PhD in Molecular, Cellular and Environmental Biology
XXXII – Academic Year 2018/2019

Investigating Phylogeny, Biogeography and speciation processes in the genus *Hycleus* Latreille 1817 (Coleoptera, Meloidae)

Filogenesi, Biogeografia e processi di speciazione nel genere *Hycleus* Latreille 1817 (Coleoptera, Meloidae)

Ph.D. Student: Alessandra Riccieri

Tutors: Prof. Marco Alberto Bologna
Dr. Emiliano Mancini

Alessandra Riccieri (2019). **Investigating phylogeny, biogeography and speciation processes in the genus *Hycleus* Latreille 1817 (Coleoptera, Meloidae)**

Doctoral thesis. Department of Science, Roma Tre University, Rome, Italy.

Alessandra Riccieri
Department of Science, Roma Tre University,
Viale Marconi, 446,
00146 Rome, Italy.
e-mail: alessandra.riccieri@uniroma3.it

In the frontispiece: *Hycleus polymorphus,* France, Var, Trigance. June 2017.

“Nothing in Biology makes sense except in the light of evolution”
Theodosius Dobzhansky, 1973

“In Biologia esiste una sola regola fondamentale:
tutte le regole hanno eccezioni.”
Ernst Mayr, 1982

INDEX

GENERAL INTRODUCTION

RIASSUNTO

I Meloidi sono una famiglia di circa 3000 specie, presente in tutto il mondo ad eccezione della Nuova Zelanda, dell'Antartide e di molte isole Polinesiane. Studi morfologici e molecolari ne hanno definito la divisione in tre sottofamiglie (Eleticinae, Nemognathinae, Meloinae) e in 16 tribù. I Meloidi sono noti per lo più per la produzione di cantaridina, una sostanza altamente tossica ed irritante utile a questi insetti come difesa naturale e probabilmente legata anche ad aspetti riproduttivi, nota all'uomo sin dall'antichità per le sue proprietà mediche. Sinapomorfie di questa famiglia sono il ciclo di sviluppo di tipo ipermetabolico e la presenza di larve parassitoidi di Imenotteri Aculeati o di Ortotteri Acridoidei.

In questa tesi è stato studiato per la prima volta in maniera approfondita e con un approccio molecolare il più numeroso tra i 130 generi appartenenti alla famiglia: il genere *Hycleus* Latreille 1817.

Hycleus appartiene alla sottofamiglia delle Meloinae e alla tribù dei Mylabrini e, con almeno 430 specie descritte (più di 500 dopo questo lavoro), può essere definito un genere iper-diverso. La sua distribuzione comprende quasi tutta la regione Paleartica, la regione Afrotropicale (escluso il Madagascar) e gran parte della regione Orientale. Probabilmente a causa di questa elevata diversità, le relazioni tra le specie appartenenti a questo genere non sono mai state esplorate.

Negli anni cinquanta, lo spagnolo Pardo Alcaide, basandosi su alcune caratteristiche morfologiche del mesosterno, suddivise il genere in tre sezioni: Mesoscutatus (mesosterno con uno scuto largo); Mesotaeniatus, (mesosterno con uno scuto stretto); Mesogorbatus, (mesosterno privo di scuto). Successivamente, all'interno di queste sezioni, sono stati descritti alcuni gruppi di specie sulla base di caratteri morfologici comuni. Più di recente, lavori di filogenesi molecolare sulla famiglia e sulla tribù dei Mylabrini hanno evidenziato una stretta affinità tra i generi *Ceroctis* e *Paractenodia* e *Hycleus*, tanto da mettere in dubbio la monofilia di quest'ultimo.

La prima parte di questa tesi è stata dedicata alla ricostruzione della filogenesi di *Hycleus* utilizzando 125 specie e cinque marcatori (nuDNA: CAD, Arginin Chinasi, 28S e Wingless; mtDNA: COI) con lo scopo di: (a) ricostruire le relazioni tra le specie di *Hycleus*; (b) chiarire la posizione filogenetica di *Ceroctis* e *Paractenodia*; (c) comprendere se le tre sezioni descritte su basi morfologiche possano ancora avere un valore tassonomico

ed essere utilizzate per definire dei sottogeneri; (d) delineare la storia biogeografica di *Hycleus*.

I risultati ottenuti hanno evidenziato la presenza di quattro cladi principali uno dei quali include *Ceroctis* e *Paractenodia*, che sono quindi da considerarsi sinonimi di *Hycleus*. Le sezioni descritte su basi morfologiche non hanno ottenuto supporto nelle analisi molecolari, risultando polifiletiche e pertanto non possono essere prese in considerazione come sottogeneri di *Hycleus*. Al contrario, molti dei gruppi di specie descritti su basi morfologiche, sono stati confermati anche dalle analisi molecolari. Per quanto riguarda la storia biogeografica di *Hycleus*, i risultati hanno permesso di ipotizzare una sua origine Miocenica (20 Ma) nel paleo-continente Africano da cui, a seguito di diversi eventi di dispersione probabilmente favoriti dalla presenza della connessione tra Africa e Asia rappresentata dalla Penisola Arabica, sono state colonizzate in tempi diversi le regioni Paleartica, Saharo Sindica e Orientale.

Nella seconda parte della tesi è stata approfondita la storia biogeografica di *Hycleus polymorphus*, una specie con un'interessante distribuzione geografica: l'areale, infatti, si estende dalle steppe del Kazakhstan fino alle praterie d'alta quota delle catene montuose dell'Europa meridionale ed è continuo in Asia centrale e in Europa orientale, mentre risulta frammentato sui Pirenei, sulle Alpi e sulle catene montuose della Penisola Balcanica. Tale distribuzione potrebbe essere legata alle oscillazioni climatiche del Pleistocene che potrebbero quindi aver lasciato tracce nella struttura genetica di *H. polymorphus*. Nel precedente studio sulla filogenesi di *Hycleus* era stata confermata l'attribuzione di *H. polymorphus* ad un gruppo di specie già individuato su base morfologica. Sia precedenti lavori morfologici, sia le più recenti analisi molecolari hanno però evidenziato una serie di incertezze tassonomiche relative alle specie appartenenti a questo complesso che necessitano di essere chiarite. Lo studio della storia biogeografica di *Hycleus polymorphus,* è stato quindi preceduto da un'analisi tassonomica per chiarire l'identità della specie in esame. Attraverso un'analisi filogenetica preliminare, sono state studiate 8 delle 14 specie appartenenti al gruppo *polymorphus*. Quasi tutte sono risultate monofiletiche ad eccezione di *H. humerosus* e di *H. zebraeus*, rispettivamente probabili sinonimi di *H. polymorphus* e di *H. atratus*. Uno studio dedicato che includa tutte le specie del gruppo rimane necessario per descriverne in maniera esaustiva la tassonomia e la sistematica. Una volta identificata correttamente l'unità tassonomica corrispondente a *H. polymorphus*, 103 individui appartenenti a

27 popolazioni sono stati utilizzati per descriverne la storia biogeografica attraverso i marcatori nucleari CAD e ITS2. Mentre il gene CAD, altamente polimorfico, non ha mostrato alcun segnale filogeografico, l’ITS2 ha invece permesso di definire quattro aplogruppi ben distinti da un punto di vista genetico e geografico, ma caratterizzati da una bassa variabilità genetica intra-gruppo. Sulla base dei risultati ottenuti in questo lavoro e delle datazioni molecolari ottenute in precedenza, è stato possibile ipotizzare che quella di *H. polymorphus* sia una storia recente. L’origine della specie è da collocarsi nel Pleistocene molto probabilmente in Asia centro-occidentale, regione da cui, durante uno dei glaciali, l’areale di *H. polymorphus* ha potuto espandersi fino a raggiungere l’Europa occidentale e meridionale seguendo l’avanzamento degli ambienti steppici. Con l’innalzamento delle temperature durante le fasi interglaciali, e la conseguente riduzione degli ambienti steppici, le popolazioni di *H. polymorphus* presenti nell’area Mediterranea sono rimaste confinate prevalentemente ad alta quota sulle catene montuose, dove si sono potuti riadattare ad ambienti di prateria montana xerica con condizioni simili a quelli steppici.

Nella terza ed ultima parte di questa tesi è stato studiato il gruppo di specie di *Hycleus sexmaculatus*. Come per il gruppo di *H. polymorphus*, anche in questo caso il complesso di specie era stato descritto su basi morfologiche e poi confermato attraverso dati molecolari nella prima parte di questa tesi. Al gruppo *sexmaculatus* appartengono 12 specie distribuite prevalentemente nella regione di transizione Saharo-Sindica, con ampia estensione in Medio Oriente e Asia centrale. Lo scopo di questo lavoro è stato di fornire una dettagliata descrizione della morfologia di tutte le specie, tra cui una specie nuova, e delle caratteristiche ecologiche e biogeografiche di ciascuna. Per il riconoscimento dei maschi e delle femmine, che mostrano un accentuato dimorfismo, sono state prodotte nuove chiavi dicotomiche e sono state preparate figure di dettagli morfologici diagnostici. Nel tentativo di definire in maniera più approfondita la sistematica del gruppo, sono stati prima proposti sottogruppi di specie in base a caratteristiche morfologiche e successivamente sono state effettuate analisi filogenetiche molecolari basate sulla Maximum Likelihood e Inferenze Bayesiane, utilizzando una matrice di cinque geni e cinque specie. Le analisi molecolari, seppur basate su un basso numero di specie, sembrano confermare i sottogruppi definiti su base morfologica.

SUMMARY

Among Meloidae, a beetle family of about 3000 species, *Hycleus* (Tribe: Mylabrini) is the most speciose genus with ~430 species described, widely distributed in the Old World. Relationships among those species have never been investigated, but morphological studies divided *Hycleus* in three main sections (Mesogorbatus, Mesoscutatus and Mesotaeniatus) and in several species groups. Recent molecular studies about the family and the Mylabrini tribe highlighted a possible paraphyly of *Hcyleus* with respect to the genera *Ceroctis* and *Paractenodia*.

In the first part of this work, we built a time-calibrated phylogenetic tree based on five genes (nuDNA: CAD, Arginin Chinasi, 28S e Wingless; mtDNA: COI) in order to: (a) explore the phylogenetic relationships among the species of *Hycleus*; (b) define the relationships among *Hycleus*, *Ceroctis* and *Paractenodia*; (c) understand if the three sections may have a taxonomic value; (d) delineate a plausible scenario describing the biogeographic history of the genus. According to our results, four main clades were identified one of which included the species belonging to *Ceroctis* and *Paractenodia*: both taxa are now referred to *Hycleus* as new synonyms. The three sections resulted polyphyletic and do not have taxonomic value, whereas several species groups were confirmed. Biogeographic results indicated a Miocene origin (20 Mya) of *Hycleus* in the African paleo-continent. Subsequently, several dispersal events determined the spread of the genus in the Palaearctic, Saharo Sindian and Oriental Regions in different times.

In the second part of the thesis, we have dealt more closely with the species *Hycleus polymorphus*, which is of particular interest because of its geographic distribution. Its range, in fact, goes from central Asia, where it is probably distributed with continuity, to South-Western Europe, where it shows a mountain distribution with fragmented populations. This distribution might be related to the climatic Pleistocenic fluctuations. *H. polymorphus* belongs to a group of 14 species widely distributed in the Palaearctic region. The systematics of the species of this group is controversial and needs to be clarified to better understand the species boundaries, included those of *H. polymorphus*. Therefore, aim of this work was first to define the species boundaries of *H. polymorphus* and of other species belonging to the *H. polymorphus* group, then to investigate the genetic structure of 27 populations of *H. polymorphus*. Phylogenetic results based on three molecular markers (COI, CAD and ITS2) confirmed the necessity of a taxonomic revision of this group, as species like *H.*

humerosus and *H. zebraeus* resulted synonyms respectively of *H. polymorphus* and *H. atratus* . The biogeographic history of *H. polymorphus* was investigated with the markers CAD, which resulted highly polymorphic and did not show a phylogeographic structure, and ITS2, which, on the contrary, allowed to recognize four well distinct geographic groups, showing low intra-group genetic diversity. According to our results and previously obtained molecular dating, we can hypothesize a recent origin of *H. polymorphus*, likely occurred in the Pleistocene in central Asia. During glacial phases, following the expansion of steppe environments, the range of *H. polymorphus* expanded as well till western Europe. During interglacials, suitable environments for this species in the Mediterranean basin were mainly confined to mountain ranges, forcing populations to survive at higher altitudes.

In the last part of this work we focused on the species group of *Hycleus sexmaculatus*, described according to morphological characters and confirmed by molecular analysis in the first part of this thesis. The *sexmaculatus* complex comprises 12 species, one of which described in this work, and primarily distributed in the Saharo-Sindian Transitional Region. In this third work we produced a morphological revision of the species of this group, proposed new species and new synonymies and published keys and figures of both males and females. Moreover, to better understand the systematic of the species, we explored their phylogenetic relationships using both morphological characters and molecular data. Molecular analyses were conducted using five genes and five species. Even if performed with a reduced number of species, molecular results seem to confirm the relationships among species inferred by morphological data.

GENERAL INTRODUCTION

Meloidae and Mylabrini tribe

Meloidae is a family of Coleoptera Tenebrionoidea including about 3000 species and 130 genera, commonly known as blister beetles (Bologna, 1991; Bologna et al., 2010). This beetle family has a nearly worldwide distribution with no records only in New Zealand, Antarctica and many Polynesian islands. Species can be found mainly in arid and semiarid areas, and in particular the highest diversity of this family occurs in steppe and savannah of tropical and subtropical zones, as well as in the Mediterranean subregion (Bologna, 1991; Bologna et al., 2010).

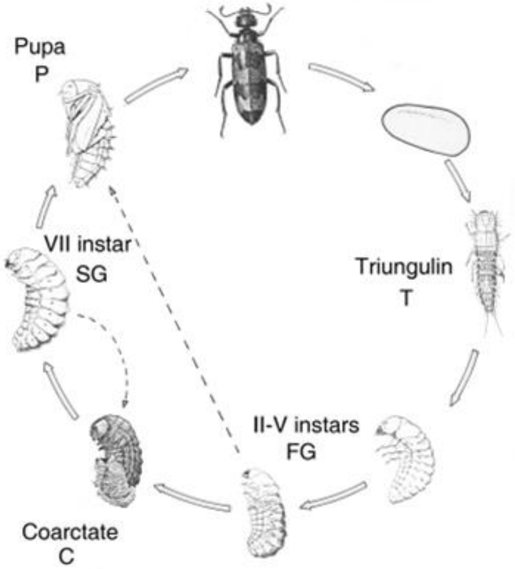


Fig. 1 Blister beetles life cycle (from Bologna et al., 2010)

Blister beetles are known worldwide for the production of Cantharidin, a terpenoid present in the haemolymph and softer parts of the body of the adults, released through reflex bleeding when the beetles are disturbed and probably used also as aggregative substance (Bologna, 1991; Bologna et al., 2010). The medical properties of cantharidin were discovered independently in several parts of the world as early as 2000 years ago (Bologna et al., 2010). Nowadays, it is still of large use in the Traditional Chinese Medicine and is also studied for its anticancer properties (Wu et al., 2018). In some parts of the world, mainly in Asia and Africa, Meloidae are also known as pests for agricultural cultivations, due to the phytophagous diet and gregarious habits of the adults (e.g. Adams and Selander, 1979; Lebesa et al., 2012). Another peculiar characteristic of blister beetles is the hypermetabolic larval development, absent only in the primitive subfamily Eleticinae (Bologna, 1991; Bologna et al., 2008c; Bologna et al., 2010). Meloid hypermetaboly is characterized mainly by seven (I-VII) larval instars, with at least five morphologically and biologically distinct phases (Fig. 1; Bologna, 1991; Bologna et al., 2008c; Bologna et al., 2010): 1. the "triungulin" phase, which actively reach the host's nest, in some cases by phoresy; 2. the second phase includes the feeding larval instars II-V; 3. the hypnotheca, a quiescent sixth instar; 4. the posthypnothecal instar VII; 5. the pupa.

Blister beetle larvae are also known because of their parasitoid biology: they, in fact, feed on grasshoppers (Orthoptera, Acridoidea) egg pods or on the provisions or immature larval stages of various species of Hymenoptera (mainly Aculeata) (Bologna, 1991; Bologna et al., 2010).

The evolutionary history and the systematics of the family were deeply studied both with morphological and molecular approach (Bologna and Pinto, 2001; Bologna et al., 2008c). According to these studies, Meloidae have been divided into three subfamilies and 16 tribes: the primitive subfamily Eleticinae, distributed in South America, Africa south of Sahara, India and southeastern Asia, including the tribes Derideini, Morphozonitini, Spasticini and Eleticini; the cosmopolitan subfamily Nemognathinae, the only one present also in Australia, including the tribes Stenoderini, Palaestrini, Horinii and Nemognathini; and finally the most diverse subfamily Meloinae, widely distributed as well, including the tribes Lyttini, Pyrotini, Eupomphini, Cerocomini, Epicautini, Mylabrini, Meloini, Tetraonycini.

Mylabrini, is the largest tribe of Meloidae (Fig. 2; Bologna, 1991; Bologna and Pinto, 2002; Bologna et al., 2005; Pan et al., 2013; Salvi et al, 2019), with about 750 species assigned to 11 genera, distributed only in the Old World and centred in the Afrotropical region. Species of this tribe are generally parasitoids of Acridoidea, or, in few cases, of megachilid bees (Bologna and Pinto, 2002).

Fig. 2 Some Mylabrini blister beetles. First row, left to right: *Mylabris calida* (by D. Salvi), *Mylabris variabilis* (by D. Salvi), *Mylabris intermedia* (by P. Gorbunov), *Hycleus hemprichi* (by O. Rittner). Second row, left to right: *Hycleus tricolor* (by R. Perissinotto), *Ammabris elegans* (by O. Rittner), *Ceroctis capensis* (by R. Perissinotto), *Croscherichia sanguinolenta* (by O. Rittner). (From Salvi et al., 2019)

Within the subfamily Meloinae, Mylabrini are distinguishable by antennal and mesosternal morphology (Bologna and Pinto, 2002; Bologna et al., 2008a; Pan et al., 2013; Salvi et al., 2019). In particular, antennomeres are distinctly widened to apex (except in *Lydoceras* Marseul 1870 and in a few *Hycleus* Latreille 1817) and are variable in number from seven to 11. The anatomy of mesosternum, instead, presents a modified anterior portion distinct from the remaining area by the absence of punctures (Bologna and Pinto, 2002). Salvi et al. (2019) rearranged the taxonomy of mylabrine genera, elevating *Ammabris* to the genus level and considering *Pseudabris* as subgenus of *Mylabris.*

The monophyly of this tribe is strongly supported by morphological and molecular studies (Bologna, 1991; Pinto and Bologna, 1999; Bologna and Pinto, 2001, 2002; Bologna et al., 2005; Bologna et al., 2008c; Salvi et al., 2019), and in the last decades the revision of six out of 11 mylabrine genera have been published: *Croscherichia* Pardo Alcaide 1950 (Bologna and Coco, 1991), *Mimesthes* Marseul 1872 (Bologna, 2000), *Actenodia* Laporte de Castelnau1840 (Bologna et al., 2008b), *Lydoceras* (Bologna et al., 2011), *Pseudabris* Fairmaire (Pan et al., 2013; now included in *Mylabris* as subgenus), *Mylabris* Gyllenhal 1810 (Salvi et al., 2019).

The genus *Hycleus*

Among the 11 described genera of Mylabrini there is *Hycleus*, the largest of the entire family of Meloidae with about 430 species described (Bologna and Pinto, 2002; Bologna et al., 2005). *Hycleus* is widespread in the Palaearctic Region, in the Oriental Region (East to Timor) and in the Afrotropical Region (Madagascar excluded) where the highest number of species occurs (Bologna and Pinto, 2002). As most of Meloidae, species of this genus inhabit mainly open habitats as temperate steppe and arid regions or tropical and subtropical savannahs (Bologna and Pinto, 2002). Almost all the species are parasitoids of Acrididae's egg pods, only two (*H. wagneri, H. octodecimmaculatus*) are recorded as parasitoids of *Osmia* bees (Megachilidae) (Bologna, 1991; Bologna and Pinto, 2002).

Probably, because of its large number of species, the systematics and the evolutionary history of the genus *Hycleus* have never been investigated.

Due to their ostensible similarity, *Mylabris* and *Hycleus* have been often confused and considered as a single taxonomic unit (Bologna et al., 2005), but can be morphologically distinguished for the presence in *Hycleus* of a mesepisternum delimited by a furrowed border, and an aedeagus with two apical hooks (Bologna and Pinto, 2002).

According to the mesosternal morphology, and in particular to the presence or absence of a "scutum", *Hycleus* has been divided by Pardo Alcaide (1950, 1954, 1955, 1958) into three main sections: Mesoscutatus, including about 300 species with a prominent scutum; Mesotaeniatus, including about 60 species with a less evident scutum; and Mesogorbatus, including about 70 species without the scutum. In the genus *Mylabris* the shape of mesosternum has been recognized as a fundamental character to delimit subgenera (Salvi et al., 2019), and an accurate phylogenetic study will clarify if that is true even for *Hycleus*.

Within the three sections, several groups of species were described according to morphological features [e.g. group of *Hycleus polymorphus* (Bologna, 1991); group of *Hycleus phaleratus* (Pan et al. 2014); group of *Hycleus amoenus* (Bologna et al., 2018); group of *Hycleus sexmaculatus*

(Bologna and Turco, 2007); group of *Hycleus brunnipes* (Bologna and Turco, 2007) etc.], but the phylogenetic value of these species groups has never been investigated.

A controversial debate involving the genus *Hycleus* (Bologna, 1991; Bologna and Coco, 1991; Bologna, 2000; Bologna et al., 2005; Bologna et al., 2008c; Salvi et al., 2019), regards the phylogenetic position of two other Mylabrini genera: *Ceroctis* Marseul 1870 and *Paractenodia* Péringuey 1904. *Ceroctis* includes about 59 species, distributed in Africa (except the western regions, Madagascar and the inner Sahara), western Arabia, Sinai and Palestine; *Paractenodia* is a small genus of only four species endemic to Namibia and western South Africa (Bologna and Pinto, 2002). Both *Ceroctis* and *Paractenodia* share the same mesepisternal and aedeagal characteristics of *Hycleus*, but are differentiated by the antennal shape and palpal structure of first instar larvae (Bologna et al., 2005). In particular, species belonging to *Ceroctis* have a very peculiar flabellate antennal aspect (Fig. 3C) whereas *Paractenodia* species have antennae with only seven antennomeres instead of those of *Hycleus* which varies from eight to 11 (Bologna and Pinto, 2002). The strict affinity between *Hycleus* and *Ceroctis* and *Paractenodia* was pointed out by both morphological (Bologna and Pinto, 2001; Bologna et al., 2005) and molecular preliminary works (Bologna et al., 2005; Bologna et al., 2008c; Salvi et al., 2019), but further dedicated analysis with a larger number of samples of the three genera are required to disentangle the phylogenetic relations among them.

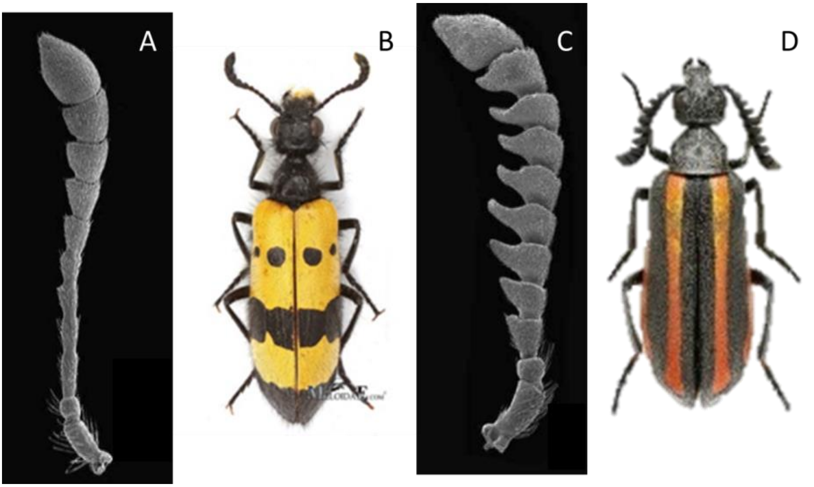


Fig. 3. Antennal morphology (A, C; from Bologna and Pinto, 2002) and habitus (B, D) of *Hycleus* and *Ceroctis*. A-B *Hycleus tripunctatus*; C. *Ceroctis exclamationis*; D. *Ceroctis* sp.

Aims

The main aim of this PhD thesis is to investigate the evolutionary and biogeographic history of the genus *Hycleus* with a molecular approach. Moreover, we tested the taxonomic value of the sections (Mesoscutatus, Mesotaeniatus and Mesogorbatus) and the species groups proposed within this genus, and clarified the relationships among *Hycleus*, *Ceroctis* and *Paractenodia*.

In the second part of this thesis, we choose to explore the biogeographic history of *Hycleus polymorphus*, to understand if the climatic fluctuations of Pleistocene might have affected its peculiar distribution. However, since the taxonomy of its species complex is poorly resolved, we first needed to clarify the species boundaries, in particular those of *H. polymorphus*, with a phylogenetic approach.

Finally, the third objective of this work was that to revise morphologically the species group of *Hycleus sexmaculatus*, and to investigate phylogenetic relationships among species using morphological and molecular data.

REFERENCES

Adams, C. L., & Selander, R. B., 1979. The biology of blister beetles of the vittata group of the genus Epicauta (Coleoptera, Meloidae). Bulletin of the AMNH; v. 162, article 4.

Bologna, M.A., 1991. Coleoptera Meloidae. Fauna d'Italia. XXVIII, Calderini, Bologna.

Bologna, M.A., 2000. Revision of the genus *Mimesthes* (Coleoptera: Meloidae: Mylabrini). European Journal of Entomology 97, 75–84.

Bologna, M.A., Amore, V., Pitzalis M., 2018. Meloidae of Namibia (Coleoptera): taxonomy and faunistics with biogeographic and ecological notes. Zootaxa 4373, 1–14.

Bologna, M.A., Coco, E., 1991. Revisione del genere *Croscherichia* Pardo Alcaide, 1950 (Coleoptera, Meloidae). Memorie della Società Entomologica Italiana. 69 (1990), 97-180.

Bologna, M.A., D'Inzillo, B., Cervelli, M., Oliverio, M., Mariottini, P., 2005. Molecular phylogenetic studies of the Mylabrini blister beetles (Coleoptera, Meloidae). Molecular Phylogenetics and Evolution 37, 306–311.

Bologna, M.A., Di Giulio, A., Pitzalis, M., 2008a. Examples of disjunct distributions between Mediterranean and southern or eastern Africa in Meloidae (Coleoptera, Tenebrionoidea). Biogeographia (n.s.) 29, 81-98.

Bologna, M. A., Di Giulio, A., & Pitzalis, M., 2008b. Systematics and biogeography of the genus *Actenodia* (Coleoptera: Meloidae: Mylabrini). *Systematic Entomology*, *33*(2), 319-360

Bologna, M.A., Oliverio, M., Pitzalis, M., Mariottini, P., 2008c. Phylogeny and evolutionary history of the blister beetles (Coleoptera, Meloidae). Molecular Phylogenetics and Evolution 48, 679–693.

Bologna, M.A., Pinto, J.D., 2001. Phylogenetic studies of the Meloidae (Coleoptera), with emphasis on the evolution of phoresy. Systematic Entomology 26, 33–72.

Bologna, M.A., Pinto, J.D., 2002. The Old World genera of Meloidae (Coleoptera): a key and synopsis. Journal of Natural History 36, 2013–2102.

Bologna, M.A., Pitzalis, M. & Viglioglia, V., 2011. Revision of the Afrotropical mylabrine genus *Lydoceras* Marseul, 1870 (Coleoptera: Meloidae). African Entomology, 19, 70–87.

Bologna, M.A., Turco, F. & Pinto, J.D., 2010. 11.19. Meloidae Gyllenhal, 1810. Handbook of Zoology. Coleoptera, Beetles, Volume 2: Morphology and Systematics (Elateroidea, Bostrichiformia, Cucujiformia partim) (Vol. ed. by R.A.B. Leschen, R.G. Beutel and J.F. Lawrence), Arthropoda: Insecta (ed. by N.P. Kristensen and R.G. Beutel), pp. 681–693. De Gruyter, Berlin/New York, New York.

Lebesa, L.N., Khan, Z.R., Krüger, K., Bruce, T.J.A., Hassanali, A., Pickett, J.A., 2012. Farmers' knowledge and perceptions of blister beetles, *Hycleus* spp. (Coleoptera: Meloidae), as pest herbivores of *Desmodium* legumes in western Kenya. International Journal of Pest Management 58, 165–174.

Pan, Z., Ren, G. D., Wang, X. P., Bologna, M. A., 2013. Revision of the genus Pseudabris Fairmaire (Coleoptera, Meloidae), an endemic to the Tibetan Plateau, with biogeographical comments. Systematic entomology, 38, 134-150.

Pan, Z., Carosi, M., Bologna, M.A., 2014. A new Eastern Asian Hycleus and key to the Chinese species of the phaleratus group (Coleoptera, Meloidae, Mylabrini). ZooKeys 463, 11–19.

Pardo Alcaide 1950. Estudios sobre "Meloidae". II. Los "Mylabrini" de la Peninsula Iberica. Boletin de Patóologia vegetal y de Entomologia Agricola. 17, 61-82.

Pardo Alcaide, A., 1954. Études sur les Meloidae. V. Les Mylabrini du Maroc et du Sahara occidental espagnol (Col. Meloidae). Bulletin de la Société des Sciences naturelles et physiques du Maroc 34, 55–88.

Pardo Alcaide, A., 1955. Estudios sobre Meloidae VI. Sobre algunos Mylabris (s. lat.) de la región etiópica. Bulletin de l'Institut royal des sciences naturelles de Belgique 31, 1–32.

Pardo Alcaide, A., 1958. Études sur les Meloidae (Col.). IX. Observations sur quelques *Mylabris* (s.l.) des régions éthiopienne et orientale. Bulletin de l'Institut royal des sciences naturelles de Belgique 35, 1–39.

Pinto, J.D. & Bologna, M.A., 1999. The New World genera of Meloidae (Coleoptera): a key and synopsis. Journal of Natural History, 33, 569–619.

Salvi, D., Maura, M., Pan, Z., Bologna, M.A., 2019. Phylogenetic systematics of *Mylabris* blister beetles (Coleoptera, Meloidae): a molecular assessment using species trees and total evidence. Cladistics 35, 243-268.

Wu, Y., Liu, Y., & Chen, X., 2018. The complete mitochondrial genomes of *Hycleus cichorii* and *Hycleus phaleratus* (Coleoptera: Meloidae). Mitochondrial DNA Part B, 3, 159-160.

Molecular Phylogenetics and Evolution 144 (2020) 106706

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogeny, biogeography and systematics of the hyper-diverse blister beetle genus *Hycleus* (Coleoptera: Meloidae)

Alessandra Riccieri[a,*], Emiliano Mancini[b], Daniele Salvi[c,d], Marco A. Bologna[a]

[a] Department of Sciences, University “Roma Tre”, Viale G. Marconi 446, 00146 Roma, Italy
[b] Department of Biology and Biotechnology “C. Darwin”, “Sapienza” University of Rome, Viale dell’Università 32, 00186 Roma, Italy
[c] Department of Health, Life & Environmental Sciences, University of L’Aquila, Via Vetoio snc, 67100 L'Aquila-Coppito, Italy
[d] CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

ARTICLE INFO

Keywords:
Molecular phylogenetics
Taxonomy
Mylabrini
Old World
BioGeoBEARS

ABSTRACT

Hycleus is a hyper-diverse genus of blister beetles including ~500 species widely distributed in the Old World, currently divided into three “sections” and into 45 “phenetic” species groups according to morphological characters. Recently the monophyly of *Hycleus* was questioned pointing out its paraphyly with respect to the genera *Ceroctis* and *Paractenodia*. In this study, we built a time-calibrated phylogenetic tree based on DNA sequence data from mitochondrial and nuclear genes obtained from 125 species, to understand the phylogenetic relationships among the species of this genus, to infer the biogeographic processes behind their diversification, and to assess their taxonomy and classification. Our results identified four main lineages one of which included the species belonging to *Ceroctis* and *Paractenodia*; therefore, both taxa are now referred to *Hycleus* as **new synonyms**. The three described sections of *Hycleus* resulted polyphyletic and are rejected, whereas several species groups represented well supported clades. *Hycleus* likely originated in Africa during the Early Miocene (~20 Mya), and subsequently spread in Europe and western Asia. Later, in the Late Miocene (~6 Mya) a Saharo-Sindian group branched off from the Palaearctic lineage, whereas the Oriental Region was colonized following a dispersal event through the Arabian Peninsula from the Afrotropical Region (~5 Mya).

1. Introduction

Meloidae is a widely distributed family of beetles characterized by some peculiar biological and ecological traits like the hypermetabolic larval development or the parasitoid habits of larval stages (Bologna, 1991; Bologna and Pinto, 2002). Blister beetles are also known for the production of cantharidin, a toxic terpene used since the ancient time to treat several diseases (e.g. Bologna, 1991; Moed et al., 2001; Bologna and Pinto, 2002) and nowadays studied for its antitumoral properties (e.g. Wang et al., 2000; Yang et al., 2007; Liu and Chen, 2009; Puerto Galvis et al., 2013; Zhang et al, 2014). The family includes 130 genera and about 3000 species, nearly half of which (~1300) belong to only five genera. Among them, the genus *Hycleus* Latreille, 1817 (subfamily Meloinae, tribe Mylabrini), with more than 450 species, is the most speciose genus of Meloidae (Bologna and Pinto, 2002; Bologna et al., 2005).

The hyper-diverse genus *Hycleus* is widely distributed in the Old World, with the highest diversity occurring in the Afrotropical region (more than 300 species, only absent in Madagascar; Bologna and Pinto, 2002; Fig. 1). *Hycleus* species are strictly associated to open habitats such as tropical and subtropical savannahs and temperate steppes (Bologna and Pinto, 2002; Bologna et al., 2018c). Like most of Mylabrini, larvae of *Hycleus* are predators of egg pods of Acrididae grasshoppers and only two species are known to be parasitoid of wild bees (Apoidea; Bologna, 1983, 1991; Bologna and Pinto, 2002).

The reconstruction of the evolutionary and biogeographic history of *Hycleus* may provide insights into the processes underlying the origin of such a great biodiversity and clarify the internal relationships of a hyper-diverse taxon - a great challenge in systematics. Because of the high species richness of *Hycleus*, phylogenetic relationships within the genus are currently unknown, though the need of a revision has been repeatedly emphasized (Bologna et al., 2008c; Bologna and Pinto, 2002; Pan et al., 2014; Salvi et al., 2019). According to the shape of mesosternum, and particularly to the presence of a modified fore area called “scutum”, Pardo Alcaide (1954, 1955) formerly divided *Hycleus* into three main “sections”: Mesoscutatus, Mesotaeniatus and Mesogorbatus. Furthermore, several “phenetic species groups” were defined within each of these intra-generic sections according to various morphological

---

[*] Corresponding author.
E-mail address: alessandra.riccieri@uniroma3.it (A. Riccieri).

https://doi.org/10.1016/j.ympev.2019.106706
Received 9 May 2019; Received in revised form 1 October 2019; Accepted 8 December 2019

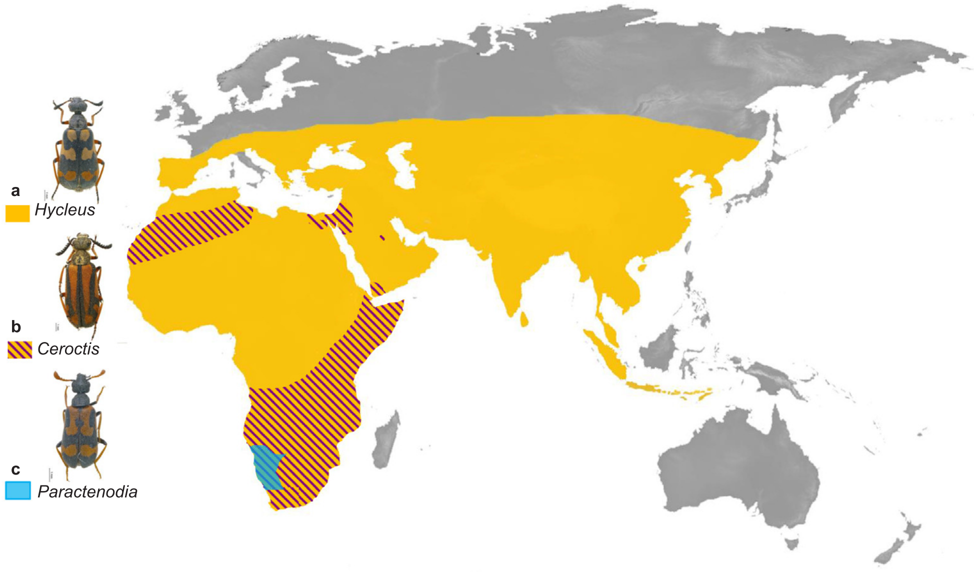


Fig. 1. Geographic distribution of *Hycleus*, *Ceroctis* and *Paractenodia*; a. *Hycleus deserticolus*; b. *Ceroctis angolensis*; c. *Paractenodia parva*;

characters of adults (Bologna, 1978, 1979, 1990, 1991, 1994a, 1994b; Bologna et al., 2018c; Bologna and Turco, 2007; Pardo Alcaide, 1954, 1955, 1958a, 1958b, 1963, 1966, 1968, 1969; for details on species groups see Table S3). However, both sections and species groups have never been assessed using a phylogenetic approach, thus it is still unclear whether they represent natural groups (i.e. evolutionary lineages) or not. Moreover, a recent molecular phylogeny based on multiple loci (Salvi et al., 2019; see also Bologna et al., 2008c) questioned the monophyly of *Hycleus* itself, as this genus resulted paraphyletic to both *Ceroctis* Marseul, 1870 (59 species, with a disjunct distribution in the tropical Africa and the Saharan-Arabic deserts: Bologna et al., 2008b; Fig. 1) and *Paractenodia* Péringuey, 1904 (5 species, endemic to Namibia and north-western South Africa: Bologna et al., 2018c; Fig. 1) genera. Hence, understanding the phylogenetic relationships among these three genera based on extensive taxon sampling is required to assess their taxonomy and classification (Salvi et al., 2019).

In this work, we reconstruct a time-calibrated phylogeny of the hyper-diverse genus *Hycleus* based on a multilocus approach, including both mitochondrial and nuclear genes, with the main aim to understand the phylogenetic relationships within this genus and to infer the biogeographic processes behind its diversification. We also aimed to test the taxonomic value of the sections and the species groups proposed within this genus, and to resolve the relationships among *Hycleus, Ceroctis* and *Paractenodia* in order to provide an evolutionary framework for the classification of these taxa.

2. Materials and methods

2.1. Taxon sampling

Our sampling included 101 species of *Hycleus*, 18 species of *Ceroctis* and 3 species of *Paractenodia*, all stored in the collection of Marco A. Bologna (MAB), housed at Roma Tre University (Table S1). We gathered molecular data from at least one individual per species. However, when available, for taxa with a wide (and/or fragmented) distribution range, additional specimens belonging to the most distant populations available within their geographic ranges were considered [i.e. *Ceroctis trizonata* (Reiche, 1866), *Hycleus brunnipes* (Klug, 1845)]. Similarly, two or more specimens were included in our analyses for those species whose morphological identification was not straightforward [i.e. *H. humerosus* (Escherich, 1899), *H. polymorphus* (Pallas, 1771), *H. zebraeus* (Marseul, 1870)]. Specimens were preserved in ethanol 96% and identified based on morphological characters by a stereomicroscope Olympus SZX12 by MAB. Three additional specimens from other genera and tribes were used as outgroups, according to previous phylogenetic studies (Bologna et al., 2005, 2008c; Salvi et al., 2019): *Mylabris schreibersi* Reiche, 1866, *M. connata* (Rey, 1892) (Mylabrini); *Oenas sericeus* (A.G. Olivier, 1795) (Lyttini).

2.2. Laboratory procedures

Total genomic DNA was extracted from one or more legs of each sample, following the salting out protocol (Sambrook et al., 1989), and eluted in 100 µl H_2O milliq and stored at −20 °C. Sequences were obtained from one mitochondrial gene fragment, the Cytochrome Oxidase subunit I (COI) and four nuclear gene fragments: the Arginine Kinase (ARGK); a portion of the rudimentary gene (CAD), the Carbamoylphosphate Synthetase domain; the gene Wingless (WG); and a portion of the 28S rDNA gene.

Polymerase Chain Reactions (PCR) amplifications were carried out in a final volume of 25 µl, containing 3 µl of 10x reaction buffer, 1/1,5/3 µl of $MgCl_2$ (50 mM), 0,5/1 µl dNTPs (10 mM), 0,2 µl of TaqDNA polymerase (5 U/µl; BIOTAQ Bioline), 0,5µl of each primer (25 mM) and 1 µl of DNA template. Information about the PCR primers and thermal cycles used are reported in Table S2. Three µl of PCR products were used to determine amplification success by agarose (1%) gel run stained with 1 µl of SimplySafe (EurX). Amplified products were purified and sequenced by Macrogen.

GenBank accession numbers are listed in Table S1. Our final dataset was completed with the addition of CAD and COI sequences of six species (see Table S1) deposited in GenBank by Salvi et al. (2019).

2.3. Alignment, model selection and phylogenetic analysis

Sequences were edited using Staden Package v4.11.2 (Staden et al., 2000), and aligned with MAFFT v7 (Katoh et al., 2019). The number of variable, parsimony-informative and singleton sites was calculated with MEGA v7 (Kumar et al., 2016) in the concatenated dataset.

Phylogenetic analyses were first performed using single-gene alignments, then building combined datasets. Two different combined datasets were used in order to verify the effect of missing data at certain loci (i.e. loci that failed to be amplified in some specimens) on the resulting trees: one dataset excluding specimens with missing loci, i.e. including only specimens for which data from all the five genes were obtained (102 species), and a second dataset including all specimens for which at least two out of the five genes were successfully sequenced (125 species; 5.5% missing sequences). Since these preliminary phylogenetic trees showed similar topologies and node supports, we used the larger dataset with 125 species for final downstream analyses.

Phylogenetic analyses were performed with Maximum Likelihood (ML) and Bayesian Inference (BI) methods. For all the five genes, the GTR + G + I was selected as the best substitution model with JModeltest v2.1.6 (Posada, 2008) according to the Akaike Information Criterion (AIC). ML analyses were carried out using RAxML-HPC v8.2.10 (Stamatakis, 2006) with a partitioned GTRGAMMA model and a rapid-bootstrap analysis with 1000 replicates. BI analyses were performed with two independent MCMC runs, with four chains each, on Mr Bayes v3.2.6 (Ronquist et al., 2012) for 30 million generations with a burn-in of 3 million generations (10%). Trees were sampled every 1000 generations and Tracer v1.6 (Rambaut et al., 2014) was used to assess convergence. Phylogenetic analyses were carried out through the CIPRES web portal (http://www.phylo.org). FigTree v1.3.1 (Rambaut and Drummond, 2009) was used to depict the trees.

2.4. *Topology test*

To test for the topological congruence between the concatenated tree and single-gene trees, we independently enforced the main nodes recovered in the multilocus tree (Table 1) in single-gene topologies, using the command –g in RaxML-HPC v8.2.10 (Stamatakis, 2006). Gene trees with enforced nodes were compared with the best gene trees recovered by ML analyses using the Shimodaira-Hasegawa (SH; Shimodaira and Hasegawa, 1999) and the approximately unbiased (AU; Shimodaira, 2002) tests through the IQ-TREE web server (Trifinopoulos et al., 2016).

2.5. *Molecular clock and biogeographic analysis*

To estimate the age of divergence of lineages, a molecular clock analysis was performed in BEAST v1.8.4 (Drummond et al., 2012) as implemented in the CIPRES web portal, using the combined dataset. The input file was built using BEAUTi with the following settings: unlinked substitution models (HKY + G + I), unlinked molecular clock model (Uncorrelated relaxed lognormal), linked tree model with the fixed topology previously obtained by Bayesian analysis. Molecular clock was calibrated according to the rates estimated by Papadopoulou et al. (2010) for the COI of Tenebrionidae, a family related to Meloidae (lognormal prior, mean in real space = 0.00178, stdev = 0.075). For all other genes we used an uninformative prior on clock rate (uniform prior from 0 to 0.25). BEAST was run two times for 200 million generations, sampling every 20.000 generations; convergence of runs and ESS values higher than 200 were assessed with Tracer v1.6 (Rambaut et al., 2014) discarding the first 50 million generations as burn-in. Subsequently, Log Combiner v1.8.4 was used to combine log files and tree files retaining the first 10,000 states and trees, and Tree Annotator v1.8.4 was employed to produce the MCC tree (maximum clade credibility) that was used for biogeographic analyses.

Table 1
Results of the SH and AU tests. Values marked with * show the congruence between unconstrained and constrained topologies ($p > 0.05$).

Constrained Nodes		28S	ARGK	CAD	COI	WG
A	**ΔLnL**	9.96	60.52	73.33	199.81	55.88
	SH	0.599*	0.072*	0.028	0.000	0.009
	AU	0.347*	0.000	0.003	0.000	0.005
B	**ΔLnL**	4.91	26.80	14.91	84.22	30.87
	SH	0.722*	0.452*	0.608*	0.052*	0.121*
	AU	0.388*	0.078*	0.200*	0.003	0.070*
C	**ΔLnL**	43.70	62.13	155.35	141.38	67.77
	SH	0.032	0.075*	0.000	0.000	0.002
	AU	0.006	0.002	0.000	0.000	0.000
C1	**ΔLnL**	18.16	36.69	29.31	5.23	73.67
	SH	0.230*	0.043	0.138*	0.587*	0.000
	AU	0.124*	0.025	0.111*	0.403*	0.000
D	**ΔLnL**	55.53	117.19	149.70	199.91	46.09
	SH	0.008	0.001	0.000	0.000	0.024
	AU	0.000	0.000	0.000	0.000	0.006
H	**ΔLnL**	2.04	1.87	19.40	1.53	0.54
	SH	0.634*	0.661*	0.235*	0.593*	0.758*
	AU	0.528*	0.474*	0.093*	0.496*	0.454*

Biogeographic analyses considered the following biogeographic regions: Palaearctic Region; Oriental Region; Saharo-Sindian Transitional Region; Afrotropical Region (Fig. 3). The time calibrated tree obtained from BEAST was pruned to exclude the outgroups and to include only one individual per species. The analysis was performed using the R package BioGeoBEARS (Matzke, 2013a; Matzke, 2013b) that implements three models for the reconstruction of ancestral range: DEC (Dispersal-Extinction-Cladogenesis, Ree et al., 2005); DIVALIKE (a likelihood version of dispersal-vicariance DIVA; Ronquist, 1997); and BAYAREALIKE (a likelihood version of BayArea model; Landis et al., 2013). All models were applied both including or excluding the +j parameter (emulating the founder effect speciation; Matzke, 2014). The obtained models were compared in BioGeoBEARS to choose the best fitting model given the data.

3. Results

3.1. *Phylogenetic analyses and taxonomic assessment of Hycleus*

Our final dataset consisted of 3271 bp (28S: 831 bp, ARGK: 671 bp, CAD: 726 bp, COI: 597 bp, WG: 446 bp) obtained from 134 specimens. Overall, we found 1239 variable sites (38%), 942 parsimony-informative sites (28%) and 295 singletons (9%). For 79% of specimens we obtained the whole targeted sequences for all genes; 17% of specimens had one missing locus, 2% had two missing loci, and 2% had three missing loci.

The trees obtained from the concatenated dataset by ML and BI showed congruent topologies, therefore, only the Bayesian tree topology is shown in Fig. 2. Nodes were considered supported when bootstrap (BS) values were ≥70 and Posterior Probabilities (PP) ranged between 0.95 and 0.98, and highly supported if BS ≥90 and PP ≥0.98.

Phylogenetic results showed four main clades (clades A, B, C, D in Fig. 2). Clade A is strongly supported (BS = 94, PP = 1) and is represented by Afrotropical species and the Saharo-Sindian *C. trizonata*. *Hycleus tripunctatus* (Thunberg, 1791) and *H. hemprichi* (Klug, 1845) have a sister relationship with clade A, these nodes received low statistical support (BS = 50, PP = 0.53). Clade B is well supported (BS = 94, PP = 1) and includes Saharo-Sindian and Palaearctic species (clades R and S). Clade C shows high support (BS = 92, PP = 1), and includes Afrotropical species [clades O and P, and *H. maculiventris* (Klug, 1845)]. Within clade C the subclade C1 contains Palaearctic species [mainly in clades K, L, and N) and Saharo-Sindian species (mainly in clade M). Clade D is well supported in both ML (BS = 95) and BI (PP = 0.98), although the relationships among species are better resolved in the Bayesian tree. Clade D is represented by Afrotropical species and a small subclade of Oriental species (clade H).

Single-gene trees based on BI and ML methods presented topological differences among them and with the multilocus tree (Fig. S1a–j). Topology tests (AU and SH) showed that single-gene trees enforcing the monophyly of clades B and H are not significantly different from the best ML gene trees. The monophyly of clades A, C and C1 is supported by some single-gene datasets, whereas significant differences are recovered between any gene tree enforcing the monophyly of clade D compared with the relative (unconstrained) ML gene tree (see Table 1).

Species belonging to *Ceroctis* and *Paractenodia* are intermixed with

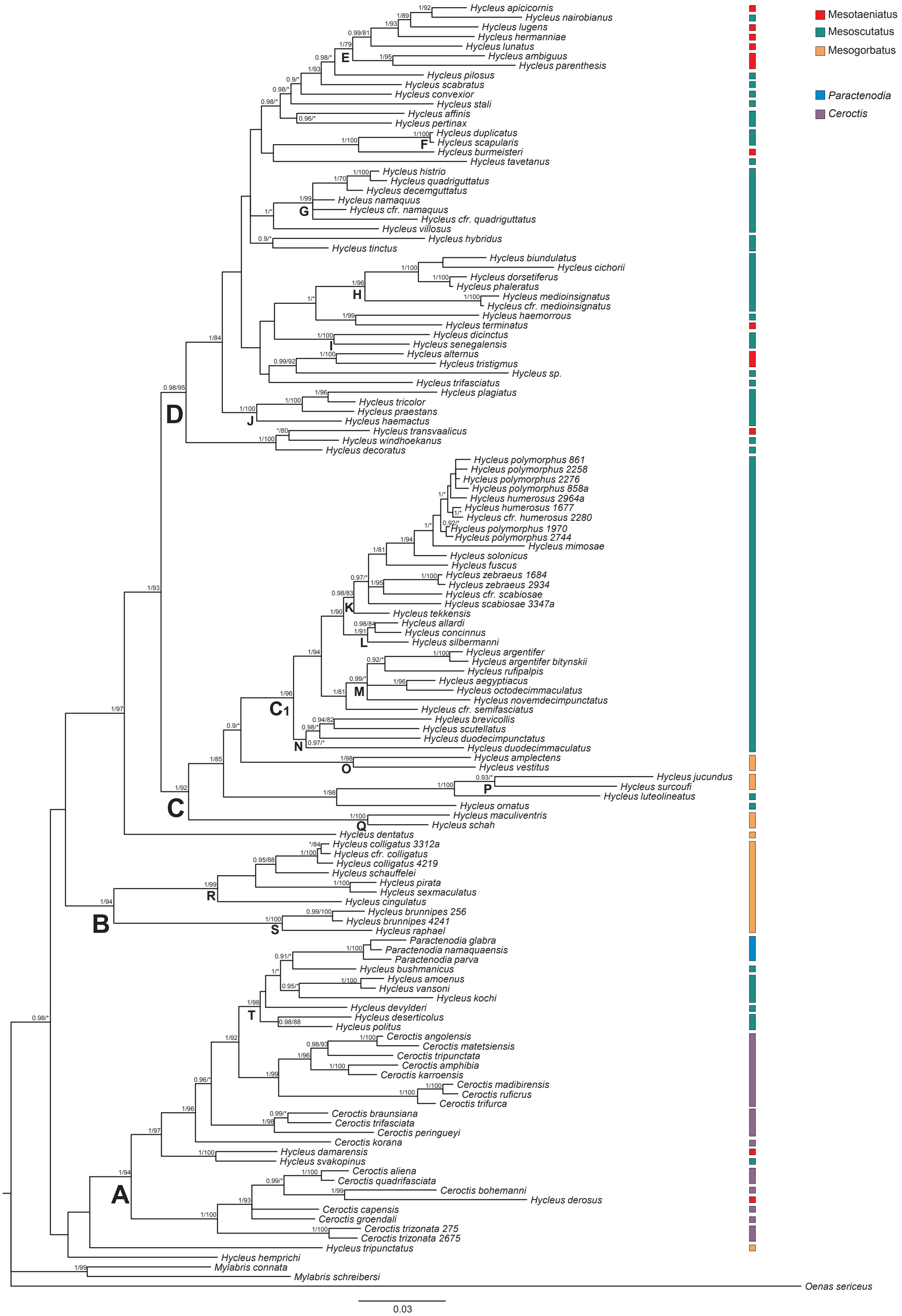


Fig. 2. Bayesian tree based on the concatenated dataset of COI, CAD, 28S, ARGK, and WG gene fragments. Support for Bayesian (BI) and Maximum Likelihood (ML) analyses is reported at each node: on the left Bayesian Posterior Probability (PP); on the right Bootstrap values (BS). Not supported values are indicated with *. Main clades are indicated with letters A-D; species groups are indicated with letters E-T (see Table S3 for further details). Species belonging to the three sections and to *Ceroctis* and *Paractenodia* are indicated with different colours (see Legend).

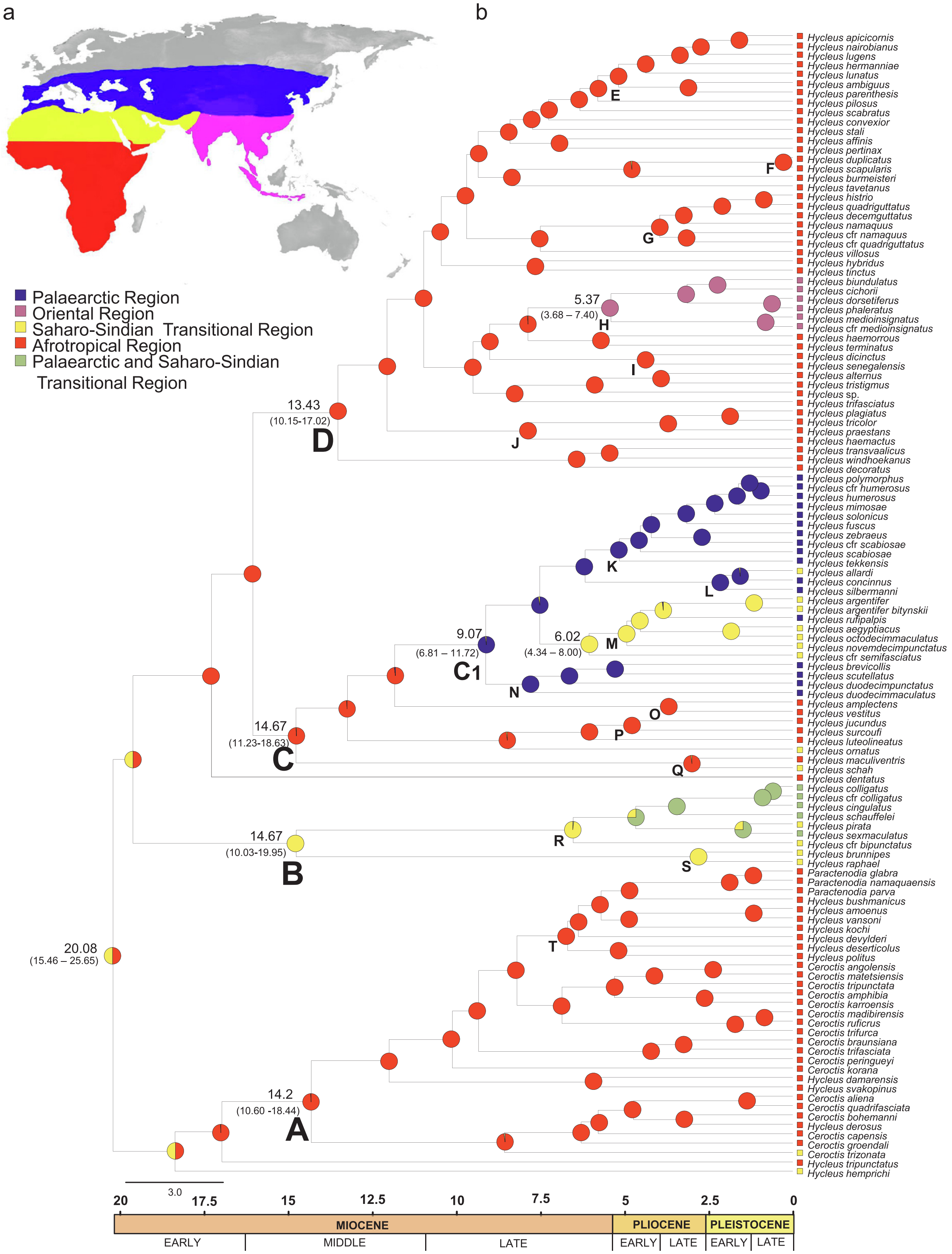


Fig. 3. a. Map of the regions selected for biogeographic analyses; b. Biogeographic analyses results based on the BAYAREALIKE model in BioGeoBEARS.

those of *Hycleus* in both concatenated and single phylogenetic trees (Fig. 2, see Clade A; Fig. S1a–j). Due to such a paraphyletic condition of *Hycleus,* we propose the following new synonymies: *Ceroctis* Marseul, 1870 = *Hycleus* Latreille, 1817, **syn. nov.**; *Paractenodia* Péringuey, 1904 = *Hycleus* Latreille, 1817, **syn. nov.**.

Species assigned to the three morphological-based sections of *Hycleus* (i.e. Mesoscutatus, Mesogorbatus and Mesotaeniatus) do not form monophyletic assemblages in any of the resulting trees (Fig. 2; Fig. S1a–j), and therefore cannot be considered as subgenera of *Hycleus*. On the other hand, the combined-dataset tree points to several well-supported groups of species, some of which correspond to phenetic groups described in literature (for further details see Fig. 2 and Table S3).

3.2. *Temporal and spatial diversification of Hycleus*

BioGeoBEARS analyses selected the BAYAREALIKE + J as the best model for our concatenated dataset according to AIC. Accrding to this model, the four main clades recovered in the multilocus tree (Fig. 2, Fig. 3) showed a possible origin either in the Afrotropical Region (*P* = 0.5) or in the Saharo-Sindian Transitional Region (*P* = 0.5) in the Early Miocene (about 20 million years ago, Mya; 95% High Posterior Density intervals, 95% HPD: 15.46–25.65 Mya; Fig. 3, Fig. S2). Clade A likely originated in the Middle Miocene (14.23 Mya; 95% HPD: 10.60–18.44 Mya) in the Afrotropical Region. Clade B and C originated at about the same time in the Middle Miocene, [B: ~14.67 Mya (95% HPD: 10.03–19.95), C: ~14.67 Mya (95% HPD: 11.23–18.63)] but in different regions: clade B in the Saharo-Sindian Transitional Region, clade C in the Afrotropical Region. Within clade C, the Palaearctic lineage C1 branched off in the Late Miocene (9.07 Mya; 95% HPD: 6.81–11.72), and, within this lineage, a Saharo-Sindian lineage diverged a few million years later (~6.02 Mya; 95% HPD: 4.34–8.00). Clade D originated in the Middle Miocene (~13.43 Mya; 95% HPD: 10.15–17.02) in the Afrotropical Region, and subsequently a sub-clade of species from the Oriental Region (clade H) diversified in the Early Pliocene (~5.37 Mya; 95% HPD: 3.68–7.40).

4. Discussion

Studying hyper-diverse taxa is a challenge in phylogenetics and systematics, and a few examples concerning insect genera have been published until now. These are represented, for example, by the louse genus *Bruuelia* Kéler, 1936 (Bush et al., 2016), the ant genus *Pheidole* Westwood, 1839 (Moreau, 2008), the moss beetle genus *Hydraena* Kugelann, 1794 (Trizzino et al., 2013). *Hycleus*, with more than five hundred species, provides an example of a speciose beetle genus with a wide geographic distribution covering different biogeographic regions and owning some of the most noted medically-exploited Meloidae species [e.g. *H. cichorii* (Linnaeus, 1758) and *H. phaleratus* (Pallas, 1781)] and crop pests [e.g. *H. apicicornis* (Guérin-Ménéville, 1847), *H. dubiosus* (Marseul, 1870); Bologna, 1991; Lebesa et al., 2012]. Here we provided a phylogenetic and biogeographic hypothesis that allow explaining the current diversity and distribution patterns as well as updating the systematics of the genus.

Phylogenetic analyses showed a few topological incongruences among gene trees and between them and the multilocus tree (see Table 1). Discordance between gene trees and species history is expected because different genealogical histories may exist in different genes throughout the genome (Degnan and Rosenberg, 2006, 2009). Our multilocus analysis showed four main clades strongly supported (Fig. 2), as well as most of the nodes within these clades. The close relationships between clade C and D is well supported, whereas phylogenetic relationships between the remaining clades are poorly supported. Poorly supported relationships mainly regard deep nodes and the position of *H. tripunctatus* and *H. hemprichi*. The unresolved phylogenetic position of these latter species might be due to suboptimal taxon sampling. Low support at deep nodes could be either indicative of a soft polytomy due to a lack of data, or rather represent a fast radiation of this group resulting in a hard polytomy. Indeed, according to our molecular dating estimates, all cladogenetic events at deep nodes took place in a relatively short time span of about 3 million years in the Early Miocene (Fig. 3). Additional data (both in terms of markers and taxa) would allow disentangling between these two hypothesyes (see e.g. Mendes et al., 2016;, García-Porta et al., 2019).

All our phylogenetic analyses (Fig. 2, Fig. S1–S10), clearly show that *Ceroctis* and *Paractenodia* are nested within *Hycleus*, confirming results of a previous phylogenetic study that employed fewer representatives of these genera (Salvi et al., 2019). *Ceroctis* and *Paractenodia* were previously described according to differences in antennal morphology (Pardo Alcaide, 1961; Kaszab, 1969; Bologna and Pinto, 2002). In particular, *Ceroctis* presents serrate antennae and *Paractenodia* has seven antennomeres, unlike *Hycleus* species, which own from eight to eleven antennomeres. Serrate antennae like *Ceroctis* are also found in a few *Hycleus* species, such as *H. brincki* (Kaszab, 1956) and *H. derosus* (Péringuey, 1909), that in our analysis showed strong phylogenetic affinities with *Ceroctis* (Fig. 2). The polyphyly of *Ceroctis,* as revealed from all phylogenetic reconstructions (Fig. 2, Fig. S1a–j), suggests that the serrate antennae do not represent a synapomorphy, but instead a character state gained (or lost) multiple times in clade A (Fig. 2). The number of antennomeres has been shown to be a character affected by convergent evolution in Mylabrini (Salvi et al., 2019) and shows intrageneric variability in some genera of this tribe: *Mylabris* (11 and rarely 10 antennomeres), *Actenodia* Laporte de Castelnau, 1840 (eight or nine) and *Croscherichia* Pardo Alcaide, 1950 (11 and rarely 10) (Bologna, 1978, 1991, 2000; Bologna and Coco, 1991; Bologna and Pinto, 2002). Our phylogenetic result confirmed that this character is poorly informative to discriminate genera: in fact, though having seven antennomeres, the analysed *Paractenodia* species resulted a monophyletic group nested within a clade of small-sized species of *Hycleus* strictly endemic to the south-western Africa (Group of *H. amoenus* Marseul, 1872*,* clade T, Fig. 2; see Bologna et al., 2018c).

Members ascribed to the morphologically-based sections i.e. Mesogorbatus, Mesotaeniatus and Mesoscutatus - suggested by Pardo Alcaide (1954, 1955) according to the shape of the mesosternal scutum - did not constitute monophyletic groups in our molecular analyses (Fig. 2 and Fig. S1a–j), and therefore they can not be considered subgenera within *Hycleus*. On the contrary, in a recent study, mesosternal morphology was demonstrated to be a valid diagnostic character to delimit Mylabrini genera (including the genus *Hycleus*), and to differentiate subgenera in the genus *Mylabris* (150 species, 16 subgenera; Salvi et al., 2019).

According to our biogeographic analyses, *Hycleus* evolved in the Early Miocene (about 20 Mya) in the Afrotropical or in the Saharo Sindian Transitional Region. Most parsimoniously - minimizing the number of dispersal events required to achieve the current geographic distribution and diversification – *Hycleus* likely originated in the African continent.

Miocene and Pliocene are known to be two epochs in which several events of rapid speciation of different taxa occurred in Africa (e.g. Axelrod and Raven, 1978; Wieczorek et al., 2000; Voje et al., 2009), especially in southern Africa (e.g. Endrödy-Younga, 1978; White, 1983; Bologna et al., 2018c). Miocene climatic conditions favoured the expansion of species typical of savannahs and Sahel (Voje et al., 2009), whereas Pliocene climatic oscillations caused continuous range shifts in plant and animal populations, triggering numerous events of allopatric divergence followed by speciation (Bologna, 1990; Bologna et al., 2008a; Micheels et al., 2009). Those events might have affected cladogenesis also within *Hycleus*, producing the current high diversity in Africa, especially in the Afrotropical Region (423/518 species, 39 of which from the Saharo-Sindian area).

In our tree (Fig. 3), species from the Saharo-Sindian Transitional Region, included in the clades B and C1, originated by distinct cladogenetic and biogeographic events: a first event in the Early Miocene (about 14.6 Mya) gave origin to the Saharo-Sindian and Palaearctic (Iranian) species of clade B, whereas in the Late Miocene (about 6 Mya), a second, more recent, Saharo-Sindian group branched off from the Palaearctic lineage (C1) of clade C. Palaeoclimate data provide a plausible scenario for the Miocene differentiation of lineages within the Saharo-Sindian Transitional Region, currently occupied mainly by deserts. In fact, Miocene climate was warmer and more humid than today: during the Early Miocene, the northernmost part of Africa was occupied by forests, progressively changing into savannahs and grasslands through the end of the same epoch (Micheels et al., 2009). Desertification started at the boundary between Miocene and Pliocene, and about 7 Mya the Sahara Desert originated (Micheels et al., 2009). The Miocene climatic and environmental conditions, together with the presence of the land bridge between Arabian Peninsula and Eurasia (established ~20–35 Mya; Rögl, 1999; Jolivet and Faccenna, 2000; Allen and Armstrong, 2008; Koshnaw et al., 2018), likely favoured the dispersal of species towards the actual Sind limit (clade B), and also promoted colonization of the Palaearctic Region (~9 Mya, clade C1). From this latter region, as mentioned above, a more recent (~6 Mya) event of dispersal originated a distinct group of Saharo-Sindian species (Group of *H. novemdecimpunctatus*, clade M and Fig. 2).

Clade D originated in the Afrotropical Region in the Middle Miocene (about 13 Mya), and the nested position of Oriental *Hycleus* (clade H, Fig. 2, Fig. 3) within this clade, suggests an Afrotropical origin of the group in the Late Miocene (about 6 Mya). Four main patterns have been described in literature to explain Afrotropical-Oriental disjoint distributions: the Indian rafting following the breakup of Gondwana (100 Mya-40 Mya; Conti et al., 2002), the dispersal of a high-latitude boreotropical palaeoflora (50 Mya; Davis et al., 2002), the transoceanic long-distance dispersal (Yuan et al., 2005), and the Miocene dispersion across the Arabian Peninsula (Kosuch et al., 2001; Zhou et al., 2011). The first two hypotheses can be rejected due to their incongruence with our date estimates, and the transoceanic long-distance dispersal can also be excluded according to the limited dispersal capabilities of *Hycleus*. A Late Miocene migration of an Afrotropical lineage across the Arabian Peninsula seems to be the more plausible scenario to explain the Afrotropical-Oriental disjoint distribution of *Hycleus* species (Fig. 3; clade D).

In summary, our molecular phylogenetic analyses refused the distinction of *Hycleus* from *Ceroctis* and *Paractenodia* and the taxonomic value of the three morphological “sections” and provide a first support to some previously morphologically defined species-groups (Table S3). Our results also suggested a Miocene origin of *Hycleus* in the African continent and that subsequent events of colonization toward the Palaearctic, Saharo-Sindian and Oriental Regions likely generated the large distribution and diversification we observe today.

Acknowledgements

This study was supported by a PhD scholarship to A.R. and by grants to M.A.B. from MIUR, Ministero dell’Istruzione, dell’Università e della Ricerca, Italy (Programmi di ricerca scientifica di rilevante interesse nazionale: PRIN 99C5271884-007; PRIN 2004057217 “Zoogeography of Mediterranean-southern African disjunct distributions by a multimethod approach’; CRUI “Programma di mobilità Pietro della Valle” 2017) (Departments of Excellence - L. 232/2016 - art.1, commi 314–337 awarded to Dept. of Science - University Roma Tre - Rome - Italy for 2018–2022), and from the University Roma Tre, Department of Sciences. D.S. is currently supported by the program ‘Rita Levi Montalcini’ (MIUR) for the recruitment of young researchers at the University of L’Aquila.

We are indebted to people who provided samples for this study, some of which collaborates with us in the field collection: V. Amore (Roma, Italy), F. Angelini (Francavilla Fontana, Italy), P. Audisio (Roma, Italy), L. Bartolozzi (Firenze, Italy), D. Bergamaschi (Roma, Italy), M. Biondi (L’Aquila, Italy), Marta Bologna (Roma, Italy), P. Bombi (Roma, Italy), A. Campanaro (Mantova, Italy), A. Carapezza (Palermo, Italy), S. Carboni (Roma, Italy), G. Carpaneto (Roma, Italy), F. Cerini (Roma, Italy), L. Černý (Ceske Budejovice, Czech Rep.), P. Cerretti (Roma, Italy), Z.-L Chen (Hbei, China), L. Čížek (Ceske Budejovice, Czech Rep.), A. De Biase (Roma, Italy), A. Di Giulio (Roma, Italy), S.H. Dong (Hebei, China), L. Fekrat (Mashhad, Iran), M. François (Bar sur Aube, France), N. Gompel (Marseille, France), G. Gracceva (Amsterdam, The Netherlands), S. Kolov (Alma Ata, Kazakhstan), M. Lorenzetti (Roma, Italy), E. Marais (Windhoek, Namibia), C. Marangoni (Roma, Italy), B. Massa (Palermo, Italy), F. Mensa (Roma, Italy), F. Montalto (Roma, Italy), W. Moore (Tucson, USA), Mor (Iran), P. Moretto (Toulon, France), G. Nardi (Mantova, Italy), Z. Pan (Hebei, China), R. Perissinotto (Port Elizabeth, South Africa), M. Pitzalis (Cerveteri, Italy), P. Rapuzzi (Cialla di Prepotto, Italy), I. Renan (Sde Boqer, Israel), O. Rittner (Tel Aviv, Israel), M. Romano (Capaci, Italy), G. Sabatinelli (Prévessin, France), V. Sbordoni (Roma, Italy), L. Saltini (Modena, Italy), S. Serri (Tehran, Iran), C. Settanni (Roma, Italy), I.C. Sharp (South Africa), F. Turco (Canberra, Australia), G. Venturini (Roma, Italy), L. Vignoli (Roma, Italy), J.-Y. Wang (Hebei, China), H. Xu (Hebei, China), N. Zader (Tehran, Iran), M. Zapparoli (Viterbo, Italy).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2019.106706.

References

Allen, M.B., Armstrong, H.A., 2008. Arabia-Eurasia collision and the forcing of mid-Cenozoic global cooling. Palaeog. Palaeoclim. Palaeoecol. 265, 52–58.
Axelrod, D.I., Raven, P.H., 1978. Late Cretaceous and Tertiary vegetation history of Africa. In: In: Werger, M.J.A. (Ed.), Biogeography and Ecology of Southern Africa. Monographiae Biologicae, vol. 31. Springer, Dordrecht, pp. 77–130.
Bologna, M.A., 1978. Alcuni Meloidi dell'Africa orientale e meridionale e descrizione di una specie nuova (Coleoptera Meloidae). Quad. Accad. Naz. Lincei, Roma 243 (1), 137–189.
Bologna, M.A., 1979. Meloidae di Turchia. I contributo (Coleoptera). Fragm. Entomol. 15, 143–199.
Bologna, M.A., 1983. Utilizzazione dei dati biologici nella sistematica dei Meloidae (Coleoptera). Atti XII Congr. Naz. Entomol., Roma 2 (1980), 21–36.
Bologna, M.A., 1990. Faunistica e zoogeografia dei Meloidae (Coleoptera) della Somalia. Biogeographia (n.s.) 13, 375–457.
Bologna, M.A., 1991. Coleoptera Meloidae. Fauna d'Italia. XXVIII, Calderini, Bologna.
Bologna, M.A., 1994a. I Meloidae della Grecia (Coleoptera). Fragm. Entomol. 25 (suppl.), 1–119.
Bologna, M.A., 1994b. The Coleoptera Meloidae of Sierra Leone. Quad. Accad. Naz. Lincei, Roma, “Ricerche Biologiche in Sierra Leone. (IV) 247, 357–379.
Bologna, M.A., 2000. Revision of the genus *Mimesthes* (Coleoptera: Meloidae: Mylabrini). Eur. J. Entomol. 97, 75–84.
Bologna, M.A., Audisio, P.A., Biondi, M., Casale, A. (Eds.), 2008. The mediterranean-southern African disjunt distribution pattern. Biogeographia (n.s.) 29, 181 pp.
Bologna, M.A., Di Giulio, A., Pitzalis, M., 2008b. Examples of disjunct distributions between Mediterranean and southern or eastern Africa in Meloidae (Coleoptera, Tenebrionoidea). Biogeographia (n.s.) 29, 81–98.
Bologna, M.A., Oliverio, M., Pitzalis, M., Mariottini, P., 2008c. Phylogeny and evolutionary history of the blister beetles (Coleoptera, Meloidae). Mol. Phyl. Evol. 48, 679–693.
Bologna, M.A., Coco, E., 1991. Revisione del genere *Croscherichia* Pardo Alcaide, 1950 (Coleoptera, Meloidae). Mem. Soc. Entomol. Ital. 69 (1990), 97–180.
Bologna, M.A., Pinto, J.D., 2002. The Old World genera of Meloidae (Coleoptera): a key and synopsis. J. Nat. Hist. 36, 2013–2102.
Bologna, M.A., Turco, F., 2007. The Meloidae (Coleoptera) of the United Arab Emirates with an updating of the Arabian checklist. Zootaxa 1625, 1–33.
Bologna, M.A., D’Inzillo, B., Cervelli, M., Oliverio, M., Mariottini, P., 2005. Molecular phylogenetic studies of the Mylabrini blister beetles (Coleoptera, Meloidae). Mol. Phyl. Evol. 37, 306–311.
Bologna, M.A., Amore, V., Pitzalis, M., 2018. Meloidae of Namibia (Coleoptera): taxonomy and faunistics with biogeographic and ecological notes. Zootaxa 4373, 1–14.
Bush, S.E., Weckstein, J.D., Gustafsson, D.R., Di Blasi, J.A.E., Shreve, Boldt, R., Skeen, H.R., Johnson, K.P., 2016. Unlocking the black box of feather louse diversity: A molecular phylogeny of the hyper-diverse genus *Brueelia*. Mol. Phyl. Evol. 94 (B), 737–751. https://doi.org/10.1016/j.ympev.2015.09.015.
Conti, E., Eriksson, T., Schonenberger, J., Sytsma, K.J., Baum, D.A., 2002. Early Tertiary out-of-India dispersal of Crypteroniaceae: evidence from phylogeny and molecular dating. Evolution 56, 1931–1942.
Davis, C.C., Bell, C.D., Fritsch, P.W., Mathews, S., 2002. Phylogeny of *Acridocarpus-Brachylophon* (Malpighiaceae): implications for Tertiary tropical floras and AfroAsian biogeography. Evolution 56, 2395–2405.
Degnan, J.H., Rosenberg, N.A., 2006. Discordance of species trees with their most likely gene trees. PLoS Genet. 2, e68.
Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends Ecol. Evol. 24, 332–340.
Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.
Endrödy-Younga, S., 1978. Coleoptera. In: In: Werger, M.J.A. (Ed.), Biogeography and Ecology of Southern Africa Vol. 2. Junk, The Hague, pp. 799–821. https://doi.org/10.1007/978-94-009-9951-0_26.
García-Porta, J., Irisarri, I., Kirchner, M., Rodriguez, A., Kirchhof, S., Brown, J., MacLeod,

A., Turner, A., Ahmadzadeh, F., Albaladejo, G., Crnobrnja-Isailovic, J., de la Riva, I., Fawzi, A., Galan, P., Göçmen, B., Harris, D.J., Jiménez-Robles, O., Joger, U., Jovanović, O., Karış, M., Koziel, G., Künzel, S., Lyra, M., Miles, D., Nogales, M., Oğuz, M., Pafilis, P., Rancilhac, L., Rodríguez, N., Rodríguez, Concepción B., Sanchez, E., Salvi, D., Slimani, T., S'khifa, A., Qashqaei, A., Žagar, A., Lemmon, A., Lemmon, E., Carretero, M.A., Carranza, S., Philippe, H., Sinervo, B., Müller, J., Vences, M., Wollenberg, K.C., 2019. Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. Nat. Commun. 10, 4077.

Jolivet, L., Faccenna, C., 2000. Mediterranean extension and the Africa-Eurasia collision. Tectonics 19, 1095–1106.

Kaszab, Z., 1969. The system of the Meloidae (Coleoptera). Mem. Soc. Entomol. Ital. 48, 241–248.

Koshnaw, R.I., Stockli, D.F., Schlunegger, F., 2018. Timing of the Arabia-Eurasia continental collision — Evidence from detrital zircon U-Pb geochronology of the Red Bed Series strata of the northwest Zagros hinterland, Kurdistan region of Iraq. Geology 47, 47–50.

Kosuch, J., Vences, M., Dubois, A., Ohler, A., Bohme, W., 2001. Out of Asia: mitochondrial DNA evidence for an oriental origin of tiger frogs, genus *Hoplobatrachus*. Mol. Phylogen. Evol. 21, 398–407.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Katoh, K., Rozewicki, J., Yamada, K.D., 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief. Bioinform. 20, 1160–1166.

Landis, M.J., Matzke, N.J., Moore, B.R., Huelsenbeck, J.P., 2013. Bayesian analysis of biogeography when the number of areas is large. System. Biol. 62, 789–804.

Lebesa, L.N., Khan, Z.R., Krüger, K., Bruce, T.J.A., Hassanali, A., Pickett, J.A., 2012. Farmers' knowledge and perceptions of blister beetles, *Hycleus* spp. (Coleoptera: Meloidae), as pest herbivores of *Desmodium* legumes in western Kenya. Inter. J. Pest Manag. 58, 165–174.

Liu, D., Chen, Z., 2009. The effects of cantharidin and cantharidin derivates on tumour cells. Med. Chem. 9, 392–396.

Matzke, N.J., 2013a. BioGeoBEARS: BioGeography with Bayesian (and Likelihood) Evolutionary Analysis in R Scripts. R package, version 0.2.1, published July 27, 2013 at: http://CRAN.R-project.org/package=BioGeoBEARS.

Matzke, N.J., 2013b. Probabilistic Historical Biogeography: New Models for Founder-Event Speciation, Imperfect Detection, and Fossils Allow Improved Accuracy and Model-Testing. Front. Biogeogr. 5, 242–248.

Matzke, N.J., 2014. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. Syst. Biol. 63, 951–970.

Mendes, J., Harris, D.J., Carranza, S., Salvi, D., 2016. Evaluating the phylogenetic signal limit from mitogenomes, slow evolving nuclear genes, and the concatenated approach. New insights into the Lacertini radiation using fast evolving nuclear genes and species trees. Mol. Phylog. Evol. 100, 254–267.

Micheels, A., Eronen, J., Mosbrugger, V., 2009. The Late Miocene climate response to a modern Sahara Desert. Glob. Planet. Change 67, 193–204.

Moed, L., Shwayder, T.A., Chang, M.W., 2001. Cantharidin revisited: a blistering defence of an ancient medicine. Arch. Dermatol. 137, 1357–1360.

Moreau, C.S., 2008. Unravelling the evolutionary history of the hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae). Mol. Phylog. Evol. 48, 224–239. https://doi.org/10.1016/j.ympev.2008.02.020.

Pan, Z., Carosi, M., Bologna, M.A., 2014. A new Eastern Asian *Hycleus* and key to the Chinese species of the *phaleratus* group (Coleoptera, Meloidae, Mylabrini). ZooKeys 463, 11–19. https://doi.org/10.3897/zookeys.463.8261.

Papadopoulou, A., Anastasiou, I., Vogler, A.P., 2010. Revisiting the insect mitochondrial molecular clock: the Mid-Aegean trench calibration. Mol. Biol. Evol. 27, 1659–1672.

Pardo Alcaide, A., 1954. Études sur les Meloidae. V. Les Mylabrini du Maroc et du Sahara occidental espagnol (Col. Meloidae). Bull. Soc. Sci. Nat. Phys. Maroc 34, 55–88.

Pardo Alcaide, A., 1955. Estudios sobre Meloidae VI. Sobre algunos *Mylabris* (s. lat.) de la región etiópica. Bull. Inst. R. Sci. Nat. Belg. 31 (49), 1–32.

Pardo Alcaide, A., 1958a. Études sur les Meloidae (Col.). IX. Observations sur quelques *Mylabris* (s.l.) des régions éthiopienne et orientale. Bull. Inst. R. Sci. Nat. Belg. 35 (13), 1–39.

Pardo Alcaide, A., 1958b. Études sur Meloidae (Col.) X. Quelques *Mylabris* nouveaux ou intéressants de la faune éthiopienne. Boll. Mus. Civ. St. Nat. Trieste 21, 63–76.

Pardo Alcaide, A., 1961. Estudios sobre 'Meloidae'. XIII. Meloideos del Sahara occidental español y de la región du Drâa (Marueccos) (Coleoptera). EOS Rev. Españ. Entomol. 37, 91–111.

Pardo Alcaide, A., 1963. Coléoptères Méloïdes récoltés par M. J. Mateu dans l'Ennedi et au Tchad. Etudes sur les Meloidae. XII. Bull. Inst. Fondam. Afr. Noire (A) 25, 572588.

Pardo Alcaide, A., 1966. Contribution à la faune du Congo (Brazzaville). Mission A. Villiers et A. Descarpentries. XXX. Coléoptères Meloïdae. Bull. Inst. Fondam. Afr. Noire (A) 38, 1196–1207.

Pardo Alcaide, A., 1968. Étude sur les Meloidae (Coleoptera). XX. Sur le genre *Gorrizia* Pardo et une nouvelle espèce soudanaise du groupe du *brunnipes* (Klug). EOS Rev. Españ. Entomol. 43, 623–629.

Pardo Alcaide, A., 1969. Études sur les Meloidae. XXI. Matériaux pour une révision des Mylabrini de l'Afrique du Nord et du Moyen Orient (2ème partie) (Coleoptera). EOS Rev. Españ. Entomol. 44, 367–376.

Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.

Puerto Galvis, C.E., Vargas Mendez, L.Y., Kouznetsov, V.V., 2013. Cantharidin-based small molecules as potential therapeutic agents. Chem. Biol. Drug Des. 82, 477–499.

Rambaut, A., Drummond, A., 2009. FigTree, version 1.3. 1. Institute of Evolutionary Biology, University of Edinburgh.

Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer 1.6. Available at http://beast.bio.ed.ac.uk/Tracer.

Ree, R.H., Moore, B.R., Webb, C.O., Donoghue, M.J., 2005. A Likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evolution 59, 2299–2311.

Rögl, F., 1999. Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography (short overview). Geol. Carpath. 50, 339–349.

Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Syst. Biol. 46, 195–203.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Salvi, D., Maura, M., Pan, Z., Bologna, M.A., 2019. Phylogenetic systematics of *Mylabris* blister beetles (Coleoptera, Meloidae): a molecular assessment using species trees and total evidence. Cladistics 35, 243–268.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114.

Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. 51, 492–508.

Staden, R., Beal, K.F., Bonfield, J.K., 1998. 2000. The staden package. In: Misener, S., Krawetz, S.A. (Eds.), Computer Methods in Molecular Biology, Bioinformatics Methods and Protocols. Humana Press Inc., Totowa, NJ, pp. 115–130.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.

Trifinopoulos, J., Nguyen, L.T., von Haeseler, A., Minh, B.Q., 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 44.

Trizzino, M., Jäch, M., Audisio, P., Alonso, R., Ribera, I., 2013. A molecular phylogeny of the cosmopolitan hyperdiverse genus *Hydraena* Kugelann (Coleoptera, Hydraenidae). Syst. Entomol. 38, 192–208.

Voje, K.L., Hemp, C., Flagstad, Ø., Sætre, G.P., Stenseth, N.C., 2009. Climatic change as an engine for speciation in flightless Orthoptera species inhabiting African mountains. Mol. Ecol. 18, 93–108.

Wang, C.C., Wu, C.H., Hsieh, K.J., 2000. Cytotoxic effects of cantharidin on the growth of normal and carcinoma cells. Toxicology 147, 77–87.

White, F., 1983. The vegetation of Africa. Unesco, Paris.

Wieczorek, A.M., Drewes, R.C., Channing, A., 2000. Biogeography and evolutionary history of *Hyperolius* species: application of molecular phylogeny. J. Biogeogr. 27, 1231–1243.

Yang, H.Y., Guo, W., Xu, B., Li, M., Cui, J.R., 2007. Anticancer activity and mechanisms of norcantharidin-Nd3II on hepatoma. Antican. Drugs 18, 1133–1137. https://doi.org/10.1097/CAD.0b013e3282eeb1c5.

Yuan, Y.M., Wohlhauser, S., Möller, M., Klackenberg, J., Callmander, M.W., Küpfer, P., 2005. Phylogeny and biogeography of *Exacum* (Gentianaceae): a disjunctive distribution in the Indian Ocean Basin resulted from long distance dispersal and extensive radiation. Syst. Biol. 54, 21–34.

Zhang, W., Ma, Y.Z., Song, L., Wang, C.H., Qi, T.-G., Shao, G.-R., 2014. Effect of cantharidins in chemotherapy for hepatoma: a retrospective cohort study. Am. J. Chin. Med. 42, 561–567. https://doi.org/10.1142/S0192415X14500360.

Zhou, L.L., Su, Y.C.F., Thomas, D.C., Saunders, R.M.K., 2011. 'Out-of-Africa' dispersal of tropical floras during the Miocene climatic optimum: evidence from *Uvaria* (Annonaceae). J. Biogeogr. 39, 322–335.

MOLECULAR PHYLOGENETICS AND EVOLUTION

Phylogeny, biogeography and systematics of the hyper-diverse blister beetle genus *Hycleus* (Coleoptera: Meloidae)

Alessandra Riccieri, Emiliano Mancini, Daniele Salvi, Marco A. Bologna

Corresponding author: Alessandra Riccieri; e-mail: alessandra.riccieri@uniroma3.it

SUPPLEMENTARY MATERIAL

Fig. S1a

Bayesian tree based on 28S sequences. Only supported values of posterior probability are reported at each node (PP>0.9).

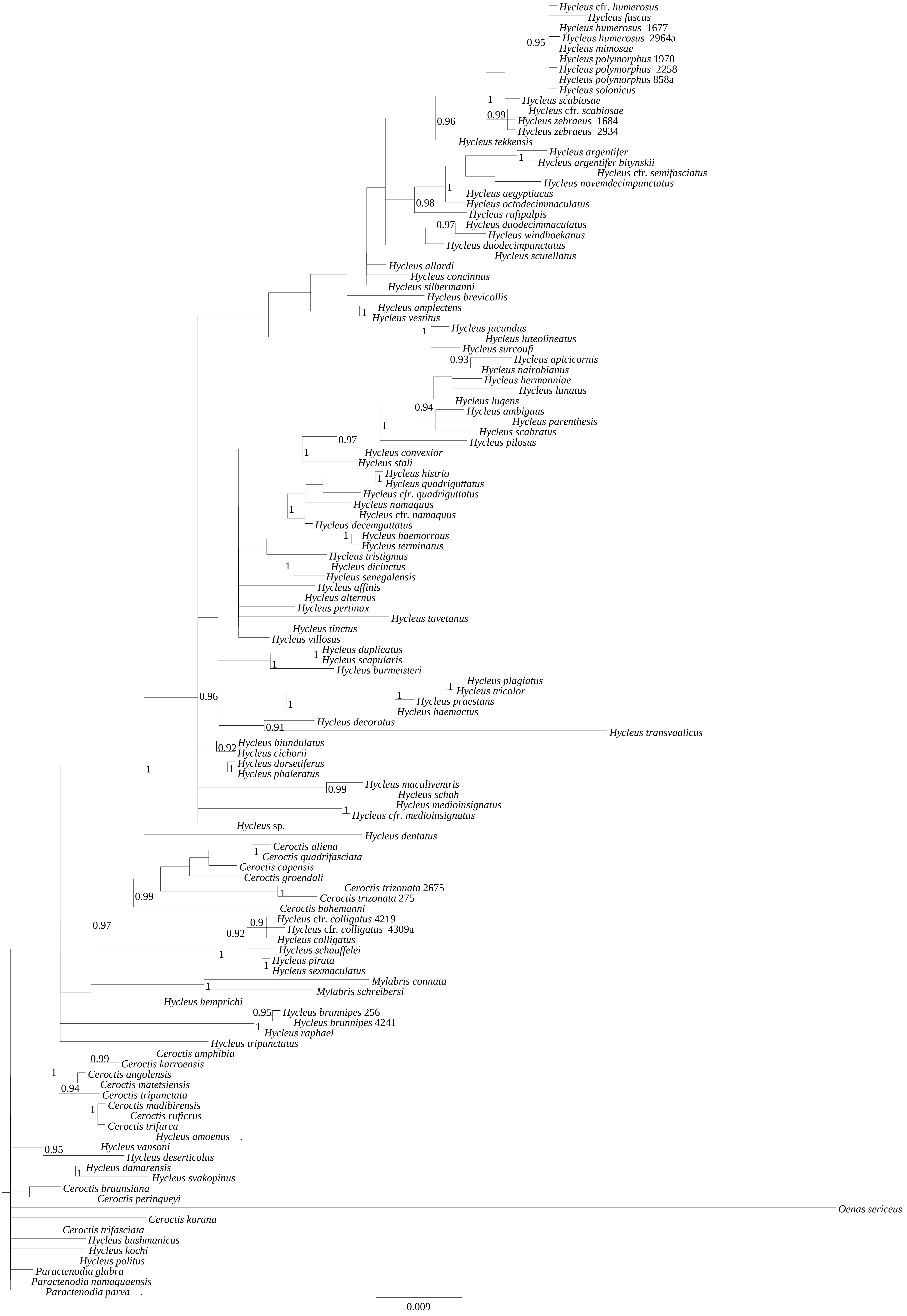


Fig. S1b

Maximum likelihood tree based on 28S sequences. Only supported values of bootstrap are reported at each node (BP>70).

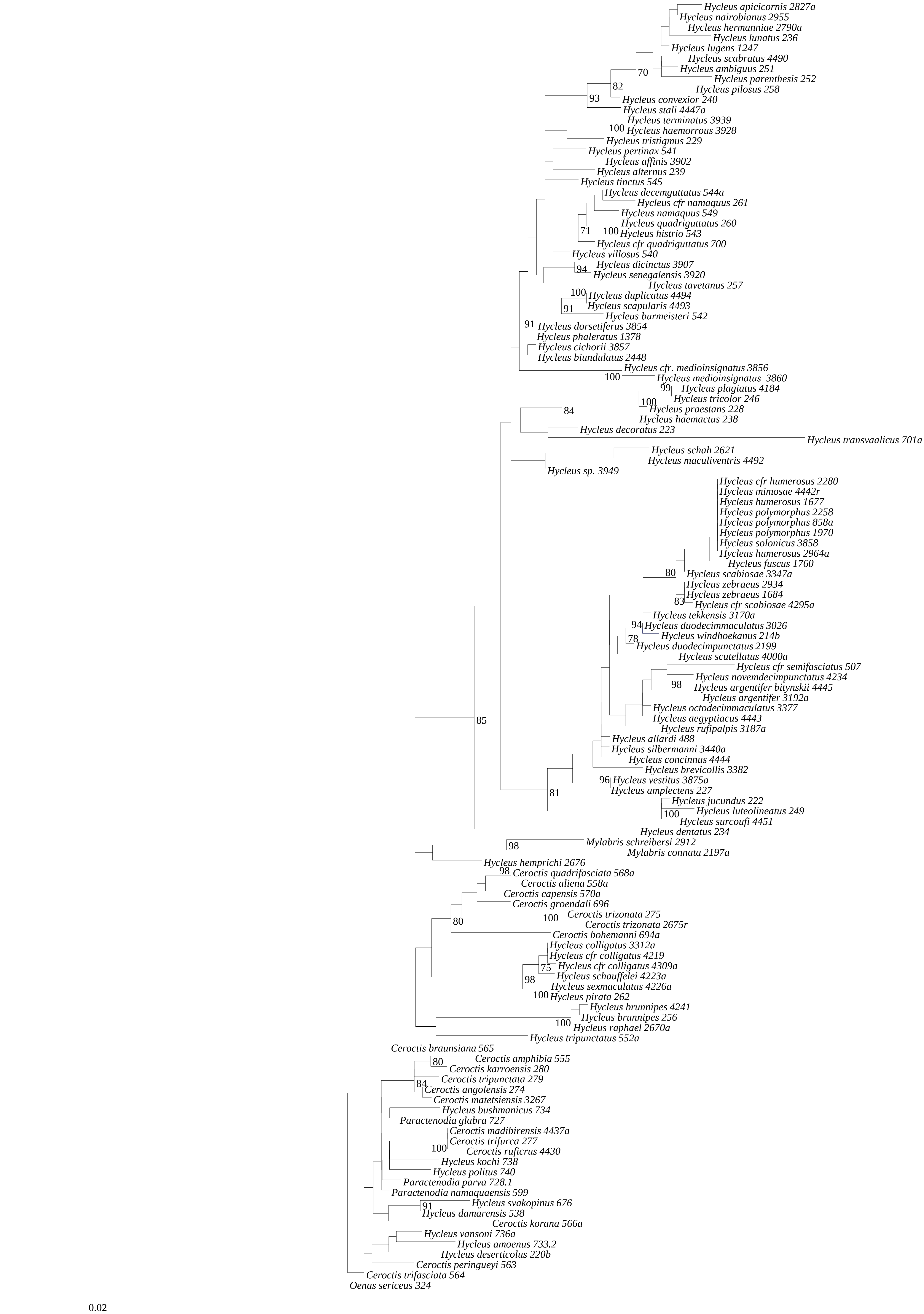


Fig. S1c

Bayesian tree based on ARGK sequences. Only supported values of posterior probability are reported at each node (PP>0.9).

Fig. S1d

Maximum likelihood tree based on ARGK sequences. Only supported values of bootstrap are reported at each node (BP>70).

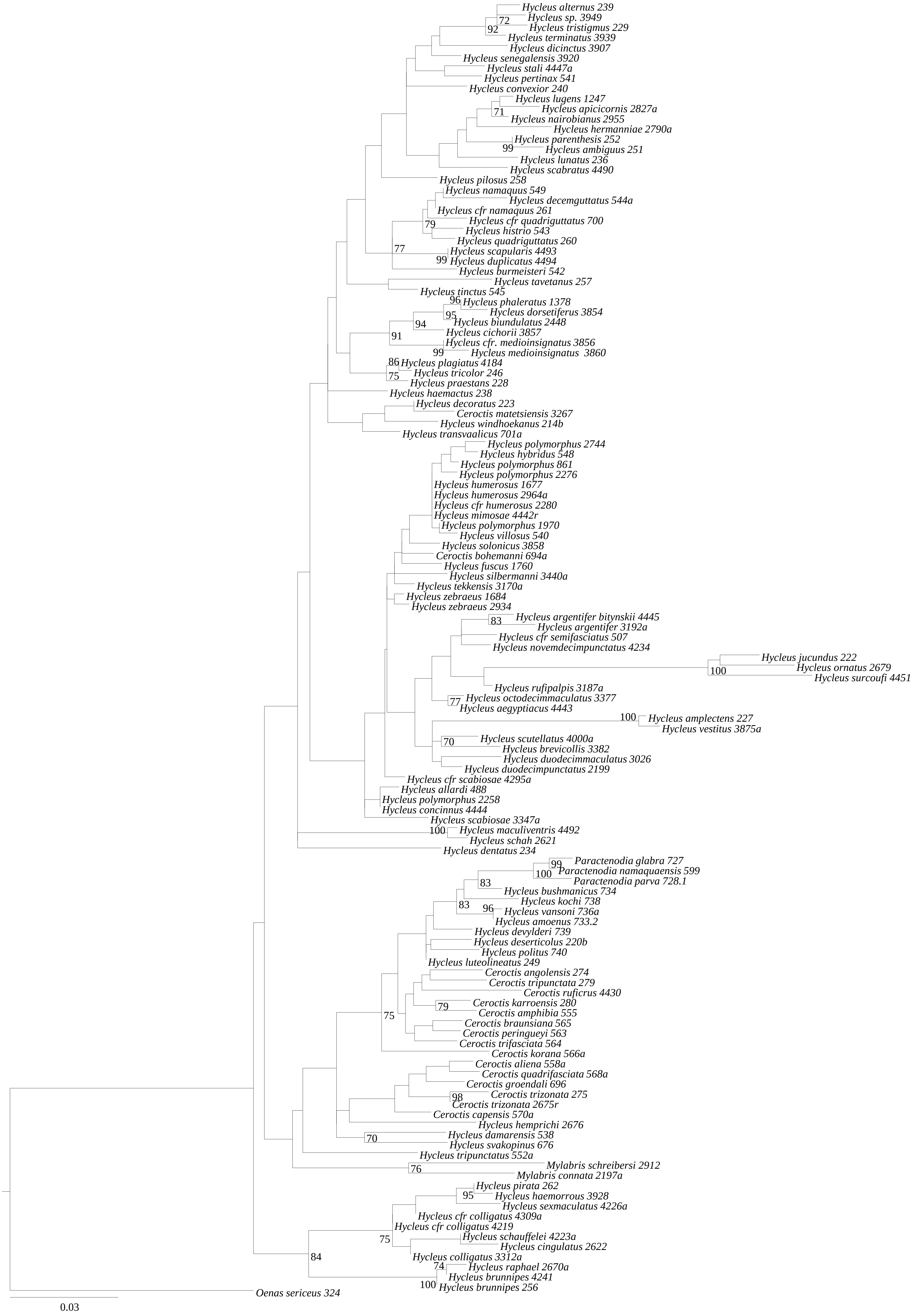


Fig. S1e

Bayesian tree based on CAD sequences. Only supported values of posterior probability are reported at each node (PP>0.9).

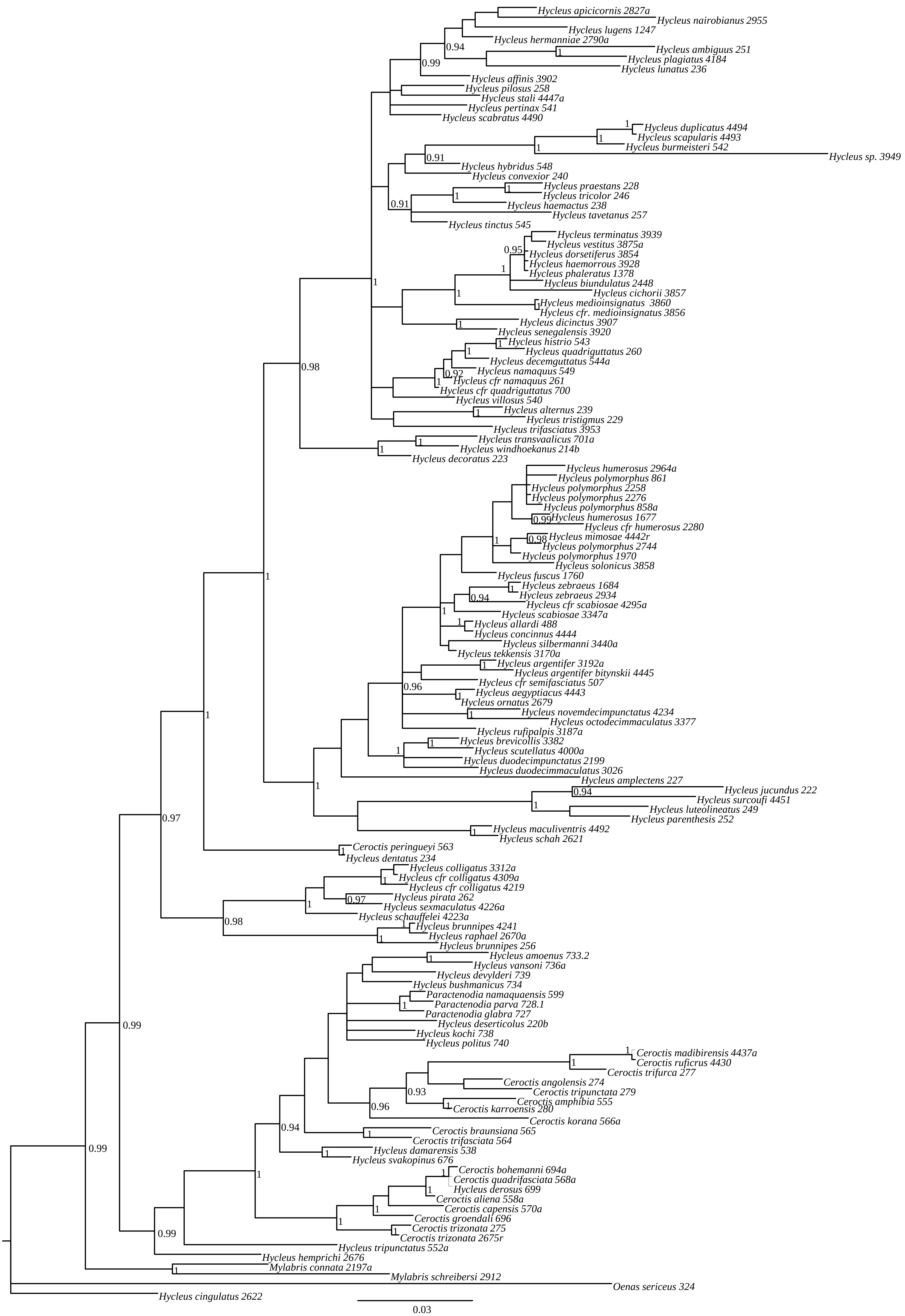


Fig. S1f

Maximum likelihood tree based on CAD sequences. Only supported values of bootstrap are reported at each node (BP>70).

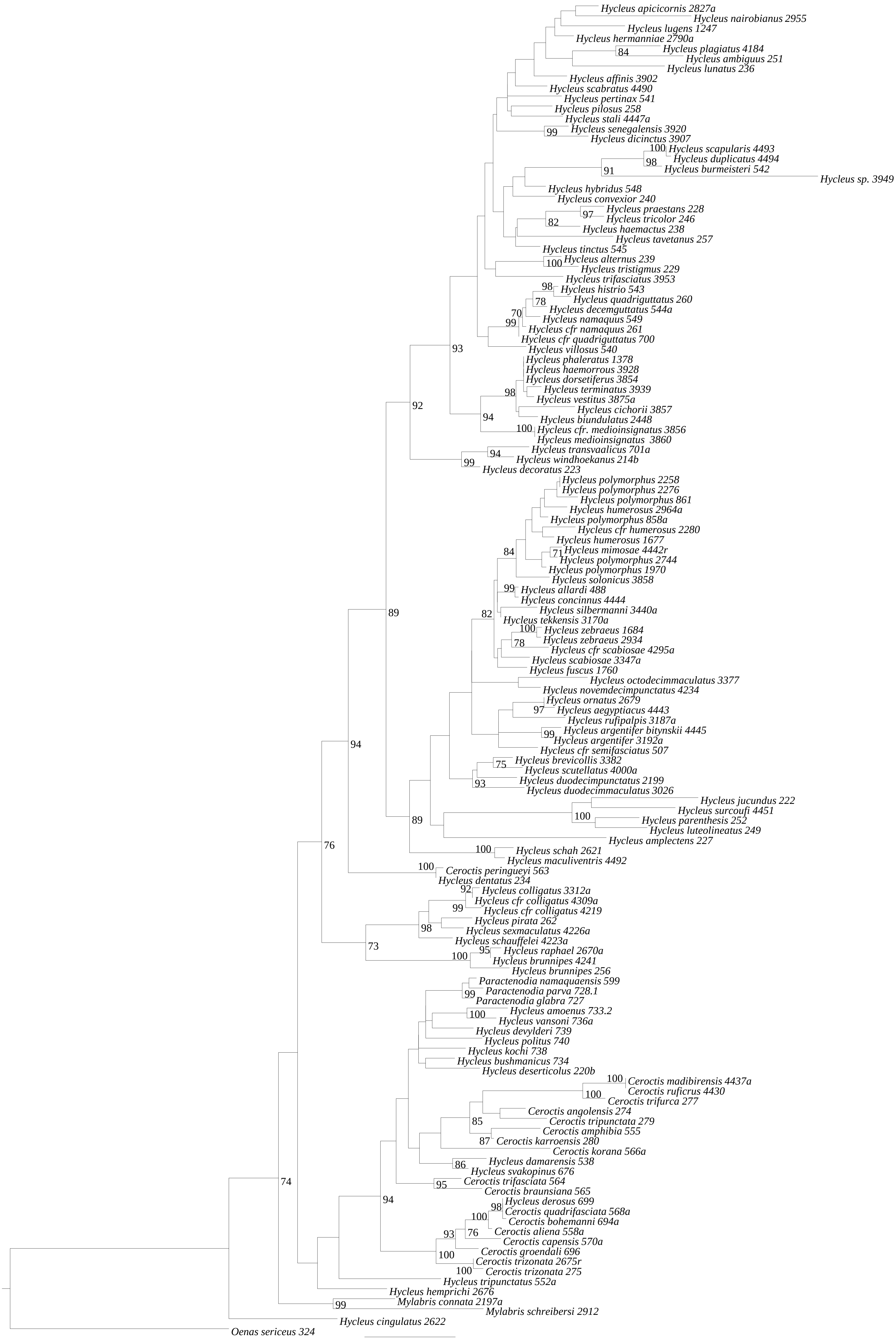


Fig. S1g

Bayesian tree based on COI sequences. Only supported values of posterior probability are reported at each node (PP>0.9).

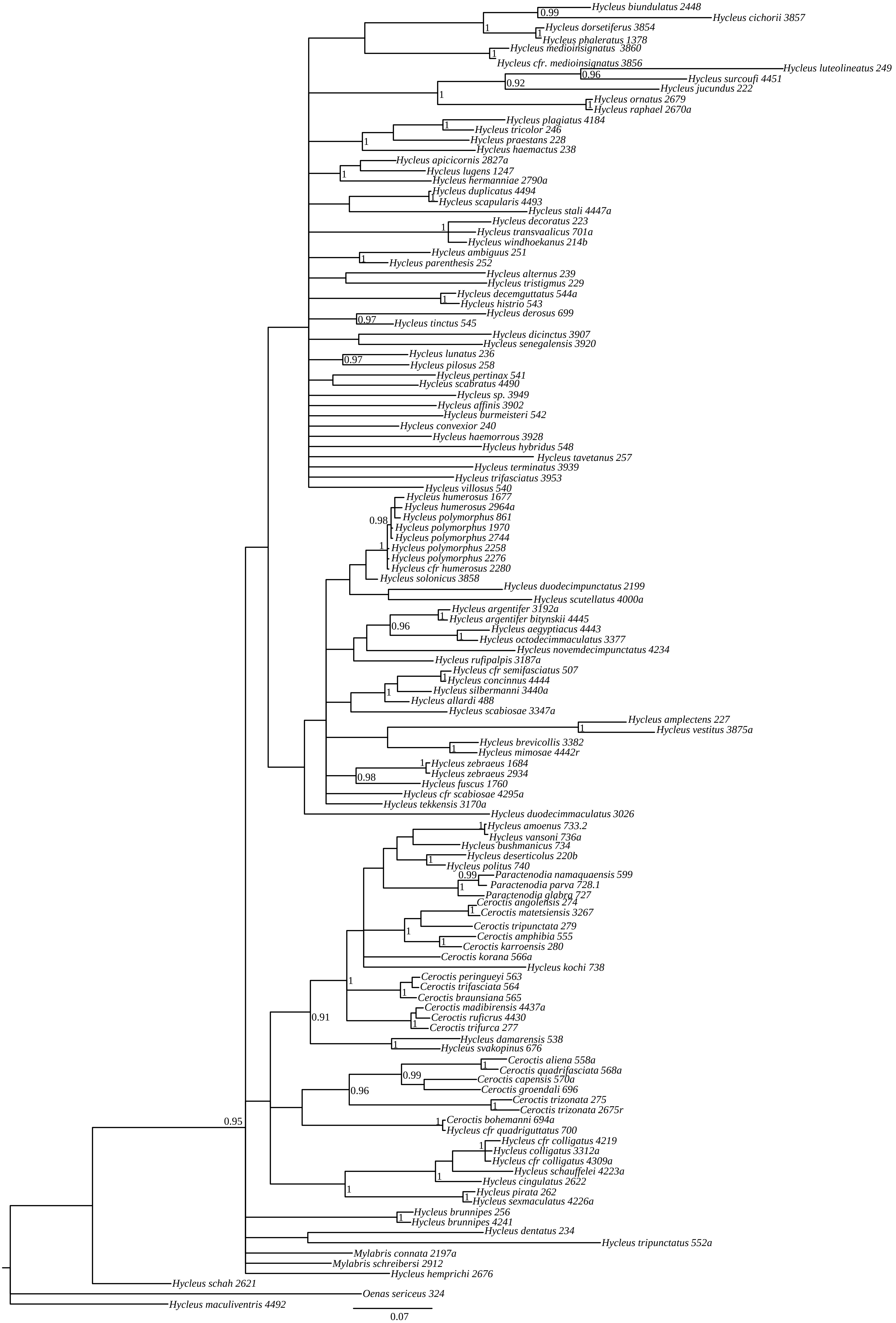


Fig. S1h

Maximum likelihood tree based on COI sequences. Only supported values of bootstrap are reported at each node (BP>70).

Fig. S1i

Bayesian tree based on WG sequences. Only supported values of posterior probability are reported at each node (PP>0.9).

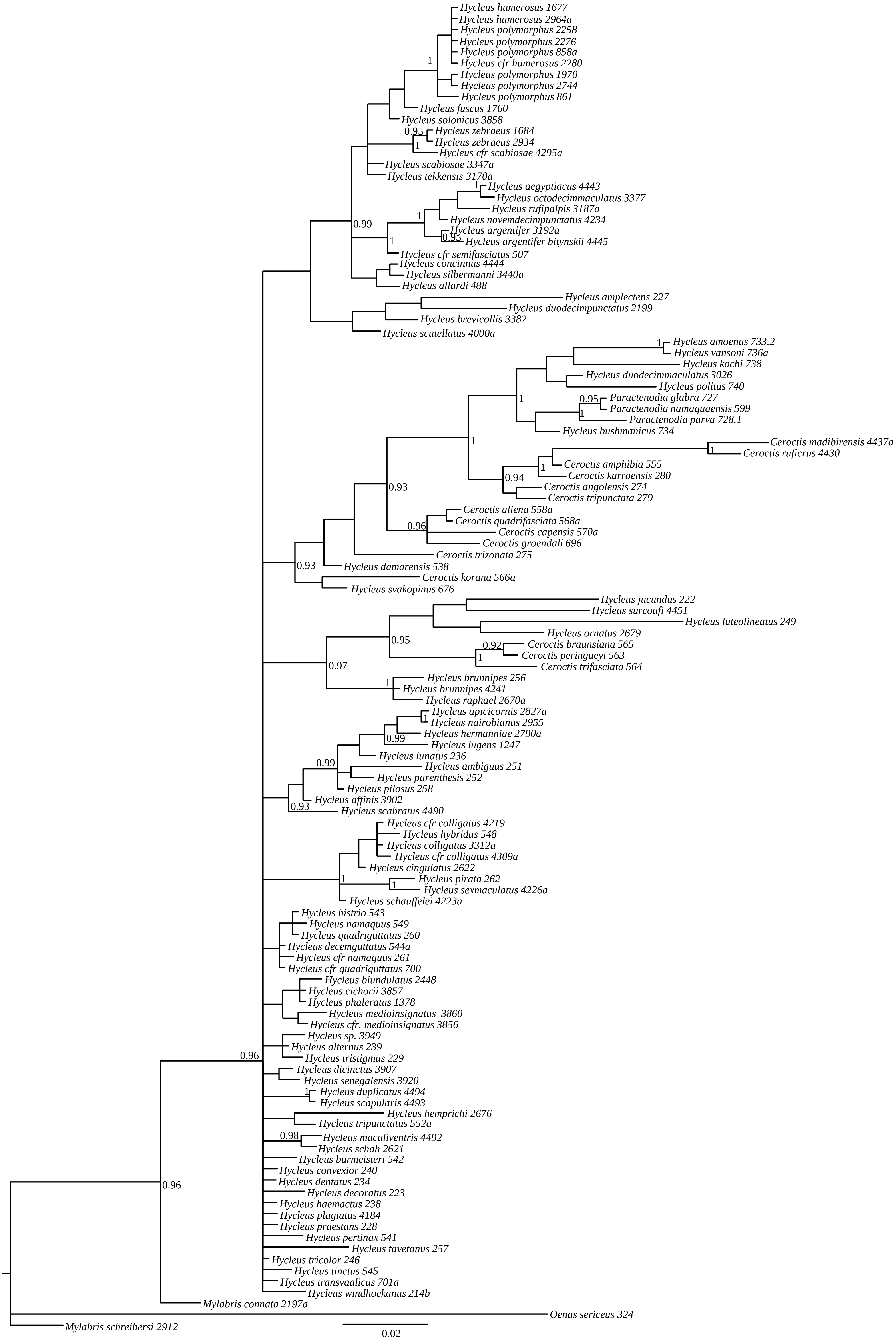


Fig. S1j

Maximum likelihood tree based on WG sequences. Only supported values of bootstrap are reported at each node (BP>70).

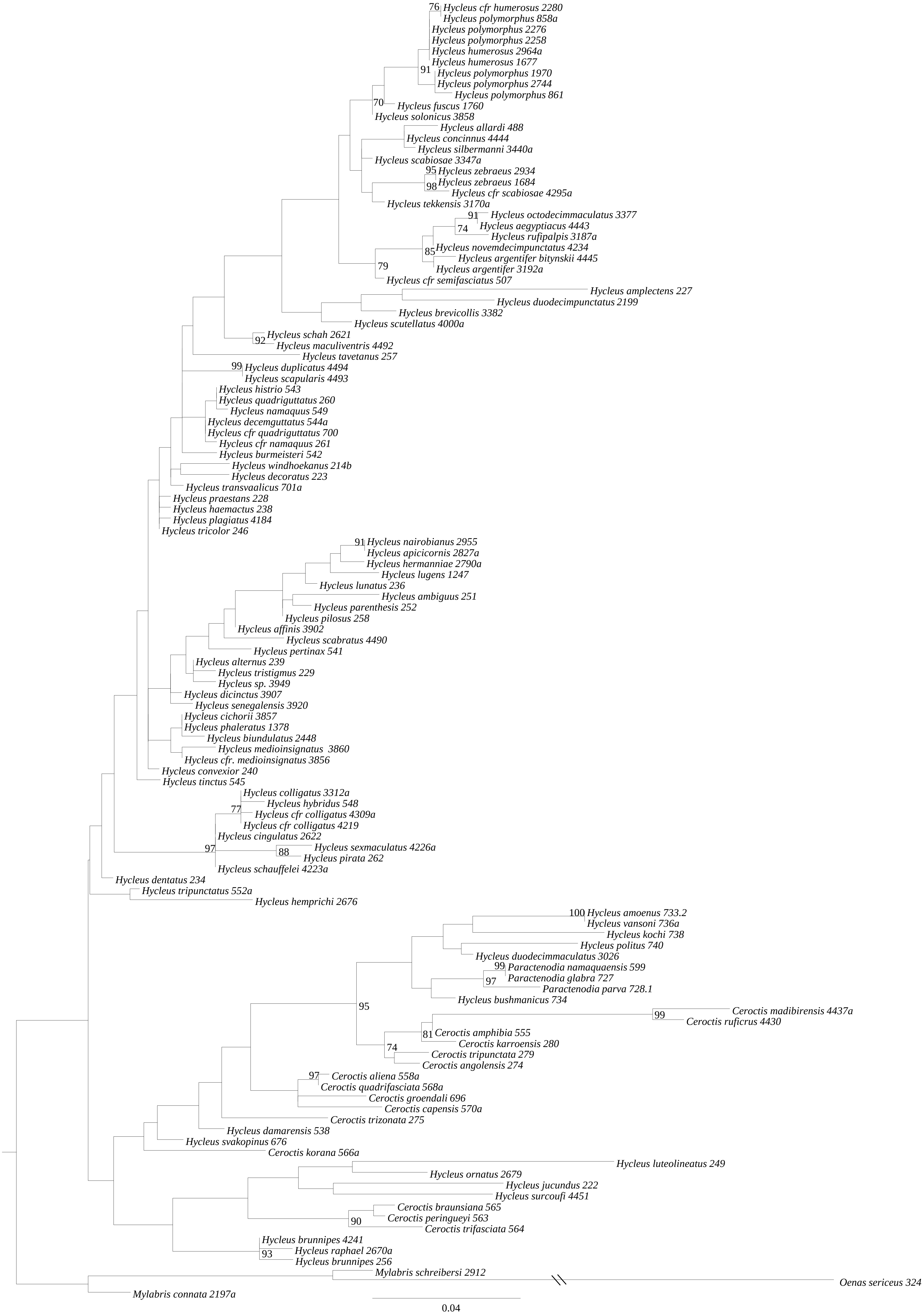


Fig. S2

Molecular clock analyses resulting tree, with confidence intervals at each node represented as bars.

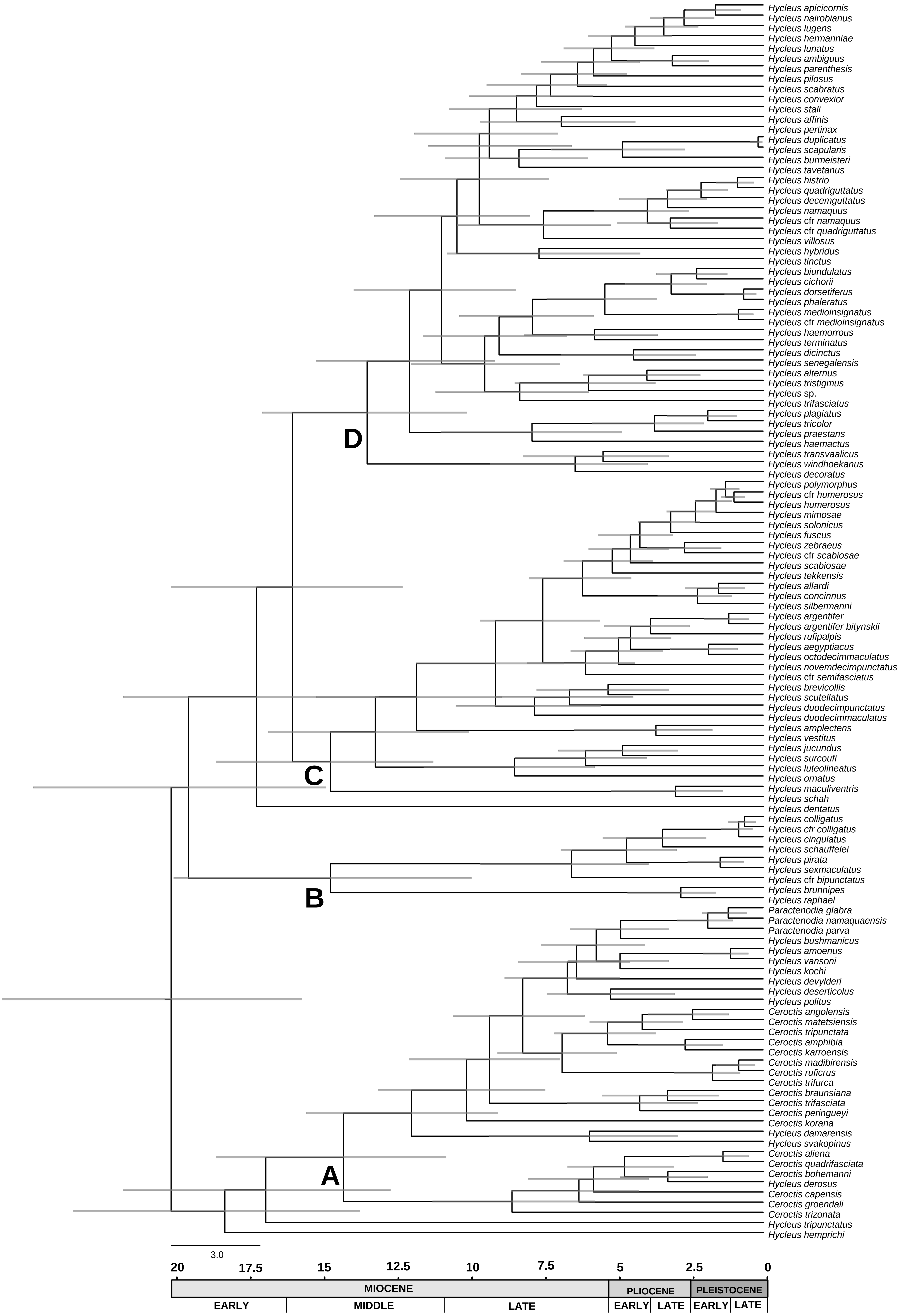


Table S1

Species, specimen code, sample localities, genes and GenBank accession numbers for the 134 specimens analysed in this study. Genbank accession numbers marked with * indicates sequences derived from Salvi et al. (2019).

SPECIES	SPECIMEN CODE	SAMPLE LOCALITIES	COI	28S	ARGK	CAD	WG
Ceroctis aliena	558a	Namibia, 44.5 km N Noordoewer, Grape Valley	MN849932	MN848613	MN845227	MN845361	MN845494
Ceroctis amphibia	555	Namibia, 17 km SE Messum Valley	MN849933	MN848614	MN845228	MN845362	MN845495
Ceroctis angolensis	274	Namibia, 10 km N di Aais	MH668504.1*	MN848615	MN845229	MN845363	MN845496
Ceroctis bohemanni	694a	South Africa, 17 km NE Bloemhof	MN849934	MN848616	MN845230	MN845364	-
Ceroctis braunsiana	565	Namibia, 8 km E Witvlei	MN849935	MN848617	MN845231	MN845365	MN845497
Ceroctis capensis	570a	Sudafrica, Cederberg, Sneeuberg	MH668505.1*	MN848618	MN845232	MH668480.1*	MN845498
Ceroctis groendali	696	South Africa, Free State, Olivershoek Pass	MN849936	MN848619	MN845233	MN845367	MN845499
Ceroctis karroensis	280	South Africa, 10 km E Calitzdorp	MN849937	MN848620	MN845234	MN845368	MN845500
Ceroctis korana	566a	Namibia, 44.5 km N Noordoewer, Grape Valley	MN849938	MN848621	MN845235	MN845369	MN845501
Ceroctis madibirensis	4437a	Tanzania, Dodoma Reg., Mapinduzi	MN849940	MN848622	-	MN845370	MN845502
Ceroctis matetsiensis	3267	Zambia, 5 km W Masese	MN849939	MN848623	MN845236	-	-
Ceroctis peringueyi	563	Namibia, 10 km S Waltevrede	MN849941	MN848624	MN845237	MN845371	MN845503
Ceroctis quadrifasciata	568a	South Africa, 12.8km S Clanwilliam	MN849942	MN848625	MN845238	MN845372	MN845504
Ceroctis ruficrus	4430	Mozambique, Parco Limpopo	MN849943	MN848626	MN845239	MN845373	MN845505
Ceroctis trifasciata	564	Namibia, 68 km E Windhoek	MN849944	MN848627	MN845240	MN845374	MN845506
Ceroctis trifurca	277	Mozambique, Matutuine district, Tinti Gala Lodge	MN849948	MN848628	-	MN845378	-
Ceroctis tripunctata	279	South Africa, Mpumalanga, Kruger NP, Pretoriuskop	MN849945	MN848629	MN845241	MN845375	MN845507
Ceroctis trizonata	275	Morocco, 30 Km W Guercif	MN849946	MN848631	MN845242	MN845376	MN845508
Ceroctis trizonata	2675	Israel, Dead Sea, Masada jct	MN849947	MN848630	MN845243	MN845377	-
Hycleus aegyptiacus	4443	Israel, Midreshet Ben Gurion	MN849960	MN848632	MN845255	MN845390	MN845520
Hycleus affinis	3902	Ivory Coast, Touba, Biémasso	MN849952	MN848633	-	MN845382	MN845512
Hycleus allardi	488	Tunisia, 10 Km Tamerza, road Tamerza to Redeyef	MN849950	MN848634	MN845246	MN845380	MN845510
Hycleus alternus	239	South Africa, Mpumalanga Berlin forest	MN849959	MN848635	MN845254	MN845389	MN845519
Hycleus ambiguus	251	Kenya, 17 km N of Mombasa	MN849951	MN848636	MN845247	MN845381	MN845511
Hycleus amoenus	733.2	South Africa, Grootmis Buffels riverbed	MN849957	MN848637	MN845252	MN845387	MN845517
Hycleus amplectens	227	Kenya, Masai-Mara	MN849956	MN848638	MN845251	MN845386	MN845516
Hycleus apicicornis	2827a	Burundi, Kabuyenge	MN849958	MN848639	MN845253	MN845388	MN845518
Hycleus argentifer	3192a	Morocco, Meknes reg., Errachidia prov., Goulmima	MN849953	MN848640	MN845248	MN845383	MN845513
Hycleus argentifer bitinski	4445	Israel	MN849955	MN848641	MN845250	MN845385	MN845515
Hycleus biundulatus	2448	Pakistan, Islamabad	MN849961	MN848642	MN845256	MN845391	MN845521
Hycleus brevicollis	3382	Morocco, Oujda, 20 km SW	MN849964	MN848643	MN845259	MN845394	MN845524
Hycleus brunnipes	256	United Arab Emirates, Al Jazirat Al Hamra	MN849962	MN848644	MN845257	MN845392	MN845522
Hycleus brunnipes	4241	Morocco, 30km E of Foum Zguid	MN849963	MN848645	MN845258	MN845393	MN845523
Hycleus burmeisteri	542	South Africa, 70 km NE Kuruman	MN849966	MN848646	MN845261	MN845396	MN845526
Hycleus bushmanicus	734	Namibia, 23 km N Velloorsdrif	MN849965	MN848647	MN845260	MN845395	MN845525
Hycleus cfr. colligatus	4309a	Iran, Kerman, Dbhakri	MN849992	MN848649	MN845289	MN845424	MN845549
Hycleus cfr. humerosus	2280	Russia, Altai rep., Dzhaztor env	MN850027	MN848650	MN845332	MN845465	MN845589

Hycleus cfr medioinsignatus	3856	China, Sichuan, Luding, Jiaoziping	MN850016	MN848700	MN845318	MN845453	MN845577
Hycleus cfr. namaquus	261	South Africa, 28 km S Sutherland	-	MN848651	MN845298	MN845433	MN845557
Hycleus cfr. quadriguttatus	700	South Africa, 6 km NW Grahamstown	MN850017	MN848652	MN845320	MN845455	MN845579
Hycleus cfr. scabiosae	4295a	Iran, Kerman, road Sirch	MN850022	MN848653	MN845326	MN845460	MN845584
Hycleus cfr. semifasciatus	507	Syria, Palmyra	MN849954	MN848654	MN845249	MN845384	MN845514
Hycleus cichorii	3857	China, Yunnan, Lvchun, Mt. Huanglianshan, Xiaoheijiang	MN849968	MN848655	MN845263	MN845398	MN845528
Hycleus cingulatus	2622	Iran, Khorasan, Mashhad	MN849969	MN848648	MN845264	MN845399	MN845529
Hycleus colligatus	3312a	Iran, Elborz Mts., Above Rudehen	MN849991	MN848656	MN845288	MN845423	MN845548
Hycleus colligatus	4219	Iran, Kordestan, Sarvabad, Piyazeh mt	MN849970	-	MN845265	MN845400	MN845530
Hycleus concinnus	4444	Israel, Nahal Gmalim	MN849967	MN848657	MN845262	MN845397	MN845527
Hycleus convexior	240	Kenya, Galana	MN849971	MN848658	MN845266	MN845401	MN845531
Hycleus damarensis	538	Namibia, 8 km W Usakos	MN849972	MN848659	MN845267	MN845402	MN845532
Hycleus decemguttatus	544a	South Africa, 8km S Clanwilliam	MN849973	MN848660	MN845269	MN845403	MN845533
Hycleus decoratus	223	Namibia, 5 km W di Bullsport	MN849980	MN848661	MN845275	MN845410	MN845538
Hycleus dentatus	234	Namibia, Omaruru	MN849977	MN848662	MN845272	MN845407	MN845536
Hycleus derosus	699	South Africa, 12 km NE Wolmanransstad	MN849974	-	-	MN845404	-
Hycleus deserticolus	220b	Namibia, 16 km W di Karibib	MH668512.1*	MN848663	MN845276	MH668470.1*	-
Hycleus devylderi	739	Namibia, 33km NNW Usakos	-	-	MN845278	MN845413	-
Hycleus dicinctus	3907	Ivory Coast, Comoe, Zamou	MN849975	MN848664	MN845270	MN845405	MN845534
Hycleus dorsetiferus	3854	China, Xizang, Medog, Renqin	MN849978	MN848665	MN845273	MN845408	-
Hycleus duodecimmaculatus	3026	Morocco, Marrakech province	MN849981	MN848666	MN845277	MN845412	MN845539
Hycleus duodecimpunctatus	2199	France, Bouche du Rhone, Marseille, La Couronne	MN849979	MN848667	MN845274	MN845409	MN845537
Hycleus duplicatus	4494	Oman, Dhofar, Wadi Mugshail	MN849976	MN848668	MN845271	MN845406	MN845535
Hycleus fuscus	1760	Turkey, Adana, E Küçükgezbeli Geç.	MN849982	MN848669	MN845279	MN845414	MN845540
Hycleus haemactus	238	South Africa, Bowesdorp	MN849986	MN848670	MN845283	MN845418	MN845543
Hycleus haemorrous	3928	Ivory Coast, Comoe, Zamou	MN849983	MN848671	MN845280	MN845415	-
Hycleus hemprichi	2676	Israel, 5 km NW Yeroham	MN849987	MN848672	MN845284	MN845419	MN845544
Hycleus hermanniae	2790a	Togo, Dzegba, Konoji, Assaoun, Lomé	MN849984	MN848673	MN845281	MN845416	MN845541
Hycleus histrio	543	South Africa, 8 km E De Rust	MN849985	MN848674	MN845282	MN845417	MN845542
Hycleus humerosus	1677	Turkey, 20.5 km NE Zara	MN849988	MN848675	MN845285	MN845420	MN845545
Hycleus humerosus	2964a	Turkey, 10 km E Kırkarya	MN849989	MN848676	MN845286	MN845421	MN845546
Hycleus hybridus	548	Namibia, 25 km E Khorixas	MN849990	-	MN845287	MN845422	MN845547
Hycleus jucundus	222	Namibia, Oshakati, Ongenga	MN849993	MN848677	MN845290	MN845425	MN845550
Hycleus kochi	738	Namibia, 6 km N Otjiwarongo	MN849994	MN848678	MN845291	MN845426	MN845551
Hycleus lugens	1247	South Africa, KwaZulu-Natal	MN849995	MN848679	MN845292	MN845427	MN845552
Hycleus lunatus	236	South Africa, Gauteng, nr Bronkhorspruit	MN849997	MN848680	MN845294	MN845429	MN845554
Hycleus luteolineatus	249	Kenya, Tsavo East, between Aruba and Voi	MN849996	MN848681	MN845293	MN845428	MN845553
Hycleus maculiventris	4492	Oman, Dhofar, Wadi ayun	MN849998	MN848682	MN845295	MN845430	MN845555
Hycleus medioinsignatus	3860	China, Hebei, Zhuolu, Yangjiaping	MN849999	MN848683	MN845296	MN845431	MN845556
Hycleus mimosae	4442	Israel, Nahal Zenifim	MN850000	MN848684	MN845297	MN845432	-
Hycleus nairobianus	2955	Kenya	-	MN848685	MN845300	MN845435	MN845559
Hycleus namaquus	549	South Africa, 4,7 km W Spingbok	-	MN848686	MN845299	MN845434	MN845558

Hycleus novemdecimpunctatus	4234	Morocco, 20kmN of Taznakht road	MN850001	MN848687	MN845301	MN845436	MN845560
Hycleus octodecimmaculatus	3377	Morocco, Ain Bni Mathar 10 km N	MN850002	MN848688	MN845303	MN845437	MN845561
Hycleus ornatus	2679	Israel, Nizzanim dunes	MN850003	-	MN845304	MN845438	MN845562
Hycleus parenthesis	252	Kenya, Chakama	MN850004	MN848689	MN845305	MN845439	MN845563
Hycleus pertinax	541	South Africa, Limpopo, Strydpoortberge Pass	MN850011	MN848690	MN845312	MN845446	MN845570
Hycleus phaleratus	1378	Buthan, Jomkhar	MN850006	MN848691	MN845307	MN845441	MN845565
Hycleus pilosus	258	Mozambique, Matutuine dist., Maputo S.R., Milibangalala	MN850008	MN848692	MN845309	MN845443	MN845567
Hycleus pirata	262	United Arab Emirates, Abu Dhabi, Al Aïn, 3-4 Km E Al Selimat	MN850007	MN848693	MN845308	MN845442	MN845566
Hycleus plagiatus	4184	South Africa, EC, Compassberg	MN850005	MN848694	MN845306	MN845440	MN845564
Hycleus politus	740	Namibia, 10 km S Waltevrede	MN850009	MN848695	MN845310	MN845444	MN845568
Hycleus polymorphus	861	Grecia, Metsovo, Peristéri Mts, str. Anilio e Haliki	MH668513.1*	-	MN845313	MN845447	MN845571
Hycleus polymorphus	1970	Italy, Val d'Aosta PN Gran Paradiso, Valnontey	MN850012	MN848696	MN845314	MH668469.1*	MN845572
Hycleus polymorphus	2258	Russia, Altai rep., Samacha steppe	MN850013	MN848697	MN845315	MN845449	MN845573
Hycleus polymorphus	2276	Russia, Altai rep., Dzhaztor env	MN850014	-	MN845316	MN845450	MN845574
Hycleus polymorphus	2744	Italy, Liguria, Col di Nava	MN850015	-	MN845317	MN845451	MN845575
Hycleus polymorphus	858a	Grecia, Metsovo, Peristéri Mts, str. Anilio e Haliki	-	MN848698	-	MH668492.1*	MN845576
Hycleus praestans	228	Kenya, Masai-Mara	MN850010	MN848699	MN845311	MN845445	MN845569
Hycleus quadriguttatus	260	South Africa, 10 km E Calitzdorp	-	MN848701	MN845319	MN845454	MN845578
Hycleus raphael	2670a	Israel, 500 m W Nave	MN850018	MN848702	MN845321	MN845456	MN845580
Hycleus rufipalpis	3187a	Morocco, Sidi Allal-el-Bahraui	MN850019	MN848703	MN845322	MN845457	MN845581
Hycleus scabiosae	3347a	Iran, Shahrood	MN850021	MN848704	MN845325	MN845459	MN845583
Hycleus scabratus	4490	Oman, Dhofar, Wadi ayun	MN850020	MN848705	MN845323	MN845458	MN845582
Hycleus scapularis?	4493	Oman, Dhofar, Wadi Mugshail	MN850029	MN848706	MN845334	MN845467	MN845591
Hycleus schah	2621	Iran, S Khorasan, Ferdos	MN850025	MN848707	MN845330	MN845463	MN845587
Hycleus schauffelei	4223a	Iran, Kerman Khabr, Baghmolla	MN850024	MN848708	MN845329	MN845462	MN845586
Hycleus scutellatus	4000a	Spagna, Tabernas, Almeria	MN850032	MN848709	MN845337	MN845470	MN845593
Hycleus senegalensis	3920	Ivory Coast, Touba, Biémasso	MN850023	MN848710	MN845327	MN845461	MN845585
Hycleus sexmaculatus	4226a	Iran, Hamedan, E Malayer, Zangeneh, Lashgardar p.r.	MN850034	MN848711	MN845339	MN845472	MN845595
Hycleus silbermanni	3440a	Morocco, Ouarzazate	MN850026	MN848712	MN845331	MN845464	MN845588
Hycleus solonicus	3858	China, Hebei, Mt. Xiaowutaishan, Zhangjiayao	MN850028	MN848713	MN845333	MN845466	MN845590
Hycleus sp.	3949	Cameroon, N.W. Santa	MN849949	MN848714	MN845244	MN845379	MN845509
Hycleus stali	4447a	South Africa, Limpopo, York	MN850031	MN848715	MN845336	MN845469	-
Hycleus surcoufi	4451	South Africa, Limpopo, Zandspruit	MN850030	MN848716	MN845335	MN845468	MN845592
Hycleus svakopinus	676	Namibia, Fish River Canyon, Hiker Point	MN850033	MN848717	MN845338	MN845471	MN845594
Hycleus tavetanus	257	Kenya, Tsavo East, between Aruba and Voi	MN850035	MN848718	MN845340	MN845473	MN845596
Hycleus tekkensis	3170a	Kazakhstan, Sayak	MN850039	MN848719	MN845344	MN845478	MN845599
Hycleus terminatus	3939	Ivory Coast, Comoe, Zamou	MN850037	MN848720	MN845342	MN845475	-
Hycleus tinctus	545	Namibia, 25 km E Khorixas	MN850040	MN848721	MN845345	MN845479	MN845600
Hycleus transvaalicus	701a	South Africa, 17 km NE Bloemhof	MN850042	MN848722	MN845347	MN845481	MN845602
Hycleus tricolor	246	Mozambique, Matutuine distr., Maputo S.R., 4.5 km N of Gala Gate	MN850036	MN848723	MN845341	MN845474	MN845597
Hycleus trifasciatus	3953	Cameroon, NW Santa	MN850038	-	-	MN845476	-

Hycleus tripunctatus	552a	South Africa, 5 km N Rooihoogte Pass	MH668515.1*	MN848724	MN845343	MH668479.1*	MN845598
Hycleus tristigmus	229	Kenya, Masai-Mara	MN850041	MN848725	MN845346	MN845480	MN845601
Hycleus vansoni	736a	South Africa, 11.6 km jct Wallekraal	MN850043	MN848726	MN845348	MN845482	MN845603
Hycleus vestitus	3875a	Ivory Coast, Comoe, Zamou	MN850044	MN848727	MN845349	MN845483	-
Hycleus villosus	540	South Africa, Mpumalanga, Volksrust	MN850045	MN848728	MN845350	MN845484	-
Hycleus windhoekanus	214b	Namibia, D1525 to Bodenhausen	MN850046	MN848729	MN845351	MN845485	MN845604
Hycleus zebraeus	1684	Turkey, 20.5 km NE Zara	MN850047	MN848730	MN845352	MN845486	MN845605
Hycleus zebraeus	2934	Ukraina, NE Krym, Prozracne, Dzankoj	MN850048	MN848731	MN845353	MN845487	MN845606
Mylabris connata	2197a	France, Bouche du Rhone, Marseille, La Couronne	MN849930	MN848611	MN845225	MN845359	MN845492
Mylabris schreibersi	2912	Italy, Sicilia, PA, Capaci, Contrada Zarcati	MN849931	MN848612	MN845226	MN845360	MN845493
Oenas sericeus	324	Morocco, 5 Km S Settat	MN849929	MN848610	MN845224	MN845358	MN845491
Paractenodia glabra	727	Namibia, 17 km SE Messum Valley	MN850049	MN848732	MN845354	MN845488	MN845607
Paractenodia namaquaensis	599	Namibia, S Karasburg, Ortmaunsbaum	MH668591.1*	MN848733	MN845355	MH668487.1*	MN845608
Paractenodia parva	728.1	Namibia, 20 km S Aus	MN850050	MN848734	MN845356	MN845490	MN845609

Table S2

List of primer used in this work and their sequences. PCR Thermal Cycle conditions for each gene are also reported.

GENE	PRIMER	SEQUENCE	THERMAL CYCLE
COI	LCO1490 [1]	GGTCAACAAATCATAAAGATATTGG	94°C 3' (94°C 1'- 46°C 1'30" - 72°C 1'30")x5 (94°C 1'- 48°C 1'30" - 72°C 1'30")x35 72° 6'
	HCO2198 [1]	TAAACTTCAGGGTGACCAAAAAATCA	
	COIMelZ2 [2]	GGGTCAAAGAARGATGTATT	
ARGK	AK168F [3]	CAGGTTTGGARAAYCAACGAYTCYGG	94°C 2' (94°C 30"- 60°C 30" - 72°C 1')x8 (94°C 30"- 57°C 30" - 72°C 1')x35 72° 3'
	AK939R [3]	GCCNCCYTCRGCYTCRGTGTGYTC	
	AK183F [3]	GATTCTGGAGTCGGNATYTAYGCNCCYGAYGC	
	AKHY1F [4]	GCTCCTGATGCTGAAGCCTA	
	AKHY1R [4]	GACGRTCATGRTGGGAGAAYG	
CAD	CD439F [3]	TTCAGTGTACARTTYCAYCCHGARCAYAC	94°C 2' (94°C 30"- 55°C 30" - 72°C 1')x5 (94°C 30"- 50°C 30" - 72°C 1')x5 (94°C 30"- 45°C 30" - 72°C 1')x35 72° 3'
	CD688R [3]	TGTATACCTAGAGGATCDACRTTYTCCATRTTRCA	
	CAD_int2_R [5]	AATTATCATAAGCATCACGTAC	
WG	Wg550F [3]	ATGCGTCAGGARTGYAARTGYCAYGGYATGTC	94°C 2' (94°C 30"- 59°C 30' - 72°C 1')x35 72° 3'
	WgAbRZ-R [3]	CACTTNACYTCRCARCACCARTG	
28S	28S01 [6]	GACTACCCCCTGAATTTAAGCAT	94°C 2' (94°C 1'- 56°C 1' - 72°C 2')x35 72° 3'
	28SR01 [6]	GACTCCTTGGTCCGTGTTTCAG	

References

[1] Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotech. 3, 294–299.

[2] Salvi, D., Maura, M., Pan, Z., Bologna, M.A., 2019. Phylogenetic systematics of Mylabris blister beetles (Coleoptera, Meloidae): a molecular assessment using species trees and total evidence. Cladistics 35, 243-268.

[3] Wild, A.L., Maddison, D.R., 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. Mol. Phyl. Evol. 48, 877–891.

[4] This study

[5] Riccieri, A., Maura, M., Salvi, D., Bologna, M. A., Mancini, E., 2017. Messinian Salinity Crisis and Quaternary glacial events shaped genetic diversification in Siculo-Maghrebian blister beetles (Coleoptera: Meloidae). Biol. J. Linn. Soc. 122, 455–468.

[6] Kim, C.G., Zhou, H.Z., Imura, Y., Tominaga, O., Su, Z.H., Osawa, S., 2000. Pattern of morphological diversification in the Leptocarabus ground beetles (Coleoptera: Carabidae) as deduced from mitochondrial ND5 gene and nuclear 28S rDNA sequences. Mol. Biol. Evol. 17, 137–145.

Table S3

List of the groups of species described in literature, for each section of *Hycleus* and for the genera *Ceroctis* and *Paractenodia*, according to morphological characters (see references). In the fourth column we summarized the results of the phylogenetic assessment of each group relatively to the analysed species, which are indicated with an asterisk (*).

Sections and genera, with distinctive characters	Phenetic species group		Phylogenetic assesment according to the present work
Mesogorbatus [1,2] (mesosternum without a clear modified fore portion or scutum)	*Hycleus borchmannianus*[3]	*H. borchmannianus*	-
	Hycleus maculiventris[3, 4]	*H. maculiventris** *H. abiadensis* *H. lateplagiatus* *H. rutilicollis* *H. thomasi* *H. schah**	Monophyletic (clade Q)
	Hycleus sexmaculatus[3, 4]	*H. sexmaculatus** *H. apicipennis* *H. trianguliferus* *H. bipunctatus* *H. schauffelei** *H. balteatus* *H. pirata** *H. cingulatus**	Monophyletic (clade R)

		*H. colligatus** *H. tenuepictus*	
	Hycleus brunnipes[3, 6]	*H. brunnipes** *H. raphael** *H. ghorfii* *H. kaszabi* *H. varius?* *H. pintoi*	Monophyletic (clade S)
	Hycleus quatuordecimsignatus[3, 4, 7]	*H. quatuordecimsignatus* *H. saharicus* *H. rungsi* *H. wagneri* *H. pseudobrunnipes*	-
	Hycleus dunalis[3]	*H. dunalis*	-
	Hycleus gratiosus[3]	*H. gratiosus*	-
	Hycleus rubricollis[3]	*H. rubricollis*	-
	Hycleus dentatus[8]	*H. dentatus**	
	Hycleus tripunctatus[8]	*H. tripunctatus** *H. coecus?*	

	H. connexus?	
Hycleus argentatus[4, 5, 8]	*H. argentatus*	Monophyletic (clade P)
	*H. jucundus**	
	H. peringueyi	
	*H. surcoufi**	
	H. bicoloricornis	
	H. egregius	
	H. gabonensis	
	H. guineensis	
	H. ocellaris	
	H. quadrinotatus	
	H. amabilis	
	H. salaamensis	
	H. upembanus	
	H. rungsianus	
Hycleus brincki[8]	*H. brincki*	-
Hycleus congoensis[8]	*H. congoensis*	-
	H. arabicus?	
Hycleus amplectens[4]	*H. amplectens**	Monophyletic (clade O)
	*H. vestitus**	

Mesoscutatus[1,2] (mesosternum with a wide fore modified portion named scutum)	*Hycleus duplicatus*[3]	*H. duplicatus**	Monophyletic (clade F)
		H. aestuans	
		H. andreinii	
		H. angyrosticus	
		*H. scapularis**	
	Hycleus scabratus[3]	*H. scabratus**	
	Hycleus pallipes[3, 8, 9, 10]	*H. pallipes*	Not monophyletic
		H. nigriplantis	
		H. pakistanus?	
		H. hilaris	
		*H. villosus**	
		H. rufitarsis	
		*H. pertinax**	
		H. andrei	
		H. dahomeyensis	
	Hycleus dubiosus[10]	*H. boranus*	
		H. dubiosus	
		H.liquidus	
		*H. trifasciatus**	
	Hycleus fraudulentus[3]	*H. fraudulentus*	-

Hycleus pitcheri[3]	*H. pitcheri*	-
Hycleus adamantinus[8]	*H. adamantinus*	-
Hycleus amoenus[8]	*H. amoenus** *H. bushmanicus** *H. deserticolus** *H. devylderi** *H. kochi** *H. politus** *H. vansoni** *H. planitiei* *H. afrotrpicus?* *H. yemenicus?*	Monophyletic only with the inclusion of *Paractenodia* (clade T)
Hycleus lavaterae[8]	*H. lavaterae* *H. haemactus** *H. plagiatus** *H. testudo* *H. capeneri* *H. tettensis*	Monophyletic (clade J) only with the inclusion of *H. tricolor*, previously referred to the group of *H. dicintus* (see below) and of *H. praestans*.Renamed Group of *H. tricolor* after this work.
Hycleus lactimalus[8]	*H. lactimalus* *H. maculicornis*	-

	H. maxillaris	
Hycleus dicinctus[8]	*H. dicinctus** *H. scalaris* *H. tricolor** *H. versutus*	Not monophyletic (clade I): must include *H. senegalensis* and exclude *H. tricolor* (see above)
Hycleus hybridus[8, 10]	*H. hybridus** *H. herero* *H. convexior** *H. ligatus* *H. cruentatus*	Not monophyletic
Hycleus tinctus[8]	*H. tinctus** *H. matabele*	
Hycleus africanus[8]	*H. africanus* *H. namaquus** *H. quadriguttatus**	Monophyletic (clade G) with the new inclusion of *H. histrio* and *H. decemguttatus* previously not considered but phenetically similar
Hycleus svakopensis[8]	*H. svakopensis*	-
Hycleus dvoraki[8]	*H. dvoraki* *H. aridus*	-
Hycleus phaleratus[11]	*H. phaleratus** *H. hirtus*	Monophyletic (clade H)

		*H. biundulatus** *H. marcipoli* *H. cichorii** *H. parvulus* *H. brevetarsalis* *H. dorsetiferus** *H. medioinsignatus** *H. mannehinsi*	
	Hycleus polymorphus[11, 12]	*H. polymorphus** *H. atratus* *H. tenerus* *H. zebraeus** *H. fuscus** *H. scabiosae** *H. pierrei* *H. humerosus** *H. solonicus** *H. chodschenticus* *H. hokumanensis* *H.*	Monophyletic (clade K)

		quatuordecimpunctatus *H. tekkensis** *H. biguttatus*	
	Hycleus brevicollis[13]	*H. brevicollis** *H. duodecimpunctatus** *H. scutellatus**	Monophyletic (clade N) with the inclusion of *H. duodecimmaculatus*
	Hycleus duodecimpunctatus[14]	*H. rufipalpis** *H. novemdecimpunctatus** *H. linnavuori** *H. argentifer** *H. octodecimmaculatus** *H. ornatus**	Monophyletic only after the exclusion of *H. duodecimpunctatus*, which refers to the group of *H. brevicollis* (see above), and of *H. ornatus*, and the inclusion of *H. aegyptiacus.* Here renamed as group of *H. novemdecimpunctatus* (clade M)
	Hyclue silbermanni[1, 14]	*H. silbermanni** *H.allardi** *H. concinnus**	Described in this work (clade L)
Mesotaeniatus[1,2] (mesosternum with a narrow fore modified portion, named scutum)	*Hycleus lunatus*[8]	*H. lunatus** *H. bissexnotatus*	*H. lunatus* resulted part of the group of *H. apicicornis* (see below)
	Hycleus tristigmus[8]	*H. tristigmus** *H. ertli?*	*H. tristigmus* resulted close to *H. alternus*

	Hycleus apicicornis[10]	*H. apicicornis** *H. parenthesis** *H. ambiguus** *H. burmeisteri** *H. catenatus* *H. auritinus* *H. lugens?** *H. kersteni* *H. mylabroides* *H. tigrinus*	Monophyletic group (clade E) with the exclusion of *H. burmeisteri*, never defined before, and with the inclusion of *H. nairobianus*, phenetically similar (Bologna, unpublished) and of *H. lunatus* (see above)
Paractenodia	[8]	*P.parva** *P. namaquensis** *P. freyi* *P. damarensis* *P. glabra**	To be referred to *Hycleus*. Included in the group of *H. amoenus* (see above)
Ceroctis	*Ceroctis angolensis*[8]	*C. angolensis** *C. callicera* *C. delagoensis* *C. exclamationis* *C. matetsiensis**	To be referred to *Hycleus*. Not monophyletic

	C. pilosicollis *C. pubicollis* *C. ruficrus* *C. trifurca** *C. tripunctata** *C. bilineata?* *C. bunkeyana?* *C. foveithorax?* *C. vittata?*	
Ceroctis aurantiaca[8]	*C. aurantiaca* *C. bohemani** *C. ovamboana* *C. paolii* *C. rufimembris*	To be referred to *Hycleus*. Not monophyletic
Ceroctis gyllenhalli[8]	*C. gyllenalli* *C. aliena** *C. peringuey** *C. trifasciata**	To be referred to *Hycleus*. Not monophyletic
Ceroctis interna[8]	*C. interna* *C. angolana*	To be referred to *Hycleus*

	C. blanda	
	C. congoana	
Ceroctis korana[8]	*C. korana**	To be referred to *Hycleus*. Not monophyletic
	*C. amphibia**	
	*C. karroensis**	
Ceroctis capensis[8]	*C. capensis**	To be referred to *Hycleus*. Not monophyletic
Ceroctis subtrinotata[8]	*C. subtrinotata*	To be referred to *Hycleus*
	C. trispila?	
	C. bisbilunata?	
	C. seabrai?	

References

[1] Pardo Alcaide, A., 1954. Études sur les Meloidae. V. Les Mylabrini du Maroc et du Sahara occidental espagnol (Col. Meloidae). Bull. Soc. Sci. nat. phys. Maroc 34, 55–88.

[2] Pardo Alcaide, A., 1955. Estudios sobre Meloidae VI. Sobre algunos Mylabris (s. lat.) de la región etiópica. Bull. Inst. R. Sci. nat. Belg. 31(49), 1–32.

[3] Bologna, M.A., Turco, F., 2007. The Meloidae (Coleoptera) of the United Arab Emirates with an updating of the Arabian checklist. Zootaxa 1625, 1–33.

[4] Pardo Alcaide, A., 1963a. Coléoptères Méloïdes récoltés par M. J. Mateu dans l'Ennedi et au Tchad Etudes sur les Meloidae, XII. Bull. Instit. Fondam. Afr. Noire (A) 25, 572588.

[5] Pardo Alcaide, A., 1963b. Études sur les Meloidae. XIV. Trois Nouvelles espèces de Mylabris Fabricius (Coleoptera) EOS, Rev. Españ. Entomol. 39, 295–306

[6] Pardo Alcaide, A., 1968. Étude sur les Meloidae (Coleoptera). XX. Sur le genre Gorrizia Pardo et une nouvelle espèce soudanaise du groupe du brunnipes (Klug). EOS, Rev. Españ. Entomol. 43, 623–629.

[7] Ruiz, J.L., 2004. Distribución y morfología de Hycleus rungsi (Peyerimhoff, 1935), un endemismo de Marruecos meridional (Coleoptera, Meloidae). Bol. Soc. Entomol. Arag. 34, 95-100.

[8] Bologna, M.A., Amore, V., Pitzalis M., 2018. Meloidae of Namibia (Coleoptera): taxonomy and faunistics with biogeographic and ecological notes. Zootaxa 4373, 1–14.

[9] Pardo Alcaide, A., 1963c. Études sur les Meloidae. XVI. Sur quelques Mylabris Fabricius des collections de l'IFAN. Bull. Instit. Fondam. Afr. Noire (A) 25 697-709.

[10] Pardo Alcaide, A., 1958. Études sur les Meloidae (Col.). IX. Observations sur quelques Mylabris (s.l.) des régions éthiopienne et orientale. Bull. Inst. R. Sci. nat. Belg. 35(13), 1–39.

[11] Pan, Z., Carosi, M., Bologna, M.A., 2014. A new Eastern Asian Hycleus and key to the Chinese species of the phaleratus group (Coleoptera, Meloidae, Mylabrini). ZooKeys 463, 11–19. doi: 10.3897/zookeys.463.8261 http://zookeys.pensoft.net.

[12] Bologna, M.A., 1991. Coleoptera Meloidae. Fauna d'Italia. XXVIII, Calderini, Bologna.

[13] Pardo Alcaide 1950. Estudios sobre "Meloidae". II. Los "Mylabrini" de la Peninsula Iberica. Bol. Patol. Veg. Entomol. Agr. 17, 61-82.

[14] Pardo Alcaide 1965. Études sur les Meloidae. XVII. Matériaux pour une révision des Mylabrini de l'Afrique du nord et du Moyenne Orient (Coleoptera). EOS, Rev. Españ. Entomol. 40, 529-544.

CHAPTER 2

Phylogenetics and population structure of the steppe species Hycleus polymorphus reveals multiple refugia in Mediterranean mountain ranges

Alessandra Riccieri[1]; Emiliano Mancini[2]; Daniele Salvi[3,4]; Marco Alberto Bologna[1]

[1] Department of Sciences, University "Roma Tre", Viale G. Marconi 446, 00146 Roma, Italy
[2] Department of Biology and Biotechnology "C. Darwin", "Sapienza" University of Rome, Viale dell'Università 32, 00186, Roma, Italy
[3] Department of Health, Life & Environmental Sciences, University of L'Aquila, Via Vetoio snc, 67100 L'Aquila-Coppito, Italy
[4] CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

Corresponding author: Alessandra Riccieri; e-mail: alessandra.riccieri@uniroma3.it

Keywords

phylogeography – continental elements - Pleistocene climate oscillations - cold adapted species - Mediterranean mountains - fragmented distribution – ITS2 – sequence signature – CAD - COI

Ready for submission to The Biological Journal of The Linnean Society

Abstract

Continental elements distributed in steppe environments from Asia to central Europe often show relict populations in the mountain ranges of Western Europe. Their response to Quaternary climate changes is poorly studied, but it is thought they may have acted alike cold-adapted species.-In this work we investigated the biogeographic history of a typical steppe element, the blister beetle *Hycleus polymorphus,* from the family of Meloidae, using two nuclear markers (CAD and ITS2).-Since the taxonomy of the species group of *H. polymorphus* is poorly resolved, we first performed a molecular phylogenetic assessment to define species boundaries within this group. This lead us to include respectively within *H. polymorphus* and *H. atratus* specimens that were assigned to *H. humerosus* and *H. zebraeus* based on morphology. Unlike CAD, ITS2 data analyses revealed a strong phylogeographic structure of *H. polymorphus* populations, with the presence of four main isolated haplogroups. Our outcomes suggest that *H. polymorphus* responded to Pleistocene climate oscillations expanding its range from central Asia to South - Western Europe, following the spread of steppe environments. On the contrary, during interglacials, populations in the Mediterranean area underwent contraction of their range and altitudinal shifts becoming confined to higher altitudes in different mountain ranges.

1. Introduction

The current distribution of organisms and their genetic diversity has been mostly shaped by Pliocene and Pleistocene climatic fluctuations, especially in the northern hemisphere (Taberlet *et al.*, 1998; Hewitt, 1999, 2000, 2004; Petit *et al.*, 2003). In the Palaearctic Region, two types of biota have been documented by different authors according to their response to Pleistocene climate fluctuations (Schmitt, 2007, 2017; Stewart *et al.*, 2010; Bartonova *et al.*, 2018): the warm-adapted biota, which ranges underwent a contraction during cold periods and an expansion during interglacial phases (Hewitt, 1999; Seddon *et al.*, 2001; Habel *et al.*, 2011; Korabek *et al.*, 2018); and the cold-adapted biota, which showed a reverse pattern (Schmitt & Hewitt, 2004; Mardulyn *et al.*, 2009; Smith *et al.*, 2017; but see Salvi *et al.*, 2014 and Senzuck *et al.*, 2019 for notable exceptions). Recently, phylogeographic studies also focused on a third kind of biota, the so called "continental elements", currently distributed in steppes and steppe-like environments in plain and mountain areas from central Asia to eastern-central Europe

(Schmitt, 2007, 2017; Stewart *et al.*, 2010; Kajtoch *et al.*, 2016). Among these continental elements, few species present also isolated populations in the mountain ranges of South-Western Europe, which are considered as relicts of a continuous distribution occurred during a more favourable climate stage (e.g. *Parnassius apollo*, Todisco *et al.*, 2010; *Vipera ursinii-renardi complex*, Ferchaud *et al.*, 2012). Continental elements are expected to widen their ranges according to a longitudinal gradient (instead of latitudinal) from east to west during glacials and to retreat eastward during interglacials, following steppe expansions and contractions (Schmitt, 2007, 2009, 2017; Stewart et al., 2010; Bartonova *et al*., 2018). Consequently, they should display a longitudinal decreasing genetic diversity from east to west (Schmitt, 2007; Stewart *et al*., 2010). However, the review by Kajtoch *et al.* (2016) pointed out that steppe species do not exhibit a generalizable east-west expansions and contractions pattern, but rather show several separate interglacial refugia across their range, acting more alike cold-adapted species.

The blister beetle *Hycleus polymorphus* (Pallas, 1771) is one of the very few examples of steppe species with a disjunct Central Asiatic - Western European distribution, and represents an interesting opportunity to explore the biogeographic processes underlying this distribution pattern. *Hycleus polymorphus* belongs to the largest genus of the family of Meloidae (tribe Mylabrini) and it is part of a group of 14 species (Fig. 1) mainly centred in steppes and grasslands of western-central Asia (Bologna, 1991; Pan *et al.*, 2017; Riccieri *et al.* 2020). According to the current distribution of this species group, we can assume an Asiatic ancestral range of *H. polymorphus*. Among its allied taxa, *H. polymorphus* is the only species showing a disjunct pattern with isolated populations on Mediterranean mountain ranges (Bologna, 1991; Bologna & Pinto, 2002). Specifically, its current distribution includes with continuity steppes from Central Asia to Eastern Europe, usually at both middle- and high-altitudes, whereas in South-Western Europe it shows isolated populations mainly related to prairies and xeric pastures on the Pyrenees, the Alps, and mountain ranges of the Balkan peninsula (between ~300 and ~2000 m a.s.l.; Bologna, 1991, 1994).

Fig. 1 Six of the fourteen blister beetles from the species complex of Hycleus polymorphus. First row left to right: *Hycleus fuscus*, *H. tekkensis*, *H. cfr zebraeus* (by F. Cerini). Second row left to right: *H. polymorphus* (by A. Riccieri), *H. scabiosae*, *H. solonicus* (by Z. Pan).

Our hypothesis is that during glacial phases, *H. polymorphus* underwent possibly repeated expansions of its range from Central Asia to Western Europe, following the spread of steppe ecosystems. On the contrary, during interglacials, like the one we are presently experiencing, suitable environments for *H. polymorphus* in the Mediterranean basin were mainly confined to mountain ranges, forcing the species to survive at higher altitudes, originating the current fragmented and mountain distribution of populations. The main aim of this work is to test this hypothesis and describe a plausible biogeographic scenario at the origin of the peculiar distribution of *H. polymorphus*. In doing so, we investigated the population genetic structure of this species with particular emphasis on the

Mediterranean portion of its range where altitudinal shifts plausibly played a major role in adaptation during Pleistocene glacial cycles. However, since some species of the *H. polymorphus* group are often confused based on morphological features, especially when in syntopy [e.g. *H. zebraeus* (Marseul, 1870) with *H. polymorphus*; *H. chodschenticus* (Ballion, 1878) with *H. solonicus* (Pallas, 1782) (Bologna, 1991, 1994; Pan *et al.*, 2017)], our biogeographic investigation was preceded by a phylogenetic systematic assessment of the *H. polymorphus* species group to better define species boundaries.

2. Material and Methods

2.1 *Taxon Sampling and dataset preparation*

Samples used for this study were collected from 1999 to 2018. Specimens were stored in ethanol 96% at 4 °C. Only one examined specimen was killed in ethyl-acetate and pinned. Specimens identification was carried out according to dichotomous keys published by Marseul (1870) and Soumakov (1915) and, and to the taxonomic study by Bologna (1994).

Two different datasets were used: a first dataset for phylogenetic inference, including 53 specimens belonging to different populations of the following taxa defined based on morphological characters (Table S1): *Hycleus atratus* (Pallas, 1773) (n = 5), *H. fuscus* (A.G: Olivier, 1811) (n = 6), *H. humerosus* (Escherich, 1899) (n = 4), *H. polymorphus* (n = 18), *H. scabiosae* (A.G. Olivier, 1811) (n = 7), *H. solonicus* (n = 2), *H. tekkensis* (Heyden, 1883) (n = 1), *H. zebraeus* (n = 7), *H.* cfr. *zebraeus* (n = 2), and *Mylabris sinuata* Klug 1845 as outgroup. A second dataset was used for phylogeographic analyses, and included 103 specimens of *H. polymorphus* from 27 localities (Fig. 2; Table 1), plus 4 specimens of *H. humerosus*, which resulted nested within the *H. polymorphus* clade based on phylogenetic investigations (see Results).

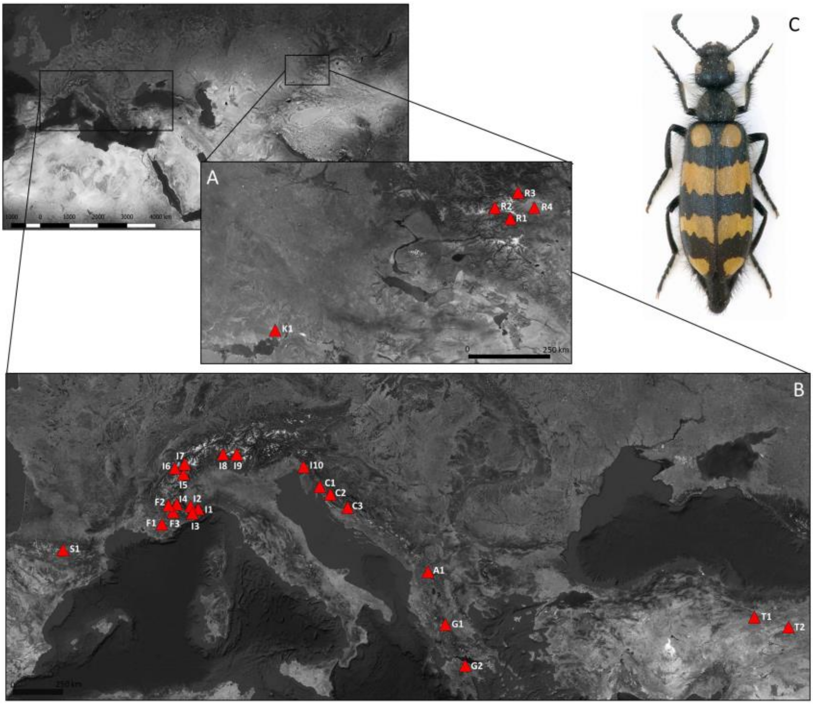


Fig. 2 Geographical distribution of sampled localities of *Hycleus polymorphus*. **A**. Central Asia **B**. Mediterranean Europe and Anatolia **C**. *Hycleus polymorphus*.

Table 1. Sampled localities and relative altitude, coordinates, collection date and number of individuals analysed for each marker. (n.d. = not detected)

SPECIES	**LOCALITIES**			**ALTITUDE** (m a.s.l.)	**COORDINATES**	**DATES** (dd/mm/yy)	**CAD**	**ITS2**
H. humerosus	**TURKEY**	**T1**	Sivas	1720	39°57'29.88"N 37°56'33.04"E	03/07/2010	1	1
		T2	Tunceli	1680-1850	39°27'10.71"N 39°46'40.06"E	22/06/2013	3	2
H. polymorphus	**CROATIA**	**C1**	Krk Island	350	45°01'25.48"N 14°20'26"E	21/05/2017	1	1
		C2	Krasno Polje	1020	44°51'43"N 14°53'33"E	18/07/2017	1	1
		C3	Vučipolje	832	44°15'17"N 15°56'59"E	15/06/2018	5	5
	ALBANIA	**A1**	Korab Mts.	1700	41°48'07"N 20°29'56"E	07/2017	4	4
	GREECE	**G1**	Ioannina, Metsovo, Peristéri Mts	1750	39°42'18.90"N 21°12'37.83"E	06/07/2009	5	5
		G2	Kalavrita, Aroania Mts	1610	38° 0'27.03"N 22°11'56.61"E	08/07/2009	5	5
	ITALY	**I1**	Col di Nava (IM)	850	44° 6'17.44"N 7°52'13.86"E	28/07/2012 - 23/6/2017	9	7
		I2	Carnino (CN)	1700	44° 8'52.20"N 7°44'6.79"E	27/07/2018	1	1
		I3	Upega (CN)	1350	44°07'56"N 7°42'54"E	23-24/06/2017	2	0
		I4	Sambuco (CN)	1300-1500	44°20'39.4"N 7°04'15.5"E	22/06/2004-29/06/2011	7	6
		I5	Valnontey (AO)	1899	45°35'8.48"N 7°19'56.42"E	10/07/2010	10	8
		I6	Chécrouit (AO)	2200	45°47'22.83"N 6°57'41.80"E	07/08/2018	4	3
		I7	Blavy (AO)	1460	45°46'28"N 7°20'28"E	15/07/2017	5	5
		I8	Montemezzo (CO)	1050	46°12'16.74"N 9°21'39.67"E	14/07/2017	5	4
		I9	Vervio (SO)	1430-1520	46°15'26"N 10°13'10"E	16/07/2017	5	4
		I10	Sgonico (TS)	270-300	45°43'59"N 13°45'15" E	03/06/2012	3	1
	FRANCE	**F1**	Var, Trigance env.	995	43°44’57”N 6°24’56”E	25/06/2017	5	5
		F2	Alpes d’Haute Provence, Villard d’Abbas	1535	44°19’22”N 6°39’45”E	29/06/2017	3	3
		F3	Alpes Maritimes Entraunes	1200	44°10’25”N 6°44’60”E	29/06/2017	5	5
	SPAIN	**S1**	Lerida, Valencia de Aneu	1700	42°37'21.72"N 1°4'54.68"E	n.d.	0	1
	KAZAKHSTAN	**K1**	Aktogay, Aâköz	393	47° 6'36"N 79°18'36"E	29/06/2002	0	1
	RUSSIA	**R1**	Altai rep, Belyashi	n.d.	49°42'60"N 87°24'59.99"E	12/06/2011	3	0
		R2	Altai rep., Samacha steppe	1600	49°43'46"N 87°15'25"E	08/06/2011	6	4
		R3	Altai rep., Kuray	n.d.	50°12'60"N 87°54'59.99"E	03/06/2011	2	2
		R4	Altai rep., Ortolyk	1750	50°04'18"N 88°25'30"E	25/06/2011	1	1
	Tot. Populations		**27**			**Tot.**	**101**	**85**

2.2 *DNA extraction, gene amplification, sequencing and alignment*

Following the salting out protocol (Sambrook *et al.*, 1989), we extracted total genomic DNA from one to three legs of each specimen, eluted in 100 µl of pure H_2O and stored at -20 C. DNA extraction from the dried sample required the procedure described by Gilbert *et al.* (2007) and modified by Giordani (2019).

Three genetic markers were amplified by PCR: the Cytochrome Oxidase subunit I (COI); the Carbamoylphosphate Synthetase domain of the rudimentary gene (CAD); and the Internal transcribed spacer (ITS2).

PCR amplifications were carried out in a total volume of 25 µl with 3 µl of 10x reaction buffer, 1/1,5/2 µl of $MgCl_2$ (50mM), 0,5/1 µl dNTPs (10 mM), 0,2 µl of TaqDNA polymerase (5 U/µl; BIOTAQ Bioline), 0,5µl of each primer (25 mM) and 1 µl of DNA template. PCR primers and thermal cycles followed Salvi et al. (2019) and Riccieri et al. (2017). Purification and sequencing of amplified products was carried out by Macrogen. In addition, 16 sequences for CAD and 15 for COI were downloaded from GenBank.

Sequences were edited with the program Staden Package v4.11.2 (Staden *et al.*, 2000), and aligned with MAFFT (Katoh *et al.*, 2017). Due to the presence of many indels in the ITS2 alignment including different species (dataset 1), ambiguous and poorly aligned positions were removed with the GBLOCKS server v0.91b (Castresana, 2000) for phylogenetic analyses

2.3 *Phylogenetic analysis of the species group*

Phylogenetic analyses were performed on single genes and multilocus datasets, by means of the web portal CIPRES (http://www.phylo.org). The best substitution model for each gene partition was selected with JModeltest v2.1.6 (Posada, 2008) according to the Akaike Information Criterion (AIC). Maximum Likelihood (ML) analysis was carried out with RAxML-HPC v8.2.10 (Stamatakis, 2006) with a partitioned GTRGAMMA model and a rapid-bootstrap analysis with 1000 replicates. Bayesian Inference (BI) was performed with the software Mr Bayes v3.2.6 (Ronquist *et al.*, 2012), with the following settings: two independent runs with four Markov chains each were run for 10 million generations sampling trees every 1000 generations with a 10% burn in. Tracer v1.6 (Rambaut *et al.*, 2014) was used to confirm the convergence of run, and FigTree v1.3.1 (Rambaut & Drummond, 2009) was used to visualize the tree.

2.4 *Phylogeography of* Hycleus polymorphus

Reconstruction of haplotypes was performed with the PHASE algorithm implemented in DNAsp v6 (Rozas *et al.*, 2017). Gap positions recovered in the ITS2 alignment were treated as 5th state characters. In order to infer relationships among haplotypes, we used the statistical parsimony network approach implemented in the software TCS v1.21 (Clement *et al.*, 2000), and tcsBU (Santos *et al.*, 2015) was used to depict the network of CAD and ITS2. For downstream analyses, sequences were grouped according to the haplogroups observed in the Network of ITS2. To do this, we excluded from CAD dataset those specimens that were not correctly amplified for ITS2. Number of segregating sites (S) and haplotypes (H), haplotype diversity (Hd) and nucleotide diversity (π) were computed with DNAsp v6 (Rozas *et al.*, 2017) for the entire dataset and for each haplogroup (Table 1). Arlequin v3.5 (Excoffier and Lischer, 2010) was used to estimate F_{st} values based on CAD, between haplogroups.

3. Results

3.1 *Phylogeny of the* H. polymorphus *species group*

Our final dataset consisted of 1731 bp (CAD: 782 bp, 50 sequences; COI: 577 bp, 53 sequences; ITS2: 372 bp, 40 sequences). Some COI chromatograms showed a few double picks, probably due to heteroplasmy. Therefore, we only used COI sequence to estimate interspecific relationships where the impact of these few heterozygotic sites is likely small compared to intraspecific comparisons.

Maximum Likelihood and Bayesian Inference results were rather consistent among multilocus (Fig. 3) and single-gene datasets (Fig. S1). In almost all the resulting trees, samples were grouped in well supported clades, but deeper relationships received lower support. In particular, seven clades were recovered in multilocus BI tree (Fig. 3a) corresponding to each nominal species, with two exceptions: specimens of *H. zebraeus* were included in the clade of *H. atratus* (clade F), and specimens of *H. humerosus* were nested within the clade of *H. polymorphus* (clade A). In the multilocus ML tree (Fig. 3b), and also in the COI ML tree (Fig. S1), two out of four samples of *H. humerosus* formed a sister group of a clade including all *H. polymorphus* and the remaining *H. humerosus*. Only in the phylogenetic trees based on the ITS2 *H. solonicus* and *H. polymorphus* are intermixed in the same clade (Fig. S1).

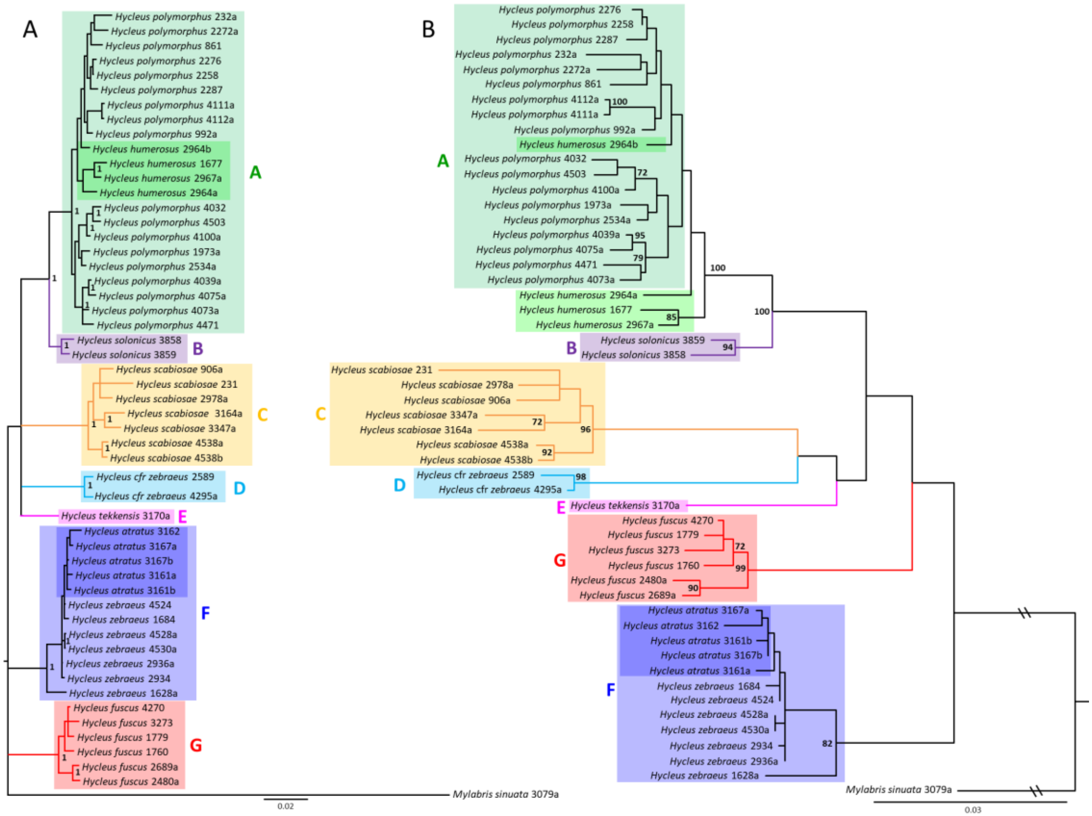
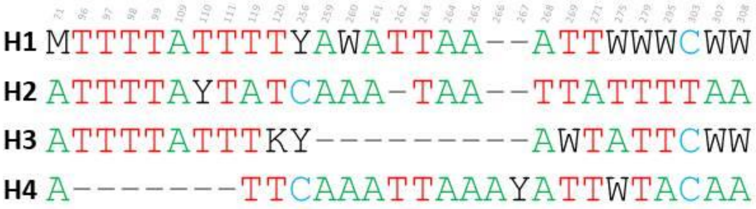


Fig. 3 A Bayesian Inference and **B** Maximum Likelihood multilocus (CAD, COI, ITS2) trees. Different colours are assigned to species clades.

3.2 *Phylogeography of* Hycleus polymorphus

The phylogeographic dataset of *Hycleus polymorphus* consisted of 170 (phased) sequences of ITS2 (376 bp) and 202 (phased) sequences of CAD (748 bp).

Along the ITS2 alignment some population-specific signature sequences [i.e. indel shared among specific OTUs (see Trizzino *et al.*, 2009)] were detected (Fig. 4).



	71	96	97	98	99	109	110	111	119	120	256	259	260	261	262	263	264	265	266	267	268	269	271	275	279	295	303	307	308
H1	M	T	T	T	T	A	T	T	T	T	Y	A	W	A	T	T	A	A	-	-	A	T	T	W	W	W	C	W	W
H2	A	T	T	T	T	A	Y	T	A	T	C	A	A	A	-	T	A	A	-	-	T	T	A	T	T	T	T	A	A
H3	A	T	T	T	T	A	T	T	T	K	Y	-	-	-	-	-	-	-	-	-	A	W	T	A	T	T	C	W	W
H4	A	-	-	-	-	-	-	-	T	T	C	A	A	A	T	T	A	A	A	Y	A	T	T	W	T	A	C	A	A

Fig. 4 Partial ITS2 alignment showing variable positions and sequence signature (i.e. indels). On the left, for each sequence is indicated the corresponding haplogroup observed in the network.

The ITS2 haplotypes network showed the presence of four main haplogroups (Fig. 5): (i) haplogroup 1 (H1) includes haplotypes from Kazakh, Russian Altai, and Italian Alpine populations; (ii) haplogroup 2 (H2) includes two haplotypes shared among populations from Western Alps (France and one sample from SW Italian Alsp) and Pyrenees (Spain); (iii) haplogroup 3 (H3) includes haplotypes from Southern Balkan mountains (Greece and Albania), North Eastern Pontic mountains (Turkey) and Altai mountains (Russia); (iv) haplogroup 4 (H4) includes haplotypes from Dinaric Alps (Croatia). H1 represents the core of the network, i.e. is connected with all the other haplogroups that have a terminal position and are separated from H1 respectively by: H2 = 6 mutational steps; H3 = 7 mutational steps; H4 = 9 mutational steps. On the contrary, we did not observe any phylogeographic structure (i.e., no clear haplotype/haplogroup segregation) in the haplotypes network of CAD.

Overall CAD resulted highly polymorphic (189 haplotypes; Table 2) with 157 heterozygous positions (21%), two third of which (66%) in third position, whereas ITS2 showed a lower level of polymorphism (22 haplotypes; Table 2). and a lower number of heterozygous positions (2.6%).

CAD F_{st} values scored between haplogroups pairs were all statistically supported ($p>0.005$) and ranged between 0.07-0.20, with the highest value observed between H2 and H3.

Table 2. DNA polymorphism of CAD and ITS2.Sequences were grouped according to haplogroups observed in ITS2 Network. (n.d. = not detected)

	Geographic groups	2N	S	H	Hd (%)	π (%)
***CAD* (748 bp)**	Overall	202	174	189	99.9	2.3
	H1	88	111	81	99.0	2.0
	H2	28	56	26	99.0	2.0
	H3	36	80	35	99.0	2.0
	H4	14	58	14	1	2.0
***ITS2* (376 bp)**	Overall	170	29	22	91.0	2.0
	H1	90	8	10	80.0	0.5
	H2	30	1	2	51.0	0.1
	H3	36	5	7	54.0	0
	H4	14	2	3	71.0	0.2

N, number of alleles (phased for CAD); **S**, number of segregating sites; **H**, number of haplotypes; **Hd**, haplotype diversity; **π**, nucleotide diversity.

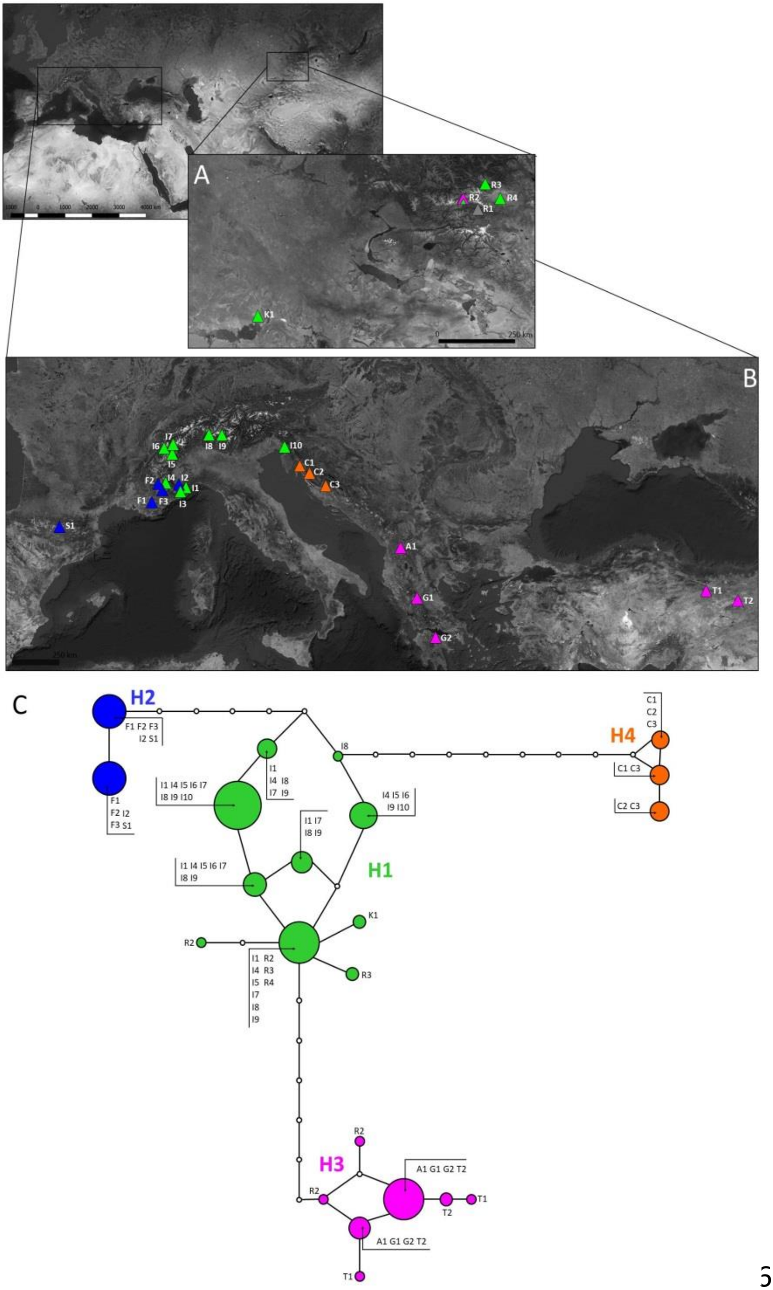
4. Discussion

Palaearctic steppe is among the largest continuous biomes on earth, which have existed as a wide belt connecting Asia and Europe that underwent expansions and contractions throughout the ice ages (Kajtoch *et al.*, 2016; Wesche *et al.*, 2016, Bartonova *et al.*, 2018). Particularly in Europe, during interglacial phases, steppe environments became confined to the eastern and south eastern regions, to spread again westward during colder and dryer glacial phases (Kajtoch *et al.*, 2016). Following this pattern, steppe species underwent severe range shifts during glacial cycles, which affected the genetic structure of their populations (Kajtoch *et al.*, 2016).

In this study, we investigated the evolutionary and biogeographic history of a typical steppe species, the blister beetle *Hycleus polymorphus*.

This species belongs to a primarily Centro-Asiatic species group and shows a disjunct Asiatic-European distribution with a continuous range in central Asia and fragmented and isolated populations along the mountain ranges of southern Europe (Pyrenees, Alps, Balkan ranges, Carpathians). Phylogenetic relationships among members of this species group revealed that specimens from Turkey, morphologically assigned to *H. humerosus*, are nested within the clade of *H. polymorphus* (Fig. 3a, 3b), as well as specimens of *H. atratus* and *H. zebraeus* are intermixed in the same clade. These results allowed better defining species boundaries within this species group, particularly of *H. polymorphus*, and provided a first molecular taxonomic assessment of the *H. polymorphus* species group.

Fig. 5 Maps showing the geographic distribution of the main haplogroups in **A.** Central Asia and **B.** Mediterranean Europe an Anatolia. **C.** Haplotype parsimony network of ITS2. Haplotypes are represented by circles with size proportional to their frequency. The geographic origin of the samples included in each haplotype is indicated with the codes reported in Table 1.


A
R3
R2
R4
R1
K1
0
250 km
B
I7
I6
I5
I8
I9
I10
C1
C2
C3
F2
I4
I2
I1
F1
F3
I3
S1
A1
G1
G2
T1
T2
250 km
C
H2
F1 F2 F3
I2 S1
F1
F2 I2
F3 S1
I8
I1
I4 I8
I7 I9
I1 I4 I5 I6 I7
I8 I9 I10
I4 I5 I6
I9 I10
I1 I7
I8 I9
I1 I4 I5 I6 I7
I8 I9
H1
K1
R2
I1 R2
I4 R3
I5 R4
I7
I8
I9
R3
H4
C1
C2
C3
C1 C3
C2 C3
R2
H3
A1 G1 G2 T2
R2
T2
T1
A1 G1 G2 T2
T1

Our results from ITS2 data analyses revealed a strong phylogeographic structure of *Hycleus polymorphus* populations, concordant with the fragmented distribution of the species. In particular we observed four main isolated haplogroups representing as many geographically distinct areas (if excluding central Asiatic haplotypes, see below) corresponding to the main mountain ranges: H1: Italian Alps, H2: SW Alps, including French and one Italian population, and Pyrenees; H3: South Balkan mountains and North East Pontic mountains; H4: North Dinaric Alps. The obtained genetic structure is in line with our hypothesis of a post glacial relict condition of the mountain populations from the Mediterranean area (Schmitt, 2009, 2017). In particular, haplogroups 2 and 3 showed the lowest haplotype diversities (Hd = 0.51, 0.54 respectively, Table 2) accordingly with a plausible recent isolation during an interglacial stage and consequent genetic drift, after the occurrence of glacial connections favouring gene flow both between SW Alps and Pyrenees (Albre et al., 2008; Schmitt, 2009, 2017), and between SW Balkan and Pontic mountains.

The isolated haplogroup 4, including Dinaric Alps (Croatia) populations (C1-3; Fig. 5), showed a surprisingly high genetic distance from the other sampled in the Balkan Peninsula (A1, G1, G2; Fig. 5). This is in contrast with previous hypothesis about the presence of a uniform glacial refugium in the Western Balkans (Steinfartz et al. 2000; Seddon et al. 2001; Heuertz et al. 2004; Michaux et al. 2004; Randi et al. 2004; Deffontaine et al. 2005), but a similar pattern has been already observed for example in the Martino’s vole (*Dinaromys bogdanovi* Martino V & Martino E, 1922), in which a strong North-South segregation of genetic lineages was found in this area, providing the evidence of at least three independent differentiation centres occurred within this mountain range (Krystufek *et al.*, 2007). Also in the steppe butterfly *Proterebia afra* (Fabricius, 1787) a clear distinction of lineages between North and South Balkans was observed, and, in this case, .a reduced climatic connection between these two areas was hypothesized (Bartonova et al., 2018). Interestingly, no genetic affinities were observed between Croatian (C1-3, H4; Fig. 5) and Italian (I10; Fig. 5) populations from Karst region, with the latter more related to the other Italian Alpine populations (H1; Fig.5).This strong segregation found in such a contiguous area might indicate that even during glacial stages no gene flow connected these two areas, increasing the genetic isolation of populations.

The only exception to this strong phylogeographic structure, is represented by the few analysed specimens (n = 13) from Central Asiatic populations, which show haplotypes intermixed with those from Italian Alps (H1) and from South Balkan and Pontic mountains (H3). Such a retention of haplotypes, referable to two highly divergent ITS2 signature sequences (Fig 4), might indicate: (a) an ancestral origin in the Asiatic populations of the genetic diversity currently observed in the two main haplogroups (H1, H3) of *H. polymorphus*, or (b) the introduction of this genetic variability following at least two colonization events from Italian Alps and South Balkans/Anatolia. Based on our network only, it is not possible to draw any conclusion on the polarization of the colonization routes, however, according to the current distribution of the species complex, mainly centred in western-central Asia, the first hypothesis (a) seems more plausible.

In conclusion, it is reasonable to speculate that *H. polymorphus* reacted to climate oscillations as a cold-adapted species (Kajtoch et al., 2016): during glacial phases it expanded its range from central Asia to South - Western Europe, following the spread of steppe environments, whereas, during interglacials, populations in the Mediterranean area underwent altitudinal shifts and became confined to higher altitudes in different mountain ranges.

The absence of phylogeographic structure in CAD, may indicate a recent origin of the disjunct pattern observed in *Hycleus polymorphus*. This gene, in fact, was successfully used in a previous phylogeographic analysis on three blister beetle species to highlight a strong genetic segregation among Maghrebian and Sicilian populations occurred after the Messinian Salinity Crisis, at least in *Mylabris schreibersi* Reiche, 1866 and *Cabalia segetum* (Fabricius, 1792) (Riccieri *et al.* 2017). Although very likely, unfortunately this hypothesis cannot be confirmed only based on our data: since we were prevented to use mitochondrial markers due to the presence of heteroplasmy, we could not perform any molecular dating estimates. Further analyses based on population genomic approaches will be helpful to better understand tempo and modes of evolution of *Hycleus polymorphus*.

Acknowledgements

This study was supported by a PhD scholarship to A.R. and by grants to M.A.B. from MIUR, Ministero dell’Istruzione, dell’Università e della Ricerca, Italy (Programmi di ricerca scientifica di rilevante interesse nazionale: PRIN 99C5271884-007; PRIN 2004057217 “Zoogeography of Mediterranean-southern African disjunct distributions by a multimethod approach'; CRUI "Programma di mobilità Pietro della Valle" 2017), and from the University Roma Tre, Department of Science. D.S. is currently supported by the program 'Rita Levi Montalcini' (MIUR) for the recruitment of young researchers at the University of L'Aquila.

We would like to thank people who provided samples for this study, or helped us in field collection: P. Audisio (Roma, Italy), G. Belmonte (Lecce, Italy), W. Beier (Germany), F. Cerini (Roma, Italy), P. Cerretti (Roma, Italy), L. Čížek (Ceske Budejovice, Czech Rep.), G. Cristiani (Alassio, SV, Italy), S.H. Dong (Hebei, China), L. Fekrat (Mashhad, Iran), G. Gardini (Genova, Italy), C. Giusto (Genova, Italy), S. Kolov (Alma Ata, Kazakhstan), S.S. Liu (Hebei, China), G. Mariani (Italy), Z. Pan (Hebei, China), R. Paoloni (Italy), R. Poggi (Genova, Italy), P. Rapuzzi (Cialla di Prepotto, UD, Italy), R. Sindaco (Torino, Italy), A. Vigna Taglianti + (already Roma, Italy), V. Volpe (Roma, Italy), M. Zapparoli (Viterbo, Italy), S. Zoia (Milano, Italy).

References

Albre J, Gers C, Legal L 2008. Molecular phylogeny of the *Erebia tyndarus* (Lepidoptera, Rhopalocera, Nymphalidae, Satyrinae) species group combining CoxII and ND5 mitochondrial genes: A case study of a recent radiation. *Molecular phylogenetics and evolution* 47, 196-210.

Bartonova A, Konvicka M, Korb S, Kramp K, Schmitt T, Faltynek Fric Z. 2018. Range dynamics of Palaearctic steppe species under glacial cycles: the phylogeography of Proterebia afra (Lepidoptera: Nymphalidae: Satyrinae). *Biological Journal of the Linnean Society* 125: 867-884.

Bologna MA, 1991. *Coleoptera Meloidae. Fauna d'Italia. XXVIII.* Bologna: Calderini.

Bologna MA, 1994. I Meloidae della Grecia (Coleoptera). *Fragmenta Entomologica* 25: 1-119.

Bologna MA, Pinto J D. 2002. The Old World genera of Meloidae (Coleoptera): a key and synopsis. *Journal of Natural Hystory* 36: 2013–2102.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540-552.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1660.

Deffontaine V, Libois R, Kotlík P, Sommer R, Nieberding C, Pradis E, Searle J.B. Michaux J.R. 2005. Beyond the Mediterranean peninsulas: evidence of central European glacial refugia for temperate forest mammal species, the bank vole (*Clethrionomys glareolus*). *Molecular Ecology* 14, 1727–1739.

Excoffier L, Lischer HE. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564-567.

Ferchaud AL, Ursenbacher S, Cheylan M, Luiselli L, Jelić D, Halpern B, Major A, Kotenko T, Keyan N, Crnobrnja-Isailović J, Tomović L, Ghira I, Ioannidis Y, Arnal V, Montgerald C. 2012. Phylogeography of the Vipera ursinii complex (Viperidae): mitochondrial markers reveal an east–west disjunction in the Palaearctic region. *Journal of Biogeography* 39: 1836–1847.

Gilbert MTP, Moore W, Melchior L, Worobey M. 2007. DNA extraction from dry museum beetles without conferring external morphological damage. *PloS one* 2: e272.

Giordani G. 2019. Non-invasive approaches to morphological and molecular identification of insects from museum, archaeo-funerary and forensic contexts. Unpublished D. Phil. Thesis, University of Huddersfield.

Habel JC, Lens L, Rodder D, Schmitt T. 2011. From Africa to Europe and back: refugia and range shifts cause high genetic differentiation in the Marbled White butterfly *Melanargia galathea*. *BMC Evolutionary Biology* 11: 215.

Heuertz M, Fineschi S, Anzidei M, Pastorelli R, Salvini D, PAule L, Frascaria-Lacoste N, Hardy O.J., Vekemans X., Vendramin G.G. 2004. Chloroplast DNA variation and postglacial recolonization of common ash (*Fraxinus excelsior* L.) in Europe. *Molecular Ecology* 13, 3437–3452.

Hewitt GM, 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* 68:87–112.

Hewitt GM, 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405:907–913.

Hewitt GM, 2004. Genetic consequences of climatic oscillation in the Quaternary. *Philosophical Transactions of the Royal Society of London B* 359:183–195.

Kajtoch Ł, Cieślak E, Varga Z, Paul W, Mazur MA, Sramkó G, Kubisz D. 2016. Phylogeographic patterns of steppe species in Eastern Central Europe: a review and the implications for conservation. *Biodiversity and Conservation* 25: 2309-2339.

Katoh K, Rozewicki J, Yamada KD. 2017. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in bioinformatics*.

Korabek O, Petrusek A, Jurickova L. 2018. Glacial refugia and postglacial spread of an iconic large European land snail, *Helix pomatia* (Pulmonata: Helicidae). *Biological Journal of the Linnean Society* 123: 218–234.

Krystufek B, Buzan E.V., Hutchinson W.F., HÄNFLING B. 2007. Phylogeography of the rare Balkan endemic Martino's vole, *Dinaromys bogdanovi*, reveals strong differentiation within the western Balkan Peninsula. *Molecular ecology* 16, 1221-1232.

Louy D, Habel JC, Abadjev S, Rakosy L, Varga Z, Rödder D, Schmitt T. 2014. Molecules and models indicate diverging evolutionary effects from parallel altitudinal range shift in two mountain Ringlet butterflies. *Biological Journal of the Linnean Society* 112: 569–583.

Louy D, Habel JC, Abadjiev S, Schmitt T. 2013. Genetic legacy from past panmixia: high genetic variability and low differentiation in disjunct populations of the Eastern Large Heath butterfly. *Biological Journal of the Linnean Society* 110: 281-290.

Mardulyn P, Mikhailov YE, Pasteels JM. 2009. Testing phylogeographic hypotheses in a Euro-Siberian cold-adapted leaf beetle with coalescent simulations. *Evolution* 63: 2717–2729.

Michaux JR, Libois R, Paradis E, Filippucci M-G 2004. Phylogeographic history of the yellow-necked fieldmouse (*Apodemus flavicollis*) in Europe and in the Near and Middle East. *Molecular Phylogenetics and Evolution* 32, 788–798.

Pan Z, Bai QQ, Wang J, Ren GD. 2017. Revision of *Hycleus solonicus* (Pallas, 1782)(Coleoptera: Meloidae, Mylabrini), with larval description and DNA barcoding. *Entomologica Fennica* 28: 219-232.

Petit RJ, Aguinagalde I, de Beaulieu JL, Bittkau C, Brewer S, Cheddadi R, Ennos R, Fineschi S, Grivet D, Lascoux M, Mohnty A, Müller-Starck G, Demesure-Musch B, Palmé A, Pedro Martin J, Rendell S, Vendramin GG. 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* 300:1563–1565

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*. 25: 1253–1256.

Rambaut A, Drummond A. 2009. FigTree, version 1.3. 1. Institute of Evolutionary Biology, University of Edinburgh.

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer 1.6. Available at http://beast.bio.ed.ac.uk/Tracer.

Randi E, Alves P.C., Carranza J., Milošević-Zlatanović S., Sfougaris A., Mucci N 2004.. Phylogeography of roe deer (*Capreolus capreolus*) populations: the effects of historical genetic subdivisions and recent nonequilibrium dynamics. *Molecular Ecology* 13, 3071-3083.

Riccieri A, Maura M, Salvi D, Bologna MA, Mancini E. 2017. Messinian Salinity Crisis and Quaternary glacial events shaped genetic diversification in Siculo-Maghrebian blister beetles (Coleoptera: Meloidae). *Biological Journal of the Linnean Society* 122: 455-468.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA Sequence

Polymorphism Analysis of Large Datasets. *Molecular Biology and Evolution* 34: 3299-3302.

Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.

Salvi D., Schembri P. J., Sciberras A., Harris D. J., 2014. Evolutionary history of the Maltese wall lizard Podarcis filfolensis: insights on the 'Expansion-Contraction' model of the Pleistocene biogeography. Molecular Ecology, 23: 1167–1187

Santos AM, Cabezas MP, Tavares AI, Xavier R, Branco M. 2015. tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics*, btv636.

Schmitt, T. 2007. Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. *Frontiers in zoology* 4, 11.

Schmitt T. 2009. Biogeographical and evolutionary importance of the European high mountain systems. *Frontiers in Zoology* 6: 9.

Schmitt T. 2017. Molecular biogeography of the high mountain systems of Europe: an overview. In: Catalan J, Ninot JM, Mercè Aniz M, eds. *High Mountain Conservation in a Changing World*. Cham: Springer, 63-74.

Schmitt T, Hewitt GM. 2004. Molecular biogeography of the arctic-alpine disjunct burnet moth species *Zygaena exulans* (Zygaenidae, Lepidoptera) in the Pyrenees and Alps. *Journal of Biogeography* 31: 885–893.

Seddon JM, Santucci F, Reeve NJ, Hewitt GM. 2001. DNA footprints of European hedgehogs, *Erinaceus europaeus* and *E. concolor*: Pleistocene refugia, postglacial expansion and colonization routes. *Molecular Ecology* 10: 2187–2198.

Senczuk G., Harris D. J., Castiglia R., Litsi Mizan V., Colangelo P., Canestrelli Salvi D., 2019. Evolutionary and demographic correlates of Pleistocene coastline changes in the Sicilian wall lizard Podarcis wagleriana. *Journal of Biogeography*, 46: 224-237

Smith S, Sandoval-Castellanos E, Lagerholm VK, Napierala H, Sablin M, Von Seth J, Fladerer FA, Germonpre M, Wojtal P, Miller R, Stewart JR, Dalen L. 2017. Nonreceding hare lines: genetic continuity since the Late Pleistocene in European mountain hares (*Lepus timidus*). *Biological Journal of the Linnean Society* 120: 891–908.

Staden R, Beal KF, Bonfield JK. 2000. The staden package. In: Misener S, Krawetz SA, eds. *Computer Methods in Molecular Biology,* *Bioinformatics Methods and Protocols*. Totowa: Humana Press Inc., 115–130.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.

Steinfartz S, Veith M, Tautz D 2000. Mitochondrial sequence analysis of Salamandra taxa suggests old splits of major lineages and postglacial recolonizations of Central Europe from distinct source populations of *Salamandra salamandra*. *Molecular Ecology* 9, 397–410.

Stewart, J. R., Lister, A. M., Barnes, I., Dalén, L. 2009. Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences* 277: 661-671.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cossons JF. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* 7:453–464.

Todisco V, Gratton P, Cesaroni D, Sbordoni V. 2010. Phylogeography of Parnassius apollo: hints on taxonomy and conservation of a vulnerable glacial butterfly invader. *Biological Journal of the Linnean Society* 101: 169-183.

Trizzino M, Audisio P, Antonini G, De Biase A, Mancini E. 2009. Comparative analysis of sequences and secondary structures of the rRNA internal transcribed spacer 2 (ITS2) in pollen beetles of the subfamily Meligethinae (Coleoptera, Nitidulidae): potential use of slippage-derived sequences in molecular systematics. *Molecular Phylogenetics and Evolution* 51: 215-226.

Wesche K, Ambarl D, Kamp J, Torok P, Treiber J, Dengler J. 2016. The Palaearctic steppe biome: a new synthesis. *Biodiversity and Conservation* 25: 2197–2231.

Appendix

Fig. S1a

Bayesian tree based on COI sequences. Only supported values of posterior probability are reported at each node (PP>0.9).

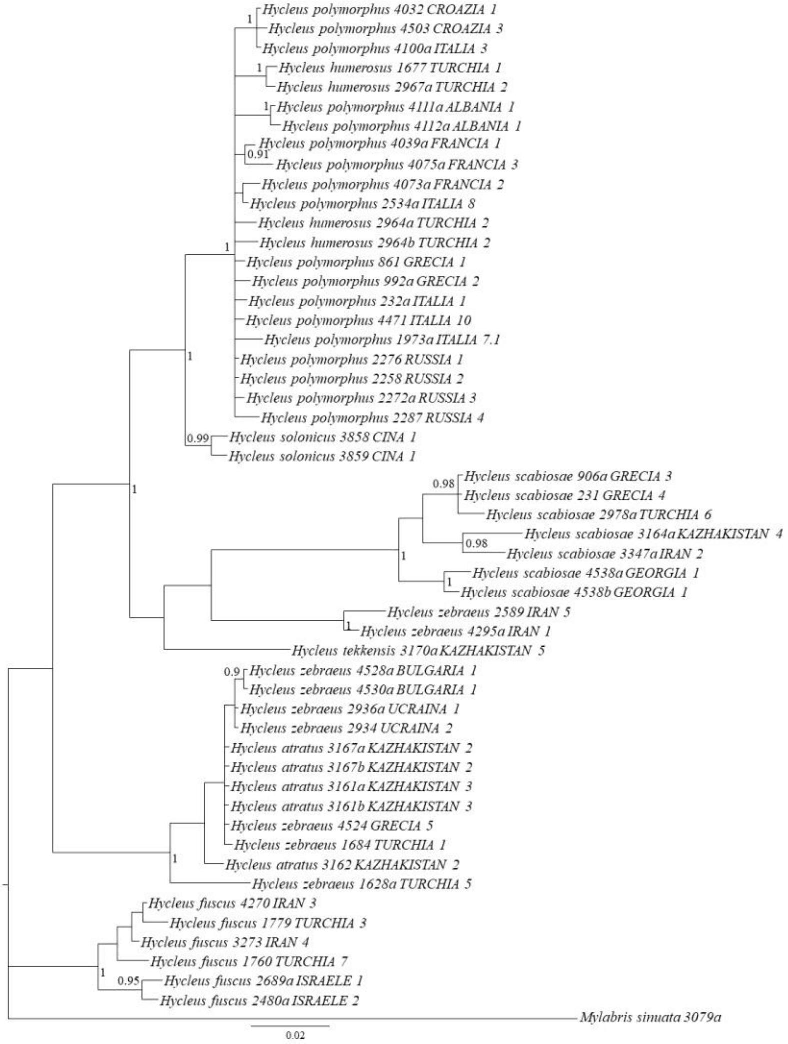


Fig. S1b

Bayesian tree based on ITS2 sequences. Only supported values of posterior probability are reported at each node (PP>0.9).

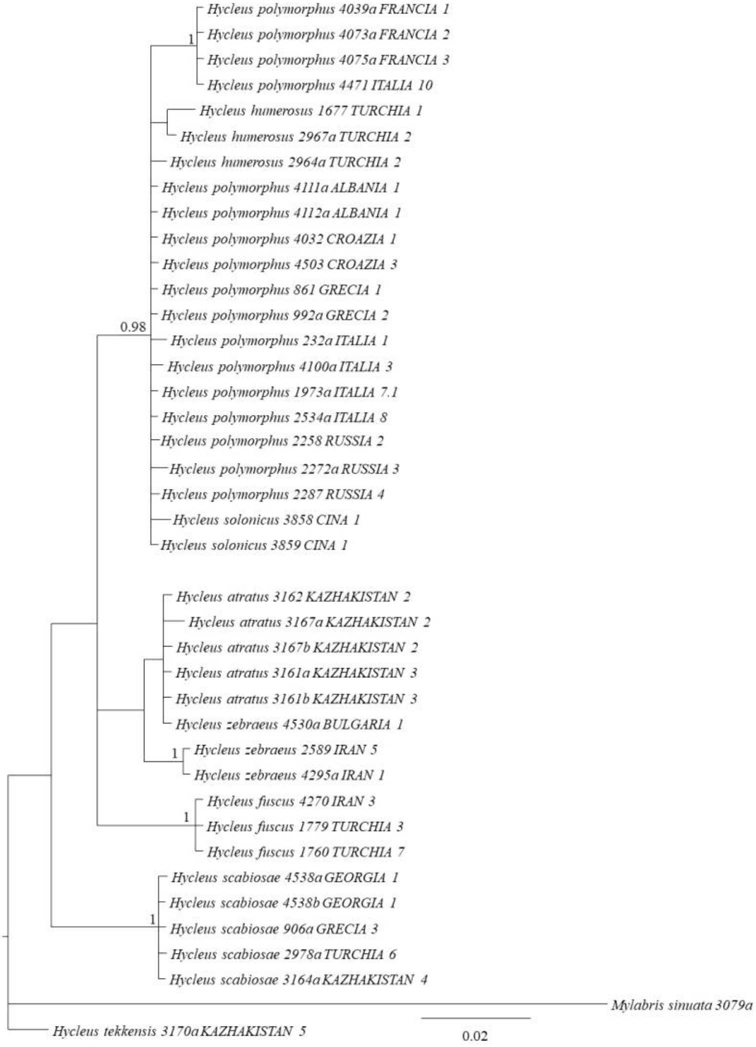


Fig. S1c

Bayesian tree based on CAD sequences. Only supported values of posterior probability are reported at each node (PP>0.9).

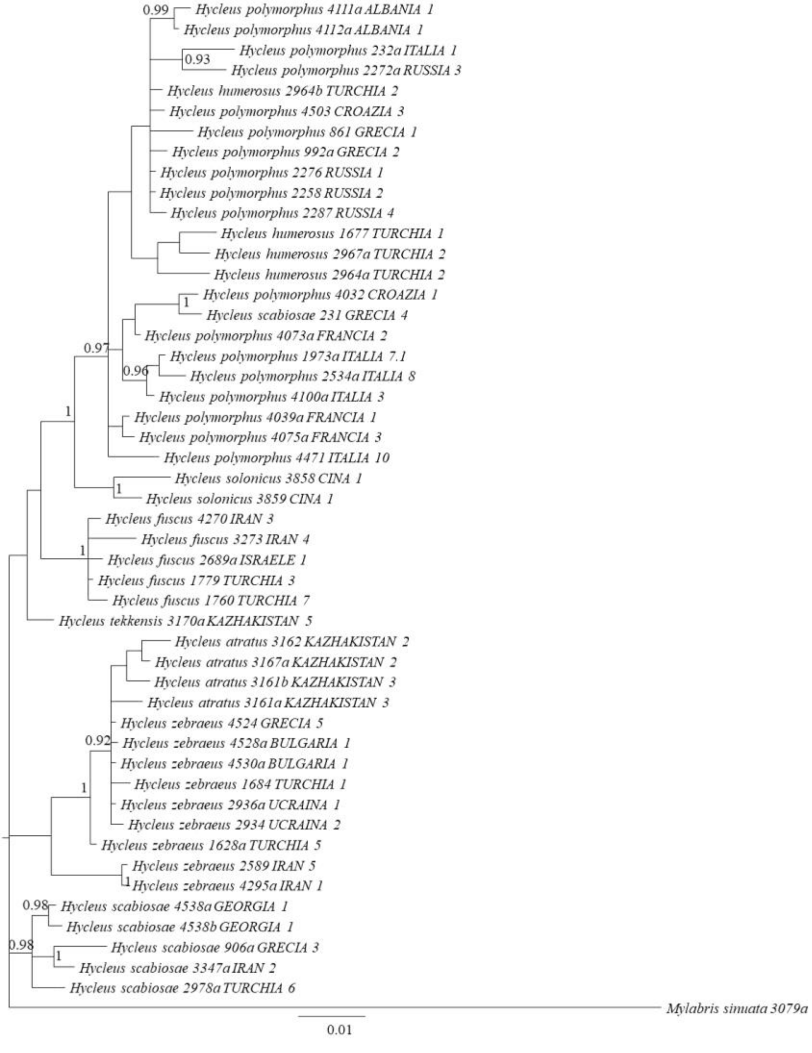


Fig. S1d

Maximum Likelihood tree based on COI sequences. Only supported values of bootstrap are reported at each node (BP>70).

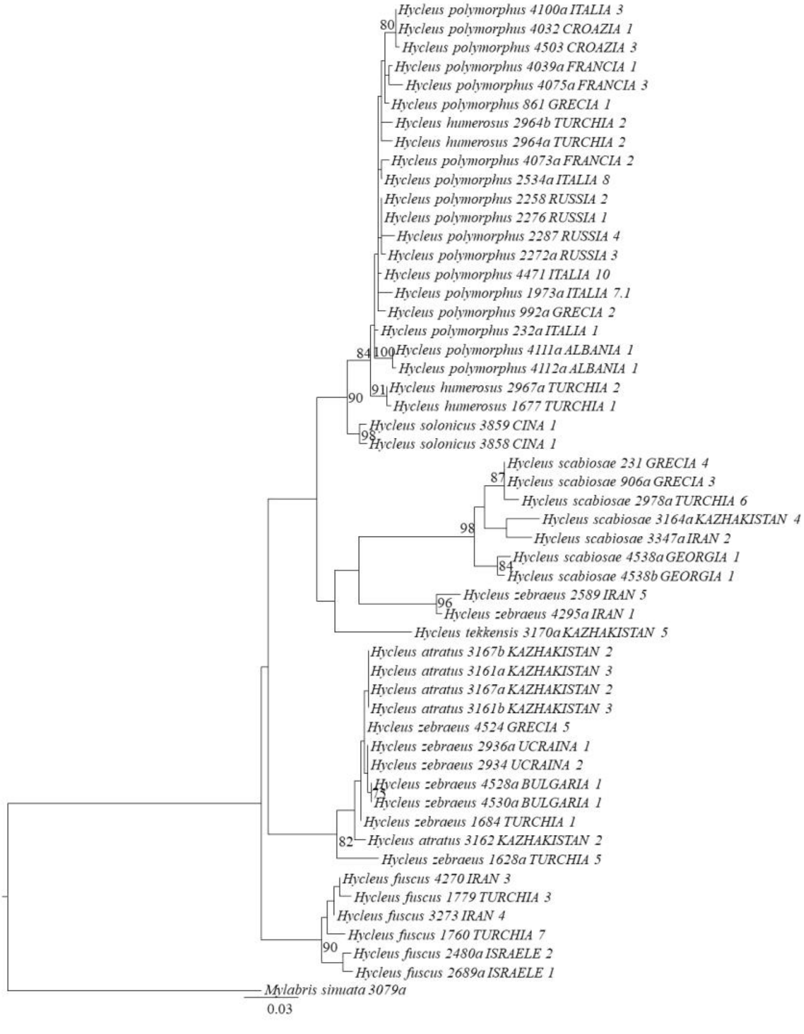


Fig. S1e

Maximum Likelihood tree based on ITS2 sequences. Only supported values of bootstrap are reported at each node (BP>70).

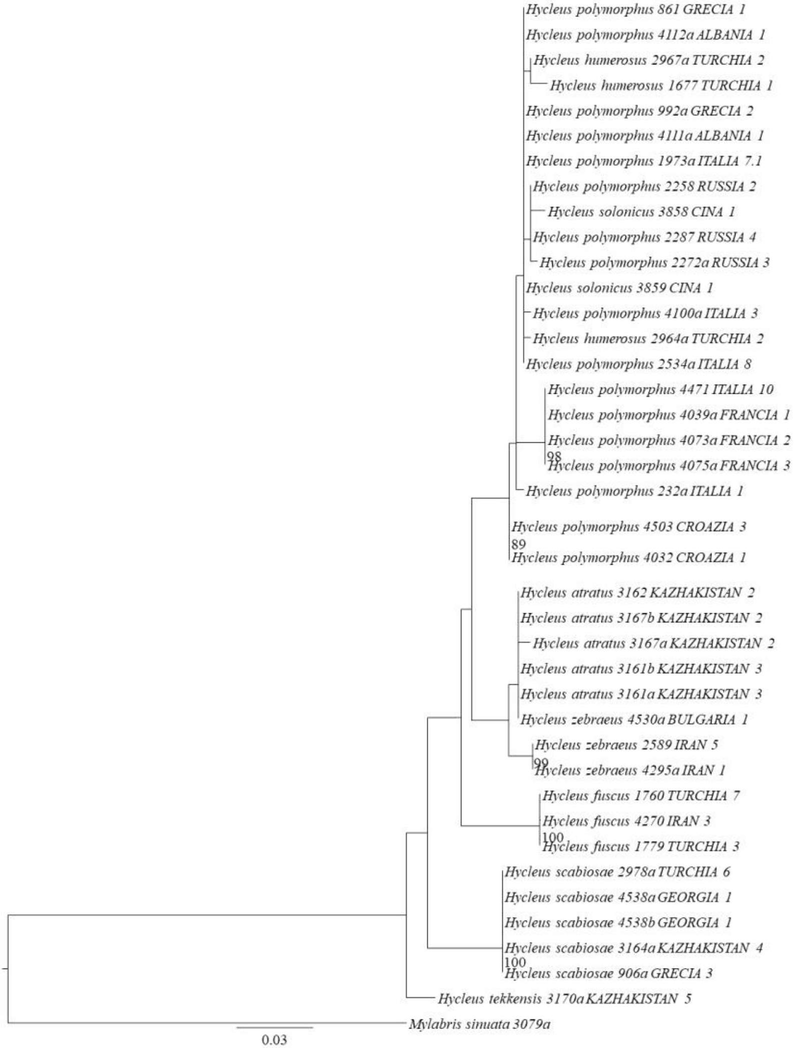


Fig. S1f

Maximum Likelihood tree based on CAD sequences. Only supported values of bootstrap are reported at each node (BP>70).

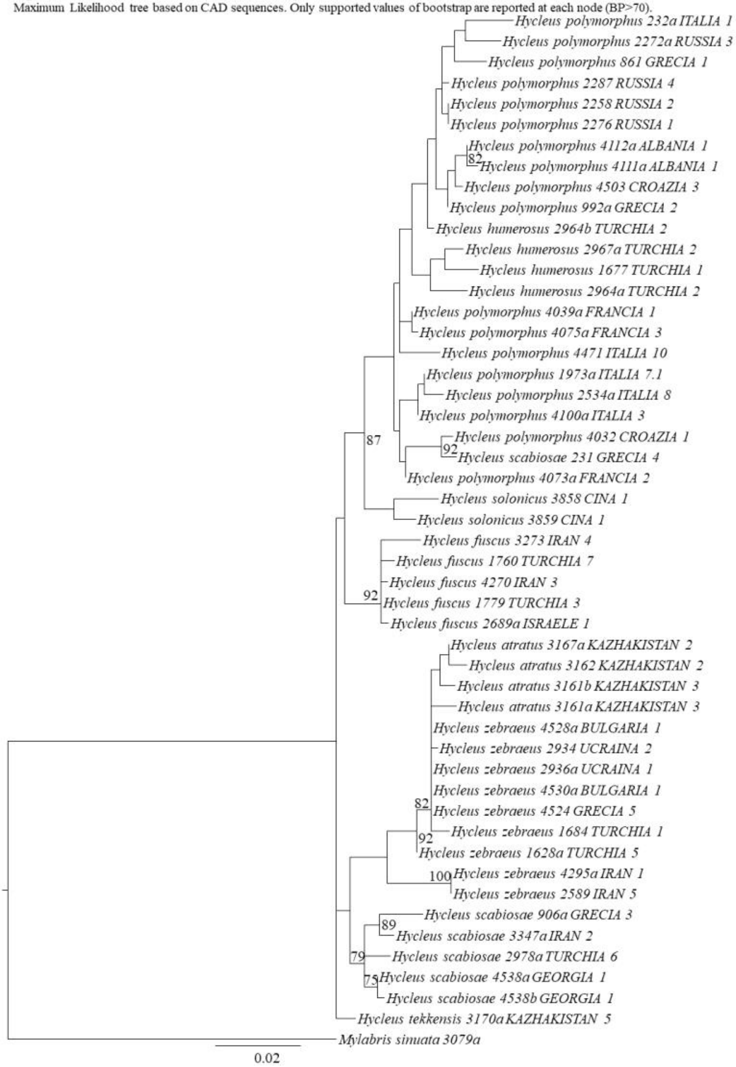


Table S1. Specimens used for phylogenetic analyses, with relative code, sampling locality and genbank accession number for the three markers CAD, ITS2 and COI. Genbank accession numbers marked with * indicates sequences derived from Salvi et al. 2019 and Riccieri et al. 2020.

Species	Code	Locality	CAD	ITS2	COI
Mylabris sinuata	3079a	Turkey, Pozanti mts.	This study	This study	This study
H. atratus	3162	Kazakhstan, Saryshaghan env.	This study	This study	This study
H. atratus	3167a	Kazakhstan, Saryshaghan env.	This study	This study	This study
H. atratus	3167b	Kazakhstan, Saryshaghan env.	-	This study	This study
H. atratus	3161a	Kazakhstan, Qulanbasy	This study	This study	This study
H. atratus	3161b	Kazakhstan, Qulanbasy	This study	This study	This study
H. fuscus	4270	Iran, Esfahan, Kashan, Nashalj Barikarafs	This study	This study	This study
H. fuscus	3273	Iran, Elborz Mts., Danavand Mt.	This study	-	This study
H. fuscus	2689a	Israel, Sayeret Shaqed Park	This study	-	This study
H. fuscus	2480a	Israel, Aloon	-	-	This study
H. fuscus	1779	Turkey, Kirşehir	This study	This study	This study
H. fuscus	1760	Turkey, Adana	MN845414*	This study	MN849982*
H. humerosus	1677	Turkey, Sivas	MN845420*	This study	MN849988*
H. humerosus	2964a	Turkey, Tunceli	MN845421*	This study	MN849989*
H. humerosus	2964b	Turkey, Tunceli	This study	-	This study
H. humerosus	2967a	Turkey, Tunceli	This study	This study	This study
H. polymorphus	4111a	Albania, Korab Mts., Radomir	This study	This study	This study
H. polymorphus	4112a	Albania, Korab Mts., Radomir	This study	This study	This study
H. polymorphus	4032	Croatia, Krk island	This study	This study	This study
H. polymorphus	4503	Croatia, Vučipolje	This study	This study	This study
H. polymorphus	4039a	France, Var, Trigance env.	This study	This study	This study
H. polymorphus	4073a	France, Alpes d’Haute Provence, Villard d’Abbas	This study	This study	This study
H. polymorphus	4075a	France, Alpes Maritimes, Entraunes	This study	This study	This study
H. polymorphus	861	Greece, Ioannina, Metsovo, Peristéri Mts.	MN845447*	This study	MH668513.1*

H. polymorphus	992a	Greece, Kalavrita, Aroania Mts.	This study	This study	This study
H. polymorphus	232a	Italy, Piemonte, Sambuco (CN)	This study	This study	This study
H. polymorphus	4471	Italy, Piemonte, Carnino (CN)	This study	This study	This study
H. polymorphus	4100a	Italy, Lombardia, Montemezzo (CO)	This study	This study	This study
H. polymorphus	1973a	Italy, Val d'Aosta, Valnontey (AO)	This study	This study	This study
H. polymorphus	2534a	Italy, Friuli Venezia Giulia, Sgonico (TS)	This study	This study	This study
H. polymorphus	2276	Russia, Altai rep., Belyashi	MN845450*	-	MN850014*
H. polymorphus	2258	Russia, Altai rep., Samacha steppe	MN845449*	This study	MN850013*
H. polymorphus	2272a	Russia, Altai rep., Kuray	This study	This study	This study
H. polymorphus	2287	Russia, Altai rep., Ortolyk	This study	This study	This study
H. scabiosae	4538a	Georgia, Kvemo Kartli reg.	This study	This study	This study
H. scabiosae	4538b	Georgia, Kvemo Kartli reg.	This study	This study	This study
H. scabiosae	906a	Greece, Trikala	This study	This study	This study
H. scabiosae	231	Greece, Trikala	This study	-	This study
H. scabiosae	2978a	Turkey, Tunceli	This study	This study	This study
H. scabiosae	3164a	Kazakhstan, Qokishbay	-	This study	This study
H. scabiosae	3347a	Iran, Shahrood	MN845459*	-	MN850021*
H. solonicus	3858	China, Hebei, Mt. Xiaowutaishan, Zhangjiaya	MN845466*	This study	MN850028*
H. solonicus	3859	China, Hebei, Mt. Xiaowutaishan, Zhangjiaya	This study	This study	This study
H. tekkensis	3170a	Kazakhstan, Sayak	MN845478*	This study	MN850039*
H. cfr zebraeus	2589	Iran, Mahalat Merkazi	This study	This study	This study
H. cfr zebraeus	4295a	Iran, Kerman	MN845460*	This study	MN850022*
H. zebraeus	4524	Greece, Vlachava	This study	-	This study
H. zebraeus	4528a	Bulgaria, Yarlovo	This study	-	This study
H. zebraeus	4530a	Bulgaria, Yarlovo	This study	This study	This study
H. zebraeus	1684	Turkey, Sivas	MN845486*	-	MN850047*
H. zebraeus	1628a	Turkey, Dağyolu	This study	-	This study
H. zebraeus	2936a	Ukraine, Solontsi	This study	-	This study

H. zebraeus	2934	Ukraina, Prozracne	MN845487*	-	MN850048*

Table S2. Specimens used for phylogeographic analyses, with relative code, sampling locality and genbank accession number for the two markers CAD and ITS2. Genbank accession numbers marked with * indicates sequences derived from Salvi et al. 2019 and Riccieri et al. 2020.

Species	Code	Population Code	Locality	CAD	ITS2
H. polymorphus	4111a	A1	Albania, Korab Mts., Radomir	This study	This study
H. polymorphus	4111b	A1	Albania, Korab Mts., Radomir	This study	This study
H. polymorphus	4112a	A1	Albania, Korab Mts., Radomir	This study	This study
H. polymorphus	4112b	A1	Albania, Korab Mts., Radomir	This study	This study
H. polymorphus	4032	C1	Croatia, Krk island	This study	This study
H. polymorphus	4110	C2	Croatia, Krasno Polje	This study	This study
H. polymorphus	4502	C3	Croatia, Vučipolje	This study	This study
H. polymorphus	4503	C3	Croatia, Vučipolje	This study	This study
H. polymorphus	4504	C3	Croatia, Vučipolje	This study	This study
H. polymorphus	4505	C3	Croatia, Vučipolje	This study	This study
H. polymorphus	4506	C3	Croatia, Vučipolje	This study	This study
H. polymorphus	4039a	F1	France, Var, Trigance env.	This study	This study
H. polymorphus	4039b	F1	France, Var, Trigance env.	This study	This study

H. polymorphus	4040a	F1	France, Var, Trigance env.	This study	This study
H. polymorphus	4040b	F1	France, Var, Trigance env.	This study	This study
H. polymorphus	4041a	F1	France, Var, Trigance env.	This study	This study
H. polymorphus	4074	F2	France, Alpes d’Haute Provence, Villard d’Abbas	This study	This study
H. polymorphus	4073a	F2	France, Alpes d’Haute Provence, Villard d’Abbas	This study	This study
H. polymorphus	4073b	F2	France, Alpes d’Haute Provence, Villard d’Abbas	This study	This study
H. polymorphus	4075a	F3	France, Alpes Maritimes, Entraunes	This study	This study
H. polymorphus	4075b	F3	France, Alpes Maritimes, Entraunes	This study	This study
H. polymorphus	4076a	F3	France, Alpes Maritimes, Entraunes	This study	This study
H. polymorphus	4076b	F3	France, Alpes Maritimes, Entraunes	This study	This study
H. polymorphus	4077a	F3	France, Alpes Maritimes, Entraunes	This study	This study
H. polymorphus	861	G1	Greece, Ioannina, Metsovo, Peristéri Mts.	MN845447*	This study
H. polymorphus	862	G1	Greece, Ioannina, Metsovo, Peristéri Mts.	This study	This study
H. polymorphus	1250	G1	Greece, Ioannina, Metsovo, Peristéri Mts.	This study	This study
H. polymorphus	1251	G1	Greece, Ioannina, Metsovo, Peristéri Mts.	This study	This study
H. polymorphus	858a	G1	Greece, Ioannina, Metsovo, Peristéri Mts.	MH668492.1*	This study
H. polymorphus	1026a	G2	Greece, Kalavrita, Aroania Mts.	This study	This study
H. polymorphus	1026b	G2	Greece, Kalavrita, Aroania Mts.	This study	This study

H. polymorphus	1027a	G2	Greece, Kalavrita, Aroania Mts.	This study	This study
H. polymorphus	1027b	G2	Greece, Kalavrita, Aroania Mts.	This study	This study
H. polymorphus	992a	G2	Greece, Kalavrita, Aroania Mts.	This study	This study
H. polymorphus	4033a	I1	Italia, Col di Nava (IM)	This study	-
H. polymorphus	4033b	I1	Italia, Col di Nava (IM)	This study	-
H. polymorphus	4034a	I1	Italia, Col di Nava (IM)	This study	This study
H. polymorphus	4034b	I1	Italia, Col di Nava (IM)	This study	This study
H. polymorphus	4035a	I1	Italia, Col di Nava (IM)	This study	This study
H. polymorphus	2744	I1	Italia, Col di Nava (IM)	MN845451*	This study
H. polymorphus	2745	I1	Italia, Col di Nava (IM)	This study	This study
H. polymorphus	2746	I1	Italia, Col di Nava (IM)	This study	This study
H. polymorphus	2747	I1	Italia, Col di Nava (IM)	This study	This study
H. polymorphus	4471	I2	Italia, Carnino (CN)	This study	This study
H. polymorphus	4038a	I3	Italia, Upega (CN)	This study	-
H. polymorphus	4038b	I3	Italia, Upega (CN)	This study	-
H. polymorphus	232a	I4	Italia, Sambuco (CN)	This study	This study
H. polymorphus	232b	I4	Italia, Sambuco (CN)	This study	This study
H. polymorphus	233a	I4	Italia, Sambuco (CN)	This study	-

H. polymorphus	233b	I4	Italia, Sambuco (CN)	This study	This study
H. polymorphus	2216	I4	Italia, Sambuco (CN)	This study	This study
H. polymorphus	2215a	I4	Italia, Sambuco (CN)	This study	This study
H. polymorphus	2215b	I4	Italia, Sambuco (CN)	This study	This study
H. polymorphus	1962	I5	Italia, Valnontey (AO)	This study	This study
H. polymorphus	1963	I5	Italia, Valnontey (AO)	This study	This study
H. polymorphus	1966	I5	Italia, Valnontey (AO)	This study	This study
H. polymorphus	1967	I5	Italia, Valnontey (AO)	This study	This study
H. polymorphus	1970	I5	Italia, Valnontey (AO)	MH668469.1*	This study
H. polymorphus	1973a	I5	Italia, Valnontey (AO)	This study	This study
H. polymorphus	1973b	I5	Italia, Valnontey (AO)	This study	-
H. polymorphus	1974a	I5	Italia, Valnontey (AO)	This study	This study
H. polymorphus	1974b	I5	Italia, Valnontey (AO)	This study	This study
H. polymorphus	1975a	I5	Italia, Valnontey (AO)	This study	-
H. polymorphus	4468a	I6	Italia, Chécrouit (AO)	This study	-
H. polymorphus	4468b	I6	Italia, Chécrouit (AO)	This study	This study
H. polymorphus	4469a	I6	Italia, Chécrouit (AO)	This study	This study
H. polymorphus	4469b	I6	Italia, Chécrouit (AO)	This study	This study

H. polymorphus	4094a	I7	Italia, Blavy (AO)	This study	This study
H. polymorphus	4094b	I7	Italia, Blavy (AO)	This study	This study
H. polymorphus	4095a	I7	Italia, Blavy (AO)	This study	This study
H. polymorphus	4095b	I7	Italia, Blavy (AO)	This study	This study
H. polymorphus	4096a	I7	Italia, Blavy (AO)	This study	This study
H. polymorphus	4100a	I8	Italia, Montemezzo (CO)	This study	This study
H. polymorphus	4100b	I8	Italia, Montemezzo (CO)	This study	This study
H. polymorphus	4101a	I8	Italia, Montemezzo (CO)	This study	This study
H. polymorphus	4101b	I8	Italia, Montemezzo (CO)	This study	-
H. polymorphus	4102a	I8	Italia, Montemezzo (CO)	This study	This study
H. polymorphus	4106a	I9	Italia, Vervio (SO)	This study	This study
H. polymorphus	4106b	I9	Italia, Vervio (SO)	This study	This study
H. polymorphus	4107a	I9	Italia, Vervio (SO)	This study	This study
H. polymorphus	4107b	I9	Italia, Vervio (SO)	This study	-
H. polymorphus	4108a	I9	Italia, Vervio (SO)	This study	This study
H. polymorphus	2534a	I10	Italia, Sgonico (TS)	This study	This study
H. polymorphus	2534b	I10	Italia, Sgonico (TS)	This study	-
H. polymorphus	2535r	I10	Italia, Sgonico (TS)	This study	-

H. polymorphus	674	K1	Kazakhstan, Aktogay, Aâköz	-	This study
H. polymorphus	2276	R1	Russia, Altai rep., Belyashi	MN845450*	-
H. polymorphus	2279	R1	Russia, Altai rep., Belyashi	2279	-
H. polymorphus	2280	R1	Russia, Altai rep., Belyashi	MN845465*	-
H. polymorphus	2258	R2	Russia, Altai rep., Samacha steppe	MN845449*	This study
H. polymorphus	2260	R2	Russia, Altai rep., Samacha steppe	This study	This study
H. polymorphus	2262	R2	Russia, Altai rep., Samacha steppe	This study	-
H. polymorphus	2266	R2	Russia, Altai rep., Samacha steppe	This study	-
H. polymorphus	2268	R2	Russia, Altai rep., Samacha steppe	This study	This study
H. polymorphus	2269	R2	Russia, Altai rep., Samacha steppe	This study	This study
H. polymorphus	2272a	R3	Russia, Altai rep., Kuray	This study	This study
H. polymorphus	2273a	R3	Russia, Altai rep., Kuray	This study	This study
H. polymorphus	2287	R4	Russia, Altai rep., Ortolyk	This study	This study
H. polymorphus	01D	S1	Spain, Lerida, Valencia de Aneu	-	This study
H. humerosus	1677	T1	Turkey, Sivas	MN845420*	This study
H. humerosus	2964a	T2	Turkey, Tunceli	MN845421*	This study
H. humerosus	2964b	T2	Turkey, Tunceli	This study	-
H. humerosus	2967a	T2	Turkey, Tunceli	This study	This study

CHAPTER 3

Revision of the *Hycleus sexmaculatus* species group (Coleoptera: Meloidae)

Sayeh Serri[1], Marco A. Bologna[2], Alessandra Riccieri[2]

[1]*Hayk Mirzayans Insect Museum (HMIM), Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization, Tehran, 19395-1454, Iran.*
e-mail: serri@iripp.ir / serrisayh@gmail.com
[2]*Dipartimento di Scienze, Università Roma Tre, Viale G. Marconi 446, 00146 Roma, Italy,* e-*mail:alessandra.riccieri@unirom3.it*.

Corresponding author: Marco A. Bologna; e-mail: marcoalberto.bologna@uniroma3.it

Keywords
Mylabrini – taxonomy - phylogenetic relationships - biogeography

Zootaxa – Minor revision

Abstract.

The blister beetles belonging to the *Hycleus sexmaculatus* species group are revised. Morphological characters are described and figure and keys to both male and female are presented. *Hycleus hayki* **new species** from Southwest Iran is described and some new synonymies are proposed: *Mylabris triangulifera* ab. *reitterioides* Mader 1929 = *Hycleus bipunctatus* (Olivier, 1811) **new synonymy**; *Mylabris triangulifera* ab. *bushirensis* Mader 1929 = *Hycleus bipunctatus* (Olivier, 1811) **new synonymy**; *Mylabris javeti* Marseul, 1870 = *Hycleus colligatus* (Redtenbacher, 1850) **new synonymy**; *Mylabris javeti umbilicatus* Kaszab, 1958 = *Hycleus colligatus* (Redtenbacher, 1850) **new synonymy**; *Hycleus amrishi* Makhan, 2012 = *Hycleus cingulatus* (Faldermann, 1837) **new synonymy**. Additional remarks which clarify some errors in the identification of the species are provided. The taxonomic positions of *Zonabris soumakovi* and *Zonabris sialanus* are also discussed. Phylogenetic relationships of the species, based on a subset of available species inferred from molecular data are proposed, and some morphologically defined subgroups of species were distinguished with a molecular support. The geographic and ecological distributions of all species are defined, and detailed list of localities are published. The ecological information (phenology, elevation and host plants) of the species is summarized in a table. Based on literature records, collections and recent collecting data, the distribution of the *sexmaculatus* species and their biogeographic characteristics are discussed.

1. Introduction

Hycleus Latreille, 1817, with about 500 species, is the most speciose genus of the family Meloidae, which is well known in the literature because of its hypermetabolic development and the production of cantharidin, a terpenoid used in pharmacology (for a synthesis on this family see: Bologna, 1991; Pinto & Bologna, 1999; Bologna & Pinto 2002; Bologna et al., 2011, 2013).

The recent phylogenetic study of *Hycleus* (Riccieri et al., 2020), evidenced that no subgenera are distinguishable in this genus, differently than in *Mylabris* Fabricius, 1775, a close taxon belonging to the same tribe Mylabrini (Salvi et al., 2018). The three “sections”, Mesogorbatus, Mesotaeniatus and Mesoscutatus, previously described by Pardo Alcaide (1954, 1955), are not discrete units but only a morphological continuum, while *Ceroctis* Marseul, 1870 and *Paractenodia* Péringuey, 1904, considered as genera in the previous literature (e.g. Kaszab, 1969; Bologna, 1991; Bologna et al., 2018), actually represent only *Hycleus* species characterized by modified but not synapomorphic features of antennomeres (Bologna et al., 2005, 2008; Salvi et al., 2018; Riccieri et al., 2020). Moreover, Riccieri et al. (2020) pointed out that some groups of *Hycleus* species, previously defined morphologically, are confirmed molecularly and represent monophyletic lineages. One of these groups, the *H. sexmaculatus* (Olivier, 1811) group, was well defined morphologically by Escherich (1899), and confirmed by Sumakov (1915), Pardo Alcaide (1963), Kaszab (1973) and more comprehensively by Bologna & Turco (2007). Twelve species are referred to this group in the present paper, one of which is new. The most evident morphological synapomorphies of this group, are: (a) the last antennomere narrower than X at base and distinctly narrowed in the apical portion, differently than in other *Hycleus*, and similarly than in the genus *Lydoceras* Marseul, 1870); (b) the mesosternum without a modified fore portion and without tracks of longitudinal suture in this fore portion (similarly than in some other group of species previously referred to the section Mesogorbatus, which have different antennomeres).

Aims of this paper are: (a) to revise this group of species morphologically, to publish diagnostic characters and figures, to describe intraspecific variability, and to propose new species and new synonymies; (b) to publish keys to both males and females of each species; (c) to explore phylogenetic relationships among species by using morphological and molecular characters; (d) to summarize ecological and biogeographic information.

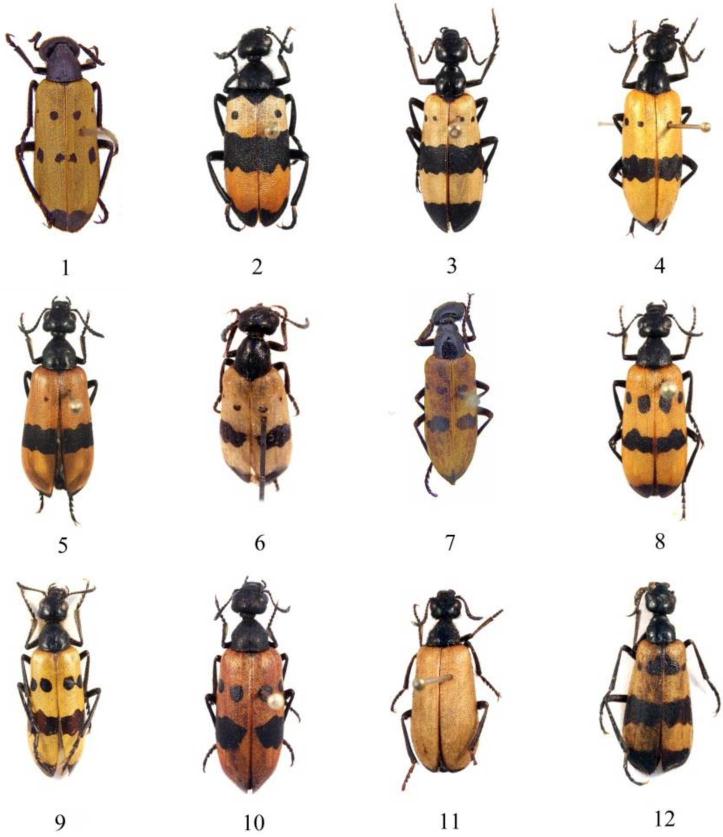


Fig.1–12. Habitus of: 1. *Hycleus apicipennis*; 2. *H. balteatus*; 3. *H. bipunctatus*;4. *H. cingulatus*; 5. *H. colligatus*; 6. *H. hayki*; 7. *H. lindbergi*; 8. *H. pirata*; 9. *H. schauffelei*; 10. *H. sexmaculatus*; 11. *H. tenuepictus*; 12. *H. trianguliferus.*

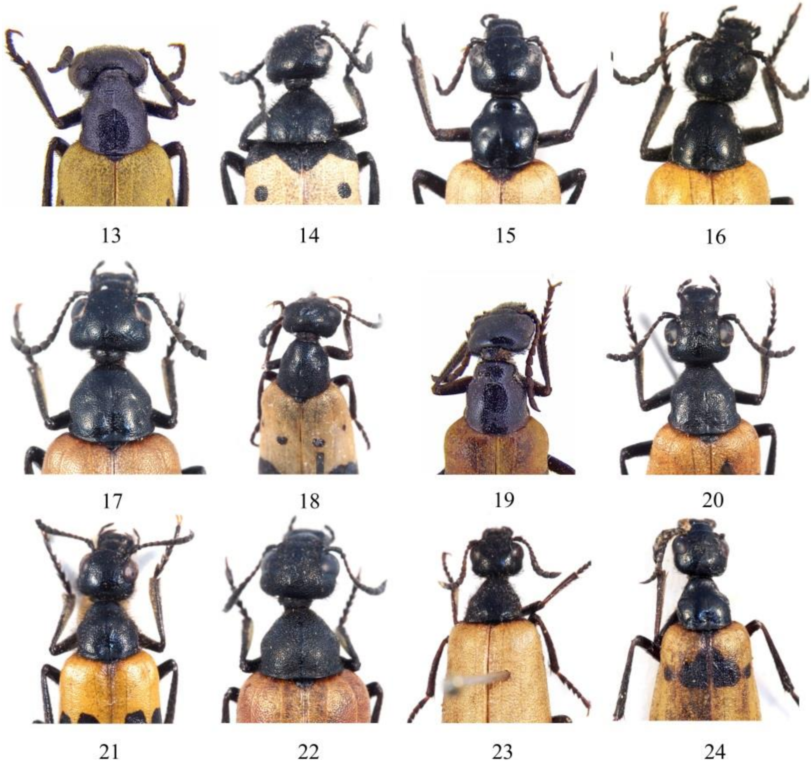


Fig. 13–24. Head and pronotum of: 13. *Hycleus apicipennis*; 14. *H. balteatus*; 15 *H. bipunctatus*; 16. *H. cingulatus*; 17. *H. colligatus*; 18. *H. hayki*; 19. *H. lindbergi*; 20. *H. pirata*; 21. *H. schauffelei*; 22. *H. sexmaculatus*; 23. *H. tenuepictus*; 24. *H. trianguliferus.*

2. Material and methods

2.1 *Morphological analysis*

A complex of 2476 adult specimens of this group of *Hycleus* species were examined for this study: 30 *Hycleus apicipennis* (including the holotypes of two varieties); 64 *H. balteatus*; 618 *H. bipunctatus*; 203 *H. cingulatus*; 897 *H. colligatus* (including holotype and 3 paratypes; the holotype and at least 4 certain syntypes of *Mylabris javeti*; holotype and 3 paratypes of *M. javeti umbilicata*); 6 *H. lindbergi* (all paratypes); 18 *H. hayki* (holotype and 17 paratypes); 13 *H. pirata* (including holotype and 11 paratypes); 384 *H. schauffelei* (including 9 paratypes); 108 *H. sexmaculatus* (including holotype and 4 syntypes of the synonym *Mylabris bimaculata*);

51 *H. tenuepictus* (including 1 syntype); 67 *H. trianguliferus* (including 1 syntype).

The following abbreviations used in the text, represent the studied collections (acronyms of collections in alphabetical order): ALC = A. Liberto, Rome, Italy; BMNH = Natural History Museum, London, United Kingdom; FUUZM = Federal Urdu University, Zoological Museum, Rawalpindi, Pakistan; HMIM = Hayk Mirzayans Insect Museum, Iranian Research Institute of Plant Protection, Teheran, Iran; HNHM = Hungarian Natural History Museum, Budapest, Hungary; JCRC = J.-C. Ringenbach, Pau, France; JPC = late J. Probst, Wien, Austria; LCC = L. Černý, České Budějovice, Czech Republic; MABC = M. A. Bologna coll., Università degli studi Roma Tre, Rome, Italy (some material preserved in alcohol 95%); MCNC = Museo civico di Storia naturale, Carmagnola, Torino, Italy; MCNV = Museo Civico di Storia Naturale, Venice, Italy; MCZR = Museo Civico di Zoologia, Rome, Italy; MFUM = Museum of Ferdowsi University, Mashhad, Iran; MHNG = Muséum d'Histoire Naturelle, Geneva, Switzerland; MNHN = Muséum National d'Histoire Naturelle, Paris, France; MRSN = Museo Regionale di Storia Naturale, Torino, Italy; MSNG = Museo Civico di Storia Naturale "G. Doria", Genoa, Italy; MSNM = Museo Civico di Storia Naturale, Milan, Italy; MSNV = Museo Civico di Storia Naturale, Verona, Italy; MUH = Zoological Museum of the Haifa University, Haifa, Israel; MUSE = Museo delle Scienze, Trento, Italy; MZUF = Museo Zoologico de "La Specola", Università di Firenze, Florence, Italy; NHMW = Naturhistorisches Museum, Wien, Austria; NMPC = Czech National Museum, Department of Entomology, Prague, Czech Republic; SDEI = Senckenberg Deutsches Entomologisches Institut Müncheberg; SKC = S. Krejcik, Unicov, Czech Republic; TAU = Tel Aviv University Museum, Tel Aviv, Israel; TGC = T. Garuzek, Warszawa, Poland; VVC = Vladimir Vrabec, Prague, Czech Republic; ZMHB = Museum für Naturkunde der Humboldt Universität, Berlin, Germany; ZSM = Zoologische Staatssammlung, Munich, Germany.

Figures were drawn by hand, using a stereomicroscope Zeiss Stemi SV8 equipped with a Zeiss drawing tube 474622-9901. Photographs of habitus and pronotum were taken by an Olympus OM-D-E-M5 camera, equipped by Olympus M. Zuiko Digital ED 60 d/2.8 macro mm objective, using Lupo Repro 1 stand, and also a 650D Canon digital camera through an Olympus SZH stereomicroscope.

In the treatment for each species we listed: synonyms and most relevant citations (relevant taxonomic contributions), information on type specimens and locality, examined material, records from literature and collections, synthesis of the distribution and a short diagnostic description with figures of diagnostic characters. One new species, *Hycleus hayki* is described more extensively. Information on habitat preferences is discussed in the Chapter of Biogeography and Ecology, while that on phenology, elevation and host plants are summarized in Table 3.

2.2 *Molecular analysis*

Taxon sampling and Laboratory procedures

Twenty specimens preserved in ethanol 96% belonging to five species were employed for phylogenetic analyses (Table 1). The species *Oenas sericeus* from Lyttini tribe was added as outgroup.

Genomic DNA was extracted from one to three legs of each specimen following the salting out protocol (Sambrook et al., 1989). We amplified fragments of one mitochondrial gene, the Cytochrome Oxidase subunit I (COI) and of four nuclear genes: the Arginine Kinase (ARGK); the Carbamoylphosphate Synthetase domain (CAD); the gene Wingless (WG); and a portion of the 28S rDNA gene.

Polymerase chain reactions (PCR) amplifications were carried out in a final volume of 25 µl, containing 3 µl of 10x reaction buffer, 1/1,5/3 µl of MgCl2 (50mM), 0,5/1 µl dNTPs (10 mM), 0,2 µl of TaqDNA polymerase (5 U/µl; BIOTAQ Bioline), 0,5µl of each primer (25 mM) and 1 µl of DNA template. For information about primers and thermal cycles see Riccieri et al. (2020). Amplification success was determined by agarose (1%) gel run, stained with 1µl of SimplySafe (EurX), using 3 µl of PCR products. Amplified products were purified and sequenced by Macrogen, and GenBank accession numbers for each sequence are provided in Table 1.

Alignment, model selection and phylogenetic analysis

Each gene region was edited using the software Staden Package v4.11.2 (Staden et al., 2000) and aligned with MAFFT (Katoh et al., 2019).

Phylogenetic analysis were carried out on the single genes and on the concatenated matrix partitioned by genes, through the web portal CIPRES (http://www.phylo.org). JModeltest v2.1.6 (Posada, 2008) was used to select the best substitution model for each gene partition, according to the Akaike Information Criterion (AIC) (Table 2). With MEGA7 (Kumar et al., 2016) we calculated the number of variable and singleton sites for each gene (Table 2). Maximum Likelihood (ML) analysis was performed with RAxML-HPC v8.2.10 (Stamatakis, 2006) with a partitioned GTRGAMMA model and a rapid-bootstrap analysis with 1000 replicates. Mr Bayes v3.2.6 (Ronquist et al., 2012) was used for Bayesian Inference (BI): two independent runs with four Markov chains each were run for 20 million generations sampling trees every 1000 generations with a 10% burn in. By means of Tracer v1.6 (Rambaut et al., 2014) convergence of run was confirmed and FigTree v1.3.1 (Rambaut and Drummond, 2009) was used to visualize the trees.

Fig. 25. Habitat of *Hycleus colligatus*, South Iran

Table 1. Species, specimen code, sample localities, genes and GenBank accession numbers for thespecimens analysed in this study.

SPECIES	SPECIMEN CODE	SAMPLE LOCALITY	28S	ARGK	CAD	COI	WG
Oenas sericeus	324	Morocco, Settat, Settat	MN848610	MN845224	MN845358	MN849929	MN845491
Hycleus cingulatus	2622	Iran, Khorasan, Mashhad	-	MN845264	MN845399	MN849969	MN845529
Hycleus cfr colligatus	4309a	Iran, Kerman, Dbhakri	MN848649	MN845289	MN845424	MN849992	MN845549
Hycleus colligatus	4219	Iran, Kordestan, Piyazeh mt.	MN848648	MN845265	MN845400	MN849970	MN845530
Hycleus colligatus	2579	Iran, Khorasan, Mashhad	-	-	This study	This study	-
Hycleus colligatus	2587	Iran, Khorasan, Mashhad	This study	This study	This study	This study	This study
Hycleus colligatus	3336	Iran, Nord Khorasan, Koppe Mts.	This study	This study	This study	This study	This study
Hycleus colligatus	3311a	Iran, Māzandarān, Elborz Mts.	-	-	This study	-	This study
Hycleus colligatus	3311b	Iran, Māzandarān, Elborz Mts.	This study	-	This study	This study	This study
Hycleus colligatus	3312a	Iran, Māzandarān, Elborz Mts.	MN848656	MN845288	MN845423	MN849991	MN845548
Hycleus colligatus	3312b	Iran, Māzandarān, Elborz Mts.	This study	This study	This study	This study	-
Hycleus colligatus	3346a	Iran, Khorasan, Golmakan	This study	This study	-	This study	-
Hycleus pirata	262	United Arab Emirates, Abu Dhabi, Sayh Al Daay	MN848693	MN845308	MN845442	MN850007	MN845566
Hycleus schauffelei	4231	Iran, Kerman, Khabr	This study	This study	This study	This study	-
Hycleus schauffelei	4223a	Iran, Kerman, Khabr	MN848708	MN845329	MN845462	MN850024	MN845586
Hycleus schauffelei	4223b	Iran, Kerman, Khabr	This study	-	This study	This study	-
Hycleus schauffelei	4223c	Iran, Kerman, Khabr	This study	This study	-	This study	-
Hycleus sexmaculatus	4226a	Iran, Hamedan, Lashgardar protected region	MN848711	MN845339	MN850034	MN845472	MN845595
Hycleus sexmaculatus	4226b	Iran, Hamedan, Lashgardar protected region	This study	This study	This study	This study	This study
Hycleus sexmaculatus	4227a	Iran, Hamedan, Lashgardar protected region	This study	This study	This study	This study	This study
Hycleus sexmaculatus	4227b	Iran, Hamedan, Lashgardar protected region	This study	This study	This study	This study	This study

3. Results

3.1 Taxonomy

3.1.1 Description and distribution of the species

Here we synthetically revised the species recognized in this group, with diagnostic characters, figures, and intraspecific variability. We describe a new species and propose new synonymies.

Bologna & Turco (2007) referred *Zonabris soumakovi* Pic, 1930 to the *H. sexmaculatus* group, and considered it as a doubtful species. After receiving some photos of two syntypes *of Z. soumakovi* preserved in Paris Museum, we consider it as a species of the genus *Mylabris*.

Hycleus apicipennis **(Reiche, 1866)**
Figs 1, 13, 26–32

Mylabris apicipennis Reiche, 1866 (1865): 635
Zonabris apicipenis var. innesi Pic, 1912: 1
Zonabris apicipenis var. *fortuai* Pic, 1921: 45

Type material. We did not find in MNHN the type of *H. apicipennis*, which probably is lost, but we examined in that Museum specimens identified by Marseul, who surely were compared with the Reiche's type. We also examined the types of Pic' varieties.

Additional material examined. **Egypt.** 4 exx. Egypt (MSNV, MRSN); 2 ♂♂ Egypten (HMHN); 1 ♀ Aegypten, coll. Germar (HNHM); 3 ♂♂ Aegyptus, coll. Reitter (HNHM); 1 ♀ Egypte, R. Oberthür, Eing. Nr. 4, 1956 (HNHM); 2 ♀♀ Egitto, dint. Cairo, VII.1936, leg. S. Patrizi, det. M. Bologna (one identified by Kaszab, 1975) (MABC 2 ♂♂ 3 ♀♀ Le Caire, Henon, R. Oberthür (Coll. E. Martin), Eing. Nr. 4, 1956 (HNHM); 1 ♀ Meadi (Cairo), 11.VII.33, leg. W. Wittmer; 1♀ Ägypten, Piramiden (HNHM); 1 ex. Hebah (MCNV); 1 ex. Tell el Kebir (MCNV); 2 ♀♀ [illegible label] (HNHM); 1 ♀ Ägypten, Bitterseegebiet, 17.VI.1946, leg. L. Dieckmann (HNHM). **Israel-Palestine:** 1 ♂ Israel, Zofar, 10.VI.1997, leg. A. Freidberg (MABC). **Saudi Arabia.** 1 ♂ Arabia, Rawdat Khraim, 6.VI.83 (HNHM); 1 ♀ Arabia, Wadi al Hawshah, Najd, 27.V.87 (HNHM).

Records from literature. Egypt. Egypt (Reiche, 1866, type loc.; Baudi, 1878a; 1878b; Soumakov, 1915; 1930: Mader, 1927; Kuzin, 1954; Bologna, 2008); Alexandrie, Maryout (Andrès, 1911); Cairo (Marseul, 1870; 1872); near Cairo (Reiche, 1866; Alfieri, 1976); Wadis S of Cairo (Alfieri, 1976); Kirdasa and Abu Rauwash (Iconomopoulos, 1916; Alfieri, 1976); El Hayat and Maghagha (Alfieri, 1976); Sinai (Mader, 1927); Sinai, Wadi Karam, Cheikh Hamid and El Krieg (Pic, 1920; Alfieri, 1976); Sinai (Chikatunov, 1999; Bologna, 2008); Sakkara (Bologna, 2009). **Israel-Palestine.** Israel-Palestine (Bologna, 2008); Judean Hills, Dead Sea area, northern Negev, central Negev (Chikatunov, 1999); Dead Sea area and northern Arabe valley (Bytinsky-Salz, unpubl.). **Jordan**. Jordan (Bologna, 2008).

The record from Libya (Beccari & Gerini, 1979; Bologna, 2008) refers to Egypt (Bologna, 2009). Chikatunov (1999) recorded this species generically from northern Africa. Heyden (1892) recorded a mylabrine species from Turkestan as *Zonabris apicipennis* which is distinctly different in colouration.

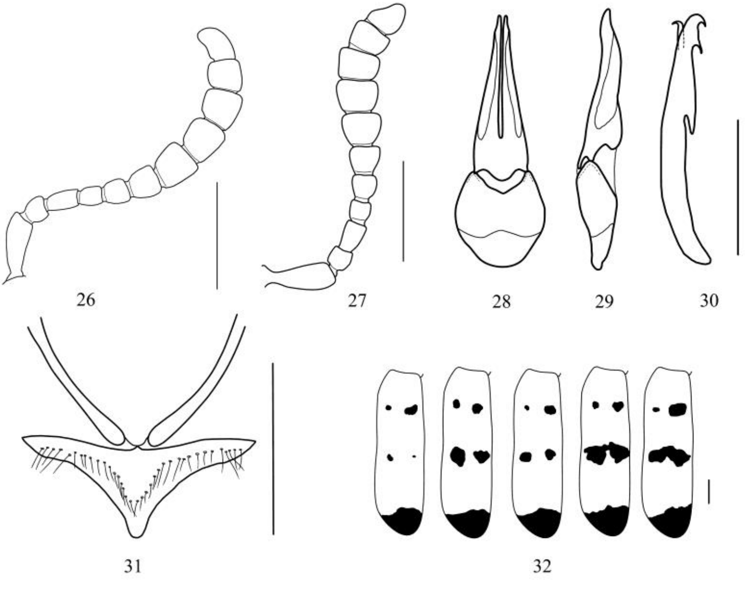


Fig. 26–32. *Hycleus apicipennis* (Reiche): 1. Male antenna; 2.female antenna; 3. tegmen, ventral view; 4.tegmen, lateral view; 5. aedeagus, lateral view; 6. mesosternum; 7. elytral pattern. Scale bar = 1 mm.

Description. Body length 10–15 mm. Body black, but elytra reddish yellow with black pattern; setation black and short; head with deep punctures;

antennomere III ca. 1.5 times as long as IV, V–VII progressively widened, VIII equal or very slightly narrower than VII, IX–XI progressively narrowed, male XI (Fig. 26) distinctly narrower and slightly longer than X, curved and obtuse at apex, in female (Fig. 27) shorter, curved, more conical, at base narrower than X. Pronotum (Fig. 13) longer than wide, with shallow anterior and basal depressions; mesosternun as in Fig. 31. Each elytron with two round black spot on anterior third, two irregular spot after the middle and a big apical black spot (Figs. 32). Last male ventrite convex and with posterior margin relatively deep emarginated; male genitalia as in figures 28–30; gonoforceps slender both in dorsal and lateral view; aedeagus with two distanced, subequal dorsal hooks, with the same inclination.

Taxonomic remarks. In some specimens the following variations exist: male last antennomere more curved and slightly longer than in most specimens; anterior spots of elytra sometimes very small, middle spots in some specimens fused and forming a fascia, sometimes inner middle spots close or reaching the suture. Pic (1912) described the variety *innesi* with the median sinuate elytral fascia which extends toward suture, and a wide apical spot. In the specimens of the variety *fourtani* both elytral anterior spots are reduced.

Despite of the antennal variations, the shape of male genitalia is constant among populations. This species looks extremely close to *H. pirata*, because of the similarity in shape of genitalia and the elytral pattern, but mostly differs because of the length of antennomere IV which is submoniliform and clearly shorter than V; in most specimens of *apicipennis* elytra are more opaque, due to the denser punctures.

According to the description of *Zonabris sialanus* Pic, 1929, we suspect that this species, described from Egypt (Fayum, Siala), can be referred to *H. apicipennis* as synonym and represents only a variation of *H. apicipennis* with different elytral pattern; this variation was already examined in some specimens (MNHN). We did not examine the holotype of *Z. sialanus*, preserved in the Alfieri’s collection (probably in Egypt, Cairo), but the shape of last antennomere, pronotal shape, punctation, and elytral pattern are extremely similar to those of *H. apicipennis*.

Distribution. *Hycleus apicipennis* was described from Cairo, Egypt (Reiche, 1866), and later recorded from other north-eastern Egyptian localities (including Sinai), southern Israel and Jordan. The records of Rawdat Khraim and Najd are the first of this species in Saudi Arabia.

Hycleus balteatus* (Pallas, 1782)
Figs 2, 14, 33–39

Meloe balteata Pallas, 1782: 88, tav. F, f. E14
Mylabris indica Herbst in Füessly, 1784: 147, Tav. 30 f. 6
Mylabris bicolor Thunberg, 1791: 111, f. 2
Mylabris punctum Fabricius, 1792: 89
Mylabris coromanda Lichtenstein, 1795: 75
Mylabris laticlavia Lichtenstein, 1795: 75
Mylabris fasciata Voet, 1806: 20
Zonabris punctum var. *puttalamamensis* Pic, 1912: 13
Zonabris punctum var. *singularipennis* Pic, 1912: 13

Type material. The type was housed in the Pallas' collection of insects which is lost. We considered the specimens described and figured by Billberg (1813) almost coeval, as well as those identified by several specialists of the XIX century, such as Marseul (MNHN), as reference specimens.

Additional material examined. **India:** 10 ♂♂ 10 ♀♀ Kerala, Nilgiri Hills, Kovalam, VIII.1998, leg. Rautensterauch (HMIM; MABC); 1 ♂ Nilgiri Hills, VI.1990, leg. Rautensterauch (MABC); 2 exx. Tamil-Nadu, Ama distr., Dindigul, Nilakkotai env. (MZUF); 4 exx. Tamil-Nadu, Ambekdar distr., 20 km S Vellore (MZUF); 1 ♂ India orientalis Tamil Nadu, Shenbagamur (HNHM) 1 ♂ Tamil Nadu, Madura district, Sirumalai Hills, 16.VII.1937 (HNHM), 1 ♂ 1 ♀ India Orientalis, Tamil Nadu, Madura (HNHM); 1 ♀ Madura, Coimbatore Or., IV.1975, 1400 ft., leg. T.R.S. Nalhan (HNHM); 1 ♂ Madura, Coimbatore (HNHM); 1 ♀ Tamil Nadu, Madura district, Sirumalai Hills, 16.XII.1957 (HNHM); 6 ♂♂ 6 ♀♀ Tamil Nadu, Padukkottai, Vamban, Uniu Tamil, VII–VIII.2013, leg. Narayanasami (MABC); 2 ♀♀ Tiruchi Dt., Padukkottai, X.1973, leg. T.R.SA. Nalhan (HNHM); 2 exx. Tamil-Nadu, Padukkotai distr., 29 km N Padukkotai (MZUF); 2 ♂♂ Madras (HNHM); 2 ♂♂ 1 ♀ South India, Karikal Terr., Kurumbagaram, III.1951 (HMHM); 5 ♂♂ Trichinopoly, leg. Biro, 1902 (HNHM); 3 ♀♀ Nedungadu, Tanjore distr., India orientalis (HNHM); 1 ♂ South India, Ranisaram, III.1961 (HNHM).

Records from literature. India. India gangetica (Pallas, 1782, loc. typ.); India (Fischer, 1827 as *punctum*; Marseul, 1870, 1872 both as *indica*; Kuzin, 1954; Bologna et al., 2018); Coromandel coast (Olivier, 1811), Tranquebar (Olivier, 1811); Tranquebar (Fabricius, 1801 as *punctum* loc. typ.); Madurai (Fairmaire, 1896; Chockalingam & Mangharan, 1979, 1980 as *indica*); Vadakkankulam (Haniffa & Fransis Sekar, 1993); Bihar, Kerala, Tamil Nudu (Anand, 1989); Andhra Pradesh: Cuddapah dist, Kodar Ca.; Vempati; Tamil Nadu (Madras): Rameswaram; Tuticorin, Pearl Bank Fisheries; Salem Town; Salem dist. Harur; Kambalai; 10 miles SE Harur, foot of Chitteri 'Hills; Kattur; Yelagiri Hills; Palni, foot of Palni Hills; Bapat1a; Tirunelvelli; Pamben (Saha, 1979). **Pakistan.** Pakistan (Hashmi and Tashfeen, 1992; Bologna et al., 2018); doubtful record. **Sri Lanka.** Sri Lanka (Pic, 1912a as *punctum*; Kuzin, 1954; Anand, 1989); Puttalam (Pic, 1912a as *punctum*); Kandy Distr., Kandy (Mohamedsaid, 1982).

Billberg (1813) erroneously recorded this species (as *punctum*) from the Cape of Good Hope. As for some very old records from India (especially from Thunberg), the species was reported as collected in the Cape, in South Africa, where all ships stopped for the supplies during their navigation to India.

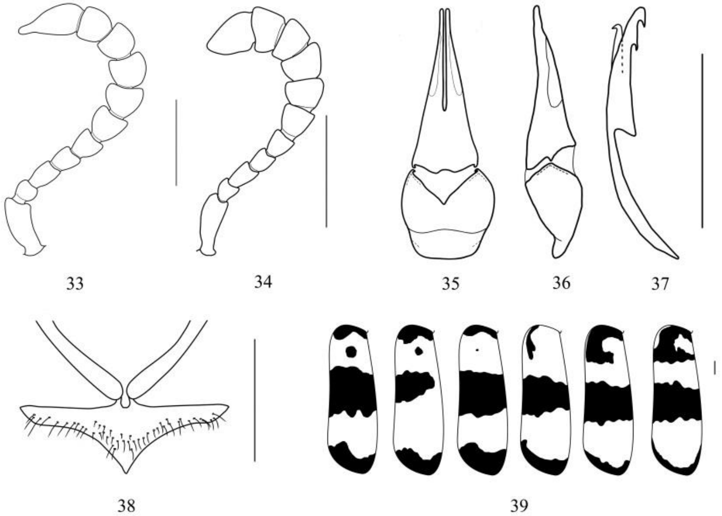


Fig. 33–39. *Hycleus balteatus* (Pallas): 33. Male antenna; 34. female antenna; 35. tegmen, ventral view; 36. tegmen, lateral view; 37. aedeagus, lateral view; 38. mesosternum; 39. elytral pattern. Scale bar = 1 mm.

Description. Body length 8–19 mm. Body black, but elytra reddish yellow with black pattern, anteriorly almost yellowish; head with deep punctures; antennomere III ca. 1/4 time longer than IV, V–VIII progressively widened with broader apical part, male XI (Fig.33) two times as long as X, with curved and elongated apex; in female (Fig.34) shorter and not elongated. Pronotum (Fig. 14) almost as long as wide, with anterior and basal depressions and a middle longitudinal furrow, punctuation relatively deep; mesosternun as in Fig. 38. Each elytron with a narrow black basal fascia, stretched to scutellum, one black spot on anterior third, a wide middle fascia with sinuate posterior margin, and a black margin on apical part (Figs. 39). Ventrites with red margin on sides; last male ventrite convex, posterior margin with a shallow rounded emargination. Aedeagus (Fig. 37) with endophallic hook small, aedeagal hooks quite close, distal hook slightly greater; gonoforceps (Figs. 35–36) very slender in both ventral and lateral vision.

Taxonomic remarks. In some specimens the following elytral variation exist (Fig. 39): anterior narrow fascia connected to frontal spot forming a wide black anterior colouration; black anterior spots reduced or totally absent; middle fascia shortened or narrowed near suture; apical margin with different width and shape of anterior margin. The shape of the male genitalia (Figs. 35–37) seems to be constant among populations. Anand (1980), compared male genitalia of Meloinae and figured the IX tergum and genitalia but the shape of gonoforceps figured by this author is very approximate and does not match with the material we examined.

Pic (1914) synonymised *Mylabris japonica* Sumakov, 1913 with *M. balteata* and mentioned it as a simple variation. According to the description of *M. japonica* it has a big antennal club formed by antennomeres IX–XI, which differs totally from the synapomorphic condition of the *Hycleus sexmaculatus* species group. Consequently, we consider the Pic’s synonymy as erroneous.

Pardo Alcaide (1958, 1963) did not include *balteatus* in the *sexmaculatus* group, but in a separate group with *H. orientalis* (Marseul, 1872) and *H. thunbergi* (Billberg, 1813), which both species do not have modified male antennomere XI.

Distribution. *Hycleus balteatus* is cited from Pakistan, but this record is doubtful and its range is confirmed only in southern India and Sri Lanka. The species was never recorde from western India.

Hycleus bipunctatus **(Olivier, 1811)**

Figs 3, 15, 40–46

Mylabris bipunctata Olivier, 1811: 94
Mylabris triangulifera ab. *reitterioides* Mader, 1929: 108 **syn. n.**
Mylabris triangulifera ab. *bushirensis* Mader, 1929: 109 **syn. n.**
Mylabris matthiesseni Reitter *in litteris,* according to Kaszab, 1957

Type material. The type of *Mylabris bipunctata,* described from Arabia, seems to be lost, but we studied the old material of the Marseul's collection from Arabia, Syria and Iraq, which probably has been compared by the French specialist with the Olivier's types already housed at the Paris Museum.

Additional material examined. Iran. 5 ♂♂ 8 ♀♀ Bushehr prov. 10 m, 30.V.2001, leg. Osten, Ebrahmi& Mofidi (HMIM); 1 ♂ 2 ♀♀ Esfahan prov., Natanz, 5.VI.1970, leg. Mirzayans& Abai (HMIM); 1 ♀ Fars prov., Kazeroun, Gav Koshsk, 5.V.1975, leg. Abai (HMIM); 1 ♂ Fars prov., Lar, 4.IV.1953, leg. Manuchehri (HMIM); 17 ♂♂ 14 ♀♀ Ghom prov., Ghom, 20.VI.1969, leg. Abai (HMIM); 1 ♂ Tehran, Lac Ghom, 20.VI.1969, leg. Abai (HNHM); 3 ♂♂ 3 ♀♀ Ghom prov., Ghom lake, 5.IV.1970, leg. Abai (HMIM); 3♂♂ 1 ♀ Hormozgan prov., Bandar abbas, 25.IV.1949, leg. Esfandiari (HMIM); 2♂♂ 1 ♀ Hormozgan prov., Bandar abbas, Rezvan, 22.IV.1949, leg. Esfandiari (HMIM); 1 ♂ 11 ♀♀ Hormozgan prov., Bandar abbas, 7.V.1950, leg. Fasihi (HMIM); 1 ♀ Hormozgan prov., Bandar abbas, 15 .IV.1950, leg. Fasihi (HMIM); 4 ♂♂ 3 ♀♀ Hormozgan prov., Bandar abbas, 17. V.1950, leg. Fasihi (HMIM); 1 ♂ Hormozgan prov., Farur Island, 25.IV.2001, leg. Ghayourfar (HMIM); 26 ♂♂ 30 ♀♀ Hormozgan prov., Bandar abbas, Khamiran, 25,30.IV.1950, leg. Fasihi (HMIM); 1 ♂ Hormozgan prov., 10 km E Dehbarez, N 27 27 E 57 19, 350 m., 16–17.IV.2000, leg. Hajek & Mikat (NMPC); 1 ♀ Hormozgan prov., Lengeh, 4.IV.1949, leg. Fasihi (HMIM); 11 ♂♂ 8 ♀♀ Hormozgan prov., Lengeh, 23–25,28.IV.1950, leg. Fasihi (HMIM); 1 ♀ Hormozgan prov., Lengeh, Chah Moslem, 14.V.1940, leg. Fasihi (HMIM); 1 ♂ 2 ♀♀ Minab, 31.III.1965, Mission Franco- Iranienne (HNHM); 3 ♀♀ Hormozgan prov., Minab, 3.III.1949, leg. Fasihi (HMIM); 1 ♂ Hormozgan prov., Minab, 18–

22.V.1973, leg. Hashemi & Broumand (HMIM); 4 ♂♂ 3 ♀♀ Hormozgan prov., Minab- Fariab, 18.V.1973, leg. Hashemi & Broumand (HMIM); 1 ♀ Hormozgan prov., Minab,: 26.IV.1949, leg. Fasihi (HMIM); 10 ♂♂ 12 ♀♀ Kerman prov., Jiroft, IV.1951, leg. Farmanara (HMIM); 14 ♂♂17 ♀♀ Kerman prov., Jiroft, 2.V.1951, leg. Farmanara (HMIM); 1 ♂ 1 ♀ Kerman prov., Jiroft, Aliabad, 4.V.1968, 700 m, leg. Hashemi & Pazuki (HMIM); 1 ♀ Kerman prov., Jiroft, 9.V.1969, leg. Hashemi & Pazuki (HMIM); 3 ♂♂ 4 ♀♀ Kerman prov., Jiroft, IV.1951, leg. Farmanara (MABC), 1 ♂ 1 ♀ Kerman prov., Jiroft, 2.V.1951, leg. Farmanara (MABC), 1 ♂ Kerman prov., Jiroft: 7.X.1967 (HMIM); 1 ♀ Kerman prov., Jiroft, Mohammadabad, 3–4.V.1973, leg. Borumand (HMIM); 1 ♂ 1 ♀ Kerman prov., Kahnou, 1.V.1969, leg. Hashemi & Pazuki (MABc); 1 ♂ 1 ♀ Kerman prov., Kerman- Sirjan, 24.V.1953, leg. Salavatian (HMIM); 3 ♂♂ 1 ♀ Kerman prov., Kerman, Sirjan Soghan, 8.V.1973, leg. Broumand & Hashemi (HMIM); 3 ♀♀ Kermanshah prov., Ghasr-e Shirin, 6.VI.1951, leg. Vakilian (HMIM); 2 ♂♂ 6 ♀♀ Khuzestan prov., Ahvaz: 10.VI.1954, leg. Salavatian (HMIM), 1 ♂ Khuzestan prov., Ahvaz: 10.VI.1954, leg. Salavatian (HNHM); 1 ♀ Sistan & Baluchestan prov. (HMIM); 6 ♂♂ 19♀♀ Sistan & Baluchestan prov., Alidar [Iranshahr], 800–1000 m, 1.V.1969, leg. Pazuki & Hashemi (HMIM); 1♂ Sistan & Baluchestan prov., Bampour, 18.IV.1950, leg. Sarkisian (HMIM); 2♀♀ Belutschistan, Südwestl. Iranschahr, Bampurufer, 21.V.1954, leg. Richter u. Schäuffele (HNHM); 1♀ Sistan & Baluchestan prov., Bampour, Razvan, 12–14.IV.1973, leg. Safavi & Borumand (HMIM); 9 ♂♂ 17 ♀♀ Sistan & Baluchestan prov., Damen, 24.IV.1969, leg. Hashemi & Pazuki (HMIM); 2 ♂♂ 2 ♀♀ Sistan & Baluchestan prov., Hodar, 1.IV.1950, leg. Famouri (HMIM); 7 ♂♂ 3 ♀♀ Sistan & Baluchestan prov., Aliabad, 833 m, 15.IV.2005, leg. Ebrahimi& Moghaddam (HMIM); 1 ♀ Sistan & Baluchestan prov., Iranshahr Lab. 1993, leg. Omara (HMIM); 5 ♂♂ 1 ♀ Sistan & Baluchestan prov., Iranshahr, 10./25.1V.1949, leg. Mirsalavatian (HMIM); 7 ♂♂3 ♀♀ Sistan & Baluchestan prov., Iranshahr, 5.V.1949, leg. Mirsalavatian (HMIM); 12 ♂♂ 7 ♀♀ Sistan & Baluchestan prov., Iranshahr, 14.IV.1965, leg. Safavi (HMIM); 4 ♂♂ 1 ♀ Sistan & Baluchestan prov., Ghasreghand, 7.VI.1950, leg. Famouri (HMIM); 28 ♂♂ 45 ♀♀ Sistan & Baluchestan prov., Kahnou, 800 m, 1.V.1969, leg. Pazuki & Hashemi (HMIM); 4 ♂♂2♀♀ Sistan & Baluchestan prov., [Khash], Karvandar, 23.IV.1969, leg. Hashemi & Pazuki (HMIM); 1 ♀ Sistan & Baluchestan prov., Karvandar, 23.1V.1969, leg. Pazuki & Hashemi (HMIM); 2♂ ♂ 1 ♀ Sistan & Baluchestan prov., Karvandar, Alidar [Iranshahr], 24.1V.1969, leg. Pazuki & Hashemi

(HMIM); 1 ♀ Sistan & Baluchestan prov., Nikshahr, 8–10.IV.1973, leg. Broumand & Safavi (HMIM); 5 ♂♂ 3 ♀♀ Sistan & Baluchestan prov., Nikshahr, Spake, 4,12.IV.1965, leg. Safavi (HMIM); 3 ♂♂ 1 ♀ Sistan & Baluchestan prov., Pishin, 8,22.IV.1950, leg. Famuri (HMIM); 1 ♂ Sistan & Baluchestan prov., Rask, 24.IV.1968, leg. Hashemi & Pazuki (HMIM); 2 ♂♂ 1 ♀ [Sistan & Baluchestan prov.], SE Iran, Rask, vall r. Sarbaz, 3–4.IV.1973, loc. No. 146, Exp. Nat. Mus, Praha (NMPC); 1 ♀ Sistan & Baluchestan prov., Rimdan [Chabahar], 4.IV.1950, leg. Famouri (HMIM); 1 ♂ Sistan & Baluchestan prov., Saravan, Bamposht, 23.IV.1950, leg. Salavatian (HMIM); 1 ♀ Sistan & Baluchestan prov., Sarbaz, 24.IV.1950, leg. Salavation (HMIM); 2 ♀♀ Sistan & Baluchestan prov., Sarbaz-Rask, 24.IV.1968, leg. Hashemi & Pazuki (HMIM); 1 ♂ Sistan & Baluchestan prov., Spakeh, 13.IV.1965, leg. Safavi (HMIM); 1 ♀ Sistan & Baluchestan prov., Spakeh, 24.IV.1969, leg. Pazuki & Hashemi (HMIM); 2 ♀♀ Sistan & Baluchestan prov., Tangesarhe, 10.IV.1973, leg. Safavi & Broumand (HMIM); 10 ♂♂ 9 ♀♀ Sistan & Baluchestan prov., Zabol, leg. Mirzayans (HMIM); 2 ♂♂ Semnan prov., Khar [Garmsar], V.1948, leg. Mirzayans (HMIM); 21 ♂♂ 25 ♀♀ Yazd prov., Abarghou, Aliabad, 25.VI.1972, leg. Safavi (HMIM); 1 ♂ 1 ♀ Yazd prov., Abarghou, Aliabad, 25.VI.1972, leg. Safavi (MABC). **Iraq**. 1 ♂ 1 ♀ Mesopotamia, Mosul, Coll. Hauser, V.1909 (HNHM); 2 ♂♂ Mesopotamia, Assur, VI.1908 (HNHM); 4 ♂♂ Baghdad, 1913, leg. Mattanovic (HNHM). **Pakistan.** 3 ♂♂ 4 ♀♀, Baluchestan, Nushki, 16.VI.2012 (MABC); 4 ♂♂ 5 ♀♀ Baluchestan, Nushki, 16.VII.2015 (MABC); 2 ♂♂ 2 ♀♀ Baluchestan, Nushki, 8.X.2016 (MABC); 1 ♂ Baluchestan, Nushki, 16.VI.2016 (MABC); 1 ex., Baluchistan, Nushki, Kili Jamaldini, 25.V.2016 (MABC); Baluchistan, Nushki, Kili Jamaldini, 3 exx. 22.VI.2013, 1 ex. 22.VII.2016 (FUUZM); 1 ex., Baluchistan, Tump, 15.4.1993 (SKC); 30 exx., western Baluchistan, Tump, 90 km west of Turbat, 13–15.IV.1993, S. Bečvář (LCC); 2 exx., western Baluchistan, Turbat, 8–19.IV.1993, S. Bečvář (LCC); 1 ex., Khyber Pakhtoon Khwa, Dera Ismail Khan, 12.V.2016 (FUUZM).

Records from literature. Iran. Iran (Koçak & Kemal, 2015a; Bologna et al., 2018; Ghahari & Campos-Soldini, 2019); Khuzestan, Ahvaz (Mirzayans, 1970; Serri, 2015); Khuzestan (Modaress Awal, 1997, 2012; Serri, 2015 as *trianguliferus trianguliferus*; Ghahari & Campos-Soldini, 2019); NW Iranshar, Trockental (Kaszab, 1957 as *triangulifera reitterioides* and ab. *bushirensis*); Belutschistan, NW Iranshar, Bampurufer (Kaszab, 1957 as *triangulifera reitterioides*); Iranshar (Kaszab, 1957, 1968 both as *triangulifera reitterioides*); Minab (Kaszab, 1968 as *triangulifera reitterioides*); Bushehr (Mader, 1929 as *triangulifera bushirensis*); Bandar Abbas (Kaszab, 1968 *as triangulifera reitterioides*); 25 km W Bandar Abbas (Kaszab, 1968 as *triangulifera reitterioides*); 80 km S Jiroft (Kaszab, 1968 as *triangulifera reitterioides*); Esfahan, W Ná-in (Kaszab, 1968 as *triangulifera reitterioides*); Fars, Sarvestan (Kaszab, 1968 as *triangulifera reitterioides*); Fars, Bandar-Langeh (Kaszab, 1968 as *triangulifera reitterioides*); Fars (Kaszab 1968b; Serri, 2015 as *trianguliferus trianguliferus*); Djiroft, Anbar Abad (Kaszab, 1959 as *triangulifera reitterioides*); Hormozgan, Kerman, Sistan & Baluchestan, Tehran (Modaress Awal, 1997, 2012; Fekrat & Modaress Awal, 2012; Ghahari & Campos-Soldini, 2019 all as *triangulifera* or *triangulifera reitterioides*); Khorasan (Modaress Awal, 1997, 2012; Fekrat & Modaress Awal, 2012 *as triangulifera reitterioides*); Khorasan, Razavi prov., Bajestan (Fekrat & Modaress Awal, 2012); Khuzestan (Serri, 2015), Sistan & Baluchestan (Kaszab, 1957, 1968b; Mirzayans, 1970; Modaress Awal, 1997, 2012 all as *triangulifera reitteroides*); Sistan, Zabol; Tehran, Ghazvine; Kohandehlj; Kerman, Bahramdjerd; Balaouchetan, Iranshahr, Pichine,; Abassi, Lemgueh, Tschah-mosslem, Minab (Mirzayans, 1970 as *triangulifera*); Khorassan, Torbat-heydarieh; Abassi: Minab, Lengueh, Bandar-abbass; Baluchestan, Iranshahr, Spakeh, Nikchahr, Khach, Karvandar, Sarbaz, Rask, Hodar, Rimdah, Ghasrghand, Pichine, Saravan, Bampocht; Sistan, Zabol,; Tehran, Garmsar, Ghotogh, Ghom; Kerman, Bardsair, Sirdjan; Fijroft, Alibad; LKermanchahan, Ghasrchirine (Mirzayans, 1970 as *triangulifera reitterioides*). **Iraq.** Iraq (Koçak & Kemal, 2015a; Bologna et al., 2018; Ghahari & Campos-Soldini, 2019); Baghdad (Al-Alì, 1977); Hilla (Al-Alì, 1977). **Pakistan.** Baluchistan, Nushki; Nushki, Kili Jamaldini; Baluchistan, Tump; Tump, 90 km west of Turbat; W Baluchistan, Turbat; Khyber Pakhtoon Khwa, Dera Ismail Khan (Bologna et al., 2018). **Saudi Arabia**. Arabia (Fischer, 1827; Marseul, 1870, 1872; Baudi, 1878a, 1878b; Mader, 1927; Kaszab, 1983; Bologna et al. 2018); Saudi Arabia (Batelka & Geisthardt, 2009; Koçak & Kemal, 2015a; Bologna & Turco, 2007; Ghahari & Campos-Soldini, 2019); Jeddah-Taif, (Kaszab, 1983); Wadi Hanifa (Kaszab, 1983).

Records from United Arab Emirates published by Gillet & Howarth (2004), Gillet & Gillet (1996, 2004) and by Batelka & Geisthardt (2009) as *Mylabris bipunctata* Olivier, actually must be referred to *H. pirata* (Bologna & Turco, 2007).

Description. Body length 8–27 mm; black, but elytra reddish brown with black pattern. Head subquadrate; antennomere III ca. 1/4 time longer than IV, V–VII pregressively widened, male XI (Fig. 40) with curved and elongated narrowed apex, narrowed portion of XI as long as the basal portion; in female short and not elongated (Fig. 41). Pronotum (Fig. 15) almost as long as wide, with anterior and basal depressions and a middle longitudinal furrow, anteriorly smooth, laterally punctuated; mesosternun as in Fig. 45. Each elytron with two black spot on anterior third, one wide middle fascia, and a black fascia on apical part (Figs 46). Last male ventrite widely and deeply concave and depressed, with widely emarginated posterior margin; aedeagus (Fig. 44) with endophallic hook narrow and slender, distal aedeagal hook far from proximal one and almost at apex, proximal hook slightly greater; gonoforceps (Figs 42–43) very narrow and curved apically in lateral view.

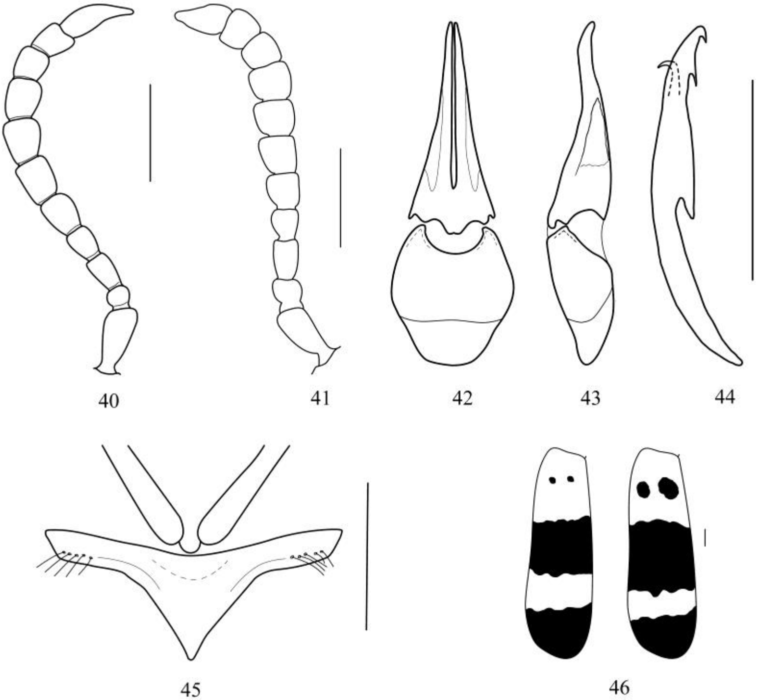


Fig. 40–46. *Hycleus bipunctatus* (Olivier): 40. Male antenna; 41. female antenna; 42. tegmen, ventral view; 43. tegmen, lateral view; 44. aedeagus, lateral view; 45. mesosternum; 46. elytral pattern. Scale bar = 1 mm.

Taxonomic remarks. In few specimens the narrowed portion of antennomere XI is shorter. The size of anterior black spots of elytra and middle fascia differs in some populations. The anterior and basal depressions and the middle longitudinal furrow of pronotum have different depth even among members of a population.

Although the types of *Mylabris triangulifera* ab. *reitterioides* and ab. *bushirensis* were not available for us, we studied topotypic specimens of this intraspecific form from Busher (Mader, 1929) and found out that they are distinct from *Hycleus trianguliferus* (Heyden, 1883) and totally similar to *H. bipunctatus* (Olivier, 1811) in the shape of last ventrite, elytral pattern, pronotal punctation and male genitalia. As mentioned in Bologna et al. (2018), here we formally propose the following synonymy:

Mylabris triangulifera ab. *reitterioides* Mader, 1929) and ab. *bushirensis* Mader, 1929 = *Hycleus bipunctatus* (Olivier, 1811) **new synonymies**.

Distribution. This species is distributed in Saudi Arabia, Iraq, Iran and Southwest Pakistan (Bologna et al., 2018). It has a wide distribution mostly in southern Iran.

Hycleus cingulatus (Faldermann, 1837)

Figs 4, 16, 47–54

Mylabris cingulata Latreille, *in litteris*
Mylabris cingulata Faldermann, 1837: 122
Mylabris succincta Faldermann, 1837: 365
Zonabris cingulata var. *flavipennis* Dokhtouroff, 1889: 157
Mylabris cingulata ab. *flavis* Mader, 1927a: 196 (new name for *flavipennis*)
Hycleus amrishi Makhan, 2012:1 **syn. n.**
Hycleus colligatus sensu Auct. nec Hycleus colligatus (Redtenbacher, 1850)

Type material. The type of this species is lost. We examined in MRSN (Baudi's collection from the Dejan's collection) specimens identified as *succinctus,* which were possibly sent by Faldermann.

Additional material examined. Azerbaijan. 1 ex. Lankaran (MUSE). **Iran.** 1 ♀ Iran, Alborz prov., Karadj, VIII.1947 (HMIM); 1 ♀ Esfahan prov., Delijan, 2170 m, 13.VI.2004, leg. Serri & Frisch (HMIM); 1 ♂ East Azarbaijan provinc, Azarshahr, 1.VI.1976, leg. Damanabi (HMIM); 1 ♂ Elburz, 30 km S di Ab Ali, 9.VII.1965, leg. Giordani Soika, Mavromoustakis (MABC); 2 ♂♂ 1 ♀ Dasht-e Kavir, Kashan dint., 10.VIII.2000, leg.Crucitti, Vignoli, Facheris (MABC); 8 ♂♂ 8 ♀♀ Esfahan prov., Golpayegan, 15.VII.1974, leg. Hashemi & Zairi (HMIM); 38 ♂♂ 48 ♀♀ (HMIM) 1 ♂ 1 ♀ (MABC) Esfahan prov., Kashan, Karkas, 2800 m, 29.VII.1974, leg. Hashemi & Zairi; 5 ♂♂ 1 ♀ Esfahan prov., 25 km E Khomein, 1860 m, 2.VII.1983, leg. Mirzayans & Brumand (HMIM); 6 ♂♂ 2 ♀♀ Esfahan prov., Khomein- Delijan, 1780 m, 30.VII.1981, leg. Pazuki & Brumand (HMIM); 1 ♀ Esfahan prov., Natanz, S Slope of Karkas, 21.VII.2003, leg. Rajaiee (HMIM); 1 ♂ Fars prov., Firouzabad, 27.VI.1963, leg. Mirzayans (HMIM); 3 ♂♂ Fars prov., Sepidan, Sibkhalaj- Shiraz, 29.VI.1963, leg. Mirzayans (HMIM); 1 ♂ Fars prov., Mehkou, 9.VII.1971, leg. Safavi (HMIM); 2 ♂♂ 1 ♀ Fars prov., Shiraz, 2.VIII.1949, leg. Mirzayans (HMIM); 5 ♂♂ 10 ♀♀ (HMIM), 1 ♂ 1 ♀ (MABC) Ghazvin prov., Ghazvin, 4.VIII.1950, leg. Mirzayans; 1 ♂ 2 ♀♀ Ghom prov., Aliabad, 10.VI.1970, leg. Rajabi (HMIM); 1 ♀ Mazandaran prov., Amol, Ramsar, Behshahr, VIII.2002, leg. Ghahari (MABC); 1 ♀ Hamedan prov., Nahavand, Garmasiab, 1750 m, 7.VIII.1997, leg. Barari & Mofidi (HMIM); 3 ♀♀ Hamedan prov., Asadabad, 11.VIII.1968, leg. Mirzayans & Mortazaviha (HMIM); 1 ♀ Hamedan prov., Asadabad, 2100m, 17.VII.1978, leg. Hashemi & Zairi (HMIM); 2 ♂♂ 1 ♀ Hamedan prov., Asadabad, 2200 m, 30.VII.1987, leg. Mirzayans & Hashemi (HMIM); 1 ♂ Kavir Schutzgeb., Siahkuh geb. (MABC); 1 ♂ Kerman prov., Baft, Lalehzar, 4.VI.1971, leg. Naim & Hashemi (HMIM); 1 ♂ Kerman prov., Sirjan, 15.VIII.1964, leg. Safavi (HMIM); 1 ♂ Kerman prov., Rayen, 2400 m, 17.VI.1988, leg Hashemi & Badii (HMIM); 1 ♂ Kordestan prov., Sanandaj, 14.VII.1969, leg. Pazuki & Hashemi (HMIM); 1 ♂ Kordestan prov., 10 km N Sarvabad, 2230 m, 31.VII.2015, leg. Montreuil (HMIM); 2 ♂♂ Persien, Sabzvaran (HNHM); 1 ♂ 1 ♀ Tehran prov., Abali, 18.IV.1946, leg. Mirzayans (HMIM); 1 ♂ Tehran prov., Abahar, 8.VII.1947, leg. Salavatian (HMIM); 1 ♀ Tehran prov., Eschtehard, 3.IX.2000, leg. Serri (MABC); 1 ♀ Tehran prov., Golhak, 1400 m, b. Tehran, IX–X.1961, leg Klapperich (HNHM); 1 ♂ Tehran prov., Vanak, 11.VII.1947, leg. Salavatian (HMIM); 1 ♂ Tehran prov., Varamin, VII.1949, leg. Abaspour (HMIM); 1 ♀ Tehran prov., Varamin, 2.VIII.1972, leg. Abai (HMIM); 1 ♂ Tehran prov., Damavand, Marunak, 12.VIII.1948, leg. Vaezi (HMIM); 2 ♂♂ Razavi Khorasan prov., Mashhad, 5.VI.1965, leg. Sharif (HMIM); 1 ♀ Razavi Khorasan prov., Neishabur, N. Sabzevar, 1000m., 15.VI.1977, leg. Pazuki & Abai (HMIM); 1 ♂ Semnan, 31.VII.1972, leg. Iranshahr (HMIM); 1 ♀ (HMIM) 1 ♂ (HNHM) Semnan prov., Shahrud, Mayamay, 8.VII.1972, leg. Iranshahr; 1 ♂ Belutschistan, Sangun 1650 m, ostl. Kuhi Taftan, 4–8.VI.1954, leg. W. Richter (HNHM); 2 ♂♂ 2 ♀♀Strauss 91, Persien (HNHM); 2 ♂♂ 3 ♀♀ Persien (143), Mahmudieh, Tehran, Stepp, 1250 m, 21.VIII.1948, coll. P. Aellen (HNHM); 1 ♀ Persien, Sudl. Dorf (131), Damavand, steppe, 11.VIII.1948, coll. P. Aellen (HNHM). **Syria.** 1 ex. Faliah env., Palmyra oasis, N 34°333.196 E 38°17.150 (MZUF). **Turkey**. 1 ♂ Malataya, Kale, 17.VIII.1982, leg. Pavesi (MABC).

Records from literature. The taxonomy of this species is very confused, and we prefer consider only confirmed or congruent records from the literature. **Iran**. Iran (also as Perse) (Marseul, 1870, 1872; Baudi, 1878a, 1878b also as *colligatus* and *succinctus*; Soumakov, 1915, 1930; Mader, 1927; Kuzin, 1954; Özbek & Szaloki, 1998); Fars (Serri, 2015 as *colligatus*); Fars, Ghazvin, Hormozgan, Kerman, Kordestan, Sistan & Baluchestan (Mirzayans, 1970; Modarres Awal, 1997, 2012 as *colligata*); Semnan, Damghan, Ahvanu (Makhan, 2012 as *amrishi*); Semnan (Ghahari & Campos-Soldini, 2019); S Iran (Özbek & Szaloki, 1998). **Syria**. Syria (Özbek & Szaloki, 1998). **Transcaucasia**. Transcaucasia (Faldermann, 1837, 1839; Soumakov, 1930; Kuzin, 1954; Kaszab, 1983; Özbek & Szaloki, 1998; Ghahari & Campos-Soldini, 2019. **Saudi Arabia**. Arabia (Soumakov, 1930; Bologna & Turco, 2007; Ghahari & Campos-Soldini, 2019). Oriente (Baudi, 1878a). Probably *M. husseini sensu* Marseul 1870 and 1872 cited from northern Egypt and southern Iran refers to *cingulatus*. **Turkmenistan**. Turkmenia (Dokhtouroff, 1890; Kaszab, 1983; Ghahari & Campos-Soldini, 2019). **"Turkestan".** "Turkestan" (Dokhtouroff, 1890; Kaszab, 1983; Ghahari & Campos-Soldini, 2019). **Turkey**. Turkey (Koçak & Kemal, 209); Ankara, Delice (Özbek & Szaloki, 1998).

Description. Body length 12–22 mm. Body black, but elytra yellowish brown with black pattern. Head subquadrate; antennomere III ca. 1/4 time longer than IV, V–VII transverse and progressively widened, antennomeres VIII–X progressively narrowed, male XI (Fig. 47) elongated apically, with an angulated step before the elongated portion, small setae on apex and on the angulated point before the apex; in female (Fig. 48) last antennomere short and spindle-shaped. Pronotum (Fig. 16) longer than wide, distinctly strangulate on fore third, deeply depressed on fore sides, with fine sparse punctures; mesosternun as in Fig. 53. Each elytron with two black spots on anterior third, one wide middle fascia, and a narrow black fascia on apical portion (Fig. 54). Last male ventrite narrowly concave and depressed, posterior margin with a deep middle emargination; aedeagus (Figs 51–52) with slightly narrowed endophallic hook, distal aedeagal hook distinctly curved, far from the proximal one and almost at apex, gonoforceps (Figs 49–50) progressively narrow and straight in both ventral and lateral view.

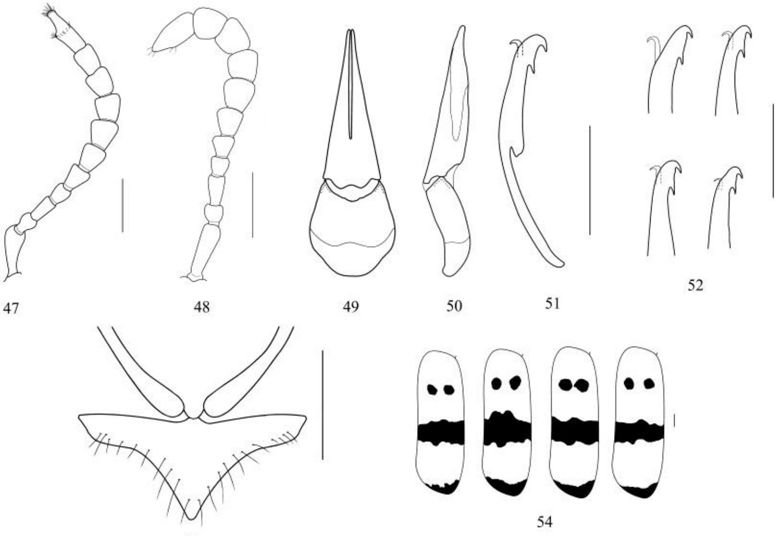


Fig. 47–54. *Hycleus cingulatus* (Faldermann): 47. Male antenna; 48. female antenna; 48. tegmen, ventral view; 50. tegmen, lateral view; 51. aedeagus, lateral view; 52. variations of aedeagus; 53. mesosternum; 54. elytral pattern. Scale bar = 1 mm.

Taxonomic remarks. This species represents a taxonomic *rebus*. The name *Mylabris cingulata* Latreille is a *nomen in litteris* which seems just to be mentioned in Dejean (1836). Faldermann (1837) considered this name and described formally *cingulata* and *M. succincta*, which is a synonym of *M. cingulata*, and consequently became the Author. Checking the Faldermann's description in detail, we attributed a species to *H. cingulatus* which is uncommon out of Iran, and which was confused by all blister beetle specialists.

Baudi (1878a, 1878b) mentioned both *M. cingulata* Latreille and *M. cingulata* Faldermann, indicating that both correspond to the Marseul (1870) description of this species. However, the specimens of *M. cingulata* in Marseul's collection (MNHN) corresponds to *Mylabris parumpicta* (Heyden, 1883) rather than to the Faldermann's description. Probably this is the reason why Borchmann (1917) erroneously considered *M. parumpicta* as synonym of *M. cingulata* Faldermann, while *parumpicta* belongs to the nominate subgenus of *Mylabris* (Pan & Bologna, 2014; Serri & Bologna, 2018). Baudi (1878a, 1878b) also mentioned another species as "*colligata*?", the same with *M. succincta* Faldermann, which is synonym of *M. cingulata*. In the Baudi's collection (MRSN), which includes the Dejan' collection, is housed one specimen of this species from Iran, possibly sent by Faldermann (see above). According to the description of *colligata*, the pronotum is extremely stangulate and the last antenomere internally with a projected part. Baudi (1878a, 1878b) also mentioned that because of the shape of antennae he considered it as a distinct species. This distinct species with the specific features of antennae and pronotum has been usually named "*colligatus*" in the literature and collections by some specialists such as Sumakov, Borchmann, Kaszab. After the examination of syntypes of both *colligatus* and *javeti* (MNHN, NHMW), we concluded that they are synonyms (see below under *H. colligatus*). In conclusion, the specimens identified as *Mylabris colligata* in most literature probably must be referred to *H. cingulatus*, while records of *Mylabris javeti*, correponds to *H. colligatus*.

We did not examine types of varieties of *cingulatus* described by Dokhtouroff (1889), so their specific attribution remains doubtful.

Kaszab identification of this species was erroneous, and it seems that he ignored the strangulate shape of pronotum and the shape of male XI antennomere, which is very characteristic in this group of species and in his *colligata*, *javeti* and *schauffelei* identification. In particular, two Kaszab's paratypes of varieties, one identified as *Mylabris schauffelei* ab. *postmediojuncta* Kaszab and the other as *M. javeti* ab. *heratensis* Kaszab, actually refer to *H. cingulatus* with the typical last male antennomere and stangulate and soft punctuated pronotum.

We noted small differences in the shape of aedeagal hooks (Figs. 52) in different populations of this species but we believe that the diagnostic shape of last antennomere and pronotum are synapomorphic characteristics.

According to the photos and description published by Makhan (2012), we propose the following taxonomic act:

Hycleus amrishi Makhan, 2012 = *Hycleus cingulatus* (Faldermann, 1837) **new syn**onymy

Distribution. The distribution of this species is difficult to define with certainty, because it was largely confused in the literature and several citations have been erroneously referred to it. Bologna (2008), considered *H. cingulatus* endemic to Iran, but we studied scattered records also from Azerbaijan, Syria and Turkey. Records from Saudi Arabia (Sumakov, 1930) need confirmation as well as the old citation from the late “Turkestan”. In Iran, this species is distributed at higher elevation unlike to its two sympathric species *H. colligatus* and *H. schauffelei*.

***Hycleus colligatus* (Redtenbacher, 1850)**
Figs 5, 17, 55–62

Mylabris colligata Redtenbacher, 1850: 49
Mylabris javeti Marseul, 1870: 88 **syn. n.**
Mylabris javeti umbilicatus Kaszab, 1958: 291 **syn. n.**

Type material. 2 ♂♂ 1 ♀ syntypes, “*colligata*// Kots./ m” (NHMW).

Holotype and 3 syntypes of Mylabris javeti (possibly other specimens close in the box in the Marseul’s collection to the holotype could be syntypes) (MNHN); 1 ♂ syntype, “Persia / settent./ 1862–63/ Col. G. Doria// Collect./ Türk// Javeti Mars./ type/ Persia Heyd. // Javeti” (NHMW);

Paratypes of *Mylabris javeti umbilicata*, 1 ♂ “Nuristan (Afghan.) 1935/ Expedit. Dr. Sceibe//Pargil n./ Patschligil/ 12.7.35// Myl. colligata Redtb.// Deutsch. Entomol./ Institut Berlin//Paratypus/ *Mylabris javeti/* ssp. *umbilicata* m./ det. Dr. Kaszab, 1956”; 1 ♂” J. Klapperich/ Umgeb. Kabul/ 1740 m, 24.6.52/ O- Afghanistan// Paratypus ♂/ *Mylabris javeti*/ ssp. *umbilicata* m./ det. Dr. Kaszab, 1956”; 1 ♀ “J. Klapperich/ Bashgul Tal 1200 m/ Nuristan 11.5.53/ Afghanistan// Paratypus/ *Mylabris javeti/* ssp. *umbilicata* m./ det. Dr. Kaszab, 1956” (HNHM).

Additional material examined. Afghanistan. 5 ♂♂ 6 ♀♀ Borgh, Logar, 19.VII.1957, no. 246, leg. Lindberg (HNHM); 1 ♂ Ghazni, 30 km SW Moqur, 1900 m, 10.V.1973, leg. Kabakov (HNHM); 1 ♀Ghazni, 50 km NW Moqur, 2200 m, 10.VI.1972, leg. Kabakov (HNHM); 1 ♂ E Afghanistan, Gulbahar, 1700 m, 15.VI.1956, leg. Amsel (HNHM); 1 ♂ Heart, 970 m, 15.V.1956, leg. H. G. Amsel (HNHM); 1 ♀ Kouh- Djaouz, 20 km de Kaboul,1820 m, 31.V.1960, no. 847, leg. Lindberg (HNHM); 1 ♂ umgeb. Kabul, 1740 m, 24.VI.1952, leg. Klapperich (HNHM); 1 ♀ Qal′eh Lakou, aff. Val. Kaboul, 7.VII.1957, no. 254, leg. Lindberg (HNHM); 1 ♂ Laghman, Samakat, 900 m, 24.IV.1972, leg. Kabakov (HNHM); 1 ♀ Nangarhar prov., (112) Darunta, 580–600 m, 3.V.1967, leg. Povolny (HNHM); 1 ♀ Nuristan, Bashgaltal, 120 0m, 11.V.1953, leg. Klapperich (HNHM);1 ♂ Nuristan, Parigil n., Patschligil, 12.VII.1935, leg. Scheibe

(HNHM); 1 ♂ Nangarhar, Dasht-e Gamberay, 650 m, 8.V.1974, no. 82, leg. Papp (HNHM); 2 ♂♂ 1 ♀ Nuristan, Parigil n. Patsdeligil, 12.VII.1935, Exp. Dr. Scheibe (HNHM); 3 ♀♀ Darreh Gazak, 20 km S Oubeh, 1370 m, 10.VI.1962, leg. K. Lindberg (HNHM). **Caucasus.** 1 ♂. R. Oberthur, Eing. Nr. 4. 1956 (HNHM). **Iran.** 1 ♂, Alborz prov., Aderan, Karaj, 23.VII.1946 (HMIM); 1 ♀ Alborz prov., Eshtehard, 26.V.1968, leg. Ayatollahi (HMIM); 1 ♂ Bushehr prov., Borazjan, Asaluyeh, 40 m, 23.IV.1977, leg. Pazuki & Hashemi (HMIM); 1 ♂ 2 ♀♀ Bushehr prov., Bushehr, Ahram, Tangestan, 100 m, 4–5.V.1995, leg. Parchami, Badii & Ardeh (HMIM); 1 ♂ Bushehr prov., Borazjan, 19.III.1953, leg. Mirsalavatian (HMIM); 6 ♂♂ 7 ♀♀ Bushehr prov., Dylam, 1,9.V.1969, leg. Zairi & Ayatollahi (HMIM); 14 ♂♂ 2 ♀♀ Esfahan prov., Delijan, 2.VI.1970, leg. Mirzayans & Abai (HMIM); 3 ♂♂ 1 ♀ Esfahan prov., Delijan, 1700 m, 8.VI.1986, leg. Mirzayans & Hashemi (HMIM); 1 ♂ Esfahan prov., Esfahan, V.1948, leg. Makoui (HMIM); 1 ♀ Esfahan prov., Khansar, 16.VI.1948, leg. Alexandrov (HMIM); 1 ♂ 1 ♀ Esfahan prov., Khansar, Golestan kuh, 2700 m, 3/4.VII.1983, leg. Mirzayans & Broumand (HMIM); 20 ♂♂ 18 ♀♀ Esfahan prov., Kashan- Niasar, Sericheh, 1650 m, 9–11.VI.1984, leg. Pazuki & Hashemi (HMIM); 2 ♂♂ 4 ♀♀ Esfahan prov., Kashan, Rahagh, 2350 m, 25.VI.1981, leg. Hashemi (HMIM); 15 ♂♂ 7 ♀♀ Esfahan prov., 25 km E Khomein, 1860 m, 2.VII.1983, leg. Mirzayans & Borumand (HMIM); 1 ♂ 1 ♀ Esfahan prov., Zagros Mt. Shahreza, Koruyeh, 11.VI.2011, leg. Dalihod (MABC); 2 ♂♂ [Fars prov.], zentral Iran. 20 km nordl. v. Abadeh, 2000 m, leg. Vartaian (HNHM); 1 ♂ Perisa, [Fars prov.], Abadeh, V–VI.1916, leg. P. Paschen, coll. P. Gough (HNHM); 5 ♂♂ 5 ♀♀ Fars prov., Dadin, 8, 11.V.1968, leg. Mojib (HMIM); 1 ♀ Fars prov., Dadin, 7.V.1965, leg. Mojib (HMIM); 2 ♂♂ Fars prov., Dascht-e Arjan, 27.VI.1976, leg. Sbordoni & Rampini (MABC); 1 ♀ Fars prov., Eghlid, 3.VI.1969, leg. Termeh & Izadyar (HMIM); 3 ♂♂ 1 ♀ Fars prov., Farashband, Hosseinabad, 4.VI.1968, leg. Mirzayans (HMIM); 3 ♂♂ Iran, Fars prov., Izadkhast, N 31 31 E 52 08, leg. A. Senglet (HNHM); 1 ♂ Fars prov., Kazeroun, V.1953, leg. Mirzayans (HMIM); 1 ♂ Fars prov., Kazeroun, 12.V.1967, leg. Gharib (HMIM); 1 ♀ Fars prov., Kazeroun, Mamasani, V.1951, leg. Manuchehri (HMIM); 1 ♂ Fars prov., Lar, 19.V.1969, leg. Pazuki & Hashemi (HMIM); 3 ♂♂ 2 ♀♀ Fars prov., Shiraz, Kandar, Sibkhalaj, 29.VI.1963, leg. Mirzayans (HMIM); 1 ♀ Fars prov., Kazeroun, Sarmashad, 16.V.1969; leg. Mojib (HMIM); 4 ♂♂ 1 ♀ Fars prov., Kakan, 7.VIII.1949, leg. Mirzayans (HMIM); 1 ♂ 5 ♀♀ Ghazvin prov., 9.VIII.1950, leg. Mirzayans (HMIM); 2 ♂ ♂ 1 ♀ Ghom prov., Lake Ghom, 27.VI.1969, leg. M.A.B (HMIM); 1 ♂ 3♀ ♀ Ghom prov., Ghom, 900 m, 2.VI.1990, leg. Mirzayans & Abai (HMIM); 2 ♂♂ 1 ♀ Hamedan prov., Asadabad, 2200m, 30.VII.1987, leg. Mirzayans & Hashemi (HMIM); 3 ♂♂ 4 ♀♀ Hamedan prov., Bandarabbas, 16.V.1969, leg. Pazuki & Hashemi (HMIM); 1 ♂ 1 ♀ Hamedan prov., Bandarabbas, Geno Mt., 320 m, 14.VI.1992, leg. Mirzayans & Badii (HMIM); 4 ♂♂ 2 ♀♀ Hamedan prov., Bandarabbas, Geno Mt., 900 m, 8.IV.2004, leg. Moghaddam & Serri (HMIM); 2 ♀♀ Hamedan prov., Bandarabbas, Hajiabad, 1050 m, 3.V.1996, leg. Badii, Ardeh & Nazari (HMIM); 1 ♂ Hormozgan prov., Bashagerd, Senderk, 220 m, 12.VI.1977, leg. Safavi & Pazuki (HMIM); 1 ♂ Hormozgan prov., Minab, 23.III.1949, leg. Fasihi (HMIM); 1 ♂ 4 ♀♀ Hormozgan prov., Minab, 12, 13.V.1969, leg. Pazuki & Hashemi (HMIM); 2 ♂♂ 3 ♀♀ Kerman prov., Bardsir, 12.V.1952, leg. Famouri (HMIM); 1 ♂ 1 ♀ Kerman prov., Dehbakri, 2500 m, 15.VI.1967, leg. M.A. (HMIM); 1 ♂ Kerman prov., Sirjan, 6.IV.1973, leg. Abai (HMIM); 1 ♂ 1 ♀ Kerman prov., 50 km S Sirjan, 1500 m, 2.V.1986, leg. Mirzayans & Boroumand (HMIM); 8 ♂♂ 9 ♀♀ Kerman prov., Jebalbarez, 14.VI.1967, leg. Mirzayans & Pazuki (HMIM); 1 ♂ 4 ♀ Kerman prov., Jiroft, 11.V.1969, leg. Pazuki & Hashemi (HMIM); 2 ♂♂ 1 ♀ Kerman prov., Jiroft, Asminon, 11.V.1969, leg. Pazuki & Hashemi (HMIM); 9 ♂♂ 9 ♀♀ Kerman prov., Jiroft, Mijan, 1250 m, 12.V.1968, leg. Hashemi & Pazuki (HMIM); 3 ♀♀ Kerman prov., Jiroft, Mijan, 1000 m, 9.V.1969, leg. Pazuki & Hashemi (HMIM); 1 ♂ Kermanshah prov., 12.VIII.1968, leg. Mirzayans & Mortazaviha (HMIM); 1 ♀ Kermanshah prov., Ghasre Shirin, 27.V.1961, leg. Farahbakhsh (HMIM); 1 ♀ Kermanshah prov., Kerend, 1948, leg. Alexandrov (HMIM); 1 ♂ Kermanshah prov., Mehran, V.1945, leg. Sarkissian (HMIM); 2 ♂♂ 2 ♀♀ Kordestan prov., Bijar, 28.VII.1968, leg. Dezfulian & Iranshahr (HMIM); 3 ♂♂ 1 ♀ Khuzestan prov., Aghajari, 1,3.V.1969, leg. Ayatollahi & Zairi (HMIM); 4 ♂♂ 2 Khuzestan prov., Andimeshk, 8_9.V.1969, leg. Zairi & Ayatollahi (HMIM); 4 ♂♂ 6 ♀ Khuzestan prov., Andimeshk, Bidrubeh, Ghalerezeh, 500 m, 30.IV.2001, leg. Gilasian, Hajesmailian & Mofidi (HMIM); 1 ♀ Khuzestan prov., Behbahan, 11.VI.1952, leg. Vakilian (HMIM); 7 ♂♂ 11 ♀♀ Khuzestan prov., Behbahan, 1,2,6.V.1969, leg. Zairi & Ayatollahi (HMIM); 3 ♂♂ 1 ♀ Khuzestan prov., Behbahan, 3.VI.1969, leg. Zairi & Ayatollahi (HMIM); 1 ♂ Khuzestan prov., Behbahan, Kheirabad, 3.V.1968, leg. Zairi (HMIM); 2 ♂♂ 6 ♀♀ Kohgiluyeh & Boyerahmad prov., Dogonbadan, 9.V.1967, leg. Zairi & Ayatollahi (HMIM); 13 ♂♂ 12 ♀♀ Kohgiluyeh & Boyerahmad prov., Dogonbadan, 8,11.V.1968, leg. Zairi & Ayatollahi (HMIM); 6 ♂♂ 1

♀ Kohgiluyeh & Boyerahmad prov., Dogonbadan, 15, 28.IV.1969, leg. Ayatollahi (HMIM); 1 ♂ Lorestan prov., Azna, Oshtorankuh, Kamandan, 2050–2300 m, 23–24.VIII.1994, leg. Mirzayans & Sarafrazi (HMIM); 3 ♂♂ 1 ♀ Markazi prov., km 80 Ghom- Arak, near Easyjan, VI.1978, leg. Mash. (HMIM); 2 ♂♂ 2 ♀♀ Mazandaran, Elborz Mts., above Rudehen, 1900–2200 m, 11.VI.2014, leg. Bologna (MABC); 2 exx Qom prov., Neyzar, 34°16’N 50°36’E, Takht-e-Chekab (JCRC); 2 ♂♂ 2 ♀♀Persia [Razavi Khorasan prov.], Sabsavaran (HNHM); 1 ex Khorasan [e- Razavi] , Fazel, 9 km W Mashad (MCNV); 1 ♀ Sistan & Baluchestan prov., Bampour, 21.IV.1968, leg. Hashemi & Pazuki (HMIM); 1 ♂ 2 ♀♀ Sistan & Baluchestan prov., Hodar, 1.IV.1950, leg. Famouri (HMIM); 5 ♂♂ 2 ♀♀ Sistan & Baluchestan prov., Iranshahr, 5, 25.VI.1949, leg. Morsalavatian (HMIM); 1 ♂ 4 ♀♀ Sistan & Baluchestan prov., Iranshahr, 550 m, 25.IV.1969, leg. Hashemi & Pazuki (HMIM); 1 ♀ Sistan & Baluchestan prov., Iranshahr, 20.IV.1969, leg. Hashemi & Pazuki (HMIM); 3 ♂♂ 1 ♀ Sistan & Baluchestan prov., Iranshahr-Sarbaz, 22.IV.1968, leg. Hashemi & Pazuki (HMIM); 5 ♂♂ 5 ♀♀ Sistan & Baluchestan prov., Iranshahr, Damen, 24.IV.1969, leg. Hashemi & Pazuki (HMIM); 2 ♂♂ 3 ♀♀ Sistan & Baluchestan prov., Iranshahr, Damen, 711.IV.1950, leg. Sarkissian (HMIM); 4 ♂♂ Sistan & Baluchestan prov., Iranshahr, 25, 30.III.1951, leg. Sarkissian (HMIM); 1 ♂ 4 ♀♀ Sistan & Baluchestan prov., Iranshahr, Hamont kouh, 1100 m, 20.IV.1968, leg. Pazuki & Hashemi (HMIM); 1 ♂ Sistan & Baluchestan prov., Iranshahr, 21.IV.1968, leg. Hashemi & Pazuki (HMIM); 1 ♀ Sistan & Baluchestan prov., Iranshahr, Spake, Aliabad, 833m, N 26 47 28.9 E 60 10 46.8, 15.IV.2005 leg. Ebrahimi & Moghaddam (HMIM); 12 ♂♂ 15 ♀♀ Sistan & Baluchestan prov., Isin, 1500 m, 17.V.1969, leg. Hashemi & Pazuki (HMIM); 7 ♂♂ 5 ♀♀ Sistan & Baluchestan prov., Kahnou, 800 m, 1–4.V.1969, leg. Pazuki & Hashemi (HMIM); 1 ♂ 6 ♀♀ Sistan & Baluchestan prov., Karvandar, 18.IV.1968, leg. Hashemi & Pazuki (HMIM); 51 ♂♂ 47 ♀♀ Sistan & Baluchestan prov., Karvandar, 22, 23.IV.1969, leg. Hashemi & Pazuki (HMIM); 28 ♂♂ 23 ♀♀ Sistan & Baluchestan prov., Karvandar, 1140 m, 22–23.IV.1969, leg. Hashemi & Pazuki (HMIM); 8 ♂♂ 9 ♀♀ Sistan & Baluchestan prov., Karvandar, Alidar, 24.IV.1969, leg. Hashemi & Pazuki (HMIM); 3 ♀♀ Sistan & Baluchestan prov., Karvandar, Alidar, 800–1000 m, 1.V.1969, leg. Pazuki & Hashemi (HMIM); 1 ♂ Sistan & Baluchestan prov., Karvandar, Iranshahr, 16.VII.1972, legAbai (HMIM); 1 ♀ Sistan & Baluchestan prov., Pishin, 17.IV.1950, leg. Famouri (HMIM); 4 ♂♂ 4 ♀♀ Sistan & Baluchestan prov., Pishin, 22.V.1950, leg. Famouri (HMIM); 1 ♀ Sistan &

Baluchestan prov., Negor, 7.III.1997, leg. Mirzayans, Badii & Sarafrazi (HMIM); 1 ♂ 2 ♀♀ Sistan & Baluchestan prov., Saravan, Bamposht, 26.IV.1950, leg. Mirsalavatian (HMIM); 3 ♂♂ 6 ♀♀ Sistan & Baluchestan prov., Sarbaz, 1200 m, 23.IV.1968, leg. Pazuki & Hashemi (HMIM); 10 ♂♂ 7 ♀♀ Sistan & Baluchestan prov., Sarbaz- Rask, 24.IV.1968, leg. Hashemi & Pazuki (HMIM); 2 ♂♂ 3 ♀♀ Sistan & Baluchestan prov.,Sarbaz-Zangian, 13.IV.1949, leg. Mirsalavatian (HMIM); 2 ♂♂ 2 ♀♀ Sistan & Baluchestan prov., Rask, 24.IV.1968, leg. Pazuki & Hashemi (HMIM); 1 ♂ Sistan & Baluchestan prov., Tang-e Sarhe Nikshahr, 23. III. 1949, leg. Sharif (HMIM); 3 ♀♀ Sistan & Baluchestan prov., Tangesarhe, 10.IV.1973, leg. Safavi & Broumand (HMIM); 1 ♀ Sistan & Baluchestan prov., Nikshahr, 23.IV.1968, leg. Hashemi & Pazuki (HMIM); 2 ♂♂ Sistan & Baluchestan prov., Nikshahr, 28.IV.1969, leg. Pazuki & Hashemi (HMIM); 16 ♂♂ 13 ♀♀ Sistan & Baluchestan prov.,Tang-e Sarhe,1000 m, 27, 30.IV.1969, leg. Pazuki & Hashemi (HMIM); 1 ♀ Sistan & Baluchestan prov., Zabol, 5.VI.1949, leg. Mirzayans (HMIM); 2 ♂♂ Sistan & Baluchestan prov., Zabol, 25.V.1950, leg. Mirzayans (HMIM); 1 ♂ Sistan & Baluchestan prov., Zahedan, Nosratabad, 21.IV.1969, leg. Pazuki & Hashemi (HMIM); 1 ♂ 1 ♀ Persien, Baluchistan, IV. 1949, leg. Sharif (HNHM); 1 ♂ Beluchistan, nrdwestl. Iranshahr (Trocken Tal), 15.III.1954, leg. Richter & Schäuffele (HNHM); 2 ♂♂ [Semnan prov.], Shrud, 9.V.1956, leg.Schmid (HNHM); 1 ex Semnan prov., 5 km NE Agareh (TGC); 1 ♂ 2 ♀♀ Tehran prov., Abshar, 10.VII.1947, leg. Mirsalavatian (HMIM); 1 ♂ 1 ♀ Tehran prov., Evin, 18.VII.1948 leg. Mirzayans (HMIM); 8 ♂♂ 14 ♀♀ (HMIM); 1 ♀ (HNHM) Tehran prov., Evin, 6.VI.1965, leg. Mirzayans/Daneshpazhouh; 4 ♂♂ 2 ♀♀ (MABC), 2 ♂♂ 2 ♀ (HNHM) Tehran prov., Golhak, bei Teheran, 1700 m, 9–23.VI.1961, leg. Klapperich; 1 ♂ Tehran prov., Lashgarak, 1650 m, 18.VI.2004, leg. Serri & Frisch (HMIM); 1 ♂ 1 ♀ Persien, Tehran, Mahmudieh, steppe, 15.VII.1948, coll. P. Allen (HNHM); 1 ♂ Tehran prov., Marounak, 12.VIII.1948, leg. Vaezi (HMIM); 1 ♀ Tehran prov., Pachalak, 29.VI.1948, leg. Mirzayans (HMIM); 1 ♂ Tehran prov., Sohanak, 16.VI.1998, leg. Zareiee & Razi (HMIM); 1 ♂ 1 ♀ Tehran prov., Tehran, 11.VIII.1947, leg. Mirsalavatian (HMIM); 65 exx. Tehran, Golhak (MSNV); 1 ♂ Tehran prov., Varamin, VI.1947, leg. Alexandrov (HMIM); 1 ♂ 1 ♀ Tehran prov., Varamin, IV.1950, leg. Eghlidi (HMIM); 1 ♀ Yazd prov., Abarghou, Aliabad, 25.VI.1972, leg. Safavi (HMIM); 1 ♂ Strauss Persien (HNHM); 1 ♂ Persia, 1862–63, coll. Soloris,. R. Oberthür, Eing. Nr. 4, 1956 (HNHM). **Iraq.** 3 ♂♂ 6 ♀♀ Assur, Mesopotamia, coll. Hauser (HNHM); 1 ♀ Assur, Mesopotamia, V.1908 (HNHM); 1 ♂ Assur, Mesopotamia, Exp. Nat. O.V., 1910, leg. Pietschmann (HNHM); 1 ♂ Mesopotamia, Mosul, V.1909, coll. Hauser (HNHM); 1 ♂ Tekrit, 7.V.1910, Mesopot. Exp. Nat. O.V. (HNHM). **Pakistan.** 1 ex., NWFP, 85 km NE Chitral, Chitral-Mastuj road (VVC). **Turkmenistan.** 1 ♂ coll. Reitter (HNHM); 2 ♀♀ Kopet Dag Mt., 10 km S Aldere, N38 14 E 56 46, 600–1000 m, 27.VI.1992, leg. G. Fabian (HNHM); 1 ♂ Kopet Dagh, Germab, 1894, coll. Hauser (HNHM); 3 ♂♂ 1 ♀ Chuli, 20.VI.1963, leg. Kozolvo (MABC).

Records from literature. Being deeply confused the taxonomy of this species, we prefer consider only confirmed or congruent records from the literature. **Afghanistan.** Afghanistan (Kaszab, 1968 as *javeti*); Darreh-Gazak (Kaszab, 1973); Hérat (Kaszab, 1961 as *javeti* and ab. *heratensis* nov.); Nuristan, Bashgultal, Parigil-Patscheigil (Kaszab, 1958, 1973 both as *javeti umbilicata*); near Kabul (Kaszab, 1958, type locality, 1973 both as *javeti umbilicata*); Kabul West (Kaszab, 1965); Nuristan (Borchman, 1936); E Afghanstan, Neugrahar, Darunta (Kaszab, 1973 as *javeti umbilicata*); Borgh, Logar (Kaszab, 1973 as *javeti umbilicata*); Qal'eh Lakou nr. Kabul valley (Kaszab, 1973 as *javeti umbilicata*); Kouh-Djaouz, nr. Tang-Saldan 20 km W Kaboul (Kaszab, 1973 as *javeti umbilicata*); Khairou Khil, 30 km NW Djelalabad (Kaszab, 1973 as *javeti umbilicata*); Laghman, Samakat (Kaszab, 1981as *javeti umbilicata*); Ghazni, 50 km NW Moqur ((Kaszab, 1981 as *javeti umbilicata*); Nangarhar, Dast-e-Gamberay (Kaszab, 1981 as *javeti umbilicata*); Kabul-Sarobi (Kaszab, 1981); Parigil and Patscheigil (Borchmann, 1936 as *colligata*). **Iran.** 1 Iran (also as Perse) (MSNV; Redtenbacher, 1867; Marseul, 1870, 1872, both also as *javeti*; Soumakov, 1915, 1930 also as *javeti*; Mader, 1927 also as *javeti;* Kuzin, 1954 also as *javeti*; Kaszab,1968 as *javeti*; Fekrat & Modaress Awal, 2012 also as *javeti*; Bologna et al, 2018 as *javeti*; Ghahari & Campos-Soldini, 2019 also as *javeti*); S Iran (Redtenbacher, 1850 type locality); Fars (Koçat & Kemal, 2015a); Bushehr, Fars, Khuzestan, Kohgiluyeh & Boyerahmad (Serri, 2015); Fars, Hormozgan, Kerman, Kermanshah, Kordestan, Khuzestan, Qazvin, Sistan & Baluchestan, Zanjan (Mirzayans, 1970; Modaress Awal, 1997, 2012; Ghahari & Campos-Soldini, 2019, all as *javeti*); Esfahan, W Ná-in (Kaszab, 1968; Mohammad-Momeni et al., 2016; Ghahari & Campos-Soldini, 2019; Ghahari & Campos-Soldini, 2019 as *javeti*); Hamedan, Nahavand Co. (Nikbakhtzadeh & Tirgari, 2002 as *Mylabris schreibersi*); 100 km N Iranshar (Kaszab, 1968 as *javeti*); Khorasan (Fekrat & Modaress Awal, 2012; Ghahari & Campos-Soldini, 2019; also as *javeti*);

Kopet dag (Kuzin, 1954 as *javeti*); Markazi (Faraji et al., 2012; Moslemi et al., 2015 both alsa as *javeti*); Shiraz (Sumakov, 1930); Tehran (Mirzayans 1970; Modaress Awal 1997, 2012; Ezzoddinloo & Mohammadian, 2012 as *javeti*); Balouchestan, Fars, Ghazvin, Hormozgan, Kerman, Kordestan, Zanjan, Khuzestan, Tehran (Mirazyans, 1970; Moraress Awal, 2012; Fekrat & Modaress Awal, 2012 also as *javeti*); Khorasan, Razavi prov., Mashhad (Fekrat & Modaress Awal, 2012 also as *javeti*); S Khorasan Birjand (Fekrat & Modaress Awal, 2012 also as *javeti*); Sistan & Baluchestan (Kaszab, 1968b as *javeti*). **Iraq.** Iraq (Derwesh, 1965 as *javeti*; Fekrat & Modaress Awal, 2012 as *javeti;* Bologna et al., 2018, as *javeti*; Ghahari & Campos-Soldini, 2019 as *javeti*); Assur (Holdhaus, 1920). **Transcaucasia**. Transcaucasia (Faldermann, 1837, 1839 as *superba*). **«Turkestan».** (Dokhtouroff, 1889 as *javeti*; Soumakov, 1915 as *javeti*; Fekrat & Modaress Awal, 2012 as *javeti*; Ghahari & Campos-Soldini, 2019 as *javeti*). **Turkmenistan**. Turkmenia (Soumakov, 1915 as *javeti*; Mader, 1927 as *javeti;* Fekrat & Modaress Awal, 2012 as *javeti*; Bologna et al, 2018 as *javeti*; Ghahari & Campos-Soldini, 2019 as *javeti*); Transcaspia (Mader, 1927 as *javeti;* Kaszab, 1968 as *javeti*); Askhabad (Reitter, 1889; Soumakov, 1930 as *javeti*); Kopet Dag (Kuzin, 1954 as *javeti*) ; Askhabad and Tekke (Dokhtouroff, 1889 as *javeti*); Samaná Syr (Heyden, 1883 as *javeti*); Nokhur (Reichardt, 1934 as *javeti*); Arman saad-Kizil arvat (Reichardt, 1934 as *javeti*); Gaudan (Reichardt, 1934 as *javeti*); Kaaşka (Reichardt, 1934 as *javeti*); Firyuza (Reichardt, 1934 as *javeti*); Tejen (Reichardt, 1934 as *javeti*).

Description. Body length 7–24 mm. Body black, but elytra yellowish or reddish brown, sometimes dark red, with black pattern. Head subquadrate; antennomere III less than 1/4 time longer than IV, V–VII transverse and widest, antennomeres VII–IX progressively narrowed, male XI (Fig. 55) elongated apically, spindle shaped; in female last antennomere short (Fig. 56). Pronotum (Fig. 17) as long as wide or slightly longer, with anterior and middle depression, a middle longitudinal furrow, distinct punctures; mesosternun as in Fig. 61; each elytron with two black spot on anterior third, one wide middle fascia, and a black fascia on apical part (Figs 62). Last male ventrite convex, except a very narrow depression in the middle, posterior margin deeply emarginated in the middle; aedeagus (Figs 59–60) with endophallic hooks slightly narrow, distal aedeagal hook small and curved, very close to proximal one and both almost at apex, proximal hook short and nearly straight; gonoforceps (Figs 57–58) narrow and straight in noth ventral and lateral view.

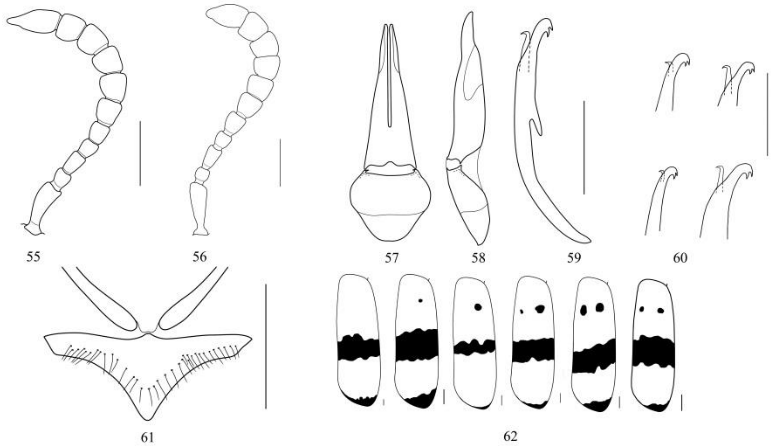


Fig. 55–62. *Hycleus colligatus* (Redtenbacher): 55. Male antenna; 56. female antenna; 57. tegmen, ventral view; 58. tegmen, lateral view; 59. aedeagus, lateral view; 60. variations of aedeagus; 61. mesosternum; 62. elytral pattern. Scale bar = 1 mm.

Taxonomic remarks. As explained in the taxonomic remarks of *H. cingulatus*, after checking the syntypes of both *Mylabris colligata*” and *Mylabris javeti* (MNHN, NHMW) we concluded that these species are synonyms and *colligata* has the priority of name.

We propose the following taxonomic act:

Mylabris javeti Marseul, 1879 = *Hycleus colligatus* (Redtenbacher, 1850) **new synonymy**.

Kaszab (1958) described *Mylabris javeti umbilicatus* as a subspecies endemic to Afghanistan, and the variety *subcordata*. This subspecies differs from the nominate form mostly by its dense and robust puctation. Checking some hundred specimens of this species from the whole range, we found this variation also among some populations of Iran, sympathric with the nominate form, so we consider this subspecies only as a variation without taxonomic value. Apart of the pronotal punctures, the shape of the last antennomere of males and aedeagal hooks show intrapopulation variations (Fig. 62). The molecular study of this complex confirms this huge varation.

We propose the following taxonomic act:

Mylabris javeti umbilicatus Kaszab, 1958 =*Hycleus colligatus* Redentbacher, 1850 **new synonymy**.

Distribution. This species is largely spread in Iran and in northern Afghanistan, and marginally distributed in the Transcaucasian area, Iraq, Northwest Pakistan and Turkmenistan.

Hycleus hayki Serri & Bologna n. sp.

Figs 6, 18, 63–69

Hycleus pirata, Serri, 2015: 235, nec Bologna & Turco, 2007

Type material. Holotype ♂ (HMIM) " [Iran] Dogonbadan/ 15.V.68/ [leg.] Zairi, Ayat". Paratypes: 4 ♂♂ 2 ♀♀ (HMIM) with the same label of holotype; 3 ♂♂ 1♀(HMIM) "Dogonbadan/ 11.V.68 [leg.] Ayat., Zairi"; 3 ♂♂ 2 ♀♀(1 ♂ MABC; HMIM) "Dogonbadan/ 9.V.67/ [leg.] Zairi, Ayat‘; 1 ♂ (HMIM): Behbahan/ 1.V.69/[leg.] Zairi, Ayat“; 1 ♂ (HMIM): Behbahan/ 3.VI.69/ [leg.] Zairi, Ayat".

Records from literature. Iran. Khuzestan, Kuhgiloyeh & Boyerahmad (Serri, 2015 as *pirata*; Ghahari & Campos-Soldini, 2019 as *pirata*).

Description. Body black, elytra testaceous with black pattern. setation black, short, sparser shorter and obliquely recumbent on elytra. Golden dense pubescence on the ventral side of fore tibiae and tarsi of male, only on tibiae of female. Body length 8–14 mm. Head subquadrate (Fig. 18); with the maximum width at the level of eye; punctures relatively wide, deep and irregular, surface among punctures relatively shiny; eye globose, with the antero-dorsal margin slightly sinuate, just behind the antennal insertion. Temples parallel, only slightly curved posteriad and shorter than the longitudinal diameter of eye; frons almost flat; clypeus transverse, convex, with slightly rounded anterior and lateral margins, fronto-clypeal suture clearly visible; labrum only very slightly shorter than clypeus, anterior margin slightly sinuate, weakly depressed in middle; mandibles robust, curved, longer than clypeus and labrum together; maxillary palpomeres subcylindrical, IV flattened; antennae (male Fig. 63, female Fig. 64) not extending to posterior margin of pronotum, antennomere III more than 1/3 time longer than IV, IV–VII progressively longer and widened, VIII as long as IX, IX very slightly narrower than VIII, X narrower than IX, IX widest before middle and narrowed to apex, with a transverse depressed line right at the middle of the curved part; in female last antennomere short. Pronotum

(Fig. 18) convex, very slightly longer than wide, bulging on sides and slightly narrowing anteriad, fore portion without depression, posterior margin slightly depressed just in front of mesonotum; with a middle longitudinal furrow, punctation deep, interstices shiny, mesosternun as in Fig. 53. Elytral pattern as in Fig. 69, each elytron with two black spot on anterior third, middle fascia with sinuate posterior margin, and a wide black fascia on apical part with straight fore margin. Last male ventrite convex, posterior margin with a deep middle emargination; aedeagus (Fig. 67) with endophallic hooks slightly narrow, distal aedeagal hook scarcely curved, far from proximal one and almost at apex; gonoforceps (Figs 65–66) in ventral view regularly narrowed, in lateral view sinuate in the last third but not curved.

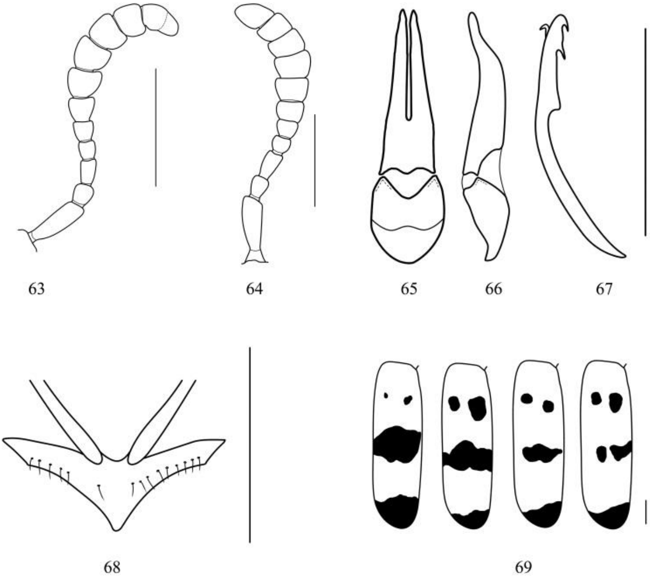


Fig. 63–69. *Hycleus hayki* Serri & Bologna: 63. Male antenna; 64. female antenna; 65. tegmen, ventral view; 66.tegmen, lateral view; 67. aedeagus, lateral view; 68. mesosternum; 69. elytral pattern. Scale bar = 1 mm.

Taxonomic remarks. This new species is very similar to *H. sexmaculatus* and somehow to *H. pirata*, but can be distinguished by the shape of last antennomere of male which has a transverse depressed line at the middle of the curved part, by the shape of the pronotum which is slightly longer than wide, by the elytral pattern which middle fascia is wide and has always sinuate hindmargin, and by the shape of male genitalia. We didn’t succeed in finding fresh material of this species for DNA extraction, but we hope future molecular studies may reveal the phylogenetic relationships among these closely related species.

Distribution. *Hycleus hayki* sp. n. is hitherto known from southwestern Iran.

Etymology. The species is named after Hayk Mirzayans, orthopterist and founder of Hayk Mirzayans Insect Museum (HMIM), the largest insect collection of Iran. He was the first who sorted the blister beetles of the HMIM coleoptera collection and published a contribution to the knowledge of Meloidae of Iran.

Hycleus lindbergi (Kaszab, 1973)

Figs 7, 19, 70–76

Mylabris lindbergi Kaszab, 1973: 294.

Type material. 1 ♂ 1 ♀ paratypes “Afghanistan / K. Lindberg//Herat, Gozar Gah / No. A 1130/ 8.6.1962// Paratypus 1972 ♂/ *Mylabris / lindbergi* Kaszab”; 1 ♂ paratype “Afghanistan/ No. 925, 19.VI.1960/ Dr. K. Lindberg//Orozgan// Paratypus 1972/ *Mylabris/ lindbergi* Kaszab”; 3 ♀♀ paratypes “Afghanistan/ No. 754, 1959 / Dr. K. Lindberg// Herat, 920 m/ Hotel Parc, 19.VI// Paratypus 1972/ *Mylabris/ lindbergi* Kaszab” (HNHM).

Records from literature. Afghanistan. Hérat (Kaszab, 1973); Koushk, N Hérat (Kaszab, 1973); N Kandahar (Kaszab, 1981); NO Kandahar, Baghtu (Kaszab, 1981).

Description. Body length 13.5–16.5 mm. Body black, but elytra yellowish brown, with black pattern. Head subquadrate; antennomere III less than 1/3 time longer than IV, IV–VII progressively longer and widened, VIII slightly narrower than VII, XI wider, with parallel sides next to middle and narrowes apex; in female last antennomere short. Pronotum (Fig. 19) longer than wide, distinctly strangulate on fore third, depressed on fore sides, with fine punctures; with a middle longitudinal furrow; mesosternun as in Fig.

75. Each elytron with two black spots on anterior third, shortened middle fascia, and a narrow black fascia on apical part (Fig. 76). Last male ventrite narrowly concave and depressed, posterior margin with a deep middle emargination; aedeagus (Fig. 74) with endophallic hooks slightly narrow, distal aedeagal hook sligthly curved, far from proximal one and almost at apex, both pointed and similarly inclined; gonoforceps (Figs 72–73) quite robust and straight in both ventral and quite wide in lateral view.

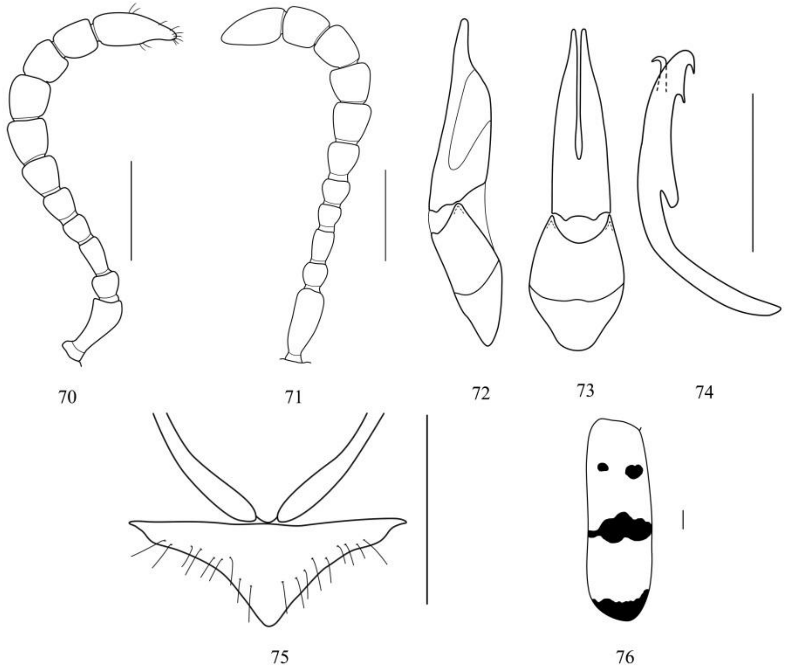


Fig. 70–76. *Hycleus lindbergi* (Kaszab): 70. Male antenna; 71. female antenna; 72. tegmen, ventral view; 73. tegmen, lateral view; 74. aedeagus, lateral view; 75. mesosternum; 76. elytral pattern. Scale bar = 1 mm.

Taxonomic remarks. *Hycleus lindbergi* seems to be very close to *H. cingulatus*, especially in the shape of pronotum and aedeagus, but it can be easily identified by the shape of last male antennomere which has not the angulate pre-apical portion.

Among the studied material there are specimens from Kandahar, Afghanistan which have been identified by Kaszab as *lindbergi* and have the same elytral pattern and pronotal shape, but the last male antennomere has the shape of *cingulatus* and must be reffered to this species. Unfortunately, we didn’t have access to fresh material for molecular study and here we consider it as a distinct species because of the differences in male antennomeres.

Distribution. This species is endemic to Afghanistan collected only from two discrete localities in the eastern and central parts of this country (Kaszab, 1973).

Hycleus pirata Bologna & Turco, 2007

Figs 8, 20, 77–83

Hycleus pirata Bologna & Turco, 2007: 16

Type material. Holotype ♂ and 2 ♀♀ paratypes:” United Arab Emirates: Abu Dhabi Emirate, EA4, Al /Bateen dunes, 4 Km W Al Ain on the road to Abu Dhabi, 195 m a.s.l. / 24,20491° N 55,61263° E, 15.III.2005, leg. M. Bologna & F. Turco (MABC)”; 4 ♂♂ 4 ♀♀ paratypes (MABC), 1 ♂ paratype (HMIM): “United Arab Emirate, Abu Dhabi Emirate, EA10, Al Aïn, 3–4 Km E/ Al Selimat on road Al Aïn-Abu Dhabi, 24,19263° N 55,58861° E, 220 m, 12–14.III.2005, leg. M. Bologna & F. Turco”.
Additional material examined. 1 ♀ Oman, Wadi Air, 7.V.1957, leg. Popov & Wood (MABC).
Records from literature. Oman. Oman (Koçat & Kemal 2015a). **UAE.** UAE (Gillet & Gillet, 2005 as *bipunctata;* Koçat & Kemal 2015a; Ghahari & Campos-Soldini, 2019); Abu Dhabi Emirate, EA4, Al Bateen dunes, 4 Km W Al Aïn on the road to Abu Dhabi, 24,20491° N 55,61263° E (Bologna & Turco, 2007, type locality); Abu Dhabi Emirate, EA10, Al Aïn, 3–4 Km E Al Selimat on road Al Aïn-Abu Dhabi, 24,19263° N 55,58861° E (Bologna & Turco, 2007); Jebel Hatif (Gillet & Howarth, 2004 as *bipunctata*); Al Aïn area (Gillet & Gillet, 1996 as *bipunctata*); Wadi Bih; Wadi Shawkah (Batelka & Geistardt, 2009 as *bipunctatus*).
Description. Body length 12–16.5 mm. Body black, but elytra yellowish bright brown, sometimes red, with black pattern. Head subquadrate; male antennomere (Fig. 77) III less than 1/2 time longer than IV, V–VIII

transverse and widened, IX–X narrower than VIII, male XI narrower and longer than X; in female (Fig. 78) shorter than X. Pronotum (Fig. 20) longer than wide, with anterior depression; mesosternun as in Fig. 82.Each elytron with two black spot on anterior third, one sinuate middle fascia, and a black oblique fascia on apical part (Fig. 83). Male last ventrite regularly concave and depressed, posterior margin with a wide and deep middle emargination; aedeagus (Fig. 81) with endophallic hook narrow, distal aedeagal hook curved, far from proximal one and close to apex, proximal hook slightly curved; gonoforceps (Figs 79–80) narrow and elongate both in ventral and lateral view (see Bologna & Turco 2007 for a detailed description).

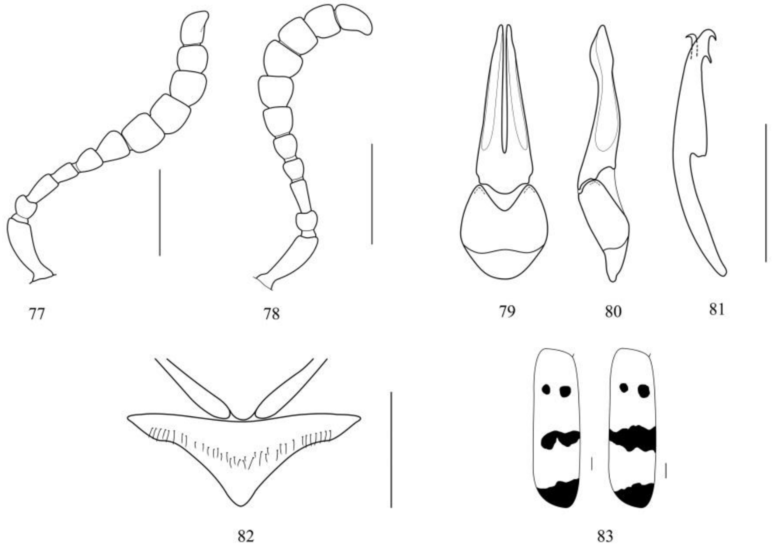


Fig. 77–83. *Hycleus pirata* Bologna & Turco: 77. Male antenna; 78. female antenna; 79. tegmen, ventral view; 80. tegmen, lateral view; 81. aedeagus, lateral view; 82. mesosternum; 83. elytral pattern. Scale bar = 1 mm.

Taxonomic remarks. This species is closely related to *H. apicipennis* but it can be distinguished by the elongate shape of antennomere IV, which is subequal in length to V, the shape of last male antennomere, which is strikingly curved, and by the male genitalia. The shape of last antennomere is very similar also to that of the new species *H. hayki* and of *H.*

sexmaculatus, but *H. pirata* differs because of the pronotum and aedeagus shape.

Distribution. This species seems to have restricted distribution in the United Arab Emirates and Oman (Bologna & Turco, 2007). The record of this species from south Iran (Serri, 2015) actually refers to the new species *H. hayki*.

***Hycleus schauffelei* (Kaszab, 1957)**
Figs 9, 21, 84–90

Mylabris schauffelei Kaszab, 1957: 58
Mylabris schauffelei ab. anticebiconjuncta Kaszab, 1957: 58
Mylabris schauffelei ab. bisbisconjuncta Kaszab, 1957: 58
Mylabris schauffelei ab. postmediojuncta Kaszab, 1957: 58
Mylabris husseini Sumakov1915, 1930, Kuzin, 1954 and Axentiev, 1985, nec Redtenbacher, 1850

Type material. 1 ♂ 1 ♀ paratypes, ”Iran, Beluchestan/ Iranshahr, 800 m/ 1–10.III.1954, Richeter u. Schäuffele”// Paratypus *Mylabris*/ *schauffelei*/ det. Dr. Kaszab, 1956“ (HNHM); 1 ♂ paratype,”Iran, Beluchestan/ nordwestl. Iranshahr/ Trockental/ 1.III.1954, Richter u. Schäuffele“ (HNHM); 1 ♀ paratype, “Iran, Beluchestan/ nordwestl. Iranshahr/ Trockental/ 1.III.1954, Richter u. Schäuffele” (HNHM); 1 ♀ paratype, “Iran, Beluchestan/ nordwestl. Iranshahr/ Trockental/ 9.IV.1954/ Richter u. Schäuffele” (HNHM); 2 ♀ ♀ paratypes, “Iran, Beluchestan/ nordwestl. Iranshahr/ Trockental, 4.IV.1954/ Richter u. Schäuffele” (HNHM); 1 ♀ Paratype, “Iran (Makran)/ am Kahuran bei/ Putab, 25.III.1954/ Richter u. Schäuffele//Paratypus *Mylabris* /*schaiffelei* m./ ab. *anticotriconjuncta*” (HNHM).

Additional material examined. **Iran.** 1 ♂ Iran, 27.III.1950, Ost. Iran-Exped, Pollei, Dschaz Morian (HNHM); 1 ex. Iran (MNHN); 6 ♀♀ 62 km 300 S Iranshahr, 14.IV.1965, Museum Paris, Mission Franco Iranienne, 1965 (HNHM); 2 ♀♀ 80 km Sud Jiroft, 2.IV.1965, Museum Paris, Mission Franco Iranienne, 1965 (HNHM); 1 ♀, Shah Bahar, 30 km N, 9.IV.1965, Museum Paris, Mission Franco Iranienne, 1965 (HNHM); 4 ♂♂ 1 ♀ Fars, Izad Khast, N 31 31 E 52 08, 12.VI.1974, leg. Senglet (HNHM); 1 ♂ 1 ♀

SE Iran, Sands, 13–17 km N Espakeh, 11.IV.1973, Loc. No. 156, Exp. Nat. Mus. Praha (NMPC); 2 ♂♂ SE Iran, 44 km NE Zaboli, 30–31.III.1973, Loc. No. 141, Exp. Nat. Mus. Praha (NMPC); 1 ♂ Sistan & Baluchestan prov., Gegan env. 640 m, N 28 23 E 59 26, 8–9.IV.2000, Iran 2000 Czech Biological Expedition, leg. Hajek & Mikat (NMPC); 1 ♂ 1 ♀ Fars prov., Fasa, Mianjangal, 12.V.1966, leg. Gharib (HMIM); 1 ♂ 1 ♀ [Ghom prov.]: Teheran dint. Qom, 5.V.1976, leg. Brignoli (MABC); 1 ♀ Hormozgan prov., Bandarabbas, 20.III.1951, leg. Fassihi (HMIM); 1 ♀ Hormozgan prov., Minab, 15.IV.1949, leg. Sarkissian (HMIM); 1 ♂ 4 ♀♀Hormozgan prov., Minab- Jiroft, 2.IV.1965, leg. Safavi (HMIM); 1 ♂ Hormozgan prov., Minab- Jiroft, 14.III.1971, leg. Termeh & Iranshahr (HMIM); 2 ♂♂ 1 ♀ Kerman prov., Shahdad, 18.III.1949, leg. Sarkissian (HMIM); 1 ♀ Kerman prov., 7.VI.1951, leg. Mirsalavatian (HMIM); 1 ♂ 1 ♀ North Khorasan prov., Bojnourd, VI.1964, leg. Kheiri (HMIM); 1 ♂ (HMIM) 1 ♂ (HNHM): Semnan prov., Mayamay, 8.VII.1972, leg. Iranshahr; 1 ♂ 4 ♀♀ Sistan & Baluchestan prov., Bampour, 3.IV.1950, leg. Sarkissian (HMIM); 22 ♂♂ 18 ♀♀ Sistan & Baluchestan prov., Bampour, Bazman, 12–15.IV.1973, leg. Safavi & Broumand (HMIM); 1 ♂ 5 ♀♀ Sistan & Baluchestan prov., Bampour, 580m, 19.IV.1968, leg. Hashemi & Pazuki (HMIM); 1 ♂ 1 ♀ Sistan & Baluchestan prov., Bampour, 11.IV.1973, leg. Safavi & Broumand (HMIM); 6 ♂♂ 10 ♀♀ Sistan & Baluchestan prov., Iranshahr, 20,23,24,26.III.1950, leg. Sarkissian (HMIM); 1 ♂ 4 ♀♀ Sistan & Baluchestan prov., Iranshahr, 1/7.IV.1950, leg. Sarkissian (HMIM); 1 ♂ 6 ♀♀ (HMIM) 1♂(MABC): Sistan & Baluchestan prov., Iranshahr, 30.III.1951, leg. Sarkissian; 5 ♂♂ 23 ♀♀ Sistan & Baluchestan prov., Iranshahr, 14.IV.1965, leg. Safavi (HMIM); 1 ♀ Sistan & Baluchestan prov., Iranshahr, 21.IV.1968, leg. Hashemi & Pazuki (HMIM); 2 ♂♂ 3 ♀♀ Sistan & Baluchestan prov., Iranshahr, 25/26.IV.1969, leg. Pazuki & Hashemi (HMIM); 1 ♂ Sistan & Baluchestan prov., Iranshahr, Hamont kouh, 1700 m, 20.IV.1968, leg. Hashemi & Pazuki (HMIM); 1 ♂ 1 ♀ Sistan & Baluchestan prov., Karvandar, Alidar, 30.IV.1968, leg. Hashemi & Pazuki (HMIM); 1 ♀ (HMIM) 1 ♂ (HNHM): Sistan & Baluchestan prov., Khash, 16.IV.1965, leg. Safavi; 1 ♀ Sistan & Baluchestan prov., Khash, 13.V.1972, leg. Abai (HMIM); 1 ♀ Sistan & Baluchestan prov., Nikshahr, 13.IV.1965, leg. Safavi (HMIM); 1 ♀ Sistan & Baluchestan prov., Nikshahr, 8/16.IV.1973, Broumand & Safavi (HMIM); 1 ♀ Sistan & Baluchestan prov., Nikshahr, 13.IV.1965, leg. Safavi (HMIM); 15 ♂♂ 11 ♀♀ Sistan & Baluchestan prov., Nikshahr, Spake, 4/12/13.IV.1965, leg. Safavi (HMIM); 2 ♂ ♀♀ (HMIM) 1 ♂ (HNHM) Sistan & Baluchestan prov., Nikshahr, 29.IV.1968, leg. leg. Hashemi & Pazuki; 17 ♂♂ 16 ♀♀ (HMIM) 1 ♂ (HNHM): Sistan & Baluchestan prov., Jalgh, 16.IV.1950, leg. Mirsalavatian; 8 ♂♂ 8 ♀♀ Sistan & Baluchestan prov., Jalgh, Sinoukan, 15.IV.1950, leg. Mirsalavatian (HMIM); 1 ♂ Sistan & Baluchestan prov., Sarhe, Nikshahr, 23.III.1949, leg. Sharif (HMIM); 10 ♂ ♂ 16 ♀♀ Sistan & Baluchestan prov., Saravan, 13,21.V.1950, leg. Mirsalavatian (HMIM); 1 ♀ Sistan & Baluchestan prov., Saravan, Nahok, 12.IV.1950, leg. Mirsalavatian (HMIM); 1 ♀ Sistan & Baluchestan prov., Sarbaz, 24.IV.1950, leg. Mirsalavatian (HMIM); 1 ♀ Sistan & Baluchestan prov., Rask, 24.IV.1968, leg. Pazuki & Hashemi (HMIM); 1 ♀ Sistan & Baluchestan prov., Sarbaz-Rask, 24.IV.1968, leg. Hashemi & Pazuki (HMIM); 3 ♀♀Sistan & Baluchestan prov., Zabol, 25.V.1950, leg. Mirzayans (HMIM); 37 ♂♂ 50 ♀♀ Yazd prov., Abarghou, Aliabad, 25.VI.1972, leg. Safavi (HMIM). 4 ♂♂ 3 ♀♀ SE Iran, Rask, vall r. Sarbaz, 3–4.IV.1973, Loc. No.146, Exp. Nat. Mus. Praha (NMPC). **Uzbekistan:** 1 ♂ 1 ♀Buchara (HNHM).

Records from literature. Iran. Iran (Soumakov, 1915 as *husseini*; Kuzin, 1954 as *husseini*; Kaszab, 1968; Axentjev, 1985 as *husseini*); Bologna, 2008; Bologna et al. 2018); 80 km SE Tehran, 5 km E Aradan (Kaszab, 1965); NW Iranshar, Trockental (Kaszab, 1957); Iranshar (Kaszab, 1957, 1968; Ghahari & Campos-Soldini, 2019); SE Iranshar, Hamant Kuh (Kaszab, 1957); Makran, Am Kahuran bei Putab (Kaszab, 1957); Sangun, E Kuh i Taitan (Kaszab, 1957); Makran, SE Nahu (Kaszab, 1957); Khash (Kaszab, 1968); Shiraz (Soumakov, 1930 as *husseini*); 62 km S Iranshar (Kaszab, 1968); 80 km S Jiroft (Kaszab, 1968); 50 km N Iranshar (Kaszab, 1968); 100 km N Iranshar (Kaszab, 1968); 30 km N Shah-Bahar (Kaszab, 1968); Minab (Kaszab, 1968); 80 km SE Tehran, 5 km E Aradan (Kaszab, 1965); Fars, Khorasan, Khuzestan, Tehran (Modaress Awal 1997, 2012; Ghahari & Campos-Soldini, 2019); Hormozgan, Kerman (Kaszab, 1968; Modaress Awal, 1997, 2012; Ghahari & Campos-Soldini, 2019); Sistan & Baluchestan (Kaszab, 1957; Modaress Awal,1997, 2012; Ghahari & Campos-Soldini, 2019); Balouchestan, Iranshahr, hamant-kouh, Spakeh, Neichhahr, Tange-sasheh,Bampour, Khach, Alifdaer Karvandar, Tchahbahar, Rsk, Saravan, Djalgh,; Abassi, Minab, Sarkhoum, Bandar-abbass; Kermn, Chhdad, Djiroft; Sistan, zabol; Khouzestan, behbahan, Dogonbadan; Fars, Mandjangal (Mirzayans, 1970). Soumakov (1930) and Kuzin (1954) (see below) cited *husseini* from Egypt and Arabia (see Axentjev, 1985), but probably these records refer to other Mylabrini species.

Description. Body length 8.8–19 mm. Body black, but elytra yellowish red, with black pattern. Head subquadrate; antennomere III twice as long as wide, slightly longer than IV, V-VIII transverse and widened, IX-X narrower than VIII, male (Fig. 84) XI very elongated and 2 times longer than X; female XI shorter than X (Fig. 85). Pronotum (Fig. 21) as long as wide or only slightly longer than wide, with anterior depression; mesosternun as in Fig. 88. Each elytron with two black spots on anterior third, one sinuate middle fascia which sometimes shortened, and a black fascia on apical part (Fig. 90). Last male ventrite very convex and posterior margin with a shallow middle emargination; aedeagus (Fig. 88) with endophallic hooks narrow, distal aedeagal hook only very slightly curved, far from proximal one and close to apex, proximal hook as long as distal one and equally inclined; gonoforceps (Figs 86–87) very slender and progressively narrowed from middle in both lateral and ventral view.

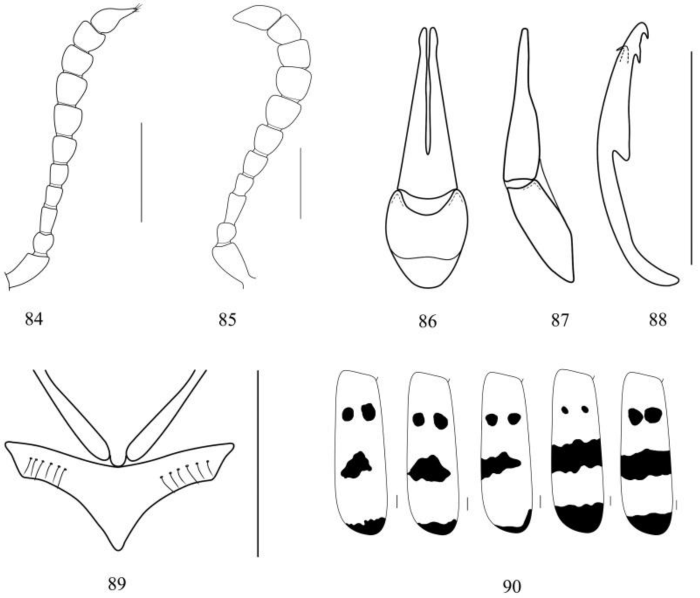


Fig. 84–90. *Hycleus schauffelei* (Kaszab): 84. Male antenna; 85. female antenna; 86. tegmen, ventral view; 87.tegmen, lateral view; 88. aedeagus, lateral view; 89. mesosternum; 90. elytral pattern. Scale bar = 1 mm.

Taxonomic remarks. *Hycleus schauffelei* has a great variability in the elytral pattern, punctuations, pronotum size and depressions, and also the shape and size of male XI antennomere. We studied a large number of specimens and noticed that the variations clearly exist among each population.

Axentjev (1985) erroneously synonymised *Mylabris schauffelei* Kaszab, 1957 with *Mylabris husseini* Redtenbacher, 1850, which was referred to *Sphenabris* by this Author. Axentjev did not examine types of *husseini* but only specimens identified by Soumakov and Kuzin which are housed at the St. Pietersburg Museum. We examined types of *M. husseini* (NHMW) and found out that they refer to *Mylabris (Mylabris) quadripunctata* (Linnaeus, 1767) (Pan & Bologna, 2014). Consequently, the synonymy proposed by Axentjev (1985) is erroneous and the old citations under the name *husseini* must be reffered to *H. schauffelei*. One specimen identified in the Marseul's collection (MNHN) as *M. husseini* also belongs to *H. schauffelei.*

Distribution. This species is endemic to Iran (Bologna, 2008). The single record from Bukhara (Uzbekistan), not so far from those from North Khorasan (Iran), could evidence a wider distribution in central Asia.

***Hycleus sexmaculatus* (Olivier, 1811)**
Figs 10, 22, 91–97

Mylabris sexamaculata Olivier, 1811: 98.
Mylabris superba Faldermann, 1837: 123
Mylabris bimaculata Klug, 1845: 32.
Mylabris ledereri Marseul, 1870: 57.
Zonabris ledereri var. *onerata* Escherich, 1899: 109.

Type material. The type of this species is lost, but we examined at MNHN specimens in the Marseul's collection from the XIX century, which contain samples probably compared with the Olivier's types.

Holotype and 4 possible syntyes of *Mylabris bimaculata* Klug, 1845, housed at ZMHB, which was always considered as a distinct species of the same group, were studied by one of us (MAB) and synonymized with *H. sexmaculatus* (Bologna & Turco, 2007). One female (with left antennae broken) along with four other specimens (some without antennae, one without head and pronotum), probably Syntypes (two from Arabia and two from Syria), have the following labels: “*bimaculata* N. Arab. Ol.” [yellow, original, handwritten], “28658” [printed]; “Hist. Coll. Nr. 28658” [printed]; Zool. Mus. Berl.” [printed]

Additional material examined. **Armenia**. 4 exx. Armenia (MRSN). **Azerbaijan.** 1 ex. Elisabetpol (SMNS). **Caucasus.** Caucasus, 2 exx. (MRSN), 2 exx. (MCZR), 10 exx. (MNHN). **Iran.** 1 ♂ 1 ♀ NW Iran, 26 km, SSE Khoy, 6.VII.1973, Loc. No. 267, Exp. Nat. Mus. Praha (NMPC); 1 ♀ NW Iran, 25 km, SE Shahpus, 27–28.VII.1977, Loc. No. 406, Exp. Nat. Mus. Praha (NMPC); 2 ♂ East Azarbaijan prov., Tabriz, VII.1959, leg. Manuchehri (HMIM); 2 ♂♂ East Azarbaijan prov., Tabriz, VII.1959, leg. Goudarzi (HMIM); 5 ♂♂ 1 ♀ Hamedan prov., E Malayer, Zangeneh, Lashgardar protected region, Golparabad area, up to the end of the road, nr. Department of environment station, N 34 12 25.9 E 49 00 57.5, 2404 m, 6.VII.2017, leg. Serri (HMIM); 2 ♂♂ 1 ♀ Kordestan prov., 23 km NE Baneh, Saghez-Baneh, 1750 m, 2.VII.1975, leg. Pazuki (HMIM); 1 ♂ Kordestan prov., Marivan, Dare Toofi, 1370 m, 21.VIII.2011, leg. Mofidi & Ebrahimi (HMIM); 1 ♂ Semnan prov.: Tape Gerayeli, Dasht-e Danial, 10.VI.1957, leg. Mirzayans (HMIM); 1 ex. Perse (MNHN). **Israel-Palestine**. 1 ex. Arad-Mazada rd. (MUH); 2 exx. Jericho (MSNV); 12 exx. Jericho (MNHN); 1 ex. Maale Hdanmin (Haadnmin) (MUH); 1 ex. H. Fasayil, Lower Jordan valley (MUH); 1 ex. Mash’abbe Sade (MUH). **Russia**. 1 ex. Crimea (MSRSN). **Syria:** 1 ♂ 1 ♀ Akbes, coll Reitter (HNHM); 2 ♂♂ 1 ♀ Akbès, summer 1890, leg. Delagrange, Slg. R. Oberthür, coll. E. Martin (HNHM); 1 ex. Syrie (MNHN). **Turkey.** 2 exx. Asia Minor (MRSN); 1 ex Adana (BMNH); 1 ex. Adana, Aladağg Büyüksofulu Köyü 12.VII (COZ); 1 ex. Adana, Gülek; 2 exx. Adana, Osmanye, *Pinus* forest; 2 ♂♂ 3 ♀♀Turkey, Adiyaman prov., 21 Gölbazi dint., 900 m, 14.6.1986, leg. Bologna (MABC, HMIM); 4exx Akşhehir (SMNS); 1 ♂ 1 ♀ SE Anatolia, Osmanye (*Pinus* Forest), VII.1976, leg. A. Casale (MABC); 1 ex. Antakya (ZSM); 1 ♂ Antalia, 7.VI.1969 (MABC); 1 ex. Antalya (ZSM); 1 ex. Beylik Köprü (ZMHB); 1ex. Elazig (BMNH); 2 exx. Kayseri (ZMJB); 8 exx. Malatya (MCNV); 1 ex. Malatya, Eskimalatya (MABC); 1♂ 1 ♀ Malatya, 23.VI.1965, leg. Pierotti & Perissinotto (MABC); 1 ♀Turkey, Mersin prov., 10 Mut, ca 400 m, 8.6.1980, leg. Bologna (MABC); 1 ♂ SE Turkey, Gülek (Adana), 8.VIII.1976, leg. Casale (MABC); 1 ♀ Gaziantep, 16.07.1984 (MABC); 1 ♀ Bitlis prov., Tatvan Benckli köyu, 5.VIII.2000, leg. Crucitti, Vignoli & Facheris (MABC); 1 ♀

Tauro, 1300 m, 12.VIII.1966, leg. Sbordoni (MABC); 1 ♀ Elazig prov., Gözeli, 24.VI.1968, leg. Sbordoni (MABC); 3 ♂♂ 3 ♀♀Turkey, Van, 1700 m, 5.VIII.67, leg.G. Gardini (MABC, HMIM).

Records from literature. Armenia. Armenia (Baudi, 1878a as *ledereri*; Baudi, 1878b as *superba* and *ledereri*; Ghahari & Campos-Soldini, 2019). **Azerbaijan.** Azerbaijan (Ghahari & Campos-Soldini, 2019). **Central Asia.** Central Asia (Bologna, 1979); Turkestan (Dokhtouroff, 1889 as *ledereri*; Soumakov, 1930; Ghahari & Campos-Soldini, 2019). **Caucasus**. Caucasus (Marseul, 1870, 1872, both also as *ledereri*; Soumakov, 1915, 1930 also as *bimaculata*; Mader, 1927; Kuzin, 1954; Bologna, 1979; Chikatunov, 1999, 2003; Bologna & Turco, 2007; Koçat & Kemal, 2015a). **Egypt.** Sinai (Chikatunov, 2003). **Georgia**. Georgia (Ghahari & Campos-Soldini, 2019). **Iran**. (Marseul, 1870, 1872, both also as *bimaculata*; Soumakov, 1915, 1930 also as *bimaculata*; Mader, 1927 as *bimaculata*; Ghahari & Campos-Soldini, 2019); Iran (Bologna, 1979; Chikatunov, 1999 also as *bimaculata*, 2003; Bologna & Turco, 2007; Koçat & Kemal, 2015a); East Azarbaijan, Khorasan, Khuzestan (Mirzayans, 1970; Modarres Awal, 1997, 2012); Khorasan (Farahbakhsh, 1961); Khorassan, Chahroud, Dacht-danial; Azarbaidjan, Tabriz; Khouzestan, Dagionbadan (Mirzayans, 1970). **Iraq**. Assur; Badija (Holdhaus, 1920 also as *bimaculata*). **Israel-Palestine**. Syro-Palaestinian region (Bologna, 1988 also as *bimaculata*); Golan Heights, Central Coastal plain, Southern Coastal plain, Judean desert, valley of Yizre'd, Dead Sea area, northern Negev, central Negev (Chikatunov, 1999 also as *bimaculata*, 2003); Israel-Palestine (Ghahari & Campos-Soldini, 2019); Levant (Bologna & Turco, 2007). **Jordan.** Jordan (Ghahari & Campos-Soldini, 2019). **Kazakhstan.** Kazakhstan (Bologna & Turco, 2007; Ghahari & Campos-Soldini, 2019). **Saudi Arabia.** Arabia (Olivier, 1811; Holotype of *Mylabris bimaculata* Klug ZMHB; Baudi, 1878a as *superba*; Baudi, 1878b also as *superba*; Bologna, 1979, doubtful record, which could refer to some areas of the northern Arabian Desert in Jordan or Iraq); Saudi Arabia (Ghahari & Campos-Soldini, 2019). **Syria.** (Baudi, 1878a as *ledereri* and *bimaculata*; Baudi, 1878b also as *ledereri*; Marseul, 1870, 1872, both also as *ledereri* and *bimaculata*; Mader, 1927; Kuzin, 1954; Chikatunov, 1999; Koçat & Kemal, 2015a; Ghahari & Campos-Soldini, 2019) Soumakov, 1915 also as *bimaculata*; Bologna, 1979). **Russia**. Southern Russia (Olivier, 1811, type loc.; Bologna, 1979; Bologna & Turco, 2007; Koçat & Kemal, 2015a; Ghahari & Campos-Soldini, 2019); Crimea (Baudi, 1878a, 1878b); Cherson (Soumakov, 1930); Dagestan (Kuzin, 1954).

Turkey. Turkey (Bologna, 1979; Chikatunov, 1999 also as *bimaculata*; Koçak & Kemal, 2009 also as *bmaculata*; Koçat & Kemal, 2010; 2015a); Anatolia (Baudi, 1878a as *bimaculata*; Bologna & Turco, 2007); Asia Minor (Marseul, 1870, 1872, both also as *ledereri*; Baudi, 1878a as *superba*; Baudi, 1878b also as *superba*; Soumakov, 1915, 1930 also as *bimaculata*; Mader, 1927; Kuzin, 1954); Kisilgye-Aolle (Marseul, 1870, 1872, both as *ledereri*); Malatya prov. ((Koçak & Kemal, 2015); Elazig, Harput (Özbek & Szaloki, 1998 as *bimaculata*); Içel, Silifke (Özbek & Szaloki, 1998 as *bimaculata*). **Ukraine.** Ukraine (Kuzin, 1954; Bologna & Turco, 2007; Ghahari & Campos-Soldini, 2019); Tauria (Soumakov, 1930 as *bimaculata*).

Chikatunov (2003) generically cited this species from SE Mediterranean, and Koçat & Kemal (2015a) erroneously recorded it from United Arab Emirates.

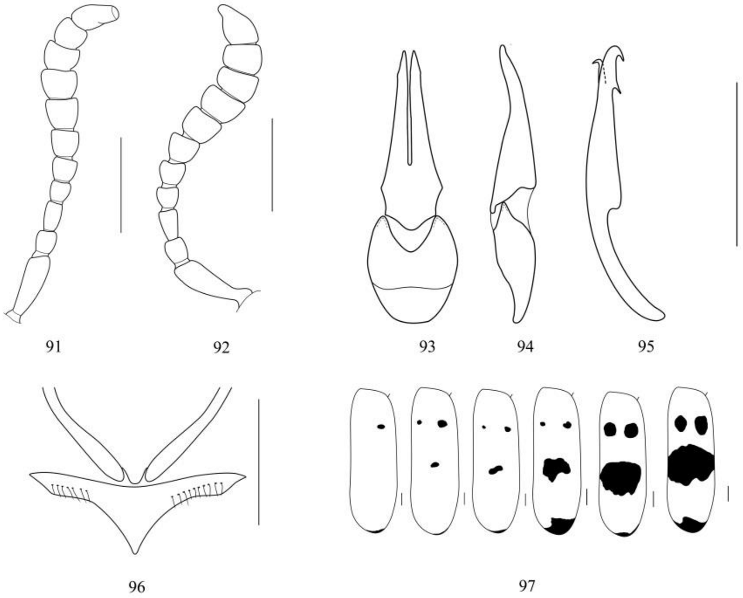


Fig. 91–97. *Hycleus sexmaculatus* (Olivier): 91. Male antenna; 92. female antenna; 93. tegmen, ventral view; 94. tegmen, lateral view; 95. aedeagus, lateral view; 96. mesosternum; 97. elytral pattern. Scale bar = 1 mm.

Description. Body length 9–17 mm. Body black, but elytra yellowish brown (in some specimens more reddish), with black pattern. Head subquadrate; antennomere III 1/2 times longer than IV, V–VIII transverse and progressively widened, IX–X narrower than VIII, male (Fig.91) XI curved and elongated and longer than antennomere X; in female (Fig. 92)shorter than X. Pronotum (Fig. 22)as long as wide and very convex; mesosternun as in Fig. 96. Each elytron with two black spot on anterior third, one sinuate middle fascia, and a black narrow fascia on apical part (Fig. 97); male last ventrite regularlyconvex, posterior margin with a middle deep emargination; aedeagus (Fig. 95) with endophallic hook narrow, distal aedeagal hook curved, far from proximal one and close to apex, proximal hook short and slightly curved; gonoforceps (Figs 93–94) very narrow from middle both ventral and lateral view, in which is sinuate posteriad on the apical third.

Taxonomic remarks. Bologna (1979) figured and described male genitalia, antennae and mesosternum of specimens from Turkey. The variability of elytral pattern, with the apical black margin more or less extended, was described by Escherich (1899) who synonymized *ledereri* Marseul with *sexmaculatus*; this pattern of variability was evidenced also in other populations. The variability concerns the shape of pronotum, more or less parallel in front, but were unable to define discrete differences among populations.

This species is very close to *Hycleus pirata* and to *H. hayki* described in this paper. It can be distinguished by the shape of antennae, pronotum and the male genitalia. The shape of pronotum is a constant feature, but the shape of last antennomere and of apical fascia of elytra show variability among specimens.

The synonymy of this species is quite complex. One of us (Bologna & Turco, 2007) studied types of *Mylabris bimaculata* Klug and synonymized it with *H. sexmaculatus*; moreover, the name *bimaculata* is preoccupied by *Mylabris bimaculata* Olivier, 1811 belonging to the complex of *M.* (*Eumylabris*) *calida* (Pallas, 1871).

Distribution. This species is widely distributed in the eastern Mediterranean regions (Turkey, Levant, Sinai), southern Ukraine and southern Russia, Caucasian region, Iraq and possibly northern Arabia peninsula, Iran; it is recorded generically from Kazakhstan.

***Hycleus tenuepictus* (Fairmaire, 1892)**

Figs 11, 23, 98–104

Mylabris tenuepicta Fairmaire, 1892: 154.
Zonabris tenuepicta ab. *marsabensis* Pic, 1898b: 3.

Type material. Lectotype, Syrie, Akbès, C.D. 1891/ *Mylabris tenuepicta* n.sp. Fairm. Syria (MNHN; Fairmaire, 1892, type loc.); 1 paralectotypus, *idem* (MNHN). We also examined (MNHNN) three syntypes of the variey *marsabensis* Pic.

Actually, the type locality, Akbès is not in Syria but in the Turkish province of Hatay.

Additional material examined. **Egypt.** 1 ex. Fayum (MNHN). **Israel.** 1 ex. Mt. Hermon (CPR); 1 ♂ Syrien, Kaifa (Haifa), coll. Reitter (HNHM); 1 ex. Jerusalem-Jericho rd. km 32 (MUH); 1 ♂ Jerusalem, Jericho road km 22, 18.IV.1942, leg. Bytinski & Salz (MABC); 1 ex. Jerusalem-Jericho rd. km 18 (MUH); 1 ex., *idem* without information on km (MUH); 1 ♂ Israel, Jerusalem (MABC); 3 exx. Mar Saba (MNHN) syntypes of var. *marsabensis* Pic; Pic, 1898); 1 ex. Arad-Mezada rd. (MUH); 4 exx. Bor Mushah (MUH); 1 ex. Mizp e Shalem Palms, East (MUH); 1 ex. Ejn Gedi (MUH); 1 ex. Boker (MUH); 1 ex. Judean desert, Mitape Jericho (CL); 1 ex. Dead Sea area and northern Arabe valley (Bytinsky-Salz, 1957); 1 ♂ (MABC), 3 exx. (NHMW) Dead Sea Area, Sea level; 1 ex. Jericho, Midbaryahuda (MHNG); 1 ♂ Ein Ovdat, Negev, 5.VIII.1975, leg. Fedeschi (MABC); 1 ♂ Israel, Nahal Qrdron, 7.VI.1996, leg. Freidberg (MABC); 1 ♂ Aradmejada road, 2.VII.71, leg. Bytinski & Salz (HMIM). **Turkey**. 2 exx. Akbès (MNHN det. as *sexmaculatus*); 43 exx. Akbès (MNHN).

Records from literature. Israel-Palestine. Palestine (Mader, 1927; Koçat & Kemal, 2015a); Dead Sea area, northern Negev, central Negev (Chikatunov, 1999, 2003); Judean desert (Chikatunov, 2003); Syro-Palaestinian region (Bologna, 1979). **Jordan.** Jordan (Koçat & Kemal, 2015a). **Syria.** Syria (Mader, 1927; Chikatunov, 1999, 2003; Koçat & Kemal, 2015a). **Turkey.** Turkey **(**Koçat & Kemal, 2010, 2015a)**.**

Description. Body length about 15 mm. Body black, but elytra yellowish red, with soft black pattern. Head subquadrate; antennomere III 1/2 times longer than IV, V–VIII transverse and progressively widened, IX–X narrower or with the same width of VIII, male (Fig. 98)XI slightly elongate, spindle shaped and ½ times longer than X; in female (Fig. 99) last antennomere spindle shape but shorter. Pronotum (Fig. 23) as long as wide, transversely depressed anteriorly, with deep middle longitudinal furrow and depression; mesosternun as in Fig. 103. Each elytron sometimes with two black small spot or without spot, and one narrow fascia on apical part (Fig. 104). Last male ventrite convex, except a narrow fore depression posterior margin with deep narrow middle emargination; aedeagus (Fig. 102) with endophallic hook narrow, distal aedeagal hook relatively curved, far from proximal one and close to apex, proximal hook slightly curved and nearly as long as distal; gonoforceps (Figs 100–101) very narrow in both ventral and lateral view; in lateral view scarcely sinuate in apical third but not turned posteriad.

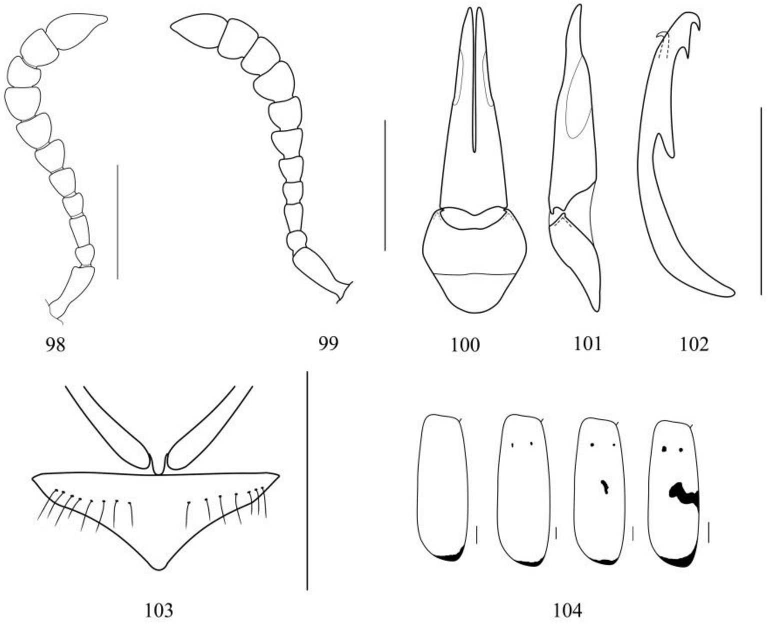


Fig. 98–104. *Hycleus tenuepictus* (Fairmaire): 98. Male antenna; 99. female antenna; 100. tegmen, ventral view; 101. tegmen, lateral view; 102. aedeagus, lateral view; 103. mesosternum; 104. elytral pattern. Scale bar = 1 mm**.**

Taxonomic remarks. Differently than in other species of this group, *H. tenuepictus* shows less variation among populations. At MNHN we examined several topotypic specimens belonging to the nominate form, to one form with two fore spots and nothing posteriorly, and to another form with one spot in the middle; we examined also the var. *marsabensis*. Additional variation concerns differences of the elytral spots size.

Distribution. This species is distributed in the southeastern Turkish Hatay province, Syria, Israel-Palestine and Jordan, with an isolated record from northern Egypt (Fayum), which needs confirmation.

***Hycleus trianguliferus* (Heyden, 1883)**

Figs 12, 24, 105–111

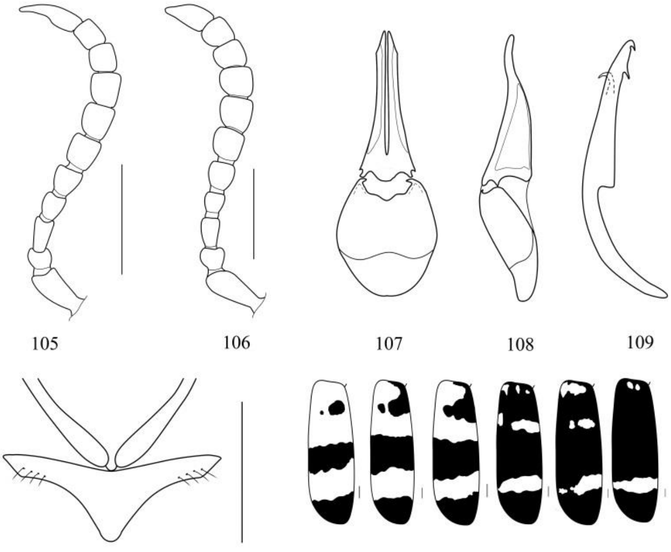
Zonabris triangulifera Heyden, 1883: 359.

Type material. 1 ♀ Syntype, Tekke, Turcmenia, Staudinger (DEI).

Additional material examined. **Afghanistan:** 2 ♂♂ Orozgan, No.496, 1.V.1958, leg. Dr. Lindberg (HNHM); 1 ♀ Aqtchah, No.774, 19.V.1959, leg. Dr. Lindberg (HNHM); 1 ♂ 1 ♀ Kouchk, au nord de Herat, 960 m, No.794, 29.VI.1959, leg. Dr. Lindberg (HNHM); 1 ♂ 2 ♀ ♀ Kadjakhai, No.925, 19.VI.1960, leg. Dr. Lindberg (HNHM); SW Afghanistan, Darweshan, 500 m, Registanwüste, 21.V.1961, leg. Ebert (HNHM); 4 ♂♂ 4 ♀ ♀ Orozgan, Gezab, 1300m, 11.VI.1970, leg. Kabakov (HNHM); 2 ♂♂ 1 ♀ Dahlah, No.554, 6.V.1958, leg. Dr. Lindberg (HNHM). **Iran.** 1 ♂ 1 ♀ Persien, Sistan, Malek Haidari, 31.V.1949, leg. Sharif (HNHM); 3 ♂♂ 4 ♀♀ Ghazvin prov., Kouhandej, VII.1950, leg. Mirzayans (HMIM); 3 ♂♂ 4 ♀♀ Sistan & Baluchestan prov., Zabol, 26.V.1950, leg. Mirzayans (HMIM); 2 ♀♀ Sistan & Baluchestan prov., Zabol, Bonjan, 29.V.1950, leg. Mirzayans (HMIM); 4 ♂♂ 8 ♀♀ Sistan & Baluchestan prov., Zabol, 31.V.1960, leg. Mirzayans (HMIM). **Tajikistan:** 1 ♂ S Tajikistan, near Shaartus, near Dzharkurgan vill., 300 m, 3.V.2005, leg. Pak. (MABC). **Turkmenistan:** 1 ♀Transcaspica, Merw, V.1900, leg. Hauser (HNHM); 4 ♂♂ 1 ♀ Transcaspica, Aschabad (HNHM); 1 ♂ 1 ♀ Transcaspica, Imam baba (HNHM); 2 ♂♂ Achkhabad, 7.VI.1964, Koslov (MABC); 1 ex Turcmenia (MSNV); 1 ex Kurtlinskojo Ozoro (CL).

Records from literature. Afghanistan. Afghanistan (Kuzin, 1954; Kaszab, 1958; Kolov, 2003; Nikolaev & Kolov, 2005Koçat & Kemal, 2015a; Ghahari & Campos-Soldini, 2019); Dahlah (Kaszab, 1973); Aqtachach (Kaszab, 1973); Kouchk N Hérat (Kaszab, 1973); Darreh-Gazak, 20 km S Qubeh (Kaszab, 1973); Djanada Khan, 50 km E Hérat (Kaszab, 1973); Kadjahkaï (Kaszab, 1973); Orozgan (Kaszab, 1973); Oruzgan, Gezab (Kaszab, 1981); N Kandahar (Kaszab, 1981). **Iran**. Iran (also as Perse) (Soumakov, 1915; Mader, 1927 as *javeti;* Kuzin, 1953, 1954; Kaszab, 1958, 1973; Kolov, 2003; Nikolaev & Kolov, 2005; Koçat & Kemal, 2015a; Ghahari & Campos-Soldini, 2019). **Kazakhstan**. Kazakhstan, (Kuzin, 1953, 1954; Kolov, 2003; Nikolaev & Kolov, 2005; Koçat & Kemal, 2015a; Ghahari & Campos-Soldini, 2019); Qyzylorda (Kuzin, 1953). **Tajikistan.** Tajikistan **(**Kuzin, 1953; Koçat & Kemal, 2015a; Ghahari & Campos-Soldini, 2019); Kabadin (Prispinova, 1987); mouth of Kafirnigan River, Ayvadzh (Prispinova, 1987); Kulyab (Prispinova, 1987); Tigrovaya Balka Reserve (Prispinova, 1987); Ramit reserve Hissar rangeBoshkent (Prispinova, 1987). **Turkmenistan**. Turcmenia (Soumakov, 1915; Kuzin, 1953, 1954; Kaszab, 1958, 1973; Koçat & Kemal, 2015a; Ghahari & Campos-Soldini, 2019); Trancaspia (Mader, 1927 as *javeti*); Tekke (Heyden, 1883 type loc.; Dokhtouroff, 1889); Merv, Bayramaly (Hauser, 1894); Merv (Reichardt, 1934); Merv, Geok-Tepe (Suoumakov, 1930); Nukhur (Reichardt, 1934); Mollakara (Reichardt, 1934); Kaşka (Reichardt, 1934); Tejen (Reichardt, 1934); Komarovskiy (Reichardt, 1934); Karakum (Reichardt, 1934). **Uzbekistan**. Uzbekistan (Kuzin, 1954; Koçat & Kemal, 2015a; Ghahari & Campos-Soldini, 2019); Bukhara Tschardzhuj (Soumakov, 1930).

Description. Body length 9–25 mm. Body black, but elytra yellowish red, with black pattern. Had subquadrate; antennomere III 1¼ times longer than IV, V as long as III, VI–VIII progressively widened, IX–X than VIII, male (Fig. 105) XI very long and narrowed apically; in female(Fig. 106) narrowed apical portion longer than basal one. Pronotum (Fig. 24) longer than wide, with anterior and basal depressions; mesosternun as in Fig. 110. Eachelytron sometimes with a very wide fascia or two black small spots on anterior third, one middle and one wide fascia on apical part (Fig. 111); last male ventrite widely and deeply concave and depressed, posterior margin with very wide and deep middle emargination; aedeagus (Fig. 109) with endophallic hook narrow, distal aedeagal hook distinctly curved, short, far from proximal one and close to apex, proximal hook slightly curved and shor; gonoforceps (Figs 107–108) distinctly narrowed from the basal third to apex, in ventral view; narrow and slightly sinuate in apical third in lateral view.


105 106 107 108 109

110 111

Fig. 105–111. *Hycleus trianguliferus* (Fairmaire): 105. Male antenna; 106. female antenna; 107. tegmen, ventral view; 108. tegmen, lateral view; 109. aedeagus, lateral view; 110. mesosternum; 111. elytral pattern. Scale bar = 1 mm.

Taxonomic remarks. Species strictly related to *Hycleus bipunctatus* because of the synapomorpgic condition of last ventrite.

The elytral pattern includes variation in the shape of anterior spots, and in some population looks very similar to that of *H. bipunctatus* which makes the identification very difficult. The anterior depression of pronotum also varies lightly. *Hycleus trianguliferus* differs from the latter species by the shape of last antennomere, emargination of last sternite, and the aedeagal hooks.

We already discussed the synonymy between *H. trianguliferus* ab. *reitterioides* (Mader) and ab. *bushirensis*, and *H. bipunctatus* (Olivier).

Distribution. This species is widely distributed in Iran, Kazakhstan, Turkmenistan, Uzbekistan, Tajikstan and Afghanistan (Bologna, 2008).

3.1.2 Key to males

1 Last ventrite widely and deeply concave and depressed; apical elytral black fascia very large, extended on the apical fourth ……………………..……………………………………….……… **2**

- Last ventrite convex, with or without middle depressed concave part …………………………………………………………………… **3**

2 Antennomere XI narrowed apical portion longer than the basal portion (Fig.105); elytral fore black colouration forming one fascia, frequently divided in irregular spots, triangularly extended along the suture until the scutellum (Fig. 111); depression of last ventrite extended until external sides; apical lobiform portion of gonoforceps very slender in lateral view (Fig. 108) (N and E Iran,Turkmenistan, Kazakhstan, Tajikistan, Uzbekistan, Afghanistan)…………………**trianguliferus**

- Antennomere XI narrowed apical portion as long as the basal portion (Fig. 40); elytral fore black pattern composed by two well distinct rounded spots never forming one fascia and never extended triangularly to the scutullum (Fig.46); depression of last ventrite not extended on the whole surface and never reaching external sides; apical lobiform portion of gonoforceps less slender in lateral view (Fig. 43) (Arabia, Iraq, S Iran, SW Pakistan) (syn. trianguliferus ab. reitterioides Mader) …………….………………………… **bipunctatus**

3 Last ventrite convex at base with middle depressed concave part **4**

- Last ventrite regularly convex ……………………………………..… **7**

4 Pronotum distinctly strangulate on fore third, deeply depressed on fore sides, without transverse anterior depression………….……….….. **5**

- Pronotum not strangulate on fore third, variously depressed on fore sides, with transverse anterior depression………………..………... **6**

5 Pronotum with dispersed and fine punctures; antennomere XI greatly slender and in lateral view with an angulated step before the last portion; apical portion of antennomere XI abruptly narrowed, apically subfiliform (Fig.47); last ventrite concave and deeply emarginated; aedeagus (Fig. 51) (E Turkey, Iran) (= colligatus sensu Auct. nec Redt., nec cingulatus Marseul) ………………...……..……**cingulatus**

- Pronotum with distinct and deeper puncture; antennomere XI without an angulated step before the last portion, apical portion of antennomere XI progressively narrowed, apically elongated (Fig. 70); last ventrite concave and deeply emarginated; aedeagus (Fig. 74) (NW and central Afghanistan) ………..…………………………… **lindbergi**

6 Aedeagal hooks distanced, one at apex and the second distinctly subapical (Fig. 102); fore sides of pronotum almost distinctly depressed; black elytral colouration greatly reduced to tracks of two fore minute spots and a narrow incomplete middle strip extended from the suture to half of elytron, sometimes completely lacking, and a very narrow apical margin (Fig.104); antennomere XI shortly conical as in Fig.98 (SE Turkey, Syria, Israel-Palestine, Jordan) ……………..…………………………...…………..... **tenuepictus**

- Aedeagal hooks closer, short and apical in position (Figs 59–60); fore sides of pronotum scarcely depressed; black elytral colouration including two fore distinct spots, a middle transverse fascia and a small and apical fascia, more or less extended (Fig. 62); antennomere XI pyriform as in Fig.55 (Caucasus and Transcaucasia, Iraq, N and central Iran, Turkmenistan, “late Turkestan”, Afghanistan, W Pakistan) (syn. javeti Marseul and javeti umbilicatus Kaszab, nec colligatus Auct.) …………………………..…….….…… **colligatus**

7 Apical portion of antennomere XI suddenly and greatly narrowed and filiform (Fig. 84); last ventrite strongly convex with shallow emargination; aedeagus slender, hooks distanced, both distal and proximal hooks elongate and distanced from the aedeagal body (Fig. 88); middle transverse fascia of elytra shortened in most populations (Fig. 90) (central and E Iran, S Uzbekistan) ……………………..……………………………………….... **schauffelei**

- Apical portion of antennomere XI progressively narrowed …..……… **8**

8 Last antennomere more or less pointed apically, antennomere XI short and stout, shortly conical pointed and slightly curved (Fig. 33); elytral pattern tricoloured (black, yellow and orange), base of elytra with a large, sinuate black fascia, one yellow fascia with one black spot, one black middle fascia, one large orange fascia, apex largely black (Fig.39); last ventrite convex with shallow emargination (Pakistan?, India, Sri Lanka) ..……..……………………………..……..…. **balteatus**

- Last antennomere not pointed but more or less distinctly truncate apically …………………………………………………………….…… **9**

9 Pronotum almost as wide as long; antennomere XI slender, at apex a few curved and conically truncate (Fig. 91); apical black fascia or totally lacking or very narrow (Fig. 97); aedeagus as in Fig. 95 (S Russia, Caucasus, Trancaucasia, S and E Turkey, Levant, Iraq, W Iran, Kazakhstan, Uzbekistan, N Arabia) (syn. bimaculatus, ledereri) ……………………………………………..………..... **sexmaculatus**

- Pronotum longer than wide; antennomere XI variously shaped but quite short, truncate but not conical at apex; apical black fascia wide ………………………..…………………………..…………..…. **10**

10 Fore portion of pronotum almost convex dorsally in lateral view, slightly depressed on sides in dorsal view; last antennomere bean-like, curved in the fore half, with a transverse depressed line at the middle of the curved part, obtusely truncate apically as in Fig. 63; middle black elytral fascia mostly wide, (Fig.69); genitalia as in Figs 65– 67 (SW Iran) …..…………………………………………… **hayki n. sp.**

- Fore portion of pronotum in lateral view slightly depressed dorsally, distinctly depressed on sides in dorsal view; last antennomere variously shaped; middle black elytral fascia narrow ………..…………………………..…………………...……..…... **11**

11 Body integument, including elytra, subopaque; elytra punctures wide and deep; last antennomere as in Fig. 26; antennomere IV and V short, IV usually submoniliform and V subtrapezoidal (Fig. 26); punctures on head and pronotum fine and less approached; fore tarsomeres short; genitalia as in Figs 28– 30 (S Israel, S Jordan, NE Egypt and Sinai, N Arabia)…….………………………………….…..…… **apicipennis**

- Body integument, including elytra, shiner; elytra punctures smaller and finer; last antennomere as in Fig. 77; antennomere IV and V slender, only slightly widened anteriorly (Fig. 77); punctures on head and pronotum lesser fine and more approached, quite deep; fore tarsomeres more elongate; genitalia as in Figs 79– 81 (United Arab Emirates, Oman) …………..………..…………………….……………. **pirata**

3.1.3 Key to females

1 Elytral apex unicolour brown or with a black fascia wide less than 1/5 of the elytral length …….. **2**

- Elytral apex black wide at least 1/5 of the elytral length ..……………. **4**

2 Elytral apex with a very narrow black margin, the remaining surface unicolour brown or with indistinct tracks of two fore spots (SE Turkey, Syria, Israel, Palestine, Jordan) ……………………………….……………………….**tenuepictus**

- Elytral apex unicolour brown or with a narrow or slightly wider black margin, the remaining black pattern includes distinct spots or fasciae ……………………………………………………….….………… **3**

3 Elytral black pattern, other than apex, composed by a row of two rounded spots on the same level, and one middle large oval transverse spot not reaching suture and lateral margin (S Russia, Caucasus, Trancaucasia, S and E Turkey, Levant, Iraq, W Iran, Kazakhstan, Uzbekistan, N Arabia) (syn. bimaculatus, ledereri) …………………………….……..………………..... **sexmaculatus**

- Elytral black pattern, other than apex, composed by a row of two rounded spots slightly inclined the external more anterior, and one middle large transverse and complete fascia, slightly inclined from suture to external margin. Punctures on head and pronotum variable, quite fine and dense or great and spaced (Iraq, N and central Iran, Turkmenistan, "late Turkestan", Afghanistan) (syn. javeti Marseul, nec colligatus Auct.) ………..……………………………….…... **colligatus**

4 Elytra tricoloured, black at base, two fore spots, a wide middle complete fascia and apex, orange between the black middle fascia and apex (Pakistan?, India, Sri Lanka) ……………….............…… **balteatus**

- Elytra brown-orange with black spots or fasciae …………………… **5**

5 Middle and apical fasciae wide, apical fascia wide, about ¼ of the whole elytra ……………………………………………………..……… **6**

- Middle fascia usually narrow or fragmented in spots, apical fascia less than ¼ of the whole elytra ……………..…………..………… **7**

6 Fore black elytral pattern composed by two well distinct rounded spots never forming one fascia and never extended triangularly to the

scutellum; pronotum in dorsal view in the middle on sides with less distinct sub-gibbose area (Arabia, Iraq, S Iran, SW Pakistan) (syn. trianguliferus abb. reitterioides and bushirensis)...……. **bipunctatus**

- Fore black pattern forming one fascia, frequently divided in irregular spots, triangularly extended along the suture until the scutellum; pronotum in dorsal view in the middle of sides with an area sub-gibbose and shiner (N and E Iran, Turkmenistan, Kazakhstan, Tajikistan, Uzbekistan, Afghanistan)………...…..….. **trianguliferus**

7 Last antennomere bean-like, more or less distinctly truncate apically; pronotum not strangulate anteriorly ………..…………..……..….. **8**

- Last antennomere conically narrowed at apex; pronotum strangulate anteriorly **10**

8 Fore portion of pronotum almost convex dorsally in lateral view, slightly depressed on sides in dorsal view, with reddish margin or spots laterally; middle black elytral fascia wide (SW Iran) …………………..……………..........................….…..… **hayki n. sp.**

- Fore portion of pronotum in lateral view slightly depressed dorsally, distinctly depressed on sides in dorsal view, usually without reddish margin or spots; middle black elytral fascia narrow …..…………………………………………………….…………... **9**

9 Body integuments, including elytra, subopaque; elytral punctures wide and deep; punctures on head and pronotum fine and less approached; fore tarsomeres short (S Israel, S Jordan, NE Egypt and Sinai, N Arabia) …………………………………………………… **apicipennis**

- Body integument, including elytra, shiner; elytral punctures smaller and finer; punctures on head and pronotum lesser fine and more approached, quite deep; fore tarsomeres more elongate (United Arab Emirates, Oman) …………………………………..……….. **pirata**

10 Pronotum with very dispersed and finer punctures; middle black elytral fascia usually complete (E Turkey, Syria, Transcaucasia, Azerbaidjan, Iran, Turkmenistan, N Arabia?) (= colligatus sensu Auct. nec Redt., nec cingulatus Marseul) ……………………………..…… **cingulatus**

- Pronotum with denser and deeper punctures; middle black elytral fascia more or less incomplete or fragmented…………..…………….……… **11**

11 Antennomere XI ca as long as IX–X together (central and E Iran, SW Uzbekistan)…………………………………………….…... **schauffelei**

\- Antennomere XI shorter than IX–X together (NW and central Afghanistan)…………………………..………...…...….. **lindbergi**

3.2 Phylogenetics

3.2.1 *Morphological evidences*

According with morphological characters, especially of male, we can distinguish six phenetic subgroups of species inside of this lineage of *Hycleus*:

(i) subgroup of *H. cingulatus*, monotypic: well characterized by the pronotum distinctly strangulate anteriorly and the male antennomere XI distinctly slender and in lateral view with an angulated step before the last portion.

(ii) subgroup of *H. schauffelei*, incuding *H. schauffelei* and *H. lindbergi*: characterized by the strangulate pronotum and male antennomere XI very narrowed in the apical portion.

(iii) subgroup of *H. bipunctatus*, including *H. bipunctatus* and *H. trianguliferus*: these species are very close; characterized by the last ventrite widely and deeply concave and depressed,

(iv) subgroup of *H. sexmaculatus*, including *H. apicipennis, H. hayki, H. pirata, H. sexmaculatus*: charcaterized by male antennomere XI not pointed but more or less distinctly truncate apically, pronotum not or scarcely strangulate on fore third.

(v) subgroup of *H. colligatus*, monotypic: characterized by both aedeagal hooks short and apical in position, approached each other, pronotum scarcely strangulate on fore third fore sides of scarcely depressed

(vi) subgroup of *H. balteatus*, monotypic: characterized by male antennomere XI short and stout, shortly conical pointed and slightly curved, elytra tricoloured; pronotum scarcely strangulate on fore third.

(vii) subgroup of *H. tenuepictus*, monotypic: characterized by aedeagal hooks distanced, one at apex and the second distinctly subapical; pronotum scarcely strangulate on fore third.

Relationships among these subgroups are not clear. According with the pronotum shape, possibly the subgroups of *H. cingulatus* and that of *H. schauffelei* which have also the very narrowed last antennomere in common, could be related.

3.2.2 Molecular evidences

Overall, we analysed 89 unphased sequences from 21 samples (Table 1) and our final concatenated dataset consisted of 3079 bp. Information about length, variable sites and substitution models selected for each gene are listed in Table 2.

Bayesian and Maximum Likelihood trees were identical, but the topology provided by Bayesian Inference showed higher values of nodal supports (Fig 112 and 113). Four main well supported clades were recovered from our phylogenetic analyses: Clade A, including only specimens belonging to *H. colligatus*; Clade B, including individuals of *H. sexmaculatus* and *H. pirata*; Clade C, including specimens of *H. schauffelei*; and Clade D, including the only analysed specimen of *H. cingulatus*.

These results, even if based on a subset of the morphological one, seems to confirm at least the same subgroups defined morphologically: subgroup of *H. colligatus* (Clade A); subgroup of *H. sexmaculatus* (Clade B); subgroup of *H. schauffelei* (Clade C); subgroup of *H. cingulatus* (Clade D). Of course, the lacking of other species analysed only morphologically, do not permit additional inferences on the definition of subgroups of species and on the relationships among them

Table 2. Number of sequences for each gene fragment, length of alignments (bp, base pairs), models of nucleotide substitution and number of variable positions and singleton mutations

	N°Sequences	**Lenght (bp)**	**Substitution Model**	**Variable Sites**	**Singleton**
28S	18	807	GTR+G	88	77
ARGK	17	507	GTR+G	52	38
CAD	19	726	HKY+G	170	95
COI	20	597	GTR+G+I	162	67
WG	15	442	GTR+G	50	40

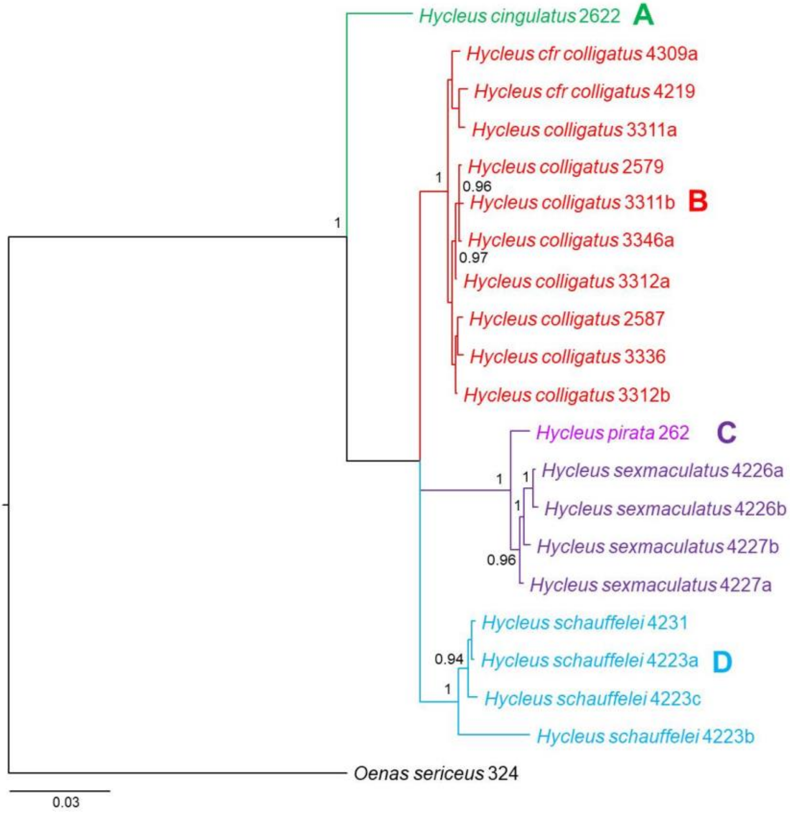


Fig. 112. Bayesian tree based on the concatenated dataset of COI, CAD, ARGK, WG and 28S gene fragments. Only supported values (PP>0.9) are reported at each nodes. Clades corresponding to morphological subgroups are indicated with letters (A-D) and different colours.

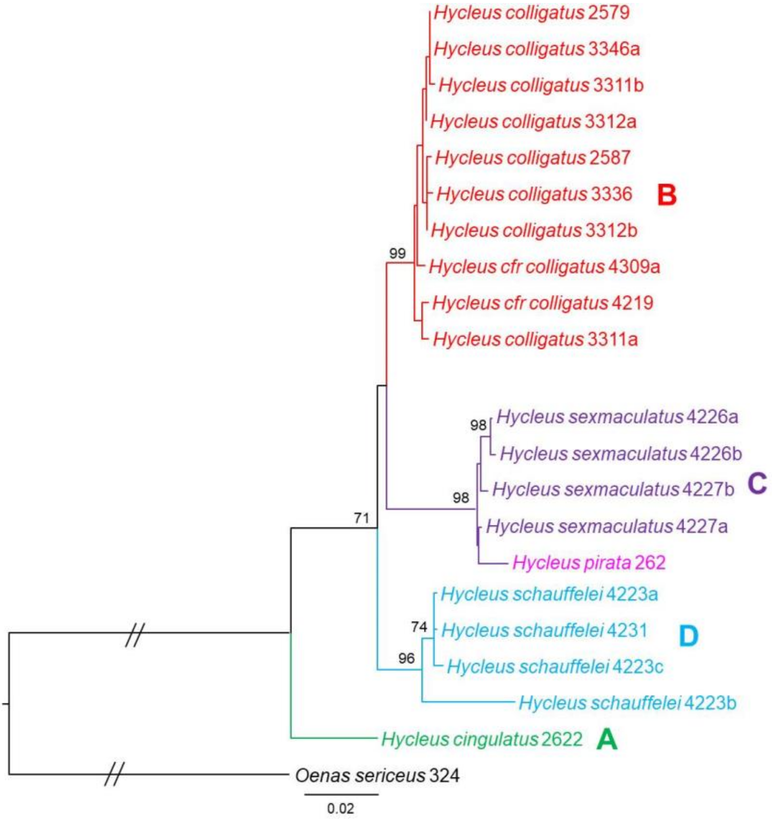


Fig. 113. Maximum Likelihood tree based on the concatenated dataset of COI, CAD, ARGK , WG and 28S gene fragments. Only supported values (PP>0.9) are reported at each nodes. Clades corresponding to morphological subgroups are indicated with letters (A-D) and different colours.

3.3 Biogeography and Ecology

3.3.1 *Geographic distribution*

The group of species of *H. sexmaculatus* is distributed primarily in the Saharo-Sindian Transitional Subregion, particularly in its eastern portion, with large extension to the the whole Near and Middle East and partially to central Asia but also in India with a single species. The western limit of distribution is represented by central Anatolia (*H. cingulatus, H. sexmaculatus*) and northern Egypt; (*H. apicipennnis*); the eastern limit is in India and Sri Lanka (*H. balteatus*); the northern limit is in Crimea and southern Russia (*H. sexmaculatus*), while the southern limit is in Oman (*H. pirata*).

Few species are endemic of small regions: for example, *H. apicipennis* is distributed only in northern Egypt, Sinai and Negev and northern Arabia; *H. hayki* is endemic to the southwestern Iran; *H, pirata* is endemic to the eastern horn of the Arabian peninsula; *H. teneuepictus* is endemic to the Levant, from Hatay province to northern Negev. On the contrary, the remaining eigth species have wide range which includes large portions of Middle East, Sind or Central Asia

3.3.2 *Ecological distribution*

Information about ecological preferences of the species are scarce. Few of them are related to steppe ecosystems: *H. sexmaculatus* in southern Russia, Anatolia, Caucausus, northern Syria, northern Iraq and western Iran; *H. trianguliferus* in southern Central Asia; *H. teneupictus* in the Levant. Other species are related to dry steppe or semidesert in the Middle East (Fig. 25): *H. cingulatus, H. colligatus, H. schauffelei, H. lindgergi* . Finally, some other species are related to more xeric ecosystems or true deserts: *H. apicipennis, H. hayki, H. pirata, H. bipunctatus*.

As for the altidudinal distribution, data recognized from the literature and collections are summarized in Table 3. Most species have a wide rage of distribution from about 300 m a.s.l. to about 1800 m a.sl. Few species (*H. bipunctatus, H. hayki, H. pirata, H. apicipennis*) are distributed from the sea level to few hundreds m a.s.l., while *H, tenuepictus* and *H. sexamculatus* are distributed also in the Dead Sea area under the sea level.

3.3.3 *Larval and adult host*

Data on the pre-imaginal biology of the species are extremely scarce. The only information concerns the description of the first instar larva of *H. trianguliferus*, a predator of grasshopper Eggs, provided by Priamikova & Iukhnevich (1958).

Adults are phytopagous and apparently polyphagous; most records concern feeding on flowers of different families, and rarely also on leaves. All available data, deriving from personal field collection in Turkey, Iran and UAE, and from literature, are summarized in Table 3. Occasionally few species are considered pest of cultivated plants, especially *H. balteatus* in India; in particular, Murugesan (1988) tested the food preference of this species using flowers of different families.

Table 3. Ecological information on the species of the Hycleus sexmaculatus group

Species	**Phenology**	**Elevation** (m a.s.l.)	**Host plants**
H. apicipennis	IV/VIII		*Linaria* sp. (Plantaginaceae)
H. balteatus	III/VIII, X, XII	180-640	*Hibiscus rosasinensis, H. esculantus, H. canalinus, Gossipyum spp.(Malvaceae), Dolichos lablab, D. tribolus, Taphrosia purpurea, Arachis hypogaea, Cajanus cajan (Fabaceae), Cucurbita peo, Trichosanthus angunia (Cucurbitaceae), Spinifer squamosa (Poaceae)*
H. bipunctatus	III/VII, X	1–1800	*Prosopis* (Mimosaceae), *Abelmoschus esculentus* (Malvaceae)
H. cingulatus	IV/X	1000–2800	
H. colligatus s.l.	III/VIII	40–3000	Malvaceae
H. lindbergi	VI	920	
H. hayki	V		
H. pirata	III, V	125–1200	*Tribulus omanense* (Zygophyllaceae)
H. schauffelei	III/VII	580–1950	*Beta vulgaris* (Amaranthaceae)
H. sexmaculatus	IV/VIII	-280–2450	*Beta vulgaris* (Amaranthaceae)
H. tenuepictus	IV/VIII	-350–800	
H. trianguliferus	IV/VIII	300–1300	*Apocynum* (Apocynaceae), *Alhagi* sp., Glycyrrhiza sp. (Fabaceae), Matricaria sp. (Asteraceae)

Acknowledgements

We thanks the following colleagues, for providing material and information from their institutions or private collections: M. Barclay (BMNH), L. Bartolozzi (already MZUF), L. Behne (SDEI); L. Černý (LCC); G. Cuccodoro (MHNG), G. Curletti (MCNC), L. Fekrat (MFUM), A. Freidberg (TAU), T. Garuzek, (TGC), P. Giachino (already MRSN), C. Girard and A. Mantillieri (MNHN), S. Krejcik (SKC), L. Latella (MSNV), A. Liberto (ALC), O. Merkl (HNHM), T. Pavlicek (already MUH), late F. Pesarini (already MSNM), R. Poggi (MSNG), late J. Probst (JPC), J.-C. Ringenbach (JCRC), E. Ratti (MCNV), V. Vrabec (VVC), A. Zilli (already MCZR), A. Zubair (FUUZM), H. Schilhammer and H.Schönmann (NHMW), M. Ühlig and B. Jāger (ZMHB).

Our thanks also to all colleagues who helped us collecting in the field: P. Audisio and F. Cerini (Roma, Italy), A. Casale (Torino, Italy), Ebrahim Gilasian and, F. Mozaffarian (Tehran, Iran), L. Fekrat (Mashhad, Iran), Sara Amjadi (Kordestan, Iran). F. Turco (Canberra, Australia). A special thank to M. Muzzi (Roma, Italy) for his hep with photos and to M. Bennet for improving the English revision.

This research was partially supported by funds from: CRUI, Progetto Della Valle (MAB, AR), MIUR, Italian Ministry of University and Research; Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization, Tehran, Iran..

References

Al-Ali, A.S. (1977) Phytophagous and entomophagous insects and mites of Iraq. Baghdad, Ministry of High Education Sciences Research, Univ. Baghdad, Natural history Research Center, Publ. 33, 142 pp..

Alfieri, A. (1976) The Coleoptera of Egypt. Memoires de la Société entomologique d'Egypte, 5, i-XVI, 1–362.

Anand, R. K. (1980) Male gential apparatus of Meloinae (Coleoptera: Meloidae) and their taxonomic significance. Journal of entomological Research, 4, 15–24.

Anand, R. K. (1989) Indian Meloidae (Coleoptera): a Taxonomic Review. Bulletin of Entomology, 30, 24–34.

Andrès, A. (1911) Note sur quelques espèces de Zonabris de la région de Mariout (environs d'Alexandrie) et description de M. Maurice Pic d'une espèce nouvelle. Bulletin de la Société Entomolologique d'Egypte, 1911: 129–135.

Axentjev, S.I. (1985) Meloidae from Iran (Insecta: Coleoptera). Senkenbergiana Biologica, 85 (1984), 245–250.

Batelka, J. & Geisthardt M. (2009) Order Coleoptera, family Meloidae. Arthropod fauna of the UAE, 2, 187–206.

Baudi di Selve, F. (1878a) Europaeae et circummediterraneae faunae Heteromerum specierum, quae Comes Dejean in suo Catalogo, editio 3a, consignavit, ex ejusdem collectione in R. Taurinensi Musaeo asservata, cum auctorum hodierne recepta denominatione collatio. Deutsche Entomologische Zeitschrift, 22: 337–376.

Baudi di Selve, F. (1878b) Coleotteri eteromeri esistenti nelle collezioni del R. Museo zoologico di Torino ed in altre italiane. Atti della Reale Accademia delle Scienze Torino, 13: 1027–1183.

Billberg, G.J. (1813) Monographia Mylabridum. Caroli Delén, Holmiae, 74 80, 7 pls.

Beccari, F. & Gerini V. (1979) Catalogo della collezione entomologica. Vol.II – Coleoptera. Fasc. VI-VII, 81–112. Firenze, Istituto Agronomico per l'Oltremare.

Bodenheimer, F.S. (1934) Studies on the Ecology of Plestinean Coleoptera: II Seasonal and diurnal appearance and activity. Bulletin de la Societé Royale d'Entomologie d'Egypte, 1934, 211–241.

Bologna, M.A. (1979) Meloidae di Turchia. I Contributo (Coleoptera). Fragmenta Entomologica, 15, 143–199.

Bologna, M.A. (1988) Il popolamento di aree di transizione zoogeografica: i Meloidae (Coleoptera) della regione siro-palestinese. Biogeographia, (n.s.) 12 (1986): 75–96.

Bologna, M.A. (1991) Coleoptera Meloidae. Fauna d'Italia. XXVIII. Bologna, Calderini

Bologna, M.A. (2008) New nomenclatorial and taxonomic acts, and comments, Meloidae, pp. 45-49; family Meloidae Gyllenhal, 1810, pp. 370-412. In: Löbl, I. & Smetana, A. (Eds.), Catalogue of Palaearctic Coleoptera. Vol. 5. Stenstrup, Apollo Books.

Bologna, M.A. (2009) The Meloidae (Coleoptera) of Libya: an annotated catalogue and description of three new species. Annales de la Societé Entomologique de France, (N.S.) 45, 345–364.

Bologna, M.A. & Pinto J.D., (2002). The Old World genera of Meloidae (Coleoptera): a key and synopsis. Journal of Natural History, 36: 2013–2102.

Bologna, M.A., D'Inzillo, B., Cervelli, M., Oliverio, M. & Mariottini, P. (2005) Molecular phylogenetics of the Mylabrini blister beetles (Coleoptera, Meloidae). Molecular Phylogenetic and Evolution, 37, 306–311.

Bologna, M.A. & Turco, F. (2007) The Meloidae (Coleoptera) of the United Arab Emirates with an updated Arabian checklist. Zootaxa 1625, 1–33.

Bologna, M.A., Oliverio, M., Pitzalis, M. & Mariottini, P. (2008) Phylogeny and evolutionary history of the blister beetles (Coleoptera, Meloidae). Molecular Phylogenetics and Evolution, 48, 679–693.

Bologna, M.A., Turco, F. & Pinto, J.D. (2010) 11.19. Meloidae Gyllenhal 1810. In: Leschen, R.A.B., Beutel, R.G. & Lawrence, J.F. (Vol. Eds.), Coleoptera, Beetles. Vol. 2. Morphology and Systematics (Elateroidea, Bostrichiformia, Cucujiformia partim). In: Kristensen, N.P. & Beutel, R.G. (Eds.), Arthropoda: Insecta. Handbook of Zoology. De Gruyter, Berlin/New York, 681–693.

Bologna, M.A., Turco, F. & Pinto, J.D. (2013) The Meloidae (Coleoptera) of Australasia: a generic review, descriptions of new taxa, and a challenge to the current definition of subfamilies posed by exceptional variation in male genitalia. Invertebrate Systematics, 27, 391–427.

Bologna, M.A., Černy, L. & Zubair, A. (2018). Meloidae (Coleoptera) of Pakistan and Kashmir with the description of three new species, new faunistic and taxonomic records, and a zoogeographic analysis. Turkish Journal of Zoology, 42, 637–660.

Borchmann, F. (1917) Meloidae, Cephaloidae. Pars 69. In: Schenkling S. (Ed.), Coleopterorum Catalogus. W. Junk, Berlin.

Borchmann, F. (1936) Entomologische Sammelergebnisse der Deutsche Hindukusk.Expedition 1935 der Deutschen Forschungsgemeinschaft. Meloidae. Arbeiten über morphologische und taxonomische entomologie aus Berlin-Dahlem, 3, 190–191 (in German).

Chikatunov, V. (1999) Catalogue of the beetles (Coleoptera) of Israel and adjacent areas. Tel Aviv, Dept. Zoology, Tel Aviv University.

Chikatunov, V. (2003) Catalogue of beetles (Coleoptera) from the National Collection of Insects of Tel Aviv, Museum of Natural History, Tel Aviv University. Department of Zoology, George S. Wise Faculty of Life Sciences.

Chockalingam, S. & Monogharan, T. (1979) Studies on the damage to the flower of Hibiscus rosasinensis by beetle, Mylabris indica and utilization of the food by the insect. Indian Zoology, 3, 83–87.

Chockalingam, S. & Mongharan, T. (1980) Dietary water budget un Mylabris indica (Coleoptera: Meloidae). Entomon, 5, 265–268.

Dejean, P.F.M.A. (1836) Catalogue des coléoptěres de la collection de M. le Comte Dejean. Troisième edition revue, corrigée et augmentée. Livraisons 1-4. Méquignon-Marvis Pere et Fils, Paris.

Derwesh, A.I. (1965) A preliminary list of identified insects and some arachnids of Iraq., The Government Press, Baghdad.

Dokhtouroff, W. (1889) Faune coléoptérologique aralo-caspienne. XIII partie. Meloïdes et Cantharides (Mylabrides). Horae Societatis Entomologicae Rossicae, 24, 121–192, 1 pl.

Escherich, K.L. (1899) Zur kenntniss der Coleopteren-Gattung Zonabris Harold. Wiener Entomologische Zeitung, 18, 84–92, 97–109 (in German).

Ezzoddinloo, L. & Mohammadian, H., 2012. Faunistic investigations on Coleoptera in Khojeer and Sorkhe Hesar National Parks. Proceedings of the 17th National and 5th International Iranian Biology Conference, Kerman, September 5–7, 2012, 1–9.

Fabricius, J.C. (1775) Systema entomologicae, systens insectorum classes, ordines, genera, species, adiectis synonymis, locis, descriptionibus, observationibus. Libraria Kortii, Flensburgi et Lipsiae.

Fabricius, J.C. (1801) Systema Eleutheratorum secundum ordines, genera, species adiectis synonymis, locis, observationibus, descriptionibus. Tomus II. Bibliopolii Academici Novi, Kiliae.

Fairmare, L. (1892) Descriptions de Coléoptères des environs d’Akbès (Syrie). Annales de la Société Entomologique du Belgique, 36, 144–192.

Faimaire, L. (1896) Hétéromères de l’Inde recueillis per M. Andrewes. Annales de la Société Entomologique du Belgique 40: 6–62.

Faldermann, F. (1837) Fauna entomologica Trans-caucasica. Pars II. Coleoptera Trans-caucasica. Nouvelles Mémoires de la Société Imperiale des Naturuealistes Moscou, 5, 1–433, 15 pls.

Faldermann, F. (1839) Fauna entomologica Trans-Caucasica. Coleoptera Pars III. Vésicantes. Mémoires de la Société Imperiale des Natururalistes Moscou, 6, 1–338.

Faraji, A., Pashaie Rad, S. & Shayestehfar A. (2012) Taxonomic study of blister beetles (Coleoptera: Meloidae) in Arak county, Iran. Experimental Animal Biology, 1, 55–68.

Fekrat, L. & Modaress Awal, M. (2012) A contribution to the knowledge of Meloidae (Insecta: Coleoptera) fauna of Northeastern Iran. Journal of Crop Protection,1, 303–311.

Fischer, J.B. (1827) Tentamen conspectus Cantharidiarum. Dissertatio inauguralis quam pro summis in medicina et chirurgia honoribus legitime obtinendis eruditorum examini. Lindauer, Monachi.

Ghahari, H. & Campos-Soldini, M.P. (2019) An annotated catalogue of blister-beetles (Coleoptera: Tenebrionoidea: Meloidae) of Iran. Entomofauna, 40, 59–138.

Gillet, M.P.T. & Gillet, J. (1996) A large blister beetle Mylabris maculiventris Klug, 1845 from Al Ain and neighbouring Oman (Coleoptera: Meloidae). Tribulus, 6, 20–21.

Gillet, M.P.T. & Gillet, C. (2005) Insects and other arthropods. In: Hellyer, P. & Aspinall, S. (Eds.) The Emirates. A Natural History. Trident Press Limited, Abu Dhabi, 169–196.

Gillet, M.P.T. & Howarth, B. (2004) Insects. In: Aspinall, S. & Hellyer, P. (Eds.) Jebel Hafit, a natural history. Emirates Natural History Group, Abu Dhabi, 94–143.

Haniffa, M.A. & Fransis Sekar, M. (1993). Effect of food quality on the energy budget in the blister beetle Mylabris indica (Coleoptera: Meloidae). Zeitschrift für angewandte Zoologie, 79, 329–334.

Hashmi, A.H. & Tashfeen, A. (1992) Coleoptera of Pakistan. Proceedings of the Pakistan Congress of Zoology, 12, 133–170.

Hauser, F. (1894) Beitrag zur Coleopteren-Fauna von Transcaspien und Turkestan. Zusammengestelt unter Mitwirkung der Herren Dr.

Eppelsheim, Escherich, Faust, Dr. von Heyden, Kuwert, Reitter und Weise. Deutsche Entomologische Zeitschrift, 1894, 17–74 (in German).

Heyden, L.F.J.D. von (1883) Käfer aus Tekke-Turkmenien. Wiener Entomologische Zeitung, 8, 354–360 (in German).

Heyden, L.F.J.D. von (1892) XIV. Beitrag zur Coleopteren-Fauna von Turkestan. Unter Mitwirkun der Herren J. Faust, A. Kuwert und J. Weise. Deutsche entomologische Zeitschrift, 1892, 99–104 (in German).

Heyden, L.F.J.D. von & Kraatz, G. (1883) Käfer aus Osch (in Turkestan). Deutsche entomologische Zeitschrift, 27, 337–353 (in German).

Holdhaus, K. (1920) Koleopteren aus Mesopotamien. Annalen des K.K. Naturhistorischen Hofmuseums Wien, 33, 39–58, 1 tav.

Iconomoupolos, M.L.D. (1916). La faune Coléoptérologique d'Abou-Rouache et du plateau de Kerdaché. Bulletin de la Société entomologique d'Egypte, 1916, 109-113.

Kaszab, Z. (1957) Neue Meloiden aus Iran 1954 (Coleopt.) (Ergebnisse der Entomologischen Reisen Willi Richter, Stuttgart, in Iran 1954 und 1956 - Nr. 6). Jahreshefte des Vereins für vaterländische Naturkunde in Württemberg, 112, 50–59 (in German).

Kaszab, Z. (1958) Die Meloiden Afghanistans (Coleoptera). Acta Zoologica Hungarica, 3, 245–312 (in German).

Kaszab, Z. (1959) Neue Meloiden aus Iran 1954, 1956 (Coleoptera). Stuttgarter Beiträge zur Naturkunde, 20, 1–5 (in German).

Kaszab, Z. (1961) Ergebnisse der Deutschen Afghanistan-Expedition 1956 der Landessammlungen für Naturkunde Karlsuhe. Beiträge zur naturkundlichen Forschung in Südwestdeutschland, 19, 239–240, 1 map (in German).

Kaszab, Z. (1965) Österreichsche entomologische Expeditionen nach Persien und Afghanistan. Beiträge zur Coleopterenfauna. Teil I: Tenebrionidae, Meloidae und Pedilidae. Annalen des Naturhistorischen Museums in Wien, 68, 667–670.

Kaszab, Z. (1968) Contribution à la faune de l'Iran, 8. Coléoptères Meloidae. Annales de la Société entomologique de France, (N. S.) 4, 749–776 (in German).

Kaszab, Z. (1969) The system of the Meloidae (Coleoptera). Memorie dela Società entomol. italiana, 48, 241–248.

Kaszab, Z. (1973) Beiträge zur Kenntnis der Fauna Afghanistans (Sammelergebnisse von O. Jakes 1963-64, D. Povolny 1965, D. Povolny and Fr. Tenora 1966, J. Simek 19665-66, D. Povolny, J. Gaisler, Z.

Sebenek and Fr. Tenora 1967). Meloidae. Col. Acta Musei Moraviae, 56-57, 267–308 (in German).

Kaszab, Z. (1981) Faunistische und taxonomische studien über Meloiden (Coleoptera). Annales historico-naturales Musei nationalis Hungarici, 73, 159–181.

Kaszab, Z. (1983) Insects of Saudi Arabia. Coleoptera: Fam. Meloidae – A synopsis of the Arabian Meloidae. Fauna of Saudi Arabia, 5, 144– 204.

Koçak, A.O. & Kemal, M. (2009) Results of the entomofauna projects of the Cesa. 6 –List of the 11322 Pterygot insect species of Turkey hitherto recorded in the database of the Cesa. Cesa News 47, 47–197.

Koçak, A.O & Kemal, M. (2010) A list of the species of the family Meloidae ecorded in Turkey with a nomenclatural note (Coleoptera). Miscellaneous papers Cesa, 152, 1-7.

Koçak, A.O. & Kemal, M. (2015) Initial list of the Pterygota species of Malatya Prov., based upon the info-system of the Cesa (East Turkey). Cesa News, 115, 1–150.

Koçak, A.O & Kemal, M. (2015a) Initial results of the Entomofauna of SW Asia, based upon the info-system of the Cesa (excl. Lepidoptera). Priamus, Serial Publication of the Centre for Entomological Studies Ankara Supplement, 35, 1–1185.

Kollar, V. & Redtenbacher L. (1850) Ueber den Character der Insecten-Fauna von Südpersien. Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe, 1, 42–53.

Kolov, S.V. (2003) Taxonomic composition of blister-beetles (Coleoptera, Meloidae) of Kazakhstan. Tethys Entomological Research, 8, 157–168 (in Russian).

Katoh, K., Rozewicki, J. &Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics, 20, 1160–1166.

Kumar, S., Stecher, G. & Tamura K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biologìy and Evolution, 33, 1870-1874.

Kuzin, V.S. (1953) The blister beetles of Kazakhstan. Trudy Respublikanskoy Stantsii Zashchity Rasteniy Kazakhstanskiy Filial Vaskhnil (Alma-Ata), 1, 72–154 (in Russian).

Kuzin, V.S. (1954) K poznanyyu systemy narybnikov (Coleoptera, Meloidae, Mylabrini). Trudy Vsesoyznogo Entomologicheskogo Obshchestva, 44, 336–379 (in Russian).

Latreille, P.A. (1817) [new taxa]. In: Cuvier, G., Le régne animal distribué d’après son organisation, pour servir de base a l'histoire naturelle des animaux et d'introduction a L’ anatomie comparée. Tome III, contenant les crustacés, les arachnides et les insectes., Deterville, Paris

Mader, L. (1927) Meloidae. In: Winkler, A. (Ed.), Catalogus Coleopterorum regionis palaearcticae. A. Winkler, Wien, 851–880.

Mader, L. (1927a) Coleopterologische Notizen. Entomologische Anzeiger, 17, 193-197 (in German).

Mader, L. (1929) Zur variabilität der Meloiden und Coccinelliden. Entomologischer Anzeiger, 9, 108–114.

Makhan, D. (2012) Hycleus amrishi sp. nov. (Coleoptera: Meloidae), an additional new blister beetle from Ahvanu, Damghan, Semnan Prov., Iran. Calodema, 229, 1–3.

Marseul, S.A. de (1870) Monographie des Mylabrides d'Europe et des contrées limitrophes en Afrique et en Asie. L'Abeille, Mémoires d’'Entomologie, 7 (2), 1–204, 2 pls.

Marseul, S.A. de (1872) Monographie des Mylabrides. Mémoires de la Société Royale des sciences de Liège, 1872, 363–662, 9 pls.

Mirzayans, H. (1970) Contribution a la connaissance de la fauna des Clérides et Méloides de l’Iran. Entomologie et Phytopathologie Appliquées, 29, 25–37.

Modaress Awal, M. (1997) Family Meloidae, pp. 175–183. In: Modarres Awal, M. (Ed.), List of agricultural pests and their natural enemies in Iran.Ferdowsi University Press, Mashhad.

Modaress Awal, M. (2012) Family Meloidae, pp. 346-357. In: Modarres Awal, M. (Ed.), List of agricultural pests and their natural enemies in Iran. Ferdowsi University Press, Mashhad.

Mohammad-Momeni, Z., Jalali-Zand, A.R. & Abtahi, S.M. (2016) Fauna of blister beetles (Coleoptera: Meloidae) in Isfahan township. Plant Protection Journal, 8, 43–55.

Mohamedsaid, M.S. (1982) Supplementary records of Meloid beetles (Coleoptera) from Sri Lanka. Ceylon Journal of Science, 15, 155–159.

Moslemi, R., Pashaie Rad, Sh. & Serri S. (2015) An identification guide to meloid beetles (Coleoptera: Meloidae) (Insecta; Coleoptera) of Markazi prov. Journal of Animal Research (Iranian Journal of Biology) 28, 105–115.

Murugesan, S. (1988) Some Biochemical correlates in relation to the quantitative food utilization and reproduction in two species of Meloids

(Insecta: Coleoptera: Meloidae). Proceedings of the Indian national. Science Academy, B54, 155–160.

Nikbakhtzadeh, M.R. & Tirgari, S. (2002) Blister Beetles (Coleoptera: Meloidae) in Nahavand County (Hamedan Prov., Iran) and Their Ecological Relationship to Other Coleopteran Families. Iranian Journal of Public Health, 31, 55–62.

Nikolaev, G.V. & Kolov, S.V. (2005) Blister beetles (Coleoptera, Meloidae) from Kazakhstan: Biology, Systematics, Determinant. Almaty, Cossack University.

Olivier, A.G. (1811) Encyclopédie méthodique, ou par ordre de matières; par une société de gens de lettres, desavans et d'artistes; précédée d'un vocubulaire üniversel, servant de table pour tout Vouvrage, ornée des portraits de Mm. Diderot & d'Alembert, premiers éditeurs de VEncyclopédie. Histoire naturelle. Insectes.Tome huitième. H. Agasse, Paris.

Özbek, H. & Szaloki, D. (1998) A contribution to the knowledge of the Meloidae (Coleoptera) fauna of Turkey along with new records. Turkish Journal of Zoology, 22, 23–40.

Pallas, P.S. (1782) Icones Insectorum praesertim Rossiae Sibiriaeque peculiarum quae collegit et descritionibus illustravit. Fasciculus secundus. W. Waltheri, Erlangae, 57–96, A–F.

Pan, Z. & Bologna, M.A. (2014) Taxonomy, Bionomics and Faunistics of the Nominate Subgenus of Mylabris Fabricius, 1775, with the description of five new species (Coleoptera: Meloidae: Mylabrini). Zootaxa, 3806 (1), 1–78.

Pardo Alcaide, A. (1954) Études sur les Meloidae. V. Les Mylabrini du Maroc et du Sahara occidental espagnol (Col.Meloidae). Bulletin de la Société de Sciences Naturelles et physiques du Maroc, 34, 55–88.

Pardo Alcaide, A. (1955) Estudios sobre Meloidae VI. Sobre algunos Mylabris (s. lat.) de la región etiópica. Bulletin de l'Institur royal des Sciences Naturelles du Belgique, 31 (49), 1–32.

Pardo Alcaide, A., 1963. Coléoptères Méloïdes récoltés par M. J. Mateu dans l'Ennedi et au Tchad. Etudes sur les Meloidae, XII. Bulletin de l'Institut fondamental de l'Afrique Noire, (A) 25, 572–588.

Pemberton, R.W. & Edna, M.H. (1980) Insects associated with wild plants in Europe and the Middle East. Biol. Control of Weeds Survey, USDA Miscellaneous Publications, 1382.

Pic, M. (1898) Variétés et Nouvelles espèces de Coléoptères. Miscellanea Entomologica, 6, 2–4.

Pic, M. (1912) Descriptions ou diagnoses et notes diverses (Suite). L'Echange, Revue Linnéenne, 28, 1–3, 9–10.

Pic, M. (1912a) Coléoptères exotiques nouveaux ou peu connus (Suite). L'Echange, Revue Linnéenne, 28, 29-32, 46-47.

Pic, M. (1914) Notes synonymiques sur divers Hétéromères (Col.). Bulletin de la Société. entomologique de France, 1914, 203–204.

Pic, M. (1920) Contribution à la faune entomologique du Nord-Est du Sinai. Bulletin de la Société entomologique d'Egypte, 13, 40–53.

Pic, M. (1921): [new taxa]. In: Alfieri A., Contributions â la faune entomologique du nord-est du Sinai. Bulletin de la Société entomologique d'Egypte, 6 (1919-1920), 40–53.

Pinto, J.D. & Bologna, M.A. (1999) The New World Genera of Meloidae (Coleoptera): a key and synopsis. Journal of Natural History, 33, 569–619.

Posada, D. (2008) jModelTest: phylogenetic model averaging. Molecular Biology and Evolution, 25, 1253–1256.

Priamikova, M.A. & Iuchnevich L.A. (1958) Key tot the triungulins of blister beetles (Coleoptera, Meloidae) of the Mylabrini in the USSR fauna. Entomologicheskoye Obozreniye, 37, 139-145 (in Russian).

Pripisnova, M.G. (1987) Fauna and Ecology of the Meloidae (Coleoptera) of Tajikistan. Entomologicheskoye Obozreniye, 66, 555–571 (in Russian).

Rambaut, A., &Drummond, A. (2009) FigTree, version 1.3. 1. Institute of Evolutionary Biology, University of Edinburgh.

Rambaut, A., Suchard, M.A., Xie, D. &Drummond, A.J. (2014) Tracer 1.6. Available at http://beast.bio.ed.ac.uk/Tracer.

Reichardt, A.N. (1934) Opredelitel i spisok zhukov naryvnikov v Turkmenii. Trudy Sovieta po Izutcheniu Proizvoditelnykh Sil. Seriya Turkmenskaya 6, 205–239 (in Russian).

Reiche, L.J. (1866) Etude sur les epĕces de mylabrides de la collection de L. Reiche, suivie d'une note sur le genre Trigonurus Mulsant et description d'une espĕce nouvelle. Annales de la Société entomologique de France, (4) 5 [1865], 627–642.

Reitter, E. (1889) Coleopterologische Ergebnisse der im Jahre 1886 und 1887 in Transcaspien von Dr. G. Radde, Dr. A. Walter und A. Konchin ausgeführten Expedition. Verhandlungen des Naturforschenden Vereines in Brünn, 27, 95–133 (in German).

Riccieri, A., Mancini, E., Salvi, D., & Bologna, M. A. (2020). Phylogeny, biogeography and systematics of the hyper-diverse blister beetle genus

Hycleus (Coleoptera: Meloidae). Molecular Phylogenetics and Evolution, 144, 106706.

Robert, C.E. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32, 1792–1797. (https://doi.org/10.1093/nar/gkh340)

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61, 539–542.

Saha, G.N. (1979) Revision of Indian blister beetles (Coleopetra: Meloidae: Meloinae). Records of the Zoological Survey of India, 74, 1–146.

Salvi, D., Maura, M., Pan, Z., & Bologna, M. A. (2019) Phylogenetic systematics of Mylabris blister beetles (Coleoptera, Meloidae): a molecular assessment using species trees and total evidence. Cladistics, 35 (2018 online), 243–268.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Serri, S. (2015) Blister beetles (Coleoptera: Meloidae) of Southwest Iran. Proceedings of the 1st Iranian Internationa Congress of Entomology, 232–237.

Serri, S. & Bologna, M.A. (2018) Description of the male of Mylabris (Mylabris) parumpicta (Heyden, 1883) (Coleoptera: Meloidae) and new distributional data of this species. Journal of Entomological Society of Iran, 37, 405–410.

Shukla, G.S. & Upadhayaya, V.K. (1973) Tecoma stans (L.) H.B. and K. as a new host record for Mylabris pustulata (Thunb) (Coleoptera: Meloidae). Indian Journal of Entomology, 35, 71.

Staden, R., Beal, K.F. & Bonfield J.K. (2000) The staden package, pp. 115–130. In: Misener, S., Krawetz, S.A. (Eds.), Computer Methods in Molecular Biology, Bioinformatics Methods and Protocols. Humana Press Inc., Totowa, NJ (1998).

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 22, 2688–2690.

Sumakov, G.G. (1913) Deux nouvelles espèces du genre Mylabris F. Revue russe d’Entomologie, 13, 473–475.

Sumakov, G.G. (1915) Les espèces paléarctiques du genre Mylabris Fabr. (Coleoptera, Meloidae). Horae Societatis Entomologicae Rossicae, 42, 1–73.

Sumakov, G.G. (1930) Catalogue des espèces paléarctiques de tribu Mylabrina (Coleoptera, Meloidae). Acta Instituti et Musei Zoologici Universitatis Tartuensis, 37, 1–114.

Vasantharaj, D.B. & Kumaraswami, T. (1978) Insect pests of crops, pp. 44–166. In: Elemets of Economic Entomology. Popular Book Depot, Madras.

Zaripova, D. (1972) The blister-beetles (Coleoptera, Meloidae) of the Ramit Natural Reservation (Hissar range Tadjikistan) and their zonal distribution. Revue d’Entomologie de l’Urss, 51, 585–587.

FINAL DISCUSSION AND CONCLUSIONS

This work is part of a broader line of research aimed at studying through morphological and molecular analyses the evolutionary history of Meloidae, whose relevance is known from both medical and agricultural perspective (Adams and Selander, 1979; Bologna, 1991; Lebesa et al., 2012; Wu et al., 2018).

The first outcome of this thesis regards the phylogenetic relationships of the species of *Hycleus*. In particular, we clarified the phylogenetic position of *Ceroctis* and *Paractenodia* that resulted nested within *Hycleus*, confirming previous hypothesis (Bologna et al., 2008c; Salvi et al., 2019). According to this result, we suggested a new synonymy between *Hycleus* and *Ceroctis* and *Paractenodia*. The three morphologically-based sections (i.e. Mesogorbatus, Mesotaeniatus and Mesoscutatus) described by Pardo Alcaide (1954, 1955) did not constitute monophyletic groups in our molecular analyses and should not be used to define subgenera in *Hycleus*. Therefore, unlike for the genus *Mylabris* (150 species, 16 subgenera; Salvi et al., 2019), mesosternal morphology do not represent a useful character to differentiate taxonomic units in *Hycleus.*

Biogeographic results showed that *Hycleus* likely originated in the African paleo-continent in the Early Miocene, about 20 Mya. Miocene climatic conditions and Pliocene climatic oscillations favoured the succession of contractions and expansions of species ranges, plausibly triggering numerous events of allopatric divergence followed by speciation (Bologna et al., 2008a; Micheels et al., 2009). These events might be at the origin of the high diversity observed in *Hycleus*. Furthermore, during Miocene, forests occupying the northernmost part of Africa progressively changed into savannahs and grasslands (Micheels et al., 2009), favouring, together with the presence of the land bridge constituted by the Arabian Peninsula, the dispersal of species towards the Saharo Sindian Transitional region (~14.6 Mya) and also the colonization of the Palaearctic Region (~9 Mya). From this latter region, ~6 Mya a second event of colonization of the Saharo-Sindian Transitional region occurred. Finally, a Late Miocene migration (about 6 Mya) of an Afrotropical lineage across the Arabian Peninsula, gave origin to the Oriental species of *Hycleus*.

The second part of this thesis consisted of a phylogeographic work involving the species *Hycleus polymorphus*. Because the taxonomy of the *H. polymorphus* group is not well clarified, we first investigated the phylogeny of eight of the 14 species of the complex, in the attempt to clarify the species boundaries, particularly those of *H. polymorphus*.

Phylogenetic results showed that almost all the species analysed were monophyletic, except *Hycleus humerosus* and *Hycleus zebraeus* that resulted synonyms respectively of *H. polymorphus* and *H. atratus*. This preliminary analysis, however, confirmed that a taxonomic revision is needed to better define the relationships among the species of the group.

Phylogeographic analysis were carried out with the genetic markers CAD and ITS2 (mtDNA was unavailable because of widespread heteroplasmy). CAD resulted highly polymorphic, and its network did not show any geographic structure; on the contrary, even if not highly variable, ITS2 resulted more informative from a phylogeographic perspective. The results obtained in this work allow to hypothesize an Asiatic origin in the Pleistocene of *Hycleus polymorphus*, also supported by previously obtained molecular dating. During cold stages, steppe environments underwent an expansion toward western Europe, followed by the extension of cold-tolerant species ranges. On the contrary, during interglacials, those species suffered the contraction of their ranges (Kajtoch et al., 2016). Similar events, likely characterized the biogeographic history of *H. polymorphus*, which, during interglacials, like the one we are presently experiencing, remained confined to mountain ranges in western Europe. Moreover, the current distribution pattern observed in this species seems related to the last glacial/interglacial cycle. In fact, the presence of a single haplotype shared by one Italian individual and Spanish and French populations, might be related to the presence of a glacial connection between Pyrenees and Western Alps favouring the gene flow among populations during the last glacial, as hypothesized for other taxa (Schmitt, 2009). At the same time, the uniform genetic constitution found in the South-Balkan Peninsula was already observed in other species (e.g. butterflies species *Erebia ottomana*, *Coenonympha rhodopensis*; Louy et al., 2013, 2014), and explained as the result of gene flow connecting populations in the Balkan mountain ranges during the last glacial. Surprisingly, Croatian populations showed a strong genetic divergence from all others, and in particular from other Balkan populations. A similar result was observed in the butterfly *Proterebia afra* (Fabricius, 1787), and explained as probably related to a reduced climatic connection between Northern and Southern Balkans (Bartonova et al., 2018).

The last part of this PhD thesis, focused on the species group of *H. sexmaculatus*. Each species belonging to this group was described in detail according to morphological, ecological and biogeographical features. Moreover, the new species *Hycleus hayki* was described and new synonymies were proposed (e.g. that of *H. javeti* and *H. colligatus*). New dichotomous keys were provided for males and females of each species. Furthermore, morphological evidences allowed to describe six phenetic subgroups of species, some of which were also confirmed by molecular results that, however, should be enhanced by improving the number of species and samples in the analysis.

In conclusion, this thesis contributed to improve the knowledge about Meloidae by exploring the evolutionary history of the largest genus of the family, the genus *Hycleus,* and clarifying several issues related to its systematics. Moreover, by focusing on *H. sexmaculatus* and allied species, we provided a detailed description and a phylogenetic background on this species group. Finally, we analysed some taxonomic aspects of the *H. polymorphus* species group and explored the biogeographic history of a peculiarly-distributed species in this complex, the nominal species *H. polymorphus*.

This work raised novel interesting questions on the taxonomy and the biogeography of meloidae, which are worth to be explored in the next future with high-throughput approaches in molecular ecology, such as phylogenomics and population genomics.

References

Adams, C. L., & Selander, R. B., 1979. The biology of blister beetles of the vittata group of the genus *Epicauta* (Coleoptera, Meloidae). Bulletin of the AMNH; v. 162, article 4.

Bartonova, A., Konvicka, M., Korb, S., Kramp, K., Schmitt, T., Faltynek Fric, Z., 2018. Range dynamics of Palaearctic steppe species under glacial cycles: the phylogeography of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae). Biological Journal of the Linnean Society 125, 867-884.

Bologna, M.A., 1991. Coleoptera Meloidae. Fauna d'Italia. XXVIII, Calderini, Bologna.

Bologna, M.A., Di Giulio, A., Pitzalis, M., 2008a. Examples of disjunct distributions between Mediterranean and southern or eastern Africa in Meloidae (Coleoptera, Tenebrionoidea). Biogeographia (n.s.) 29, 81-98.

Bologna, M.A., Oliverio, M., Pitzalis, M., Mariottini, P., 2008b. Phylogeny and evolutionary history of the blister beetles (Coleoptera, Meloidae). Molecular Phylogenetics and Evolution 48, 679–693.

Kajtoch, Ł., Cieślak, E., Varga, Z., Paul, W, Mazur, M.A., Sramkó, G., Kubisz, D., 2016. Phylogeographic patterns of steppe species in Eastern Central Europe: a review and the implications for conservation. Biodiversity and Conservation 25, 2309-2339.

Lebesa, L.N., Khan, Z.R., Krüger, K., Bruce, T.J.A., Hassanali, A., Pickett, J.A., 2012. Farmers' knowledge and perceptions of blister beetles, *Hycleus* spp. (Coleoptera: Meloidae), as pest herbivores of *Desmodium* legumes in western Kenya. International Journal of Pest Management 58, 165–174.

Louy, D., Habel, J.C., Abadjev, S., Rakosy, L., Varga, Z., Rödder, D., Schmitt, T., 2014. Molecules and models indicate diverging evolutionary effects from parallel altitudinal range shift in two mountain Ringlet butterflies. Biological Journal of the Linnean Society 112, 569–583.

Louy, D., Habel J.C., Abadjiev, S., Schmitt, T., 2013. Genetic legacy from past panmixia: high genetic variability and low differentiation in disjunct populations of the Eastern Large Heath butterfly. Biological Journal of the Linnean Society 110, 281-290.

Micheels, A., Eronen, J., Mosbrugger, V., 2009. The Late Miocene climate response to a modern Sahara Desert. Global and Planetary Change 67, 193-204.

Pardo Alcaide, A., 1954. Études sur les Meloidae. V. Les Mylabrini du Maroc et du Sahara occidental espagnol (Col. Meloidae). Bulletin de la Société des Sciences naturelles et physiques du Maroc 34, 55–88.

Pardo Alcaide, A., 1955. Estudios sobre Meloidae VI. Sobre algunos *Mylabris* (s. lat.) de la región etiópica. Bulletin de l'Institut royal des sciences naturelles de Belgique 31, 1–32.

Salvi, D., Maura, M., Pan, Z., Bologna, M.A., 2019. Phylogenetic systematics of *Mylabris* blister beetles (Coleoptera, Meloidae): a molecular assessment using species trees and total evidence. Cladistics 35, 243-268.

Schmitt, T., 2009. Biogeographical and evolutionary importance of the European high mountain systems. Frontiers in Zoology 6, 9.

Wu, Y., Liu, Y., & Chen, X., 2018. The complete mitochondrial genomes of *Hycleus cichorii* and *Hycleus phaleratus* (Coleoptera: Meloidae). Mitochondrial DNA Part B, 3, 159-160.

ACKNOWLEDGMENTS

“Have you ever considered the idea of doing a PhD?” Honestly? No. Never before that day in June 2015, when during an expedition in Sicily for collecting samples for my master thesis Professor Marco Bologna asked me that question. So if I am here today, writing this thesis at the end of these three years, the first person I have to thank is him, for this opportunity and his trust, patience, continuous support and for all the amazing experiences made in these years. Thank you Marco!

Then I want to thank Dr. Emiliano Mancini and Prof. Daniele Salvi for their support and help in writing this thesis (and papers!!), and for all the advices about my future.

A big thank goes to Dr. Sayeh Serri, for the scientific collaboration, but most of all for her help, guidance and friendship during our wonderfoul sampling trip to Iran, my first experience in an Islamic country. For the same reasons I would like to thank also Dr. Fariba Mozzafarian, thank you both for your being women and scientist in such a difficult moment in your country.

I would like to thank also Prof. Stefano Vanin, and all his team from Huddersfield University (UK), Dr. Giorgia Giordani, Dr. Fabiola Tuccia, Dr. Jennifer Pradelli, for their kindness and friendship, for welcoming me in their lab and for teaching me new molecular techniques that will be helpful for my future.

Thanks to my two reviewers, Prof. Pierfilippo Cerretti and Prof. Giuliana Allegrucci, for their suggestions and advices.

Thanks to my colleagues and friends for all the things we did together.

Thanks to Francesco, for these three years side by side, and for making me rediscover the beauty of being a biologist.

As always, thanks to my parents, to whom I owe everything.

And finally, thanks to Iran, that *“unties the knots”*.