PALACKÝ UNIVERSITY OLOMOUC

FACULTY OF SCIENCE

DEPARTMENT OF ZOOLOGY

PHYLOGENY AND PHYLOGEOGRAPHY OF METRIORRHYNCHINI (COLEOPTERA: LYCIDAE) IN THE PAPUAN REGION

Ph.D. thesis

Mgr. Matěj Boček

P1527 – Biology

Zoology

Supervisor: prof. Ing. Ladislav Bocák, Ph.D.

Olomouc 2019

I hereby declare that I prepared and wrote the Ph.D. thesis entitled "Phylogeny and phylogeography of Metriorrchynchini (Coleoptera: Lycidae) in the Papuan region" under the guidance and supervision of prof. Ladislav Bocak and I used literature cited in this study.

Olomouc, 7th June 2019 ……

Acknowledgements

I am very grateful to my supervisor prof. Ladislav Bocak for his patience, motivation, usefull advices and guidance during writing this thesis. I am also indebted to all my former and present colleagues from the Laboratory of Molecular Systematics for their support, useful comments and for the opportunity working together in such a greatly prospering team. Besides my supervisor and labmates, I deeply thank to all other schoolmates from the Palacky University in Olomouc for helping me succesfully reach the final destination of my Ph.D. study. Last but not the least, my sincere thanks goes to my family, primarily to my parents Jana and Petr, my brother Petr and to my girlfriend Sabina for supporting me spiritually under all circumstances on my long-lasting journey at Palacky University.

ABSTRACT

The Ph.D. thesis contains four separate studies dealing with the molecular phylogeny, Müllerian mimicry, morphology-based taxonomy, and phylogeography of trichaline net-winged beetles from Australo-Indonesian Archipelago. These beetles belong to the tribe Metriorrhynchini (Coleoptera: Elateroidea: Lycidae). Tribe Metriorrhynchini is a most diverse group of Lycidae which encompasses over a third of the Lycidae alpha-taxonomic diversity. Most net-winged beetles have a limited dispersal ability, but the members of tribe Metriorrhynchini are slightly better colonists compared to the most of other close relatives. Moreover, another weakly sclerotized elateroid lineage, Elateridae: Omalisinae, was studied to recover ancient distribution patterns in the Mediterranean.

The phylogeny of trichaline genera was used to recover generic limits. Further, phylogeography and dispersal capacity was studied in detail also here. Such information is needed to properly understand the biological processes which leads to colonization of new ranges in poorly dispersing animal groups like Lycidae or Omalisinae. Ancestral distribution of trichaline genera was inferred in New Guinea and adjacent islands where the highest species diversity occurs. Additionally, unexpected direction of colonization events points to secondary range expansion to Australia. The trichaline genera used the islands of the Moluccas, Philippines, Sulawesi and Sundas as stepping stones for their colonization of continental Asia.

Another study deals with Müllerian mimetic complexes which play an important role in a recent radiation of *Eniclases* communities in New Guinea. In contrast to widely accepted theory of purifying selection leading to convergent evolution of a single aposematic signal, sympatrically occurring species of *Eniclases* are characterized by the presence of multiple patterns in a place and intraspecific color polymorphism.

The result of phylogenetic analyses of Omalisinae are also unexpected. The females are wingless, incompletely sclerotized with retained larval characters when mature. I document that most species are distributed exclusively in coastal refugia around the Mediterranean Sea and only a single species is widespread. Understanding species diversity and phylogeography in the Mediterranean can extraordinarily improve the knowledge on natural processes and provide information for species conservation in this highly disturbed region.

ABSTRAKT

Dizertační práce obsahuje čtyři nezávislé studie týkající se molekulární fylogeneze, Müleriánských mimikry, tradiční taxonomie založené na morfologickém popisu nových druhů a fylogeografie. Jako modelová skupina byla vybrána skupina trichaliních rodů, jež je terminální linií tribu Metriorrhynchini (Coleoptera: Elateroidea: Lycidae) a vyskytuje se v oblasti indonéských ostrovů mezi Austrálií a kontinentální částí jihovýchodní Asie. Tribus Metriorrhynchini je nejdiverzifikovanější skupinou čeledi Lycidae a čítá více než třetinu dosud popsané druhové diverzity. Navzdory omezené schopnosti disperze u většiny zástupců čeledi Lycidae, zástupci tribu Metriorrhynchini jsou lepšími kolonizátory než jejich blízcí příbuzní. Vzhledem k dosažení dlouho nedostupného materiálu byla studována taktéž další málo sklerotizovaná elateroidní linie Elateridae: Omalisinae, která sdílí starodávné rozšíření v oblastech glaciálních refugií v okolí Mediteránu.

Fylogeneze trichaliních rodů byla použita k vymezení jednotlivých rodů. Dále byla zkoumána jejich fylogeografie a schopnost šíření druhů, díky kterým se vědci snaží pochopit procesy vedoucí k osídlování nových oblastí zejména pro druhy s omezenými schopnostmi šíření, jakými jsou zástupci Lycidae i Omalisinae. Ancestrální distribuce trichaliních rodů je situována do oblasti Nové Guinei a blízkých ostrovů, odkud byli její zástupci schopni prostřednictvím ostrovů Moluk, Filipín, Sulawesi a Velkých i Malých Sund kolonizovat kontinentální Asii.

Další studie řeší problematiku Müleriánských mimikry hrající důležitou roli v druhové radiaci rodu *Eniclases* na Nové Guinei. Na rozdíl od přijímané teorie purifikující selekce vedoucí ke konvergenci pro jeden přijímaný aposematický signál, sympatricky se vyskytující zástupci rodu *Eniclases* se vyznačují přítomností mnoha barevných vzorů na lokalitě a intraspecifickým druhovým polymorfismem.

Výsledky fylogenetické analýzy Omalisinae jsou rovněž neočekávané. Samice jsou bezkřídlé, nekompletně sklerotizované se zachovanými larválními znaky v jejich dospělosti. Většina druhů se vyskytuje výhradně v oblasti příbřežních refugií v okolí Středozemního moře a pouze jeden druh je široce rozšířen. Pochopení diverzity a způsobu šíření Omalisinae může významně pomoci k poznání přírodních procesů, které mohou poskytnout informace pro ochranu přírody v této disturbované oblasti.

CONTENTS

PREFACE

The phylogeny, origins of species diversity, evolutionary novelties and interactions between organisms and environment are major fields of contemporary biology. Different groups of insects serve as model organisms in such studies due to their extreme biodiversity and ecological plasticity. Traditional morphology-based taxonomical studies and later morphological cladistic analyses had been for most of the 20th century in the centre of systematic entomology, but a methodological revolution came with molecular techniques not only in the fields of transition zones between biochemistry and biology, but also in the systematic research. Recent state-of-art studies in molecular systematics are based on methods with an extreme power to resolve even very recent species evolution, interactions and the origin of adaptations. Many relationships crucial for understanding evolution of insects have remained poorly understood due to the absence of appropriate markers and high phenotypical plasticity which compromised morphology-based analyses (Whiting et al., 1997; Bocek & Bocak, 2016). In species delimitation, species polymorphism and the cryptic diversity represented further challenge to be solved (Larsen, 2001; Porco et al., 2012; Bocek & Bocak, 2017). Regardless amount of information and explanatory power, also molecular data occasionally failed in the species delimitation due to incomplete lineage sorting and hybridization. Thus, any conclusion on the evolution of species should be preferably validated by independent information. Therefore, the combination of morphological traits and molecular phylogeny are widely used side by side to reveal the relationships among problematic groups and to construct phylogenetic hypotheses (Giribet et al., 2001; Glenner et al., 2004; Bocek & Bocak, 2016; 2017). In some fields of systematic biology, the molecular data became an obligatory part of a species description (e.g., Journal of Nematology).

Similarly, in the explosive development of molecular techniques, the morphology-based systematics explores the technological advancement. Traditional external phenotype-based analysis has been expanded by the detailed study of internal structures. Since WWII, the male genitalia are almost obligatory used for the description of new species (Beal, 1959; Young, 1967; Kimsey, 1979). Although highly valuable for species delimitation and as a one of the most reliable reproductive isolation mechanisms, their permanent and rapid evolution due to sexual selection makes them unreliable in higher classification. Some very similar

structures are preserved in species without contact in sympatry and in contrary, many closely related species display substantial morphological disparity due to strong selection (Serb et al., 2011; Bocek & Bocak, 2016; Bocak et al., 2018; Bocek et al., 2018; Kusy et al., 2018; 2019). The digital documentation of diagnostic traits, high resolution electron microphotography and advanced methods in morphometry, all have become an integral part of morphology-based systematic research. Similarly, a high importance has been given to data basing of taxonomical acts, e.g. an obligatory registration of all new species in the ZooBank database if the publication is electronically disseminated (http://zoobank.org) or archives of morphological information in MorphoBank (https://morphobank.org/).

Under current situation, no further progress can be reached without new techniques and rigorous consideration of all evidence. Historically, two or more different phenotypical manifestations were usually considered as an evidence for delimitation of a separate species. However, the early phase of differentiation is none but simple. The long-lasting gene flow can result in a very gradual separation of gene pools and some differences can be manifested by a few crucial genes which could make emerging biological lineage separate (Reiseberg et al., 1999; Parsons & Shaw, 2001; Petit & Excoffier, 2009; Stölting et al., 2013). Conversely, different phenotypes can be produced under different environmental conditions without genomic differentiation.

Enormous insect's diversity represents both the challenge and opportunity. Among large insect orders, beetles are the most diverse group with about 400,000 formally described species and they represent over a fifth of all known organisms. Besides their importance concerning the alpha-diversity, they represent an important part of ecosystems especially in the dynamic and highly structured environment of tropical rainforests. Comprehensive world-wide studies at order level or even higher are impossible and researchers usually concentrate on a single lineage and a limited study area. The currents set of studies deals with elateroid beetles, especially with the families Lycidae and Elateridae: Omalisinae *sensu* Kusy et al. (2018). The family Lycidae, commonly known as net-winged beetles, have been chosen as a model group for molecular, ecological and phylogeographical studies in the Australo-Indonesian Archipelago (Simpson 1977; Lohman et al., 2011). The Omalisinae were used for a study dealing with Tertiary vicariance and current distribution of Omalisinae in the Mediterranean.

The beetle family Lycidae (Coleoptera: Elateriformia: Elateroidea) has an almost cosmopolitan distribution with the only exception of species absence in polar areas and severe deserts (Bocak & Bocakova, 2008, Masek et al., 2018). The highest species diversity is known in the humid tropical rainforests especially in the Oriental and Australian regions, namely, the Greater Sundas, Wallacea, Moluccas, New Guinea and in the Neotropical region, *i.e.*, humid tropical forests of the Southern America (Bocak & Bocakova, 2008). In total, net-winged beetles encompasses more than 4,200 formally described species recorded from all zoogeographical realms (Masek et al., 2018). The number of newly described species grows each year particularly from the areas of intensive research efforts in Sulawesi, the Moluccas and New Guinea (*e.g.*, Dvorak & Bocak, 2007; 2009; Kazantsev, 2010; 2013; Bocek, 2017; Kalousova & Bocak, 2017; Bocek & Adamkova, in press) or from South America (*e.g.*, Ferreira & Ivie, 2017; 2018; Bocakova & Nascimento, 2013; Nascimento & Bocakova, 2016; Kazantsev, 2017). The most diversified lineage of net-winged beetles is a tribe Metriorrhynchini which contains about a third of diversity of the family Lycidae as a whole (Bocak, 2002; Masek et al., 2018). Here, I specifically use the former subtribe Trichalina, recently reclassified as a terminal lineage in tribe Metriorrhynchini and nowadays referred simply as trichaline net-winged beetles (Sklenarova et al., 2013; 2014), to infer molecular phylogeny, ancestral areas and dispersal routes in their colonization events towards continental Asia.

The study area is the Australo-Indonesian Archipelago, a region where first zoogeographic principles were recognized, and which is still in the focus of zoogeographers. The species diversity of insects in this area is immense with several tens of thousands described beetle species and more than a thousand of recognized net-winged beetles (Riedel et al., 2013; 2014; Toussaint et al., 2015; Masek et al., 2018; Riedel & Narakusumo, 2019). At present, phylogeographic studies in Southeastern Asia seriously consider historical processes like plate tectonics (Hall, 2002; 2012), submerging and emerging landmasses and their exact origin that might be an important factor when ranges of extant species were established (Riedel et al., 2013; 2014; Tanzler et al., 2016; Bocek & Bocak, 2019). For these studies, net-winged beetles have some advantages. Similar to vast majority of beetles, they are fully winged but unlike most of their relatives, they are soft-bodied and incapable to fly over long distances. Hence, their diversity is established more often due to

vicariance and not because of their power to colonize new ranges (Sklenarova et al., 2013). The knowledge on net-winged beetles dispersal capabilities is continuously growing. For example, very limited dispersal capacity was identified in neotenic lineages (Li et al., 2015; Bray & Bocak, 2016). Slightly higher capacity, but still incomparably low to many other insects is known in Metriorrhynchinae owing to their clumsy flight (Jiruskova et al., 2019). Moreover, the dispersal capacity of Lycidae is also affected by propensity of their bodies to drying out. Therefore, net-winged beetles usually sit inactive under the forest canopy and because of this unique behaviour, the dispersal propensity of the model group is very low and thus most trichaline net-winged beetles are characterized by *in situ* speciation and a very low proportion of widely distributed species (Bray & Bocak, 2016; Motyka et al., 2018; Jiruskova et al., 2019).

The beetle family Omalisidae was added to this project as another interesting model group which has been earlier considered as a sister lineage to Lycidae (Kleine, 1933; Crowson, 1972; Lawrence, 1988; Lawrence et al., 2011). Recently, they have been transferred to Elateridae as a subfamily Omalisinae (Kusy et al., 2018). Females of Omalisinae are incompletely metamorphosed with larval characteristics retained when adults are sexually mature (Bocek et al., 2018; Kusy et al., 2019). As a non-flying group, they are an additional very interesting group for zoogeographical studies due to their predominantly Mediterranean distribution with the ancient distribution patterns. The molecular data can be employed to answer various questions on evolution, classification and zoogeography.

Thesis focus

The here presented Ph.D. thesis encompasses four studies dealing with molecular phylogeny, phylogeography, dispersal, mimicry and taxonomy. First study is a still unpublished manuscript dealing with the complex question of the persistence of multiple mimetic patterns in New Guinean communities of *Eniclases* net-winged beetles (**PART I**). It is based on a well-resolved phylogeny recovered from the large dataset of random restriction site genomic marker data. The study questions the suitability of mitochondrial markers for species delimitation in recently split species clusters with still considerable gene flow between emerging species-rank entities. Therefore, I have studied population structure in detail to obtain an insight into *Eniclases* differentiation and to recover the history of mimetic pattern adoption.

Two more studies (**PARTS II–III**) included in the thesis use molecular phylogenetic approach based on Sanger markers, *i.e.*, the analysis of mitochondrial and nuclear DNA fragments. These markers have a potential for reconstruction of deep to shallow phylogenies, ancestral distribution states of selected taxa or dating analyses indicating the presumable age of important splits between principal lineages. A single study (**PART IV**) deals exclusively with morphology-based description of four new species of *Diatrichalus* from New Guinea.

The substantial part of this thesis has already been published and passed through rigorous peer review process in various international journals, such as Zoological Journal of the Linnean Society, Systematic Entomology, and Zootaxa. The **PART I** dealing with a nextRAD dataset has not yet been published in a journal and it is presented in a form prepared for submission to an evolutionary or ecological journal. All presented studies except the morphological description of new species combined the analyses based on molecular phylogeny, species diversity and zoogeography of local groups. The analyses of phylogeography and dispersal history of trichaline net-winged beetles in the Australo-Indonesian Archipelago are fully based on a comprehensive dataset containing five-genes (**PART II**). This study confirms a low dispersal capacity of trichaline genera where only a limited number of lineages are capable to colonize a new range. Moreover, the presumable direction of the trichaline colonization leads from New Guinea and the Moluccas towards continental Asia using the islands of Wallacea, Philippines and the Sundas as the stepping stones. These results are contra intuitive concerning area differences and tectonical age.

During my Ph.D. study, I had an opportunity to examine the first known female of *Thilmanus obscurus* when this species was collected after hundred years since a previous record. The new DNA-grade material enabled a comprehensive study about the zoogeography of Omalisidae (Coleoptera: Elateriformia: Elateroidea) in the Mediterranean (**PART III**). The study presents phylogeographical analyses resolving the ancient patterns in species distribution along the shores of former Mediterranean basin. Such study was not planned originally and is included here as it uses the same methodology and fits with the main goals of the Ph.D. project. The insect's diversity in Mediterranean has been studying for decades and the here recovered relict distribution of an ancient group can be considered as unexpected. The last study (**PART IV**) deals with the traditional morphology-based taxonomy of

Diatrichalus from New Guinea with the description of four species new for science. Primary alpha-taxonomic studies are very important to understand the diversity and distribution of species and their evolutionary directions.

Conclusions

The set of studies relates to the uniting a single goal: I wanted to recognize and document the diversity of the selected groups of beetles and to identify processes leading to their evolutionary success in the case of highly diverse trichaline beetles, or to their supposed evolutionary failure, as in the case of extremely rare Omalisidae which till today persist in a few refugia in the Mediterranean. Besides the self-evident goal to document diversity and construct the classification and name some species earlier unknown to science, the studies can classify the centres of diversity and regions where the genetic diversity has been preserved despite climatic and tectonic turbulences. It is more than likely that a huge amount of species richness is still unknown and probably will never been recognized. The effort of researchers from all over the world is often surpassed by the pace of industrial development or deforestation to obtain palm oil as an ecological fuel or a food resource for continually growing population (Sodhi et al., 2004; Fitzherbert et al., 2008). Nevertheless, I believe that any contribution to our knowledge on nature is valuable and may persuade people to protect it.

Acknowledgements

The Ph.D. thesis contains selected publications published or prepared during my studies. It is my honour to thank to all colleagues with whom I studied evolution of insects and had an opportunity to produce further studies, not included in my dissertation. I am also obliged to Ms. Renata Bilkova who provided ongoing flow of data and supported my work in the laboratory. The study was supported by ESF grant from EU and Ministry of Education, Internals grants from the Faculty of Science and research grant from the Czech Science Foundation. The supervisor, Ladislav Bocak, and my colleagues Michal Motyka and Dominik Kusy, ignited discussions on various aspects of insect evolution during my stay in the laboratory and they contributed to many ideas published in my studies.

References

Beal, R. S. (1959) Notes on the Biology and Systematics of the Dermestid Beetle Genus *Aspectus* with Descriptions of Two New Species. *Annals of the Entomological Society of America*, **52**, 132–137.

Bocak, L. (2002). Revision and phylogenetic analysis of Metriorrhynchinae. *European Journal of Entomology*, **99**, 315–351.

Bocak, L. & Bocakova, M. (2008) Phylogeny and Classification of the Family Lycidae (Insecta: Coleoptera). *Annales Zoologici*, **58**, 695–720.

Bocak, L., Motyka, M., Bocek, M. & Bocakova, M. (2018) Incomplete sclerotization and phylogeny: The phylogenetic classification of *Plastocerus* (Coleoptera: Elateroidea). *PLoS ONE*, **13**: e0194026.

Bocakova M. & Nascimento E. A. (2013) A new species of *Brasilycus* (Coleoptera: Lycidae) from Peru. *Zootaxa*, **3637**, 597–599.

Bocek, M. (2017) New species of *Diatrichalus* (Coleoptera: Lycidae) from New Guinea and the Moluccas. *Zootaxa*, **424**, 577–584.

Bocek, M. & Adamkova, K. (in press) New species of Moluccan trichaline net-winged beetles, with remarks on the phylogenetic position and distribution of *Schizotrichalus* (Coleoptera: Lycidae: Metriorrhynchinae). *Zootaxa*.

Bocek, M. & Bocak, L. (2016). Species limits in polymorphic mimetic Eniclases net-winged beetles from New Guinean mountains (Coleoptera: Lycidae). *ZooKeys*, **593**, 15–35.

Bocek, M. & Bocak, L. (2017) The comparison of molecular and morphology-based phylogenies of trichaline net-winged beetles (Coleoptera: Lycidae: Metriorrhynchini) with description of a new subgenus. *PeerJ*, **5**, e3963.

Bocek, M. & Bocak, L. (2019) The origins and dispersal history of the trichaline net-winged beetles in South East Asia, Wallacea, New Guinea and Australia. *Zoological journal of the Linnean Society*, **185**, 1079–1094.

Bocek, M., Fancello, L., Motyka, M., Bocakova, M. & Bocak, L. (2018) The molecular phylogeny of Omalisidae (Coleoptera) defines the family limits and demonstrates low dispersal propensity and ancient vicariance patterns. *Systematic Entomology*, **43**, 250–261.

Bray, T. C., & Bocak, L. (2016) Slowly dispersing neotenic beetles can speciate on a penny coin and generate space-limited diversity in the tropical mountains. *Scientific Reports*, **6**, 33579.

Crowson, R. A. (1972) A review of classification of Cantharoidea (Coleoptera), with the definition of two new families, Cneoglossidae and Omethidae. *Revista Universidad de Madrid*, **21**, 35–77.

Dvorak, M. & Bocak, L. (2007) *Sulabanus* gen. nov., a new genus of Lycidae (Coleoptera) from Sulawesi. *Zootaxa*, **1611**, 1–24.

Dvorak, M. & Bocak, L. (2009) Ten new species of *Wakarumbia* Bocak, 1999 from Sulawesi (Coleoptera: Lycidae), with a key to males of the genus. *Zootaxa*, **2282**, 51–61.

Ferreira, V. S. & Ivie, M. A. (2017) The First Fossil Species of the Extant Genus *Cessator* Kazantsev (Coleoptera: Lycidae): A New Leptolycini from Dominican Amber. *The Coleopterists Bulletin*, **71**, 57–60.

Ferreira, V. S. & Ivie, M. A. (2018) A revision of *Lycinella* Gorham, 1884 with the description of six new species (Coleoptera, Lycidae, Calopterini). *ZooKeys*, **792**, 69–89.

Fitzherbert, E. B., Struebig, M. J., More, A., Danielsen, F., Brüh, C. A., Donald, P. F. & Phalan, B. (2008) How will oil palm expansion affect biodiversity? *Trends in Ecology & Evolution*, **23**, 538–545.

Giribet, G., Edgecombe, G. D. & Wheeler, W. C. (2001) Arthropod phylogeny based on eight molecular loci and morphology. *Nature*, **413**, 157–161.

Glenner, H., Hansen, A. J., Sørensen, M. V., Ronquist, F., Huelsenbeck, J. P. & Willerslev E. (2005) Bayesian Inference of the Metazoan Phylogeny. *Current Biology*, **15**, 392–393.

Hall, R. (2002) Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. *Journal of Asian Earth Sciences*, **20**, 353–431.

Hall, R. (2012) Late Jurassic-Cenozoic reconstructions of the Indonesian region and the Indian Ocean. *Tectonophysics*, **570–571**, 1–41.

Jiruskova, A., Motyka, M., Bocek, M. & Bocak, L. (2019) Does the Malacca Strait separate distinct faunas of poorly flying *Cautires* net-winged beetles? *PeerJ*, **7**, e6511.

Kalousova, R., & Bocak, L. (2017) Species delimitation of colour polymorphic *Cladophorus* (Coleoptera: Lycidae) from New Guinea. *Zootaxa*, **4320**, 505–522.

Kazantsev, S. V. (2010) New taxa of Omalisidae, Drilidae and Omethidae, with a note on systematic position of Thilmaninae (Coleoptera). *Russian Entomological Journal*, **19**, 51–60.

Kazantsev, S. V. (2012) A New Lycid Genus from the Dominican Amber (Insecta, Coleoptera, Lycidae, Leptolycinae, Leptolycini). *Psyche*, **2012**, 982141.

Kazantsev, S. V. (2013) New and little known taxa of neotenic Lycidae (Coleoptera), with discussion of their phylogeny. *Russian Entomological Journal*, **22**, 9–31.

Kazantsev, S. V. (2017) New leptolycines from Ecuador and Peru (Coleoptera: Lycidae). *Russian Entomological Journal*, **26**, 127–146.

Kimsey, L. S. (1979) An illustrated key to the genus *Exaerete* with descriptions of male genitalia and biology (Hymenoptera: Euglossini, Apidae). *Journal of Kansas Entomological Society*, **52**, 735–46.

Kleine, R. (1933) *Coleopterorum catalogus auspiciis et auxilio W. Junk editus S. Schenkling*. Pars 128: Lycidae. W. Junk, Berlin.

Kusy, D., Motyka, M., Bocek, M., Masek, M. & Bocak, L. (2019) Phylogenomic analysis resolves the relationships among net-winged beetles (Coleoptera: Lycidae) and reveals the parallel evolution of morphological traits. *Systematic Entomology* (early view).

Kusy, D., Motyka, M., Bocek, M., Vogler, A. P. & Bocak, L. (2018) Genome sequences identify three families of Coleoptera as morphologically derived click beetles (Elateridae). *Scientific Reports*, **8**, 17084.

Larsen, K. (2001) Morphological and molecular investigation of polymorphism and cryptic species in tanaid crustaceans: implications for tanaid systematics and biodiversity estimates, *Zoological Journal of the Linnean Society*, **131**, 353–379.

Lawrence, J. F. (1988) Rhinorrhipidae, a new beetle family from Australia, with comments on the phylogeny of Elateriformia. *Invertebrate Taxonomy*, **2**, 1–53.

Lawrence, J. F., Slipiński, A., Seago, A. E., Thayer, M. K., Newton, A. F. & Marvaldi, A. E. (2011) Phylogeny of the Coleoptera based on morphological characters of adults and larvae. *Annales Zoologici*, **61**, 1–217.

Li, Y., Gunter, N., Pang, H. & Bocak, L. (2015) DNA-based species delimitation separates highly divergent populations within morphologically coherent clades of poorly dispersing beetles. *Zoological Journal of the Linnean Society*, **175**, 59–72.

Lohman, D. J., de Bruyn, M., Page, T., von Rintelen, K., Hall, R., Ng, P. K. L., Shih, H. T., Carvalho, G. R. & von Rintelen, T. (2011) Biogeography of the Indo-Australian Archipelago. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 205–226.

Masek, M., Motyka, M., Kusy, D., Bocek, M., Li, Y. & Bocak, L. (2018) Molecular Phylogeny, Diversity and Zoogeography of Net-Winged Beetles (Coleoptera: Lycidae). *Insects*, **9**, 154.

Motyka, M., Kampova, L., & Bocak, L. (2018). Phylogeny and evolution of Müllerian mimicry in aposematic *Dilophotes*: evidence for advergence and size-constraints in evolution of mimetic sexual dimorphism. *Scientific Reports*, **8**, 3744.

Nascimento, E. A. & Bocakova, M. (2016) A new species of neotenic *Neolyrium* from Peru (Coleoptera: Lycidae). *Acta Entomologica Musei Nationalis Pragae*, **56**, 721–724.

Parsons, Y. M. & Shaw, K. L. (2001) Species boundaries and genetic diversity among Hawaiian crickets of the genus *Laupala* identified using amplified fragment length polymorphism. *Molecular Ecology*, **10**, 1765–1772.

Petit, R. J. & Excoffier, L. (2009) Gene flow and species delimitation. *Trends in Ecology & Evolution*, **24**, 386–393.

Porco, D., Bedos, A., Greenslade Penelope, Janion, C., Skarżyński, D., Stevens, M. I., Jansen van Vuuren, B. & Deharveng, L. (2012) Challenging species delimitation in Collembola: cryptic diversity among common springtails unveiled by DNA barcoding. *Invertebrate Systematics*, **26**, 470–477.

Riedel, A. & Narakusumo, R. P. (2019) One hundred and three new species of *Trigonopterus* weevils from Sulawesi. *ZooKeys*, **828**, 1–153.

Riedel, A., Sagata, K., Surbatki, S., Tanzler, R. & Balke, M. (2013) One hundred and one new species of *Trigonopterus* weevils from New Guinea. *Zookeys*, **280**, 1–150.

Riedel, A., Tanzler, R., Balke, M., Rahmadi, C. & Suhardjono, Y.R. (2014) Ninety-eight new species of *Trigonopterus* weevils from Sundaland and the Lesser Sunda Islands. *ZooKeys*, **467**, 1–162.

Rieseberg, L. H, Whitton, J. & Gardner, K, (1999) Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. *Genetics*, **152**, 713–727.

Serb, J. M., Alejandrino, A., Otárola-Castillo, E. & Adams, D. C. (2011) Morphological convergence of shell shape in distantly related scallop species (Mollusca: Pectinidae), *Zoological Journal of the Linnean Society*, **163**, 571–584.

Simpson, G. G. (1977) Too many lines; the limits of the Oriental and Australian zoogeographic regions. *Proceedings of the American Philosophical Society*, **121**, 107–120.

Sklenarova, K., Chesters, D. & Bocak, L. (2013) Phylogeography of poorly dispersing net-winged beetles: A role of drifting India in the origin of Afrotropical and Oriental fauna. *PLoS One*, **8**, e67957.

Sklenarova, K., Kubecek, V. & Bocak, L. (2014) Subtribal classification of Metriorrhynchini (Insecta: Coleoptera: Lycidae): an integrative approach

using molecular phylogeny and morphology of adults and larvae. *Arthropod Systematics & Phylogeny*, **72**, 37–54.

Sodhi, N. S., Koh, L. P., Brook, B. W. & Ng, P. K. L. (2004) Southeast Asian biodiversity: an impending disaster. *Trends in Ecology & Evolution*, **19**, 654–660.

Stölting, K. N., Nipper, R., Lindtke, D., Caseys, C., Waeber, S., Castiglione, S. & Lexer, C. (2013) Genomic scan for single nucleotide polymorphisms reveals patterns of divergence and gene flow between ecologically divergent species. *Molecular Ecology*, **22**, 842–855.

Tanzler, R., Van Dam, M. H., Toussaint, E. F. A, Suhardjono, Y. R., Balke, M. & Riedel, A. (2016) Macroevolution of hyperdiverse flightless beetles reflects the complex geological history of the Sunda Arc. *Scientific Reports*, **6**, 18793.

Toussaint, E.F.A., Tanzler, R., Rahmadi, C., Balke, M. & Riedel, A. (2015) Biogeography of Australasian flightless weevils (Curculionidae, Celeuthetini) suggests permeability of Lydekker's and Wallace's Lines. *Zoologica Scripta*, **44**, 632–644.

Whiting, M. F., Carpenter, J. C., Wheeler, Q. D. & Wheeler, W. C. (1997) The Strepsiptera Problem: Phylogeny of the Holometabolous Insect Orders Inferred from 18S and 28S Ribosomal DNA Sequences and Morphology. *Systematic Biology*, **46**, 1–68.

Young, F. N. (1967) A key to the genera of American bidessine water beetles with descriptions of three new genera (Coleoptera: Dytiscidae, Hydroporinae). *Coleopterists' Bulletin*, **21**, 75–84.

PART I

Phylogeny and phylogeography of Metriorrhynchini (Coleoptera: Lycidae) in the Papuan region

Matej Bocek

Persistence of multiple patterns and intraspecific polymorphism in net-winged beetles due to variable signal perceptions and community structures

(unpublished manuscript)

Persistence of multiple patterns and intraspecific polymorphism in net-winged beetles due to variable signal perceptions and community structures

Matej Bocek

Laboratory of Molecular Systematics, Faculty of Science, Palacky University, tr. 17. listopadu 50, 771 46 Olomouc, Czech Republic

Abstract

In contrast to traditional models of purifying selection and a single aposematic signal in Müllerian complexes, some communities of unprofitable prey contain members with multiple aposematic patterns. Processes responsible for diversity in aposematic signaling are poorly understood and large multi-species communities are seldom considered. Therefore, I analyzed the phylogeny and aposematic patterns of closely related *Eniclases* net-winged beetles in central New Guinea using morphology, mtDNA, and nextRAD data. Our results confirm the complexities of early phase speciation. I suggest three clades of *Eniclases* representing groups of closely and incompletely reproductively isolated lineages, detail the extent of polymorphism among *Eniclases*, and categorize their low-contrast aposematic patterns. Field observations suggest that perception of the aposematic signal is affected by beetle behavior, weather, time of day, and vegetation density. The warning signal of *Eniclases* consists of body shape and color, with ambiguous color perception under some circumstances, *i.e.*, when resting on the undersides of leaves. As a result, purifying selection for patterns of similar color may be relaxed in net-winged beetle communities. I hypothesize that environmental factors in our study area are too

variable to allow for strong, constant purifying selection over time, resulting in multiple color patterns and polymorphism in closely related species in single localities. In addition, *Eniclases* occur in highly diverse multi-species communities of other net-winged beetles, which implies changing selection pressure in space and time. Variable environmental conditions and diverse community composition are suggested to be favorable for the persistence of multiple patterns, imperfect mimics, and intraspecific polymorphism.

Keywords. Müllerian mimicry, NextRAD, mtDNA, phylogeny, Lycidae, New Guinea

Short title: Multi-pattern Müllerian rings and polymorphism

Introduction

Müllerian mimicry is among the best-studied examples of evolution, yet some theoretical predictions stand in contrast with observed mimetic communities in nature (Alatalo & Mappes, 1996; Kikuchi & Pfennig, 2013; Mallet & Barton, 1989; Müller, 1879; Sherratt, 2008). Although the number of exhibited patterns should be quickly reduced by purifying selection (Chouteau & Angers, 2012; Chouteau, Arias & Joron, 2016; Mallet & Joron, 1999; Mappes, Marples & Endler, 2005; Poulton, 1890), I commonly observe high variation in mimetic signals in a single place, or intraspecific polymorphism (Abbott & Sherratt, 2012; Briolat et al., 2019; Edmunds, 2000; Mallet, 1999; Penney, Hassall, Skevington, Sherratt & Peet-Paré, 2017; Speed & Ruxton, 2004). Evidently, some factors must diminish predator learning of the association between the visual warning signal and negative stimulus produced by unprofitable prey, thereby decreasing the effectiveness of purifying selection. Inquiry into potential factors has included differences in unpalatability (quasi-Batesian

mimicry), the effects of multimodal signals, environmental conditions and community structure (Aronsson & Gamberale-Stille, 2009; Beatty, Beirinckx & Sherratt, 2004; Briolat et al., 2019; Mallet, 1999; Skelhorn, Holmes, Hossie & Sherratt, 2016; Speed, 1993). Non-adaptive genetic constraints may correlate with observed long-term persistence of a high number of distinct aposematic patterns in one place (Edmunds, 2000; Sherratt & Peet-Paré, 2017), where such constraints include the inability to produce pigments necessary for advergence to the dominant, most effective pattern, or considerable delays in pigment production (Arenas, Troscianko & Stevens, 2014; Motyka, Kampova & Bocak, 2018). Mimics may differ in body structure such that only imperfect mimicry can be produced (Edmunds, 2000; Sherratt, 2008; Wilson, Jahner, Williams & Forister, 2013). Differing interactions between genes and the environment among species may further prevent the dominance of a single signal in one community, for example, different melanization levels in response to a cold, humid climate in closely related *Cautires* beetles (Jiruskova, Motyka, Bocek & Bocak, 2019). The origins and processes of imperfect mimicry have recently been reviewed (Sherratt & Peet-Paré, 2017), and in this study, I attempted to identify processes that may produce multi-pattern communities and counterbalance the hypothesized effects of purifying selection in models of mimicry evolution.

Net-winged beetles (Coleoptera: Lycidae) are considered Müllerian mimics, known for their unprofitability and aposematic coloration (Eisner et al., 2008; Eisner, Kafatos & Linsley, 1962; Linsley, Eisner & Klots, 1961; Moore & Brown, 1981). I focused on closely related species of *Eniclases*, a trichaline genus of Metriorrhynchina (Sklenarova, Kubecek & Bocak, 2014). This approach avoids the constraints that commonly result from considering related but phylogenetically

distant members of mimetic systems, and the possibility that focal species will differ substantially in the levels of their protection (Edmunds, 2000; Motyka et al., 2018; Speed, 1993). Patterns exhibited by trichalines are simple, combining two colors at most, namely, black and shades of yellow and orange in *Eniclases*. Unlike well-studied mimetic systems, few data are available regarding predation of *Eniclases*, but field observations suggest birds, spiders, assassin bugs, and mantids (Eisner et al., 2008; field observations).

Eniclases belongs to the clade of Australian Metriorrhynchina, which dominates lycid communities in New Guinea (Bocek & Bocak, 2017, 2019; Kusy, Motyka, Bocek, Masek & Bocak, 2019; Masek et al., 2019). More than 300 Metriorrhynchina have been described from this region, but preliminary analyses of molecular data suggest even greater species diversity. Most Metriorrhynchina are brightly colored, with patterns combining high-contrast red and black areas, metallic blue or green coloration, various patches and bands, and examples of tri-colored elytra (Figs. 1–2; Kalousova & Bocak, 2017; Kleine, 1926). In total, 36 species of *Eniclases* are known and their relationships with *Trichalus* and related genera (hereafter, trichaline genera) have been clearly established using molecular data and morphology (Bocak, 2002; Bocek & Bocak, 2016; 2019; Supplementary Text). In New Guinea, trichalines such as *Eniclases* and *Microtrichalus* dominate mimetic systems at low elevations but are uncommon at elevations >1500 m (Bocek & Bocak, 2019). All net-winged beetles depend on humid forest, remaining mostly inactive under forest canopies. As a result, their dispersal propensity is low and no Metriorrhynchina species have been simultaneously recorded from landmasses separated by open sea (Jiruskova et al., 2019).

I focused on multi-pattern and multi-species New Guinean Müllerian

communities containing *Eniclases*, their co-mimics, and other lycid beetles. Currently, mimicry literature is biased to butterflies, the best studied model group (*e.g.*, Briolat et al., 2019; Mallet & Joron, 1999; Nadeau, 2014), with limited representations of mimicry in other animals (*e.g.*, Fabricant & Herberstein, 2015; McCornack, Koch & Ragsdale, 2007; Michie, Mallard, Majerus & Jiggins, 2010; Stuckert, Venegas & Summers, 2018). Our focus on net-winged beetles helps to address this bias, as these species are known as unprofitable and aposematic, yet have only recently been studied (Bocak & Yagi, 2010; Linsley et al., 1961; Motyka et al., 2018). The high diversity of *Eniclases* species, aposematic patterns, and ecosystems in our study area provide a unique opportunity to investigate the process of speciation in *Eniclases*, pattern origin, and the relevance of patterns in speciation. First, I defined species using morphology, mtDNA, and restriction-site-associated DNA (RAD) data. Next, I concentrated on mimicry under the working hypothesis that similar patterns are a result of purifying selection for a shared aposematic signal. Given the presence of multiple patterns and intraspecific polymorphism, I posed the following questions: Is purifying selection relaxed under some conditions? How dissimilar are New Guinea communities in species and pattern representation? Does variation in warning signals underpin the speciation process?

Methods

Field sampling and Eniclases specimens

We collected specimens of 12 *Eniclases* species from seven localities within a small study area (~1300 km^2) in central New Guinea in localities at the northern coast, across an elevation range from sea level to 2170 m (Tab. S1). The majority of individuals were collected in aggregations, designated AGG1-Sentani (275 m),

AGG2-Elelim (560 m), AGG3-Bokondini1 (1287 m), and AGG4-Bokondini2 (1250–1300 m). Additional specimens were collected individually in areas with low net-winged beetle abundance. Samples from each site were sorted to putative species based on color patterns and morphological traits. Coloration of the pronotum and elytra were recorded, and patterns were grouped into eight discrete categories (Tab. 1). All photographs of specimens are provided in Figs. S1–S4.

Laboratory procedures

Total DNA was extracted using a Wizard SV96 Purification System (Promega Inc.). Extraction yields were measured using a NanoDrop-1000 Spectrophotometer. The fragments *rrnL+tRNA-Leu+nad1* (~830 bp) and *nad5*+tRNAs (~1210 bp) mitochondrial DNA (mtDNA) were amplified. Primers are listed in Tab. S2 and polymerase chain reaction (PCR) settings followed Sklenarova, Chesters & Bocak (2013). PCR products were purified using PCRμ96TM Plates (Millipore Inc.) and sequenced by an ABI3130 automated sequencer using a BigDye® Terminator Cycle Sequencing Kit 1.1. Sequences were deposited in the GenBank database (accession numbers KT265092–KT265172, MF288197–MF288482 and MG844591–MF844955).

MtDNA data sampling and phylogenetic analyses

Earlier published sequences of *cox1* mtDNA (Bocek & Bocak, 2016) were merged with *rrnL* and *nad5* fragments. Fragments were aligned separately using MAFFT 7.017 (Katoh & Standley, 2013) in Geneious 7.1.9 (Biomatters, Ltd., Auckland, New Zealand) and the concatenated dataset was analyzed to infer a phylogenetic tree. I used IQ-TREE 1.6.6 (Nguyen, Schmidt, Von Haeseler & Minh,

2015) to estimate mtDNA phylogeny using an ultrafast bootstrap approximation and 5000 iterations. The best models for each fragment were selected in IQ-TREE using ModelFinder (Kalyaanamoorthy, Minh, Wong, Von Haeseler & Jermiin, 2017, Tab. S3).

A tree pruned to one representative per species was dated using a Bayesian approach implemented in BEAST 1.8.1 (Drummond, Suchard, Xie & Rambaut, 2012). I produced 5×10^7 generations with sampling every 2,500 generations. Only the *rrnL*, *cox1*, and *nad5* genes were analyzed and genes and codon positions were partitioned (Tab. S3). Each partition was provided with its own parameters. Due to an absent fossil record and young relevant splits, *i.e.*, younger than five million years, I used mtDNA rate information to calibrate our topology: 0.0115 subs/s/my/l for *cox1*, 0.0177 subs/s/my/l for *nad5*, and 0.0054 subs/s/my/l for *rrnL* (Papadopoulou, Anastasiou & Vogler, 2010). To estimate the possible effects of a higher rate of mutations, I repeated the analyses of the *cox*1 fragment with a doubled rate (0.023 subs/s/my/l). The best topology recovered from maximum likelihood analyses (Fig. S1) was fixed by a guiding tree and switching off tree operators during analyses. Convergence was assessed in Tracer v1.7 (Rambaut, Drummond, Xie, Baele & Suchard, 2018) and the first 1.25×10^7 generations were set as a burn-in.

Next-RAD sampling and analyses

Based on the mtDNA analyses, I selected 66 individuals for subsequent RAD sequencing. The samples represent 13 species of *Eniclases* net-winged beetles from seven sites and undescribed individuals of *Eniclases*. Species identification was based on morphology and *cox*1 mtDNA (Bocek & Bocak, 2016) and did not necessarily agree with the results of subsequent next-RAD analyses. Detecting a

large number of single nucleotide polymorphisms in a high number of genomic loci across closely related species and populations may provide resolution on recent species-level interactions and could support the phylogenetic hypothesis (Bray & Bocak, 2016; Lavretsky, Da Costa, Sorenson, McCracken & Peters, 2019). Given that a reference genome is unavailable for RAD data masking due to its size (3–5 GB), *de novo* assembly with clustering thresholds (Wclust, degree of sequence similarity) was used to search for orthologous sequences (Ekblom & Galindo, 2011). The nextRAD genomic sequencing was provided by SNPsaurus Inc., where the Illumina Hi-Seq system was used to generate data. RAD sequencing produces primer-based individually barcoded single-end read amplifications with an average length of ~75 bp representing loci scattered across the genome. Each read was individually assigned to the specific specimen voucher. Illumina reads were deposited in the Sequence Read Archive (ABC123456).

After demultiplexing, trimming, and filtering RAD reads, I used the software iPYRAD 0.6.24 (Eaton 2014; Eaton & Overcast, 2016) to identify a *de novo* assembly of orthologous loci. This software uses an alignment-clustering method involving indel variation, which improves the precision of recognition of global homology across different samples, and read trimming, which generates variable read lengths unlike alternative assembly methods, *e.g.*, Stacks (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011; Eaton, 2014; Takahashi, Nagata & Sota, 2014). The maximum size of the data matrix varied as I varied the Wclust parameter. I tested these matrices by analyzing five Wclust settings from 0.7–0.9, increasing by 0.05 for each filtering. The number of potential loci increased when Wclust increased, although within-individual heterozygosity decreased significantly when Wclust was set higher than 0.85 (Fig. S5). To balance the highest proportion of

potential loci accepted and the highest rate of sample heterozygosity, a Wclust value of 0.85 was used in the final analyses. A minimum depth (MinDepth) of six reads, together with a minimum number of four samples that contained data for a given locus (MinCov) was used in the final dataset. The proportion of missing data and the number of loci filtered are strongly dependent on the MinCov parameter (Huang & Knowles, 2016), therefore, I also produced additional data matrices with varied MinCov and Wclust values. Altogether, 30 datasets with unique settings were generated (Wclust from 0.7–0.9 increasing by 0.05 and MinCov of 4, 8, 16, 33, 48 and 60 for each unique filtering). Further, data were filtered independently for clades A through C, as defined by preliminary analyses. I used iPYRAD on a dataset with a Wclust of 0.7 and a MinCov of 4 to reveal population genetic structure. I inferred individual admixture coefficients based on sparse non-negative matrix factorization (sNMF) analyses using the package 'LEA' in R (Frichot & François, 2015). I further performed principal components analysis (PCA) in R, using the package 'SNPRelate' 1.6.4 (Zheng et al., 2012) to visualize the major axes of genetic variation using the above dataset, reduced by linkage-disequilibrium-based single nucleotide polymorphism (SNP) pruning (Figs. S11–S15).

Each matrix generated with a specific MinCov value was used to infer a phylogenetic tree. These matrices were again analyzed with a maximum likelihood approach using IQ-TREE, with an ultrafast bootstrap approximation and 5000 iterations. ModelFinder, implemented in IQ-TREE, estimated the optional evolution model for final matrix (Tab. S3). Resulting tree topologies from all data matrices were examined and are provided in the Supplementary Dataset. In addition to maximum likelihood trees inferred using IQ-TREE, I used SVDquartets (Chifman & Kubatko, 2014; 2015), implemented in PAUP* (v. 4.0a, build 165; Swofford, 2002),

and evaluated bootstrap support over 1000 iterations. In the final step, the PAUP* version of the QFM algorithm (Reaz, Bayzid & Rahman, 2014) was used to search for the overall tree that minimized the number of quartets that were inconsistent with it.

Results

Metriorrhynchina and community structure

In total, I collected 1914 specimens of Metriorrhynchina beetles from seven localities. The majority of specimens were collected from aggregations, but some were collected from subsamples from different altitudes (1009 spec., Tabs. 1, S1, S7). All *Eniclases* and their co-mimics showed limited flying activity and all individuals in aggregations were collected from a few trees in an area <1000 m^2. Samples contained 95–433 individuals, 24–91 species total, and up to 6 species of *Eniclases*. Cluster analyses suggested similar species composition within slopes of the Wamena valley, *i.e.*, Yiwika, Tikapura, and Napua, with Bokondini (2000 m) as sister to all communities (Fig. 3E). A cluster of low- to mid-elevation localities contained Sentani, Bokondini (1250 m), Dombomi, and Elelim. Most species were recorded from a single locality (231 species, Tab. S7), whereas a minority were recorded from two (51 species) and three (11 species) localities. Only two species were recorded from four localities. Metriorrhynchina are highly diverse in the study area; altogether, 295 species were recorded, 50 trichalines and 12 *Eniclases* species. The number of aposematic patterns among all net-winged beetles varied between 3 and 10 at each locality, where only some of these patterns were exhibited by *Eniclases* (Tab. 1). Non-lycid co-mimics represented <2% of individuals in each

community and belonged to soldier beetles, true bugs, and moths (Figs. 1C, D). They are not discussed further.

Trichaline net-winged beetles represented a majority of three aggregations from a lowland foothill forest of the Cyclops mountains (275 m) (81.4%, AGG1, sampling area of ~500 m^2 at a forest margin) and a low mountain forest of the Central Cordillera close to Bokondini (1287 m) (47.3% and 60.8% at AGG3 [300 m^2] and AGG4 [200 m^2], respectively). Further communities contained a wider spectrum of Metriorrhynchina and lower representation of trichalines. Samples from a lowland forest close to Elelim (560 m, AGG2) contained 36.3% trichalines, from an area of ~1000 m^2. In total, 763 Metriorrhynchina specimens were collected from higher-elevation mountain forests (Yiwika [2100 m], Bokondini [1700–2100 m], and Tikapura [2150 m]), places known to have lower abundance of *Eniclases* (Tab. 1). Species turnover was high among localities, including those which were geographically close (Tabs. S6, S7).

Classification of Eniclases color patterns

Color patterns of New Guinean *Eniclases* were classified into eight categories: (a) uniform black; (b) pronotum orange, elytra black, (c) pronotum and humeral patches orange, remainder of elytra black; (d) pronotum and humeral part of elytra orange, remainder of elytra black; (e) pronotum black, humeral part of elytra orange, remainder of elytra black; (f) pronotum black, the humeral part of elytra light yellow, apex infuscate; (g) pronotum and elytra orange to light yellow, the apex of elytra infuscate, and; (h) uniform yellow. All patterns were shared by both sexes and shared patterns were identified in unrelated species (Figs. 3A–B, 4). Intraspecific variation was identified in 5 of 14 species: *Eniclases niger*, *E. similis*, *E.* sp. B, *E.*

variabilis, and *E. elelimensis* (Figs. 3–4). Yellow-black and black patterns were distributed within lowlands and recorded up to ~1300 m, whereas pale yellow and pale yellow-dark patterns occurred at >1500 m. The Dombomi fauna (1150 m) was dominated by a uniform black pattern.

I recorded several discrete mimetic phenotypes in a single place: 3–10 distinct patterns of Metriorrhynchina and 1–6 patterns of *Eniclases* (Fig. 4). Color patterns in *Eniclases* were limited to pale yellow, bright yellow, and orange-yellow bright hues and dark brown to deep black on the upper-body parts. No *Eniclases* were red-colored, unlike many syntopically occurring Metriorrhynchina (*e.g.*, *Cautiromimus*, *Carathrix*, *Porrostoma*, Fig. 1). Patterns in *Eniclases* closely resembled those of the related genus *Microtrichalus* from the same region (Fig. 2). The transition between bright and dark body parts was sometimes gradual; in extreme cases, only the apices of elytra were lightly infuscated, or alternatively high contrast was present.

MtDNA phylogeny

Maximum likelihood mitochondrial analyses supported three clades of closely related species within *Eniclases*: clade A (BS 98%), clade B (BS 100%), and clade C (BS 100%; Fig. 3A). Deeper splits were poorly supported and are not discussed. These clades are suggested to be monophyletic. The age of splits among *Eniclases* species was estimated (Fig. 3C), where only splits among species within clades A–C are relevant for further discussion (4.39, 0.27, and 1.81 mya, respectively). Alternative calibration approaches produced only slightly different estimations for critical splits (Fig. S8).

Next-RAD phylogeny and genetic structure

The nuclear RAD dataset encompassed 42,000 to 138,000 clusters of possible loci with a mean depth from 5.3 to 32, mainly owing to the low quality of some isolates. As discussed, I used a Wclust threshold of 0.85 to balance the maximum number of clusters recovered against loss of individual heterozygosity. The number of clusters generated using a Wclust of 0.85 was slightly higher than the heterozygosity level (Fig. S5), however, I recovered a higher number of potential loci.

I recovered a highly similar topology in all analyses (Figs. 3A–B, 4). Deep topology based on RAD data differed slightly from the mitochondrial tree (Figs. 3, S6–7). While both methods identified the same three clades, classification based on RAD data assigned two species differently (Figs. 3–5, S10). *Eniclases* sp. A was placed as a sister to *E. tikapurensis* by mtDNA topology, but RAD data suggested that it forms a terminal branch in the *E. tikapurensis* clade, and it is therefore not considered a putative species hereafter (Fig. 4). The clade of BM0008 and BM0012 was identified by RAD data as an independent deep branch in clade C, instead of being part of the *E. variabilis* clade as in mtDNA topology. Therefore, this clade is designated *E.* sp. B hereafter. The RAD-based composition of the clades *E. variabilis* and *E. elelimensis* differed substantially from those defined using mtDNA (Figs. 3–4, S10). Further, I twice identified a divergent genomic structure within clades recovered by RAD analyses. The clade of *E.* sp. B contained a divergent individual, BM0008, with a genomic structure similar to *E. brancuccii*, and individual BM0012 was similar to individuals of *E. variabilis* (Fig. 5F). *E. variabilis* is represented by two pairs of individuals that share a common genomic structure. Two individuals of

E. tikapurensis from Bokondini were very distant from conspecifics collected from Yiwika and Tikapura. All PCA analyses confirmed the clusters delimited using RAD-based phylogeny (Figs. 5A–C, S9).

Discussion

Analyses of mitochondrial markers and RAD data recovered the monophyly of the three clades relevant to this analysis, each consisting of two to five closely related species (Figs. 3–5). I identified mtDNA introgression, indicated by individuals with different nuclear but identical or highly similar mtDNA sequences (Figs. 3–5, S10). Therefore, I based species delimitations on RAD analyses (Figs. 4–5), as this extensive, genome-wide data can better resolve clusters of individuals with shared ancestry, particularly among closely related species and recently separated populations (Bray & Bocak, 2016; Dupuis, Roe & Sperling, 2012; Lam et al., 2018; Leache & Oaks, 2017). Results from sNMF and PCA analyses identified some genomes with different genetic structure within the RAD phylogeny-based species-rank entities (Figs. S11–S15). In two cases, *E. variabilis* and *E.* sp. B, the genomic structure corresponded with the displayed aposematic pattern. By contrast, other phenotypically distinct individuals shared a highly similar genome (*E. elelimensis* and *E. niger*, Figs. 4–5). Collectively, RAD and mtDNA phylogeny and sNMF analyses of *Eniclases* suggested that most species represent recently split lineages, typically within the last million years (Fig. 3C).

Species classification

Alpha-taxonomy of *Eniclases* has previously been based on morphology and a single mtDNA marker, with species-rank given to sets of morphologically

distinguishable populations without intermediates, or to sets identified as genetic clusters in mitochondrial phylogeny (Bocak & Bocakova, 1991; Bocek & Bocak, 2016). Results of restricted genome-wide sequencing revealed complex scenarios of diversification that were partly incongruent with earlier species designations (Figs. 3–5, S10). The number of morphology-based diagnostic traits for *Eniclases* is limited (Tab. S4); color patterns have evolved multiple times in unrelated species, some species are color polymorphic, and all *Eniclases* have uniform genitalia (Fig. 5). As phenotypic and genotypic divergence is unlinked, I used all data to separate morphologically similar species with genetic distinctness in sympatry and to assign the status of either a species or a geographic isolate to allopatrically distributed populations.

I identified closely related but distinct genetic clusters within single localities, which would obtain species rank under most species concepts (Cracraft, 1983; Endler, 1992; Mallet, 1995; Mayr, 1942). The genetically distinct *E. bicolor* and *E. infuscatus* occur sympatrically in Elelim and besides their color patterns, they differ only in eye size (Figs. 4, 5D; Tabs. S1, S4). *E. bicolor* could not be morphologically separated from the sympatric, but distantly related, *E. similis* (Figs. 4, 5D–E). These two species differed only in mtDNA and genomic markers (Figs. 3–5, Tab. S5). In addition, I assigned species status to allopatric *E. niger* and *E. similis*. Their genetic *cox1* mtDNA divergence reached only 0.45%, but genomic data supported their distinctness (Figs. 3–5, S6) and the possibility that they represent geographic isolates was falsified by divergence in eye size (Tab. S4). A further species pair, *E. brancuccii* and the allopatrically distributed *E. elelimensis*, are morphologically indistinguishable, but genetically divergent at a similar level as other sister species (Figs. 3–5, Tabs. S4, S5). No threshold between geographic isolates and cryptic

species can be defined as shown in *Heliconius* (Rosser et al., 2019). Further, some phenotypically very close *E. variabilis* and *E. elelimensis* share highly similar mtDNA (Figs. 3A, S10, Tab. S4) and genomic data are needed to reliably separate them.

The continuous nature of diversification, a species-population continuum, complicates determining which populations should be given the rank of species, subspecies, or considered divergent populations (Coates, Byrne & Moritz, 2018; Mallet, 1995; 2007; Nater et al., 2017). Simultaneously considering genetic and phenotypic information is the only way to avoid delimiting taxonomic units that represent morphologically distinct but artificial assemblages of unrelated populations (Figs. 3–5, Tabs. S4, S5). Unfortunately, this integrative approach does not produce an easily navigable taxonomic system. Under the present state of knowledge, clear assignment of some populations to a Linnean species requires a simultaneous examination of both morphology and nuclear DNA. *Eniclases* appears to be a taxonomist's nightmare and the difficulties with practical identification are not limited to sister taxa but also include distantly related species. Our results speak to the complexity of speciation and I note that some level of arbitrariness is unavoidable, even if extensive data are available for populations in an early phase of diversification (Coates et al., 2018; Elias et al., 2007; Mallet, 1995; Rosser et al., 2018).

Mimetic patterns in Eniclases

The foundational premise of Müllerian mimicry holds that unprofitable prey benefit from convergence in warning signals (Müller, 1879; Sherratt, 2008). The phylogeny of *Eniclases* showed that resemblance among various *Eniclases* is the

product of parallel evolution rather than common ancestry (Figs. 3–5). Our data support the mimicry hypothesis by the noted sympatric occurrence of distantly related but similarly colored Metriorrhynchina with *Eniclases* (Figs. 1, 2). Aggregations of net-winged beetles in the field indicate that multiple species truly coexist in a single community and do not have a microhabitat-based mosaic distribution.

Up to six *Eniclases* aposematic patterns were recorded within single localities in our small study area and additional patterns were displayed by closely related Metriorrhynchina (altogether up to 10 patterns in a single locality; Figs. 1, 2, Tab. 1). Unlike findings from other Müllerian mimetic systems (Gompert, Willmott & Elias, 2011; Willmott, Willmott, Elias & Jiggins, 2017), these observations suggest that a dominance effect on mimetic polymorphism stemming from microhabitat preference is improbable in *Eniclases*.

Coloration of *Eniclases* is limited to pale to bright yellow, yellowish orange, and black, but these beetles display multiple distinct color forms with a putative signaling function (Figs. 4, S1–S4). Dominant color patterns combine a bright-colored pronotum and elytral humeri with apically dark elytra. Higher contrast is represented by a steeper transition between bright and black parts of the elytra or pronotum. Three species in the study area are black, two of which are polymorphic with some individuals having a yellow pronotum (Fig. 4). These patterns dominate in all communities containing high representation of trichaline net-winged beetles, generally those <1500 m in elevation. Additional patterns among high-elevation species are uncommon but included pale yellow or creamy white in combination with a black pronotum and elytral apex (Fig. 4). *Eniclases* are never red, green, or metallic blue (Figs. 1L-O, 2J–L) and never display three colors, unlike numerous

sympatric Metriorrhynchina (Kleine, 1926). It is probable that the absence of red pigment in *Eniclases* is genetically constrained. Earlier studies have shown that easily remembered, high-contrast patterns, *e.g.*, a red-black combination, provide higher protection than lower contrast ones (*e.g.*, Arenas et al., 2014; Raska, Stys & Exnerova, 2017; Roper & Redston, 1987). Relative to other net-winged beetles in the same communities, I consider color patterns in *Eniclases* as low contrast, and their effectiveness is likely further reduced by relatively high intraspecific variability, given that 5 of 12 species from Central New Guinea are polymorphic (Figs. 4, S1–S4).

Aposematic color patterns are controlled by selection (Mallet & Barton, 1989; Sherratt, 2008) and their distinctness depends on the ability of predators to discriminate among them. In contrast to the observed high signal variability, *i.e.*, a putative result of ineffective purifying selection, I recorded a clearly distinct, fine-tuned warning signal in *E. divaricatus* and its co-mimics (*E. similis*, *Trichalus* sp., and *Cautiromimus* sp.) represented by characteristic bright-colored humeral patches. The specificity of brightly colored humeral patches versus whole humeri color indicates that predators could negatively select intermediates (Aronsson & Gamberale-Stille 2008). Strong pattern similarity and an almost complete absence of transitional forms indicates effective purifying selection for some species considered here (Rowe, Lindström & Lyytinen, 2004). This suggests that under certain conditions, both polymorphism and high pattern similarity can be produced within a multi-pattern community.

Behavior and signal perception

Given that trichalines commonly occur in multi-species and multi-pattern aggregations (Tab. 1; Figs. 1–2), predators encounter a spectrum of aposematic signals related to components such as body shape and colour (Gagliardo & Guilford, 1993; Skelhorn et al., 2016; O'Hanlon, 2014). Further complexity is added to aposematic signaling by different perception of the signal in space and time (Fig. 1; Kazemi, Gamberale-Stille, Tullberg & Leimar, 2014). Our observations suggest that the warning signal of *Eniclases* consists of two principal components, body shape and size, and the color-based signaling.

A body shape and size signal are predominantly perceived when an individual sits on the underside of a translucent leaf (Figs. 1A–H). This perception depends on light intensity, microhabitat conditions, and season (Arenas et al., 2014; Endler, 1992). I observed that *Eniclases* prefer the undersides of leaves, as do other trichaline genera. I assume that convergent evolution has led to the observed morphological uniformity in body shape and size of >100 species of trichalines in New Guinea (Figs. S1–S4, Tab. S4).

Color-based signals include hue, uniform or bicolored upper bodies, color patch shape, and the level of contrast between body parts (Figs. 1, 2, 4). Color is best detected under diffuse-light conditions and when an individual is on upper leaf surfaces, but *Eniclases* and its co-mimics seldom rest on upper leaf surfaces, unlike brightly colored lycids such as *Porrostoma*, *Metriorrhynchus*, and *Cladophorus* (Kalousova & Bocak, 2017, field observation). When color pattern is not readily observable, selection for a shared color pattern is relaxed. Penny *et al*. (2012) showed the importance of relaxed selection in the origin of imperfect mimicry; I

suggest that it may also increase the persistence of a greater number of color patterns in a community (Fig. 4, Tab. 1).

Overall, I documented apparent convergence and/or advergence to shared color patterns, but I simultaneously recorded a relatively high number of distinct color types, indicating that purifying selection may be relaxed or slowed due to factors such as the generalization of multiple patterns by predators and evolutionary constraints such as the ability, or inability, to rapidly adopt the dominant local aposematic pattern (Edmunds, 2000; Holen & Johnstone, 2004; Johnstone, 2002; Kikuchi & Pfenning, 2013; Motyka et al., 2018). Based on field observations of *Eniclases*, I suggest that behavior and light conditions play a major role in signal efficacy. If signal efficacy is dynamic, *e.g*., dependent on time of day or weather, then selection would likely act to produce optimal protection under various conditions (Endler, 1992). In addition, it remains unclear if *Eniclases* cannot produce red, blue, or metallic coloration due to genetic constraints or if the lack of such pigments is a product of relaxed selection for a color signal.

Eniclases communities in space and time

I must assume that the communities in which *Eniclases* occur are dynamic due to stochastic fluctuations in the relative abundance of various species, climatic conditions, and migration among local ecosystems and across the altitudinal gradient (Tab. 1). While I found that similar communities can be expected at similar altitudes in New Guinea, I observed a distinct community composition in the Dombomi locality, which does not contain brightly colored trichalines despite being located at a low elevation. Dombomi is situated on the windward slope of a high mountain range and differs from Elelim in having high levels of precipitation and fog, which

supports dense, marshy, medium-height forests. I identified altitudinal mobility in *Eniclases*, specifically, a very recent altitudinal shift in clade C, which consists of four lowland species and the recently split *E. bokondinensis* as the only high-mountain species in the clade. However, despite a distance of less than 2 kilometers, Bokondini communities sampled from 1250 and 1850 m in elevation shared no common species (richness of 26 and 58 species, respectively). Although net-winged beetles are poor dispersers, even low levels of continuous migration could increase the number of aposematic patterns in a community, delay pattern convergence, and increase the number of color-polymorphic species simultaneously adverging to different models (Motyka et al., 2018). Further research should consider that the communities I analyze today may be products of very different histories, particularly concerning migration.

Speciation and mimicry pattern shifts

Most identified sister species of *Eniclases* showed different coloration (Fig. 4). Therefore, I suppose that further diversity of sympatrically occurring aposematic patterns generated by speciation will be related to the adoption of a different aposematic pattern. The development of reproductive isolation via a switch in mimetic patterns has often been observed in aposematically colored insects, including net-winged beetles (Bocak & Yagi, 2010; Jiruskova et al., 2019; Motyka et al., 2018). The adoption of an autochthonous aposematic signal is potentially rapid, and here is best demonstrated by the unlinked phenotypic and genetic divergence between closely related *E. niger* and *E. similis* and the color shift in *E. bokondinensis* within clade C (Figs. 3–5). In the early phases of speciation, highly unequal numbers of allochthonous and autochthonous prey occurring in a single community should

result in strong purifying selection (Bocak & Yagi, 2010; Mallet & Barton, 1989), but multi-pattern communities and temporal and local variability in community structure imply dynamic conditions and therefore complexities to the speciation process. Further, high introgression can support the transfer of genes which are responsible for the adoption of various aposematic patterns as in *Heliconius* (Enciso-Romero et al., 2017; Ray et al., 2019).

Conclusion

Broadly, the speciation process in *Eniclases* combines altitudinal shifts, evolution of mimetic patterns, neutral distance-based molecular evolution, and selection for high phenotypic similarity. Our combined approach of morphology, and mtDNA-, and RAD-based phylogenies, along with analyses of population genetic structure, provides evidence for unlinked genetic and phenotypic differentiation and complex speciation with high levels of introgression in the early phases of lineage divergence in *Eniclases* (Enciso-Romero et al., 2017). As a result, the delineation of some species is ambiguous and multiple data sources are necessary to reach clear conclusions.

I considered the diversity of aposematic signals, polymorphism, and coexistence of various patterns within a single communities or aggregations of unprofitable net-winged beetles. *Eniclases* species are similar in body size and shape and are apparently simultaneously selected for color patterns shared by unrelated species. I suggest that multiple color patterns in *Eniclases* and other net-winged beetles persist in single communities due to the presence of species that are not able to readily adopt the dominant color pattern (*i.e*., the absence of red pigment in all trichalines; Motyka et al., 2018) and behavior-dependent relaxed selection, whereby

beetles are perceived differently by predators in time and space. Further, I suggest the likelihood of limited, but continuous migration of individuals from populations with different species compositions and pattern frequencies. This hypothesis is based on observed high species turnover between communities and the presence of a large number of species and patterns. As a result of such migration, populations or species displaying low-contrast patterns and intraspecific polymorphism would not be effectively removed from large complex mimetic communities by purifying selection (Aubier & Sherratt, 2015). I cannot conclude if purifying selection is merely relaxed or is inherently unable to exclude differently colored individuals from complex communities such as those described here, and additional open questions include the possibility of multiple predators and their potentially varied effects on unprofitable species (Cuthill et al., 2017; Fabricant & Herberstein, 2015), the evolutionary history of individual communities, and migration frequency between communities. Mimicry within net-winged beetles is rarely studied, and here I show their potential as an ecologically interesting and highly diverse mimetic lineage.

Acknowledgements

I thank to colleagues who provided some samples for this study and I am also grateful to R. Bilkova for technical assistance. This study was supported by GACR project (18-14942S).

References

Alatalo, R. V., & Mappes, J. (1996). Tracking the evolution of warning signals. *Nature*, *382*, 708–710. doi: 10.1038/382708a0

Aronsson, M., & Gamberale-Stille, G. (2008). Domestic chicks primarily attend to colour, not pattern, when learning an aposematic coloration. *Animal Behaviour*, *75*, 417–423. doi: 10.1016/j.anbehav.2007.05.006

Aronsson, M., & Gamberale-Stille, G. (2009). Importance of internal pattern contrast and contrast against the background in aposematic signals. *Behavioral Ecology*, *20*, 1356–1362. doi: 10.1093/beheco/arp141

Aubier, T. G., & Sherratt, T. N. (2015). Diversity in Müllerian mimicry: the optimal predator sampling strategy explains both local and regional polymorphism in prey. *Evolution*, *69*, 2831–2845. doi: 10.1111/evo.12790

Arenas, L. M., Troscianko, J., & Stevens, M. (2014). Color contrast and stability as key elements for effective warning signals. *Frontiers in Ecology and Evolution*, *2*, 25. doi: 10.3389/fevo.2014.00025

Beatty, C. D., Beirinckx, K., & Sherratt, T. N. (2004). The evolution of Müllerian mimicry in multispecies communities. *Nature*, *431*, 63–67. doi: 10.1038/nature02818.

Bocak, L. (2002). Revision and phylogenetic analysis of Metriorrhynchinae. *European Journal of Entomology*, *99*, 315–351. doi: 10.14411/eje.2002.043

Bocak, L., & Bocakova, M. (1991). Revision of the genus *Eniclases* Waterhouse, 1879 (Coleoptera, Lycidae, Metriorrhynchinae) *Mitteilungen der Münchener Entomologischen Gesselschaft*, *81*, 203–226.

Bocak, L., & Yagi, T. (2010). Evolution of mimicry patterns in *Metriorrhynchus* (Coleoptera: Lycidae): The history of dispersal and speciation in Southeast Asia. *Evolution*, *64*, 39–52. doi: 10.1111/j.1558-5646.2009.00812.x

Bocek, M., & Bocak, L. (2016). Species limits in polymorphic mimetic *Eniclases* net-winged beetles from New Guinean mountains (Coleoptera: Lycidae). *ZooKeys*, *593*, 15–35. doi: 10.3897/zookeys.593.7728

Bocek, M. & Bocak, L. (2017). The comparison of molecular and morphology-based phylogenies of trichaline net-winged beetles (Coleoptera: Lycidae: Metriorrhynchini) with description of a new subgenus. *PeerJ*, **5**, e3963.

Bocek, M., & Bocak, L. (2019). The origins and dispersal history of the trichaline net-winged beetles in South East Asia, Wallacea, New Guinea and Australia. *Zoological Journal of the Linnean Society*, *185*, 1079–1094. doi: 10.1093/zoolinnean/zly090.

Bray, T. C., & Bocak, L. (2016). Slowly dispersing neotenic beetles can speciate on a penny coin and generate space-limited diversity in the tropical mountains. *Scientific Reports*, *6*, 33579. doi: 10.1038/srep33579

Briolat, E. S., Burdfield-Steel, E. R., Paul, S. C., Ronka, K. H., Seymoure, B. M., Stankowich, T., & Stuckert, A. M. M. (2019). Diversity in warning coloration: selective paradox or the norm? *Biological Reviews*, *94*, 388–414. doi: 10.1111/brv.12460

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait J. H. (2011). Stacks: building and genotyping loci de novo from short-read sequences. *G3: Genes Genomes Genetics*, *1*, 171–182. doi: 10.1534/g3.111.000240

Chifman, J., & Kubatko, L. (2014). Quartet inference from SNP data under the coalescent model. *Bioinformatics*, *30*, 3317–3324. doi: 10.1093/bioinformatics/btu530

Chifman, J., & Kubatko, L. (2015). Identifiability of the unrooted species tree topology under the coalescent model with time-reversible substitution processes, site-specific rate variation, and invariable sites. *Journal of Theoretical Biology*, *374*, 35–47. doi: 10.1016/j.jtbi.2015.03.006

Chouteau, M., Arias, M., & Joron, M. (2016). Warning signals are under positive frequency-dependent selection in nature. *Proceedings of the National*

Academy of Sciences of the USA, *113*, 2164–2169. doi: 10.1073/pnas.1519216113

Chouteau, M., & Angers, B. (2012). Wright's shifting balance theory and the diversification of aposematic signals. *PLoS One*, *7*, e34028. doi: 10.1371/journal.pone.0034028

Coates, D. J., Byrne, M., & Moritz, C. (2018). Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. *Frontiers in Ecology and Evolution*, *6*, 165. doi: 10.3389/fevo.2018.00165.

Cracraft, J. (1983). Species concepts and speciation analysis. *Current Ornithology*, *1*, 159–187. doi: 10.1007/978-1-4615-6781-3_6

Cuthill, I. C., Allen, W. L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M. E., ... Caro, T. (2017). The biology of color. *Science*, *357*, 470–473. doi: 10.1126/science.aan0221

Drummond, A. J., Suchard, M. A., Xie, D., Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution 29*: 1969–1973. doi: 10.1093/molbev/mss075.

Dupuis, J. R., Roe, A. D., & Sperling, F. A. H. (2012). Multi-locus species delimitation in closely related animals and fungi: one marker is not enough. *Molecular Ecology*, *21*, 4422–4436. doi: 10.1111/j.1365-294X.2012.05642

Eaton, D. A. R. (2014). PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics*, *30*, 1844–1849. doi: 10.1093/bioinformatics/btu121

Eaton, D. A. R. & Overcast, I. (2016). iPYRAD: interactive assembly and analysis of RADseq data sets. Retrieved from https://ipyrad.readthedocs.io/

Edmunds, M. (2000). Why there are good and poor mimics? *Biological Journal of the Linnean Society*, *70*, 459–466. doi: 10.1111/j.1095-8312.2000.tb01234.x

Eisner, T., Kafatos, F. C., & Linsley, E. G. (1962). Lycid predation by mimetic adult Cerambycidae (Coleoptera). *Evolution*, *16*, 316–324. doi: 10.2307/2406280

Eisner, T., Schroeder, F. C., Snyder, N., Grant, J. B., Aneshansley, D. J., Utterback, D., … Eisner, M. (2008). Defensive chemistry of lycid beetles and of mimetic cerambycid beetles that feed on them. *Chemoecology*, *18*, 109–119. doi: 10.1007/s00049-007-0398-4

Ekblom, R., & Galindo, J. (2011). Applications of next generation sequencing in molecular ecology of non-model organisms. *Heredity*, *107*, 1–15. doi: 10.1038/hdy.2010.152

Elias, M., Hill, R. I., Willmott, K. R., Dasmahapatra, K. K., Brower, A. V. Z., Mallet, J., & Jiggins, C. D. (2007). Limited performance of DNA barcoding in a diverse community of tropical butterflies. *Proceedings of the Royal Society – Biological Sciences*, *274*, 2881–2889. doi: 10.1098/rspb.2007.1035

Enciso-Romero, J., Pardo-Diaz, C., Martin, S. H., Arias, C. F., Linares, M., McMillan, W. O., ... Salazar, C. (2017). Evolution of novel mimicry rings facilitated by adaptive introgression in tropical butterflies. *Molecular Ecology*, *26*, 5160–5172. doi: 10.1111/mec.14277

Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *American Naturalist*, *139*, S125–S153. doi: 10.1086/285308

Fabricant, S. A., & Herberstein, M. E. (2015). Hidden in plain orange: aposematic coloration is cryptic to a colourblind insect predator. *Behavioral Ecology*, *26*, 38–44. doi: 10.1093/beheco/aru157

Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution, 6*, 925–929. doi: 10.1111/2041-210X.12382

Gagliardo, A., & Guilford, T. (1993). Why do warning-colored prey live gregariously. *Proceedings of the Royal Society – Biological Sciences, 251*, 69–74. doi: 10.1098/rspb.1993.0010

Gompert, Z., Willmott, K. R., & Elias, M. (2011). Heterogeneity in predator micro-habitat use and the maintenance of Müllerian mimetic diversity. *Journal of Theoretical Biology*, *281*, 39–46. doi: 10.1016/j.jtbi.2011.04.024

Holen, Ø. H., & Johnstone, R. A. (2004). The evolution of mimicry under constraints. *American Naturalist*, *164*, 598–613. doi: 10.1086/424972

Huang, H., & Knowles, L. L. (2016). Unforeseen consequences of excluding missing data from next-generation sequences: simulation study of RAD sequences. *Systematic Biology*, *65*, 357–65. doi: 10.1093/sysbio/syu046

Jiruskova, A., Motyka, M., Bocek, M., & Bocak, L. (2019). The Malacca Strait separates distinct faunas of poorly-flying *Cautires* net-winged beetles. *PeerJ*, *7*, e6511. doi: 10.7717/peerj.6511

Johnstone, R. A. (2002). The evolution of inaccurate mimics. *Nature, 418*, 524–526. doi: 10.1038/nature00845

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., Haeseler, A. Von, & Jermiin, L. S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, *14*, 587–589. doi: 10.1038/nmeth.4285

Kalousova, R., & Bocak, L. (2017). Species delimitation of colour polymorphic *Cladophorus* (Coleoptera: Lycidae) from New Guinea. *Zootaxa*, *4320*, 505–522. doi: 10.11646/zootaxa.4320.3.6

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. *Molecular Biology and Evolution, 30*, 772–780. doi: 10.1093/molbev/mst010

Kazemi, B., Gamberale-Stille, G., Tullberg, B. S., & Leimar, O. (2014). Stimulus salience as an explanation for imperfect mimicry. *Current Biology*, *24*, 965–969. doi: 10.1016/j.cub.2014.02.061

Kikuchi, D. W., & Pfennig, D. W. (2013). Imperfect mimicry and the limits of natural selection. *Quarterly Review of Biology*, *88*, 297–315. doi: 10.1086/673758

Kleine, R. (1926). Coleoptera, Lycidae. *Nova Guinea. Résultats des expéditions scientifiques à la Nouvelle Guinée*, *15*, 91–195.

Kusy, D., Motyka, M., Bocek, M., Masek, M., & Bocak, L. (2019). Phylogenomic analysis resolves the relationships among net-winged beetles (Coleoptera: Lycidae) and reveals the parallel evolution of morphological traits. *Systematic Entomology* (in press). doi: 10.1111/syen.12363

Lam, A., Toussaint, E. F. A., Kindler, C., Van Dam, M. H., Panjaitan, R., Roderick, G. K., & Balke, M. (2018). Stream flow alone does not predict population structure of diving beetles across complex tropical landscapes. *Molecular Ecology*, *27*, 3541–3554. doi: 10.1111/mec.14807

Lavretsky, P., Da Costa, J. M., Sorenson, M.D., McCracken, K. G., & Peters, J. L. (2019). ddRAD-seq data reveal significant genome-wide population structure and divergent genomic regions that distinguish the mallard and close relatives in North America. *Molecular Ecology*, in press. doi: 10.1111/mec.15091

Leache, A. D., & Oaks, J. R. (2017). The utility of single nucleotide Polymorphism (SNP) data in phylogenetics. *Annual Reviews of Ecology and Systematics*, *48*, 69–84. doi: 10.1146/annurev-ecolsys-110316-022645

Linsley, E. G., Eisner, T., & Klots, A. B. (1961). Mimetic assemblages of sibling species of lycid beetles. *Evolution*, *15*, 15–29. doi: 10.2307/2405840.

Mallet, J. (1995). A species definition for the modern synthesis. *Trends in Ecology and Evolution*, *10*, 294–299. doi: 10.1016/0169-5347(95)90031-4

Mallet, J. (1999). Causes and consequences of a lack of coevolution in Müllerian mimicry. *Evolutionary Ecology*, *13*, 777–806. doi: 10.1023/A:1011060330515

Mallet, J., & Barton, N. H. (1989). Strong natural selection in a warning colour hybrid zone. *Evolution*, *43*, 421–431. doi: 10.1111/j.1558-5646.1989.tb04237.x

Mallet, J., & Joron, M. (1999). Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance and speciation. *Annual Reviews of Ecology and Systematics*, *30*, 201–233. doi: 10.1146/annurev.ecolsys.30.1.201

Mappes, J., Marples, N., & Endler, J. A. (2005). The complex business of survival by aposematism. *Trends in Ecology and Evolution*, *20*, 598–603. doi: 10.1016/j.tree.2005.07.011

Mayr, E. (1942). *Systematics and the origin of species*. Columbia University Press, New York.

McCornack, B. P., Koch, R. L., & Ragsdale, D. W. (2007). A simple method for in-field sex determination of the multicolored Asian lady beetle *Harmonia axyridis*. *Journal of Insect Science*, *7*, 1–12. doi: 10.1673/031.007.1001

Moore, B. P., & Brown, W. V. (1981). Identification of warning odor components, bitter principles and antifeedants in an aposematic beetle - *Metriorrhynchus rhipidius* (Coleoptera: Lycidae). *Insect Biochemistry*, *11*, 493–499. doi: 10.1016/0020-1790(81)90016-0

Motyka, M., Kampova, L., & Bocak, L. (2018). Phylogeny and evolution of Müllerian mimicry in aposematic *Dilophotes*: evidence for advergence and size-constraints in evolution of mimetic sexual dimorphism. *Scientific Reports*, *8*, 3744. doi: 10.1038/s41598-018-22155-6

Michie, L. J., Mallard, F., Majerus, M. E. N., & Jiggins, F. M. (2010). Melanic through nature or nurture: genetic polymorphism and phenotypic plasticity in *Harmonia axyridis*. *Journal of Evolutionary Biology*, *23*, 1699–1707. doi: 10.1111/j.1420-9101.2010.02043

Müller, F. (1879). *Ituna* and *Thyridia*: a remarkable case of mimicry in butterflies. *Proceedings of the Entomological Society London*, *1879*, xx–xxiv.

Nadeau, N. (2014). Butterfly genomics sheds light on the process of hybrid speciation. *Molecular Ecology*, *23*, 4441–4443. doi: 10.1111/mec.12877

Nater, A., Mattle-Greminger, M. P., Nurcahyo, A., Nowak, M.G., de Manuel, M., Desai T., … Krutzen, M. (2017). Morphometric, behavioral, and genomic evidence for a new orangutan species. *Current Biology*, *27*, 3487–3498. doi: 10.1016/j.cub.2017.09.047

Nguyen, L. T., Schmidt, H. A., Haeseler, A. Von, & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, *32*, 268–274. doi: 10.1111/j.1420-9101.2010.02043

O'Hanlon, J. C. (2014). The roles of color and shape in pollinator deception in the orchid mantis *Hymenopus coronatus*. *Ethology*, *120*, 652–661. doi: 10.1111/eth.12238

Papadopoulou, A., Anastasiou, I., & Vogler, A. P. (2010). Revisiting the insect mitochondrial molecular clock: the Mid-Aegean trench calibration. *Molecular Biology and Evolution*, *27*, 1659–1672. doi: 10.1093/molbev/msq051

Penney, H. D., Hassall, C., Skevington, J. H., Abbott, K. R., & Sherratt, T. N. (2012). A comparative analysis of the evolution of imperfect mimicry. *Nature*, *483*, 461–464. doi: 10.1038/nature10961

Poulton, E. B. (1890). *The colours of animals: their meaning and use. Especially considered in the case of insects*. Kegan Paul, Trench, Tubner and Co., London.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*, 901–904. doi: 10.1093/sysbio/syy032

Raska, J., Stys, P., & Exnerova, A. (2017). How variation in prey aposematic signals affects avoidance learning, generalization and memory of a salticid spider. *Animal Behaviour*, *130*, 107–117. doi: 10.1016/j.anbehav.2017.06.012

Ray, D. A. J., Grimshaw, J. R., Halsey M. K., Korstian J. M., Osmanski AB, … Mallet J. (2019). Analysis of 19 heliconiine butterflies shows rapid TE-based diversification and multiple SINE births and deaths. *BioRxiv*. doi: 10.1101/596502

Reaz, R., Bayzid, M. S., & Rahman, M. S. (2014). Accurate phylogenetic tree reconstruction from quartets: a heuristic approach. *PLoS One*, *9*, e104008. doi: 10.1371/journal.pone.0104008

Roper, T. J., & Redston, S. (1987). Conspicuousness of distasteful prey affects the strength and durability of one-trial avoidance-learning. *Animal Behaviour*, *35*, 739–747. doi: 10.1016/S0003-3472(87)80110-0

Rosser, N., Freitas, A. V. L., Huertas, B., Joron, M., Lamas, G., Mérot, C., … Dasmahapatra, K. K. (2019). Cryptic speciation associated with geographic and ecological divergence in two Amazonian *Heliconius* butterflies. *Zoological Journal of the Linnean Society*, *186*, 233–249. doi: 10.1093/zoolinnean/zly046

Rowe, C., Lindström, L., & Lyytinen, A. (2004). The importance of pattern similarity between Müllerian mimics in predator avoidance learning. *Proceedings of the Royal Society – Biological Sciences*, *271*, 407–413. doi: 10.1098/rspb.2003.2615

Sherratt, T. N. (2008). The evolution of Müllerian mimicry. *Naturwissenschaften*, *95*, 681–695. doi: 10.1007/s00114-008-0403-y

Sherratt, T. N., & Peet-Paré, C. A. (2017). The perfection of mimicry: an information approach. *Philosophical Transactions of the Royal Society – Biological Sciences*, *372*, 20160340. doi: 10.1098/rstb.2016.0340

Skelhorn, J., Holmes, G. G., Hossie, T. J., & Sherratt, T. N. (2016). Multicomponent deceptive signals reduce the speed at which predators learn that prey are profitable. *Behavioral Ecology*, *27*, 141–147. doi: 10.1093/beheco/arv135

Speed, M. P. (1993). Müllerian mimicry and the psychology of predation. *Animal Behaviour*, *45*, 571–580. doi: 10.1006/anbe.1993.1067

Speed, M. P., & Ruxton, G. D. (2004). Imperfect Batesian mimicry and the conspicuousness costs of mimetic resemblance. *American Naturalist*, *176*, E1–E14. doi: 10.1086/652990

Swofford, D. L. (2002). PAUP* *Phylogenetic Analysis using Parsimony (and Other Methods)*. Sinauer Associates, Sunderland, MA.

Stuckert, A. M. M., Venegas, P. J., & Summers, K. (2014). Experimental evidence for predator learning and Müllerian mimicry in Peruvian poison frogs (Ranitomeya, Dendrobatidae). *Evolutionary Ecology*, *28*, 413–426. doi: 10.1007/s10682-013-9685-4

Sklenarova, K., Chesters, D., & Bocak, L. (2013). Phylogeography of poorly dispersing net-winged beetles: A role of drifting India in the origin of Afrotropical and Oriental fauna. *PLoS One*, *8*, e67957. doi: 10.1371/journal.pone.0067957

Sklenarova, K., Kubecek, V., & Bocak, L. (2014). Subtribal classification of Metriorrhynchini (Insecta: Coleoptera: Lycidae): an integrative approach using molecular phylogeny and morphology of adults and larvae. *Arthropod Systematics & Phylogeny*, *72*, 37–54. doi: 10.1371/journal.pone.0067957

Takahashi, T., Nagata, N., & Sota, T. (2014). Application of RAD-based phylogenetics to complex relationships among variously related taxa in a species flock. *Molecular Phylogenetics and Evolution*, *80*, 77–81. doi: 10.1016/j.ympev.2014.07.01

Willmott, K. R., Willmott, J. C. R., Elias, M., & Jiggins, C. D. (2017). Maintaining mimicry diversity: optimal warning color patterns differ among microhabitats in Amazonian clearwing butterflies. *Proceedings of the Royal Society – Biological Sciences*, *284*, 20170744. doi: 10.1098/rspb. 2017.0744

Wilson, J. S., Jahner, J. P., Williams, K. A., & Forister, M. L. (2013). Ecological and evolutionary processes drive the origin and maintenance of imperfect mimicry. *PLoS One*, *8*, e61610. doi: 10.1371/journal.pone.0061610

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*, 3326–332. doi: 10.1093/bioinformatics/bts606

Table 1. The characteristics of net-winged beetle alpha-diversity and the number of identified color patterns in each locality. n – number of individuals, non-trich. – the genera if Metriorrhynchina except the trichalines; trichaline genera (trichalines) – the genera *Diatrichalus*, *Flabellotrichalus*, *Eniclases*, *Microtrichalus*, *Trichalus*, and *Lobatang*. The numbers for *Eniclases*, are included always also under trichalines as a whole and then separately reported.

Locality, Altitude	AGG	Number of individuals (n)					Number of spp. (n)			# of patterns				
		Total	non-trich.	trichalines	%	*Enicl.*	%	Total	non-trich.	trich.	*Eniclases*	%	Total	*Enicl.*
Sentani 275 m	1	414	77	337	81.4	167	40.3	26	14	12	5	19.2	7	4
Sentani, 360 m	-	19	9	10	52.6	2	10.5	7	3	4	2	22.2	3	2
Elelim, 580 m	2	234	149	85	36.3	20	8.5	85	67	18	6	7.1	10	6
Dombomi (1150)	-	123	89	34	27.6	9	7.3	24	23	1	1	4.2	6	1
Bokondini (1250)	3	110	36	74	67.3	36	32.7	18	9	9	2	11.1	4	2
Bokondini (1287)	4	251	99	152	60.8	43	17.2	13	9	4	2	15.4	3	2
Bokondini (1800)	-	297	171	126	42.4	4	1.3	47	44	3	2	4.3	9	2
Bokondini (2100)	-	24	20	4	16.7	2	8.3	15	14	1	1	6.7	7	1
Tikapura (2170)	-	201	173	28	14.0	7	3.5	53	39	14	1	1.9	8	1
Yiwika (2100)	-	95	78	17	17.9	1	1.1	42	34	8	1	2.4	7	1
Napua (2300)	-	146	138	8	5.5	0	0.0	48	44	4	0	0.0	8	0

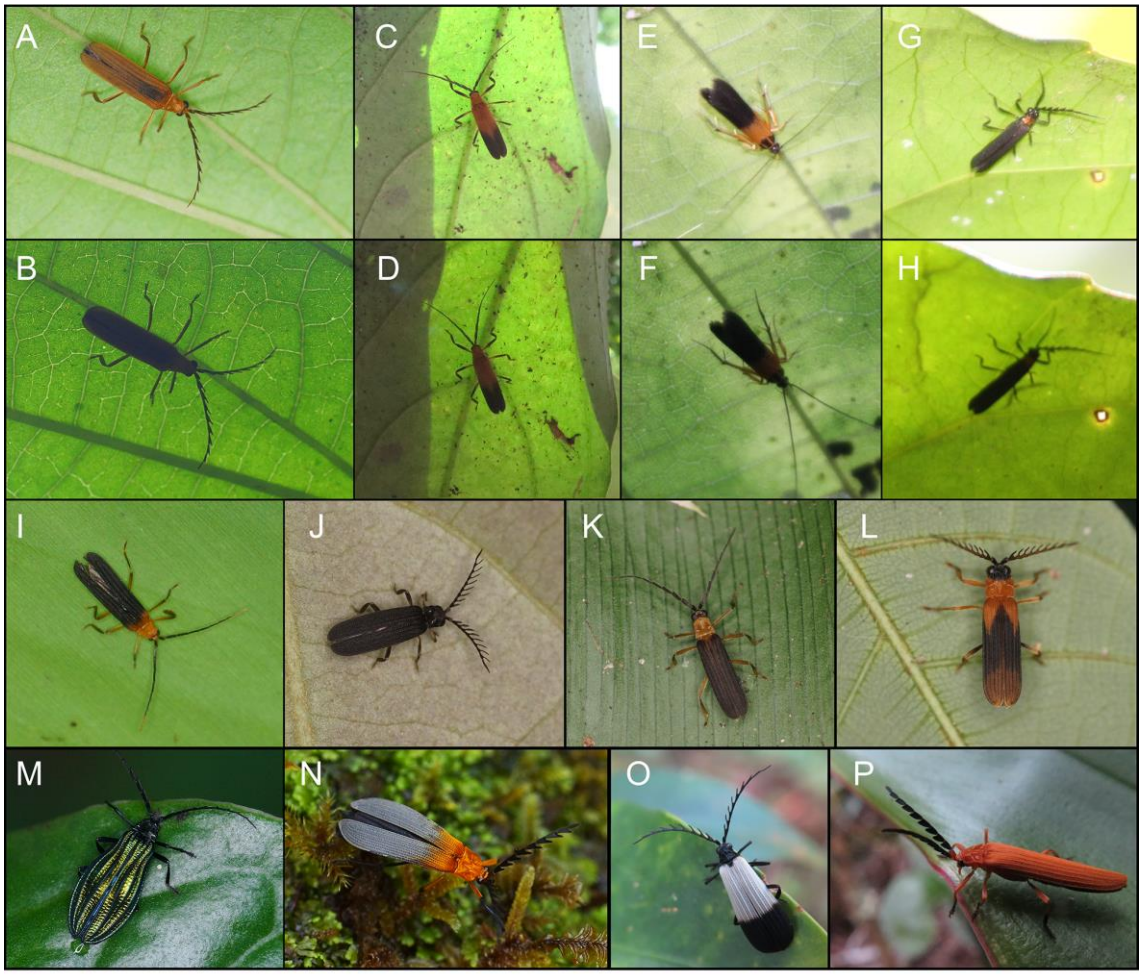


Figure 1. Trichalini and their co-mimics as observed in nature, A–H the examples of internal and external contrast of the signal of an individual depending on light conditions (the upper photo taken with flash); A–B *Eniclases* sp.; C–D *Microtrichalus* sp.; E–F unidentified moth; G–H *Metriorrhynchus* sp. Co-mimics of *Eniclases*: I, K *Microtrichalus* sp., J, L *Metriorrhynchus* sp. The representatives of further aposematic patterns recorded in the region: M *Diatrichalus aeneus* Bourgeois, N *Cladophorus* sp., O–P *Metriorrhynchus* sp.

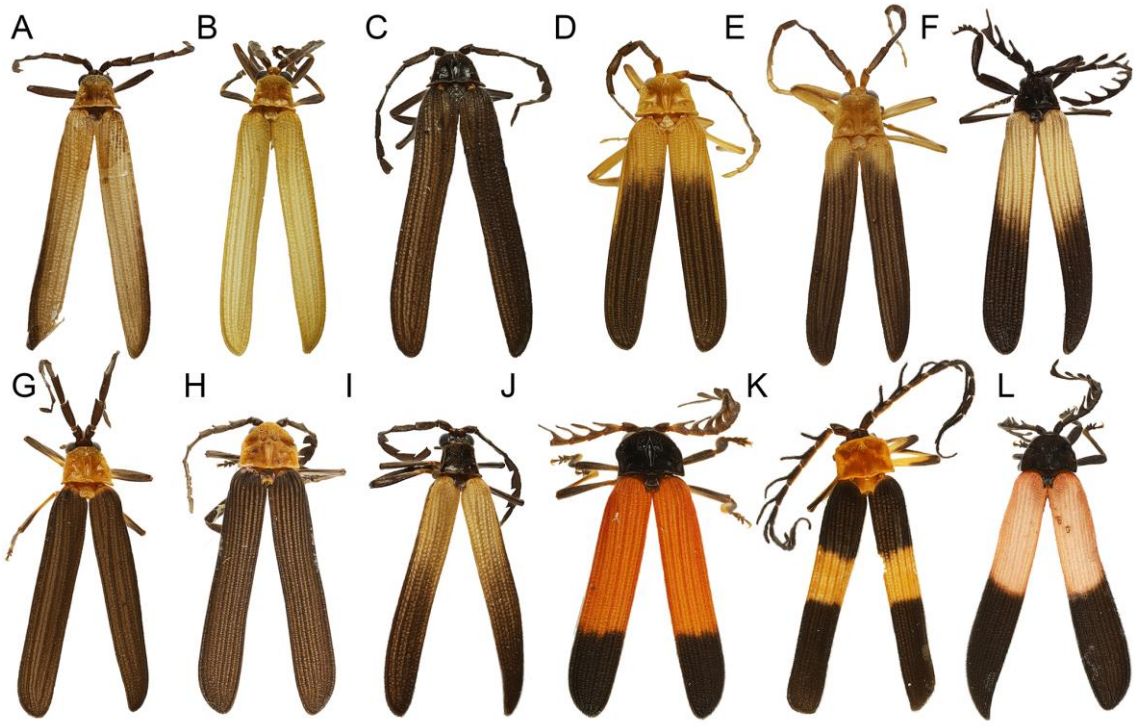


Figure 2. General appearance of trichaline co-mimics. A–H *Microtrichalus* spp. Other aposematically coloured Metriorrhynchina from New Guinea. I *Cautiromimus* sp., J. *Ditua* sp., K *Carathrix* sp., L *Metriorrhynchus* sp.

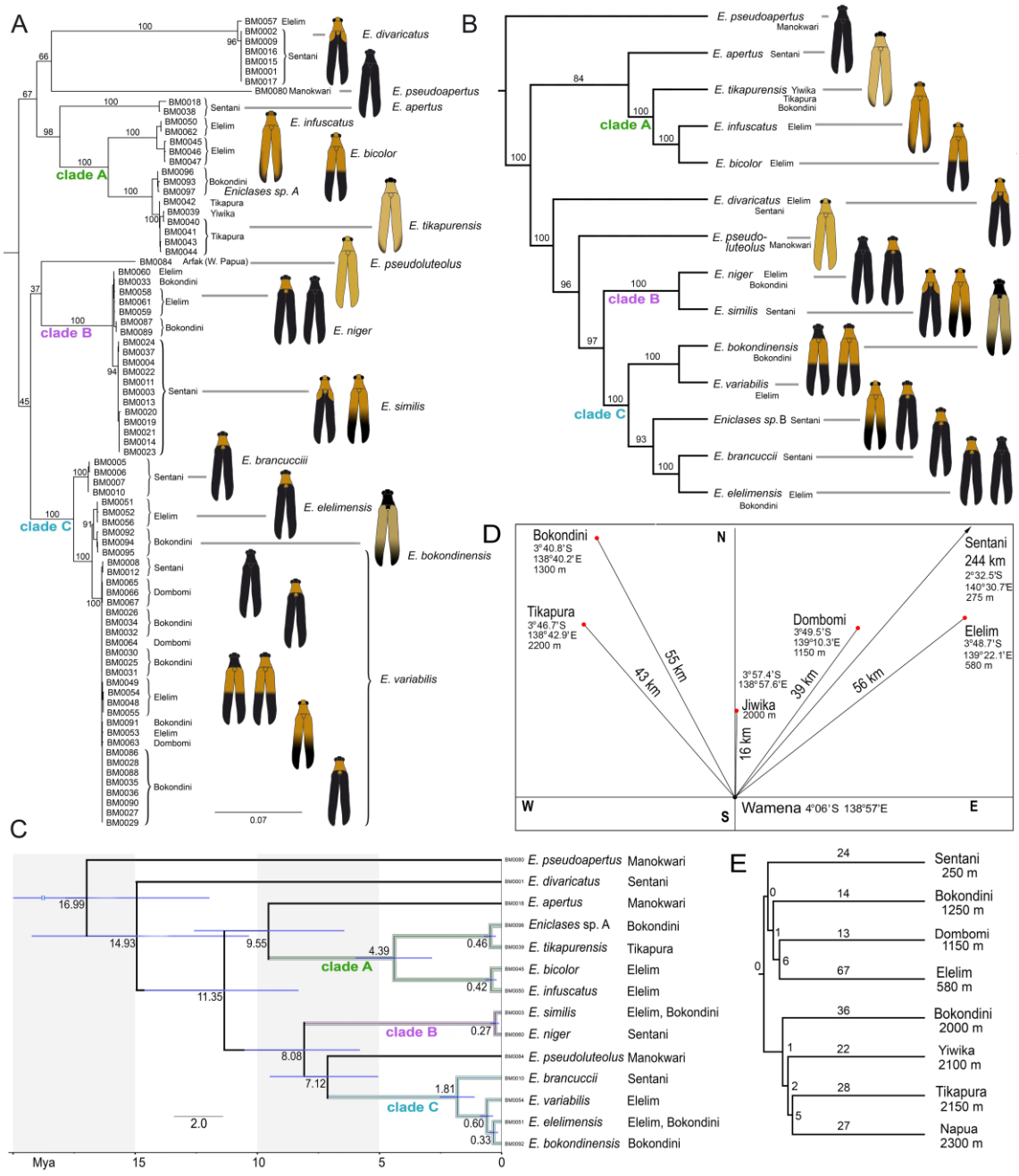


Figure 3. Phylogenetic relationships recovered by the analysis of the mtDNA dataset; B The phylogenetic relationships recovered by the quartet analysis of the RAD tree; C Dated phylogenetic tree based on mutation rates of three mitochondrial fragments; D–E Geographic positions of sampled localities.

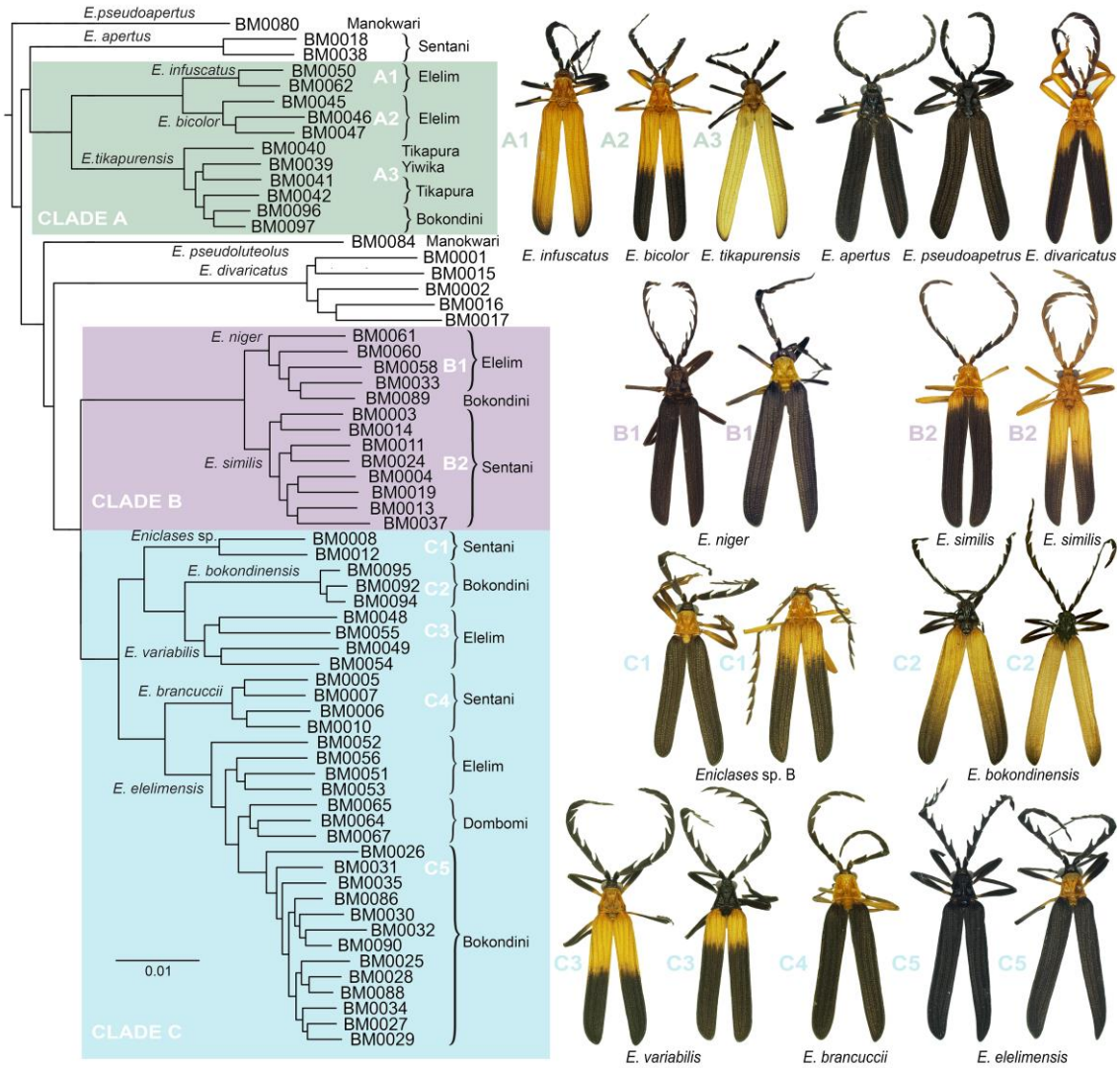


Figure 4. RAD-based maximum likelihood topology with characteristic aposematic patterns for each putative species. All individuals included in the analysis are shown in Tabs. S1–S4.

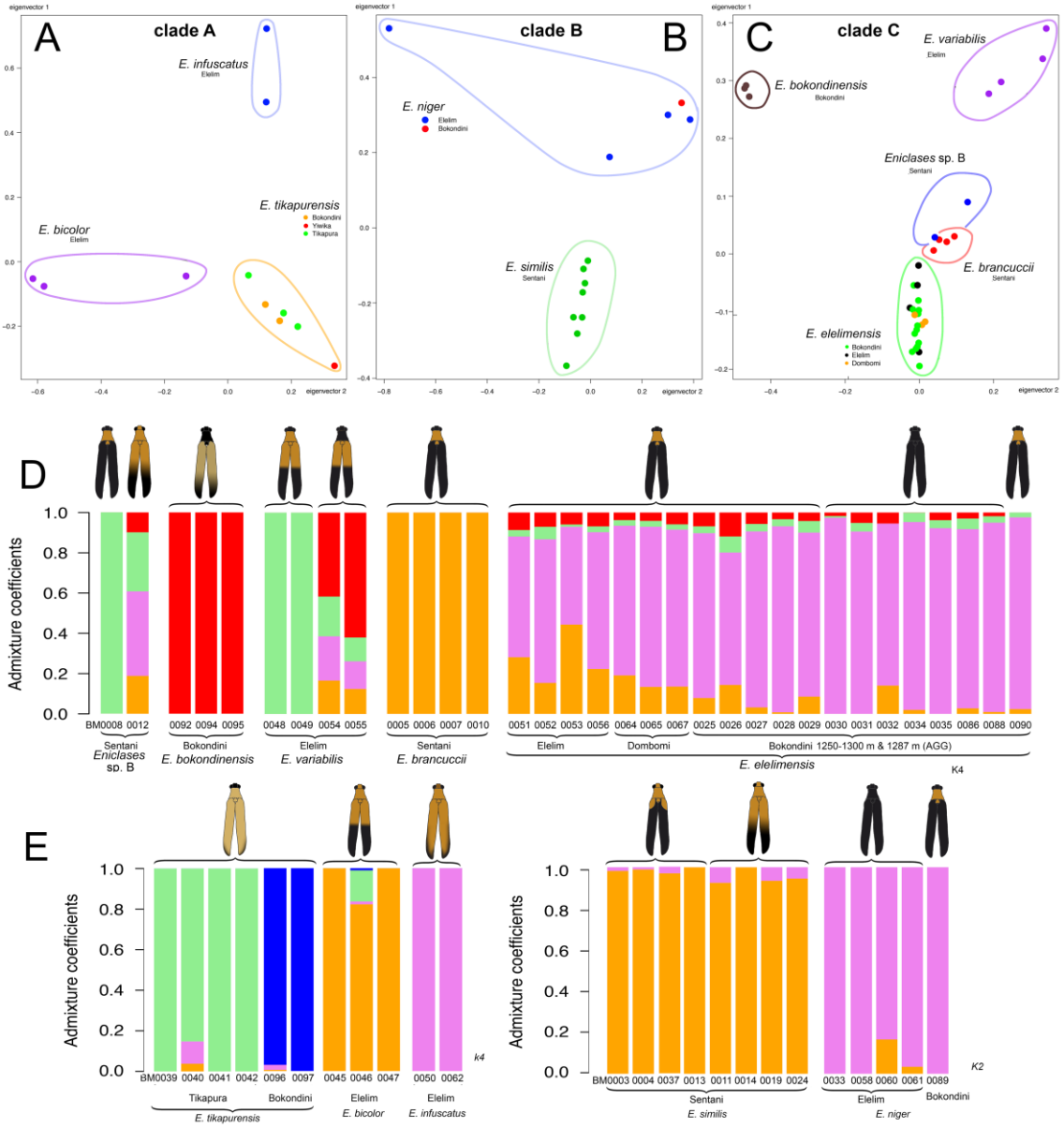


Figure 5. A-C Distribution of *Eniclases* individuals along principal component (PC) scores of genetic variation based on the analysis of the RAD dataset (PC1 for clades A, B and C 15%, 12.1% and 5.6%, respectively; PC2, 15%, 10.1% and 5.1%, respectively).
D-E Plots of inferreed individual‘s admixture coefficients based on sparse non-negative matrix factorization (sNMF) implemented in R package LEA for the clades of closely related *Eniclases* species as defined by phylogenetic analyses. Other K clusters are shown in Supplements along with entropy graphs.

Persistence of multiple patterns and intraspecific polymorphism in net-winged beetles due to variable signal perceptions and community structures

Matej Bocek

The list of supplementary material.

Supplementary Tables

Supplementary Figures

Supplementary Text

Supplementary dataset. The resulting tree topologies from all data matrices recovered from RAD data filtering. Following trees are provided in newick format each with unique settings.

Table S1. The list of species, their geographic origins, collecting circumstances and aposematic patterns localities.

Eniclases species identification Genomic		mtDNA and morph.	Voucher number	Locality (all New Guinea, Papua Province if not stated otherwise)	Aggregation	Colour pattern
E. pseudoapertus		*E. pseudoapertus*	BM0080	West Papua, Manokwari distr., Maibri vill., Arfak Mts., 1570 m	-	Fig. 7
E. pseudoluteolus		*E. pseudoluteolus*	BM0084	West Papua, Manokwari distr., Maibri vill., Arfak Mts., 1570 m	-	uniform yellow
E. divaricatus		*E. divaricatus*	BM0057	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m		similar to Fig. 6
E. divaricatus		*E. divaricatus*	BM0001	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 6
E. divaricatus		*E. divaricatus*	BM0009	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 6
E. divaricatus		*E. divaricatus*	BM0015	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 6
E. divaricatus		*E. divaricatus*	BM0017	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	no photo
E. divaricatus		*E. divaricatus*	BM0016	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	Fig. 6
E. divaricatus		*E. divaricatus*	BM0002	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 6
E. apertus		*E. apertus*	BM0038	Sentani, S slope Cyclop Mts., 02°32.320´S, 140°30.738´E, 360 m	-	Fig. 8
E. apertus		*E. apertus*	BM0018	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 8
Clade A						
E. niger	P	*E. niger*	BM0089	Bokondini (AGG), 03°40.76´S, 138°40.15´E, 1287 m	AGG3	Fig. 15
E. niger	P	*E. niger*	BM0087	Bokondini (AGG), 03°40.76´S, 138°40.15´E, 1287 m	AGG3	intermediate, as Figs 14 and 15
E. niger	P	*E. niger*	BM0060	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	similar to Fig. 14
E. niger	P	*E. niger*	BM0059	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	Fig. 14
E. niger	P	*E. niger*	BM0061	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	similar to Fig. 14
E. niger	P	*E. niger*	BM0033	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	AGG4	similar to Fig. 14
E. niger	P	*E. niger*	BM0058	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	similar to Fig. 14
E. similis	P	*E. similis*	BM0024	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	Fig. 13
E. similis	P	*E. similis*	BM0037	Sentani, S slope Cyclop Mts., 02°32.320´S, 140°30.738´E, 360 m	-	similar to Fig. 13
E. similis	P	*E. similis*	BM0020	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	intermediate, as Figs 12 and 13
E. similis	P	*E. similis*	BM0019	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	intermediate, as Figs 12 and 13
E. similis	P	*E. similis*	BM0022	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	intermediate, as Figs 12 and 13
E. similis	P	*E. similis*	BM0021	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 12
E. similis	P	*E. similis*	BM0023	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	intermediate, as Figs 12 and 13
E. similis	P	*E. similis*	BM0003	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	Fig. 12
E. similis	P	*E. similis*	BM0014	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	intermediate, as Figs 12 and 13
E. similis	P	*E. similis*	BM0013	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 12
E. similis	P	*E. similis*	BM0011	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 13
E. similis	P	*E. similis*	BM0004	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 12

Clade B					
E. infuscatus	*E. infuscatus*	BM0050	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	Fig. 11
E. infuscatus	*E. infuscatus*	BM0062	Elelim, km 6 rd to Apalapsili, 03°48.686´S, 139°21.764´E, 650 m	-	similar to Fig. 11
E. bicolor	*E. bicolor*	BM0045	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	similar to Fig. 10
E. bicolor	*E. bicolor*	BM0046	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	Fig. 10
E. bicolor	*E. bicolor*	BM0047	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	similar to Fig. 10
E. tikapurensis	✸*Eniclases* sp. A	BM0093	3 km N Bokondini, 03°39.741´S, 138°40.216´E, 1750-1900 m	-	similar to Fig. 9
E. tikapurensis	✸*Eniclases* sp. A	BM0097	3 km SW Bokondini, 03°42.51´S, 138°38.893´E, 2100 m	-	similar to Fig. 9
E. tikapurensis	✸*Eniclases* sp. A	BM0096	3 km SW Bokondini, 03°42.51´S, 138°38.893´E, 2100 m	-	similar to Fig. 9
E. tikapurensis	*E. tikapurensis*	BM0039	Yiwika, 16 km N Wamena, 03°56.883´S, 138°57.712´E, 2100 m	-	Fig. 9
E. tikapurensis	*E. tikapurensis*	BM0043	Tikapura (Rd Tagime-Kelila), 03°46.797'S, 138°42.933´E, 2170 m	-	similar to Fig. 9
E. tikapurensis	*E. tikapurensis*	BM0040	Tikapura (Rd Tagime-Kelila), 03°46.797'S, 138°42.933´E, 2170 m	-	similar to Fig. 9
E. tikapurensis	*E. tikapurensis*	BM0044	Tikapura (Rd Tagime-Kelila), 03°46.797'S, 138°42.933´E, 2170 m	-	similar to Fig. 9
E. tikapurensis	*E. tikapurensis*	BM0042	Tikapura (Rd Tagime-Kelila), 03°46.797'S, 138°42.933´E, 2170 m	-	similar to Fig. 9
E. tikapurensis	*E. tikapurensis*	BM0041	Tikapura (Rd Tagime-Kelila), 03°46.797'S, 138°42.933´E, 2170 m	-	similar to Fig. 9

Clade C					
Eniclases sp. B P	✸*E. variabilis*	BM0012	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	Fig. 26
Eniclases sp. B P	✸*E. variabilis*	BM0008	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	Fig. 25
E.brancuccii	*E.brancuccii*	BM0006	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 16
E.brancuccii	*E.brancuccii*	BM0005	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	Fig. 16
E.brancuccii	*E.brancuccii*	BM0010	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 16
E.brancuccii	*E.brancuccii*	BM0007	Sentani, S slope of Cyclop Mts., 02°32.487´S, 140°30.683´E, 275 m	AGG1	similar to Fig. 16
E. bokondinensis	*E. bokondinensis*	BM0092	3 km N Bokondini, 03°39.741´S, 138°40.216´E, 1750-1900 m	-	Fig. 18
E. bokondinensis	*E. bokondinensis*	BM0094	3 km N Bokondini, 03°39.741´S, 138°40.216´E, 1750-1900 m	-	no photo
E. bokondinensis	*E. bokondinensis*	BM0095	3 km N Bokondini, 03°39.741´S, 138°40.216´E, 1750-1900 m	-	Fig. 17
E. variabilis P	*E. variabilis*	BM0054	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	Fig. 20
E. variabilis P	*E. variabilis*	BM0055	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	Fig. 21
E. variabilis P	*E. variabilis*	BM0048	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	Fig. 23
E. variabilis P	*E. variabilis*	BM0049	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	Fig. 22
E. elelimensis P	*E. elelimensis*	BM0052	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	similar to Fig. 19
E. elelimensis P	*E. elelimensis*	BM0051	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	similar to Fig. 19
E. elelimensis P	*E. elelimensis*	BM0056	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	Fig. 19
E. elelimensis P	✸*E. variabilis*	BM0053	Elelim, km 5 rd to Apalapsili, 03°48.700´S, 139°22.088´E, 580 m	AGG2	Fig. 24

E. elelimensis P	✹*E. variabilis*	BM0035	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	-	Fig. 27
E. elelimensis P	✹*E. variabilis*	BM0029	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	AGG4	similar to Fig. 28
E. elelimensis P	✹*E. variabilis*	BM0028	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	AGG4	similar to Fig. 28
E. elelimensis P	✹*E. variabilis*	BM0027	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	AGG4	similar to Fig. 28
E. elelimensis P	✹*E. variabilis*	BM0091	Bokondini (AGG), 03°40.76´S, 138°40.15´E, 1287 m	AGG3	similar to Fig. 25
E. elelimensis P	✹*E. variabilis*	BM0090	Bokondini (AGG), 03°40.76´S, 138°40.15´E, 1287 m	AGG3	no photo
E. elelimensis P	✹*E. variabilis*	BM0088	Bokondini (AGG), 03°40.76´S, 138°40.15´E, 1287 m	AGG3	similar to Fig. 27
E. elelimensis P	✹*E. variabilis*	BM0086	Bokondini (AGG), 03°40.76´S, 138°40.15´E, 1287 m	AGG3	no photo
E. elelimensis P	✹*E. variabilis*	BM0036	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	AGG4	similar to Fig. 27
E. elelimensis P	✹*E. variabilis*	BM0063	Dombomi, Lower Pass vall., 03°49.477´S, 139°10.251´E, 1150 m	-	similar to Fig. 29
E. elelimensis P	✹*E. variabilis*	BM0065	Dombomi, Lower Pass vall., 03°49.477´S, 139°10.251´E, 1150 m		similar to Fig. 29
E. elelimensis P	✹*E. variabilis*	BM0066	Dombomi, Lower Pass vall., 03°49.477´S, 139°10.251´E, 1150 m		similar to Fig. 2
E. elelimensis P	✹*E. variabilis*	BM0067	Dombomi, Lower Pass vall., 03°49.477´S, 139°10.251´E, 1150 m		similar to Fig. 29
E. elelimensis P	✹*E. variabilis*	BM0064	Dombomi, Lower Pass vall., 03°49.477´S, 139°10.251´E, 1150 m		Fig. 29
E. elelimensis P	✹*E. variabilis*	BM0026	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	AGG4	similar to Fig. 28
E. elelimensis P	✹*E. variabilis*	BM0025	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	AGG4	Fig. 28
E. elelimensis P	✹*E. variabilis*	BM0030	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	AGG4	similar to Fig. 28
E. elelimensis P	✹*E. variabilis*	BM0031	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	AGG4	similar to Fig. 27
E. elelimensis P	✹*E. variabilis*	BM0034	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	AGG4	similar to Fig. 27
E. elelimensis P	✹*E. variabilis*	BM0032	Bokondini, 03°40.76´S, 138°40.15´E, 1250-1300 m	AGG4	similar to Fig. 27

✹ Individuals with incongruent species placement in mtDNA and RAD trees
P Individuals belonging to a polymorphic species based on the RAD phylogeny

Supplementary Table S2. The list of primers used for mtDNA amplification.

Fragment	Code	-mer	Sequence (5' >> 3')
rrnL	16a	20	CGC CTG TTT AAC AAA AAC AT
	ND1A	27	GGT CCC TTA CGA ATT TGA ATA TAT CCT
	ND1-2	24	ATC AAA AGG AGC TCG ATT AGT TTC
cox1			
	JerryN	23	CAA CAY YTA TTY TGA TTY TTY GG
	MarcyN	24	TTC RTA WGT TCA RTA TCA TTG RTG
	JerryM	23	CAA CAY YTA TTT TGR TTY TTT GG
	Marcy	27	TAR TTC RTA TGW RCA ATA YCA YTG RTG
	SPat	21	GCA CTA WTC TGC CAT ATT AGA
	SJerry	23	CAA CAT YTA TTY TGA TTY TTT GG
	Pat	25	TCC ATT GCA CTA ATC TGC CAT ATT A
	Jerry	23	CAA CAT TTA TTT TGA TTT TTT
	Marilyn	21	TCA TAA GTT CAG TAT CAT TG
nad5			
	OF1	29	CCT ACT CCT GTT TCT GCT TTA GTT CAT TC
	R6	29	GAA ACG AAA AAT CGT ATT TAA TTT CGA CT
	R2M	29	AAT TGA ASC CAA AAA GAG GTA TAT CAC TG

Supplementary Table S3. List of best-fit models per mtDNA and nextRAD partitions.

mtDNA dataset analysis

Name	Model	LogL	AIC	w-AIC	AICc	w-AICc	BIC	w-BIC
rrnL	TIM3+F+I	-1983.3195	4226.6390	+ 0.0000	4277.8571	+ 0.0000	4834.9869	+ 0.0000
cox1	TIM2+F+I+G4	-4175.5849	8617.1699	+ 0.0000	8654.0303	+ 0.0000	9282.6984	+ 0.0000
nad5	HKY+F+I+G4	-4303.4783	8860.9565	+ 0.0000	8891.0881	+ 0.0000	9508.1350	+ 0.0000

nextRAD dataset alignment analysis (Wclust = 0.85, MinCov = 4).

Input data: 66 sequences with 6066010 nucleotide sites
Number of constant sites: 5.58659 x 10^6 (= 92.0966 % of all sites)
Number of invariant (constant or ambiguous constant) sites: 5.58659 x 10^6 (= 92.0966 % of all sites)
Number of parsimony informative sites: 154300
Number of distinct site patterns: 942024

Model of substitution: GTR+F+I+G4

Rate parameter R:
A-C: 0.9833
A-G: 3.6524
A-T: 0.9157
C-G: 0.9335
C-T: 3.7609
G-T: 1.0000

	State frequencies	Rate matrix Q:			
A	pi(A) = 0.2936	-0.9256	0.1451	0.6	0.1806
C	pi(C) = 0.2047	0.208	-1.103	0.1533	0.7419
G	pi(G) = 0.2279	0.7727	0.1377	-1.108	0.1973
T	pi(T) = 0.2737	0.1937	0.5548	0.1643	-0.9128

Model of rate heterogeneity: Invar+Gamma with 4 categories
Proportion of invariable sites: 0.6185
Gamma shape alpha: 0.7601

Category	Relative_rate	Proportion
0	0	0.6185
1	0.2282	0.09538
2	1.024	0.09538
3	2.478	0.09538
4	6.755	0.09538

Relative rates are computed as MEAN of the portion of the Gamma distribution falling in the category.

Supplementary Table S2. Sequence alignment analysis of mtDNA dataset.

Fragment Name	Number of Sequences	Sites	Unique	Informative	Constant
rrnL	63	796	99	83	679
cox1	64	1101	227	249	807
nad5	62	1207	272	299	864

Supplementary Table S5. Measurements of *Eniclases* spp. (n.a. – not available; (b) - uniformly black colored species)

Species included in the current DNA analyses

Species	Body length	Width humeri	Pronotum length	Pronotum width	EDiam/Edist male
E. pseudoapertus	6.3	1.6	0.75	1.2	1.4 (b)
E. divaricatus	6.8–9.7	2.1–2.3	1.2–1.3	1.7–1.7	0.92–0.96
E. pseudoluteolus	9.3	2.3	1.15	1.6	0.9
E. apertus	5.7–8.4	1.34–1.7	0.9	1.25	1.15–1.17
E. tikapurensis	9.5–11.1	2.0–2.5	1.1–1.3	1.4–1.7	1.11–1.40
E. bicolor	10.3	2.4	1.4	1.7	n.a.
E. infuscatus	12.1	2.5	1.25	1.6	n.a.
E. brancuccii	7.6–8.0	1.8–1.9	1.0–1.1	1.5–1.8	1.00
E. bokondinensis	9.2	2.05	1.0	1.35	n.a.
E. elelimensis	6.9–8.1	1.5–1.9	0.9–1.1	1.3–1.4	n.a.
E. variabilis	6.6–8.2	1.6–2.0	0.1–1.1	1.1–1.35	0.83–0.95
E. niger	9.2–11.6	2.2–2.8	1.3–1.6	9.0–11.5	1.17–1.28 (b)
E. similis	7.5–9.7	1.9–2.3	1.1–1.4	1.8	1.02–1.15
Eniclases sp. B					

Other species of *Eniclases* (from Bocak & Bocakova 1991)

E. efferatus	6.8-9.2	1.5–1.9	1.0
E. egregius	8.5–9.2	2.2–2.4	1.08
E. electus	6.7–8.0	1.55–1.95	1.1
E. flabellatus	8.8.–10.2	2.1–2.4	1.1
E. flavoscutellaris	6.9	1.8	1.1
E. fuscicornis	8.8–10.8	2.1–2.4	1.15
E. luteolus	9.1–10.8	2.2–2.8	1.1
E. moluccanus	9.4	2.1	n.a.
E. nicricornis	10.2–11.5	2.9	1.0
E. nigriceps	7.6–9.8	1.7–1.8	1.1
E. nigroruber	10.7	2.5	1.03
E. pallidus	9.6–11.6	2.1–2.54	1.05
E. papuensis	9.4	2.35	1.13
E. pectinicornis	9.2–11.0	2.0–2.4	1.13
E. proximus	8.9–10.2	n.a.	1.15
E. robustus	10.8–11.7	2.6–2.9	1.14
E. sedlaceki	9.3–12.1	2.3–3.1	1.13
E. serratus	8.9	2.25	1.4 (b)
E. slipinskii	7.2–8.4	1.65–1.9	0.75
E. subelectus	8.3–10.5	2.1–2.75	1.0
E. versicolor	8.3–11.1	2.0–2.85	1.2
E. wauensis	10.6–12.1	2.5–3.0	0.89

Supplementary Table S5. The *cox1* mtDNA uncorrected genetic distances among species-rank lineages identified by the NextRAD data. The designation after slash refers to the mtDNA and morphology defined species. The highlighted parts designate genetic distances within clades of closely related species and population (see Fig. 3 and text for the reference).

	divar	*simil*	*niger*	*branc*	*varia*	*B/var*	*eleli*	*bokon*	*apert*	*tikap*	*tik/A*	*bicol*	*infus*	*pslut*
BM001 *E. divaricatus*	-													
BM003 *E. similis*	12.08			clade B										
BM033 *E. niger*	12.35	0.45	-											
BM005 *E. brancuccii*	12.53	8.63	8.17	-										
BM008 *E.* sp. B/*variabilis*	12.72	8.63	8.17	3.91	-		clade C							
BM054 *E. variabilis*	12.72	8.54	8.08	4.27	0.45	-								
BM051 *E. elelimensis*	12.72	8.08	7.63	3.81	1.54	1.63	-							
BM092 *E. bokondinensis*	13.08	8.17	7.72	4.00	1.73	1.82	0.73	-						
BM018 *E. apertus*	13.17	10.26	10.17	10.08	10.35	10.54	9.72	10.08	-					
BM039 *E. tikapurensis*	13.17	10.08	9.99	9.81	9.99	10.17	8.99	9.45	6.81	-		clade A		
BM093 *E. tikapur.*/sp. A	13.62	9.81	9.72	9.90	9.90	9.99	9.08	9.54	7.27	1.18	-			
BM045 *E. bicolor*	12.90	10.54	10.45	9.63	9.81	9.90	8.99	9.08	8.72	6.45	6.27	-		
BM050 *E. infuscatus*	12.72	9.99	10.08	9.45	9.54	9.54	8.54	8.63	8.72	6.18	6.18	1.09	-	
BM084 *E. pseudoluteolus*	12.99	9.45	9.26	10.26	10.90	10.81	9.81	10.26	12.08	10.81	11.17	11.81	11.26	-
BM080 *E. pseudoapertus*	13.26	10.90	10.90	10.90	11.44	11.35	10.99	11.44	11.44	11.35	11.35	11.08	10.63	11.53

Abbreviations:
divar – *E. divaricarus*
simil – *E. similis*
niger – *E. niger*
branc – *E. brancuccii*
varia – *E. variabilis*
B/var – *Eniclases* sp. B/*E. variabilis*
eleli – *E. elelimensis*
bokon– *E. bokondinensis*
apert– *E. apertus*
tikap– *E. tikapurensis*
tik/A– *E. tikapurensis/Eniclases* sp. A
bicol– *E. bicolor*
infus– *E. infuscatus*
pslut – *E. pseudoluteolus*

Supplementary Table S6. Distances between *Eniclases* localities in kilometers. Aggregations designated as AGG1–4, see main text for further information.

		Sentani 275m	Elelim 580m	Domb 1150m	Bok1 1287m	Bok2 1250m	BokN 2100m	BokSW 1900m	Tikap 2150m	Yiwika 2100m
AGG1	Sentani	-								
AGG2	Elelim	189.3	-							
	Dombomi	205.8	22.1	-						
AGG3	Bokondini1	240.3	79.1	58.0	-					
AGG4	Bokondini2	240.4	79.4	58.3	0.3	-				
	Bokondini N	239.3	79.4	58.5	1.9	1.8	-			
	Bokondini SW	244.0	80.9	59.5	4.0	4.0	5.7	-		
	Tikapura	242.1	72.7	50.8	12.3	12.5	14.0	10.7	-	
	Yiwika	232.1	47.7	27.0	44.2	44.3	45.2	43.8	33.8	-
	Napua	252.1	64.7	46.5	54.6	54.7	56.1	52.7	42.1	21.4

Supplementary Table S7. The occurrence of Metriorrhynchinae species in the localities of central New Guinea.

Metriorrhynchini (non trichaline genera)

	S	D	Y	E	T	N	Bl	Bh	Sh
Metr. 1	-	-	-	-	-	-	-	+	1
Metr. 2	-	-	-	-	-	-	-	+	1
Metr. 3	-	-	-	-	-	-	-	+	1
Metr. 4	-	-	-	-	-	+	-	-	1
Metr. 5	-	-	+	-	-	-	-	-	1
Metr. 6	-	-	-	-	+	-	-	-	1
Metr. 7	-	-	-	-	+	-	-	-	1
Metr. 8	-	-	-	-	-	+	-	-	1
Metr. 9	-	-	-	-	+	-	-	-	1
Metr. 10	-	-	-	-	+	+	-	-	2
Metr. 11	-	-	+	-	-	-	-	+	2
Metr. 12	-	-	-	-	-	+	-	-	1
Metr. 13	+	-	-	-	-	-	-	-	1
Metr. 14	-	-	-	+	-	-	-	-	1
Metr. 15	-	-	-	-	+	-	-	-	1
Metr. 16	-	-	+	-	+	-	-	-	2
Metr. 17	-	+	-	-	-	-	-	-	1
Metr. 18	-	-	-	+	-	-	-	-	1
Metr. 19	-	-	-	-	-	-	-	+	1
Metr. 20	-	-	-	-	-	+	-	-	1
Metr. 21	-	-	-	-	-	+	-	-	1
Metr. 22	-	-	-	+	-	-	-	-	1
Metr. 23	-	-	-	+	-	-	-	-	1
Metr. 24	+	-	-	-	-	-	-	-	1
Metr. 25	-	-	-	-	-	-	-	+	1
Metr. 26	-	-	-	-	-	+	-	-	1
Metr. 27	-	-	-	-	+	-	-	+	2
Metr. 28	+	-	-	-	-	-	-	-	1
Metr. 29	+	-	-	-	-	-	+	-	2
Metr. 30	-	-	-	+	-	-	-	-	1
Metr. 31	-	-	-	-	-	-	-	+	1
Metr. 32	-	-	-	-	+	-	-	-	1
Metr. 33	-	-	-	-	-	+	-	-	1
Metr. 34	-	+	-	-	-	-	-	-	1
Metr. 35	-	-	+	-	-	-	-	-	1
Metr. 36	-	-	+	-	-	+	-	-	2
Metr. 37	-	-	-	-	-	-	-	+	1
Metr. 38	-	+	-	+	-	-	-	-	2
Metr. 39	-	-	-	+	-	-	-	-	1
Metr. 40	-	-	-	+	-	-	-	-	1
Metr. 41	-	+	+	-	-	-	-	+	3
Metr. 42	-	-	-	-	+	-	-	-	1
Metr. 43	-	-	-	+	-	-	-	-	1
Metr. 44	-	-	+	-	-	-	-	-	1
Metr. 45	+	-	-	-	-	-	-	+	2
Metr. 46	-	-	-	+	-	-	-	-	1
Metr. 47	+	-	-	-	-	-	-	-	1
Metr. 48	-	-	-	+	-	-	-	-	1
Metr. 49	-	-	-	+	-	-	-	-	1
Metr. 50	+	-	-	-	-	-	-	-	1
Metr. 51	-	-	+	-	-	-	-	-	1
Metr. 52	-	-	-	-	-	+	-	+	2
Metr. 53	-	-	-	-	+	-	-	+	2

	S	D	Y	E	T	N	Bl	Bh	Sh
Metr. 54	-	-	-	-	-	-	+	-	1
Metr. 55	-	-	-	-	-	+	-	-	1
Metr. 56	-	-	-	+	-	-	-	-	1
Metr. 57	-	-	+	-	-	-	-	-	1
Metr. 58	-	-	-	-	-	-	-	+	1
Metr. 59	-	-	+	-	-	-	-	-	1
Metr. 60	-	-	-	+	-	-	-	-	1
Metr. 61	-	-	-	-	-	+	-	-	1
Metr. 62	-	-	-	-	-	+	-	-	1
Metr. 63	-	-	+	-	-	-	-	-	1
Metr. 64	-	-	-	-	-	+	-	-	1
Metr. 65	-	+	-	-	-	-	-	-	1
Metr. 66	+	-	-	-	-	-	-	-	1
Metr. 67	-	-	-	-	-	-	+	-	1
Metr. 68	-	-	-	-	-	-	+	-	1
Metr. 69	-	-	-	+	-	-	-	-	1
Metr. 70	-	-	+	-	-	-	-	-	1
Metr. 71	-	-	-	-	-	+	-	-	1
Metr. 72	-	-	-	+	-	-	-	-	1
Metr. 73	-	-	+	-	-	-	-	-	1
Metr. 74	-	-	-	-	-	-	-	+	1
Metr. 75	-	+	-	+	-	-	-	-	2
Metr. 76	-	-	-	-	+	-	-	+	2
Metr. 77	-	-	+	-	+	-	-	-	2
Metr. 78	-	-	-	+	-	-	-	-	1
Metr. 79	-	-	-	+	-	-	-	-	1
Metr. 80	-	-	-	-	-	-	-	+	1
Metr. 81	-	-	-	-	-	+	-	+	2
Metr. 82	-	-	-	+	-	-	-	-	1
Metr. 83	-	-	-	+	-	-	-	-	1
Metr. 84	-	+	-	-	-	-	-	-	1
Metr. 85	-	-	+	-	-	-	-	-	1
Metr. 86	-	-	-	-	+	+	-	-	2
Metr. 87	-	-	+	-	-	-	-	-	1
Metr. 88	-	-	-	-	-	-	-	+	1
Metr. 89	+	-	-	-	-	-	-	-	1
Metr. 90	-	-	-	+	-	-	-	-	1
Metr. 91	-	+	-	-	-	-	-	-	1
Metr. 92	-	-	-	+	-	-	-	-	1
Metr. 93	-	+	-	-	-	-	-	-	1
Metr. 94	-	-	-	+	-	-	-	-	1
Metr. 95	-	-	-	-	-	+	-	-	1
Metr. 96	-	-	-	+	-	-	-	-	1
Metr. 97	-	-	-	+	-	-	-	-	1
Metr. 98	-	-	-	-	-	+	-	+	2
Metr. 99	-	-	-	-	-	-	-	+	1
Metr. 100	-	-	-	-	-	+	-	-	1
Metr. 101	-	-	-	-	-	-	-	+	1
Metr. 102	-	-	-	-	-	-	-	+	1
Metr. 103	-	-	-	-	-	-	+	-	1
Metr. 104	-	-	-	-	-	-	-	+	1
Metr. 105	-	-	-	-	-	-	-	+	1
Metr. 106	-	-	-	-	-	-	-	+	1

Metr. 107	-	-	-	+	-	-	-	-	1
Metr. 108	-	-	-	-	-	+	-	-	1
Metr. 109	-	-	-	-	-	+	-	-	1
Metr. 110	-	+	-	+	-	-	-	-	2
Metr. 111	-	-	-	+	-	-	-	-	1
Metr. 112	-	-	-	-	+	-	-	+	2
Metr. 113	-	-	+	-	-	-	-	-	1
Metr. 114	-	-	-	-	-	-	-	+	1
Metr. 115	-	-	-	+	-	-	-	-	1
Metr. 116	-	-	-	+	-	-	-	-	1
Metr. 117	-	-	-	+	-	-	-	-	1
Metr. 118	-	-	-	-	+	-	-	-	1
Metr. 119	-	-	-	-	+	-	-	-	1
Metr. 120	-	-	-	+	-	-	-	-	1
Metr. 121	+	-	-	-	-	-	-	-	1
Metr. 122	-	-	-	+	-	-	-	-	1
Metr. 123	-	-	+	-	-	-	-	+	2
Metr. 124	-	-	-	-	-	-	-	+	1
Metr. 125	-	-	-	-	-	-	-	+	1
Metr. 126	-	-	-	-	-	-	+	-	1
Metr. 127	-	-	-	+	-	-	-	-	1
Metr. 128	-	-	-	+	-	-	-	-	1
Metr. 129	-	-	-	-	+	-	-	-	1
Metr. 130	-	+	-	-	-	-	-	-	1
Metr. 131	-	-	-	-	-	+	-	-	1
Metr. 132	-	-	-	-	+	-	-	-	1
Metr. 133	-	-	-	+	-	-	+	-	2
Metr. 134	-	-	+	-	+	-	-	+	3
Metr. 135	-	-	-	-	-	-	+	-	1
Metr. 136	-	-	-	-	+	+	-	+	3
Metr. 137	-	-	+	-	-	-	-	-	1
Metr. 138	-	-	-	-	-	-	-	+	1
Metr. 139	-	-	-	-	+	-	-	-	1
Metr. 140	-	-	-	+	-	-	-	-	1
Metr. 141	-	-	+	-	-	+	-	-	2
Metr. 142	-	-	-	-	-	+	-	-	1
Metr. 143	-	-	-	-	-	+	-	-	1
Metr. 144	-	-	-	-	-	+	-	-	1
Metr. 145	-	-	-	-	+	-	-	-	1
Metr. 146	-	+	-	-	-	-	-	+	2
Metr. 147	-	-	-	+	-	-	-	-	1
Metr. 148	-	-	-	-	+	-	-	-	1
Metr. 149	-	-	-	+	-	-	-	-	1
Metr. 150	-	-	+	-	-	+	-	-	2
Metr. 151	-	-	+	-	+	+	-	-	3
Metr. 152	-	-	-	+	-	-	+	-	2
Metr. 153	-	-	+	-	-	-	-	-	1
Metr. 154	-	-	-	-	+	-	-	+	2
Metr. 155	-	-	-	+	-	-	-	-	1
Metr. 156	+	-	-	-	-	-	-	-	1
Metr. 157	-	-	-	+	-	-	-	-	1
Metr. 158	-	-	-	+	-	-	-	-	1
Metr. 159	-	-	-	-	+	-	-	-	1
Metr. 160	-	-	-	-	+	+	-	+	3
Metr. 161	-	-	+	-	-	-	-	+	2
Metr. 162	-	-	-	-	-	-	-	+	1
Metr. 163	-	-	-	-	-	-	-	+	1
Metr. 164	-	-	-	-	+	-	-	-	1
Metr. 165	-	-	-	-	-	-	-	+	1
Metr. 166	-	-	-	-	-	+	-	-	1
Metr. 167	-	-	-	-	-	-	+	-	1
Metr. 168	-	-	-	+	-	-	-	-	1
Metr. 169	+	-	-	+	-	-	-	-	2
Metr. 170	-	-	-	-	-	-	+	-	1
Metr. 171	-	-	-	-	+	+	-	-	2
Metr. 172	-	+	-	+	-	-	-	-	2
Metr. 173	-	-	-	-	-	-	-	+	1
Metr. 174	-	-	+	-	-	-	-	-	1
Metr. 175	-	-	-	-	+	-	-	-	1
Metr. 176	-	-	-	-	-	-	-	+	1
Metr. 177	-	-	-	-	-	-	-	+	1
Metr. 178	-	-	+	-	-	+	-	-	2
Metr. 179	-	-	-	+	-	-	-	-	1
Metr. 180	-	-	-	+	-	-	-	-	1
Metr. 181	-	-	+	-	-	-	-	-	1
Metr. 182	-	-	-	-	-	-	-	+	1
Metr. 183	-	-	+	-	+	+	-	+	4
Metr. 184	-	-	-	-	-	-	-	+	1
Metr. 185	-	-	-	+	-	-	+	-	2
Metr. 186	-	-	-	+	-	-	-	-	1
Metr. 187	-	-	-	+	-	-	-	-	1
Metr. 188	-	-	-	+	-	-	-	-	1
Metr. 189	-	-	-	-	-	-	-	+	1
Metr. 190	-	-	-	-	+	-	-	-	1
Metr. 191	+	-	-	-	-	-	-	-	1
Metr. 192	-	-	-	+	-	-	-	-	1
Metr. 193	+	-	-	-	-	-	-	-	1
Metr. 194	-	+	-	+	-	-	+	-	3
Metr. 195	-	-	-	-	+	-	-	-	1
Metr. 196	-	+	-	-	-	-	-	-	1
Metr. 197	-	+	+	-	-	-	-	-	2
Metr. 198	-	-	-	+	-	-	-	-	1
Metr. 199	-	+	-	-	-	-	-	-	1
Metr. 200	-	-	-	-	+	+	-	-	2
Metr. 201	-	-	-	-	+	-	-	-	1
Metr. 202	-	-	-	+	-	-	-	-	1
Metr. 203	-	-	-	-	-	+	-	-	1
Metr. 204	-	-	-	-	+	-	-	+	2
Metr. 205	-	-	-	-	-	+	-	-	1
Metr. 206	+	-	-	-	-	-	-	-	1
Metr. 207	-	+	-	-	-	-	-	-	1
Metr. 208	-	-	+	-	-	+	-	-	2
Metr. 209	-	-	-	+	-	-	+	-	2
Metr. 210	+	-	-	+	-	-	-	-	2
Metr. 211	-	-	-	+	-	-	-	+	2
Metr. 212	-	+	-	+	-	-	-	-	2
Metr. 213	-	-	-	+	-	-	+	-	2
Metr. 214	-	-	-	+	-	-	-	-	1
Metr. 215	-	-	-	-	+	-	-	-	1
Metr. 216	-	+	-	-	-	-	-	-	1
Metr. 217	-	+	-	+	-	-	-	-	2
Metr. 218	-	+	-	-	-	-	-	-	1
Metr. 219	-	-	-	-	-	-	+	-	1
Metr. 220	-	-	-	+	-	-	-	-	1
Metr. 221	-	-	-	-	+	-	-	-	1
Metr. 222	-	-	-	+	-	-	-	-	1
Metr. 223	-	-	-	-	-	-	-	+	1
Metr. 224	-	-	-	-	-	+	-	-	1

Metr. 225	-	-	-	-	+	-	-	-	1
Metr. 226	-	-	-	+	-	-	-	-	1
Metr. 227	-	-	+	-	-	+	-	+	3
Metr. 228	+	+	-	+	-	-	-	-	3
Metr. 229	-	-	-	+	-	-	-	-	1
Metr. 230	-	-	+	-	-	-	-	-	1
Metr. 231	-	-	-	-	-	-	-	+	1
Metr. 232	-	-	-	+	-	-	-	-	1
Metr. 233	-	-	-	-	-	-	-	+	1
Metr. 234	-	-	-	+	-	-	-	-	1
Metr. 235	-	-	+	-	-	-	-	-	1
Metr. 236	-	-	-	-	-	+	-	-	1
Metr. 237	-	-	-	+	-	-	-	-	1
Metr. 238	-	+	-	-	-	-	-	-	1
Metr. 239	+	-	-	-	-	-	-	-	1
Metr. 240	-	-	-	+	-	-	-	-	1
Metr. 241	-	-	-	-	-	+	-	-	1
Metr. 242	-	-	-	-	-	-	+	-	1
Metr. 243	+	-	-	-	-	-	-	-	1
Metr. 244	-	-	+	-	-	-	-	-	1
Metr. 245	-	-	-	-	+	+	-	+	3
Total	19	24	35	73	40	44	17	55	

Trichalini (without *Eniclases*)

Trich. 1	-	-	-	+	-	-	-	-	1
Trich. 2	-	-	+	-	+	+	-	-	3
Trich. 3	-	-	-	-	-	-	+	-	1
Trich. 4	-	-	-	+	-	-	-	-	1
Trich. 5	+	-	-	-	-	-	-	-	1
Trich. 6	-	-	+	-	-	-	-	-	1
Trich. 7	-	-	+	-	-	-	-	-	1
Trich. 8	-	-	-	-	-	-	+	-	1
Trich. 9	+	-	-	+	-	-	-	-	2
Trich. 10	-	-	-	-	-	-	+	-	1
Trich. 11	-	-	+	-	-	-	-	-	1
Trich. 12	-	-	-	-	+	-	-	-	1
Trich. 13	-	-	-	-	+	-	-	-	1
Trich. 14	-	-	-	+	-	-	-	-	1
Trich. 15	-	-	-	+	+	-	-	-	2
Trich. 16	+	-	-	-	-	-	-	-	1
Trich. 17	-	-	-	+	-	-	-	-	1
Trich. 18	-	-	+	-	+	-	-	-	2
Trich. 19	+	-	-	-	-	-	-	-	1
Trich. 20	-	-	-	-	+	-	-	-	1
Trich. 21	-	-	-	-	+	-	-	-	1
Trich. 22	-	-	-	-	+	-	-	-	1
Trich. 23	+	-	-	-	-	-	-	-	1
Trich. 24	-	-	-	-	-	+	-	-	1
Trich. 25	-	-	-	-	+	-	-	-	1
Trich. 26	-	-	-	+	-	-	+	-	2
Trich. 27	-	-	-	+	-	-	-	-	1
Trich. 28	-	-	-	+	-	-	-	-	1
Trich. 29	-	-	-	-	+	+	-	-	2
Trich. 30	-	-	-	+	-	-	-	-	1
Trich. 31	-	-	-	+	-	-	+	-	2
Trich. 32	-	-	-	-	+	-	+	-	2
Trich. 33	+	-	-	-	-	-	-	-	1
Trich. 34	-	-	-	-	-	-	+	-	1
Trich. 35	-	-	+	-	+	-	-	-	2
Trich. 36	+	-	-	-	-	-	-	-	1
Trich. 37	-	-	+	+	+	+	-	-	4
Trich. 38	-	-	-	-	-	-	-	+	1
Total	7	0	7	12	13	4	7	1	

Eniclases species

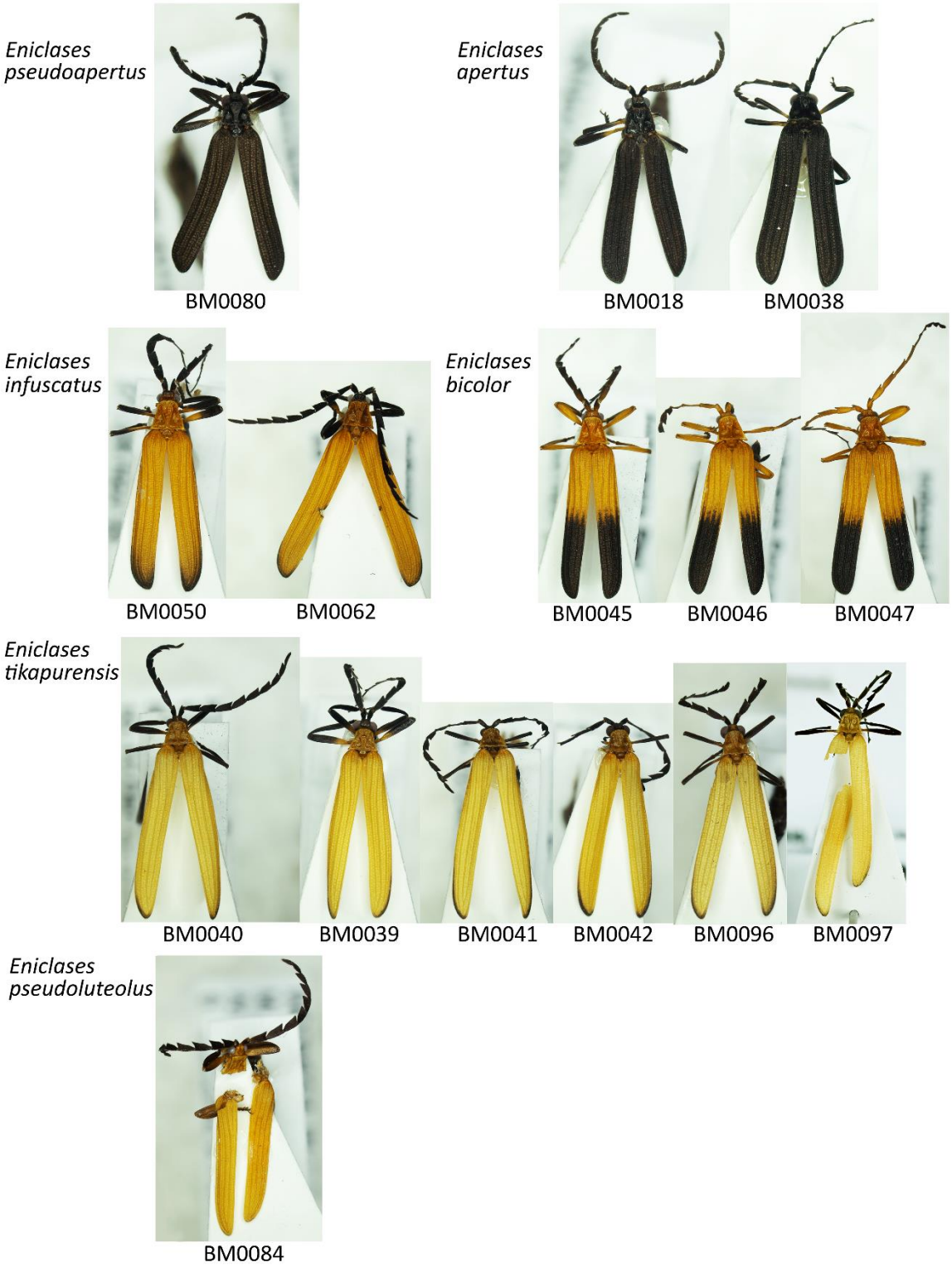
	S	D	Y	E	T	N	Bl	Bh	Sh
Enic. 1	+	-	-	-	-	-	-	-	1
Enic. 2	-	-	-	+	-	-	-	-	1
Enic. 3	-	-	-	-	-	-	-	+	1
Enic. 4	+	-	-	-	-	-	-	-	1
Enic. 5	+	-	-	+	-	-	-	-	2
Enic. 6	-	+	-	+	-	-	+	-	3
Enic. 7	-	-	-	+	-	-	-	-	1
Enic. 8	-	-	-	+	-	-	+	-	2
Enic. 9	+	-	-	-	-	-	-	-	1
Enic. 10	+	-	-	-	-	-	-	-	1
Enic. 11	-	-	+	-	+	-	-	+	1
Enic. 12	-	-	-	+	-	-	-	-	1
Total	5	1	1	6	1	0	2	2	

Abbreviations:

S – Sentani, D –Dombomi,
Y – Yiwika, E – Elelim,
T – Tikapura,N – Napua,
Bl – Bokondini 1250 m
Bh –Bokondini 2000 m
Sh –the number of localities which share the species

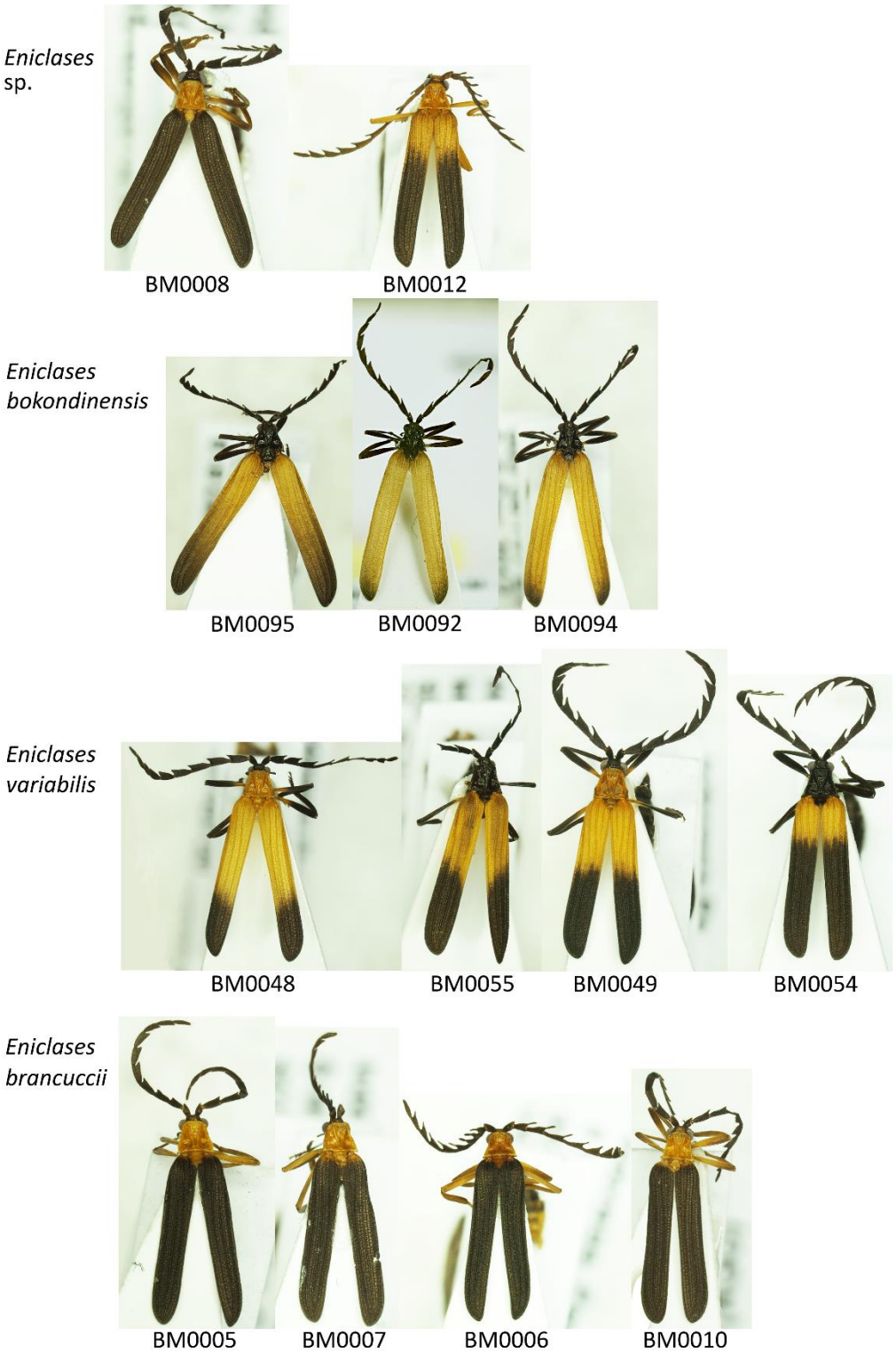
\+ – record in the locality
\- – absent in the locality

Enic. 1 – *Eniclases apertus*
Enic. 2 – *Eniclases bicolor*
Enic. 3 – *Eniclases bokondinensis*
Enic. 4 – Enicla*ses brancuccii*
Enic. 5 – *Eniclases divaricatus*
Enic. 6 – *Eniclases elelimensis*
Enic. 7 – *Eniclases infuscatus*
Enic. 8 – *Eniclases niger*
Enic. 9 – *Eniclases similis*
Enic. 10 – *Eniclases* sp. B
Enic. 11 – *Eniclases tikapurensis*
Enic. 12 – *Eniclases variabilis*



Supplementary figure S1. Aposematic patterns of sequenced specimens from northern New Guinea (part 1).

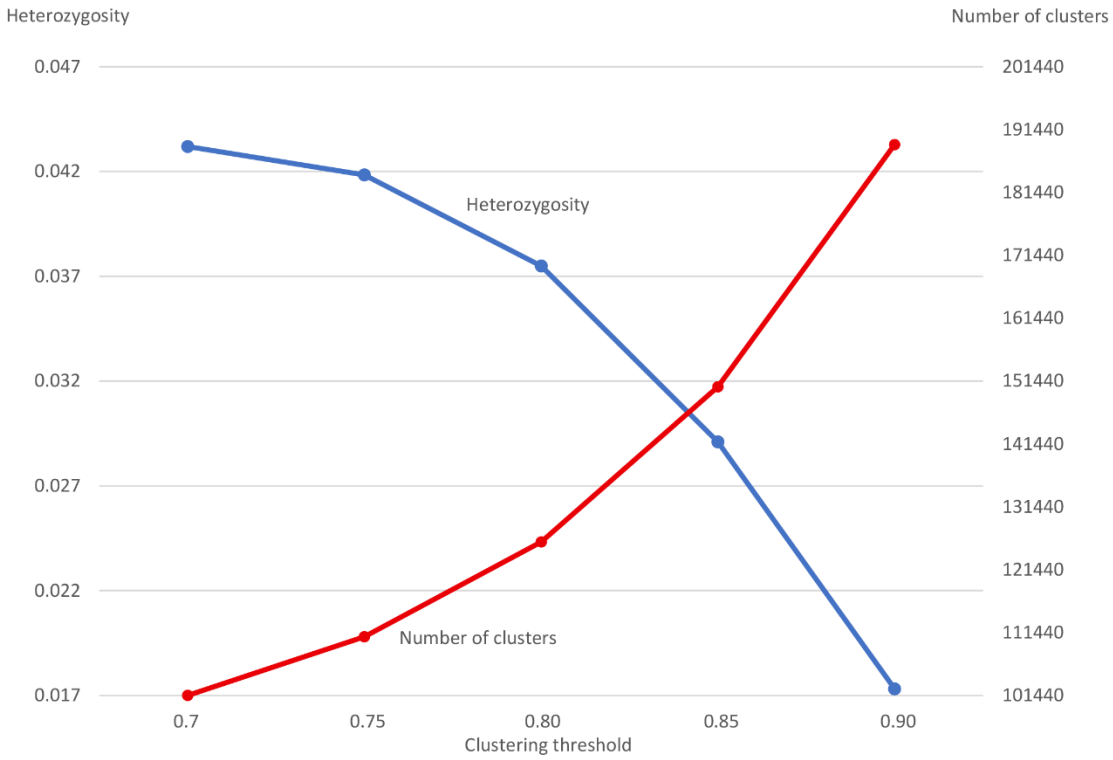
Supplementary figure S2. Aposematic patterns of sequenced specimens from northern New Guinea (part 2).



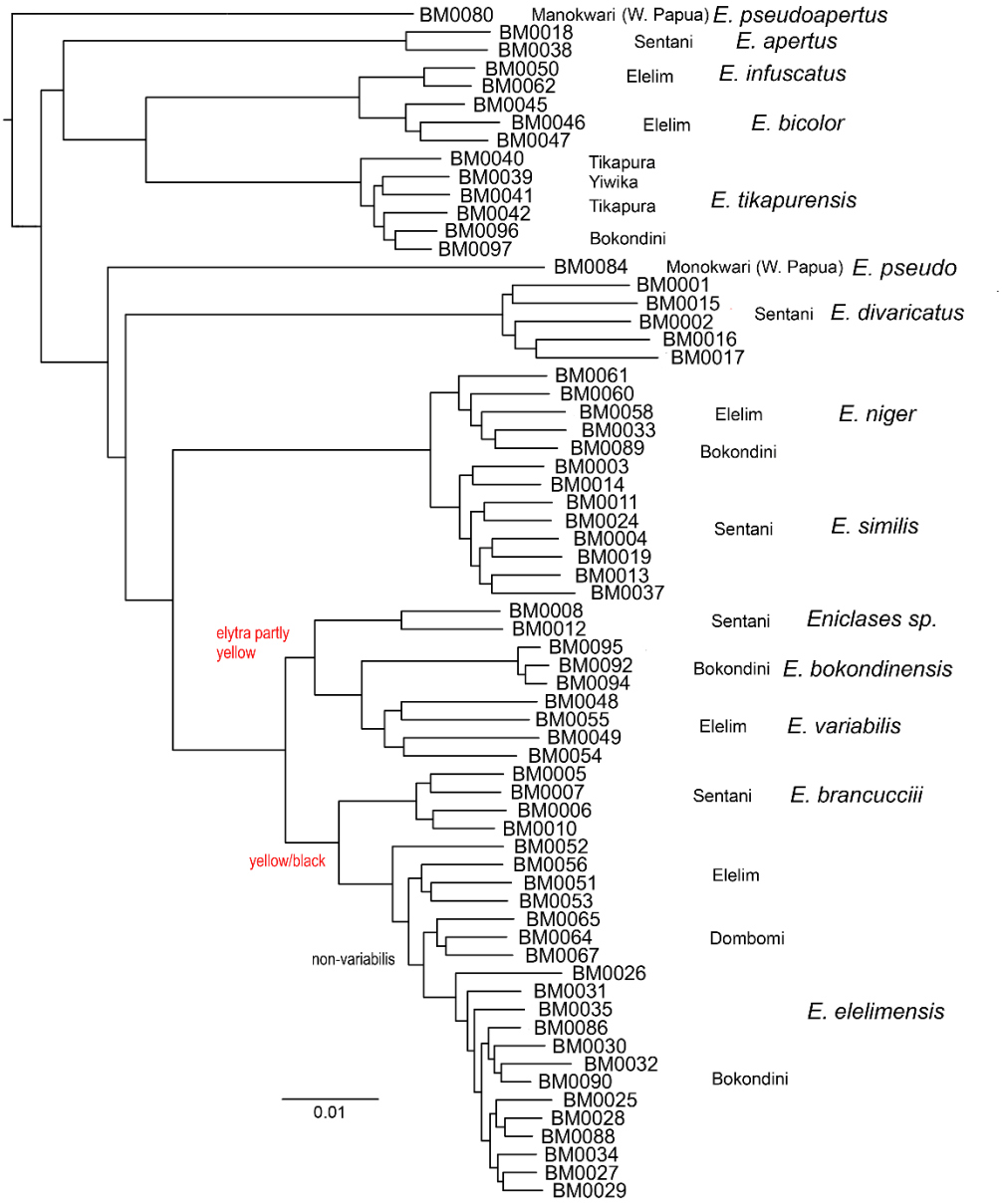
Supplementary figure S3. Aposematic patterns of sequenced specimens from northern New Guinea (part 3).

Eniclases elelimensis

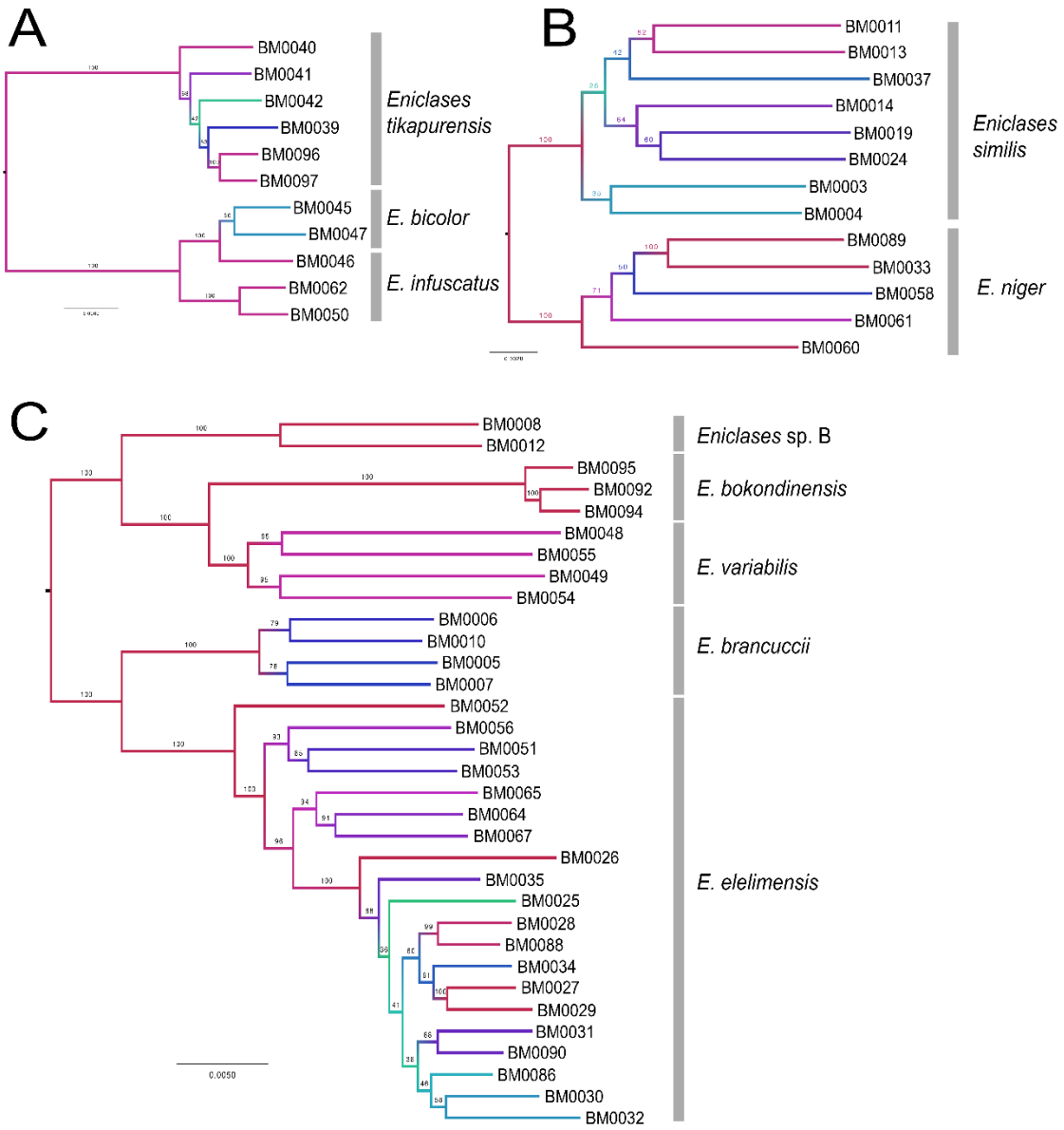
Supplementary figure S4. Aposematic patterns of sequenced specimens from northern New Guinea (part 4).



Supplementary Fig. S5 Testing the effects of clustering threshold on the individual heterozygosity and numbers of clusters produced.

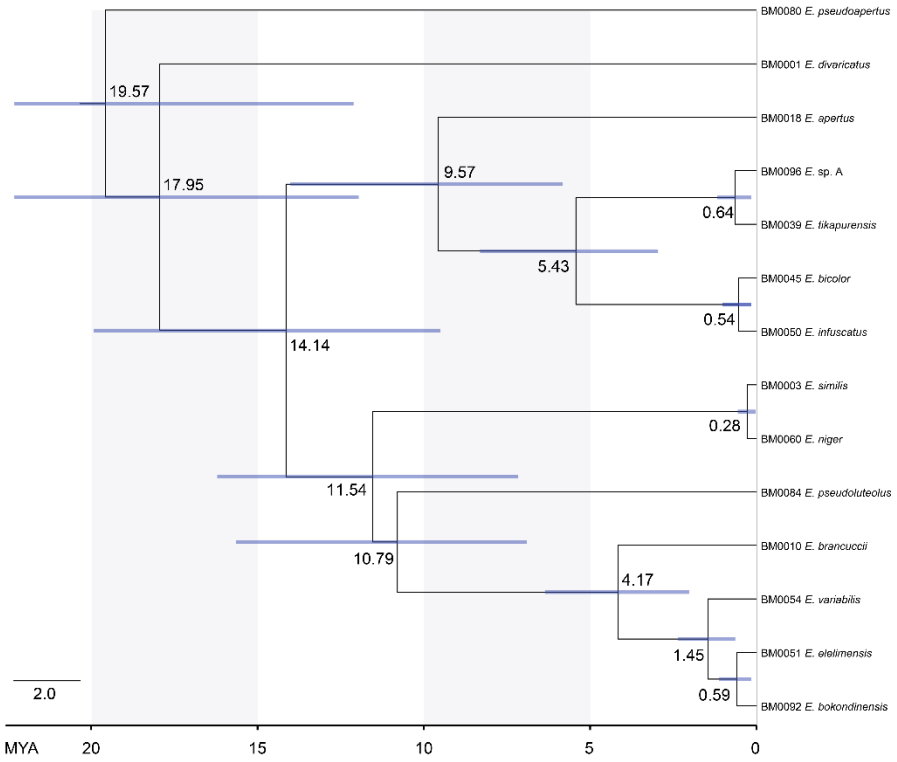


Supplementary Figure S6. Full length RAD-seq tree.

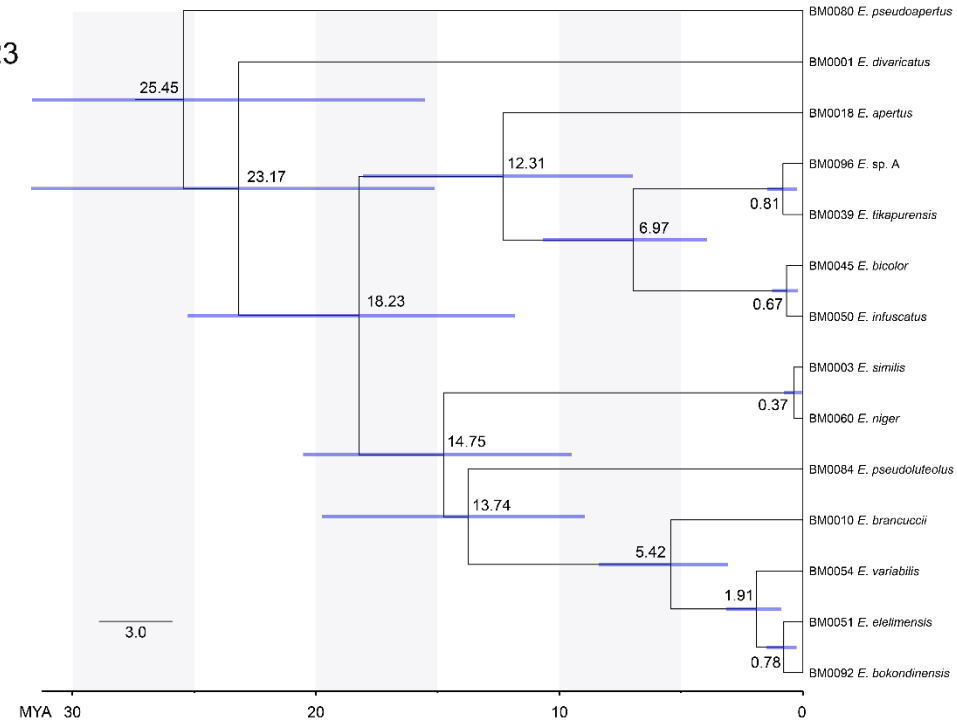


Supplementary figure S7. Subclades A, B and C individually filtered by settings used for whole RAD-seq tree reconstruction. (MinCov=4, MinDepth=6, Wclust=0.85)

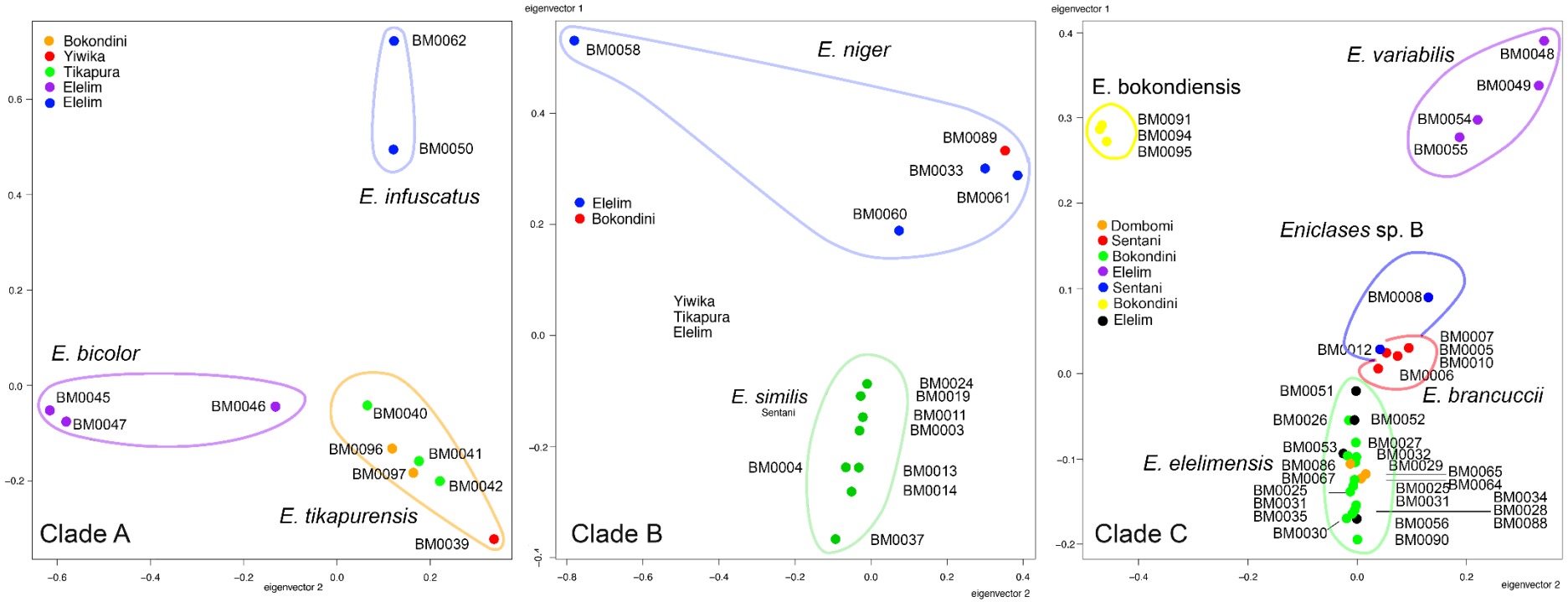
cox1 rate 0.0115



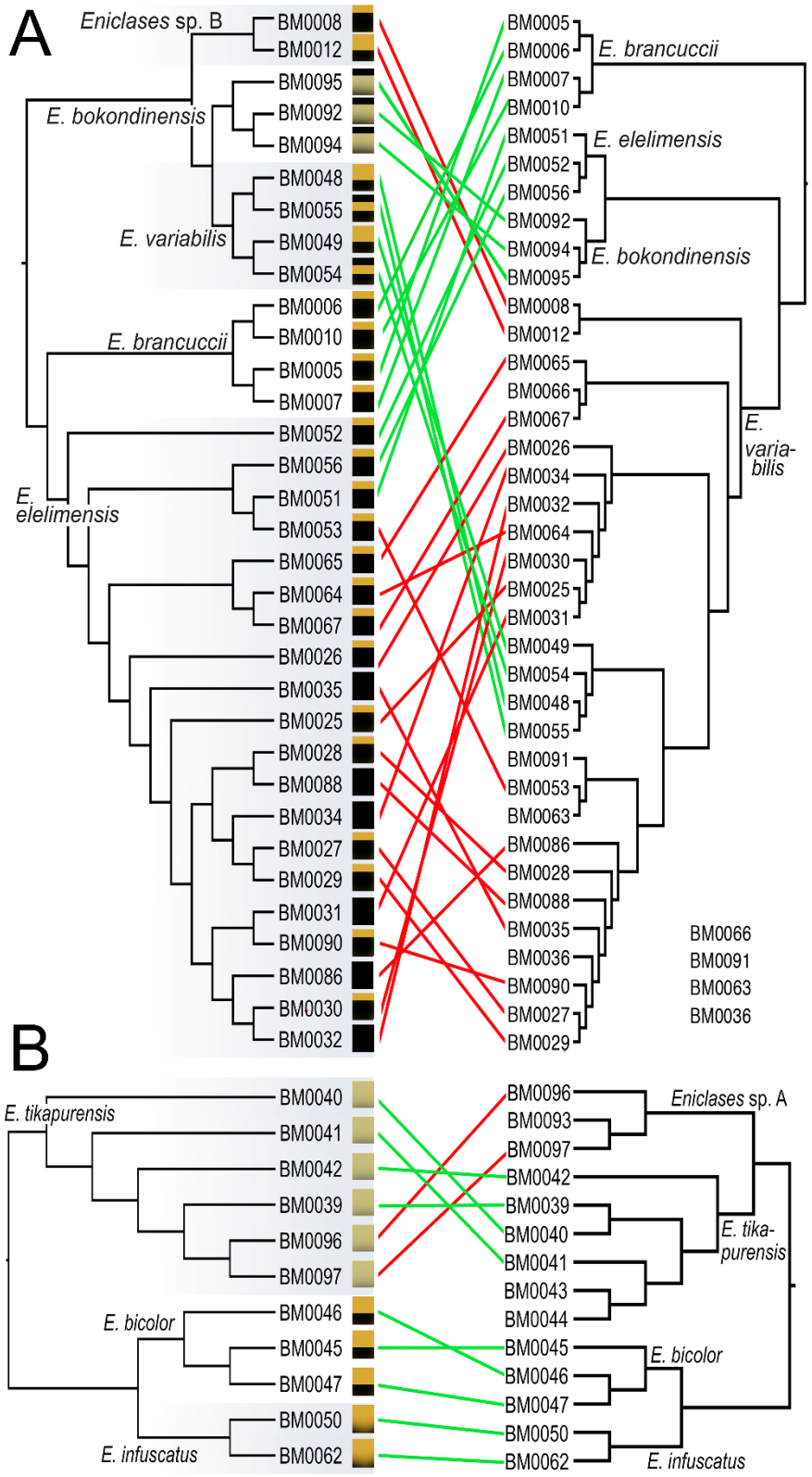
cox1 rate 0.023



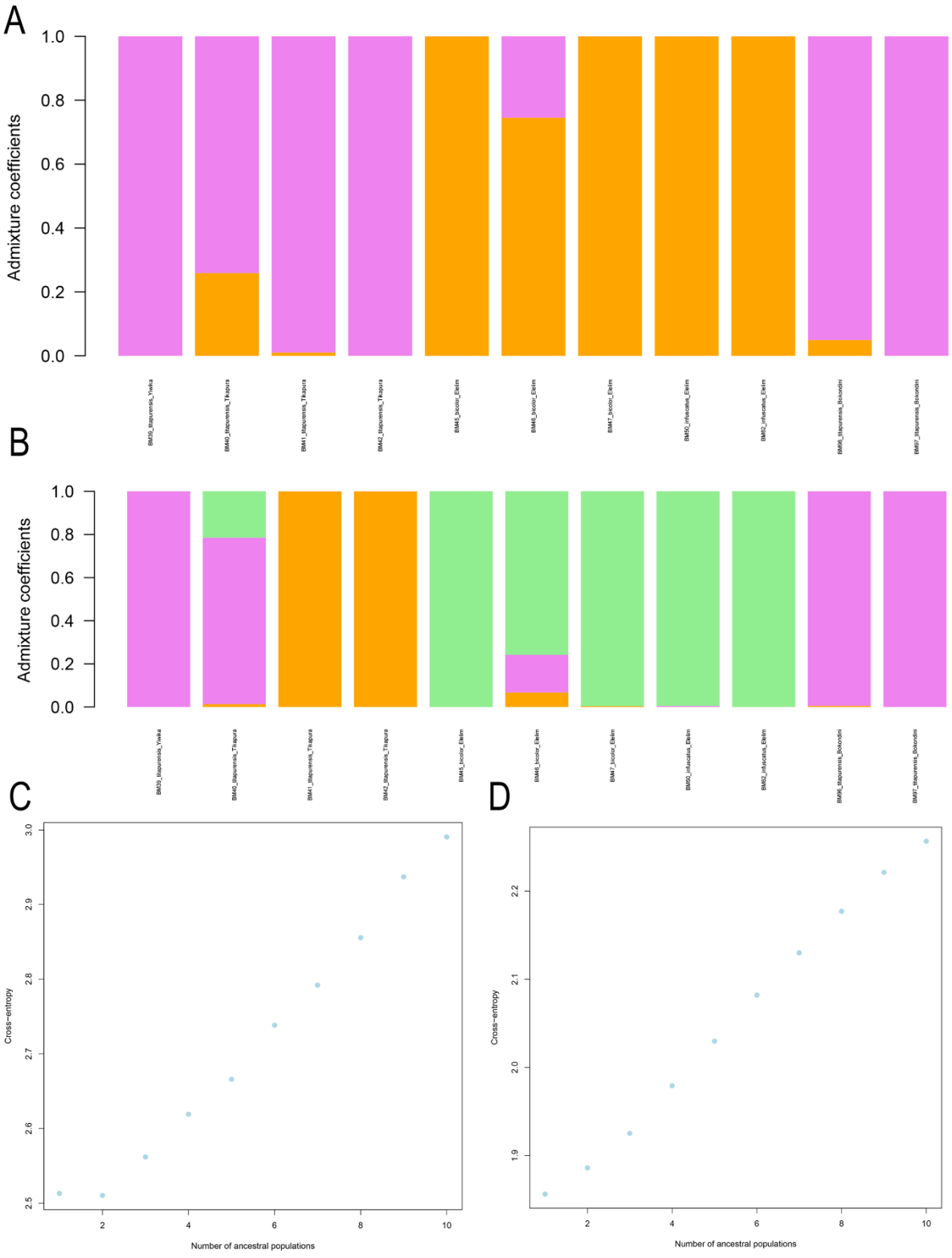
Supplementary figure S8. The dated trees based on mtDNA data dataset with two different mutation rates of *cox1*.



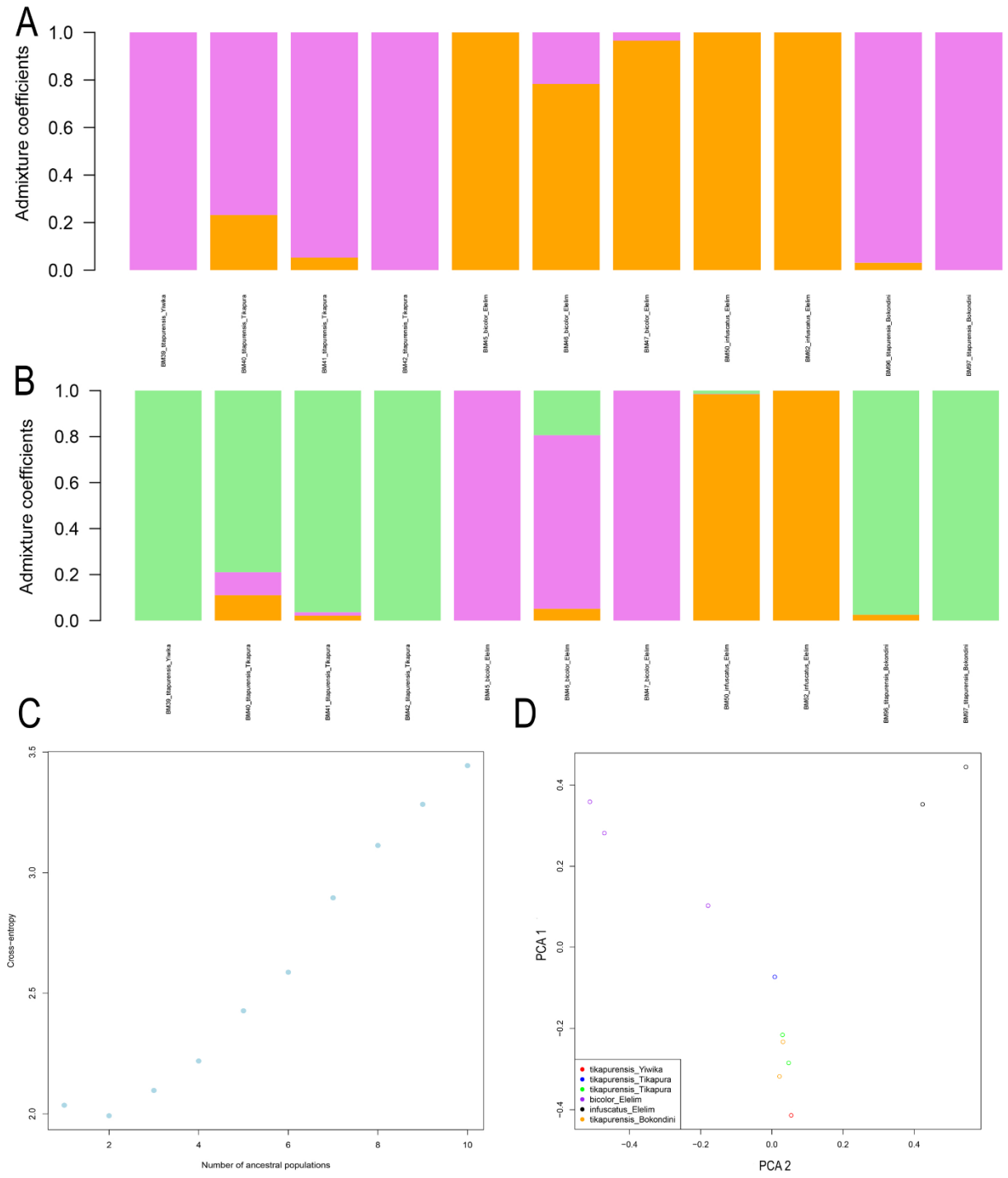
Supplementary figure S9. The principal component analysis (PCA) of the clades A, B, C inferred from RAD dataset with the localities in northern New Guinea.



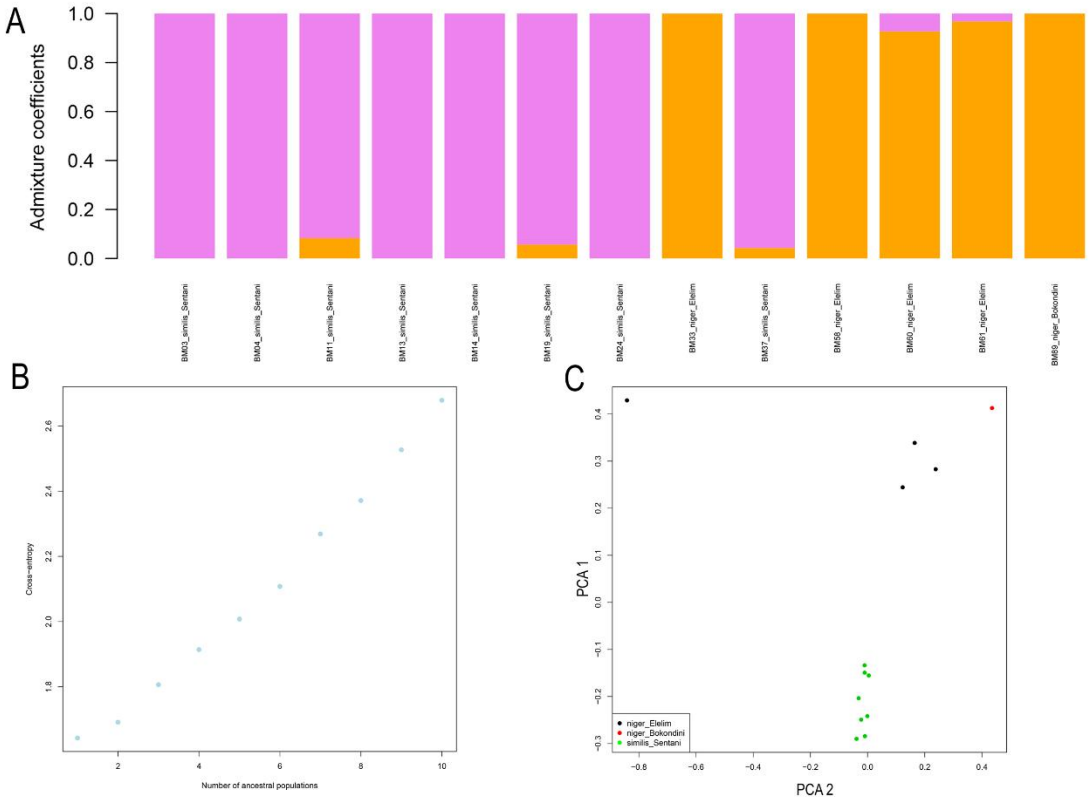
Supplementary figure S10. Comparison of RAD and mtDNA phylogenies in the selected clades (A and B) with color patterns and green/red bars indicating differences between topologies.



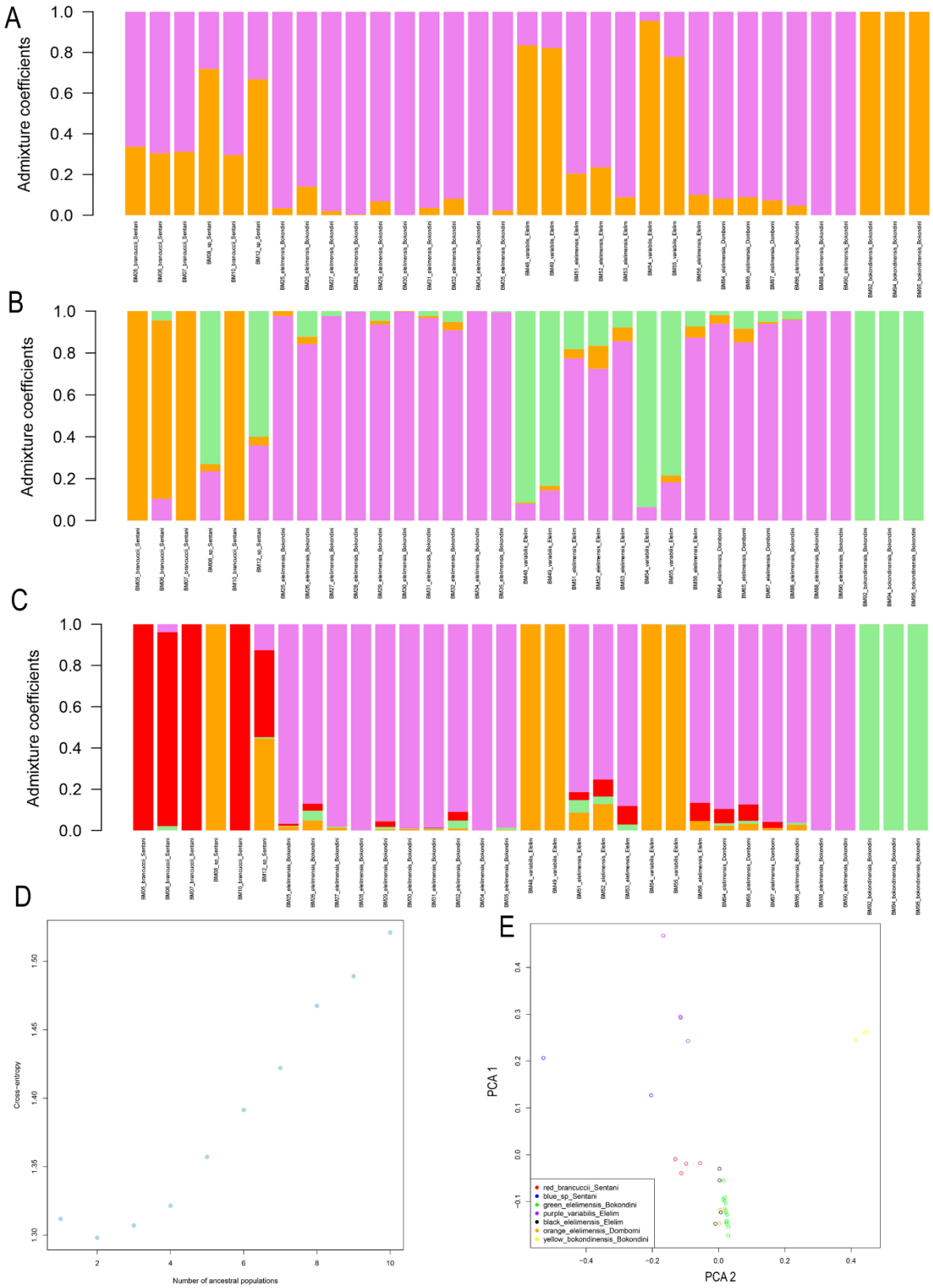
Supplementary Fig. S11 Plots of inferred individuals admixture coefficients based on sparse non-negative matrix factorization (sNMF) implemented in R package LEA for the clade A as defined by the phylogenetic analyses.The figures show the following K genetic clusters: A, K=2; B, K=3; C, cross entropy graph for the clade A; D, cross entrophy graph for the clade B.



Supplementary Fig. S13 Plots of inferred individuals admixture coefficients based on sparse non-negative matrix factorization (sNMF) implemented in R package LEA for the clade A as defined by the phylogenetic analyses. The figures show the following K genetic clusters: A, K=2; B, K=3; C, cross entropy graph for the clade A; D, distribution of Eniclases individuals from the clade A along principal component (PC) scores (PC1 17.2%; PC2 14.3%) of genetic variation based on the analysis of the RAD dataset. Both analyses based on the iPYRAD dataset calculated from individuals of the clade A only.



Supplementary Fig. S14 Plot of inferred individuals admixture coefficients based on sparse non-negative matrix factorization (sNMF) implemented in R package LEA for the clade B as defined by the phylogenetic analyses. The figure show the following K genetic cluster: A, K=2; B, cross entropy graph for the clade B; C, distribution of Eniclases individuals from the clade B along principal component (PC) scores (PC1 12.4%; PC2 9.6%) of genetic variation based on the analysis of the RAD dataset. Both analyses based on the iPYRAD dataset calculated from individuals of the clade B only.



Supplementary Fig. S15 Plots of inferred individuals admixture coefficients based on sparse non-negative matrix factorization (sNMF) implemented in R package LEA for the clade C as defined by the phylogenetic analyses. The figures show the following K genetic clusters: A, K=2; B, K=3; C, K=4; D, cross entropy graph for the clade C; E, distribution of Eniclases individuals from the clade C along principal component (PC) scores (PC1 6.1%; PC2 5.6%) of genetic variation based on the analysis of the RAD dataset. Both analyses based on the iPYRAD dataset calculated from individuals of the clade C only.

Supplementary Text

The brief taxonomic history, morphology, and diversity of Eniclases Waterhouse, 1879

The genus *Eniclases* was described by Waterhouse (1879) for a single species *Trichalus luteolus* Waterhouse, 1878. Further species were described by Kleine (1926, 1930, 1935). Additional two species were originally described in *Trichalus* by Pic (1921, 1923) and transferred to *Eniclases* by Bocak & Bocakova, 1991. The later study additionally added a number of newly described species from New Guinea. The latest study dealing with *Eniclases* added further ten species, mainly from the central part of New Guinea (Bocek & Bocak 2016).

Eniclases shares with other trichaline genera the shortened primary costa 1. Unlike related genera the pronotal carinae form a V-shaped pattern. The male genitalia are characterized by dorsal pigmented line of the phallus and are characterized by high similarity among distantly related species (Bocak & Bocakova, 1991). Most diagnostic characters have been found in the relative size of male year and the shape of male antennae. The detailed morphological characteristic of *Eniclases* was provided by Bocak & Bocakova (1991).

At present, Eniclases contains 36 species distributed mostly in New Guinea (35 spp.), a single species is recorded from the Moluccas. The present results do not result in any formal taxonomical changes despite alternative placement of individuals in different relationships (Fig. 4). The high intraspecific polymorphism and similarity makes identification of *Eniclases* species extremely difficult and in some cases neither morphology nor mitochondrial markers provide sufficient information for robust assignment of an individual to a species.

The list of species of *Eniclases* Waterhouse, 1879.

Eniclases Waterhouse, 1879: 66
Trichalus, subgenus *Trichaolus* Pic, 1923: 36; Bocak & Bocakova, 1991: 206

Eniclases apertus (Pic, 1923: 36)	New Guinea
Trichalus (Trichaolus) apertus Pic, 1923: 36	
=*Eniclases fumosus* Kleine, 1926: 181; Bocak & Bocakova, 1991: 217	
bicolor Bocek & Bocak, 2016: 23	New Guinea
bokondinensis Bocek & Bocak, 2016: 26	New Guinea
brancuccii Bocek & Bocak, 2016: 25	New Guinea
divaricatus (Pic, 1921: 10)	New Guinea
Trichalus divaricatus Pic, 1921:10	
efferatus Kleine, 1926: 181	New Guinea
egregious Kleine, 1926: 181	New Guinea
electus Kleine, 1926: 182	New Guinea
elelimensis Bocek & Bocak, 2016: 26	New Guinea
flabellatus Bocak & Bocakova, 1991: 207	New Guinea
flavoscutellaris Bocak & Bocakova, 1991: 216	New Guinea
fuscicornis Bocak & Bocakova, 1991: 208	New Guinea
infuscatus Bocek & Bocak, 2016: 23	New Guinea
luteolus (Waterhouse, 1878: 113)	New Guinea, Mysool, Aru Isl.

moluccanus Kleine, 1930: 328	Halmahera
nicricornis Bocak & Bocakova, 1991: 216	New Guinea
niger Bocek & Bocak, 2016: 29	New Guinea
nigriceps Bocak & Bocakova, 1991: 208	New Guinea
nigroruber Kleine, 1935: 318	New Guinea
pallidus Bocak & Bocakova, 1991: 209	New Guinea
papuensis Bocak & Bocakova, 1991: 213	New Guinea
pectinicornis Bocak & Bocakova, 1991: 211	New Guinea
pseudoapertus Bocek & Bocak, 2016: 21	New Guinea
pseudoluteolus Bocek & Bocak, 2016: 29	New Guinea
proximus Bocak & Bocakova, 1991: 209	New Guinea
riedeli Bocak & Bocakova, 1998: 14	New Guinea
robustus Bocak & Bocakova, 1991: 209	New Guinea
sedlaceki Bocak & Bocakova, 1991: 212	New Guinea
serratus Bocak & Bocakova, 1991: 217	New Guinea
similis Bocak & Bocakova, 1991: 210	New Guinea
slipinskii Bocak & Bocakova, 1991: 213	New Guinea
subelectus Bocak & Bocakova, 1991: 215	New Guinea
tikapurensis Bocek & Bocak, 2016: 24	New Guinea
variabilis Bocek & Bocak, 2016: 27	New Guinea
versicolor Kleine, 1926: 182	New Guinea
wauensis Bocak & Bocakova, 1991: 214	New Guinea

References

Bocak L. (2002): Revision and phylogenetic analysis of Metriorrhynchinae. *European Journal of Entomology* 99: 315 – 351.

Bocak L. (1998): A new species of the genus Eniclases Wat. (Coleoptera, Lycidae). Acta Universitae Palackianae Olomucensis 35(1997): 13 – 16.

Bocak, L., Bocakova M. (1991) Revision of the genus *Eniclases* Waterhouse, 1879 (Coleoptera, Lycidae, Metriorrhynchinae). *Mitt. Muench. Ent. Ges*. 81: 203–226.

Bocek, M., & Bocak, L. (2016). Species limits in polymorphic mimetic *Eniclases* net-winged beetles from New Guinean mountains (Coleoptera: Lycidae). *ZooKeys*, *593*, 15–35. doi: 10.3897/zookeys.593.7728.

Kleine R. (1926) Coleoptera, Lycidae. Nova Guinea. *Résultats de l'expédition scientifique néerlandaise a la Nouvelle-Guinée*. 15(2): 91–195.

Kleine, R. (1930) Bestimmungstabelle der Trichalusverwandschaft. *Treubia* 9: 325–340.

Kleine, R. (1935) Bericht uber die von Miss Chessman in British New Guinea gesammelten Brenthiden und Lyciden. *New Guinea* 17: 303–322.

Pic M. 1921a: Contribution a l'étude des Lycides. *L'Echange* 404:1-4 (hors texte).

Pic M. 1921b: Contribution a l'étude des Lycides. *L'Echange* 406: 9–12 (hors texte).

Pic M. (1923) Contribution a l'étude des Lycides. *L'Echange* 412: 36 (hors texte).

Waterhouse C. O. (1878) On the different forms occurring in the Coleopterous family Lycidae, with descriptions of new genera and species. *Trans. Entomol. Soc. Lond*. 1878: 95–118.

Waterhouse C. O. (1879) *Illustration of the typical specimens of Coleoptera in the collection of the British Museum*. Part I. - Lycidae. London: British Museum. 93 pp.

Due to the non-ownership of copyright to the Parts II–IV, the following studies are presented via hyperlinks and are freely accessible on the Internet in the electronic version of this Ph.D. thesis.

Part II

Phylogeny and phylogeography of Metriorrhynchini (Coleoptera: Lycidae) in the Papuan region

Matej Bocek

The origins and dispersal history of the trichaline net-winged beetles in Southeast Asia, Wallacea, New Guinea and Australia

(published manuscript; Zoological Journal of the Linnean Society)

https://academic.oup.com/zoolinnean/article-abstract/185/4/1079/5298314

Part III

Phylogeny and phylogeography of Metriorrhynchini (Coleoptera: Lycidae) in the Papuan region

Matej Bocek

The molecular phylogeny of Omalisidae (Coleoptera) defines the family limits and demonstrates low dispersal propensity and ancient vicariance patterns

(published manuscript; Systematic Entomology)

https://onlinelibrary.wiley.com/doi/full/10.1111/syen.12271

Part IV

Phylogeny and phylogeography of Metriorrhynchini (Coleoptera: Lycidae) in the Papuan region

Matej Bocek

New species of *Diatrichalus* (Coleoptera: Lycidae) from New Guinea and the Moluccas

(published manuscript; Zootaxa)

https://www.mapress.com/j/zt/article/view/zootaxa.4247.5.4

UNIVERZITA PALACKÉHO V OLOMOUCI

PŘÍRODOVĚDECKÁ FAKULTA

KATEDRA ZOOLOGIE

PHYLOGENY AND PHYLOGEOGRAPHY OF METRIORRHYNCHINI (COLEOPTERA: LYCIDAE) IN THE PAPUAN REGION

Fylogeneze a fylogeografie tribu Metriorrhynchini

(Coleoptera: Lycidae) v Papuánské oblasti

SUMMARY OF PH.D. THESIS / AUTOREFERÁT DIZERTAČNÍ PRÁCE

Mgr. Matěj Boček

P1527 – Biology

Zoology

Supervisor: prof. Ing. Ladislav Bocák, Ph.D.

Olomouc 2019

Summary of the Ph.D. thesis

Author:	**Mgr. Matěj Boček**
Title:	Phylogeny and phylogeography of Metriorrhynchini (Coleoptera: Lycidae) in the Papuan region
Supervisor:	prof. Ing. Ladislav Bocák, Ph.D. Department of Zoology, Faculty of Science, Palacký University Olomouc 17. listopadu 50, 771 46 Olomouc, Czech Republic
Study program:	P1527 Biology
Study field:	Zoology

Obhajoba disertační práce se koná dne..... v ..... hodin v učebně č..... na Katedře zoologie a ornitologické laboratoři Přírodovědecké fakulty Univerzity Palackého v Olomouci, 17. listopadu 50, Olomouc. Na stejné adrese se lze také seznámit s disertační prací a posudky.

Abstract

The Ph.D. thesis contains four separate studies dealing with the molecular phylogeny, Müllerian mimicry, morphology-based taxonomy, and phylogeography of trichaline net-winged beetles from Australo-Indonesian Archipelago. These beetles belong to the tribe Metriorrhynchini (Coleoptera: Elateroidea: Lycidae). Tribe Metriorrhynchini is a most diverse group of Lycidae which encompasses over a third of the Lycidae alpha-taxonomic diversity. Most net-winged beetles have a limited dispersal ability, but the members of tribe Metriorrhynchini are slightly better colonists compared to the most of other close relatives. Moreover, another weakly sclerotized elateroid lineage, Elateridae: Omalisinae, was studied to recover ancient distribution patterns in the Mediterranean.

The phylogeny of trichaline genera was used to recover generic limits. Further, phylogeography and dispersal capacity was studied in detail also here. Such information is needed to properly understand the biological processes which leads to colonization of new ranges in poorly dispersing animal groups like Lycidae or Omalisinae. Ancestral distribution of trichaline genera was inferred in New Guinea and adjacent islands where the highest species diversity occurs. Additionally, unexpected direction of colonization events points to secondary range expansion to Australia. The trichaline genera used the islands of the Moluccas, Philippines, Sulawesi and Sundas as stepping stones for their colonization of continental Asia.

Another study deals with Müllerian mimetic complexes which play an important role in a recent radiation of *Eniclases* communities in New Guinea. In contrast to widely accepted theory of purifying selection leading to convergent evolution of a single aposematic signal, sympatrically occurring species of *Eniclases* are characterized by the presence of multiple patterns in a place and intraspecific color polymorphism.

The result of phylogenetic analyses of Omalisinae are also unexpected. The females are wingless, incompletely sclerotized with retained larval characters when mature. I document that most species are distributed exclusively in coastal refugia around the Mediterranean Sea and only a single species is widespread. Understanding species diversity and phylogeography in the Mediterranean can extraordinarily improve the knowledge on natural processes and provide information for species conservation in this highly disturbed region.

Abstrakt

Dizertační práce obsahuje čtyři nezávislé studie týkající se molekulární fylogeneze, Müleriánských mimikry, tradiční taxonomie založené na morfologickém popisu nových druhů a fylogeografie. Jako modelová skupina byla vybrána skupina trichaliních rodů, jež je terminální linií tribu Metriorrhynchini (Coleoptera: Elateroidea: Lycidae) a vyskytuje se v oblasti indonéských ostrovů mezi Austrálií a kontinentální částí jihovýchodní Asie. Tribus Metriorrhynchini je nejdiverzifikovanější skupinou čeledi Lycidae a čítá více než třetinu dosud popsané druhové diverzity. Navzdory omezené schopnosti disperze u většiny zástupců čeledi Lycidae, zástupci tribu Metriorrhynchini jsou lepšími kolonizátory než jejich blízcí příbuzní. Vzhledem k dosažení dlouho nedostupného materiálu byla studována taktéž další málo sklerotizovaná elateroidní linie Elateridae: Omalisinae, která sdílí starodávné rozšíření v oblastech glaciálních refugií Mediteránu.

Fylogeneze trichaliních rodů byla použita k vymezení jednotlivých rodů. Dále byla zkoumána jejich fylogeografie a schopnost šíření druhů, díky kterým se vědci snaží pochopit procesy vedoucí k osídlování nových oblastí zejména pro druhy s omezenými schopnostmi šíření, jakými jsou zástupci Lycidae i Omalisinae. Ancestrální distribuce trichaliních rodů je situována do oblasti Nové Guinei a blízkých ostrovů, odkud byli její zástupci schopni prostřednictvím ostrovů Moluk, Filipín, Sulawesi a Velkých i Malých Sund kolonizovat kontinentální Asii.

Další studie řeší problematiku Müleriánských mimikry hrající důležitou roli v druhové radiaci rodu *Eniclases* na Nové Guinei. Na rozdíl od přijímané teorie purifikující selekce vedoucí ke konvergenci pro jeden přijímaný aposematický signál, sympatricky se vyskytující zástupci rodu *Eniclases* se vyznačují přítomností mnoha barevných vzorů na lokalitě a intraspecifickým druhovým polymorfismem.

Výsledky fylogenetické analýzy Omalisinae jsou rovněž neočekávané. Samice jsou bezkřídlé, nekompletně sklerotizované se zachovanými larválními znaky v dospělosti. Většina druhů se vyskytuje výhradně v oblasti příbřežních refugií v okolí Středozemního moře a pouze jeden druh je široce rozšířen. Pochopením diverzity a způsobu šíření Omalisinae může významně pomoci k poznání přírodních procesů, které mohou poskytnout informace pro ochranu přírody v této disturbované oblasti.

Thesis outline

The presented Ph.D. thesis entitled “Phylogeny and phylogeography of Metriorrchynchini (Coleoptera: Lycidae) in the Papuan region” comprises four parts and combine three recently published articles forming the body of this research.

PART I deals with several connected topics, mainly with the mimetic patterns and species communities of *Eniclases* net-winged beetles from New Guinea involved in an extensive Mülerian complexes. In addition, the comprehensive nextRAD dataset unveils the discrepancy between species delimitation based on mitochondrials markers and genomic data.

PART II considers dispersal routes of trichaline net-winged beetles and the directions of colonization towards continental Asia. Moreover, the study recovers the presumable age of basal and internal splits of principal lineages with rich species radiation in the terminals. Ancestral area of basal splits is situated in New Guinea.

PART III represents molecular phylogeny of the group Elateridae: Omalisinae with the first description of *Thilmanus obscurus* female which has been yet uknown for science. The results of molecular and zoogeographical analyses confirm the ancient vicariance patterns of Omalisinae and their preferences to persist in refugial areas of the Mediterranean.

PART IV offers a brief insight into traditional morphology-based description of *Diatrichalus*, the genus with a high species diversity reported from New Guinea and the Moluccas. Four new species are described here primarily using male genitalia for diagnostics and species delimitation.

Preface

The phylogeny, origins of species diversity, evolutionary novelties and interactions between organisms and environment are major fields of contemporary biology. Different groups of insects serve as model organisms in such studies due to their extreme biodiversity and ecological plasticity. Traditional morphology-based taxonomical studies and later morphological cladistic analyses had been for most of the 20th century in the centre of systematic entomology, but a methodological revolution came with molecular techniques not only in the fields of transition zones between biochemistry and biology, but also in the systematic research. Recent state-of-art studies in molecular systematics are based on methods with an extreme power to resolve even very recent species evolution, interactions and the origin of adaptations. Many relationships crucial for understanding evolution of insects have remained poorly understood due to the absence of appropriate markers and high phenotypical plasticity which compromised morphology-based analyses (Whiting et al., 1997; Bocek & Bocak, 2016). In species delimitation, species polymorphism and the cryptic diversity represented further challenge to be solved (Larsen, 2001; Porco et al., 2012; Bocek & Bocak, 2017). Regardless amount of information and explanatory power, also molecular data occasionally failed in the species delimitation due to incomplete lineage sorting and hybridization. Thus, any conclusion on the evolution of species should be preferably validated by independent information. Therefore, the combination of morphological traits and molecular phylogeny are widely used side by side to reveal the relationships among problematic groups and to construct phylogenetic hypotheses (Giribet et al., 2001; Glenner et al., 2004; Bocek & Bocak, 2016; 2017).

In some fields of systematic biology, the molecular data became an obligatory part of a species description (e.g., Journal of Nematology).Similarly, in the explosive development of molecular techniques, the morphology-based systematics explores the technological advancement. Traditional external phenotype-based analysis has been expanded by the detailed study of internal structures. Since WWII, the male genitalia are almost obligatory used for the description of new species (Beal, 1959; Young, 1967; Kimsey, 1979). Although highly valuable for species delimitation and as a one of the most reliable reproductive isolation mechanisms, their permanent and rapid evolution due to sexual selection makes them unreliable in higher classification. Some very similar structures are preserved in species without contact in sympatry and in contrary, many closely related species display substantial morphological disparity due to strong selection (Serb et al., 2011; Bocek & Bocak, 2016; Bocak et al., 2018; Bocek et al.,

2018; Kusy et al., 2018; 2019). The digital documentation of diagnostic traits, high resolution electron microphotography and advanced methods in morphometry, all have become an integral part of morphology-based systematic research. Similarly, a high importance has been given to data basing of taxonomical acts, e.g. an obligatory registration of all new species in the ZooBank database if the publication is electronically disseminated (http://zoobank.org) or archives of morphological information in MorphoBank (https://morphobank.org/).

Under current situation, no further progress can be reached without new techniques and rigorous consideration of all evidence. Historically, two or more different phenotypical manifestations were usually considered as an evidence for delimitation of a separate species. However, the early phase of differentiation is none but simple. The long-lasting gene flow can result in a very gradual separation of gene pools and some differences can be manifested by a few crucial genes which could make emerging biological lineage separate (Reiseberg et al., 1999; Parsons & Shaw, 2001; Petit & Excoffier, 2009; Stölting et al., 2013). Conversely, different phenotypes can be produced under different environmental conditions without genomic differentiation.

Enormous insect's diversity represents both the challenge and opportunity. Among large insect orders, beetles are the most diverse group with about 400,000 formally described species and they represent over a fifth of all known organisms. Besides their importance concerning the alpha-diversity, they represent an important part of ecosystems especially in the dynamic and highly structured environment of tropical rainforests. Comprehensive world-wide studies at order level or even higher are impossible and researchers usually concentrate on a single lineage and a limited study area. The currents set of studies deals with elateroid beetles, especially with the families Lycidae and Elateridae: Omalisinae *sensu* Kusy et al. (2018). The family Lycidae, commonly known as net-winged beetles, have been chosen as a model group for molecular, ecological and phylogeographical studies in the Australo-Indonesian Archipelago (Simpson 1977; Lohman et al., 2011). The Omalisinae were used for a study dealing with Tertiary vicariance and current distribution of Omalisinae in the Mediterranean region. The molecular data can be employed to answer various questions on evolution, classification and zoogeography.

Conclusion and outlook

The set of studies relates to the uniting a single goal: I wanted to recognize and document the diversity of the selected groups of beetles and to identify processes leading to their evolutionary success in the case of highly diverse trichaline beetles, or to their supposed evolutionary failure, as in the case of extremely rare Omalisidae which till today persist in a few refugia in the Mediterranean. Besides the self-evident goal to document diversity and construct the classification and name some species earlier unknown to science, the studies can classify the centres of diversity and regions where the genetic diversity has been preserved despite climatic and tectonic turbulences. It is more than likely that a huge amount of species richness is still unknown and probably will never been recognized. The effort of researchers from all over the world is often surpassed by the pace of industrial development or deforestation to obtain palm oil as an ecological fuel or a food resource for continually growing population (Sodhi et al., 2004; Fitzherbert et al., 2008). Nevertheless, I believe that any contribution to our knowledge on nature is valuable and may persuade people to protect it.

Abstracts of presented studies

Bocek, M. (unpublished manuscript) Persistence of multiple patterns and intraspecific polymorphism in net-winged beetles due to variable signal perceptions and community structures.

In contrast to traditional models of purifying selection and a single aposematic signal in Müllerian complexes, some communities of unprofitable prey contain members with multiple aposematic patterns. Processes responsible for diversity in aposematic signaling are poorly understood and large multi-species communities are seldom considered. Therefore, we analyzed the phylogeny and aposematic patterns of closely related Eniclases net-winged beetles in central New Guinea using morphology, mtDNA, and nextRAD data. Our results confirm the complexities of early phase speciation. We suggest three clades of Eniclases representing groups of closely and incompletely reproductively isolated lineages, detail the extent of polymorphism among Eniclases, and categorize their low-contrast aposematic patterns. Field observations suggest that perception of the aposematic signal is affected by beetle behavior, weather, time of day, and vegetation density. The warning signal of Eniclases consists of body shape and color, with ambiguous color perception under some circumstances, i.e., when resting on the undersides of leaves. As a result, purifying selection for patterns of similar color may be relaxed in net-winged beetle communities. We hypothesize that environmental factors in our study area are too variable to allow for strong, constant purifying selection over time, resulting in multiple color patterns and polymorphism in closely related species in single localities. In addition, Eniclases occur in highly diverse multi-species communities of other net-winged beetles, which implies changing selection pressure in space and time. Variable environmental conditions and diverse community composition are suggested to be favorable for the persistence of multiple patterns, imperfect mimics, and intraspecific polymorphism.

Bocek, M. & Bocak, L. (2019) The origins and dispersal history of the trichaline net-winged beetles in South East Asia, Wallacea, New Guinea and Australia. *Zoological journal of the Linnean Society*, 185, 1079–1094.

Trichaline net-winged beetles (Lycidae: Metriorrhynchini) are a diverse group distributed in Australia, Wallacea and Indo-Burma. The phylogenetic relationships of ~120 taxa were recovered by applying maximum likelihood and Bayesian inference using DNA fragments of the *cox1*, *rrnL* and *nad5* mitochondrial DNA and *SSU* and *LSU* ribosomal RNA genes. Divergence times and ancestral ranges were estimated using Bayesian approaches. We identified New Guinea as the ancestral region and estimated the date of dispersal events to continental Australia and Asia. Most Australian trichaline beetles diverged from New Guinean lineages during the Early Miocene to the Middle Miocene, and the fauna east of Lyddeker's Line was established by range expansion from New Guinea to the Moluccas, further on to the Philippines and then to the Greater Sunda Islands and Indo-Burma. A single species dispersed via the Lesser Sunda Islands to continental Asia. Trichaline beetles never crossed Wallace's Line between Sulawesi and Borneo. The dispersal westward started 20 Mya, after the first contact between Australian and Asian cratons, and three dispersal events led to the colonization of Sundaland. High genetic diversity and limited morphological diversification were identified

in Oriental *Diatrichalus* and *Microtrichalus*, which colonized Indo-Burma during the last 5 Myr. Geographical isolation led to the origin of cryptic genetic diversity in Southeast Asia.

Bocek, M., Fancello, L., Motyka, M., Bocakova, M. & Bocak, L. (2018) The molecular phylogeny of Omalisidae (Coleoptera) defines the family limits and demonstrates low dispersal propensity and ancient vicariance patterns. *Systematic Entomology*, 43, 250–261.

The genus-level molecular phylogeny of Omalisidae Lacordaire is presented for six of seven currently recognised genera. The monophyly and internal relationships are well-supported including the taxa which were placed in other elateroid families. We conducted molecular analyses using maximum-likelihood optimality criterion and Bayesian inference and 18S, 28S rRNA, rrnL and cox1 mtDNA markers (4038 homologous positions). Euanoma Reitter is a sister lineage to other Omalisidae. *Thilmanus* Gemminger is related to *Paradrilus* Kiesenwetter and *Phaeopterus* Costa. Thilmaninae Kazantsev (erected in Lycidae), Euanomini Kazantsev (erected in Drilidae), and Paradrilinae Kundrata et al. are removed from within omalisid classification due to widely overlapping concepts of generic and subfamilial taxa. *Pseudeuanoma* Pic, **syn.n**. was recovered as a paraphylum and is a younger synonym of *Euanoma*. *Euanoma caligo* (Kazantsev), **comb.n.**, *E. ionica* (Pic), **comb.n.**, *E. obscura* (Pic), **comb.n.** and *E. reitteri* (Pic), **comb.n.** are newly combined with *Euanoma*. The earlier classification of incompletely metamorphosed taxa was affected by the parallel evolution of morphological traits. We report on the discovery of the incompletely metamorphosed female of *Thilmanus obscurus* Baudi and compare it with the female of *O. fontisbellaquei* Geoffroy. The female is weakly sclerotised, has physogastric abdomen, vestigial elytra, no wings and simplified thoracic morphology. Furthermore, we describe allopatric ranges of ancient omalisid lineages and vicariance events resulting from geological transformations in the Mediterranean. Euanoma was split from other Omalisidae in the late Jurassic and remains restricted to the Eastern Mediterranean. *Omalisus* Geoffroy split from the Iberian genera in the Cretaceous and most species occur on southern slopes of the Alps and in the western Balkan. The separation of *Paradrilus* and *Thilmanus*+*Phaeopterus* corresponds with the isolation of the Ebro and Hesperian massifs in the Cretaceous; the fauna of Sardinia and Corsica is of Iberian origin and *Phaeopterus* dispersed from these islands to the Elba and Apennine Peninsula. The diversity of Omalisidae has an ancient origin, but survived till present only in the Mediterranean, mostly in Pleistocene refugia close to the sea.

Bocek, M. (2017) New species of *Diatrichalus* (Coleoptera: Lycidae) from New Guinea and the Moluccas. *Zootaxa*, 424, 577–584.

The recently collected material of *Diatrichalus* Kleine, 1926 from New Guinea and the Moluccas was studied. Four new species are described: *D. buruensis* **sp. n.**, *D. manokwarensis* **sp. n.**, *D. mindikensis* **sp. n.**, and *D. robustus* **sp. n.** The diagnostic morphological characters are described in detail and illustrated. New records of previously described species are reported from several localities in New Guinea and *D. mancus* (Kleine, 1926) is for the first time reported from the Australian continent. Some *Diatrichalus* have similar male genitalia and are considered as closely related. Despite supposed relationships, they differ in the presence of

secondary elytral longitudinal costae, which have been traditionally used for the delimitation of genera in net-winged beetles.

References

Beal, R. S. (1959) Notes on the Biology and Systematics of the Dermestid Beetle Genus *Aspectus* with Descriptions of Two New Species. *Annals of the Entomological Society of America*, **52**, 132–137.

Bocak, L., Motyka, M., Bocek, M. & Bocakova, M. (2018) Incomplete sclerotization and phylogeny: The phylogenetic classification of *Plastocerus* (Coleoptera: Elateroidea). *PLoS ONE*, **13**: e0194026.

Bocek, M. & Bocak, L. (2016). Species limits in polymorphic mimetic Eniclases net-winged beetles from New Guinean mountains (Coleoptera: Lycidae). *ZooKeys*, **593**, 15–35.

Bocek, M. & Bocak, L. (2017) The comparison of molecular and morphology-based phylogenies of trichaline net-winged beetles (Coleoptera: Lycidae: Metriorrhynchini) with description of a new subgenus. *PeerJ*, **5**, e3963.

Bocek, M., Fancello, L., Motyka, M., Bocakova, M. & Bocak, L. (2018) The molecular phylogeny of Omalisidae (Coleoptera) defines the family limits and demonstrates low dispersal propensity and ancient vicariance patterns. *Systematic Entomology*, **43**, 250–261.

Fitzherbert, E. B., Struebig, M. J., More, A., Danielsen, F., Brüh, C. A., Donald, P. F. & Phalan, B. (2008) How will oil palm expansion affect biodiversity? *Trends in Ecology & Evolution*, **23**, 538–545.

Giribet, G., Edgecombe, G. D. & Wheeler, W. C. (2001) Arthropod phylogeny based on eight molecular loci and morphology. *Nature*, **413**, 157–161.

Glenner, H., Hansen, A. J., Sørensen, M. V., Ronquist, F., Huelsenbeck, J. P. & Willerslev E. (2005) Bayesian Inference of the Metazoan Phylogeny. *Current Biology*, **15**, 392–393.

Kimsey, L. S. (1979) An illustrated key to the genus *Exaerete* with descriptions of male genitalia and biology (Hymenoptera: Euglossini, Apidae). *Journal of Kansas Entomological Society*, **52**, 735–46.

Kusy, D., Motyka, M., Bocek, M., Masek, M. & Bocak, L. (2019) Phylogenomic analysis resolves the relationships among net-winged beetles (Coleoptera: Lycidae) and reveals the parallel evolution of morphological traits. *Systematic Entomology* (early view).

Kusy, D., Motyka, M., Bocek, M., Vogler, A. P. & Bocak, L. (2018) Genome sequences identify three families of Coleoptera as morphologically derived click beetles (Elateridae). *Scientific Reports*, **8**, 17084.

Larsen, K. (2001) Morphological and molecular investigation of polymorphism and cryptic species in tanaid crustaceans: implications for tanaid systematics and biodiversity estimates, *Zoological Journal of the Linnean Society*, **131**, 353–379.

Lohman, D. J., de Bruyn, M., Page, T., von Rintelen, K., Hall, R., Ng, P. K. L., Shih, H. T., Carvalho, G. R. & von Rintelen, T. (2011) Biogeography of the Indo-Australian Archipelago. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 205–226.

Parsons, Y. M. & Shaw, K. L. (2001) Species boundaries and genetic diversity among Hawaiian crickets of the genus *Laupala* identified using amplified fragment length polymorphism. *Molecular Ecology*, **10**, 1765–1772.

Parsons, Y. M. & Shaw, K. L. (2001) Species boundaries and genetic diversity among Hawaiian crickets of the genus *Laupala* identified using amplified fragment length polymorphism. *Molecular Ecology*, **10**, 1765–1772.

Petit, R. J. & Excoffier, L. (2009) Gene flow and species delimitation. *Trends in Ecology & Evolution*, **24**, 386–393.

Rieseberg, L. H, Whitton, J. & Gardner, K, (1999) Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. *Genetics*, **152**, 713–727.

Serb, J. M., Alejandrino, A., Otárola-Castillo, E. & Adams, D. C. (2011) Morphological convergence of shell shape in distantly related scallop species (Mollusca: Pectinidae), *Zoological Journal of the Linnean Society*, **163**, 571–584.

Simpson, G. G. (1977) Too many lines; the limits of the Oriental and Australian zoogeographic regions. *Proceedings of the American Philosophical Society*, **121**, 107–120.

Sodhi, N. S., Koh, L. P., Brook, B. W. & Ng, P. K. L. (2004) Southeast Asian biodiversity: an impending disaster. *Trends in Ecology & Evolution*, **19**, 654–660.

Stölting, K. N., Nipper, R., Lindtke, D., Caseys, C., Waeber, S., Castiglione, S. & Lexer, C. (2013) Genomic scan for single nucleotide polymorphisms reveals patterns of divergence and gene flow between ecologically divergent species. *Molecular Ecology*, **22**, 842–855.

Whiting, M. F., Carpenter, J. C., Wheeler, Q. D. & Wheeler, W. C. (1997) The Strepsiptera Problem: Phylogeny of the Holometabolous Insect Orders Inferred from 18S and 28S Ribosomal DNA Sequences and Morphology. *Systematic Biology*, **46**, 1–68.

Young, F. N. (1967) A key to the genera of American bidessine water beetles with descriptions of three new genera (Coleoptera: Dytiscidae, Hydroporinae). *Coleopterists' Bulletin*, **21**, 75–84.

Curriculum Vitae

Matěj Boček

Ph.D. student

Department of Zoology

Faculty of Science, Palacký University Olomouc

17. listopadu 50, 771 46 Olomouc, Czech Republic

Email: bocema00@gmail.com

Education

2015 – present: **Ph.D. in Zoology,**
Palacký University Olomouc.

2013 – 2015: **Master degree in Zoology,**
Palacký University Olomouc.

2010 – 2013: **Bachelor degree in Systematic Biology and Ecology,**
Palacký University Olomouc.

Foreign internships

2016 – Malaysia – field research – duration: 1 month

Institution: Natural History Museum and Imperial College London, United Kingdom

Supervisor: prof. Alfried Vogler

2015 and 2016 - United Kingdom, London – duration: 2 months

- Natural History Museum and Imperial College London, United Kingdom

Supervisor: prof. Alfried Vogler

2015 - Poland, Warszaw – duration: 2 weeks

- Muzeum i Instytut Zoologii Polskiej Akademii Nauk, Poland

Supervisor: prof. Wioletta Tomaszewska

Conferences and oral presentations

22. 9. – 24. 9. 2017 - 8th Meeting on Insect Phylogeny in Dresden
25. 11. 2016 - Young Systematics´ Forum, Natural History Museum, London
22. 11. 2018 – Zoologia 2018, Technická Univerzita Zvolen – **oral presentation, topic:** The molecular phylogeny of Omalisidae (Coleoptera) defines the family limits and demonstrates low dispersal propensity and ancient vicariance patterns.

Research interests

- Phylogeography and dispersal of elateroid beetle lineages
- Systematics and phylogeny of family Lycidae (tribe Metriorrhynchini)
- Phylogenetic analysis, dating analysis, ancestral state reconstruction
- Assembly of genomic data

Teaching

- Biological classification of animals (tutorials)
- Applied zoology
- Systematics of invertebrates
- General zoology (larvae and pupae)

Publication record

Bocek, M. & Adamkova, K. (2019) New species of Moluccan trichaline net-winged beetles, with remarks on the phylogenetic position and distribution of *Schizotrichalus* (Coleoptera: Lycidae: Metriorrhynchinae). *Zootaxa* (in press; IF = 0.931).

Kusy, D., Motyka, M., **Bocek, M.**, Masek, M. & Bocak, L. (2019) Phylogenomic analysis resolves the relationships among net-winged beetles (Coleoptera: Lycidae) and reveals the parallel evolution of morphological traits. *Systematic Entomology*, early view, DOI:10.1111/syen.12363 (IF = 4.237)

Bocek, M. & Bocak, L. (2019) The origins and dispersal history of the trichaline net- winged beetles in South East Asia, Wallacea, New Guinea and Australia. *Zoological journal of the Linnean Society*, 185: 1079–1094, DOI: 10.1093/zoolinnean/zly090. (IF = 2.685)

Jiruskova, A., Motyka, M., **Bocek, M.** & Bocak, L. (2019) The Malacca Strait separates distinct faunas of poorly-flying *Cautires* net-winged beetles. *PeerJ*, 7 (e6511), DOI: 10.7717/peerj.6511. (IF = 2.118)

Bocak, L., Motyka, M., **Bocek, M.** & Bocakova, M. (2018) Incomplete sclerotization and phylogeny: the phylogenetic classification of *Plastocerus* (Coleoptera: Elateroidea) *PLoS ONE*, 13: e0194026, DOI: 10.1371/journal.pone.0194026. (IF = 2.766)

Bocek, M., Fancello, L., Motyka, M., Bocakova, M. & Bocak, L. (2018) The molecular phylogeny of Omalisidae (Coleoptera) defines the family limits and demonstrates low dispersal propensity and ancient vicariance patterns. *Systematic Entomology*, 43: 250–261, DOI: 10.1111/syen.12271. (IF = 4.237)

Kusy, D., Motyka, M., Andujar, C., **Bocek, M.**, Masek, M., Sklenarova, K., Kokas, F., Bocakova, M., Vogler, A. P. & Bocak, L. (2018) Genome sequencing of *Rhinorhipus* Lawrence exposes an early branch of the Coleoptera. *Frontiers in Zoology*, 15: 21, DOI: 10.1186/s12983-018-0262-0. (IF = 3.627)

Kusy, D., Motyka, M., **Bocek, M.**, Vogler, A. P. & Bocak, L. (2018) Genome sequences identify three families of Coleoptera as morphologically derived click beetles (Elateridae). *Scientific Reports*, 8: 17084, DOI: 10.1038/s41598-018-35328-0. (IF =4.122)

Masek, M, Motyka, M., Kusy, D., **Bocek, M.**, Li, Y. &; Bocak, L. (2018) Molecular Phylogeny, Diversity and Zoogeography of Net-Winged Beetles (Coleoptera: Lycidae). *Insects*, 9: 154, DOI: 10.3390/insects9040154. (IF = 1.848)

Bocek, M & Bocak, L. (2017) The comparison of molecular and morphology-based phylogenies of trichaline net-winged beetles (Coleoptera: Lycidae: Metriorrhynchini) with description of a new subgenus. *PeerJ*, 5(e3963), DOI: 10.7717/peerj.3963. (IF = 2.118)

Bocek, M. (2017) New species of *Diatrichalus* (Coleoptera: Lycidae) from New Guinea and the Mollucas. *Zootaxa*, 4247: 577–584. (IF = 0.931)

Bocek, M. & Bocak, L. (2016) Species limits in polymorphic mimetic *Eniclases* net-winged beetles from New Guinean mountains (Coleoptera, Lycidae). *ZooKeys*, 593: 15–35. (IF = 1.031)