

Subgenus *Carabus* was sister to subgenus *Eucarabus* in both trees with good support. *Eucarabus ulrichi* and a clade comprising *Eucarabus parreyssi* and *Eucarabus catenulatus* were sister groups and formed a monophyletic group with other species belonging to the subgenera *Carabus* and *Eucarabus* (Digitulati).

DISCUSSION

According to Deuve *(1, 17),* the classification based on the structure of endophallus is nowadays more widely used than classification based on the structure of larval shields. Our results showed a greater concordance of molecular data with the former system than with the later one, justifying its increasing use. This is also supported by the fact that rapid and divergent evolution of male genitalia is one of the most widespread patterns of animal evolution *(34).*

This classification includes eight subgroups (Archicarabomorphi, Arcifera, Digitulati, Lipastrimorphi, Metacarabi, Neocarabi, Spinulati and Tachypogenici) and all of them were represented in this research by at least one species.

Our results largely corresponded to the phylograms obtained from the previous studies *(3, 18, 24). Chaetocarabus* and *Platycarabus* species (classified as Arcifera) were located at the base of the tree. In previous research subgroup Arcifera was found as a sister group to the remaining *Carabus* species *(18)* whereas in our study the position of Arcifera was unresolved probably due to a small number of species included.

In both trees the species *Megodontus caelatus* was sister to the clade comprising *Megodontus violaceus* + *Procrustes coriaceus*. The same result was obtained by Deuve et al. for mtDNA (24). When nuclear DNA was analysed, subgenus *Megodontus* was found monophyletic *(18, 24).* According to Sota and Vogler *(35)* the analysis of the nuclear genes provided data more compatible with the morphological classification than those made using mitochondrial DNA. However, mitochondrial genes have many advantages in molecular analysis and therefore are often used in constructing phylogenetic trees.

Digitulati, Archicarabomorphi and Lipastrimorphi grouped together in Carabogenici division *(19)* were not supported as monophyletic neither by Sota and Ishikawa *(18)* nor by our own study, but were not rejected by Deuve *et al. (24).*

However, our results must be considered with caution because two of the eight subgroups are represented by only one species (Archicarabomorphi and Lipastrimorphi), while additional two were represented by only one subgenus (Spinulati and Tachypogenici).

Within Digitulati *C. deyrollei* and *C. arvensis* were grouped together. The first one is a mountain species; endemic to Iberian Peninsula and the second one is a Palearctic species distributed thorough Northern and Central Europe and Siberia, all the way to Sakhlin, with no presence in Iberian Peninsula *(36).* In Croatia *C. arvensis* is present in Slavonia, the North-Eastern part of the country *(2).*

Eucarabus ulrichi and clade comprising *Eucarabus catenulatus* and *Eucarabus parreyssi,* the species of special interest for this research, were sister groups and monophyletic with other species belonging to the subgenera *Carabus* and *Eucarabus*, justifying their placement within the Digitulati subgroup. These two subgenera were also obtained as sister groups in previous researches *(18, 37).*

Eucarabus ulrichi inhabits deciduous forests and open habitats, from lowlands to the hills and mountains up to 500 m a.s.l. on warmer exposures in Central and South – East Europe *(38),* and in Croatia it comes in the North and North-Eastern part of the country *(39). Eucarabus catenulatus* and *Eucarabus parreyssi* have narrow distribution. *Eucarabus catenulatus* has Alpine-Dinaric distribution (South Switzerland, Central-North and North-Eastern Alps in Italy, Slovenia, Western Croatia and Bosnia and Herzegovina) *(38). Eucarabus parreyssi* is a species endemic to Dinaric Alps and distributed in South Croatia, North-West and South Bosnia and Herzegovina to the Northern part of Montenegro. These species are considered vicariant *(36),* geographically and ecologically, and thus have undergone genotypic and phenotypic divergence. Although, their adults morphologically resemble each other, males can be separated by the shape of their *edeagus*. According to the genetic data presented in this preliminary study, these species may still hybridise on the borders of their areals. The fact that these two closely related species were not clearly separated in neither of our trees attests to this possibility. In Croatia, *Eucarabus catenulatus* is present mainly in forests from lowland to mountain zones, more south-west exposed, on calcareous soil *(36),* while *Eucarabus parreyssi*, inhabits subalpine and alpine habitats (Šerić Jelaska, personal observations on Mt.Velebit).

Further insights into their distribution, ecological constrains and genetic differences between populations may

give us more details on speciation events, as well as on some future prospects due to present environmental changes, such as climate change, that can impact the cold adapted high mountain species. The sequences for *C. ulrichi* and *C. parreyssi* have been used in this study for the first time to obtain the phylogenetic trees.

In congruence with previous studies *(3, 18, 24),* phylogenetic trees obtained in this study justify the more frequent use of the classification of the genus *Carabus* based on the structure of endophallus than of the other one, based on the frontal shields of larvae. Interpretation combining both, morphological and molecular data together is essential for obtaining the most precise phylogenetic reconstructions *(40). Carabus* species from the western parts of the Balkan Peninsula, mainly endemics, were so far underrepresented in molecular systematic analyses. As the topology of several groups still remains uncertain, further molecular phylogeny studies are required, with a larger number of species sampled, including other Balkan's endemic species, and additional molecular markers.

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ORIGINAL ARTICLE

Variations in body shape of mountain habitat specialist *Carabus croaticus* and its sister species *Carabus caelatus* (Coleoptera: Carabidae) populations across Dinaric Alps

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Abstract
There are abundant phenotypic variations of *Carabus croaticus* Dejean 1826, an endemic species of the Dinaric Alps, and its sister species *Carabus caelatus* Fabricius 1801, resulting in taxonomic inflation at intraspecific level, synonymy and inconsistency between relevant catalogues and checklists. The main aims of this research were to explore population structure based on morphology and geographic patterns of phenotypic variability, and compare the results with taxa described by nonmetric visual comparisons of morphological traits. Our study included 224 specimens of *C. croaticus* and 192 specimens of *C. caelatus*, covering most of their distributional range. Shapes of the pronotum and head were analysed using geometric morphometrics (GMM). Principal component and canonical variate analyses were used to characterize the main features of shape variation between populations and mountain ranges. GMM delimitated interspecific morphological variations but at the intraspecific level it showed many overlaps within populations for both species. Conducting the morphological analyses for the first time on most of the described phenotypic variants of studied species, we wanted to provide a new evidence for the possible solution of the taxonomic relations within these two endemic species by measuring body shape variability, and thus to enable a better understanding of the evolutionary processes in the Dinaric Mountains.

Keywords Balkan Peninsula · Ground beetles · Endemic species · Geometric morphometrics · Speciation · Morphological variability

Introduction

The Balkan Peninsula is known as a region of high endemicity (Oosterbroek and Arntzen 1992; De Jong 1998) and it is one of the 25 World's biodiversity hotspots (Myers et al. 2000). The geographic position and topography

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combined with Earth's historical events during and after the last glacial maximum resulted in numerous endemic species and the biggest species diversity among refugial areas in Europe (Huntley and Birks 1983; Birks and Line 1993). The Balkan Peninsula played one of the most important roles in European biodiversity conservation and served as a centre point for spreading species after the last glacial maximum (Taberlet et al. 1998; Hewitt 1999, 2000). Undoubtedly, one of the greatest natural treasures of the Balkan Peninsula is Dinaric karst, a place with a rich geological history and diversity of flora and fauna (Sket 1996).

Dinaric Alps, Dinaric Mountains or Dinarides is a mountain chain composed of more than 200 mountains extending from the southern edges of the Eastern Alps in Slovenia and Italy, across the western side of the Balkan Peninsula, until it reaches Pindus mountain chain in northern Albania and Sharri mountain system. Dinaric Alps are around 650–700 km long and 50–200 km wide. The height of the majority of the mountains is between 1000 and 2000 m (Papac 2014).

Numerous mountains within Dinaric chain resulted in different habitat types and consequently in the development of various microclimate conditions that contribute to the biodiversity of this area with high species and intraspecific variabilities evolved especially within species with weak dispersal power.

In this study, variations in morphological traits will be analysed in two sister species belonging to the subgenus *Megodontus, Carabus croaticus* Dejean 1826 a mountain habitat specialist, endemic to the Dinaric Alps, and *Carabus caelatus* Fabricius 1801 endemic to the Alps, Dinarides and western Balkan, along their distribution across the Dinaric Mountains. Their biology, habitat preferences, environmental conditions and conservation status have not yet been fully known (Šerić Jelaska et al. 2014). *Carabus croaticus* is distributed in the west part of the Balkan Peninsula along Dinaric Mountains

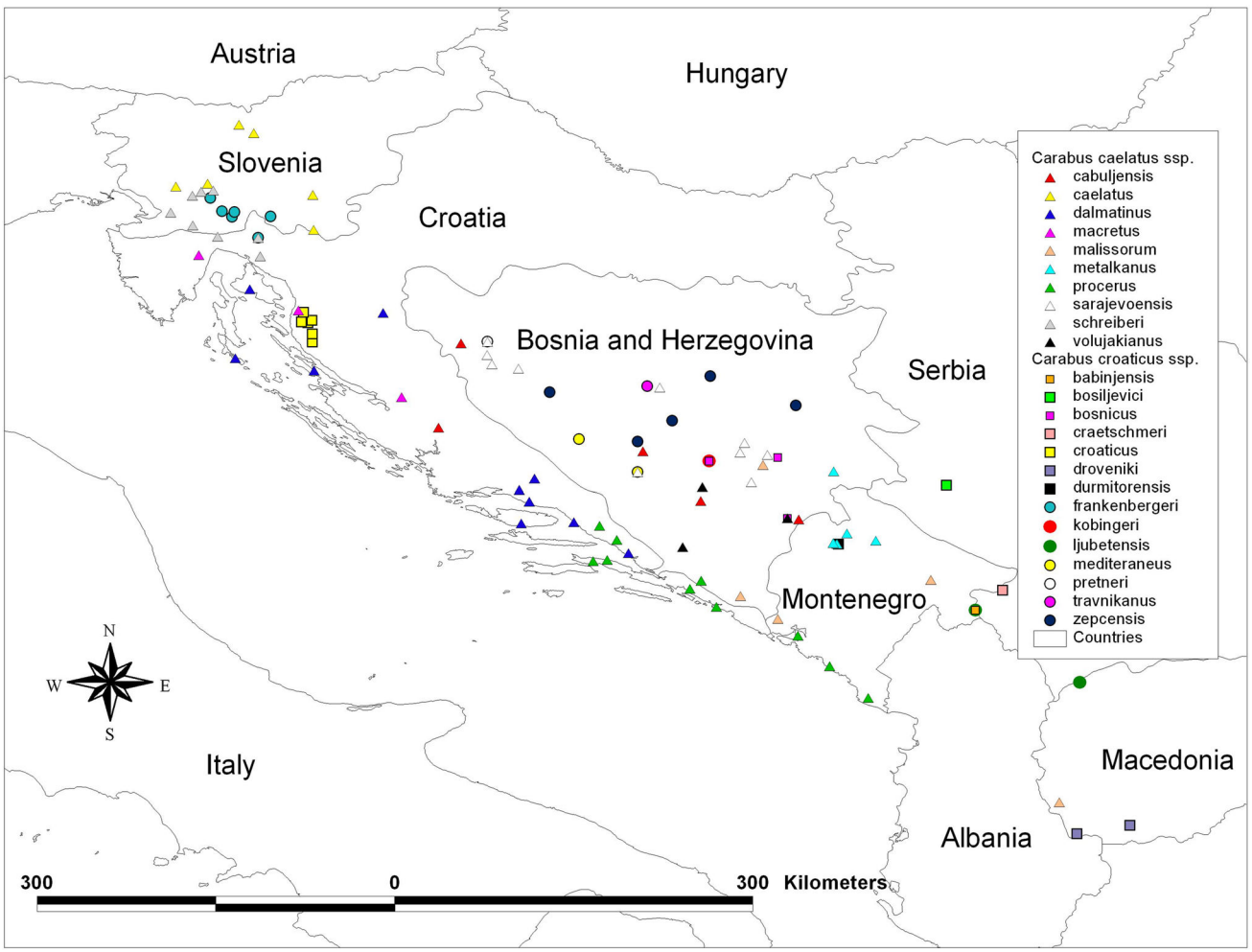


Fig. 1 Map of the study area (Dinaric Mountains, Balkan Peninsula) with sampling sites. Squares and circles represent subspecies of *Carabus croaticus*, while triangles represent subspecies of *C. caelatus* used in the analyses

Table 1 *Carabus croaticus* Dejean 1826 subspecies

Drovenik and Peks (1999)	Turin et al. (2003)	Deuve (2004)	Bousquet et al. (2017)
Subsp. *croaticus* Dejean 1826	Subsp. *croaticus* Dejean 1826 (including *frankenbergeri* Obenberger 1914)	Subsp. *croaticus* Dejean 1826 (including *albiensis* Depoli 1938, *carnicus* Csiki 1927, *carniolicus* Gehin 1885, *primarius* Lapouge 1902b, *schmidti* Apfelbeck 1890a)	Subsp. *croaticus* Dejean 1826 (including *albiensis* Depoli 1938, *frankenbergeri* Obenberger 1914, *mediterraneus* Apfelbeck 1919, *carnicus* Csiki 1927, *carniolicus* Gehin 1885, *fodori* Csiki 1927, *primarius* Lapouge 1902b, *schmidti* Apfelbeck 1890a)
Subs. *frankenbergeri* Obenberger 1914		Subs. *frankenbergeri* Obenberger 1914	
Subsp. *kobingeri* Apfelbeck 1904	Subsp. *kobingeri* Apfelbeck 1904 (described as subs. *pretneri* Krätschmer and Drovenik 1977)	Subsp. *kobingeri* Apfelbeck 1904	
Subsp. *pretneri* Krätschmer and Drovenik 1977		Subsp. *pretneri* Krätschmer and Drovenik 1977	
Subsp. *bosnicus* Apfelbeck 1890a	Subsp. *bosnicus* Apfelbeck 1890a (=*zepcensis* Reitter 1902b)	Subsp. *bosnicus* Apfelbeck 1890a (=*leonhardianus* Breuning 1932)	Subsp. *bosnicus* Apfelbeck 1890a (including *kobingeri* Apfelbeck 1904, *pretneri* Krätschmer and Drovenik 1977)
Subsp. *durmitorensis* Apfelbeck 1904	Subsp. *durmitorensis* Apfelbeck 1904	Subsp. *durmitorensis* Apfelbeck 1904	Subsp. *durmitorensis* Apfelbeck 1904 (including *babinjensis* Apfelbeck 1919, *droveniki* Krätschmer 1984, *kraetschmeri* Drovenik 1978, *ljubetensis* Apfelbeck 1918a)
Subsp. *zepcensis* Reitter 1902b		Subsp. *zepcensis* Reitter 1902b	Subsp. *zepcensis* Reitter 1902b (including *bosiljevici* Drovenik and Pavićević 1985)
Subsp. *ljubetensis* Apfelbeck 1918a	Subsp. *ljubetensis* Apfelbeck 1918a	Subsp. *ljubetensis* Apfelbeck 1918a	
Subsp. *babinjensis* Apfelbeck 1919	Subsp. *babinjensis* Apfelbeck 1919	Subsp. *babinjensis* Apfelbeck 1919	
Subsp. *bosiljevici* Drovenik and Pavićević 1985		Subsp. *bosiljevici* Drovenik and Pavićević 1985	
Subsp. *kraetschmeri* Drovenik 1978		Subsp. *kraetschmeri* Drovenik 1978	
Subsp. *mediterraneus* Apfelbeck 1919		Subsp. *fodori* Csiki 1927 (=*mediterraneus* Apfelbeck 1919)	
Subsp. *droveniki* Kraetschmer 1984		Subsp. *droveniki* Kraetschmer 1984	
			Subs. *antoniocaldoni* Rapuzzi 2014

Table 2 *Carabus caelatus* Fabricius 1801 subspecies

Drovenik and Peks (1999)	Turin et al. (2003)	Deuve (2004)	Bousquet et al. (2017)
Subsp. *caelatus* Fabricius 1801	Subsp. *caelatus* Fabricius 1801	Subsp. *caelatus* Fabricius 1801 (including *caelatus* Fabricius 1801(including *carniolicus* Crotch 1871), *schreiberi* Kraatz 1877 (including *grmecensis* Born 1910c, *sarajevoensis* Apfelbeck 1890a), *volujakianus* Apfelbeck 1894 (including *hilfi* Born 1907a), *malissorum* Apfelbeck 1919, *metalkanus* Apfelbeck 1919)	Subsp. *caelatus* Fabricius 1801 (including *carniolicus* Crotch 1871, *hilfi* Born 1907a, *malissorum* Apfelbeck 1919, *metalkanus* Apfelbeck 1919, *sarajevoensis* Apfelbeck 1890a, *volujakianus* Apfelbeck 1894)
Subsp. *schreiberi* Kraatz 1877f	Subsp. *schreiberi* Kraatz 1877f		Subsp. *schreiberi* Kraatz 1877f (including *grmecensis* Born 1911c)
Subsp. *dalmatinus* Duftschmid 1812	Subsp. *dalmatinus* Duftschmid 1812 (subsp. *procerus* Reitter 1885)	Subsp. *dalmatinus* Duftschmid 1812 (including *dalmatinus* Duftschmid 1812, *macretus* Kraatz 1887f (including *dinaricola* J. Muller 1930b), *procerus* Reitter 1885b (including *ljubinjensis* Haury 1885), *cabuljensis* Apfelbeck 1919)	Subsp. *dalmatinus* Duftschmid 1812 (including *cabuljensis* Apfelbeck 1919, *macretus* Kraatz 1877f, *dinaricola* J. Muller 1930b, *procerus* Reitter 1885b)
Subsp. *procerus* Reitter 1885			
Subsp. *sarajevoensis* Apfelbeck 1890a	Subsp. *sarajevoensis* Apfelbeck 1890a		
Subs. *volujakianus* Apfelbeck 1894			
Subs. *malissorum* Apfelbeck 1919			
Subs. *metalkanus* Apfelbeck 1919			
Subs. *macretus* Kraatz 1877f			
Subs. *cabuljensis* Apfelbeck 1919			

where it has an inland distribution (Fig. 1), (Turin et al. 2003). It is found from 900 m (750 m pers. observ.) up to 2400 m, mostly in the fir, pine and beech forests (Turin et al. 2003). Adults occur from May to September, but the most numerous are in the period of June–July (Turin et al. 2003, pers. observ.). Unlike the first species, *C. caelatus* occurs from the sea level up to 2200 m. It is widespread in the Balkan Peninsula (Fig. 1). The most abundant is in the middle forest zone and absent in the alpine steppes (Turin et al. 2003). Adults occur from May to August (Turin et al. 2003). Geographic isolation and different ecological conditions resulted in the emergence of phenotypic variants of both species and described intraspecific taxa. Comprising data from catalogues and monographs (Drovenik and Peks 1999; Turin et al. 2003; Deuve 2004; Bousquet et al. 2017) we counted 21 taxa for *C. croaticus* (Table 1) and 15 taxa for *C. caelatus* (Table 2). An incautious use of the term subspecies for every new observed and described phenotypic variant led to the existence of diverse taxonomic classifications. The absence of morphological and genetic analyses as well as the identification of subspecies through subjective visual comparisons of morphological traits (size, colour, elytral sculpture, reduction of 4th male protarsal segment, etc.) resulted in different groupings of certain variants as subspecies among various authors (Tables 1, 2). At this point, the knowledge of biology, ecology, and evolution of studied taxa is insufficient to set the basis for resolving intraspecific taxonomic relations. Therefore, we decided to perform, for the first time, morphological analysis (geometric morphometrics) on a large sample involving most of the described phenotypic variants, i.e. subspecies.

Geometric morphometrics (GMM) is a powerful analytical and graphical tool for the quantification and visualization of morphological variations (Alibert et al. 2001) that is becoming increasingly used in taxonomy and systematics (Adams and Rohlf 2000; Mutanen and Pretorius 2007; Marchiori et al. 2007; Hopkins and Thurman 2010; Worthington et al. 2012; Adams et al. 2013; Angielczyk and Feldman 2013; Milankov et al. 2013). Hence we decided to utilise GMM on these two endemic sister species to (a) compare populations along their distribution

Table 3 Number of specimens of both species included in GMM analyses

Subspecies	Country	No. of specimens (GMM-pronotum shape)		No. of specimens (GMM-head shape)	
		M	F	M	F
C. caelatus cabuljensis	SE BA	6	7	6	7
C. caelatus caelatus	N SI	10	12	10	12
C. caelatus dalmatinus	S HR and ISLANDS	11	12	11	12
C. caelatus macretus	W HR	9	6	8	5
C. caelatus malissorum	C, E ME	3	6	3	5
C. caelatus metalkanus	E BIH, N ME	10	9	10	9
C. caelatus procerus	SW ME, S HR, S BA	12	12	13	10
C. caelatus sarajevoensis	C, SE, E BA	11	10	11	10
C. caelatus schreiberi	W HR, SW SI	15	21	14	21
C. caelatus volujakianus	SE BA	4	5	3	6
Total		91	101	89	97
C. croaticus babinjensis	W, SW MC	0	3	1	2
C. croaticus bosiljevici	SW RS	2	0	2	0
C. croaticus bosnicus	C, E, SE BA	10	10	9	11
C. croaticus kraetschmeri	E ME	3	3	3	3
C. croaticus croaticus	SW HR	10	8	10	8
C. croaticus droveniki	NE, SW MD	1	1	2	0
C. croaticus durmitorensis	N ME	4	1	4	1
C. croaticus frankenbergeri	NW HR, S SI	38	42	37	41
C. croaticus kobingeri	C BA	10	10	11	9
C. croaticus ljubetensis	SW ME, S XK	2	4	2	4
C. croaticus mediterraneus	W, SW BA	13	4	13	4
C. croaticus pretneri	NW BA	7	8	7	8
C. croaticus travnikanus	C BA	4	5	4	5
C. croaticus zepcensis	C BA	10	10	10	10
Total		114	109	115	106

Subspecies were annotated by B. Drovenik, P. Durbešić and authors

Bosnia and Herzegovina (*BA*), Croatia (HR), Macedonia (MK), Monte Negro (ME), Republic of Kosovo (XK), Serbia (RS), and Slovenia (SI)

M males, *F* females

across Dinaric Alps based on morphology and explore geographic patterns of phenotypic variants and (b) to compare our results to the taxonomic classifications based on nonmetric and subjective visual comparisons of morphological traits present in the literature. The results can help to provide new evidence that contributes to the resolving intraspecific taxonomy and phylogeny, and open a possibility for developing a proper conservation strategy providing deeper knowledge of evolutionary processes that occur within the Dinaric mountain chain.

Materials and methods

For GMM a total of 192 *C. caelatus* (91 males, 101 females) and 224 *C. croaticus* specimens (115 males, 109 females), belonging to the collections of B. Drovenik, P. Durbešić, S. Hristovski, L. Šerić Jelaska, Ž. Jambrošić Vladić, were examined. The material originated from Bosnia and Herzegovina (BA), Croatia (HR), Macedonia (MK), Monte Negro (ME), Republic of Kosovo (XK), Serbia (RS), and Slovenia (SI) (Fig. 1). For each specimen, we took high-resolution digital images of the dorsal view of the head and the pronotum using a Fujifilm HS30EXR camera. Using tripod and angle headset we ensured that the objective was always parallel to the head or pronotum surface. The 2D images were double checked to avoid any distortions of the picture following Fontaneto et al. (2017). To record scale, we used a graph paper which was placed next to the specimens.

According to the linear measurements (unpublished data), the most statistically significant differences between phenotypic variants were observed in pronotum and head length and width. Therefore, we decided to check differences in the shapes of the head and pronotum using GMM analysis. To analyse the shape of the pronotum and the head, a 2D dataset was used, consisting of 186 images of the head (89 males, 97 females) and 192 images (91 males, 101 females) of the pronotum of *C. caelatus*, and 221 images (115 males, 106 females) of the head and 223 (114 males, 109 females) images of the pronotum of *C. croaticus* (Table 3). A total of ten landmarks were digitized on the pronotum and nine on the head (Fig. 2, modified from Ober and Connolly 2015) using TPSdig2 software version 2.26 (Rohlf 2001).

The shape information was extracted using a Procustes fit (Rohlf and Slice 1990; Dryden and Mardia 1998; Viscosi and Cardini 2011). To avoid any influence of measurement error, a sample of 50 individuals for head and 50 individuals for pronotum were digitized twice following the same procedure below and digitized by the same operator. Using this data, a Procustes ANOVA was calculated (Klingenberg and McIntyre 1998). The mean squares (MS) relate to the individual effect were used as an estimator of an individual's variation and compared with the MS of the digitized error 1.

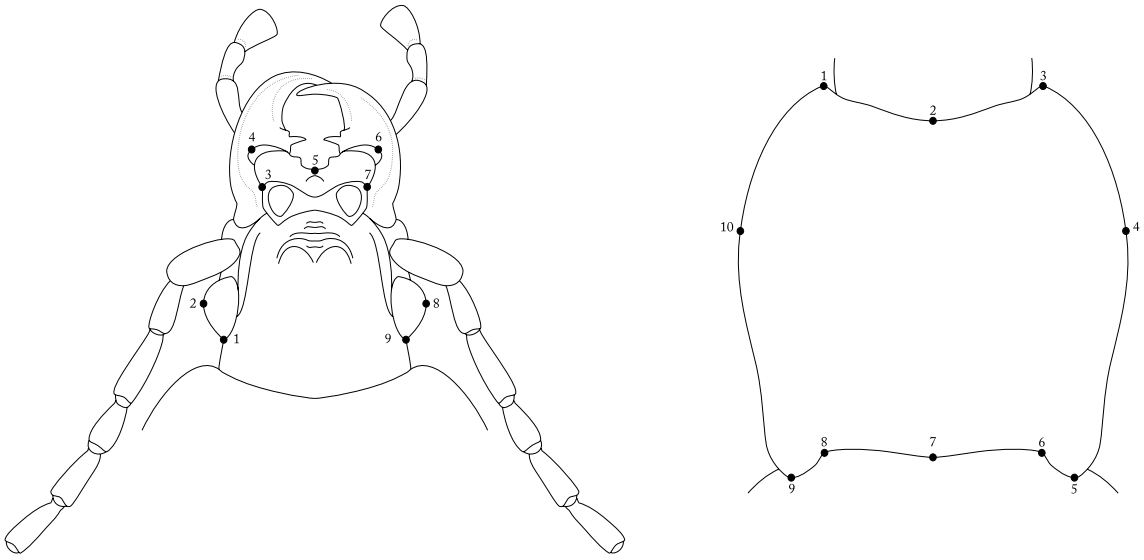


Fig. 2 Location of the nine landmarks on the head, and ten landmarks on the pronotum of the specimens of *Carabus* sp.

Table 4 Measurement error procrustes ANOVA for both centroid size and shape of *Carabus*, sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless)

Effect	SS	MS	*df*	*F*	*P* (param.)	Pillai tr.	*P* (param.)
Centroid size							
Individual (H)	42.69735	0.871374	49	133.8	< 0.0001		
Error 1 (H)	0.32566	0.006513	50				
Individual (P)	167.374436	3.415805	49	117.7	< 0.0001		
Error 1 (P)	1.450761	0.029015	50				
Shape							
Individual	0.1762633	0.000256944	686	17.95	< 0.0001	10.79	< 0.0001
Error 1	0.01002096	1.43157E−05	700				
Individual (P)	0.18536712	0.000236438	784	14.4	< 0.0001	13.14	< 0.0001
Error 1 (P)	0.01313793	1.64224E−05	800				

H head, *P* pronotum

The patterns of shape variation were visualized by carrying out a principal component analysis (PCA), computed from the covariance matrix of the individual shape (Klingenberg et al. 2002) to illustrate the shape variation between species and sex independently in a multidimensional space. To "amplify the magnitude" of the visualization and statistically assess whether there were differences between the different subspecies, a canonical variate analysis (CVA) was also performed. CVA maximizes the differences between groups relative to the variation within groups and it is consequently one of the most applied tools to discriminate among groups, nevertheless it is well-known that this method is used only when the variation is not strictly clear at the first stage on the PCA (Campbell and Atchley 1981). All multivariate analyses were performed using MorphoJ software version 1.06d (Klingenberg 2011).

Results

The Procrustes ANOVA for assessing the measurement error in head and pronotum showed that the MS for individual variation exceeded the measurement error (Table 4).

Principal component analyses (PCA) showed a good interspecific delimitation based on the shape of the pronotum (the first and the second principal components explained 56.6% and 9.2%, respectively) and on the shape of the head (the first and the second principal components explained 46.3% and 16.9%, respectively) (Fig. 3).

Sexual shape dimorphism was assessed and visualized particularly by the separation of points at the PC1 in the head and pronotum for both species. In the head shapes of both species, wider shapes were noticed in males than in females (expansion of landmarks nos. 8 and 9 in males of *C. caelatus* and less obvious expansion of the landmark no.

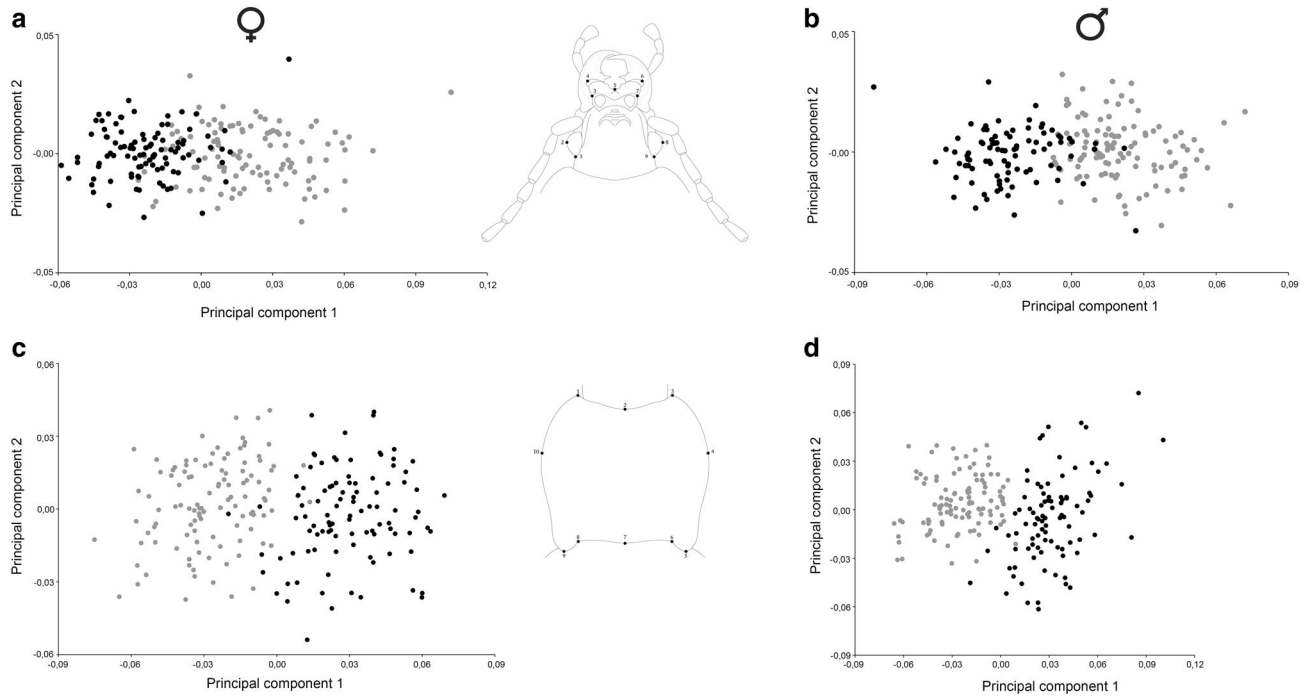


Fig. 3 Principal component analysis (PCA) showing the shape variance between the two sister species *C. croaticus* (grey) and *C. caelatus* (black) in the head (**a, b**) and pronotum (**c, d**)

2 in males of *C. croaticus*) (Fig. 4a). In the shapes of pronotum, sexual shape dimorphism was barely observable. Males of both species had slightly wider pronotum shapes than females (elongation of the pronotum landmarks nos. 1 and 3 in males of both species) (Fig. 4b).

According to the shape of the pronotum for both sexes (Fig. 5), there was a noticeable delimitation of the Velebit Mountain population in Croatia, (subspecies *C. croaticus croaticus*), from the specimens sampled in Monte Negro, Serbia and Macedonia (subspecies *C. croaticus durmitorensis, C. croaticus kraetschmeri, C. croaticus bosiljevici, C. croaticus droveniki*). Intraspecific delimitation based on the shape of the head for populations of *C. caelatus* did not show any clear separations (as well as for populations of *C. croaticus*) whereas delimitation based on the shape of the pronotum separated the eastern Bosnia and Herzegovina and northern Monte Negro populations (subspecies *C. caelatus metalkanus*) from the specimens from south Croatia (islands and mainland), southern Bosnia and Herzegovina, and south-western Monte Negro (subspecies *C. caelatus dalmatinus* and *C. caelatus procerus*) (Fig. 5).

Shape variations in the head and pronotum among populations from different mountain ranges were present in both species, but no other clear pattern was discernible.

Comparison with current classifications

Using CVA analyses of the head and pronotum shapes, we found out the best matching of our results (pronotum shape) with classification according to Bousquet et al. (2017) for populations of both species (Fig. 6). Results were compared with four classifications (Drovenik and Peks 1999; Turin et al. 2003; Deuve 2004; Bousquet et al. 2017).

GMM of the pronotum showed existence of transitional shape of populations between populations grouped in separated subspecies. Thus, the population from north-western Bosnia and Herzegovina (*C. croaticus pretneri*) seemed to connect the group of populations from Croatia and Slovenia (*C. croaticus croaticus*) with the group of populations from Bosnia and Herzegovina (*C. croaticus bosnicus*) (Online Resource 1). Populations from western Croatia (*C. caelatus macretus*) developed a transitional form between populations sampled in north-western Croatia and southern Slovenia (*C. caelatus schreiberi* and *C. caelatus caelatus*), and those sampled in southern Croatia and islands, southern Bosnia and Herzegovina and south-western Monte Negro (*C. caelatus procerus* and *C. caelatus dalmatinus*), while populations from south-eastern Bosnia and Herzegovina (*C. caelatus cabuljensis*) showed a transition form, between populations from Bosnia and Herzegovina and Monte Negro (*C. caelatus malissorum, C. caelatus metalkanus, C. caelatus sarajevoensis* and *C. caelatus volujakianus*), and those

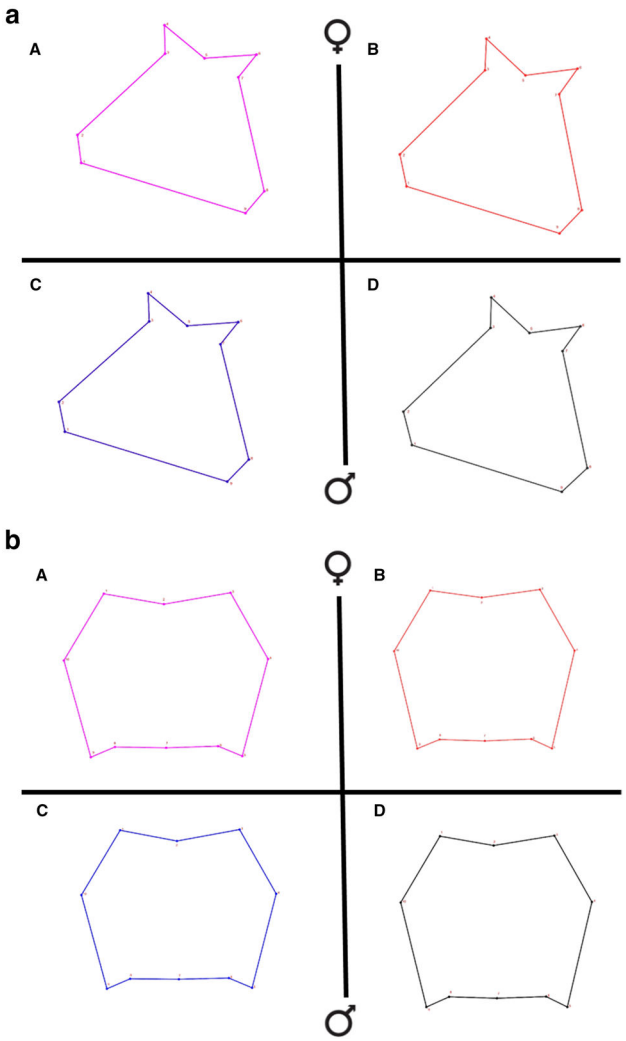


Fig. 4 Wireframe representation of the average shape between species and sex **a** head shapes (A/pink and C/blue = *C. croaticus*; B/red and D/black = *C. caelatus*), **b** pronotum shapes (A/pink and C/blue = *C. croaticus*; B/red and D/black = *C. caelatus*). (Color figure online)

from the southern part of the Balkan Peninsula (*C. caelatus procerus* and *C. caelatus dalmatinus*) (Online Resource 1).

Discussion

For the first time, geometric morphometrics of the head and pronotum shapes of *Carabus caelatus* and *C. croaticus* has been applied to compare phenotypic variants of their populations distributed across Dinaric karst, trying to clarify quandaries in their taxonomy. Our results confirmed GMM as a useful method in species delimitation, and at the intraspecific level, they indicated a continuous series of populations in both species.

GMM of the pronotum shape separated the populations from south-eastern parts of Dinaric Alps (*C. caelatus procerus* and *C. caelatus dalmatinus*) into one group while the populations from the western part of Croatia (*C. caelatus macretus*) developed a transitional shape between populations sampled in north-western Croatia and southern Slovenia (*C. caelatus schreiberi*) and populations sampled in southern Croatia and islands (*C. caelatus procerus* and *C. caelatus dalmatinus*). Regarding the distribution area and events during the last glacial maximum (sea level dropping), it could be highly likely that the phenotypic variant named as *C. caelatus macretus* is a link between costal and mainland populations of *C. caelatus* species.

As described above, the results of GMM showed the existence of populations that expressed transitional shapes between populations grouped into subspecies that have been listed in the latest catalogue of Bousquet et al. (2017), and these transitional populations are geographically situated in-between. To check for existence of partial gene flow or the recent separation of these populations, further DNA analyses may be employed.

Comparison of our result with the four classifications (Drovenik and Peks 1999; Turin et al. 2003; Deuve 2004; Bousquet et al. 2017) showed the best matching with the latest classification by Bousquet et al. (2017) for both species.

In the case of *C. croaticus* species, Bousquet et al. (2017) recognized five main subspecies (*C. croaticus croaticus, C. croaticus bosnicus, C. croaticus durmitorensis, C. croaticus zepcensis*, and *C. croaticus antoniocaldoni*), while our results, based on the GMM of the pronotum, form three groups of populations corresponding to subspecies *C. croaticus croaticus, C. croaticus bosnicus* and *C. croaticus durmitorensis*. Populations described as *C. croaticus zepcensis* were grouped together with other populations from Bosnia and Herzegovina within subspecies *C. croaticus bosnicus* (Fig. 6). Turin et al. (2003) classified *C. croaticus zepcensis* into *C. croaticus bosnicus* subspecies while Drovenik and Peks (1999) and Deuve (2004) differentiated subspecies *C. croaticus zepcensis* and *C. croaticus bosiljevici* (according to Bousquet et al. (2017) this taxon belongs into subspecies *C. croaticus zepcensis*) as detached subspecies. For further clarifications, GMM and genetic analyses should be employed on additional dataset.

Our study did not include individuals described as subspecies *C. croaticus antoniocaldoni* (Rapuzzi 2014) due to the lack of the samples. Anyway, additional analyses including ssp. *antoniocaldoni* could be intriguing since this subspecies seems to be very different from other *C. croaticus* forms and according to Rapuzzi (2014) is probably one of the most distinct subspecies (black body color without metallic hue, with thick head, and small, not sinuated pronotum).

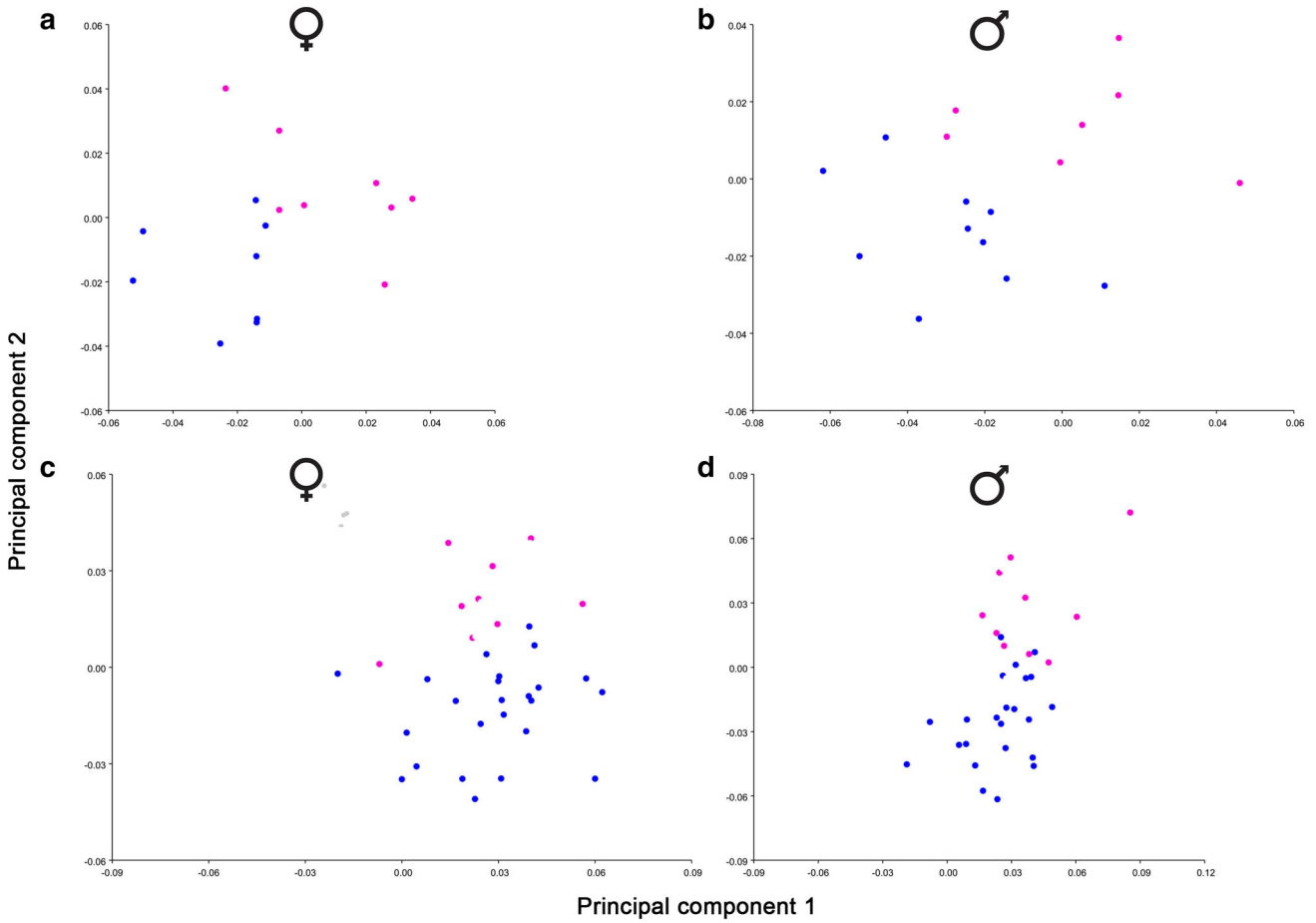


Fig. 5 Principal component analysis showing pronotum shape variations within *C. croaticus* females (**a**) and males (**b**), and *C. caelatus* females (**c**) and males (**d**). Each point represents a pronotum shape for *C. croaticus* or *C. caelatus* specimens. **a, b** blue points represent *C. croaticus* specimens from Velebit Mountain (north western HR), and pink points represent *C. croaticus* specimens from MK, ME and RS. **c, d** blue points represent *C. caelatus* specimens from southern HR, southern BA and south-western ME, and pink points represent *C. caelatus* specimens from eastern BA and northern ME. (Color figure online)

Within *C. caelatus* species Bousquet et al. (2017) distinguished three subspecies (*C. caelatus caelatus, C. caelatus schreiberi* and *C. caelatus dalmatinus*). Our results comply with this classification with the following exception—two neighbouring populations annotated as subspecies *C. caelatus caelatus* and *C. caelatus schreiberi* were grouped together. GMM of the pronotum and head did not separate northern Slovenia populations (annotated as *C. caelatus caelatus*) from populations from southern Slovenia and western Croatia (annotated as *C. caelatus schreiberi*). Besides, the geographical vicinity of these populations may go in favour of their grouping based on body shape. Comparing relevant classifications (Drovenik and Peks 1999; Turin et al. 2003; Deuve 2004; Bousquet et al. 2017), only Deuve (2004) grouped subspecies *C. caelatus schreiberi* within subspecies *C. caelatus caelatus*. Our results showed overlapping of the pronotum and head shapes for these populations pointing to the possible classification of these two phenotypic variants into a separate group, while the phenotypic variants that have so far been classified into the main subspecies *C. caelatus caelatus* might form a separate group.

GMM has been proven to be useful in resolving phylogeny, ontogeny, developmental stability and systematics at inter- and intraspecific level (Loy et al. 1993; Auffray et al. 1996; David and Laurin 1996; Naylor 1996, Klingenberg and McIntre 1998; Adams et al. 2013; Zúñiga-Reinoso and Benitez 2015). In our case GMM of the pronotum shape and the head shape delimitated two morphologically very similar sister species and proved to distinguish phenotypic variabilities within species.

More than 200 mountains within Dinaric Alps for some wingless insects may represent isolated ecosystems resulting in a large number of phenotypic variabilities within species. Long-term isolation in discrete refugia like those present in the Balkan Peninsula had caused intraspecific differentiation into multiple genetic lineages in both animals and plants

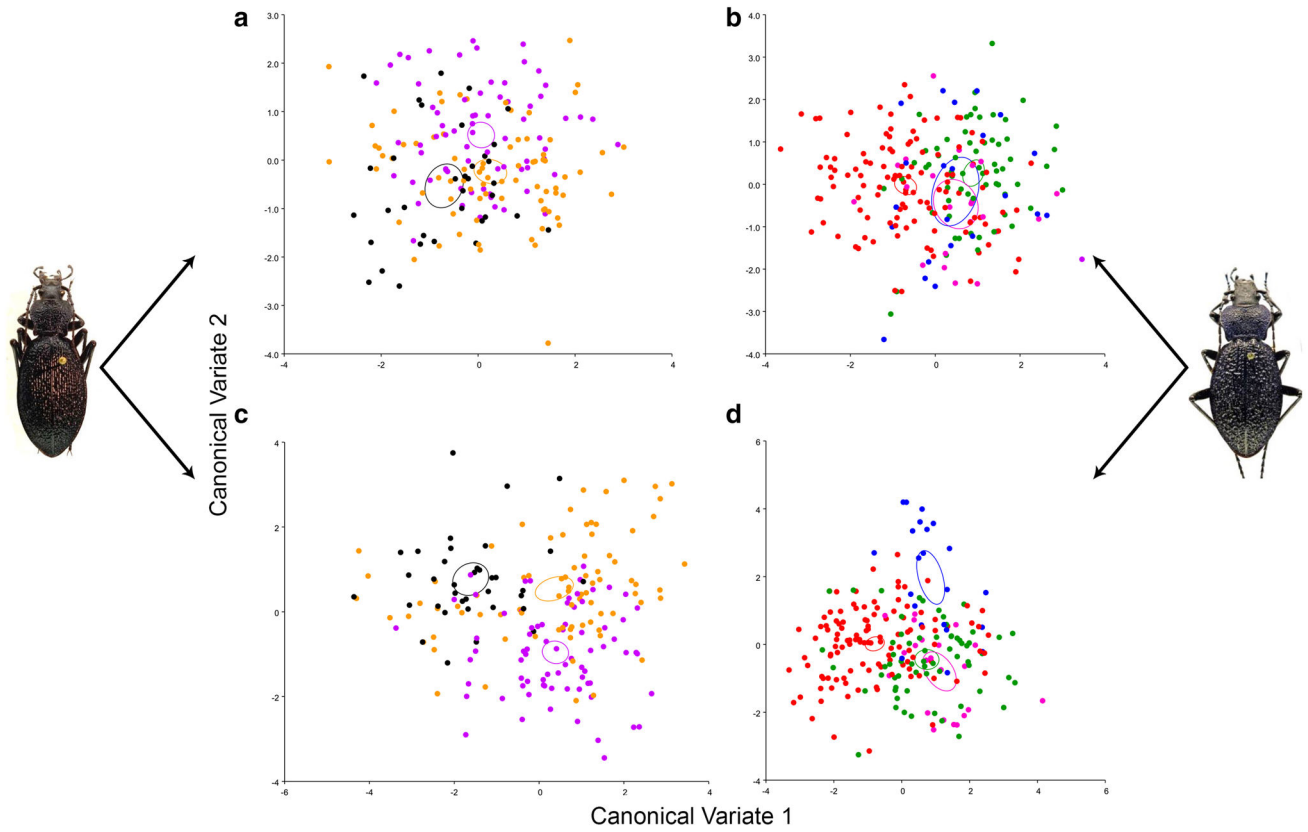


Fig. 6 Canonical variate analysis showing a concordance of the pronotum and the head shapes variations with classification according to Bousquet et al. (2017). *C. caelatus* (**a** head shape; **c** pronotum shape) and *C. croaticus* (**b** head shape; **d** pronotum shape). For *C. caelatus*: orange = *C. caelatus caelatus*; purple = *C. caelatus dalmatinus*; black = *C. caelatus schreiberi*. For *C. croaticus*: green = *C. croaticus bosnicus*; red = *C. croaticus croaticus*; blue = *C. croaticus durmitorensis*; pink = *C. croaticus zepcensis*. (Color figure online)

(Hewitt 2004). Thus, numerous phenotypic variants within species as well as speciation of these two species may be the result of allopatric speciation caused by events during and after the last glacial maximum. However, continuous distribution and the existence of great morphological similarity between closely distributed populations indicate the partial gene flow and formation of hybridisation zones. The presence of parapatric speciation or even ring species where gene flow occurs between geographically close populations but in the ends of the "ring" populations cannot interbreed (Pereira and Wake 2015) could be questioned.

Morphological analyses of *C. croaticus* and *C. caelatus* specimens, showed variations in pronotum and head shape through mountain ranges with about clear separation of geographically distinct populations pointing to a speciation in progress possibly due to geographical barriers, and therefore, GMM results should be complemented with DNA analyses to check for partial gene flow or similar environmental influences between neighbouring populations. Some of the phenotypic variants were underrepresented so it cannot be excluded that an analysis with a larger number of individuals would give a clearer picture of the geographic distribution of phenotypic variants across Dinaric Mountains.

Consistently with the result of GMM of pronotum, we suggest following classification to be tested by further analyses, particularly using molecular data for *C. croaticus* subspecies:

- *C. croaticus croaticus* (including *croaticus, frankenbergeri*, and *mediterraneus*).
- *C. croaticus bosnicus* (including *bosnicus, kobingeri, pretneri, travnikanus, zepcensis*, and *bosiljevici*).
- *C. croaticus durmitorensis* (including *durmitorensis, babinjensis, droveniki, kraetschmeri*, and *ljubetensis*).

and for *C. caelatus* subspecies:

- *C. caelatus caelatus* (including *caelatus* and *schreiberi*).

- *C. caelatus dalmatinus* (including *dalmatinus, cabuljensis, macretus*, and *procerus*).
- *C. caelatus sarajevoensis* (including *sarajevoensis, volujakianus, metalkanus*, and *malissorum*).

Revealing the ways of speciation within the studied subspecies represents an intricate process due to the uncompleted speciation processes. In both species, we can confirm the existence of three groups of populations showing the tendency toward separation into different subspecies. In relation to the size of the distribution area and the phenotypic differences between distant populations, we hypothesize that within both species there are subspecies that cannot longer interbreed. Possible hybridization zones, type/types of speciation and intraspecific variability could be clarified combining the morphological approaches performed with proper molecular analyses, some of which are in progress.

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Author contributions LSJ, ZJV and AP designed the study; ZJV, LSJ, AP and SH collected the samples, ZJV performed morphometric measurements of sampled material and data analyses, ZJV and HAB performed statistical analysis; ZJV, HAB and LSJ wrote the manuscript; all authors contributed in improving the draft of the manuscript by adding valuable comments; LSJ supervised this work from the very beginning.

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Compliance with ethical standards

Data accessibility The datasets generated during the current study are available in the figshare repository https://figshare.com/s/46665293e7390e174bc8 (doi:10.6084/m9.figshare.7415351). Details with the link will be added upon acceptance of publishing.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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ORIGINAL ARTICLE

Long term changes (1990–2016) in carabid beetle assemblages (Coleoptera: Carabidae) in protected forests on Dinaric Karst on Mountain Risnjak, Croatia

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Key words. Coleoptera, Carabidae, climatic changes, elevation gradient, endemic species, repeated study, community composition, Croatia

Abstract. Carabids, as well-known bioindicators, have been used to study the long term changes that have occurred in their communities in the Dinaric Alps. This study involved eight sites in the protected forests of the Risnjak National Park in the years 2015 and 2016 of which three were previously studied in 1990 and 1991. A total of 9, 521 individual ground beetles belonging to 17 genera and 33 species were collected. Species diversity and community composition, including percentages of species grouped according to their habitat preferences, body size, wing morphology, preferred moisture and temperature were used to compare the sites sampled in 1990 and 1991 and resampled in 2015 and 2016. Even though this study was carried out in protected forests within the National Park with minimal anthropogenic pressure and the fact that available climatic data didn't show any significant change in climate over the last 25 years, there was a reduction in the abundance of specialist species and increase in the spread of generalist species. Furthermore, the lower abundance of a mountain specialist and endemic species, *Pterostichus variolatus,* and the lack of mountain specialists *Molops alpestris*, *Pterostichus unctulatus* and *Trechus croaticus* in the catches indicate the importance of further monitoring of these mountain forest ecosystems and for a well-timed and appropriate conservation approach.

INTRODUCTION

The rapid loss of biodiversity is a threat to the stability and the existence of the ecosystems we know today. While some claim that we are in the middle of the largest extinction in the Earth's history – Sixth Mass Extinction (Ceballos et al., 2015; Payne et al., 2016), others hope that the situation is overstated (Leather, 2017). Nevertheless, the loss of any form of life in some way affects the functioning of ecosystems (Bradley et al., 2012).

One of the best ways to track changes in biodiversity is the continuous monitoring of well-known insect groups (Briggs, 2017) such as carabids (e.g. Work et al., 2008; Taylor & Morecroft, 2009; Sklodowski & Garbalinska, 2011; Blaszkiewicz & Schwerk, 2013; Homburg et al., 2019). For many years now, carabids have proved to be useful model organisms for monitoring changes in ecosystems caused by various factors (Desender et al., 1994; Heijerman & Turin, 1994; Maelfait et al., 1994; Niemelä, 2001; Niemelä et al., 2007; Šerić Jelaska et al., 2010, 2014; Schwerk, 2014). The great abundance, ecological diversity, short generation times, relatively high fecundity, sensitivity to environmental changes and well-known life history traits (Lindroth, 1961–1969; Thiele, 1977; Grum, 1986; Lövei & Sunderland, 1996; Niemelä et al., 2000; Szyszko et al., 2000) make their bioindicator role unquestionable (e.g. Müller-Motzfeld, 1989; Heijerman & Turin, 1994; Horvatovich, 1994; Maelfait et al., 1994; Pizzolotto, 1994; Eyre et al., 1996; Luff, 1996; Šerić Jelaska & Durbešić, 2009; Pizzolotto et al., 2013). Their high sensitivity to soil composition, pH and temperature (Merivee et al., 2004, 2005, 2008; Must et al., 2006) was the main reason why ground-dwelling beetles were considered to be potential early warning indicators (Koivula, 2011). Pizzolotto et al. (2014) refer to carabids as a "day-after warning" due to their ability to signal changes in vegetation before they can be visually observed. Thus, it is not surprising that carabids are being increasingly used to determine modifications in ecosystems caused by changes in climate (e.g. McCarty, 2001; Parmesan & Yohe, 2003; Harte et al., 2004; Gobbi et al., 2007, 2010, 2011; Brambilla & Gobbi, 2013; Brandmayr et al., 2013).

Long-term data could help reveal the extent of changes in species diversity, ranges, phenology of life cycles and interactions following environmental changes (such as cli-

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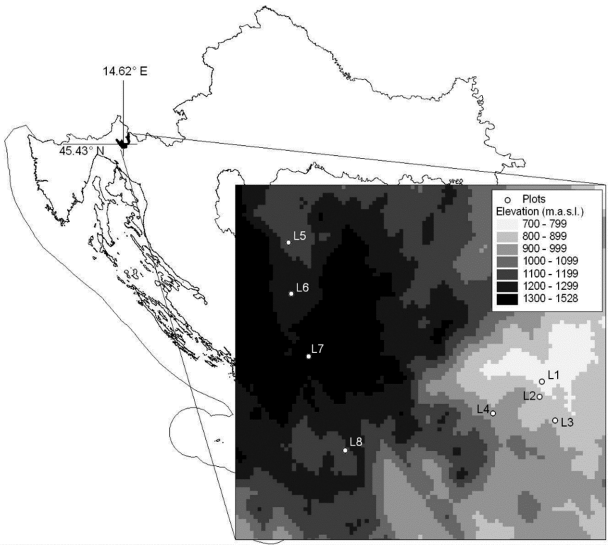


Fig. 1. Map of the area studied and the locations of the sites sampled along an altitudinal gradient on Mt. Risnjak, in Western Croatia. Different shades denote altitude (m a.s.l.).

mate change, habitat destruction, etc.). Therefore, in this paper, we aimed to compare carabid beetle assemblages sampled in the years 1990 and 1991 with those sampled 25 years later (in 2015 and 2016), at the same sites. In addition to species richness, we analysed mean individual biomass (MIB) and changes in species traits (wing morphology, preferences for particular levels of moisture affinity and temperature) assuming that the percentage of some traits within the community could have changed over the time indicating certain environmental modifications.

In this the first study on carabids over a long period in the Dinaric Alps in Croatia, in addition to changes in carabid assemblages, we checked for a possible displacement of some species along an altitudinal gradient that could point to an increased level of environmental stress due to climate change.

MATERIAL AND METHODS

Study area

This study was carried out in the region of the Risnjak mountain massif (1528 m) located in the Gorski Kotar area in western Croatia, in the northern part of the Dinaric Alps (Fig. 1). Its central part along with the Croatian Snježnik mountain range (1506 m) and the spring of the Kupa River (290 m a.s.l.) are in the Risnjak National Park. The forest communities within the Park are a part of the continuously forested area on the Dinaric Alps in Croatia (Šerić Jelaska & Durbešić, 2009). This uninhabited woodland on deep karst abounds in geographical, geological and vegetation phenomena (Prugovečki, 1997).

Of the eight sites sampled (L_1–L_8), three were surveyed in 2015 and 2016 (L_3, L_5, L_8) and five additional sites were chosen in 2016. Sites were at altitudes ranging from 704 m a.s.l. to 1277 m a.s.l. (Fig. 1, Table 1), with annual rainfall from 1919.3 mm yr^{-1} to 3708 mm yr^{-1} (values for period between 1990 and 2016), mean maximum annual temperatures from 20.07°C to 22.2°C, and mean minimum annual temperatures from –7.29°C to –2.34°C (values for period between 2006 and 2016).

The forest here is dominated by beech and includes mixed beech-silver fir forests at the lower altitudes and subalpine beech forest at the higher altitudes. Dwarf mountain pine (*Pinus mugo*) bushes occur at the treeline, above which there are subalpine calcareous grasslands.

The experimental sites (L_3, L_5, L_8) were selected within forest habitats on the upper half of the mountain right up to the top of the tree line. These three sites were at the same location as those used in the research on carabid communities carried out in 1990–1991 (Table 1). Since we did not have information on the exact location of pitfall traps at each location (e.g. geographical coordinates), traps were positioned within forest based on the descriptions of the previous field study. The sampling methods were the same as in 1990–1991.

Sampling protocol

Ground beetles were sampled between May and October, during 2015 and 2016, using pitfall traps at all eight sites (three sites in 2015 and 2016 plus an additional five sites in 2016). Plastic vessels with a diameter of 9.5 cm and a depth of 14.0 cm (0.5 L) were used as pitfall traps. In 2015 six pitfall traps per site were set (site codes: L_5, L_6, L_7), and in 2016 nine pitfall traps were set at each of the eight sites (site codes: L_1–L_8). Thus, a total of 90 traps were used during two years. At each site, traps were arranged in two rows with three traps in each row in 2015, and in a grid (3 × 3) in 2016, with about a 10-meter distance between each trap. The traps were filled up to one-third of their volume with a mixture of ethanol (96%), acetic acid (9%) and water in equal proportions (as was used in the study in 1990–1991) and reset every two to three weeks continuously throughout the sampling period (2 May 2015–10 Oct. 2015; 12 May 2016–9 Oct. 2016). Pieces of tree bark were placed above the traps to protect them from rain. All specimens were identified to species level (Trautner & Geigenmüller, 1987; Hůrka, 1996; Freude et al., 2004; Mueller collection in NHM Trieste) and stored either in a 70% alcohol solution or in a dry state.

Habitat variables

For each site, we recorded altitude (m a.s.l.), slope (degrees), aspect (i.e. the direction in which a slope faces; calculated as

Table 1. Main features of the sites sampled. The superscript 90/91 means the same site was surveyed in 1990 and 1991.

Plot	Plot mark	Forest type	Altitude (m a.s.l.)	Slope	Aspect	Number of traps per year	
						2015	2016
1	L_1	Dinaric silver fir and beech forest	704	2	37.7677		9
2	L_2	Dinaric silver fir and beech forest	742	13	80.92365		9
3	L_3 ($L_3^{90/91}$)	Dinaric silver fir and beech forest	727	19	54.23008		9
4	L_4	Dinaric silver fir and beech forest	869	9	350.4352		9
5	L_5 ($L_5^{90/91}$)	Dinaric silver fir and beech forest	1068	2	30.12193	6	9
6	L_6	Dinaric silver fir and beech forest	1160	0	269.9162	6	9
7	L_7	Subalpine beech forest	1277	16	243.5076	6	9
8	L_8 ($L_8^{90/91}$)	Subalpine beech forest	1199	4	193.4187		9

Table 2. Mann-Kendall test of the trend in maximum and minimum monthly temperatures and total monthly precipitation. *There was a statistically significant increasing trend.

Mann-Kendall trend test			
Delnice	S	Z	P
t max	284	0.42964	0.66746
t min	947	1.4092	0.15878
rain	652	0.98812	0.32309
Crni Lug	S	Z	P
t max	294	0.44906	0.65339
t min	955	1.7375	0.082308
rain	5044	2.451	0.014247*

absolute number of degrees from south), and land cover (forest types: mixed Dinaric silver fir and beech forest, and subalpine beech forest), (Table 1). The first three variables were calculated in a GIS environment, whereas the forest types were identified in the field based on the presence of particular species of plants.

The climate in the study area and how it has changed over the last few decades were analysed based on the data available from the Croatian meteorological and hydrological service (DHMZ). Precipitation data for the whole period (1990–2016) were available for Crni Lug (Risnjak National Park, at cca 800 m a.s.l.) while temperatures in the National Park were not measured before the end of 2003. Therefore, data for the nearest town Delnice (at 700 m a.s.l.) were used (Figs S1, S2). A non-parametric Mann-Kendall test was used to determine trends in the maximum and minimum monthly temperatures and total monthly precipitation (Table 2).

Species traits

Collected species were grouped with respect to their (1) wing morphology – macropterous (hind wings fully developed), brachypterous (includig apterous; hind wings reduced), and polymorphic (including dimorphic; short-and long-winged forms), (2) moisture preference – hygrophilous, xerophilous, and moisture-indifferent species, and (3) temperature preference – thermophilous, mesothermophilous (including eurytherm) and low-temperature (including oligostenotherm) species (Table 3).

Traits were compiled from the Carabids.org – online datebase (Homburg et al., 2013) and Vujčić-Karlo (1999), and amended using information from Thiele (1977), Lindroth (1985), Hůrka (1996) and Turin (2000).

Table 3. List of the carabid species recorded at the sites compared, their ecological traits and years when collected. (Wing morphology: B – brachypterous, M – macropterous, P – polypterous, X – xerophilous, H – hygrophilous, M – moisture indifferent, T – thermophilous, MT – mesothermophilous, LT – low-temperature, NA – no information available.) Sites numbers correspond to those in Table 1. *Author's note: Questionable identification (species from previous papers were not revised as identifications were retrieved as a list from the papers).

Species	Dispersal ability	Moisture preference	Temperature preference	Site	Year
Abax parallelepipedus	B	M	MT	3	2016
Abax carinatus	B	H	MT	3 3	1990–1991 2016
Abax ovalis	B	H	MT	3,5,8 3,5,8	1990–1991 2015–2016
Anisodactylus intermedius	M	H	T	3	2016
Amara eurynota	M	X	MT	3	2016
Aptinus bombarda	B	M	T	3,8 3,5,8	1990–1991 2015–2016
Calathus micropterus	B	X	LT	5	1990–1991
Calosoma inquisitor	M	M	MT	8	1990–1991
Carabus catenulatus	B	M	MT	3,5,8 3,8	1990–1991 2015–2016
Carabus convexus	B	M	T	5	1990–1991
Carabus coriaceus	B	M	MT	3,5,8 3,8	1990–1991 2015–2016
Carabus creutzeri	B	H	LT	8 5	1990–1991 2015–2016
Carabus croaticus	B	M	MT	3,5,8 3,5,8	1990–1991 2015–2016
Carabus violaceus	B	M	T	3,5,8 3,5,8	1990–1991 2015–2016
Cychrus attenuates	B	H	LT	3,5,8 3,5,8	1990–1991 2015–2016
Cymindis humeralis	P	H	LT	5	1990–1991
Harpalus sp.*	M	NA	NA	5 5	1990–1991 2015–2016
Leistus nitidus	P	H	LT	5	2015–2016
Leistus piceus	B	H	MT	3	2016
Leistus rufomarginatus	P	H	MT	3,5,8 8	1990–1991 2015–2016
Leistus spinibarbis	M	X	T	8 3	1990–1991 2016
Licinus hoffmannseggi	B	H	LT	5 3,5,8	1990–1991 2015–2016
Molops alpestris	B	H	MT	3,5,8	1990–1991
Molops elatus	B	H	LT	5,8	2015–2016
Molops ovipennis	B	M	LT	8	2015–2016
Molops piceus	B	H	LT	3,5,8	2015–2016
Molops striolatus	B	M	T	3,5,8 3,5,8	1990–1991 2015–2016
Nebria dahlii	B	H	LT	3,5,8 3,5,8	1990–1991 2015–2016
Notiophilus biguttatus	P	H	MT	3,5,8 3, 5,	1990–1991 2015–2016
Platynus scrobiculatus	B	H	MT	3 3	1990–1991 2016
Pterostichus burmeisteri	B	M	LT	3,5,8 3,5,8	1990–1991 2015–2016
Pterostichus fasciatopunctatus	B	H	LT	3 3	1990–1991 2016
Pterostichus unctulatus	B	M	LT	5,8	1990–1991
Pterostichus variolatus	B	M	LT	5,8 5,8	1990–1991 2015–2016
Reicheiodes rotundipennis	B	H	LT	5	1990
Stomis rostratus	B	M	MT	3,5,8 8	1990 2016
Trechus croaticus	B	H	LT	3,5,8	1990

Data analyses

In this study Margalef's diversity (DMg) and Menhinick's indices (DMn) were calculated as indicators of species richness, Simpsons' index as a dominance index, Shannon-Wiener's (H') as a diversity index and Pielou's (E) as an evenness index (Table 4).

To compare species composition between the sites in all the years sampled (1990, 1991, 2015, 2016), we calculated Jaccard dissimilarity coefficient and grouped the data using Euclidean

Table 4. Diversity measures recorded at the sites studied. The site codes marks correspond to those used in Table 1.

Site	L_1	L_2	L_3	($L_3^{90/91}$)	L_4	L_5	($L_5^{90/91}$)	L_6	L_7	L_8	($L_8^{90/91}$)
Total species (S)	16	16	21	18	15	16	22	15	14	17	20
Total carabids collected (N)	696	469	581	659	543	1894	4985	2973	730	1635	8688
Total carabids collected per day per trap (Nd)	0.573	0.386	0.478	0.107	0.447	0.449	0.807	0.705	0.173	1.346	1.406
Margalef's diversity index (DMg) $DMg = (S-1)/\ln(Nd)$	–26.92	–15.76	–27.11	–7.59	–17.38	–18.75	–97.73	–40.11	–7.41	53.89	55.78
Menhinick's diversity index (DMn) $DMn = S/\sqrt{Nd}$	21.14	25.75	30.37	55.12	22.44	23.87	24.49	17.86	33.64	14.65	16.86
Simpsons' index (D) $D = \sum p_i^2$	0.43	0.16	0.25	0.23	0.15	0.64	0.,23	0.40	0.14	0.51	0.41
Shannon Wiener index (H') $H' = -\sum p_i \ln p_i$	1.38	2.04	1.84	1.91	2.09	0.95	1.85	1.29	2.11	1.15	1.30
Pielou's evenness (E) $E = H/\ln(S)$	0.49	0.74	0.60	0.66	0.78	0.34	0.60	0.47	0.80	0.41	0.43

distance as distance measure and complete linkage as linkage rule for constructing dendrograms (Krebs, 1989), (Fig. 2). Shapiro-Wilk and Levene's tests were used to check for normal distribution of data and homogeneity of variance. In addition, non-parametric Kruskal Wallis one-way ANOVA and Mann-Whitney U tests were used. Since there were no significant differences in species composition and abundance among years (except for site L_3 in 1991 and 2016), we pooled data for 1990 and 1991 in one group and data for 2015 and 2016 in another group for further trait analyses.

Similarities in species composition according to the percentages of tested species traits (wing morphology, moisture and temperature affinity) recorded at the same sites in 1990–1991 and resampled in 2015–2016 were evaluated using repeated measures or one-way ANOVA. Principal Components Analysis (PCA) was used to explore variation in the distribution of the species traits among all sites. Pearson's correlation coefficient was used to measure the strength of the linear relationship between the percentages of the species traits with altitude. Multivariate linear regression analyses were used to determine the altitudinal distribution of the observed species traits.

Mean individual biomass (MIB) was calculated using a formula that describes the relationship between body length and live biomass of a carabid beetle: $\ln y = -8.928 + 2.555 \times \ln x$ (Szyszko, 1983). Data on the body length of the species caught were obtained from the Carabids.org – online datebase (Homburg et al., 2013). Differences in carabid biomass were standardise per trap per day for both of the periods 1990–1991 and 2015–2016, before further analyses.

Rarefaction curves were estimated for three of the sites in 1990–1991 and all of the sites in 2015–2016 (Fig. 3A, B).

The IBM SPSS v21; EstimateS v9.1. and Past 3 program packages were used for statistical calculations.

RESULTS

A total of 9, 521 individual ground beetles belonging to 17 genera and 33 species were collected during the two years, 2015–2016 (Table S1). The first five most abundant carabid species were brachypterous and made >80% of all the carabids captured (*Nebria dahlii* 42.43%, *Aptinus bombarda* 17.14%, *Pterostichus burmeisteri* 9.7%, *Pterostichus variolatus* 8.22% and *Abax ovalis* 6.1%). The number of species per site varied from 9 (site L_6 in 2015) to 21 (site L_3 in 2016). Species found at all of the sites were: *Abax ovalis*, *Carabus violaceus*, *Cychrus attenuatus*, *Molops striolatus*, *Nebria dahlii* and *Pterostichus burmeisteri*.

Changes in ground beetle assemblages 1990–2016

A total of 40 species were caught in 1990–1991 and 2015–2016 (sites L_3, L_5 and L_8), of which only 7 species (*Calathus micropterus*, *Calosoma inquisitor*, *Cymindis humeralis*, *Reicheiodes rotundipennis*, *Molops alpestris*, *Pterostichus unctulatus* and *Trechus croaticus*) were caught in 1990–1991 and 10 species (*Abax parallelepipedus*, *Anisodactylus intermedius*, *Amara eurynota*, *Leistus nitidus*, *Leistus piceus*, *Loricera pilicornis*, *Molops elatus*, *Molops ovipennis*, *Molops piceus*, and *Nebria brevicollis*) in 2015–2016, which indicated that the fauna recorded in 2015–2016 was richer. Species found at all of the sites in 1990–1991 and 2015–2016 were: *Abax ovalis*, *Carabus violaceus*, *Cychrus attenuatus*, *Molops striolatus*, *Nebria dahlii* and *Pterostichus burmeisteri*.

Analysing the composition and abundance of species in different years and sites, a statistically significant difference was recorded between 1991 and 2016 for site L_3 (Shapiro-Wilk W: 0.2595, Levene's test for homogeneity of variance $p < 0.01$, Kruskal-Wallis $p = 0.2799$, Mann-Withney pairwise, $p = 0.009$), while in other years and sites they did not differ significantly. The activity density (the total number of trapped specimens) of the most abundant species in 1990–1991 was for *Nebria dahlii*, which made up 48.99% of all the carabids caught. Based on their abundance the following species: *Pterostichus variolatus* (14.12%), *Pterostichus unctulatus* (11.97%), *Pterostichus burmeisteri* (6.58%) and *Abax ovalis* (6.38%), made up 88.04% of all the carabids caught.

In 2015–2016 the top five of the most numerous species (sites L_3, L_5 and L_8) were: *Nebria dahlii* (41.68%), *Aptinus bombarda* (34.09%), *Pterostichus burmeisteri* (8.2%),

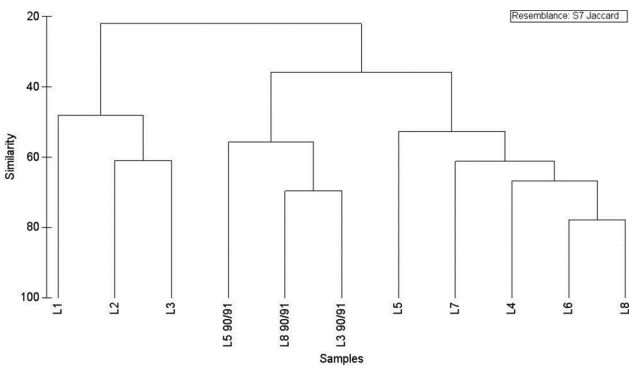


Fig. 2. Tree diagram of cluster analyses using Jaccard dissimilarity coefficient to measure similarity. The marks on the x-axes denote the sites investigated and correspond to those in Table 1.

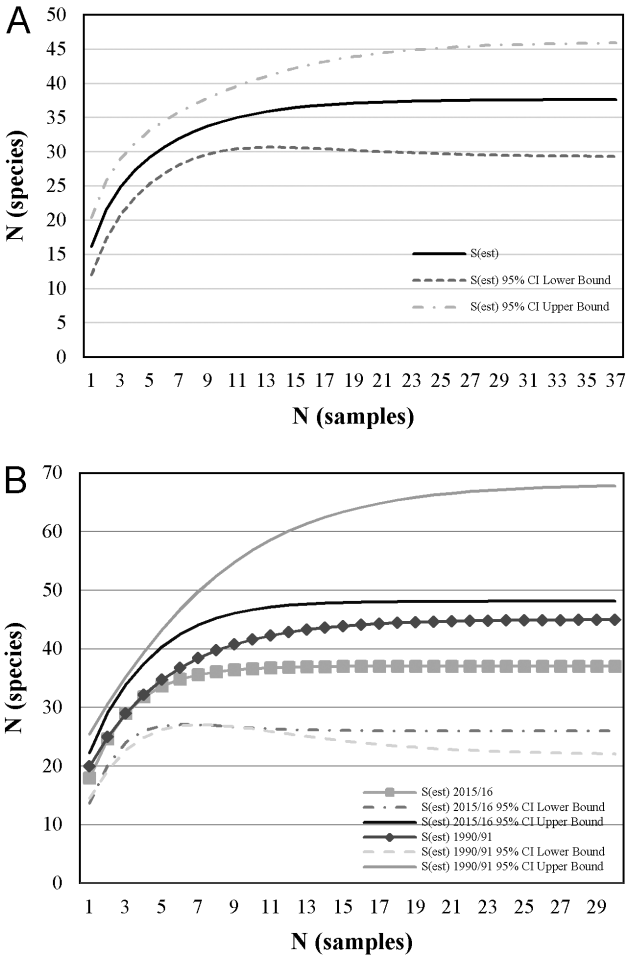


Fig. 3. A – Rarefaction curves obtained for the eight sites (L_1–L_8) studied in 2015–2016. B – Rarefaction curves obtained for the three sites (L_3, L_5, L_8) studied in 1990–1991 and again in 2015–2016.

Carabus violaceus (2.8%) and *Abax ovalis* (2.77%), which together made up 89.54% of all the carabids caught.

Of the common species caught in both periods, the biggest increase in the number caught was recorded for *Aptinus bombarda*, which increased from 0.27% in 1990–1991 to 34.09% of all carabids caught in 2015–2016 and *Pterostichus fasciatopunctatus* from 0.007% in 1990–1991 to 0.46% in 2015–2016. The highest decreases in the number of individuals caught was recorded for *Leistus rufomarginatus* from 1.86% in 1990–1991 to 0. 024% in 2015–2016, and *Stomis rostratus* from 0.32% in 1990–1991 to 0.024% in 2015–2016. A decrease was also recorded in the number of endemic species like *Pterostichus variolatus*, from 14.12% in 1990–1991 to 2.31% in 2015–2016 at the three sites that were compared.

Endemics like *Molops alpestris*, *Pterostichus unctulatus* and *Trechus croaticus* were not caught in 2015–2016.

Comparing each of the three sites that were sampled in 1990–1991 and again in 2015–2016 all of the diversity indices (except DMg for site L_5) indicate that the diversity was higher in 1990–1991 than in 2015–2016 (Table 4), and based on species composition using Jaccard similarity index the sites sampled in 1990–1991 (L_3, L_5, L_8) formed one cluster and those sampled in 2015 and 2016 (L_1–L_8) another cluster (Fig. 2).

Species trait analyses of the assemblages recorded between 1990–2016 along an altitudinal gradient

The analyses of variance (repeated measures ANOVA) did not reveal statistically significant differences in carabid assemblages based on wing morphology in the two periods compared (1990–1991 and 2015–2016). Because of the zero variance between some sites, the difference in moisture preference in the two periods sampled could not be tested using factorial ANOVA. Instead, one-way ANOVA was used and did not reveal a statistically significant differences [$F(1,13) = 0.29$, $p = 0.5946$], but some trends were recorded. The most numerous species, in both periods, were brachypterous and hygrophilous or moisture indifferent. Differences were recorded in temperature preference; in 1990–1991 the first four of the most numerous species were low-temperature species (*N. dahlii*, *P. variolatus*, *P. unctulatus*, *P. burmeisteri*) while in 2015–2016 two of the four the most numerous species (*N. dahlii*, *A. bombarda*, *P. burmeisteri*, *C. violaceus*) were thermophilous, including the second most abundant.

Site L_3 in 1990–1991 and 2016

Based on the ecological traits of the different species, three macropterous species of which two were xerophilic (*Anisodactylus intermedius*, *Amara eurynota*, *Leistus spinibarbis*) were caught in 2015–2016, whereas in 1990–1991 no macropterous or xerophilic species were caught (Table 3).

A big increase in the abundance of *Aptinus bombarda* was recorded, from 4.71% in 1990–1991 to 43.72% in 2016, which is an increase of 42 times more specimens caught per day.

Site L_5 in 1990–1991 and 2015–2016

In 2015–2016, among the top five most numerous carabids caught there were two thermophilous species (*Carabus violaceus*, *Molops striolatus*), whereas in 1990–1991 only low-temperature species were dominant (*Pterostichus unctulatus*, *Nebria dahlii*, *Pterostichus variolatus*, *Pterostichus burmeisteri*), (Table 3).

Site L_8 in 1990–1991 and 2016

In 1990–1991 the top five most numerous species were low-temperature species whereas in 2016 the most numerous species was thermophilous (*Aptinus bombarda*), (Table 3). In addition, the endemic species *Pterostichus variolatus* decreased in abundance from 18.06% to 1.7% between 1990–1991 and 2016.

A high positive correlation between the percentage of low-temperature species and increase in altitude was recorded ($r = 0.76$). Pearson's correlation coefficients also showed a positive correlation between large ($r = 0.54$), brachypterous ($r = 0.33$) and moisture-indifferent species ($r = 0.47$) with increase in altitude, indicating that large, brachypterous, low-temperature and moisture-indifferent species are more likely to occur at high altitudes.

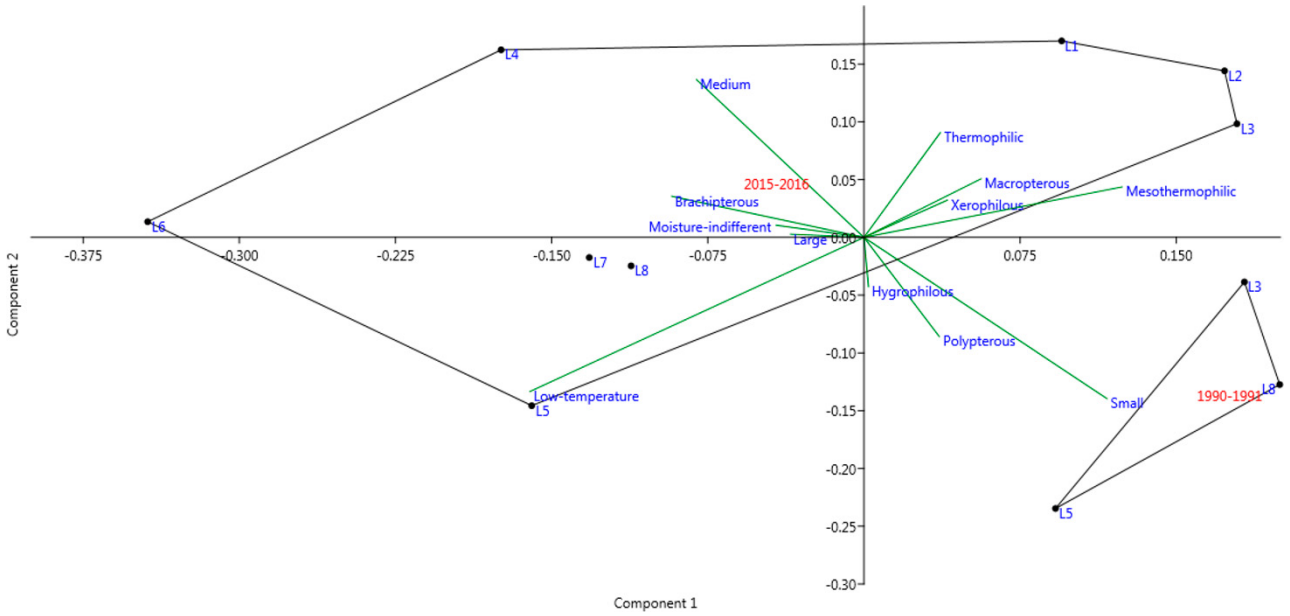


Fig. 4. A PCA correlation tri-plot showing the variation in the distribution of the species traits among sites (L_1–L_8 sampled in 2015–2016 and L_3, L_5 and L_8 sampled in 1990–1991).

The first two axes of the PCA graph (Fig. 4) represent 66.95% of the total variance (44.44% and 22.5%, respectively). The first axis separates sites L_1, L_2 and L_3 (sampled in 2015 and 2016) at low altitudes on the right side of the ordination space based on their higher percentage of thermophilic, xerophilic and macropterous species, and sites sampled in 1990 and 1991 (L_3, L_5, L_8) with smaller and polypterous species. On the left side of the ordination biplot, there are the sites L_4, L_5, L_6, L_7 and L_8 (sampled in 2015 and 2016) at high altitudes, with a high percentage of low-temperature, moisture-indifferent and large brachypterous species. Along the second ordination axis, there are sites L_5 and L_8 (sampled both in 1990–1991 and 2015–2016) that are separated on the basis of their higher percentage of low-temperature species from those sampled in 1990–1991 with more polypterous and smaller species.

Distribution of the traits analysed (Fig. 5) along the altitudinal gradient revealed some general trends in terms of brachypterous, large, cold-adapted species being more abundant at high altitudes.

Analysing mean individual biomass recorded at the sites that were compared revealed a statistically significant difference for site L_3 ($MIB^{1990–1991} = 0.0178$; $MIB^{2016} = 0.0843$; $p = 0.04$) with a markedly higher biomass of *Aptinus bombarda*, *Carabus coriaceus* and *Carabus violaceus* in 2015–2016 than in 1990–1991. The remaining two sites (L_5, L_8) differed slightly in their MIB values. (L_5: $p = 0.46$; L_8: $p = 0.97$) with lower biomass of the endemic mountain species *Pterostichus variolatus* in 2015–2016 than in 1990–1991.

Based on the percentage of different species at given altitudes we noticed a possible displacement of some species along the altitudinal gradient (Fig. 6). *Notiophilus biguttatus*, a mesothermophilous and hygrophilous species whose altitudinal range in 1990–1991 was from 742 m to 1199 m, in 2015–2016 was not recorded above 1068 m; *Licinus hoffmannseggi*, a low-temperature and hygrophilous species, in 2015–2016 seemed to expand its range at both low and high altitudes (from 742 m to 1277 m) recorded in 1990–1991, but at only one site L_6 (1060 m); *Stomis rostratus*, a mesothermophilous and moisture indifferent species, seemed to reduce its range in 2015–2016 and was caught only at site L_8 (1199 m) while 25 years ago it was recorded at altitudes from 742 m to 1199 m (sites L_3, L_5, L_8). The expected increase in abundance of cold-adapted endemic species with altitude was recorded for the species *C. creutzeri* and *P. variolatus*. Both these species were recorded at altitudes from 1068 m to 1277 m and were the most numerous at the highest site (L_7, 1277 m). This was also recorded for another endemic mesothermophilous species, *C. croaticus*. It was recorded at altitudes from 742 m to 1277 m and was by far the most numerous (20% of the total catch) at the highest site (L_7).

The 25-years moving average of minimum and maximum monthly temperatures and mean total rainfall (Figs S1, S2) did not differ significantly (independent samples T-test: Risnjak – Crni Lug rainfall: $t(46) = –1.157$; $p = 0.253$; Delnice Tmax: $t(41) = –0.933$; $p = 0.357$; Delnice Tmin: $t(41) = –0.135$; $p = 0.894$). However, results of a non-parametric Mann-Kendall test revealed a statistically significant increasing trend with time in monthly precipitation (Table 2).

DISCUSSION

Analysing the species composition of carabids assemblages and distribution of species traits between communities sampled at the same sites in 1990–1991 and again 25

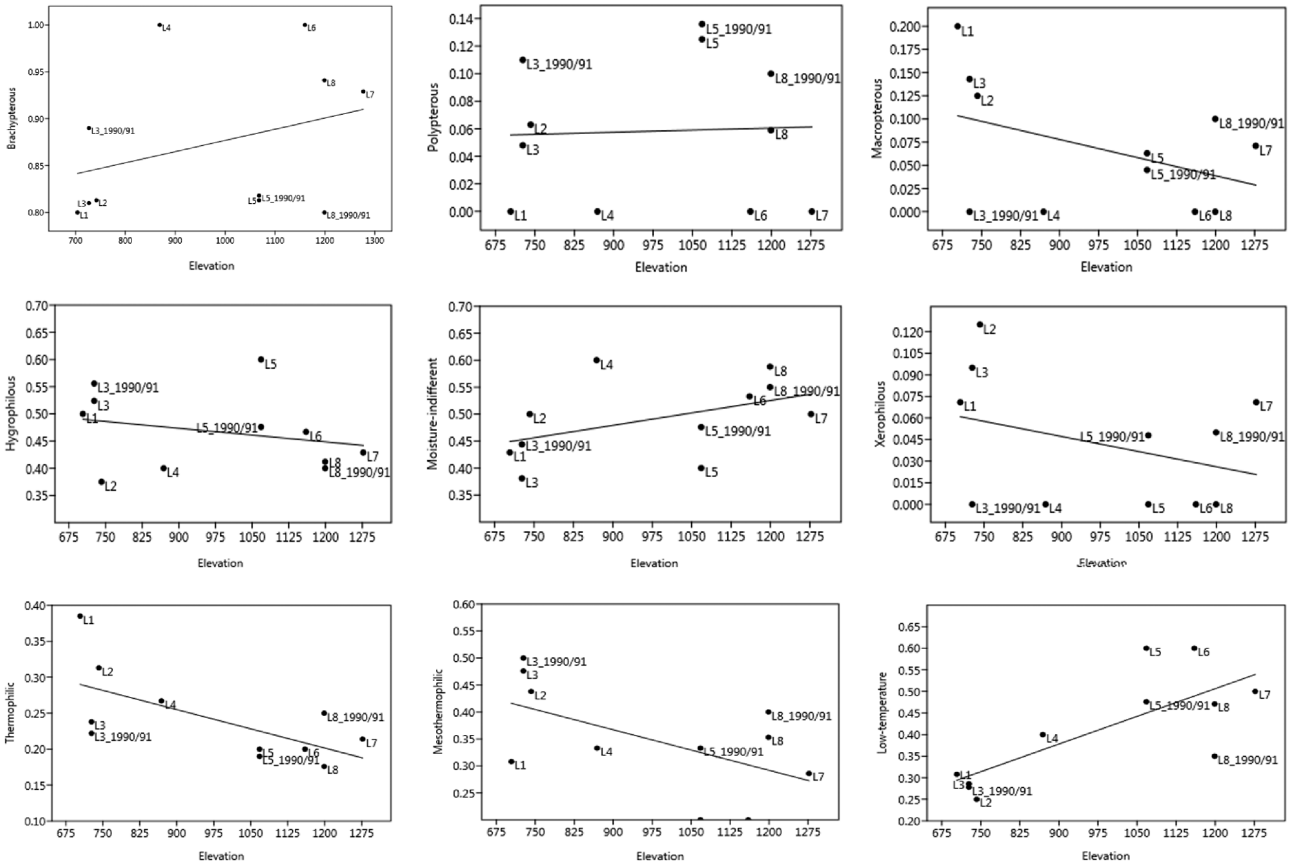


Fig. 5. Multivariate linear regression analysis of the relationship between species traits (y) and altitude (x) for each site.

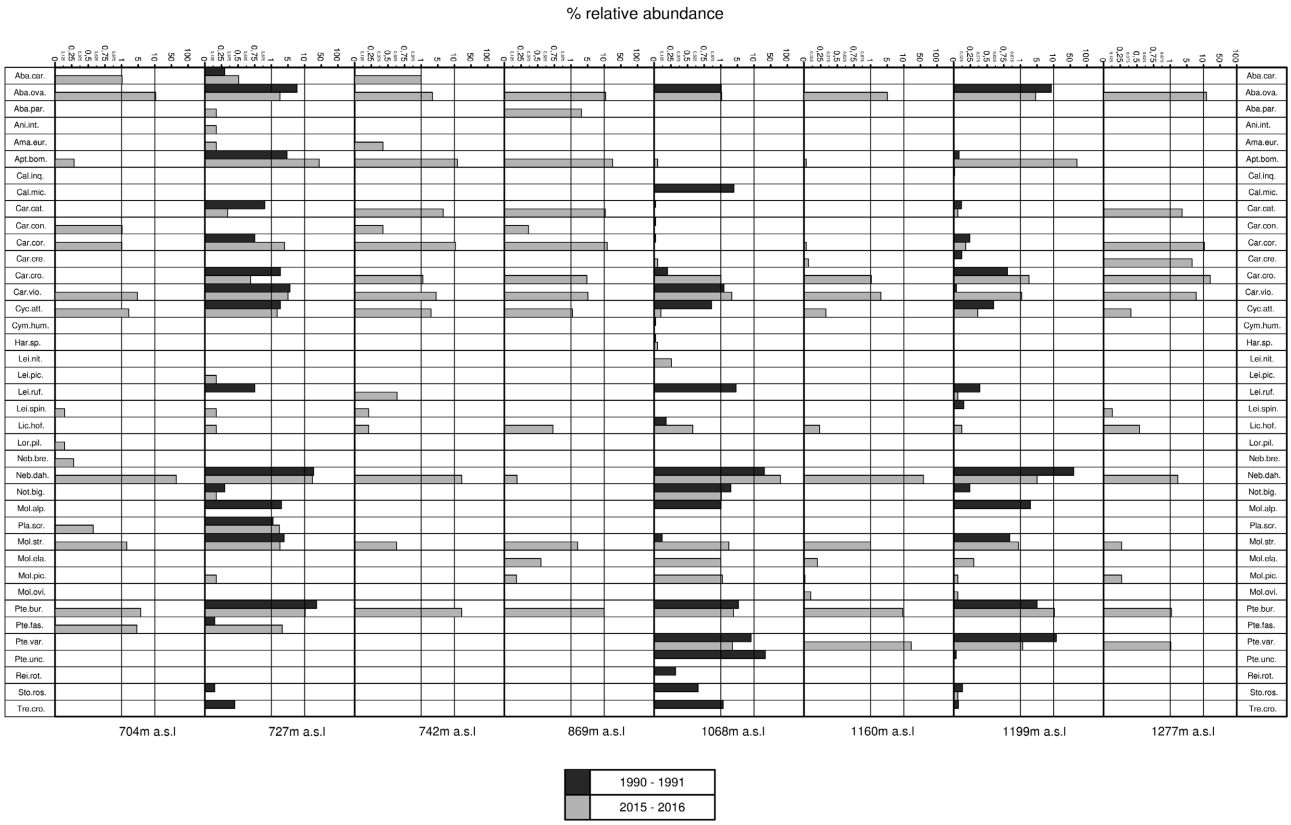


Fig. 6. Distribution and % relative abundance of species sampled in 1990–1991 (site L_3, L_5, L_8) and in 2015–2016 (site L_1–L_8). Altitudes correspond to the sites as in Table 1, and abbreviations of carabid names correspond to those in Table S1.

years later, we revealed an increase in the number of thermophilous species and decrease in the number of species preferring low temperatures at some of the sites, among them some mountain specialists (e.g. *P. unctulatus*) and species typical of the Alpine-Dinaric region (e.g. *P. variolatus*). This is an expected consequence of global warming and supported by the fact that there was a slight increase in the minimum temperature recorded on Mt. Risnjak and in the Gorski Kotar region over the last 25 years (Crni Lug: m = 0.61.; Delnice: m = 0.26). The most obvious changes were recorded at site L_{8} which is located in a subalpine beech forest, where in 1990–1991, more than 60% of the total catch consisted of species prefering low-temperatures and high humidity, such as *Nebria dahlii*, while the most numerous species at the same site 25 years later was *Aptinus bombarda* (70.1% of the total catch), which is a thermophilous and mesohigrophilic species. In addition, a big decrease in the low-temperature prefering species *Pterostichus variolatus* was also recorded at this site (an 11 fold decrease in the number of specimens caught per day). The lack of the mountain specialist, *Pterostichus unctulatus,* in the catches in 2015–2016 and the fact that this species made almost 12% of the total catch in 1990–1991 also indicate a decline in the abundance of low-temperature preferring species in this protected area. A similar decreasing trend in the abundance of low-temperature preferring species is recorded in long term studies in the Alps (Pizzoloto et al., 2014) and High Tatra Mts, where in 2004 after a windstorm, at some sites, less tolerant species, including *P. unctulatus*, disappeared (Šustek, 2013).

Certain changes can be associated with ice storms, which are characterized by freezing rain, which in February 2014 destroyed more than 680,000 m^3 of timber in the National Park (Editorial Board Šumarski list, 2014). This natural disturbance and the removal of some of the damaged trees could have stressed some parts of the ecosystem. Thinning of the forest and changes in primary habitats might account for the increase in the number of thermophilous species given that there were no significant changes in the climate in this area. But the existence of only one climatological station in the National Park is insufficient for precise monitoring of microclimatic changes in this area.

One of the most important morphological traits of carabids used for bioindication purpose, wing morphology, clearly defines the ecology and evolution occuring in a certain area (Darlington, 1943; Wagner & Liebherr, 1992). Defining the percentage of species of carabids that are wingless has proved to be a powerful method for determining the stability of an area (Dhuyvetter et al., 2007; Wagner & Liebherr, 1992; Szyszko et al., 2000; Gobbi et al., 2007; Šerić Jelaska & Durbešić, 2009; Pizzolotto et al., 2016). Therefore, we used it to compare the sites sampled in 1990–1991 and again 25 years later. The high abundance and percentage of brachypterous species (62.5% of the species, 99.5% of the individuals) caught in 2015–2016 and the absence of differences in wing morphology recorded at these three sites then and in 1990–1991 (brachypterous species: 1990–1991: 79.3% of the species, 96.8% of the individuals; 2015–2016: 75.9% of the species, 99.2% of the individuals) may indicate that the forest ecosystems in the National Park in general are stable.

Risnjak National Park as a part of the Dinaric Mountains chain has many endemic species some of which were recorded in this study (*N. dahlii, C. catenulatus, C. croaticus, C. creutzeri, M. ovipennis, P. unctulatus, P. variolatus, S. rostratus*). Most of them are mountain habitat specialists and low-temperature preferring species. Although some species may benefit from increases in availability of habitats by moving to higher altitudes, the Dinarides are so-called "mountain pyramids" on which there is a decrease in the availability of habitats with increase in altitude (Elsen & Tingley, 2015). Ultimately this can cause the local extinction of affected species (Colwell et al., 2008; Sekercioglu et al., 2008, 2012; La Sorte & Jetz, 2010).

The displacement of low-temperature species to higher and lower altitudes and that of mesothermophilous species may be due not only to extrinsic characteristics that affect the displacements' paths of certain species, but also species' intrinsic characteristics on which the response to environmental disturbances depends (Nolte et al., 2019).

It is expected that most endemic high mountain cold-adapted specialist will tend to move upwards with increase in the average annual temperature (Wilson et al., 2005; Pizzoloto et al., 2014; Scalercio et al., 2014). Although we do not have enough data to verify a possible change in temperature over the last 25 years, we noticed that two endemic low temperature species (*C. creutzeri*, *P. variolatus*) and one endemic mesothermophilous species (*C. croaticus*) are now the most numerous at the site located at the highest altitude.

According to Nolte et al. (2019), mountain habitat species are at greater risk of extinction than forest species. Thus, vegetation cover may have a mitigating role and maybe extend the time species have to adapt to new conditions. Consequently, any destruction of forest ecosystems, either by man (thinning) or indirectly by unpredictable weather conditions due to climate change, will impair habitat quality and diminishes its protective role in species conservation. Species at greatest risk of extinction include specialist predatory species, those with latitudinal and altitudinal restrictions and brachypterous species (Feehan et al., 2009). Due to stenovalence and inability to cope with sudden landscape changes (Gaston & Fuller, 2009) specialists are considered to be the most vulnerable at times of evident climate change (Kotze & O'Hara, 2003; Terzopoulou et al., 2015).

Penev (1996) points out that the composition of carabid assemblages is one of the most revealing ways of detecting changes in ecosystems. It contributes to the discovery of the effects of climate change and indicates the importance of regular and long-term monitoring (Kerr et al., 2007; Vaibhao et al., 2013). Current methods of monitoring insects assemblages require a lot of effort, so it is not surprising that there is only a small number of such long-term studies (Shortall et al., 2009; Fuentes-Montemayor et al., 2011; Dirzo et al., 2014). This is unfortunate as it makes it dif-

ficult to study the changes occurring in insect assemblages with changes in the environment. This is the case in the Risnjak National Park, which represents a natural link between the Alps and the Balkans and the most significant example of the separation of Croatia (np-risnjak.hr). The first extensive research in the field in this National Park was carried out in 1963–1964 (Durbešić, 1967) at six sites, then almost 27 years later at four different sites (Vujčić-Karlo, 1999) and then this study at eight sites.

Prediction of species-specific responses to new circumstances isn't possible without intensive and regular monitoring of carabid assemblages, especially bearing in mind the increase in anthropogenic influences and unpredictable weather conditions as a consequence of climate change. The protection of an area without further monitoring doesn't mean much in terms of protecting high mountain endemic species as they have already suffered to some extent the consequences of quick climatic changes as mountain ecosystems are among the first to suffer the consequence of global warming. Thus, despite the study of protected forests within the National Park, there was a decrease in the number of specialist species and the spread of generalist species. The necessity for continuous monitoring, in the current period of obvious climate change, is becoming unquestionable (Saunders, 2017). The high number of endemic and threatened species in mountain ecosystems such as the Dinarides requires monitoring as it is the only way to recognise and mitigate changes that can lead to the reduction of biodiversity and permanent loss of some endemic species.

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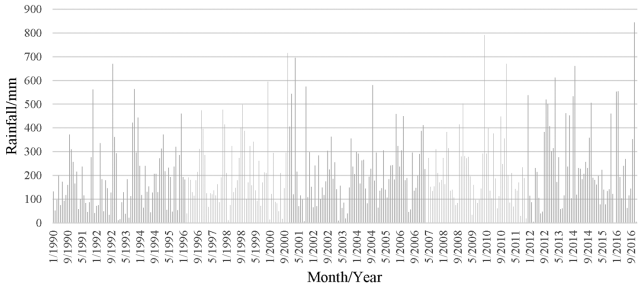


Fig. S1. The 25-years moving mean of the total rainfall recorded at meteorological station Crni Lug, NP Risnjak.

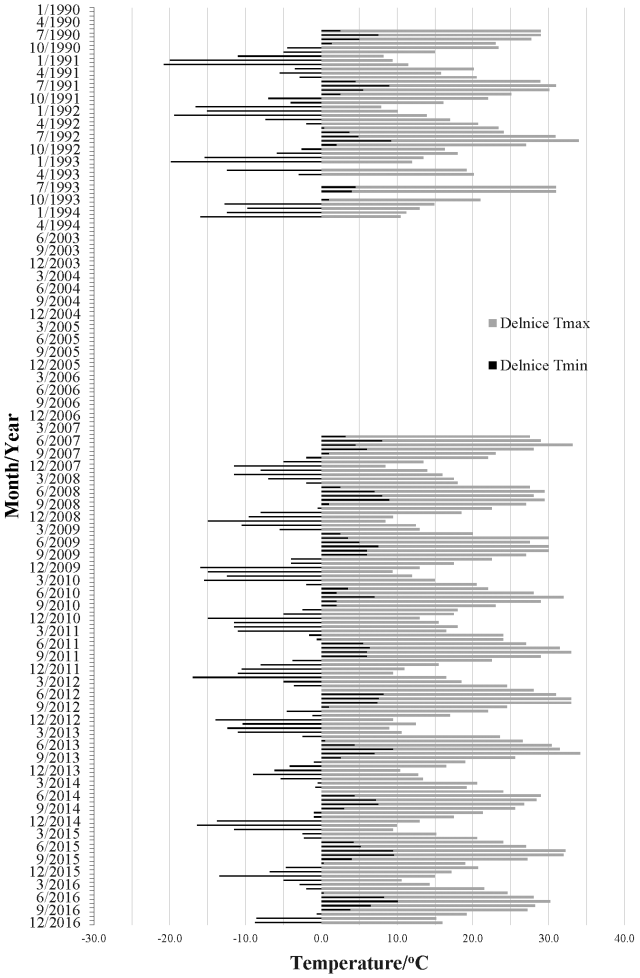


Fig. S2. The 25-year moving average of minimum and maximum monthly temperature recorded at the meteorological station Delnice.

Table S1. The percentage of individual species per site in years 1990–1991 ($L_3^{90/91}$, $L_5^{90/91}$, $L_8^{90/91}$) and 2015–2016 (L_1–L_8).

Species / Site mark	Abbreviation	Pecentage of individuals (%)										
		L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	$L_8^{90/91}$	$L_5^{90/91}$	$L_3^{90/91}$
Abax parallelepipedus (Piller & Mitterpacher 1783)	Aba. par.	0.00	0.00	0.17	3.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Abax carinatus Duftschmid 1812	Aba. car.	1.15	1.07	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30
Abax ovalis Duftschmid 1812	Aba. ova.	12.21	3.41	2.58	14.36	1.27	5.05	18.77	4.59	9.30	1.12	7.74
Agonum sp. Bonelli 1810	Ago. sp.	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Anisodactylus intermedius Dejean 1829	Ani. int.	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Amara eurynota Panzer 1797	Ama. eur.	0.00	0.43	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aptinus bombarda Illiger 1800	Apt. bom.	0.29	19.83	43.72	24.86	0.05	0.03	0.00	70.09	0.08	0.00	4.70
Calosoma inquisitor Linnaeus 1758	Cal. inq.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Calathus micropterus Duftschmid 1812	Cal. mic.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.93	0.00
Carabus catenulatus Scopoli 1763	Car. cat.	0.00	6.61	0.34	12.89	0.00	0.00	3.56	0.06	0,12	0.02	0.91
Carabus convexus Fabricius 1775	Car. con.	1.15	0.43	0.00	0.37	0.00	0.00	0.00	0.00	0.00	0.02	0.00
Carabus coriaceus Linnaeus 1758	Car. cor.	1.01	13.01	3.96	18.78	0.00	0.03	12.74	0.18	0.24	0.02	0.76
Carabus creutzeri Fabricius 1801	Car. cre.	0.00	0.00	0.00	0.00	0.05	0.07	6.58	0.00	0.13	0.00	0.00
Carabus croaticus Dejean 1826	Car. cro.	0.00	1.49	0.69	4.79	1.06	1.21	20.41	2.69	0.81	0.2	2.73
Carabus violaceus Linnaeus 1758	Car. vio.	4.74	4.48	4.99	5.71	3.33	3.09	7.81	1.41	0.05	1.95	5.61
Cychrus attenuatus Fabricius 1792	Cyc. att.	2.16	2.99	1.72	1.47	0.11	0.34	0.41	0.37	0.6	0.86	2.73
Cymindis humeralis Geoffroy 1785	Cym. hum.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
Harpalus sp. Latreille 1802	Har. sp.	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.02	0.00
Leistus nitidus Duftschmid 1812	Lei. nit.	0.00	0.00	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00
Leistus piceus Frölich 1799	Lei. pic.	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leistus rufomarginatus Duftschmid 1812	Lei. ruf.	0.00	0.64	0.00	0.00	0.00	0.00	0.00	0.06	0.39	4.57	0.76
Leistus spinibarbis Fabricius 1775	Lei. spi.	0.14	0.21	0.17	0.00	0.00	0.00	0.14	0.00	0.15	0.00	0.00
Licinus hoffmannseggi Panzer 1803	Lic. hof.	0.00	0.21	0.17	0.74	0.58	0.24	0.55	0.12	0.00	0.18	0.00
Loricera pilicornis Fabricius 1775	Lor. pil.	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Molops alpestris Dejean 1828	Mol. alp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.99	1.10	3.03
Molops elatus Fabricius 1801	Mol. ele.	0.00	0.00	0.00	0.55	1.06	0.20	0.00	0.31	0.00	0.00	0.00
Molops ovipennis Chaudoir 1847	Mol. ovi.	0.00	0.00	0.00	0.00	1.48	0.10	0.00	0.06	0.00	0.00	0.00
Molops piceus Panzer 1793	Mol. pic.	0.00	0.00	0.17	0.18	2.43	0.03	0.27	0.06	0.00	0.00	0.00
Molops striolatus Fabricius 1801	Mol. stri.	1.58	0.64	2.58	2.03	0.00	1.01	0.27	0.98	0.84	0.12	3.79
Nebria brevicollis Fabricius 1792	Neb. bre.	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nebria dahlii Duftschmid 1812	Neb. dah.	64.08	22.81	20.31	0.18	79.83	58.73	2.33	5.08	60.75	31.35	27.47
Notiophilus biguttatus Fabricius 1779	Not. big.	0.00	0.00	0.17	0.00	1.16	0.00	0.00	0.00	0.24	2.99	0.30
Platynus scrobiculatus Fabricius 1801	Pla. scr.	0.57	0.00	2.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.52
Pterostichus burmeisteri Heer 1838	Pte. bur.	5.75	21.75	11.53	9.94	3.80	9.65	14.25	12.11	5.01	5.32	36.87
Pterostichus fasciatopunctatus Creutzer 1799	Pte. fas.	4.60	0.00	3.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15
Pterostichus unctulatus Duftschmid 1812	Pte. unc.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	34.34	0.00
Pterostichus variolatus Dejean 1828	Pte. var.	0.00	0.00	0.00	0.00	3.48	20.22	11.92	1.77	18.06	9.13	0.00
Reicheiodes rotundipennis Chaudoir 1843	Rei. rot.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.00
Stomis rostratus Sturm in Duftschmid 1812	Sto. ros.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.14	0.66	0.15
Trechus croaticus Dejean 1831	Tre. cro.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	1.75	0.46

3. RASPRAVA

Cilj ovih istraživanja bio je unaprijediti saznanja o trčcima kao pouzdanim bioindikatorskim vrstama, proširiti znanje o ekologiji i biologiji naših endemskih vrsta trčaka, pobliže upoznati evolucijske i povijesne procese na području Balkanskog poluotoka te u konačnici postaviti kvalitetne temelje za primjenu potrebnih konzervacijskih mjera i buduća istraživanja trčaka na području Dinarida. U tu svrhu istraženi su međuodnosi populacija odabranih endemskih vrsta duž areala rasprostranjenosti na Dinaridima i sastav zajednica trčaka u Nacionalnom parku Risnjak te su analizirane promjene utvrđene nakon razdoblja od 25 godina.

Po prvi puta uključene su sekvence triju vrsta roda *Carabus* (*Carabus (Platycarabus) creutzeri* Fabricius, 1801, *Carabus (Eucarabus) parreyssi* Palliardi, 1825 i *Carabus (Eucarabus) ulrichii* Germar, 1824) u rekonstrukciji filogenetskog stabla ovog roda; također, po prvi puta je primijenjena metoda geometrijske morfometrije u pokušaju razrješavanja filogenetskih odnosa među sestrinskim endemskim vrstama trčaka Dinarida te je uspoređen sastav zajednica trčaka dinarskog krša nakon dugog niza godina.

Usporedba rezultata molekularnih analiza s dvije postojeće klasifikacije roda *Carabus* na temelju morfoloških karakteristika (oblik prednjeg štitića kod ličinaka i oblik endofalusa) pokazala je veće slaganje molekularnih podataka s klasifikacijom na temelju oblika endofalusa, što je u skladu s očekivanjima i prijašnjim rezultatima drugih autora (Sota i Ishikawa 2003, Andujar i sur. 2012, Deuve i sur. 2012). Sekvence triju vrsta ovoga roda, od kojih dvije pripadaju alpsko-dinarskim endemskim vrstama (*C. creutzeri*, *C. parreyssi*), po prvi puta su uključene u molekularne analize, a dobiveni rezultati također ukazuju na veće taksonomsko slaganje molekularnih podataka s klasifikacijom na temelju strukture endofalusa (Znanstveni rad br. 1).

Posebna pozornost tijekom istraživanja posvećena je dvjema sestrinskim endemskim vrstama – *Carabus (Megodonthus) croaticus* Dejean, 1826 (trčak saboriti) i *Carabus (Megodonthus) caelatus* Fabricius, 1801 (trčak smežurani). Ekologija ovih vrsta slabo je poznata, a brojni morfološki varijeteti rezultirali su neslaganjima u taksonomiji na intraspecijskoj razini. Analiza geometrijske morfometrije oblika glave i pronotuma obuhvatila je više od 400 jedinki s gotovo cijelog područja rasprostranjenja ovih vrsta. Rezultati pokazuju uspješno razdvajanje sestrinskih vrsta na temelju oblika glave i oblika pronotuma (poglavlje 3.1.2.). Na intraspecijskoj razini vidljivo je grupiranje pojedinih fenotipskih varijanti geografski bližeg areala, ali i brojna preklapanja. Usporedbom rezultata

ovog rada i triju postojećih klasifikacija predložen je manji broj podvrsta za obje vrste (Znanstveni rad br. 2).

Analiza sastava zajednica trčaka na području Nacionalnoga parka Risnjak obuhvatila je 8 ploha duž visinskoga gradijenta od 704 do 1277 m. Tri odabrane plohe podudarale su se s plohama na kojima je istraživanje provedeno prije 25 godina (Vujčić-Karlo 1999). Usporedbom brojnosti, disperzijskih sposobnosti, temperaturnog afiniteta i higrofilnosti uzorkovanih jedinki zabilježene su određene promjene kao što su: porast brojnosti generalista i smanjenje brojnosti specijalista, značajan porast brojnosti termofilnih vrsta na pojedinim plohama te izostanak nekih endemskih vrsta u ulovu (Znanstveni rad br. 3). Dostupni klimatski podaci nedostatni su za objašnjavanje navedenih promjena s obzirom da nije postajao kontinuitet u mjerenjima temperature i količine padaline na istraživanom području. Podaci s kojima raspolažemo ne pokazuju statistički značajne razlike u vrijednostima temperature i količini padalina u rasponu od 25 godina (detalji u Znanstveni rad br. 3). Određene promjene mogu se dijelom objasniti vremenskim neprilikama (ledena kiša) te pojačanim antropogenim pritiskom koji je uslijedio (pojačani ulazak ljudi i mehanizacije u područje Parka zbog sanacije šteta nastalih odronom stabala nakon vjetroloma). Uočena odstupanja naglašavaju važnost uspostave monitoringa trčaka na području Nacionalnoga parka.

3.1. Primjena morfoloških i molekularnih metoda za utvrđivanje taksonomskih odnosa unutar roda *Carabus*

Tradicionalna ili linijska morfometrija predstavlja prvi i temeljni korak u proučavanju vrsta. Detaljan vanjski opis sa pripadajućim mjerama neophodan je za daljnja istraživanja, međutim njegova primjena za rješavanje taksonomskih odnosa često nije precizna. Razlog tomu leži u činjenici da okolišni uvjeti, u manjoj ili većoj mjeri, utječu na morfologiju vrste. I dok su neki morfološki fenomeni, kao što je izgled pokrilja, još uvijek nepoznanica, veličina trčaka u većini slučajeva podudara se s abiotičkim čimbenicima. Kod većine vrsta moguće je primijeniti obrnuto Bergmanovo pravilo (s porastom nadmorske visine veličina jedinki se smanjuje). Pojavu koju je lako objasniti kraćom vegetacijskom sezonom na višim nadmorskim visinama i potrebom za bržim spolnim sazrijevanjem.

Razvojem i primjenom molekularnih metoda znatno je uznapredovalo znanje o sistematici, taksonomiji i filogeniji trčaka. DNA sekvence mitohondrijskih i nuklearnih gena omogućuju brojne analize na svim taksonomskim nivoima. Dodatnu prednost predstavlja mogućnost kombinacije filogeografije i sistematike što vodi boljem razumijevanju povijesnih događaja i evolucije vrste. Unatoč tome, zbog brojnih problema s kojima se susrećemo tijekom sekvenciranja – od same cijene postupka pa do poteškoća prilikom uzorkovanja kao i činjenice da brojne vrste unutar ove porodice još nisu otkrivene, postoje velike praznine i problemi u rješavanju taksonomije trčaka. Navedeno se stanje posebno odnosi na rod *Carabus* pa tako Assmann i sur. 2008. godine navode da katalozi izdani proteklih godina (Brezina 1999, Bousquet i sur. 2003, Deuve 1994, 2004) otkrivaju upečatljivo različita mišljenja u smislu imenovanja, sustavnosti i rangiranja taksona iznad i ispod razine vrste unutar roda *Carabus*.

Dvije najpoznatije klasifikacije roda *Carabus*, temeljene na morfologiji, su klasifikacija na temelju građe prednjeg štitića kod ličinaka te klasifikacija na temelju oblika endofalusa u odraslih jedinki. Mali broj stručnjaka koji se bave ličinkama i posljedično nedostatak opisa ličinaka za brojne vrste, posebice endemske, glavni je nedostatak klasifikacije na temelju građe prednjeg štitića ličinaka. Oblik endofalusa se općenito pokazao boljim rješenjem jer je podudaranje s podacima dobivenih molekularnim metodama znatno veće. U sklopu ove disertacije napravljeno je istraživanje koje je obuhvatilo 31 vrstu roda *Carabus*. Kao molekularni marker korišten je mitohondrijski gen za citokrom oksidazu 1 (*COI*), a dobiveni podaci također pokazuju veće slaganje s klasifikacijom na temelju oblika endofalusa (Znanstveni rad br. 1).

Sekvence vrsta *C. caelatus, C. parreyssi, C. creutzeri, C. intricatus* i *C. ulrichii* unešene su u baze BOLD (*Barcode of Life Data* Systems) i NCBI (The *National Center for Biotechnology Information*), (Prilog 3).

Unatoč neospornoj činjenici da je morfometrijski pristup koristan za sistematiku i taksonomiju u brojnim skupinama trčaka, problem predstavlja nepoznavanje do koje mjere okolišni faktori djeluju na karakteristike egzoskeleta (Maynard-Smith 1998). Fenotipske karakteristike ne mogu otkriti informacije o broju varijabilnih genskih lokusa i učestalosti alela. S druge strane isključiva upotreba DNA za rješavanje taksonomije kao što je predloženo od strane nekih autora (Tautz i sur. 2003) ne mogu zamijeniti taksonomiju i sistematiku u širem i klasičnom smislu (Valdecasas i sur. 2008, Wheeler i sur. 2004).

Nesigurnost u taksonomiji zasigurno ometa učinkoviti razvoj mjera konzervacijske biologije.

Zbog svega navedenog preporuča se integrativni pristup morfologiji, morfometriji i molekularnoj sistematici kao jedini prikladni način za pronalazak rješenja za izazovne probleme kao što je razrješavanje taksonomskih odnosa unutar kornjaša.

3.1.1. Primjena linearne morfometrije (LM) u interspecijskom i intraspecijskom razdvajanju sestrinskih vrsta *C. croaticus* i *C. caelatus*

Primjenom linearne morfometrije na 207 jedinki vrste *C. caelatus* (101 M, 106 F) i 301 jedinku vrste *C. croaticus* (149 M, 152 F) iz privatnih zbirki B. Drovenika, P. Durbešić, L. Šerić Jelaska i moje osobne zbirke pokušala se provjeriti opravdanost postojanja velikog broja opisanih podvrsta te utvrditi fenotipske razlike između populacija duž planinskog lanca Dinarida (neobjavljeni podaci; Prilog 1). Ispitivani materijal pokriva gotovo cijeli areal ovih dviju vrste (Albanija, Bosna i Hercegovina, Crna Gora, Hrvatska, Kosovo, Slovenija i Sjeverna Makedonija). Analizirane su sljedeće karakteristike: duljina glave (udaljenost od prednjeg ruba klipeusa do posteriorne margine složenih očiju; mjereno na lijevoj strani glave), širina glave (najveća poprečna udaljenost između složenih očiju), duljina pronotuma (udaljenost od prednjeg ruba do stražnjeg ruba, mjereno po središnjoj liniji), širina pronotuma (mjerena na najširem dijelu pronotuma), duljina elitre (udaljenost od stražnjeg ruba skuteluma do vrha lijeve elitre), širina elitre (mjerena na najširem dijelu elitre) i ukupna duljina tijela (zbroj duljine glave, pronotuma i elitre). Mužjaci i ženke analizirani su odvojeno zbog prisutnog spolnog dimorfizma. Značajna razlika primijećena je u svih 7 mjerenih značajki između sestrinskih vrsta ($F = 224.25$, $p < 0.05$), (Prilog 1). Općenito, jedinke *C. caelatus* su nešto veće od jedinki *C. croaticus*. Najviše je razlika uočeno u mjerenjima duljine i širine glave te duljine i širine pronotuma.

Na razini podvrsta do značajnog odvajanja došlo je samo između podvrsta uzorkovanih na suprotnim krajevima areala – gdje je i najveća geografska udaljenost. U oba slučaja značajne razlike u mjerenim veličinama mogu se objasniti obrnutim Bergmanovim pravilom jer između podvrsta koje se statistički značajno razlikuju nailazimo na razliku u nadmorskoj visini većoj od 1000 metara. Koncept poznat pod nazivom *„ring-species“* (Pereira i Wake 2015), koji govori da populacije s krajnjih dijelova areala imaju najmanje šanse za miješanje genskoga materijala zbog čega se očekuje da će upravo podvrste s krajnjih dijelova areala s vremenom postati reproduktivno izolirane, također može poslužiti kao objašnjenje pogotovo

ako uzmemo u obzir slabe disperzijske sposobnosti proučavanih vrsta trčaka (brahipterne vrste).

3.1.2. Primjena geometrijske morfometrije (GMM) u interspecijskom i intraspecijskom razdvajanju sestrinskih vrsta *C. croaticus* i *C. caelatus*

Taksonomska klasifikacija i razumijevanje biološke raznolikosti nekada je bilo bazirano isključivo na opisima vanjskih morfoloških karakteristika (Adams i sur. 2004). Problem tradicionalne morfometrije leži u nemogućnosti odvajanja učinka veličine i oblika (Zelditch i sur. 2012) iz čega proizlazi nemogućnost proučavanja oblika klasičnim metodama. Problem je posebno naglašen kod taksona u kojima su izražene razlike u veličini tijela prisutne unutar grupa, kao što su trčci. Prednost geometrijske morfometrije je zadržavanje geometrije orijentira tijekom analize i omogućavanja prikazivanje statističkih rezultata kao stvarnih oblika. U geometrijskoj morfometriji oblik je definiran kao „bilo koja geometrijska informacija koja ostaje kada su učinci translacije, skaliranja i rotacije uklonjeni iz objekta“ (Kendall 1977).

Nakon iscrpnih klasičnih morfometrijskih analiza (linijske morfometrije) uslijedile su analize geometrijske morfometrije oblika pronotuma i oblika glave s obzirom da je najviše razlika između podvrsta utvrđeno mjerenjima duljine i širine navedenih dijelova.

Primjena geometrijske morfometrije pokazala se uspješnom u razdvajanju sestrinskih vrsta na temelju oblika pronotuma i glave (Znanstveni rad br. 2). Intraspecijsko razdvajanje pokazalo se manje uspješnim s većinskim preklapanjima, poglavito populacija geografski bližeg areala. Međutim u oba slučaja, i kod vrste *C. croaticus* i *C. caelatus* uviđa se izdvajanje triju grupa fenotipskih varijanti te je na temelju rezultata GMM pronotuma predložena sljedeća klasifikacija: 3 podvrste unutar vrste *Carabus croaticus*: *C. croaticus croaticus* (uključujući: *C. croaticus croaticus*, *C. croaticus frankenbergeri* i *C. croaticus mediterraneus*), *C. croaticus bosnicus* (uključujući: *C. croaticus bosnicus*, *C. croaticus kobingeri*, *C. croaticus pretneri*, *C. croaticus travnikanus*, *C. croaticus zepcensis* i *C. croaticus bosiljevici*) i *C. croaticus durmitorensis* (uključujući: *C. croaticus durmitorensis*, *C. croaticus babinjensis*, *C. croaticus droveniki*, *C. croaticus kraetschmeri* i *C. croaticus ljubetensis*) te tri podvrste unutar vrste *Carabus caelatus*: *C. caelatus caelatus* (uključujući: *C. caelatus caelatus* i *C. caelatus schreiberi*), *C. caelatus dalmatinus* (uključujući: *C. caelatus dalmatinus*, *C. caelatus cabuljensis*, *C. caelatus macretus* i *C. caelatus procerus*) i

C. caelatus sarajevoensis (uključujući: *C. caelatus sarajevoensis*, *C. caelatus volujakianus*, *C. caelatus metalkanus* i *C. caelatus malissorum*).

Uz GMM pronotuma i glave napravljene su i analize oblika kopulatornog organa kod mužjaka (*aedeagus*). Analiza je obuhvatila 26 jedinki (10 jedinki vrste *C. croaticus* i 16 jedinki vrste *C. caelatus*) koje su pripadale trima različitim populacijama iz Slovenije, Hrvatske te Bosne i Hercegovine (neobjavljeni podaci; Prilog 2). Manji broj mužjaka u ulovu bili su ograničavajući parametri u proširivanju analize na veći broj jedinki. Ukupno je odabrano 20 orijentira (Slika P 2.1.). Rezultati analize glavnih komponenti (PCA) pokazali su jasno odvajanje vrsta na temelju oblika kopulatornog organa što je i očekivano (prva i druga PCA objašnjavaju 37.32% i 21.09%). Na intraspecijskom nivou kanonička diskriminantna analiza (CVA) je pokazala odvajanje populacija iz Slovenije i Hrvatske od udaljenijih populacija iz Bosne i Hercegovine (Slika P 2.2.). Najzanimljiviji rezultat ove analize bio je grupiranje podvrsta *C. caelatus schreiberi* (SZ Hrvatska) i podvrste *C. caelatus caelatus* (J Slovenija). Sve provedene analize, od linijske morfometrije, preko GMM pronotuma i glave i na kraju GMM kopulatornog organa ne pokazuju opravdanje za razdvajanje ovih varijeteta u dvije različite podvrste. Njihova geografska blizina također potkrepljuje navedene rezultate. Preostaju još samo analize molekularnih biljega za sigurnu potvrdu navedenih rezultata.

Opsežna linijska morfometrija pokazala je da se istraživane vrste najviše razlikuju u mjerama pronotuma i glave. Međutim daljnje analize temeljene na linijskoj morfometriji (LM) nisu pokazivale jasna razdvajanja te je ono bilo prisutno samo u slučajevima usporedbe podvrsta s najviših i najnižih nadmorskih visina. Navedeno je u izravnoj korelaciji s veličinom i obrnutim Bergmanovim pravilom, ali i za očekivati zbog udaljenosti areala većoj od 700 km. Za razliku od LM, GMM je otkrila jasno razdvajanje dviju sestrinskih vrsta i na temelju oblika glave i na temelju oblika pronotuma. Postojanje velikog broja podvrsta prema dobivenim podacima nije opravdano (Znanstveni rad br. 2).

Geometrijska morfometrija svakodnevno privlači pozornost kao koristan alat u morfološkim studijima. Sve veća upotreba tehnika geometrijske morfometrije posljedica je, između ostalog, podudaranja dobivenih podataka s rezultatima molekularnih analiza na interspecijskoj i intraspecijskoj razini (Loy i sur. 1993, Auffray i sur. 1996, David i Laurin 1996, Naylor 1996, Klingenberg i McIntre 1998, Adams i sur. 2013, Zúñiga-Reinoso i Benitez 2015). S obzirom da metode GMM predstavljaju jednostavniji i jeftiniji proces od

sekvenciranja ne čudi sve veći interes za primjenu ove metode u razrješavanju taksonomskih odnosa.

3.2. Važnost kontinuiranog istraživanja entomofaune trčaka

Sastav zajednice trčaka ocrtava stanje ekosustava pa redovito praćenje zajednica trčaka omogućuje ranu detekciju poremećaja u njemu. Redovito prikupljanje informacija kroz definirani period (Saunders (2017) preporučuje praćenje svake godine u istom periodu) važno je za bilo koje znanstveno područje. Ono omogućuje praćenje stanja i uvid u promjene, a isto tako daje mogućnost za brzo otkrivanje uzroka navedenim promjenama i pravovremeni razvoj strategija za ublažavanje istih (Saunders 2017). Kada redovno praćenje uključuje vrste koje među prvima daju odgovor na promjene u uvjetima ekosustava važnost uspostave monitoringa postaje neupitna. Trčci, a posebno rod *Carabus*, predstavljaju izvrsnu skupinu za praćenje i proučavanje promjena u fauni. Dobra zastupljenost vrsta ovog roda u većini dijelova Europe, poznavanje njihove biologije i ekologije, atraktivnost, veličina, veliki udio beskrilnih vrsta i lako proučavanje standardiziranim metodama čini ga korisnim u proučavanju biogeografije i konzervacijske biologije.

Usporedbom sastava zajednica trčaka Nacionalnoga parka Risnjak u razmaku od 25 godina zabilježene su promjene i u brojnosti pojedinih vrsta, ali i u nestanku odnosno izostanku nekih vrsta u ulovu (Znanstveni rad br. 3). Primijećen je porast brojnosti termofilnih vrsta koji na pojedinim plohama odaje potpuno drugu sliku sastava zajednice trčaka nego prije 25 godina. Najveće promjene zabilježene su na plohi zvanoj Vilje (1199 m) gdje je prije 25 godina dominirala hladno-temperaturna vrsta *Nebria dahlii*, a danas dominira termofilna vrsta *Aptinus bombarda*.

Klimatske promjene mijenjaju izgled ekosustava kroz pomicanje vrsta u potrazi za pogodnijim staništem. I dok pojedine vrste (termofilne) profitiraju globalnim zatopljenjem, vrste koje preferiraju hladnije mikroklimatske prilike povlače se na više nadmorske visine što često podrazumijeva smanjenje pogodnog areala za život. Uz navedeno prisutna je i veća izolacija što u konačnici može rezultirati izumiranjem pojedinih populacija pa i nestankom čitave vrste (Hill i sur. 2002, Midgley i sur. 2002, Williams i sur. 2003, Thomas i sur. 2004). Provjerom visinske distribucije vrsta trčaka unutar Nacionalnoga parka Risnjak prije 25 godina i danas primijećena je veća abundancija endemskih hladno-temperaturnih vrsta na višim nadmorskim visinama (npr. *Carabus creutzeri* i *Pterostichus variolatus*). Isto je

primijećeno i za endemsku vrstu *Carabus croaticus* koja je bila daleko najbrojnija na plohi smještenoj na najvišoj nadmorskoj visini.

Jedan od najvećih problema za planinske specijaliste je gubitak povoljnog staništa uslijed klimatskih promjena. Problem predstavlja i pojačani antropogeni utjecaj koji je najčešće veći na nižim nadmorskim visinama (Wilson 2005) doprinoseći time još više smanjenom vremenu za reakciju vrsta. Ubrzane promjene u načinu korištenja zemljišta (rascjepkasnost staništa, insekticidi, umjetna gnojiva i dr.) ostavljaju nesagledive posljedice na kompletnu entomofaunu. Neka od mogućih rješenja za spas ugroženih populacija ili metapopulacija uključuju koridore ili reintrodukciju. Međutim oba rješenja uključuju intenzivni monitoring radi procjene uspješnosti spomenutih metoda.

Brojne poteškoće u praćenju stanja entomofaune od onih financijske prirode do brojnih prepreka zbog „prirode posla“ koja uključuje rad na terenu u često nepogodnim uvjetima rezultirale su vrlo slabim monitoringom i malim brojem tzv. „*long-term*“ studija na području Europe. Nepotpuni podaci ostavljaju praznine u razumijevanju promjena i načina reagiranja trčaka čime se znatno otežava razvoj pravilnih konzervacijskih mjera. Sličnu situaciju nalazimo u Nacionalnom parku Risnjak. S obzirom da se radi o području visoke kategorije zaštite poznatom po očuvanim šumskim ekosistemima (Frey i sur. 2016), lako je zaključiti da su promjene, ako ih i ima, neznatne. Međutim unatoč izostanku direktnog antropogenog utjecaja, indirektni utjecaj u vidu nepredviđenih vremenskih prilika nemoguće je izbjeći, a postoje indicije da će se takvi scenariji zbog naglih klimatskih promjena sve češće ponavljati (Pachauri i sur. 2014). Upravo ekstremne vremenske prilike, kao dio globalnih klimatskih promjena, smatraju se važnim uzrokom smanjenja brojnosti populacija kornjaša (Wagner 2019).

Rezultati posljednjeg istraživanja (Znanstveni rad br. 3) ukazuju na promjene u sastavu zajednica koje upućuju na prisutnost određenih promjena u ekosustavima. Uspostava monitoringa zajednica trčaka na području Parka uvelike bi olakšala objašnjavanje uočenih, ali i predviđanje budućih promjena.

3.3. Radovi u pripremi

3.3.1. Primjena molekularnih analiza u interspecijskom i intraspecijskom razdvajanju sestrinskih vrsta *C. croaticus* i *C. caelatus*

Jedan od ciljeve ove disertacije bio je i usporediti dobivene rezultate klasične morfometrije (LM) s rezultatima molekularnih analiza za istu svrhu (intra- i interspecijsko odvajanje sestrinskih vrsta). U molekularne analize bile su uključene 124 jedinke (47 jedinki vrste *C. caelatus* i 77 jedinki vrste *C. croaticus*) s područja Republike Hrvatske, Slovenije, Bosne i Hercegovine i Sjeverne Makedonije (Prilog 4, Tablica P 4.1.).

Kod upotrebe mitohondrijskog biljega *COI* naišlo se na veliki problem kontaminacije prave i ciljane mtDNA sekvence pseudogenima unatoč korištenju manjeg broja ciklusa prilikom lančane reakcije polimerazom (30 ciklusa) te posljedično nemogućnosti interpretacije podataka (Prilog 4, Tablica P 4.2.).

Na poteškoće pri korištenju univerzalnog markera za barkodiranje trčaka naišli su i Assman i suradnici (2019) kada je primijećeno da se nekoliko vrsta, uključujući i široko rasprostranjene sestrinske vrste ne mogu identificirati DNA barkodom iz različitih razloga (npr. mlade vrste ili horizontalni protok gena).

Mali broj uspješnih *ITS-2* sekvenci (26 sekvenci) omogućio je provođenje samo dijela analiza. Na primjer: usporedbom *ITS-2* sekvenci vrste *C. croaticus* sa *ITS-2* sekvencom vrste *C. caelatus* uočen je mali broj mutacija (od 568 nukleotidnih pozicija vidljiv je jedan indel na pozicijama 157-158 te zamjena A<>G na poziciji 486), (Prilog 4, Slika P 4.1.). Mali broj mutacija ukazuje na moguću ranu specijaciju. U prilog tome govore i rezultati sekvenciranja mitohondrijskog gena *COI* koji pokazuju veliku sličnost u sekvencama sestrinskih vrsta (98,19% prema podacima iz BOLD baze (http://boldsystems.org/)).

Usporedbom bioloških sljedova (BLAST, *Basic local alignment search too*l) *ITS-2* sekvenci vrsta *C. croaticus* i *C. caelatus* sa sekvencama vrsta roda *Carabus* dostupnih u NCBI bazi, sekvence vrste *C. croaticus* najsličnije su sekvencama vrsta *C. monilis* (91,78%), *C. violaceus* (91,54%) i *C. coriaceus* (89,54%) dok su sekvence vrste *C. caelatus* najsličnije sekvencama vrsta *C. monilis* (92,01%), *C. violaceus* (91,68%) i *C. auronitens* (87,54%).

S obzirom da se radi o vrstama mladog postanka te mogućnosti hibridizacije u područjima preklapanja areala pronalazak molekularnog/ih biljega za utvrđivanje intraspecijskih odnosa

predstavlja izazov za daljnja istraživanja. U ovakvim slučajevima važnost i značaj morfoloških istraživanja postaje neupitna. Razvoj metoda geometrijske morfometrije, kao novog oblika morfoloških istraživanja, otvara nove mogućnosti u razrješavanju kompliciranih taksonomskih odnosa pogotovo kada klasične molekularne metode ne daju rezultate. Na slične probleme naišli su i Assmann i sur. (2019) te su zaključili da sve dok se ne pojave automatizirane, brze i pouzdane metode identifikacije vrsta iz uzoraka, ekološka se istraživanja moraju oslanjati na klasičnu identifikaciju svojte temeljenu na morfologiji.

3.3.2. Model rasprostranjenja vrsta

Jedan od načina za praćenje i prognoziranje promjena u životnoj sredini vrste uslijed klimatskih promjena jest izrada modela rasprostranjenja vrsta (SDM, od engleskog termina *Species Distribution Model*). Ovaj model koristi prisustvo ili odsustvo neke vrste te utvrđuje postoje li na drugim lokacijama uvjeti za postojanje te vrste.

Opsežnim pregledom literature i dostupnih zbirki prikupljeni su podaci o nalazištima endemskih vrsta na području Dinarida s ciljem izrade sadašnjeg i predviđanja budućeg modela ekoloških niša duž cijelog areala rasprostranjenja pomoću postojećih klimatskih varijabli te topografskih podataka (prema Jelaska i sur. 2013) i pretpostavki o njihovim vrijednostima u rasponu od 2041. – 2070. godine.

Ovisno o kvaliteti dobiveni ulaznih podataka o toponimima napravljeni su modeli ekoloških niša za područje Republike Hrvatske za vrste *C. caelatus* i *C. creutzeri* (Prilog 5, Slika P 5.1. i 5.2.) dok su za vrstu *C. croaticus* tablično prikazani do sada prikupljeni nalazi koji su u obradi (Tablica P 5.1.).

Za analizu i kartografski prikaz modela rasprostranjenosti korišten je model najveće entropije (*Maxent*) i SAGA GIS program.

Budući klimatski podaci (2041. – 2070.) temelje se na predviđanjima prema klimatskom modelu IPCC A2 scenarija (*Intergovernmental Panel on Climate Change*), (Nakićenović i sur. 2000), u kombinaciji s EH5OM modelom (združeni atmosfersko-oceanski model; detalji u Roeckner i sur. 2003) te podacima dinamičke prilagodbe dobivenih regionalnim klimatskim modelom (*Regional Climate Model*) treće generacije (RegCM3), (Dickinson i sur. 1989, Giorgi 1990, Pal i sur. 2007). Navedena predviđanja su: povišenje temperature tijekom zime za 1,5 ^{o}C i porast količine padalina za 10 mm, povišenje temperature u proljeće za 1,5 ^{o}C i smanjenje padalina za 45mm, povišenje ljetnih temperatura za 2,25 ^{o}C i smanjenje

padalina za 45 mm te povišenje jesenske temperature za 1,75 ^{o}C i smanjenje padalina za 45 mm.

Kombinacijom klimatskih i topografskih varijabli predviđa se smanjenje pogodnog staništa za vrstu *C. caelatus* za 53,22 % (Slika P 5.1. A i B). Za vrstu *C. creutzeri* zabilježen je porast pogodnog staništa za 22,10 % (Slika P 5. 2. A i B), od čega 45% čine nova staništa koja do prije nisu bila pogodna za život ove vrste.

Finalni cilj radova u pripremi je povezivanje genetike i morfologije te distribucije endemskih vrsta s okolišnim značajkama.

3.4. Smjernice za daljnja istraživanja

Rješavanje taksonomskih odnosa unutar porodice trčaka nemoguće je bez interdisciplinarnog pristupa koji uključuje morfologiju, molekularnu sistematiku, etologiju, ekologiju, geografsku distribuciju i bioinformatiku. Upravo je takav pristup prepoznat kao najbolje rješenje za izazove s kojima se susreću karabidolozi (Kotze i sur. 2011). Primjena pojedinačnih metoda kroz povijest (morfometrijske analize, proučavanje anatomije, molekularne analize), bilo na ličinkama ili odraslim jedinkama, taksonome je često dovodila do različitih rezultata, nerijetko oprečnih. Posljedično, nailazimo na velik broj različito definiranih taksonomskih odnosa, mnoštvo sinonima i razilaženja u mišljenjima. Problem predstavlja i činjenica da je velik broj člankonožaca zastupljen sa samo jednim primjerkom u zbirkama i o njima se ne zna ništa osim lokacije i imena (Lövei i Sunderland 1996). Navedeno za posljedicu ima postojanje "rupa" u pokušajima objašnjavanja filogenije i evolucije vrsta. Intenziviranje faunističkih istraživanja, poglavito u zemljama jugoistočne Europe koje obiluju bogatstvom vrsta, neophodno je za bolje shvaćanje filogenije trčaka. Ako uzmemo u obzir da je Balkanski poluotok smješten u klimatskoj tranziciji između umjerene zone i mediteranskih uvjeta i da će u vidu globalnih klimaskih promjena ovo područje postati suše i toplije (Grunewald i Scheithauer 2010) važnost faunističkih istraživanja postaje još naglašenija. Kombinacijom faunističkih, ekoloških i genetskih istraživanja koja obuhvaćaju različite dijelove areala vrste, kao što je u sklopu ove disertacije napravljeno za vrste *C. croaticus* i *C. caelatus*, postavili bi se temelji za rješavanje nedoumica o evolucijskoj povijesti vrsta, ali isto tako napravio korak bliže identifikaciji glacijalnih refugija i mikrorefugija na području Balkanskog poluotoka i Dinarida koji su bili ključni za preživljavanje kako trčaka tako i brojnih drugih vrsta tijekom povijesti.

Jedno od rješenja za kontinuirani monitoring vrsta planinskih, ali i drugih ugroženi ekosustava, jest odabir i uspostavljane "trajnih" ploha za istraživanje čime bi se uvelike olakšalo uočavanje trendova bitnih za procjenu stanja ekosustava i ugroženosti vrsta.

Važan naglasak potrebno je staviti i na barkodiranje faune trčaka te unos genetskih podataka u relevantne baze kao što su BOLD baza (*Barcode of Life Data* Systems) i NCBI (*The National Center for Biotechnology Information*). Problemi na koje se naišlo prilikom molekularnih analiza upućuju i na potrebu za pronalaskom najpogodnijih genetskih biljega za analize inter- i intraspecijskih odnosa unutar roda *Carabus*.

Velik broj planina unutar planinskog lanca Dinarida za pripadnike roda *Carabus* čini nepremostive prepreke. S obzirom na njihove slabe disperzijske sposobnosti i nemogućnost leta vjerojatno je da se na svakom planinskom masivu odvijaju samostalni mikro-evolucijski procesi. Ako u to uključimo različite uvjete u okolišu i različiti antropogeni pritisak na pojedine dijelove ekosustava za očekivati je da će svaki planinski masiv imati svoju morfološku varijantu. Pri tome je potrebno imati na umu da se radi o vrsti mladog postanka i da povremene migracije vrsta između ovih udaljenih metapopulacija nisu isključene. Za očekivati je da će vrste na rubnim dijelovima areala najviše odstupati u morfologiji, ali i da će prve postati reproduktivno izolirane. Opisivanje velikog broja podvrsta prilikom svakog izlaska na teren s ciljem dočaravanja raznolikosti roda dovodi do zbrke u sistematici i postojanja nerealno velikog broja podvrsta. Zbog svega navedenog potreban je naročiti oprez prilikom terenskog istraživanje entomofaune i izbjegavanje korištenja isključivo morfološkog pristupa pri definiranju novih varijeteta.

Bez poznavanja biologije i ekologije vrste kao i uvjeta u ekosustavima možemo samo nagađati o utjecaju promjena i reakciji vrsta na njih. Izrada detaljnog plana monitoringa, uspostava „trajnih" ploha, integrativni pristup i uključivanje većeg broja znanstvenika jedini je način za dobivanje kvalitetnih informacija o vrstama i stanjima ekosustava. Samo na temelju kontinuiranih istraživanja moguće je upoznati povijesne procese koji su se odvijali na ovom području, procijeniti stres koji promjene izazivaju, predvidjeti odgovor vrste s obzirom na širinu njezine ekološke valencije i poduzeti najprimjerenije konzervacijske mjere.

4. ZAKLJUČCI

1. Filogenetska stabla dobivena u ovom radu opravdavaju učestaliju upotrebu klasifikacije roda *Carabus* na temelju strukture endofalusa od klasifikacije na temelju građe prednjeg štitića ličinaka.

2. Primjenom metoda geometrijske morfometrije uspješno su razdvojene sestrinske vrste roda *Carabus (C. croaticus i C. caelatus*).

3. Primjena geometrijske morfometrije na intraspecijskoj razini ukazuje na postojanje manjeg broja podvrsta od trenutno opisanih, ali i brojna preklapanja geografski bližih fenotipskih varijanti.

4. Linijskom morfometrijom moguće je razdvojiti sestrinske vrste roda *Carabus (C. croaticus i C. caelatus*) dok su na intraspecijskoj razini statistički značajne razlike prisutne samo kod geografski najudaljenijih populacija.

5. Razrješavanje filogenetske prošlosti i točan broj podvrsta unutar sestrinskih vrsta roda *Carabus (C. croaticus i C. caelatus*) moguće je ustanoviti samo kombinacijom morfoloških i molekularnih metoda uz naglasak na pronalaženje najpogodnijeg markera za intraspecijsku deliminaciju ovih mladih vrsta.

6. Smanjenje brojnosti specijalista i povećanje brojnosti generalista na proučavanim postajama unutar Nacionalnoga parka Risnjak u odnosu na utvrđeno stanje od prije 25 godina upućuje na postojanje promjena u ekosustavu čiji se točan uzrok, zbog neredovitog praćenja entomofaune Parka, za sada ne može sa sigurnošću utvrditi.

7. Izostanak nekih endemskih vrsta u ulovu kao i smanjenje brojnosti populacija endemskih vrsta signalizira važnost uspostave monitoringa unutar Nacionalnoga parka Risnjak.

8. Praćenje stanja entomofaune treba se temeljiti na unaprijed definiranom, standardiziranom i kontinuiranom monitoringu koji, u kombinaciji s detaljnim podacima o načinu upravljanja, klimatološkim i svim ostalim relevantnim podacima o promatranom području, može prikazati ispravnu sliku promjena, njihove uzroke i razinu opasnosti koju one predstavljaju.

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PRILOG 1

Tablica P 1.1. Deskriptivna statistika (MIN-minimum, MAX-maksimum, AVER-srednja vrijednost i SD-standardna devijacija za sedam mjerenih značajki vrsta *Carabus croaticus* i *Carabus caelatus*; HL= duljina glave; PL= duljina pronotuma; EL= duljina elitre; BL= duljina tijela; HW = širina glave; PW = širina pronotuma; EW = širina elitre; F = ženke; M = mužjaci; N = broj jedinki). Vrijednosti su izražene u milimetrima.

VRSTA		HL	PL	EL	BL	HW	PW	EW
C. croaticus	MIN	2.930	4.260	14.540	21.860	2.930	6.000	9.010
F	MAX	4.220	8.750	23.070	33.470	4.110	9.610	14.000
N=152	AVER	3.588	5.800	19.927	29.315	3.601	8.209	12.135
	SD	0.246	0.482	1.671	2.235	0.275	0.743	0.966
C. croaticus	MIN	2.660	4.080	12.860	19.600	2.750	5.080	7.510
M	MAX	3.930	6.960	21.880	26.489	3.890	9.040	13.130
N=149	AVER	3.410	5.437	17.642	26.489	3.368	7.666	10.763
	SD	0.225	0.407	1.567	2.057	0.255	0.708	0.947
C. caelatus	MIN	3.500	5.660	18.830	29.300	3.340	7.170	11.960
F	MAX	5.680	8.350	28.760	41.410	5.290	10.770	16.330
N=107	AVER	4.257	7.052	23.971	35.280	3.929	8.887	14.228
	SD	0.326	0.6065	2.071	2.841	0.281	0.689	1.086
C. caelatus	MIN	3.130	5.310	15.500	24.130	2.880	6.050	9.660
M	MAX	4.560	8.110	25.310	37.880	4.120	10.520	14.330
N=101	AVER	4.043	6.609	21.546	32.159	3.659	8.332	12.605
	SD	0.267	0.563	1.776	2.495	0.268	0.732	0.984

Tablica P 1.2. Rezultati linearne morfometrije za populacije vrste ***C. croaticus***. Srednje vrijednosti su izražene u milimetrima s vrijednošću standarne devijacije navedene u zagradama. (EL= duljina elitre; PL= duljina pronotuma; HL= duljina glave; BL= duljina tijela; EW = širina elitre; PW = širina pronotuma; HW = širina glave; F = ženke; M = mužjaci; HR – Hrvatska; SI – Slovenija, BA – Bosna i Hercegovina, MK – Sjeverna Makedonija, ME – Crna Gora, AL – Albanija, RS – Srbija, Kosovo – XK). Podvrste determinirali: B. Drovenik, P. Durbešić i L. Š. Jelaska.

Lokalitet	**Država**	**Naziv podvrste**	**Br.F**	**EL (SD)**	**PL (SD)**	**HL (SD)**	**BL (SD)**	**EW (SD)**	**PW (SD)**	**HW (SD)**
Babinje	AL	*Babinjensis*	3	18,12 (0,868)	5,393 (0,146)	3,373 (0,074)	26,887 (0,809)	11,334 (0,378)	7,5 (0,401)	3,453 (0,047)
Murtenica	RS	*Bosiljevici*	0	0	0	0	0	0	0	0
Treskavica, Romanija, Jahorina, Zelengora	BA	*Bosnicus*	14	18,715 (0,774)	5,625 (0,275)	3,524 (0,152)	27,864 (1,14)	11,529 (0,615)	7,859 (0,47)	3,462 (0,187)
Velebit	HR	*Croaticus*	8	19,931 (1,03)	6,073 (0,264)	3,806 (0,187)	29,81 (1,28)	12,649 (0,802)	8,708 (0,523)	3,674 (0,188)
Pelister, Galičica	MK	*Droveniki*	1	16,37	4,97	3,1	24,44	9,98	6,77	3,2
Durmitor	ME	*Durmitorensis*	4	15,375 (0,634)	4,465 (0,17)	2,99 (0,042)	22,83 (0,757)	9,363 (0,248)	6,148 (0,178)	2,97 (0,039)
Snežnik, Javorniki, Plješivica, Risnjak	SI HR	*Frankenbergeri*	65	21,208 (0,921)	6,031 (0,472)	3,716 (0,207)	30,954 (1,263)	12,818 (0,512)	8,741 (0,404)	3,819 (0,157)
Golica, Blinje, Janj	BA	*Kobingeri*	11	19,052 (0,916)	5,739 (0,323)	3,496 (0,151)	28,287 (1,258)	11,746 (0,415)	7,805 (0,264)	3,468 (0,1)
Žljeb	ME	*Kraetschmeri*	4	18,828 (1,002)	5,605 (0,357)	3,418 (0,142)	27,85 (1,428)	11,618 (0,844)	7,875 (0,614)	3,413 (0,123)
Sarplanina	XK	*Ljubetensis*	4	17,81 (0,772)	5,355 (0,263)	3,28 (0,209)	26,445 (1,089)	10,99 (0,811)	7,355 (0,603)	3,36 (0,246)
Cincar, Vran, Šator	BA	*Mediteraneus*	4	19,277 (1,352)	5,655 (0,359)	3,403 (0,169)	28,335 (1,867)	11,56 (0,907)	7,598 (0,459)	3,31 (0,212)
Grmeč	BA	*Pretneri*	8	20,794 (0,786)	5,908 (0,195)	3,754 (0,084)	30,455 (0,996)	12,724 (0,423)	8,653 (0,418)	3,636 (0,169)
Vis	BA	*Travnikanus*	5	18,388 (0,939)	5,562 (0,059)	3,46 (0,058)	27,36 (0,976)	11,61 (0,298)	7,52 (0,202)	3,318 (0,059)
Kruščica, Žepce, Raduša, Kuljansko	BA	*Zepcensis*	21	19,306 (1,244)	5,65 (0,327)	3,46 (0,175)	28,417 (1,653)	11,501 (0,616)	7,72 (0,474)	3,411 (0,242)
			∑152							

Tablica P 1.2. Nastavak

Lokalitet	Država	Naziv podvrste	Br.M	EL (SD)	PL (SD)	HL (SD)	BL (SD)	EW (SD)	PW (SD)	HW (SD)
Babinje	AL	*Babinjensis*	0	0	0	0	0	0	0	0
Murtenica	RS	*Bosiljevici*	2	16,47 (0,283)	5,025 (0,063)	3,31 (0,198)	24,805 (0,021)	9,98 (0,156)	6,86 (0,311)	3,215 (0,12)
Treskavica, Romanija, Jahorina, Zelengora	BA	*Bosnicus*	13	16,883 (0,814)	5,393 (0,212)	3,368 (0,11)	25,644 (1,014)	10,248 (0,426)	7,405 (0,41)	3,338 (0,182)
Velebit	HR	*Croaticus*	8	17,042 (1,046)	5,587 (0,553)	3,458 (0,178)	26,087 (1,177)	10,786 (0,598)	7,538 (0,928)	3,316 (0,169)
Pelister, Galičica	MK	*Droveniki*	1	16,01	5,12	3,19	24,32	9,97	6,76	3,05
Durmitor	ME	*Durmitorensis*	1	12,86	4,08	2,66	19,6	7,51	5,46	2,75
Snežnik, Javorniki, Plješivica, Risnjak	SI HR	*Frankenbergeri*	63	19,052 (0,72)	5,702 (0,289)	3,562 (0,166)	28,317 (0,919)	11,576 (0,464)	8,237 (0,348)	3,573 (0,164)
Golica, Blinje, Janj	BA	*Kobingeri*	14	15,94 (0,782)	5,088 (0,268)	3,152 (0,132)	24,18 (0,965)	9,676 (0,44)	6,985 (0,346)	3,141 (0,118)
Žljeb	ME	*Kraetschmeri*	2	16,1 (0,721)	5,165 (0,276)	3,215 (0,05)	24,48 (0,05)	10,04 (0,255)	7,07 (0,071)	3,255 (0,191)
Šarplanina	XK	*Ljubetensis*	2	15,12 (0,099)	4,67 (0,028)	3,0 (0,057)	22,79 (0,127)	9,215 (0,134)	6,585 (0,035)	2,9 (0,057)
Cincar, Vran, Šator	BA	*Mediteraneus*	13	15,864 (0,179)	4,945 (0,232)	3,171 (0,155)	23,98 (1,041)	9,522 (0,533)	6,771 (0,399)	3,029 (0,142)
Grmeč	BA	*Pretneri*	12	17,687 (0,944)	5,393 (0,186)	3,48 (0,131)	26,56 (1,158)	10,913 (0,429)	7,851 (0,327)	3,363 (0,193)
Vis	BA	*Travnikanus*	4	16,438 (0,85)	5,253 (0,184)	3,308 (0,091)	24,998 (0,975)	10,473 (0,39)	7,353 (0,013)	3,168 (0,133)
Kruščica, Žepce, Raduša, Kuljansko	BA	*Zepcensis*	12	17,24 (1,173)	5,4 (0,229)	3,343 (0,143)	25,97 (1,451)	10,445 (0,585)	7,418 (0,424)	3,277 (0,147)
			∑149							

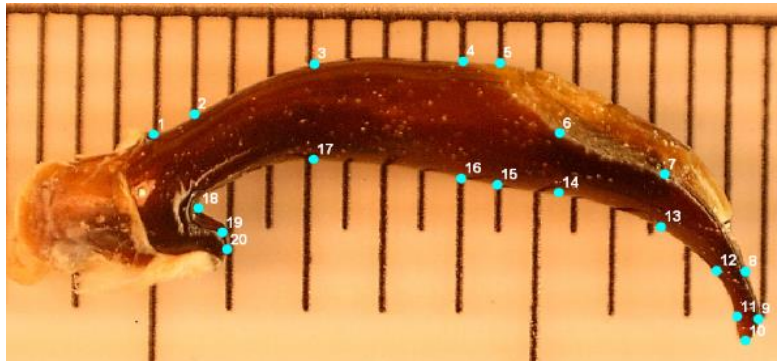
Tablica P 1.3. Rezultati linearne morfometrije za populacije vrste ***C. caelatus***. Srednje vrijednosti su izražene u milimetrima s vrijednošću standarne devijacije navedene u zagradama. (EL= duljina elitre; PL= duljina pronotuma; HL= duljina glave; BL= duljina tijela; EW = širina elitre; PW = širina pronotuma; HW = širina glave; F = ženke; M = mužjaci; HR – Hrvatska; SI – Slovenija, BA – Bosna i Hercegovina, MK – Sjeverna Makedonija, ME – Crna Gora, AL – Albanija, RS – Srbija). Podvrste determinirali: B. Drovenik, P. Durbešić i L. Š. Jelaska.

Lokalitet	**Država**	**Naziv podvrste**	**Br.F**	**EL (SD)**	**PL (SD)**	**HL (SD)**	**BL (SD)**	**EW (SD)**	**PW (SD)**	**HW (SD)**
Peručica, Prozor	BA	*cabuljensis*	7	24,529 (1,897)	7,106 (0,506)	4,247 (0,203)	35,881 (2,533)	14,661 (1,298)	9,017 (0,65)	4,029 (0,149)
Kamnik, Krvavec, Unška Koliševka, Planinska Koliševka	SI	*caelatus*	12	24,916 (1,414)	7,161 (0,667)	4,395 (0,290)	36,472 (2,290)	15,455 (0,863)	9,029 (0,731)	3,999 (0,243)
Krk, Pag, Mali Lošinj, Brač	HR otoci									
Trilj, Makarska, Omiš, Ploče,Mosor	HR kopno	*dalmatinus*	16	24,908 (1,34)	7,388 (0,538)	4,454 (0,398)	36,749 (1,882)	14,921 (0,861)	9,251 (0,506)	4,087 (0,368)
Popovo polje	BA									
Velebit		*macretus*	6	22,812 (2,223)	6,478 (0,805)	4,025 (0,421)	33,315 (3,39)	13,393 (1,461)	8,51 (1,203)	3,692 (0,483)
Bjelasica, Ilino brdo	ME	*malissorum*	6	24,002 (0,75)	7,223 (0,303)	4,403 (0,177)	35,63 (1,1)	14,662 (0,539)	8,937 (0,583)	3,887 (0,101)
Struga, Bistra planina	MK									
Goražde	BA	*metalkanus*	10	22,432 (1,396)	6,807 (0,312)	4,063 (0,196)	33,302 (1,852)	13,458 (0,743)	8,603 (0,636)	3,792 (0,165)
Durmitor	ME									
Ploče, Dubrovnik	HR	*procerus*	10	26,637 (1,255)	7,755 (0,348)	4,473 (0,08)	38,865 (1,596)	15,28 (0,631)	9,509 (0,524)	4,114 (0,144)
Uljcin	ME									
Zavale	BA									
Vran, Srnetica, Čajniče, Oštrelj, Vlašić, Ravna gora	BA	*sarajevoensis*	10	24,274 (0,942)	7,059 (0,465)	4,267 (0,222)	35,6 (1,46)	14,239 (0,357)	8,826 (0,233)	3,99 (0,132)
Risnjak, Lokve	HR	*schreiberi*	24	22,534 (2,225)	6,735 (0,528)	4,073 (0,315)	33,342 (2,856)	13,532 (1,011)	8,582 (0,606)	3,806 (0,257)
Zelen gora, Prenj	BA	*volujakianus*	5	23,14 (2,048)	6,648 (0,478)	4,198 (0,298)	33,986 (2,79)	13,964 (1,142)	8,43 (0,773)	3,8 (0,24)
			∑107							

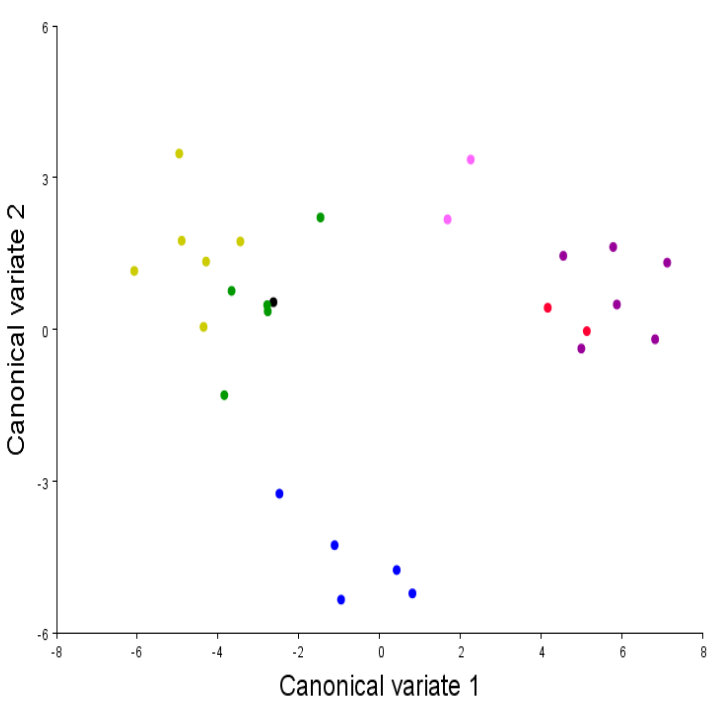
Tablica P 1.3. Nastavak

Lokalitet	Država	Naziv podvrste	Br.M	EL (SD)	PL (SD)	HL (SD)	BL (SD)	EW (SD)	PW (SD)	HW (SD)
Peručica, Prozor	BA	*cabuljensis*	6	20,892 (1,361)	6,356 (0,4)	4,001 (0,162)	31,248 (1,744)	12,207 (0,844)	8,048 (0,366)	3,585 (0,16)
Kamnik, Krvavec, Unška Koliševka, Planinska Koliševka	SI	*caelatus*	10	21,88 (0,95)	6,495 (0,497)	4,061 (0,167)	32,436 (1,249)	12,914 (0,703)	8,366 (0,496)	3,726 (0,147)
Krk, Pag, Mali Lošinj, Brač	HR otoci									
Trilj, Makarska, Omiš, Ploče,Mosor	HR kopno	*dalmatinus*	23	22,376 (1,241)	6,883 (0,416)	4,09 (0,21)	33,35 (1,58)	13,0 (0,76)	8,734 (0,644)	3,733 (0,212)
Popovo polje	BA									
Velebit		*macretus*	8	20,224 (1,186)	6,15 (0,516)	3,769 (0,267)	29,671 (1,802)	11,896 (0,803)	7,785 (0,62)	3,316 (0,274)
Bjelasica, Ilino brdo	ME	*malissorum*	3	21,307 (0,696)	6,63 (0,278)	4,183 (0,155)	32,12 (0,75)	12,413 (0,437)	8,453 (0,465)	3,583 (0,042)
Struga, Bistra planina	MK									
Goražde	BA	*metalkanus*	9	18,798 (1,945)	6,016 (0,467)	3,74 (0,311)	28,553 (2,665)	11,244 (1,139)	7,383 (0,78)	3,383 (0,254)
Durmitor	ME									
Ploče, Dubrovnik	HRV	*procerus*	13	23,346 (1,288)	7,206 (0,477)	4,251 (0,19)	34,803 (1,895)	13,293 (0,742)	8,833 (0,611)	3,842 (0,219)
Uljcin	ME									
Zavale	BA									
Vran, Srnetica, Čajniče, Oštrelj, Vlašić, Ravna gora	BA	*sarajevoensis*	11	21,376 (1,077)	6,639 (0,431)	4,007 (0,27)	32,023 (1,656)	12,781 (0,788)	8,453 (0,585)	3,678 (0,272)
Risnjak, Lokve	HR	*schreiberi*	14	21,356 (1,806)	6,478 (0,461)	4,136 (0,279)	31,97 (2,437)	12,521 (1,026)	8,203 (0,667)	3,732 (0,243)
Zelen gora, Prenj	BA	*volujakianus*	4	21,218 (1,152)	6,37 (0,708)	3,948 (0,175)	31,535 (1,985)	12,362 (0,65)	8,075 (0,655)	3,625 (0,358)
			∑101							

PRILOG 2



Slika P 2.1. Odabrani orijentiri na kopulatornom organu (*aedeagus*) vrste *Carabus caelatus schreiberi* Kraatz, 1877. (Foto: Ž. J. Vladić.)



Slika P 2.2. Kanonička diskriminantna analiza – oblik kopulatornog organa i njegove varijacije unutar vrsta *C. croaticus* i *C. caelatus*. Svaka točka predstavlja varijantu oblika kopulatornog organa. (Različitim bojama označene su populacije iz različitih država; Cae = jedinke vrste *C. caelatus*; Cro = jedinke vrste *C. croaticus*; HR = Republika Hrvatska; BA = Bosna i Hercegovina; MK = Sjeverna Makedonija; SI = Slovenija).

PRILOG 3

Tablica P 3.1. Popis sekvenci unesenih u baze BOLD (*Barcode of Life Data Systems*) i NCBI (The *National Center for Biotechnology Information)*

ProcessID	sampleID	recordID	institution_storing	bin_uri	red	porodica	podporodica	rod	vrsta	država
GBCL24384-15	KP067573	6156194	Mined from GenBank, NCBI	BOLD:ACD7337	Coleoptera	Carabidae	Carabinae	*Carabus*	*Carabus caelatus*	Hrvatska
GBCL24393-15	KP067563	6156203	Mined from GenBank, NCBI	BOLD:ACR9475	Coleoptera	Carabidae	Carabinae	*Carabus*	*Carabus creutzeri*	Hrvatska
GBCL24394-15	KP067564	6156204	Mined from GenBank, NCBI	BOLD:ACR9475	Coleoptera	Carabidae	Carabinae	*Carabus*	*Carabus creutzeri*	Hrvatska
GBCL24402-15	KP067568	6156212	Mined from GenBank, NCBI	BOLD:ACV6845	Coleoptera	Carabidae	Carabinae	*Carabus*	*Carabus parreyssi*	Hrvatska
GBCL24403-15	KP067567	6156213	Mined from GenBank, NCBI	BOLD:ACV7026	Coleoptera	Carabidae	Carabinae	*Carabus*	*Carabus parreyssi*	Hrvatska
GBCL24404-15	KP067555	6156214	Mined from GenBank, NCBI	BOLD:ACV7602	Coleoptera	Carabidae	Carabinae	*Carabus*	*Carabus ulrichii*	Hrvatska
GBCL24405-15	KP067556	6156215	Mined from GenBank, NCBI	BOLD:ACV7602	Coleoptera	Carabidae	Carabinae	*Carabus*	*Carabus ulrichii*	Hrvatska
GBCL29072-19	KP067569	9823972	Mined from GenBank, NCBI	BOLD:ADU5664	Coleoptera	Carabidae	Carabinae	*Carabus*	*Carabus intricatus*	Hrvatska
GBCL29073-19	KP067570	9823973	Mined from GenBank, NCBI	BOLD:AAO3848	Coleoptera	Carabidae	Carabinae	*Carabus*	*Carabus intricatus*	Hrvatska
GBCL29074-19	KP067571	9823974	Mined from GenBank, NCBI	BOLD:AAO3848	Coleoptera	Carabidae	Carabinae	*Carabus*	*Carabus intricatus*	Hrvatska
GBCL29075-19	KP067572	9823975	Mined from GenBank, NCBI	BOLD:AAO3848	Coleoptera	Carabidae	Carabinae	*Carabus*	*Carabus intricatus*	Hrvatska

PRILOG 4

Tablica P 4.1. DNA oznake i osnovni podaci o jedinkama vrsta *C. croaticus* i *C. caelatus* kojima je izolirana DNA (Leg. – sakupio/la).

DNA OZNAKA	Vrsta	Spol	Država	Nalazište	Datum	Leg.	Datum izolacije
CA 352	*Carabus caelatus*	F	HR	NP Risnjak	27. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 353	*Carabus croaticus*	F	HR	NP Risnjak	27. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 354	*Carabus caelatus*	F	HR	NP Risnjak	27. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 355	*Carabus croaticus*	M	HR	NP Risnjak	18. 08. 2014.	Ž. J. Vladic	06.05.2015.
CA 356	*Carabus caelatus*	F	HR	NP Risnjak	19. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 357	*Carabus caelatus*	F	HR	NP Risnjak	27. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 360	*Carabus caelatus*	M	HR	NP Risnjak	27. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 361	*Carabus caelatus*	F	HR	NP Risnjak	19. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 362	*Carabus caelatus*	F	HR	NP Risnjak	19. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 363	*Carabus caelatus*	M	HR	NP Risnjak	19. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 364	*Carabus caelatus*	F	HR	NP Risnjak	27. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 365	*Carabus caelatus*	M	HR	NP Risnjak	06. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 366	*Carabus caelatus*	M	HR	NP Risnjak	19. 07. 2014.	Ž. J. Vladic	06.05.2015.
CA 367	*Carabus croaticus*	F	HR	NP Risnjak	18. 08. 2014.	Ž. J. Vladic	10.12.2015.
CAR 430	*Carabus croaticus*	M	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 431	*Carabus croaticus*	M	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 432	*Carabus croaticus*	M	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 433	*Carabus croaticus*	M	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 434	*Carabus croaticus*	F	HR	NP Risnjak	31.05.2015.	Ž. J. Vladic	10.12.2015.
CAR 435	*Carabus croaticus*	M	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 436	*Carabus croaticus*	F	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 437	*Carabus croaticus*	F	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 438	*Carabus croaticus*	M	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 439	*Carabus croaticus*	M	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 440	*Carabus croaticus*	M	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.

Tablica P 4.1. **Nastavak**

DNA OZNAKA	Vrsta	Spol	Država	Nalazište	Datum	Leg.	Datum izolacije
CAR 441	*Carabus croaticus*	F	HR	NP Risnjak	18.07.2015.	Ž. J. Vladic	10.12.2015.
CAR 442	*Carabus croaticus*	M	HR	NP Risnjak	18.07.2015.	Ž. J. Vladic	10.12.2015.
CAR 443	*Carabus croaticus*	M	HR	NP Risnjak	31.05.2015.	Ž. J. Vladic	10.12.2015.
CAR 444	*Carabus croaticus*	M	HR	NP Risnjak	18.07.2015.	Ž. J. Vladic	10.12.2015.
CAR 445	*Carabus croaticus*	M	HR	NP Risnjak	18.07.2015.	Ž. J. Vladic	10.12.2015.
CAR 446	*Carabus croaticus*	F	HR	NP Risnjak	18.07.2015.	Ž. J. Vladic	10.12.2015.
CAR 447	*Carabus croaticus*	M	HR	NP Risnjak	18.07.2015.	Ž. J. Vladic	10.12.2015.
CAR 448	*Carabus croaticus*	F	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 449	*Carabus croaticus*	M	HR	NP Risnjak	18.07.2015.	Ž. J. Vladic	4.07.2017.
CAR 450	*Carabus croaticus*	F	HR	NP Risnjak	18.07.2015.	Ž. J. Vladic	10.12.2015.
CAR 451	*Carabus croaticus*	F	HR	NP Risnjak	12.07.2015.	Ž. J. Vladic	10.12.2015.
CAR 452	*Carabus croaticus*	F	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	4.07.2017.
CAR 453	*Carabus croaticus*	F	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	4.07.2017.
CAR 454	*Carabus croaticus*	M	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 455	*Carabus croaticus*	F	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 456	*Carabus croaticus*	M	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 457	*Carabus croaticus*	F	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 458	*Carabus croaticus*	F	HR	NP Risnjak	6.06.2015.	Ž. J. Vladic	10.12.2015.
CAR 459	*Carabus croaticus*	F	HR	NP Risnjak	29.07.2015.	Ž. J. Vladic	4.07.2017.
CAR 460	*Carabus croaticus*	F	HR	NP Risnjak	29.07.2015.	Ž. J. Vladic	4.07.2017.
CAR 461	*Carabus croaticus*	F	HR	NP Risnjak	11.08.2015.	Ž. J. Vladic	4.07.2017.
CAR 462	*Carabus croaticus*	M	HR	NP Risnjak	11.08.2015.	Ž. J. Vladic	4.07.2017.
CAR 463	*Carabus croaticus*	F	HR	NP Risnjak	09.09.2015.	Ž. J. Vladic	4.07.2017.
CAR 464	*Carabus croaticus*	M	HR	NP Risnjak	08.08.2015.	Ž. J. Vladic	10.12.2015.
CAR 465	*Carabus croaticus*	M	HR	NP Risnjak	08.08.2015.	Ž. J. Vladic	10.12.2015.
CAR 466	*Carabus caelatus*	M	HR	NP Plitvička jezera	31.07.2016.	L.Š. Jelaska	4.07.2017.
CAR 467	*Carabus caelatus*	M	HR	NP Plitvička jezera	31.07.2016.	L.Š. Jelaska	4.07.2017.

Tablica P 4.1. **Nastavak**

DNA OZNAKA	Vrsta	Spol	Država	Nalazište	Datum	Leg.	Datum izolacije
CAR 469	*Carabus caelatus*	F	HR	NP Plitvička jezera	31.07.2016.	L.Š. Jelaska	4.07.2017.
CAR 474	*Carabus caelatus*	M	BA	PP Blidinje	26.07.2016.	L.Š. Jelaska	4.07.2017.
CAR 476	*Carabus caelatus*	M	BA	PP Blidinje	26.07.2016.	L.Š. Jelaska	4.07.2017.
CAR 477	*Carabus caelatus*	F	BA	PP Blidinje	31.07.2016.	Ž.J.Vladić	4.07.2017.
CAR 481	*Carabus croaticus*	F	BA	PP Blidinje	27.07.2016.	Ž.J.Vladić	4.07.2017.
CAR 482	*Carabus croaticus*	F	BA	PP Blidinje	27.07.2016.	Ž.J.Vladić	4.07.2017.
CAR 483	*Carabus caelatus*	M	BA	PP Blidinje	27.07.2016.	Ž.J.Vladić	4.07.2017.
CAR 485	*Carabus croaticus*	F	BA	PP Blidinje	26.07.2016.	L.Š. Jelaska	4.07.2017.
CAR 493	*Carabus caelatus*	M	BA	PP Blidinje	26.07.2016.	L.Š. Jelaska	4.07.2017.
CAR 500	*Carabus croaticus*	F	BA	PP Blidinje	17.07.2016.	Ž. J. Vladic	4.07.2017.
CAR 501	*Carabus croaticus*	F	BA	PP Blidinje	17.07.2016.	Ž. J. Vladic	4.07.2017.
CAR 502	*Carabus croaticus*	F	BA	PP Blidinje	17.07.2016.	Ž. J. Vladic	4.07.2017.
CAR 503	*Carabus croaticus*	F	BA	PP Blidinje	31.07.2016.	Ž.J.Vladić	4.07.2017.
CAR 504	*Carabus croaticus*	M	BA	PP Blidinje	31.07.2016.	Ž.J.Vladić	4.07.2017.
CAR 505	*Carabus croaticus*	F	BA	PP Blidinje	31.07.2016.	Ž.J.Vladić	4.07.2017.
CAR 506	*Carabus caelatus*	F	BA	PP Blidinje	31.07.2016.	Ž.J.Vladić	4.07.2017.
CAR 508	*Carabus croaticus*	F	HR	NP Risnjak	17.07.2016.	Ž. J. Vladic	4.07.2017.
CAR 509	*Carabus croaticus*	F	HR	NP Risnjak	7.08.2016.	Ž. J. Vladic	4.07.2017.
CAR 510	*Carabus croaticus*	M	HR	NP Risnjak	7.08.2016.	Ž. J. Vladic	4.07.2017.
CAR 511	*Carabus caelatus*	F	HR	NP Plitvička jezera	14.07.2016.	L.Š. Jelaska	4.07.2017.
CAR 512	*Carabus caelatus*	F	HR	NP Plitvička jezera	15.07.2016.	L.Š. Jelaska	4.07.2017.
CAR 513	*Carabus caelatus*	F	HR	NP Plitvička jezera	15.07.2016.	L.Š. Jelaska	4.07.2017.
CAR 514	*Carabus caelatus*	M	HR	NP Plitvička jezera	14.07.2016.	L.Š. Jelaska	4.07.2017.
CAR 520	*Carabus caelatus*	F	MK		28.05.2016.	S.Hristovski	4.07.2017.
CAR 521	*Carabus caelatus*	M	MK		28.05.2016.	S.Hristovski	4.07.2017.
CAR 522	*Carabus caelatus*	F	MK		16.06.2013.	S.Hristovski	4.07.2017.
CAR 523	*Carabus caelatus*	F	HR	NP Plitvička jezera	8.08.2016.	L.Š. Jelaska	4.07.2017.

Tablica P 4.1. **Nastavak**

DNA OZNAKA	Vrsta	Spol	Država	Nalazište	Datum	Leg.	Datum izolacije
CAR 524	*Carabus croaticus*	M	HR	Snježnik	3.06.2015.	L.Š. Jelaska	4.07.2017.
CAR 525	*Carabus caelatus*	M	HR	NP Plitvička jezera	28.06.2015.	L.Š. Jelaska	4.07.2017.
CAR 526	*Carabus croaticus*	M	SI	Slovenija, Krim	15.-20.06.2017.	A. Kapla	4.07.2017.
CAR 527	*Carabus croaticus*	M	SI	Slovenija, Krim	15.-20.06.2017.	A. Kapla	4.07.2017.
CAR 528	*Carabus croaticus*	F	SI	Slovenija, Krim	15.-20.06.2017.	A. Kapla	4.07.2017.
CAR 529	*Carabus croaticus*	M	SI	Slovenija, Krim	15.-20.06.2017.	A. Kapla	4.07.2017.
CAR 530	*Carabus croaticus*	F	SI	Slovenija, Krim	15.-20.06.2017.	A. Kapla	4.07.2017.
CAR 531	*Carabus croaticus*	M	HR	NP Risnjak	1.07.2017.	Ž.J.Vladić	4.07.2017.
CAR 532	*Carabus croaticus*	M	HR	NP Plitvička jezera	1.08.2017.	L.Š. Jelaska	12.07.2018.
CAR 533	*Carabus croaticus*	F	HR	NP Plitvička jezera	1.08.2017.	L.Š. Jelaska	12.07.2018.
CAR 534	*Carabus croaticus*	M	BA	PP Blidinje; Jarebinjak	29.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 535	*Carabus caelatus*	M	BA	PP Blidinje; Jarebinjak	30.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 536	*Carabus croaticus*	M	BA	PP Blidinje; Jarebinjak	30.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 537	*Carabus caelatus*	M	BA	PP Blidinje; Jarebinjak	31.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 538	*Carabus croaticus*	F	BA	PP Blidinje; Jarebinjak	30.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 539	*Carabus croaticus*	M	BA	PP Blidinje; Jarebinjak	30.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 540	*Carabus croaticus*	M	BA	PP Blidinje; Jarebinjak	31.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 541	*Carabus caelatus*	M	BA	PP Blidinje; Jarebinjak	31.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 542	*Carabus croaticus*	F	BA	PP Blidinje; Jarebinjak	30.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 543	*Carabus caelatus*	F	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	7.09.2017.
CAR 544	*Carabus croaticus*	F	BA	PP Blidinje; Jarebinjak	30.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 545	*Carabus caelatus*	F	BA	PP Blidinje; Jarebinjak	30.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 546	*Carabus caelatus*	F	BA	PP Blidinje; Jarebinjak	31.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 547	*Carabus croaticus*	F	BA	PP Blidinje; Jarebinjak	29.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 548	*Carabus caelatus*	M	BA	PP Blidinje; Jarebinjak	30.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 549	*Carabus croaticus*	M	BA	PP Blidinje; Jarebinjak	31.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 550	*Carabus croaticus*	M	BA	PP Blidinje; Jarebinjak	30.07.2017.	Ž.J.Vladić	7.09.2017.

Tablica P 4.1. **Nastavak**

DNA OZNAKA	Vrsta	Spol	Država	Nalazište	Datum	Leg.	Datum izolacije
CAR 551	*Carabus caelatus*	M	BA	PP Blidinje; Jarebinjak	31.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 552	*Carabus caelatus*	F	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	7.09.2017.
CAR 553	*Carabus croaticus*	F	BA	PP Blidinje; Jarebinjak	29.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 554	*Carabus croaticus*	F	BA	PP Blidinje; Jarebinjak	29.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 555	*Carabus caelatus*	F	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	8.09.2017.
CAR 556	*Carabus croaticus*	M	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	8.09.2017.
CAR 557	*Carabus caelatus*	F	BA	PP Blidinje; Jarebinjak	30.07.2017.	Ž.J.Vladić	7.09.2017.
CAR 558	*Carabus caelatus*	M	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	8.09.2017.
CAR 559	*Carabus caelatus*	F	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	8.09.2017.
CAR 560	*Carabus caelatus*	M	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	8.09.2017.
CAR 561	*Carabus croaticus*	F	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	8.09.2017.
CAR 562	*Carabus croaticus*	F	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	8.09.2017.
CAR 563	*Carabus croaticus*	M	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	8.09.2017.
CAR 564	*Carabus caelatus*	M	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	8.09.2017.
CAR 565	*Carabus caelatus*	F	BA	PP Blidinje; Jarebinjak	30.07.2017.	Ž.J.Vladić	8.09.2017.
CAR 566	*Carabus caelatus*	M	BA	PP Blidinje; Jarebinjak	31.07.2017.	Ž.J.Vladić	8.09.2017.
CAR 567	*Carabus croaticus*	F	BA	PP Blidinje; Jarebinjak	29.07.2017.	Ž.J.Vladić	8.09.2017.
CAR 568	*Carabus caelatus*	M	BA	PP Blidinje; Jarebinjak	1.08.2017.	Ž.J.Vladić	8.09.2017.

Tablica P 4.2. Vremena trajanja (s) i vrijednosti temperatura (°C) na kojima su se odvijali pojedini koraci ciklusa umnožavanja fragmenata polimeraznom lančanom reakcijom (PCR), gdje su koraci: **PD** - preddenaturacija, **D** - denaturacija, **A** - sljepljivanje, **S** - sinteza, **FS** - završna sinteza, a **n** - broj ciklusa.

KOMPLET REAGENSA *Taq PCR Master Mix Kit (Qiagen)*	**PD**		**D**		**A**		**S**		**FS**		**n**
	s	**°C**	**s**	**°C**	**s**	**°C**	**S**	**°C**	**s**	**°C**	
Molekularni biljeg *COI*	180	94	30	94	30	57	60	72	600	72	30
Molekularni biljeg *ITS-2*	90	95	30	94	90	52,5	90	72	600	72	35

Score	Expect	Identities	Gaps	Strand
961 bits(520)	0.0	527/530(99%)	2/530(0%)	Plus/Plus

```
Query  1    TGGCTGAGGGTCGTGCTATATTACCAGACTGCCGGTCGTCGTTGTCGTCTGACGATGACT  60
Sbjct  41   ............................................................  100
Query  61   TCGACGATCTCGGCGACGATGGGTATCGGGGAACCAGTGGTGTTTTGTTACTGGCATATC  120
Sbjct  101  ............................................................  158
Query  121  TACTACTCGTAGTTGTCGTGTGCAATTCACATATACACTGCCCGTTTCCTTAAATCACTG  180
Sbjct  159  ............................................................  218
Query  181  TTCACACATCAAACCGAATAACACTGTTTACACAGTTTATTCACACAGTGCACATTTATG  240
Sbjct  219  ............................................................  278
Query  241  TATCGTATTGCGGTCTCCGTATGCTGTACTATATTTATGTTTTGTGTGTTAATAAGTGCG  300
Sbjct  279  ............................................................  338
Query  301  GAGTTGTCGGACGTGTCTATGAGTACCATGATAAATGTGATTATACCAACTCTAGTGCAG  360
Sbjct  339  ............................................................  398
Query  361  AGTTTATTGCATTTTGTTTGTTACCCTCGTGCCGTCCGTAGATACTTGGCTTAAGGGCAT  420
Sbjct  399  ............................................................  458
Query  421  ACAGTTTACAAACATATTATACGTATACATACACTCAGGGCTGTATCGTTTTACGTTGTG  480
Sbjct  459  ..........................G.................................  518
Query  481  AATGTTGTACGGCTAAGAATTTAGTTTTGCGACCTCAGATCAGGTGGGAT  530
Sbjct  519  ..................................................  568
```

Slika P 4.1. Usporedba (BLAST) sekvenci gena *ITS-2* vrsta *C. croaticus* i *C. caelatus*.

PRILOG 5

Tablica P 5.1. Popis nalazišta* vrste *C. croaticus* duž Dinarida. (HR – Hrvatska; SI – Slovenija, BA – Bosna i Hercegovina, MK – Sjeverna Makedonija, ME – Crna Gora, AL – Albanija, RS – Srbija, Leg. – sakupio/la, Det. – determinirao/la)

DRŽAVA	KRAJ	MJESTO	NALAZIŠTE	x-koordinata	y-koordinata	Nadmorska visina (m)	Datum pronalaska	Leg.	Det.	REFERENCE
HR	Gorski Kotar	NP Risnjak	Snježnik-ispod	45°25'59.42"N	14°35'59.37"E	1200	1963-1964	P. Durbešić	P. Durbešić	P. Durbešić, Magistarski rad,1967
HR	Gorski Kotar	NP Risnjak	Snježnik-vrh	45°26′26″N	14°35′02″E	1400-1500	1963-1964	P. Durbešić	P. Durbešić	P. Durbešić, Magistarski rad,1967
HR	Lika	Vrhovine	Borića Borik- Donji Babin potok	44°51'11.31"N	15°30'5.82"E	825	1961-1963	P. Durbešić	P. Durbešić	P. Durbešić, Magistarski rad,1967
HR	Gorski Kotar	Lokve	Z od ceste Lokve-Belo selo-Zlobin	45°19'59.14"N	14°44'36.93"E	820	1970-1974	P. Durbešić	P. Durbešić	P. Durbešić, doktorska disertacija, 1982
HR	Gorski Kotar	Ogulin	Padine Kaludjerske kose	45°13'53.87"N	15° 7'2.45"E	670	1970-1974	P. Durbešić	P. Durbešić	P. Durbešić, doktorska disertacija, 1982
HR	Gorski Kotar	Delnice	Lokve	45°21'29.68"N	14°45'2.86"E	730	1968-1969	P. Durbešić	P. Durbešić	P. Durbešić, S.Vujčić Karlo, 2001
HR	Gorski Kotar	Ogulin	Jasenak	45°13'44.29"N	15° 2'36.40"E	640	1968-1969	P. Durbešić	P. Durbešić	P. Durbešić, S.Vujčić Karlo, 2001
HR	Gorski Kotar	NP Risnjak	Bijele vodice	45°25'8.90"N	14°41'31.15"E	685	1968-1969	P. Durbešić	P. Durbešić	P. Durbešić, S.Vujčić Karlo, 2001
HR	Gorski Kotar	NP Risnjak	Sove	45°24'52.10"N	14°40'57.89"E	690	2014-2016	Ž.J. Vladic	L. Š. Jelaska	
HR	Gorski Kotar	NP Risnjak	Cajtige	45°25'40.81"N	14°36'9.58"E	1230	2014-2016	Ž.J. Vladic	L. Š. Jelaska	
HR	Gorski Kotar	NP Risnjak	Lazac	45°27'5.50"N	14°36'2.42"E	1075	2014-2016	Ž.J. Vladic	L. Š. Jelaska	
HR	Gorski Kotar	NP Risnjak	Risnik	45°24'41.97"N	14°39'50.79"E	868	2014-2016	Ž.J. Vladic	L. Š. Jelaska	
HR	Gorski Kotar	Platak	M.Platak	45°24'37.12"N	14°33'28.86"E	950	1963-1964	P. Durbešić	P. Durbešić	P. Durbešić, 1968
HR	Gorski Kotar	NP Risnjak	Rebar-Lazi	45°26'0.81"N	14°42'11.45"E	800	1963-1964	P. Durbešić	P. Durbešić	P. Durbešić, 1968
HR	Gorski Kotar	NP Risnjak	Janjičarski vrh-ispod	45°25'2.73"N	14°38'9.11"E	1200	1963-1964	P. Durbešić	P. Durbešić	P. Durbešić, 1968
HR	Gorski Kotar	NP Risnjak	Južni Mali Risnjak-ispod	45°25'28.72"N	14°37'32.36"E	1300	1963-1964	P. Durbešić	P. Durbešić	P. Durbešić, 1968
HR	Lika	Karlobag	Baške Oštarije	44°31'35.88"N	15°10'29.96"E	930				carabidae.org
HR	Gorski Kotar	Velika Kapela	Viševica	45°15'7.82"N	14°47'52.58"E	1250				Breuning 1932-36; Bruno 1966, Goidanich 1932, Muller 1924
HR	Gorski Kotar	Velika Kapela	Bitoraj	45°17'35.33"N	14°47'2.97"E	1250				Breuning 1932-36; Bruno 1966, Goidanich 1932, Muller 1924
HR	Gorski Kotar	Fužine	Fužine	45°18'23.72"N	14°43'11.35"E	750				Breuning 1932-36; Bruno 1966, Goidanich 1932, Muller 1924
HR	Mala Kapela	NP Plitvička jezera	Čorkova uvala	44°52'49.41"N	15°33'20.87"E	860-1028	2009-2009	S. Slivar	I. Ternjej	
HR	Gorski Kotar	Ravna gora	u blizini naselja Ravna Gora	45°22'7.58"N	14°56'58.94"E	945	2009-2010	S. Slivar	I. Ternjej	
HR	Velebit	Oštarija		44°31'16.65"N	15°10'45.10"E					G. Muller, 1926
HR	Gorski Kotar	Lokve		45°21'32.01"N	14°44'41.81"E					G. Muller, 1926
HR	Velebit	Karlobag		44°31'58.64"N	15° 7'41.30"E					G. Depoli, 1930
HR	Gorski Kotar	Platak		45°25'40.98"N	14°33'52.10"E					G. Depoli, 1930
HR	Gorski Kotar	Delnice	Fužine	45°18'33.53"N	14°42'32.74"E					G. Depoli, 1930
HR	Gorski Kotar	Velika Kapela	Bitoraj	45°14'2.59"N	15° 0'40.73"E					G. Depoli, 1930
HR		Plitvice		44°53'20.20"N	15°36'57.19"E		10.07.2015.	L. Š. Jelaska	L. Š. Jelaska	
SI	Javorniki	Postojna	Baba	45°46'20.32"N	14°16'13.65"E	955	28.7.2012	A. Pirnat	A. Pirnat	

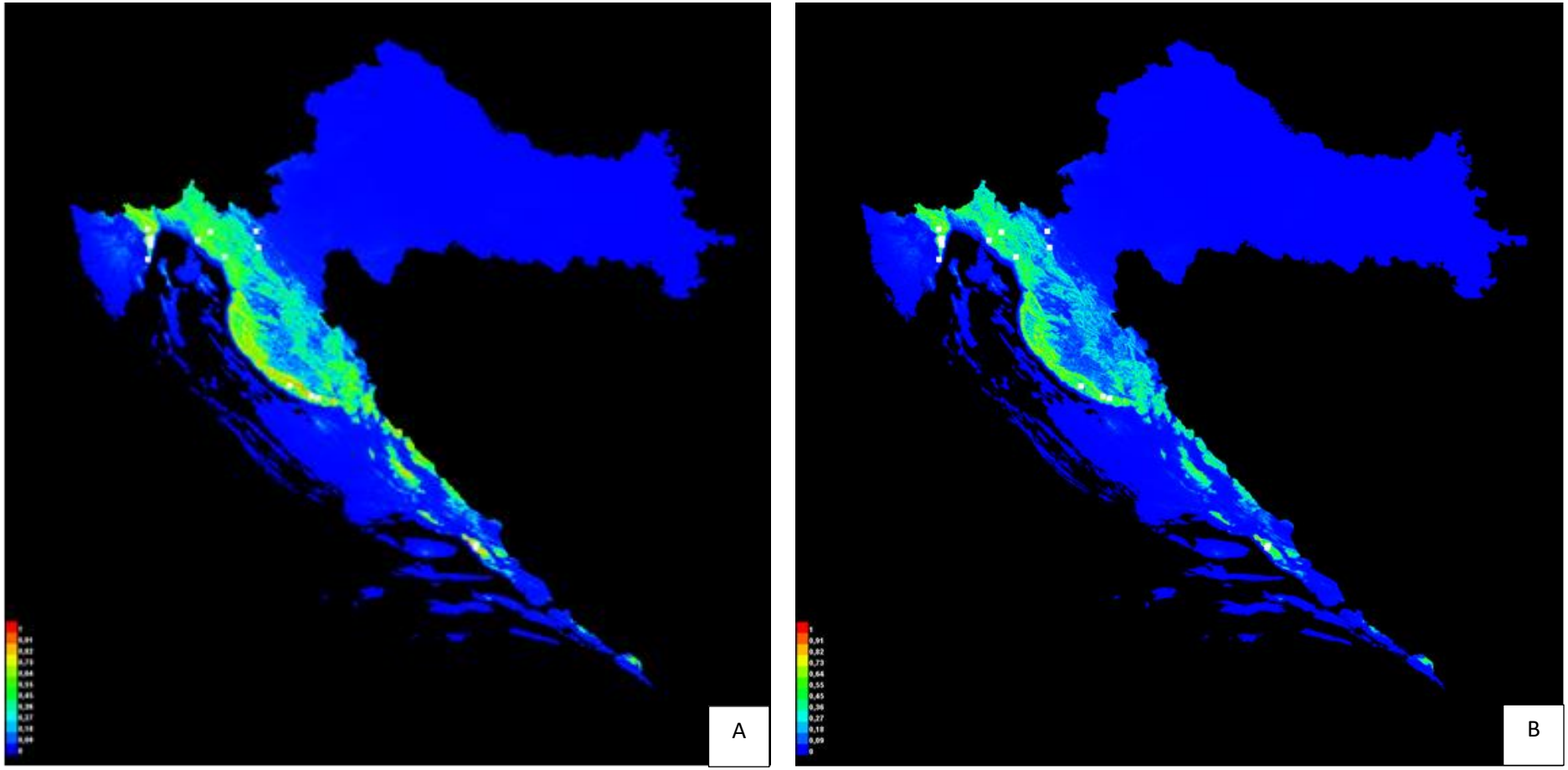
Tablica P 5.1. Nastavak

DRŽAVA	KRAJ	MJESTO	NALAZIŠTE	x-koordinata	y-koordinata	Nadmorska visina (m)	Datum pronalaska	Leg.	Det.	REFERENCE
SI	Javorniki	Trnje	Mali Kozljek	45°43'19.22"N	14°16'2.61"E	955	28.7.2012	A. Pirnat	A. Pirnat	
SI	Javorniki	Juršče	Škodovnik	45°40'7.46"N	14°25'38.96"E	1065	28.7.2012	A. Pirnat	A. Pirnat	
SI	Javorniki	Juršče	Strmi klanec	45°39'22.12"N	14°20'25.82"E	1096	28.7.2012	A. Pirnat	A. Pirnat	
SI	Snežnik	Velika Ojstrica	Velika Ojstrica	45°36'51.30"N	14°24'58.37"E	1129	29.7.2012.	A. Pirnat	A. Pirnat	
SI	Javorniki	Trnje	Debeli kamen	45°42'46.49"N	14°15'10.13"E	875	25. - 28. 7. 2012.	A. Pirnat	A. Pirnat	
SI			Krim	45°55'10.68"N	14°29'7.05"E	600	4-12.6.2003			CRP Projekt 2001-2003, Gozdarski institut Slovenije
SI			Krim	45°55'50.67"N	14°28'8.99"E	1000	5-13.6.2003			CRP Projekt 2001-2003, Gozdarski institut Slovenije
SI	Dolenjska		Mokrec	45°53'36.15"N	14°31'42.61"E		17.10.1985.	B. Drovenik	B. Drovenik	Acta entomologica slovenica, 21 (2), 2013
SI	Dolenjska	Petelinjek	Loški potok	45°40'16.20"N	14°39'44.77"E	1212	6.11.2011.	B. Kofler	B. Kofler	Acta entomologica slovenica, 21 (2), 2013
SI	Dolenjska		Travna gora	45°44'24.00"N	14°38'27.52"E		1.2.1975.	B. Kofler	B. Kofler	Acta entomologica slovenica, 21 (2), 2013
BA	Središnja BA		Travnik	44°12'49.02"N	17°38'33.01"E					carabidae.org
BA	Središnja BA		Fojnica	43°57'33.27"N	17°53'33.26"E					carabidae.org
BA	Zapad		Srnetica	44°26'59.33"N	16°36'33.81"E					carabidae.org
BA	Sjeverozapad		Oštrelj	44°28'39.46"N	16°24'23.52"E					carabidae.org
BA	Sarajevo	Bjelašnica		43°44'24.30"N	18°15'26.72"E					
BA	Hercegovina	Jablanica	Čvrsnica planina	43°38'7.82"N	17°38'40.44"E		1901		O. Leonhard	Apfelbeck, 1919
BA	Središnja BA		Treskavica planina	43°35'38.77"N	18°22'45.42"E		1888			Apfelbeck, 1920
BA	Južna Hercegovina	Mostar	Čabulja planina	43°29'47.68"N	17°34'10.76"E					Apfelbeck, 1921
BA	Hercegovina	Blidinje	Jarebinjak	43°32'47.0"N	17°28'19.0"E	1250	2017	Ž. J. Vladić	L. Š. Jelaska	
BA	Hercegovina	Blidinje	Jarebinjak	43°32'25.0"N	17°28'34.0"E	1270	2017	Ž. J. Vladić	L. Š. Jelaska	
MK	Galičica			40°57'17.50"N	20°48'21.57"E					Krätschmer i Drovenik, 1977
MK	Šar Planina		Ceripašina	42° 0'31.41"N	20°48'19.21"E	1850-2530	9.7.1995			Guéorgiuev, 1998
MK	Šar Planina		Studena Reka	42° 2'45.12"N	20°52'6.90"E	1730	10-19.7.1995			Guéorgiuev, 1998
MK	Šar Planina		Titov Vrv	41°59'33.33"N	20°47'53.01"E	2747	17.7.1995			Guéorgiuev, 1998
MK	Šar Planina	Čaušica	Čaušičko Bačilo	41°37'47.91"N	20°49'9.74"E	1600-1900	18.7.1996		S. Hristovski	Hristovski i sur. 2002
MK	Šar Planina	Bistrica	Bistričko Bačilo	40°58'20.20"N	21°16'24.03"E	1800	10.7.1996		S. Hristovski	Hristovski i sur. 2002
MK	Šar Planina	Piribeg	Kučinagledski Vrv	42°10'16.30"N	21° 3'7.50"E	2400	17.7.1997		S. Hristovski	Hristovski i sur. 2002
MK	Šar Planina	Ljuboten	Ljuboten mountain hut	42°10'52.22"N	21° 8'9.33"E	1400	13.7.1997		S. Hristovski	Hristovski i sur. 2002
MK	Šar Planina	Ljuboten	Ljuboten peak	42°11'52.95"N	21° 8'11.54"E	2000-2300	11.7.1997		S. Hristovski	Hristovski i sur. 2002
MK	Šar Planina	Govedarnik		41°19'19.27"N	23° 7'29.17"E	1900	18.7.1998		S. Hristovski	Hristovski i sur. 2002
MK	Bistra			41°18'47.16"N	20°27'18.98"E	1300	19.4.2001	S. Hristovski	S. Hristovski	Hristovski i sur. 2002
MK	Jablanica		Ajdarova Livada	41°15'1.41"N	20°31'47.25"E	2000-2050	18.7.2006	S. Hristovski	S. Hristovski	Hristovski i sur. 2010
MK	Jablanica		Krivi Viroi	41°14'47.96"N	20°32'54.74"E	1700	20.7.2006	S. Hristovski	S. Hristovski	Hristovski i sur. 2010
MK	Jablanica		Vevčanska Lokva	41°14'34.66"N	20°31'54.96"E	1970	20.7./2006	S. Hristovski	S. Hristovski	Hristovski i sur. 2010
MK	Jablanica	Vevčani	Jankovi Lazi	41°14'13.95"N	20°33'1.73"E	1700	12.8.2005	S. Hristovski	S. Hristovski	Hristovski i sur. 2010

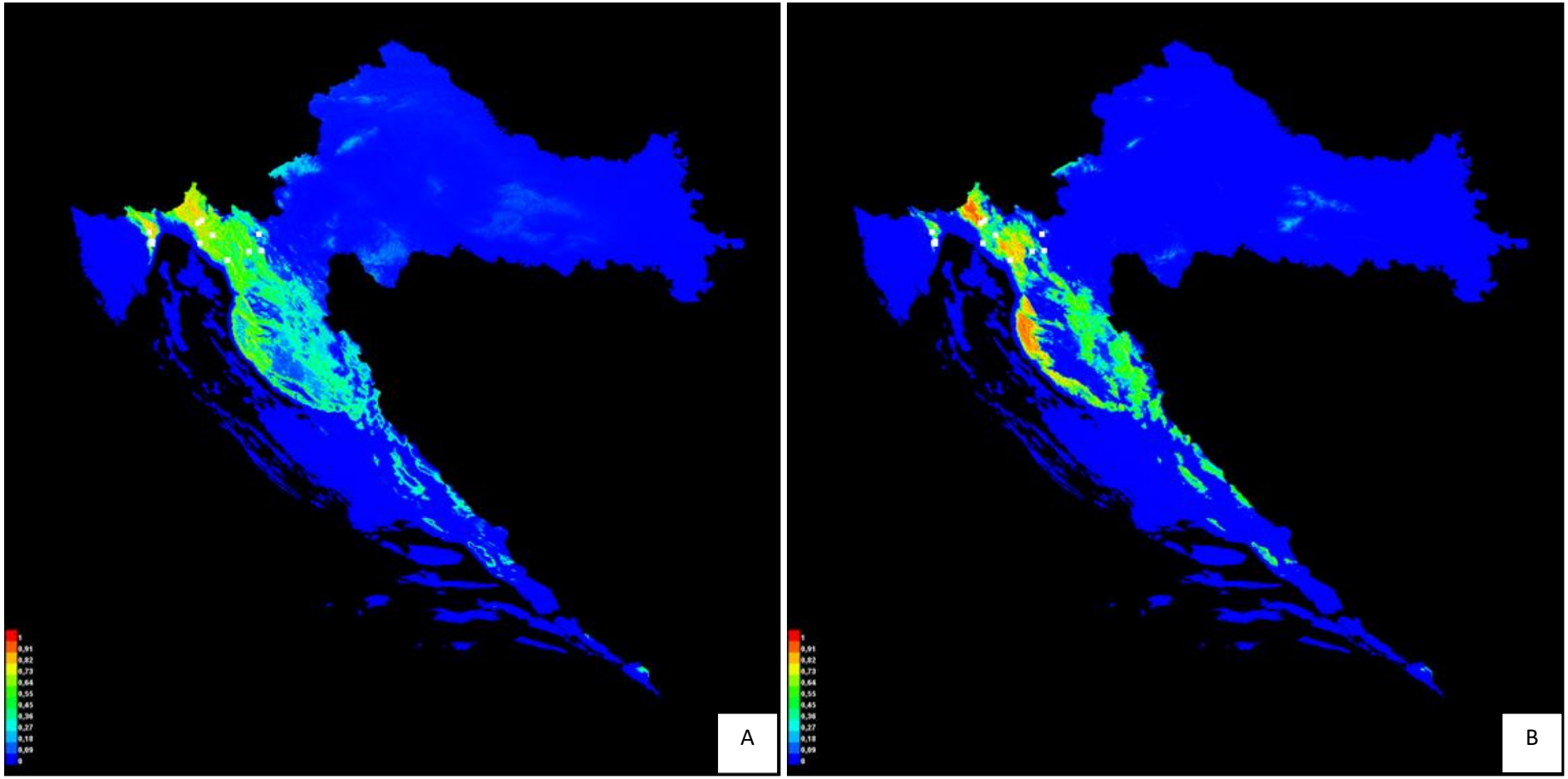
Tablica P 5.1. Nastavak

DRŽAVA	KRAJ	MJESTO	NALAZIŠTE	x-koordinata	y-koordinata	Nadmorska visina (m)	Datum pronalaska	Leg.	Det.	REFERENCE
MK	Jablanica	Gorna Belica	Belička Reka	41°13'32.69"N	20°32'53.33"E	1600	12.08-10.10.2005	S. Hristovski	S. Hristovski	Hristovski i sur. 2010
MK	Jablanica		Kokalo	41°12'12.79"N	20°31'44.87"E	1900	15.7.2006	S. Hristovski	S. Hristovski	Hristovski i sur. 2010
MK	Ilinska Planina		Ramska Padina	41°15'47.35"N	20°58'39.26"E	1300	13.7.2008	S. Hristovski	S. Hristovski	
ME		Durmitor	Sedlo	43° 5'15.55"N	19° 3'8.38"E		1971;80-82	B. Drovenik	B. Drovenik	B. Drovenik,1983
ME		Durmitor	Todorov dol	43° 8'3.12"N	18°58'27.35"E		1971;80-82	B. Drovenik	B. Drovenik	B. Drovenik,1983
ME		Durmitor	Prutaš	43° 7'39.98"N	19° 0'16.81"E		1971;80-82	B. Drovenik	B. Drovenik	B. Drovenik,1983
ME		Durmitor	Planinica	43°10'24.16"N	19° 1'40.77"E		1971;80-82	B. Drovenik	B. Drovenik	B. Drovenik,1983
ME	Sjeverozapad		Bioč	43°15'53.68"N	18°46'46.45"E		1971;80-82	B. Drovenik	B. Drovenik	
ME	Sjever		Sinjajevina	42°54'0.96"N	19°18'37.20"E		1971;80-82	B. Drovenik	B. Drovenik	
ME	Sjever	Berane/Rožaj	Turjak planina	42°41'54.03"N	19°34'14.09"E					carabidae.org
AL			Korab Mt	41°38'1.66"N	20°51'47.31"E					Breuning 1935 i 1978
RS			NP Kopaonik	43°17'46.96"N	20°48'15.79"E		2013	I.Rapuzzi, L.Caldon	I.Rapuzzi, L.Caldon	Rapuzzi., 2014

*U tablici se nalazi popis onih nalazišta za koja je bilo moguće odrediti točne ili približne koordinate.



Slika P 5.1. Kartografski prikaz rezultata predviđanja MAXENT modela za vrstu *C. caelatus* s obzirom na varijable u okolišu (klimatski podaci + topografija). A: Vremenski period (1960. – 1990.); B: Vremenski period (2041. – 2070.).
Toplije boje prikazuju područja s predviđenim boljim životnim uvjetima. Bijele točke pokazuju nalazišta vrste.



Slika P 5.2. Kartografski prikaz rezultata predviđanja MAXENT modela za vrstu *C. creutzeri* s obzirom na varijable u okolišu (klimatski podaci + topografija). A: Vremenski period (1960. – 1990.); B: Vremenski period (2041. – 2070.).

Toplije boje prikazuju područja s predviđenim boljim životnim uvjetima. Bijele točke pokazuju nalazišta vrste.

7. ŽIVOTOPIS

Rođena sam 30. ožujka 1985. godine u Čakovcu. Nakon završene osnovne škole dr. Vinko Žganec u Vratišincu 1999. godine upisujem se u jezični smjer gimnazije Josipa Slavenskog u Čakovcu gdje sam 2003. godine maturirala. Iste godine upisujem se na Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu, smjer: profesor biologije i kemije. Diplomski rad pod naslovom „Molekularna filogenija roda *Carabus*“ izradila sam 2009. godine pod mentorstvom prof. dr. sc. Mladena Kučinića. Nakon završetka studija počinjem raditi kao nastavnik biološke i kemijske skupine predmeta u Graditeljskoj, prirodoslovnoj i rudarskoj školi u Varaždinu u kojoj radim i danas. 2013. godine upisujem poslijediplomski studij biologije na Prirodoslovno-matematičkom fakultetu u Zagrebu pod mentorstvom dr. sc. Lucije Šerić Jelaska.

U sklopu poslijediplomskog studija sudjelujem na kongresima u Hrvatskoj (17^{th} European Carabidologist Meeting, 20. – 25. 9. 2015., Primošten, postersko priopćenje; 13. Hrvatski biološki kongres, 19. – 23. 9. 2018., Poreč, usmeno izlaganje); Poljskoj (15^{th} Symposium of Polish Carabidologist, 5. – 7. 6. 2017., Kiry, usmeno izlaganje), Francuskoj (18^{th} European Carabidologist Meeting, 25. – 29. 9. 2017., Rennes, postersko priopćenje) i Italiji (19^{th} European Carabidologist Meeting, 16. – 20. 9. 2019., Fiera di Primiero, usmeno izlaganje). Sudjelujem u objavi tri znanstvena rada u koautorstvu.

Autorica sam monografije kulturno umjetničke udruge „ Gornji Kraljevec“ izdane u povodu 25 godina djelovanja iste. Dobitnica sam Plakete Općine Vratišinec „dr. Vinko Žganec“ za osobita postignuća i doprinos značajan za razvitak i ugled Općine te brončane diplome Hrvatskog Sabora kulture za dvadesetogodišnji uspješan rad na širenju i razvijanju kulturno-umjetničkog amaterizma i hrvatske kulture. Članica sam Hrvatskog ekološkog društva.

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