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Phylogenetic systematics of *Mylabris* blister beetles (Coleoptera, Meloidae): a molecular assessment using species trees and total evidence

Daniele Salvi[a,b,*], Michela Maura[c], Zhao Pan[d] and Marco A. Bologna[c]

[a]*Department of Health, Life and Environmental Sciences, University of L'Aquila, Via Vetoio, 67100 Coppito, L'Aquila, Italy;* [b]*CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal;* [c]*Dipartimento di Scienze, Università Roma Tre, Viale G. Marconi 446, 00146 Roma, Italy;* [d]*College of Life Science, Hebei University, Wusidong Road 180, 071002 Baoding City, Hebei Province, China*

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Abstract

Mylabris is a diverse genus of Meloidae and includes over 170 species throughout the Palaearctic region, classified into 14 subgenera. The current classification is largely built on taxonomic works pre-dating the application of cladistic methods and based on a few morphological characters. In the present study, we use molecular data from mitochondrial and nuclear loci sampled across Mylabrini to assess the monophyly of *Mylabris* and its subgenera, and to identify which diagnostic morphological characters used for taxa delimitation represent synapomorphic features. We obtain a robust phylogeny which is consistent across datasets (3-, 4- and 5-gene datasets), methods (Bayesian vs. Maximum Parsimony), and approaches (species tree vs. total evidence). The genus *Mylabris* is monophyletic provided that *Pseudabris* is included and *Ammabris* is excluded. Most of the morphology-based subgenera are recovered as well-supported phylogenetic clades. Although previous classifications based on number and shape of antennomeres were confounded by convergent evolution of these traits, mesosternal and male genitalia features provided unambiguous apomorphies of Mylabrini genera and subgenera. We integrate these insights into an updated phylogenetic systematics of *Mylabris* and Mylabrini blister beetles, and we provide the description of two new subgenera, *Dvorabris* and *Pardabris*.

Introduction

The intimate connection among the evolutionary history of organisms, hierarchical phylogenetic tree and taxonomic classification has its roots in the inception of evolutionary thought and the translation of the first Tree of Life into a classification scheme (Lamarck, 1809; Darwin, 1859; Haeckel, 1866). The cladistic approach (Hennig, 1950), centred on a biological classification that reflects the evolutionary relationships (i.e. phylogeny) of organisms, has been applied since the last century. Much older is the practice of taxonomic classification, which has deep roots in human history (e.g. Stevens, 2003). Such asynchrony between early classification systems and the availability of cladistic techniques raises the question of whether pre-Hennigian classifications of organisms do reflect a cladistic hypothesis of organism's relationships. In other words, do the characters that have been used as evidence for grouping represent shared ancestry or only similarity?

Blister beetles of the genus *Mylabris* Fabricius, 1775 offer an interesting case study for testing the congruence between early classification of organisms and their evolutionary relationships (Fig. 1). This diverse genus of Meloidae includes over 170 species throughout the Palaearctic region, classified into 14

*Corresponding author:
E-mail address: danielesalvi.bio@gmail.com

Fig. 1. Mylabrini blister beetles (Coleoptera, Meloidae). First row, left to right: *Mylabris* (*Eumylabris*) *calida* (by D. Salvi), *Mylabris* (*Mylabris*) *variabilis* (by D. Salvi), *Mylabris* (*Micrabris*) *intermedia* (by P. Gorbunov: https://www.zin.ru/Animalia/Coleoptera/russ/ustyurt2.htm), *Mylabris* (*Chrysabris*) *hemprichi** (by O. Rittner). Second row, left to right: *Hycleus tricolor* (by R. Perissinotto), *Mylabris* (*Ammabris*) *elegans** (by O. Rittner), *Ceroctis capensis* (by R. Perissinotto), *Croscherichia sanguinolenta* (by O. Rittner). *: *Hycleus hemprichi and Ammabris elegans* following this study. [Colour figure can be viewed at wileyonlinelibrary.com]

subgenera (Bologna, 2008). The current classification of *Mylabris*, and of the tribe Mylabrini, is largely built on taxonomic works pre-dating the application of cladistic methods and is based on a few morphological characters. After the initial study of Billberg (1813), who included in *Mylabris* several species that now belong to various current genera of Mylabrini, de Marseul (1870, 1872) divided *Mylabris* into a few genera or subgenera based on the number and shape of antennomeres. Sumakov (1915) maintained these same taxa without criticism, but tried to single out distinct "*Mylabris*" lineages based also on the shape of metatibial spurs and the pronotum. These pioneering studies laid the foundation for two very different contemporary classifications of *Mylabris* and Mylabrini (Table 1), which arose earlier than the widespread use of cladistic methods (after Hennig, 1966). Russian specialist Kuzin (1953, 1954) maintained most of the taxonomy of Mylabrini as defined by the early monograph of de Marseul (1872) but added new genera such as *Semenovilia* and *Libycisca* (junior synonym of *Croscherichia*) and classified *Mylabris* into 12 new subgenera based mainly on the shape of antennae. In the same period, Spanish specialist Pardo Alcaide (1950, 1954, 1958) described various sections of *Mylabris*, which were largely similar to the subgenera contemporaneously described by Kuzin. Later, Pardo Alcaide (1969) described two new subgenera of *Mylabris* (*Zitunabris* and *Mauritabris*) and separated into three distinct genera (*Gorrizia,* which is now named *Hycleus*; *Ceroctis*; and *Rusadiria*, which is now named *Actenodia*) those taxa that were incorporated by Kuzin in five subgenera of *Mylabris* (*Ceroctis*, *Sphenabris*, *Euzonabris*, *Decapotoma* and *Tigrabris*), and in the genus *Coryna* (see Table 1). The new classification proposed by Pardo Alcaide (1950, 1954, 1955, 1958, 1968, 1969) primarily considered the shape of mesosternal structures and male genitalia. Both Kuzin and Pardo Alcaide pursued a non-cladistic approach of classification that did not

Table 1
Generic and subgeneric (in parentheses) classifications of the tribe Mylabrini by Kuzin (1953, 1954) and Pardo Alcaide (1954, 1955, 1958, 1968, 1969)

Kuzin (1953–1954)	Pardo Alcaide (1954–1969)	Bologna et al. (1978–2017)
Lydoceras	*	*Lydoceras*
Actenodia	*Paractenodia*	*Paractenodia*
Pseudabris	*	*Pseudabris*
Semenovilia	*	*Semenovilia*
		Xanthabris
Mylabris (*Mimesthes*)	*	*Mimesthes*
Lybicisca	*Croscherichia* (until 1960 considered as *Mylabris* (*Croscherichia*))	*Croscherichia*
Mylabris (*Ceroctis*)	*Ceroctis* (until 1958 considered as *Mylabris* (*Ceroctis*))	*Ceroctis*
Coryna (*partim*)	*Rusadiria* (until 1958 considered as *Mylabris* (*Rusadiria*))	*Actenodia*
Coryna (*partim*)	*Gorrizia* (until 1967 considered as *Mylabris* (*Gorrizia*))	*Hycleus*
Mylabris (*Sphenabris*)	*Gorrizia* sect. Mesogorbata (until 1967 considered as *Mylabris* (*Gorrizia*) sect. Mesogorbata)	*Hycleus* sect. Mesogorbatus
Mylabris (*Euzonabris*)	*Gorrizia* sect. Mesoscutata (until 1967 considered as *Mylabris* (*Gorrizia*) sect. Mesoscutata)	*Hycleus* sect. Mesoscutatus
Mylabris (*Decapotoma*) (*partim*)		
Mylabris (*Tigrabris*) (*partim*)		
Mylabris (*Decapotoma*) (*partim*)	*Gorrizia* sect. Mesotaeniata (until 1967 considered as *Mylabris* (*Gorrizia*) sect. Mesotaeniata)	*Hycleus* sect. Mesotaeniatus
Mylabris (*Tigrabris*) (*partim*)		
Mylabris (*Mylabris*)	*Mylabris* (*Mylabris*) (described in 1950 as *Mylabris* sect. Mesopunctata)	*Mylabris* (*Mylabris*)
Mylabris (*Ammabris*)	*Mylabris* (*Ammabris*) (described in 1954 as *Mylabris* sect. Mesorbata)	*Mylabris* (*Ammabris*)
Mylabris (*Glaucabris*)	*Mylabris* (*Mesosulcata*) (described in 1950 as *Mylabris* sect. Mesosulcata)	*Mylabris* (*Mesosulcata*)
Mylabris (*Micrabris*)	*Mylabris* (*Micrabris*) (described in 1950 as *Mylabris* sect. Mesolaevigata)	*Mylabris* (*Micrabris*)
Mylabris (*Eumylabris*)	*Mylabris* (*Eumylabris*) (described in 1950 as *Mylabris* sect. Mesolaevigata)	*Mylabris* (*Eumylabris*)
	Mylabris (*Mauritabris*) (described in 1950 as *Mylabris* sect. Mesolaevigata)	*Mylabris* (*Mauritabris*)
	Mylabris (*Zitunabris*) (described in 1950 as *Mylabris* sect. Mesolaevigata)	*Mylabris* (*Zitunabris*)
Mylabris (*Chalcabris*) (*partim*)	*Mylabris* (*Chalcabris*)	*Mylabris* (*Chalcabris*)
Mylabris (*Chalcabris*) (*partim*)	*Mylabris* (*Micrabris*) (*partim*)	*Mylabris* (*Micrabris*) (*partim*)
Mylabris (*Argabris*)	*	*Mylabris* (*Argabris*)
Mylabris (*Chrysabris*)	*	*Mylabris* (*Chrysabris*)
Mylabris (*Lachnabris*)	*	*Mylabris* (*Lachnabris*)
Mylabris (*Monabris*)	*	*Mylabris* (*Monabris*)
		Mylabris (*Calydabris*)

Current classification and nomenclature by Bologna and colleagues (Bologna, 1991, 2008; Bologna and Pinto, 2002; Bologna et al., 2005, 2008a,b) also is presented. Each row indicates the same taxon classified under distinct taxonomic levels (genus, subgenus and section) and with same or distinct names. The main changes among classifications are highlighted in grey.

*Pardo Alcaide's works did not consider species belonging to some genera of Mylabrini (*Lydoceras, Mimesthes*, *Pseudabris, Semenovilia,*), as well as some subgenera of *Mylabris* (*Argabris*, *Chrysabris*, *Lachnabris*, *Monabris*) described by Kuzin, but when Pardo Alcaide was interviewed on 1976 he confirmed to accept these taxa (M. Bologna, personal communication).

consider the pattern of character transformation and how this relates to the phylogenetic pattern. However, although Kuzin followed a simple phenetic approach to build homogenous groups from the standpoint of a few prominent morphological characters, the classification of Pardo Alcaide was explicitly based on characters that were supposed to reflect a phylogenetic pattern: "*les caractéristiques que nous avons sélectionnées* (…) *permettent à cause de la signification phylogénétique* (…) *la répartition des espèces dans des groupes naturels, finalité supérieure que jusqu'à présent on était loin d'atteindre* – the characteristics that we have selected (…), because of their phylogenetic significance (…), make it possible to partition species in natural groups, a superior goal that until now was far from being achieved" (Pardo Alcaide, 1954: p.58, note 3). The classification of Pardo Alcaide has been adopted substantially in the current taxonomy and classification of Mylabrini (Bologna, 1991, 2008; Bologna and Pinto, 2002; Bologna et al., 2005, 2008a, b; see Table 1).

To date, we still lack a phylogenetic assessment of the alternative classifications of *Mylabris* blister beetles. Morphological features that have been used in previous classifications cannot be used to test whether these classifications reflect the evolutionary history of

these organisms. There would be a lack of adequate resolution in such a cladistic analysis because too few features are available. Likewise, a larger number of additional morphological characters are not available to generate an independent morphological dataset (see, e.g., Bologna and Coco, 1991; Bologna et al., 2008b), certainly not for filling a proper number of columns of a matrix with a hundred or so Mylabrini taxa. Molecular data from multiple unlinked loci allow overcoming these drawbacks by providing a large set of independent characters.

In the present study, we generated a robust phylogenetic hypothesis based on a comprehensive multilocus dataset including most subgenera of *Mylabris* and related Mylabrini genera. We used both total evidence (i.e. supermatrix; see de Queiroz and Gatesy, 2007) and state of the art species tree phylogenetic approaches in order to account for stochastic sorting of genes embedded in a shared species phylogeny. The main aim of this study was to assess whether current and previous classifications of *Mylabris* correspond to a cladistic system and in particular to evaluate whether (i) the genus *Mylabris* is monophyletic; (ii) the morphology-based subgenera of *Mylabris* represent monophyletic clades; and finally (iii) to determine which diagnostic morphological characters used for taxa delimitation represent synapomorphic features. The final goal is to develop a well-founded phylogeny and classification, which reflect the evolution of this species-rich group of blister beetles.

Material and methods

Taxon sampling

The tribe Mylabrini currently includes 12 genera and within the nominal genus *Mylabris* 14 subgenera are described (Bologna, 2008; Pan et al., 2010). Our sampling covers more than 90% of these genus-level taxa. We included 155 terminals representing 55 species and ten genera of Mylabrini, plus two species of Lyttini. Within *Mylabris*, we analysed two to five species for each of 12 subgenera, plus an additional new subgenus, which is described in the Results. For each species, we analysed from one to four individuals per gene in order to use multi-individual phylogenetic methods (species tree). Specimen voucher information and GenBank accession numbers are provided in Table 2.

DNA extraction, amplification and sequencing

Specimens were stored in pure ethanol except five species for which only dry specimens from the Roma Tre University museum (Marco Bologna collection, henceforth CB) were available (see Table 2 for details). Total genomic DNA was extracted from preserved specimens using either a standard high-salt protocol (Sambrook et al., 1989) or the Qiagen DNeasy® Blood & Tissue extraction kit (Milan, Italy), following the manufacturer's protocol.

Initially, 3′ fragments of the mitochondrial cytochrome oxidase subunit I (*cox1*) gene were amplified through polymerase chain reaction (PCR) using the universal primers LCO1490 and HC02198 (Folmer et al., 1994); about half of the samples were amplified using LCO1490 and CO1MelZ2, a new reverse primer specifically designed for Meloidae. A fragment of the carbamoylphosphate synthetase domain of the nuclear *rudimentary* gene (*CAD*) was amplified using the primers CD439F and CD688R (Wild and Maddison, 2008). A region encompassing the first intron of the 60S acidic ribosomal protein P0 gene (*RpP0*) was amplified using the primers P0-5p and P0-3p (Gomez-Zurita et al., 2004). In *Hycleus polymorphus* (Pallas, 1771), we obtained shorter *RpP0* amplicons, whose sequence revealed they were intron-less pseudogene copies of the *RpP0* gene (see also Gomez-Zurita et al., 2004). For these taxa, we used the primers P0-5p and P0-3p in combination with the newly designed internal primers, RpP0mel-F and the complementary RpP0mel-R, annealing on the region of overlap between the first intron and the second exon. The entire region of the nuclear ribosomal internal transcribed spacer 2 (*ITS2*) was amplified using the primers ITS-3d and ITS-4r located on the 3′ end of the 5.8S rDNA and the 5′ end of the 28S rDNA regions, respectively (Oliverio and Mariottini, 2001). Primers and PCR cycling conditions are reported in Table 3. PCR products were performed in final volumes of 25 μL, containing 5 μL 5× reaction buffer, 3 mM of $MgCl_2$ (50 mM), 0.6 mM each dNTP, 0.4 μM each primer, 1 U of BIOTAQ DNA polymerase (™Bioline Ltd, London, UK) and approximately 50 ng of genomic DNA. The amount of magnesium ions and annealing temperatures were adjusted empirically to increase amplification yield and specificity on a case-by-case basis. Amplified products were sequenced by a commercial sequencing facility (Macrogen Inc., Seoul, Korea), employing the same primers used for amplification.

Molecular data matrix preparation

In addition to newly generated sequences of the genes *cox1*, *CAD*, *RpP0* and *ITS2*, we downloaded from GenBank 16 sequences of *ITS2* and 14 of the mitochondrial 16S rDNA gene fragment (*16S*) generated in previous studies (Bologna et al., 2005, 2008a, b; Table 2).

Table 2
Species, codes and sampling locality for the 155 specimens analysed in this study

Species	Specimen Code	Locality	*cox1*	*16S*	*CAD*	*RpP0*	*ITS2*
Actenodia chrysomelina	272	Mozambique, Tinti Gala Lodge	MH668497	–	MH668472	MH668713	–
Actenodia chrysomelina	273a	Namibia, 16 km S di Gobabis	MH668498	–	MH668445	MH668766–7	–
Actenodia chrysomelina	273b	Namibia, 16 km S di Gobabis	MH668499	–	MH668446	MH668715	–
Actenodia chrysomelina	Ach#1	Namibia	–	AJ633664‡	–	–	AJ635255‡
Actenodia denticulata	3113	Israel, Negev, Holot 'Agur,	MH668500	–	–	–	MH668642
Actenodia denticulata	705	United Arab Emirates, Sharjah, 6 Km N Mileiha	MH668501	–	MH668490	MH668742	MH668641
Actenodia denticulata	Ade 1	United Arab Emirates	–	–	–	–	AM712880‡
Actenodia distincta	2147	Italy, Sicily, Castelluzzo	MH668502	–	–	MH668687	–
Actenodia distincta	3044a	Morocco, Marrakech, Haut Atlas	MH668503	–	MH668476	MH668724	MH668603
Actenodia distincta	3044b	Morocco, Marrakech, Haut Atlas	–	–	MH668477	MH668725	MH668604
Actenodia distincta	Adi#1	Morocco	–	AJ633665‡	–	–	AJ635256‡
Ceroctis angolensis	274	Namibia, 10 km N di Aais	MH668504	–	–	MH668716	–
Ceroctis cfr.*angolensis*	692	South Africa, Free State, 27 km NW Lindney	MH668507	–	–	MH668740	–
Ceroctis cfr.*angolensis*	693	South Africa, Free State, 14 km NE Hoopstad	MH668508	–	–	MH668741	–
Ceroctis capensis	570a	South Africa, Cederberg, Sneeuberg	MH668505	–	MH668480	MH668734	MH668643
Ceroctis capensis	570b	South Africa, Cederberg, Sneeuberg	–	–	MH668481	MH668735	MH668644
Ceroctis capensis	691a	South Africa, E Cape, Candeboo N.P.	MH668506	–	–	–	MH668645
Croscherichia litigiosa	2775	Morocco, E region, near Mengoab	MH668509	–	MH668473	–	–
Croscherichia litigiosa	2777a	Morocco, E region, near Mengoab	–	–	–	MH668717	–
Croscherichia litigiosa	2777b	Morocco, E region, near Mengoab	MH668510	–	MH668474	MH668718	MH668605
Croscherichia litigiosa	2778a	Morocco, E region, near Mengoab	MH668494	–	–	–	–
Croscherichia litigiosa	2778b	Morocco, E region, near Mengoab	MH668495	–	–	–	–
Croscherichia paykulli	284	Morocco, 30 Km W Guercif	–	–	–	MH668719	–
Croscherichia paykulli	3008	Morocco, Marrakech prov.	–	–	MH668475	–	–
Croscherichia paykulli	3489a	Tunisia, Kairouan, 21 km SE Makhtar	–	–	MH668478	MH668733	–
Croscherichia paykulli	3489b	Tunisia, Kairouan, 21 km SE Makhtar	MH668496	–	–	–	–
Croscherichia paykulli	Cpa#1	Morocco	–	AJ633667‡	–	–	AJ635258‡
Hycleus deserticolus	220a	Namibia, 16 km W di Karibib	MH668511	–	–	–	MH668606
Hycleus deserticolus	220b	Namibia, 16 km W di Karibib	MH668512	–	MH668470	MH668690	MH668607
Hycleus deserticolus	221a	Namibia, 16 km W di Karibib	–	–	–	–	MH668608
Hycleus deserticolus	Hds#1	Namibia	–	AJ633662‡	–	–	AJ635253‡
Hycleus polymorphus	1970	Italy, Val d'Aosta, Gran Paradiso N. P., Valnontey	–	–	MH668469	–	MH668646

Table 2
(Continued)

Species	Specimen Code	Locality	*cox1*	*16S*	*CAD*	*RpP0*	*ITS2*
Hycleus polymorphus	858	Greece, Metsovo, Peristéri Mts	–	–	MH668492	–	MH668609
Hycleus polymorphus	861	Greece, Metsovo, Peristéri Mts	MH668513	–	–	–	–
Hycleus polymorphus	862	Greece, Metsovo, Peristéri Mts	MH668514	–	MH668456	–	–
Hycleus tripunctatus	552a	South Africa, W Cape, 5 km N Rooihoogte Pass	MH668515	–	MH668479	MH668778-9	MH668647
Hycleus tripunctatus	554a	South Africa, N Cape, Giftberg	–	–	–	MH668780-1	MH668648
*Lydoceras lictor**	Lyd	Ethiopia, Gamo Gofa prov., 20 km SE Konso	MH668597	–	–	–	–
Lydus unicolor	2976	Turkey, Tunceli, Çiçeklı	MH668516	–	–	–	–
Lydus unicolor	2996	Turkey, Elazig, 3 km N Gözeli	MH668517	–	MH668452	MH668770-1	–
Mimesthes holgaticus	730	South Africa, N Cape, Grootmis-Port Nolloth	MH668518	–	MH668491	MH668743	MH668649
Mimesthes holgaticus	Mho1	South Africa	–	AM712121‡	–	–	AM712364‡
Mimesthes maculicollis	589	South Africa, N Cape, 11 km N Carnarvon	MH668519	–	MH668482	–	MH668650
Mimesthes maculicollis	590	South Africa, N Cape, Giftberg	MH668520	–	MH668483	–	–
Mimesthes maculicollis	591	South Africa, Cape, 3–5 km NE Springbok	–	–	MH668484	–	MH668651
Mimesthes maculicollis	Mma#1	South Africa	–	AJ633666‡	–	–	AJ635257‡
Mylabris (*Ammabris*) *elegans*†	2725	Israel, W Negev, Holot Agur	MH668523	–	–	MH668714	–
Mylabris (*Ammabris*) *elegans*†	136	United Arab Emirates, Ras Al Khaimah, 23 Km from Airport	MH668521	–	MH668408	MH668676	MH668657
Mylabris (*Ammabris*) *elegans*†	2737a	Israel, W Negev, Holot Agur	MH668524	–	MH668444	MH668764-5	–
Mylabris (*Ammabris*) *elegans*†	156	Syria, Homs, 46 km NW Palmyra	MH668522	–	MH668410	MH668678	MH668617
Mylabris (*Ammabris*) *myrmidon*†	1285	Tunisia, 45 km E Douz, Wadi Raml	MH668525	–	MH668465	MH668673	MH668659
Mylabris (*Ammabris*) *raml*†	170	United Arab Emirates, Al Aïn, near airport	MH668526	–	MH668411	MH668679	MH668666
Mylabris (*Argabris*) *klugi*	1317	Iran, Abyaneh, S of Kashan	MH668527	–	MH668406	MH668674	MH668658
Mylabris (*Argabris*) *klugi*	2174	Iran, Kurdistan, Abidar	MH668528	–	MH668419	MH668688	–
Mylabris (*Argabris*) *klugi*	2175	Iran, Kurdistan, Abidar	MH668529	–	MH668420	MH668689	–
Mylabris (*Argabris*) *laticollis*	3253a	Iran, Kurdistan, Sanandaj Abidar	MH668530	–	MH668460	MH668729	–
Mylabris (*Argabris*) *laticollis*	3253b	Iran, Kurdistan, Sanandaj Abidar	MH668531	–	MH668459	MH668730	–
Mylabris (*Argabris*) *sedecimpunctata*	2861a	Kazakhstan	MH668532	–	–	–	–
Mylabris (*Argabris*) *sedecimpunctata*	2861b	Kazakhstan	MH668533	–	MH668451	MH668722	–
Mylabris (*Calydabris*) *allousei*	1318	Iran, Abyaneh, S of Kashan	MH668534	–	MH668407	MH668675	MH668611
Mylabris (*Calydabris*) *mirzayani*	2588	Iran, S Khorasan, Ferdos	MH668535	–	MH668439	MH668762-3	MH668628
Mylabris (*Chalcabris*) *cyaneovaria**	2835	Kazakhstan, Almty obl., Karkol vill. env.	MH668599	–	–	–	–
Mylabris (*Chalcabris*) *ledebouri*	2286	Russia, Altai, Krasnaja Gorka (Ortolyk)	MH668536	–	MH668423	MH668691	–
Mylabris (*Chalcabris*) *ledebouri*	2294a	Russia, Altai, Krasnaja Gorka (Ortolyk)	MH668537	–	MH668427	MH668693	MH668626
Mylabris (*Chalcabris*) *ledebouri*	2294b	Russia, Altai, Krasnaja Gorka (Ortolyk)	MH668538	–	–	MH668694	MH668627

Table 2
(Continued)

Species	Specimen Code	Locality	*cox1*	*16S*	*CAD*	*RpP0*	*ITS2*
Mylabris (*Chalcabris*) *marginata**	2836	Iran, Azerbayjan & Sharqi, 30 Km NE Myahneh	MH668600	–	–	–	–
Mylabris (*Chalcabris*) *speciosa*	2261	Russia, Altai, Amacha steppe	MH668539	–	MH668422	MH668748-9	MH668632
Mylabris (*Chrysabris*) *doriai**	2837	Iran, Kuh-e-Takht and Shan Mts., 25 Km SE Aliguadarz	MH668596	–	–	–	–
Mylabris (*Chrysabris*) *hemprichi*†	2674a	Israel, Negev, 1 km W jct Retavim	MH668540	–	–	MH668711	–
Mylabris (*Chrysabris*) *hemprichi*†	2676	Israel, Negev, 5 km NW Yeroham	MH668541	–	MH668443	MH668712	MH668618
Mylabris (*Eumylabris*) *aulica*	2364a	China, Ningxia, Ping-Luo County, Nuanquan	MH668542	–	MH668432	MH668756-7	MH668612
Mylabris (*Eumylabris*) *aulica*	2364b	China, Ningxia, Ping-Luo County, Nuanquan	MH668543	–	MH668433	MH668698	MH668613
Mylabris (*Eumylabris*) *aulica*	2365a	China, Ningxia, Ping-Luo County, Nuanquan	MH668544	–	MH668434	MH668699	–
Mylabris (*Eumylabris*) *calida*	2183a	Iran, Elburz, Taleghan	MH668545	–	MH668421	–	–
Mylabris (*Eumylabris*) *calida*	2389	China, Xinjiang, Fu-Hai County, Kalamagai	–	–	–	–	MH668652
Mylabris (*Eumylabris*) *calida*	2390	China, Xinjiang, Fu-Hai County, Kalamagai	MH668546	–	MH668435	MH668700	MH668614
Mylabris (*Eumylabris*) *calida*	872	Greece, Trikala, Kalambaka-Kozani, Agios Dimitros	MH668547	–	MH668457	MH668745	–
Mylabris (*Eumylabris*) *calida*	Mca#1	Greece	–	AJ633652‡	–	–	AJ635239‡
Mylabris (*Eumylabris*) *crocata*	1437a	Turkey, 17 km NW Nurhak, Nurhak Dağlari	–	–	–	–	MH668653
Mylabris (*Eumylabris*) *crocata*	1437b	Turkey, 17 km NW Nurhak, Nurhak Dağlari	–	–	–	–	MH668654
Mylabris (*Eumylabris*) *crocata*	2337a	China, Xinijang, Mu-Lei County, Diwopu	MH668548	–	MH668430	MH668697	MH668655
Mylabris (*Eumylabris*) *crocata*	2337b	China, Xinijang, Mu-Lei County, Diwopu	–	–	–	–	MH668656
Mylabris (*Eumylabris*) *crocata*	2338a	China, Xinijang, Mu-Lei County, Diwopu	MH668549	–	MH668431	MH668754-5	–
Mylabris (*Eumylabris*) *impressa*	487	Morocco, Forêet de Mamora	MH668550	–	MH668468	MH668776-7	–
Mylabris (*Lachnabris*) *mannerheimii**	3467	Kyrgyzstan, 14 km NW Taldy Bulak, Cheleke	MH668601	–	–	–	–
Mylabris (*Mauritabris*) *abdelkaderi*	128	Morocco, 31 Km N Marrakech	MH668551	–	MH668405	MH668672	MH668610
Mylabris (*Mauritabris*) *abdelkaderi*	3259a	Morocco	MH668552	–	–	–	–
Mylabris (*Mauritabris*) *abdelkaderi*	3259b	Morocco	MH668553	–	–	–	–
Mylabris (*Mauritabris*) *tenebrosa*	176	Morocco, Rich env.	MH668554	–	MH668413	MH668681	MH668667
Mylabris (*Mesosulcata*) *hieracii**	3469	Spain, Sierra de Guadarrama, Fuente Cossia	MH668493	–	–	–	–
Mylabris (*Mesosulcata*) *hieracii*	Mhi#1	Spain	–	AJ633654‡	–	–	AJ635241‡
Mylabris (*Mesosulcata*) *hirtipennis*	806	Morocco, Tizi-n-Test	–	–	–	–	MH668619
Mylabris (*Mesosulcata*) *hirtipennis*	2024a	Morocco, Taroudant, S Haut Atlas	–	–	–	–	MH668622

Table 2
(Continued)

Species	Specimen Code	Locality	*cox1*	*16S*	*CAD*	*RpP0*	*ITS2*
Mylabris (*Mesosulcata*) *hirtipennis*	2024b	Morocco, Taroudant, S Haut Atlas	–	–	–	–	MH668623
Mylabris (*Mesosulcata*) *hirtipennis*	2514	Morocco, Haut Atlas, Taroudant, NE Missirat	MH668555	–	MH668436	MH668702	MH668620
Mylabris (*Mesosulcata*) *hirtipennis*	2515	Morocco, Haut Atlas, Taroudant, NE Missirat	MH668556	–	MH668437	MH668703	–
Mylabris (*Mesosulcata*) *hirtipennis*	3046	Morocco, Marrakech prov.	–	–	–	–	MH668621
Mylabris (*Micrabris*) *connata*	1995a	Italy, Val Susa, Oulx San Marco	MH668557	–	MH668417	MH668685	MH668615
Mylabris (*Micrabris*) *connata*	1995b	Italy, Val Susa, Oulx San Marco	MH668558	–	MH668416	MH668686	MH668616
Mylabris (*Micrabris*) *frolovi*	3250a	Iran, N Khorasan, 5 Km E Kadkan, Sorb Mts	MH668559	–	MH668454	MH668726	–
Mylabris (*Micrabris*) *frolovi*	3270	Iran, Māzandarān, Elburz Mts., S slope Danavand Mt	MH668560	–	MH668458	MH668731	–
Mylabris (*Micrabris*) *frolovi*	3271	Iran, Māzandarān, Elburz Mts., Gachsar, Azadbar	MH668561	–	–	MH668732	–
Mylabris (*Micrabris*) *intermedia*	2302a	China, Xinijang, Mu-Lei County, Diwopu	MH668562	–	MH668428	MH668695	MH668624
Mylabris (*Micrabris*) *intermedia*	2302b	China, Xinijang, Mu-Lei County, Diwopu	MH668563	–	MH668429	MH668696	MH668625
Mylabris (*Micrabris*) *obsoleta*	1168a	Italy, Calabria, Castrovillari, Colloreto	MH668564	–	MH668403	MH668670	MH668629
Mylabris (*Micrabris*) *obsoleta*	1168b	Italy, Calabria, Castrovillari, Colloreto	MH668565	–	MH668404	MH668671	MH668630
Mylabris (*Micrabris*) *obsoleta*	Mob#1	Italy	–	AJ633656‡	–	–	AJ635243‡
Mylabris (*Micrabris*) *pusilla*	2656a	Italy, Abruzzo, Abruzzo N.P., Valico Acerella	MH668566	–	MH668441	MH668709	–
Mylabris (*Micrabris*) *pusilla*	2656b	Italy, Abruzzo, Abruzzo N.P., Valico Acerella	MH668567	–	MH668442	MH668710	MH668631
Mylabris (*Micrabris*) *pusilla*	Mpu#1	Italy	–	–	–	–	AJ635244‡
Mylabris (*Micrabris*) *splendidula*	2290a	Russia, Altai, Krasnaja Gorka (Ortolyk)	MH668568	–	MH668424	MH668750-1	MH668634
Mylabris (*Micrabris*) *splendidula*	2290b	Russia, Altai, Krasnaja Gorka (Ortolyk)	MH668569	–	MH668425	MH668752-3	–
Mylabris (*Micrabris*) *splendidula*	2292	Russia, Altai, Krasnaja Gorka (Ortolyk)	MH668570	–	MH668426	MH668692	–
Mylabris (*Mylabris*) *olivieri*	1406a	Turkey, K.Maraş, Göksun	MH668571	–	MH668409	MH668677	–
Mylabris (*Mylabris*) *olivieri*	1775a	Turkey, Kirşehir, Boztepe	MH668572	–	MH668414	MH668682	MH668661
Mylabris (*Mylabris*) *olivieri*	1775b	Turkey, Kirşehir, Boztepe	MH668573	–	MH668415	MH668683	MH668662
Mylabris (*Mylabris*) *olivieri*	1776	Turkey, Kirşehir, Boztepe	–	–	–	–	MH668660
Mylabris (*Mylabris*) *quadripunctata*	909a	Greece, Lamia, Lamia-Bralos, pass	–	–	–	–	MH668664
Mylabris (*Mylabris*) *quadripunctata*	909b	Greece, Lamia, Lamia-Bralos, pass	–	–	–	–	MH668665
Mylabris (*Mylabris*) *quadripunctata*	910a	Greece, Lamia, Lamia-Bralos, pass	–	–	–	–	MH668663

Table 2
(Continued)

Species	Specimen Code	Locality	*cox1*	*16S*	*CAD*	*RpP0*	*ITS2*
Mylabris (*Mylabris*) *quadripunctata*	1739	Turkey, Adana, 10 km E Tufanbeyli	MH668574	–	MH668412	MH668680	–
Mylabris (*Mylabris*) *quadripunctata*	1904a	Italy, Bari, 3 km E Gravina in Puglia	MH668575	–	MH668464	MH668684	–
Mylabris (*Mylabris*) *quadripunctata*	2591	Iran, Khorasan, Mashhad	MH668576	–	MH668463	MH668707	–
Mylabris (*Mylabris*) *quadripunctata*	Mqu#1	Greece	–	AJ633657‡	–	–	AJ635245‡
Mylabris (*Mylabris*) *variabilis*	840a	Greece, Konitsa, W Timfi, 1 km W Pápigo	–	–	–	–	MH668635
Mylabris (*Mylabris*) *variabilis*	841b	Greece, Konitsa, W Timfi, 1 km W Pápigo	–	–	–	–	MH668637
Mylabris (*Mylabris*) *variabilis*	2554a	Italy, Liguria, Sassello, Erro	MH668577	–	MH668438	MH668706	–
Mylabris (*Mylabris*) *variabilis*	2616a	Iran, Golestan, Azadshahr	MH668578	–	MH668440	MH668708	–
Mylabris (*Mylabris*) *variabilis*	841a	Greece, Konitsa, W Timfi, 1 km W Pápigo	MH668579	–	MH668455	MH668744	MH668636
Mylabris (*Mylabris*) *variabilis*	Mva#1	Greece	–	AJ633658‡	–	–	AJ635246‡
Mylabris (*Spinabris*) *spinungulata*†	2856	China, Xinjiang, Qitai County, Xibeiwan	MH668580	–	MH668448	MH668720	MH668633
Mylabris (*Zitunabris*) *interrupta*†	3239	Iran, Lorestan, Zagros Mts., road Aligūdarz-Darreye-Taht	MH668582	–	MH668453	MH668774-5	–
Mylabris (*Zitunabris*) *oleae*†	2518	Morocco, Haut Atlas, Taroudant, NE Missirat	–	–	MH668447	MH668704	–
Mylabris (*Zitunabris*) *oleae*†	2519	Morocco, Haut Atlas, Taroudant, NE Missirat	–	–	–	MH668705	–
Mylabris (*Zitunabris*) *oleae*†	2520	Morocco, Haut Atlas, Taroudant, NE Missirat	–	–	–	MH668760-1	–
Mylabris (*Zitunabris*) *oleae*†	199	Tunisia, Sidi Bouzid, 15 Km E Maknassy	MH668583	–	MH668466	MH668746-7	–
Mylabris (*Zitunabris*) *oleae*†	200	Tunisia, Sidi Bouzid, 15 Km E Maknassy	MH668584	–	MH668418	–	–
Mylabris (*Zitunabris*) cfr.*oleae*†	2799	Morocco, road 14	MH668602	–	–	–	–
Mylabris (*Zitunabris*) *suturalis*†	2857	Turkey, Tunceli, 10 km E Kırkarya	MH668585	–	MH668449	MH668768-9	–
Mylabris (*Zitunabris*) *suturalis*†	2858	Turkey, Tunceli, 10 km E Kırkarya	MH668586	–	MH668450	MH668721	–
Mylabris (*Zitunabris*) *suturalis*†	2961	Turkey, Tunceli, 10 km E Kırkarya	MH668587	–	–	MH668723	–
Mylabris maceki†	3251a	Iran, Kurdistan, Divandareh, Chehlchesme	MH668581	–	MH668462	MH668727	–
Mylabris maceki†	3251b	Iran, Kurdistan, Divandareh, Chehlchesme	–	–	MH668461	MH668728	–
Oenas crassicornis	318b	Greece, Atiki, Erythres-Thiva	MH668588	–	MH668467	MH668772-3	–
Oenas crassicornis	Ocr#1	Greece	–	AJ633672‡	–	–	AJ635263‡
Paractenodia namaquaensis	594a	Namibia, 45 km S Rosh Pinah	MH668589	–	MH668485	MH668736	MH668638
Paractenodia namaquaensis	594b	Namibia, 45 km S Rosh Pinah	MH668590	–	MH668486	MH668737	MH668639
Paractenodia namaquaensis	599a	Namibia, 31 km S Karasburg, Ortmaunsbaum	MH668591	–	MH668487	MH668738	MH668640

Table 2
(Continued)

Species	Specimen Code	Locality	*cox1*	*16S*	*CAD*	*RpP0*	*ITS2*
Paractenodia namaquaensis	Pna1	South Africa	–	AM712120‡	–	–	AM712363‡
Paractenodia parva	603	South Africa, N Cape, 13 km N Steinkopf	MH668592	–	MH668488	MH668739	MH668668
Paractenodia parva	604	Namibia, 20 km S Aus	MH668593	–	MH668489	–	MH668669
Paractenodia parva	Ppa1	Namibia	–	AM712119‡	–	–	AM712362‡
Pseudabris hingstoni†	2483	China, Tibet, Chengg Guan, Lhasa, Tiantang	MH668594	–	MH668471	MH668701	–
Pseudabris longiventris†	2500	China, Tibet, Chengg Guan, Lhasa, Tiantang	MH668595	–	–	MH668758-9	–
*Xanthabris baluchistanica**	Xan	Pakistan, Baluchistan, Nushki	MH668598	–	–	–	–

GenBank accession numbers for *cox1*, *16S*, *CAD*, *RpP0* and *ITS2* sequences are provided.
*Species for which only dry museum specimens were available.
†Species that underwent taxonomic change following this study.
‡GenBank data from previous studies.

Gene fragments including protein-coding regions (*cox1*, *CAD* and *RpP0*) were aligned with Clustal Omega (Sievers et al., 2011). Ribosomal regions (*ITS2* and *16S*) were aligned with MAFFT v.7 (Katoh and Standley, 2013) using the E-INS-i iterative refinement algorithm, and then, ambiguous and poorly aligned positions were removed by Gblocks v.0.91b (Castresana, 2000) using a relaxed selection of blocks (Talavera and Castresana, 2007). A total of 403 positions were retained in the ITS2 alignment by Gblocks (38% of the original 1054 positions).

Haplotype reconstruction for nuclear gene fragments (*CAD*, *RpP0* and *ITS2*) was performed in PHASE v.2.1 (Stephens et al., 2001; Stephens and Scheet, 2005), with 100 iterations, a phase probability threshold of 0.5 and the remaining settings by default. Input files were created in SEQPHASE (Flot, 2010; available at http://seqphase.mpg.de/seqphase/). In 18 individuals, heterozygous *RpP0* alleles defined from insertion/deletions were manually phased (GenBank accession numbers MH668746-MH668781) and incorporated as known phases to improve haplotype determination following Flot et al. (2006). PHASE was run three times to assure consistency. The absence of recombination for each phased nuclear gene dataset was verified using the Pairwise Homoplasy Index (PHI) test (Bruen et al., 2006) implemented in SPLITSTREE v.4 (Huson and Bryant, 2006).

In order to assess the effect of loci with more than 10% of missing data (i.e. *16S* and *ITS2* loci; see Table 2 for missing sequences at these loci) on phylogenetic inference, we ran preliminary analyses using three different datasets: a 3-gene dataset (*cox1*, *CAD* and *RpP0*), a 4-gene dataset (*cox1*, *16S*, *CAD* and *RpP0*) and a 5-gene dataset (*cox1*, *16S*, *CAD*, *RpP0* and *ITS2*). Because results were consistent across runs, we used the full amount of data available (the 5-gene dataset) in downstream phylogenetic analyses. For total evidence analyses, we used one sequence for each species (52 terminals) from mitochondrial and unphased nuclear alignments. Sequences of the five gene regions were concatenated into a single matrix. For species tree analyses, we used all sequences available for each of the 52 species (from one to eight sequences per species) from mitochondrial and phased nuclear alignments. Gene alignments were entered individually in the species tree analysis.

Species tree phylogenetic reconstruction

Molecular phylogenetic methods based on multiple loci have recently moved from a gene-tree perspective—where the phylogenetic history of any gene region is assumed to be the same as the phylogenetic history of the study species—to a species-tree perspective—where individual gene phylogenies are used to estimate the species phylogeny.

Table 3
Name, sequence (I: inosine) and reference of the primers, and the amplification conditions for the genes used in this study

Gene	Primer Name	Primer Sequence (5′-3′)	Reference	PCR conditions (°C (s), (…) × Number of cycles
cox1	LCO1490	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer et al. (1994)	LCO1490/HC02198 and LCO1490/CO1MelZ2R: 94 °C (180 s), (94 °C (60 s), 46 °C (90 s), 72 °C (90 s)) ×5, (94 °C (60 s), 50 °C (90 s), 72 °C (90 s)) ×35, 72 °C (600 s); alternatively: 94 °C (180 s), (94 °C (60 s), 48 °C (45 s), 72 °C (60 s)) ×35, 72 °C (600 s)
	HC02198	TAA ACT TCA GGG TGA CCA AAA AAT CA		
	CO1MelZ2R	GGG TCA AAG AAR GAT GTA TT	This study	
CAD	CD439F	TTC AGT GTA CAR TTY CAY CCH GAR CAY AC	Wild and Maddison (2008)	94 °C (180 s), (94 °C (30 s), 58↓0.4 °C * (35 s), 72 °C (150 s)) ×20, (94 °C (30 s), 55 °C (35 s), 72 °C (150 s)) ×20, 72 °C (600 s); alternatively: 94 °C (180 s), (94 °C (30 s), 56 °C (45 s), 72 °C (60 s)) ×35, 72 °C (600 s)
	CD688R	TGT ATA CCT AGA GGA TCD ACR TTY TCC ATR TTR CA		
RpP0	P0-5p	ATG GGT AGG GAG GAC AAI GCI ACI TGG	Gomez-Zurita et al. (2004)	P0-5p/P0-3p: 94 °C (180 s), (94 °C (30 s), 58↓0.4 °C * (35 s), 72 °C (150 s)) ×20, (94 °C (30 s), 55 °C (35 s), 72 °C (150 s)) ×20, 72 °C (600 s)
	P0-3p	GCD ATI GCI CCI GIA CGR GCY GGI G		
	RpP0myl-R	GCT GYA AAC AAA ATA CGR ACA AT	This study	P0-5p/RpP0myl-R and RpP0myl-F/RpP0myl-R: 94 °C (180 s), (94 °C (30 s), 52 °C (45 s), 72 °C (45 s)) ×35, 72 °C (600 s)
	RpP0myl-F	ATT GTY CGT ATT TTG TTT RCA GC		
ITS2	ITS-3d	GCA TCG ATG AAG AAC GCA GC	Oliverio and Mariottini (2001)	94 °C (180 s), (94 °C (60 s), 55 °C (90 s), 72 °C (90 s)) ×35, 72 °C (600 s)
	ITS-4r	AGT TTY TTT CCT CCG CTT AT		

*Touchdown protocol decreasing 0.4 °C each cycle.

The species tree approach allows reconciling a set of gene trees embedded in a shared species phylogeny by taking into account the stochastic sorting of lineages in gene trees (e.g. Edwards et al., 2007; Liu and Pearl, 2007). Species tree methods have been found to be superior to the sequence concatenation approach (total evidence) in estimating the species phylogeny (Kubatko and Degnan, 2007; Heled and Drummond, 2010). Here, we explored the data using the species tree and two gene tree approaches and consider as robust those results that are consistent across different approaches.

We used the multispecies coalescent method implemented in the *BEAST extension of BEAST v.1.8.4 (Drummond et al., 2012) to estimate the species tree of Mylabrini. Gene trees were *unlinked*, with the exception of the trees of the mitochondrial genes *cox1* and *16S* because these genes are genetically linked. In order to have a rough timescale of the cladogenetic events, the species tree was calibrated using relaxed assumptions of clock model (Uncorrelated Lognormal Clock) and a lognormal prior (mean in real space = 0.0178, stdev = 0.075) on the *cox1* clock rate based on the available substitution rate estimated by Papadopoulou et al. (2010) in Coleoptera Tenebrionidae (which is a family close to Meloidae; e.g. Hunt et al., 2007; Boussau et al., 2014; Du et al., 2016). The remaining settings were as follows: (*unlinked*) models of nucleotide substitution for each gene partition as selected in Partition Finder v.1.1.1 (Lanfear et al., 2012) under the corrected Akaike Information Criterion (AICc); and Yule process as species tree prior. *BEAST was run three times (for each of the 3-gene, 4-gene and 5-gene datasets), with 450 million generations, sampling every 70 000 generations. We used Tracer v.1.6 (Rambaut et al., 2014) to check the runs for convergence (burn-in = 25%), LogCombiner and TreeAnnotator (both included in the BEAST package) to combine runs and summarize the trees in a Maximum Clade Credibility Tree representing the posterior distribution. In order to assess the robustness of the species tree inference to prior choice and interactions between priors, we performed (i) different runs using alternative prior settings (such as strict clock models, fixed *cox1* rate, Birth-Death tree prior, in-group monophyly enforced) and (ii) an additional *BEAST analysis without data sampling from the prior only. From this, (i) we obtained similar results comparing runs with different prior settings and (ii) verified that the prior alone is not driving the results (i.e. is not overwhelming the posterior distribution).

Total evidence phylogenetic reconstruction

Total evidence analyses were conducted using both Bayesian and Parsimony approaches. Bayesian inference analyses were performed in BEAST with all gene trees *linked*; *unlinked* models of nucleotide substitution for each gene partition; and Yule process as tree prior. BEAST was run three times, with 100 million generations, sampling every 10 000 generations. Remaining settings and procedure were the same as in the *BEAST analysis.

Maximum-parsimony (MP) analyses were performed using TNT v.1.5 (Goloboff et al., 2008) with gaps transformed into missing data. MP tree searches were carried out using either equal weights for all characters or using the extended implied weighting (Goloboff, 2014) in which gene partitions are weighted according to their average homoplasy while accounting for missing entries (*xpiwe(** command in TNT, with concavity constant $k = 3$). *Lydus unicolor* Reitter, 1887 (Lyttini) was used as outgroup (Bologna et al., 2008a). Traditional search parameters were specified as follows: initial addseqs = 1000, tree saved per replication = 1000, swapping algorithm = TBR. Nodal support was assessed by standard bootstrap analysis with Traditional search and 1000 replicates.

Results

In total, 615 new sequences were generated in this study (phased data; Table 2). Sequences from multiple genes were obtained for all species except for those five represented only by museum dried specimens for which it was possible to obtain only the *cox1* sequence. These five species were excluded from the multilocus analyses, and their phylogenetic position

Table 4
Number of sequences for each gene fragment, length of alignments (bp, base pairs), models of nucleotide substitution and number of variable positions for the datasets with and without outgroup (in parenthesis)

Gene	N° Seq.	Alignment length (bp)	Substitution Model	Variable positions total evidence dataset	Variable positions species tree dataset
cox1	110	561	HKY+I+G	238 (235)	245 (242)
16S	17	566	HKY+G	201 (181)	201 (181)
CAD	90*	560	HKY+I+G	303 (294)	317 (310)
RpP0	94*	782	HKY+I	252 (236)	266 (253)
ITS2	83*	403	HKY+I+G	196 (193)	202 (199)

*Unphased nuclear data.

was estimated in a Bayesian *cox1* tree obtained in BEAST (see Appendix S1 for further details). The number of sequences, multiple sequence alignment length, models of sequence evolution and number of variable positions are reported for each gene in Table 4. This is the largest molecular dataset of Meloidae, and with the most comprehensive taxon set of Mylabrini, so far generated.

Species tree phylogeny

The species tree of Mylabrini is well-resolved and shows overall high levels of nodal support for subgenus-level clades, whereas relationships between subgenera and genera are poorly resolved (Fig. 2). A clade was considered to be supported if it had bootstrap support values (BS) between 80 and 90 and Bayesian Posterior Probability (BPP) between 0.95 and 0.98, and highly supported for values BS > 90 and BPP > 0.98. Most *Mylabris* species (29) cluster in a monophyletic clade including also *Pseudabris longiventris* and *P. hingstoni* (BPP = 0.90). Four *Mylabris* species of the subgenera *Ammabris* (*M. raml*, *M. elegans* and *M. myrmidon*) and *Chrysabris* (*M. hemprichi*) are not included in this clade and show closer relationships with taxa belonging to other genera of Mylabrini. *Mylabris* (*Ammabris*) species form a monophyletic clade with species of the genus *Actenodia* (BPP = 1), whereas *M.* (*Chrysabris*) *hemprichi* is deeply nested in a clade including species of the genera *Hycleus*, *Ceroctis* and *Paractenodia* (BPP = 1). A fourth clade is represented by species of the genera *Mimesthes* and *Croscherichia* (BPP = 1). These two latter genera and *Paractenodia* are monophyletic and well supported (BPP = 1), whereas species of the genera *Actenodia*, *Hycleus* and *Ceroctis* do not form monophyletic assemblages.

Within the *Mylabris* clade, seven subgenera are monophyletic and strongly supported (*Micrabris*, *Mylabris*, *Calydabris*, *Chalcabris*, *Eumylabris*, *Argabris* and *Mesosulcata*; BPP = 1 in all cases) and two subgenera (*Mauritabris* and *Zitunabris*) are not monophyletic due to the nested position of *M.* (*Zitunabris*) *oleae* within the *Mauritabris* clade. The single species analysed of the subgenus *Spinabris* (*M. spinungulata*) shows a close relationship with species of the subgenus *Eumylabris* (BPP = 1), whereas the species *M. maceki* is not included in any of these subgenus-level clades. Phylogenetic relationships among subgenus-level clades are partially resolved. The subgenera *Micrabris*, *Mylabris* and *Calydabris* show a close, although unresolved, relationship (BPP = 1). These clades, together with *Chalcabris*, *Pseudabris*, *Mauritabris* and *Zitunabris*, form a distinct lineage within the *Mylabris* clade (BPP = 0.83).

The inspection of the gene trees' topologies co-estimated within the species tree indicates that sequences of individuals morphologically identified as the same species constitute well-supported monophyletic clades at all loci with a few rare exceptions. These concern one (*CAD* and *ITS2* gene trees) or two (mtDNA and *RpP0* gene trees) instances of incomplete allele sorting between closely related species (see also Appendix S1).

The time estimated for the early cladogenetic events leading to the four main lineages of Mylabrini coincides with the early Miocene (from 20 to 18 million years ago, Ma), whereas subgenera diversification within *Mylabris* occurred mostly during the middle Miocene (15–12 Ma).

Total evidence phylogeny

The Bayesian tree estimated using the concatenated matrix (Fig. 3a) is almost identical to the species tree (Fig. 2). The same *Mylabris* clade is recovered (BPP = 0.98) and is composed of 11 supported subclades corresponding to: the subgenera *Micrabris*, *Mylabris*, *Calydabris*, *Chalcabris, Eumylabris*, *Argabris* and *Mesosulcata* (BPP = 1); the species of the genus *Pseudabris* (BPP = 1); the paraphyletic clades of *Mauritabris* and *Zitunabris*; and the branches of *Spinabris* (sister to *Eumylabris*; BPP = 1) and *M. maceki*. The subgenera *Micrabris*, *Mylabris* and *Calydabris* form a well-supported clade (BPP = 1) as well as *Chalcabris* with species of the genus *Pseudabris* (BPP = 0.95). These clades, together with *Mauritabris* and *Zitunabris*, form a well-supported lineage within the *Mylabris* clade (BPP = 0.98). Phylogenetic support for the *Mylabris* clade and the *Chalcabris*+*Pseudabris* clade is therefore higher than in the species tree. Lower support is received by the sister relationship between *Eumylabris* and *Argabris* (BPP = 0.93) and between them and *M. maceki* (BPP = 0.93). *Mylabris* species of the subgenera *Ammabris* and *Chrysabris* are sister to species of the Mylabrini genera *Actenodia* and *Hycleus,* respectively. Besides *Mylabris*, the Mylabrini genera are grouped in the same three clades as in the Bayesian tree analysis and form either monophyletic (*Paractenodia*, *Mimesthes* and *Croscherichia*; BPP = 1) or nonmonophyletic (*Actenodia*, *Hycleus* and *Ceroctis*) assemblages.

Maximum-parsimony searches under equal weighting or extended implied weighting yielded identical topologies for the main clades and lineages (coded as A–P in Fig. 2) compared with the Bayesian trees, but differences regarding the support of certain clades were found (Figs 2 and 3a,b). Parsimony bootstrap values were high (BS > 80) for all monophyletic groups representing the subgenera of *Mylabris* and the genera *Pseudabris, Paractenodia*, *Mimesthes* and *Croscherichia*, whereas deeper nodes received low (BP < 50) statistical support (Fig. 3b). Differences between the three shortest trees obtained from unweighted MP searches (tree score = 5479, CI = 0.357, RI = 0.499)

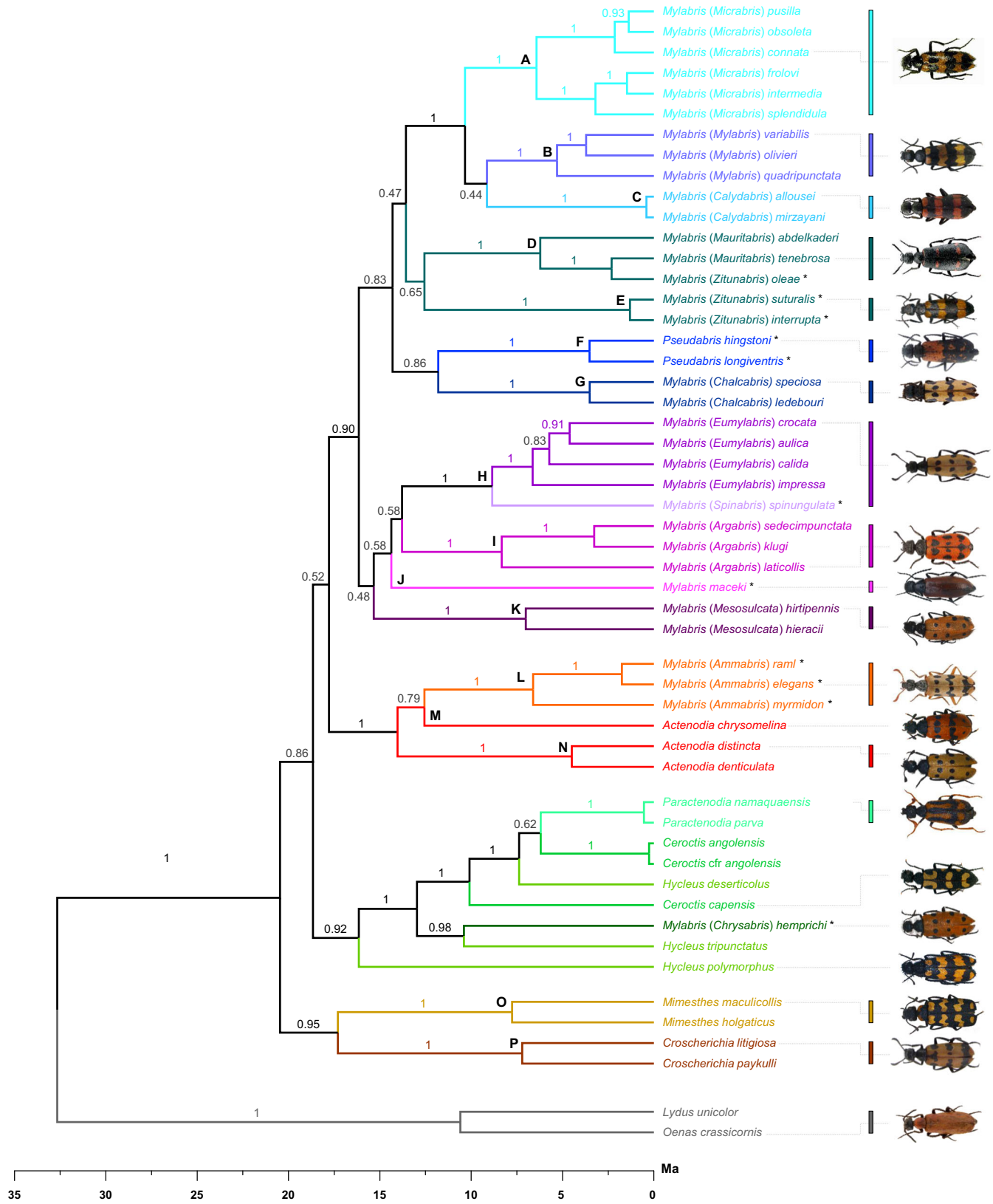

Fig. 2. Species tree phylogeny of Mylabrini based on *cox1*, *16S*, *RpP0*, *CAD* and *ITS2* DNA sequence data. Node support (Bayesian posterior probability, BPP) is reported above branches. Below the tree is represented the time axis, in million years (Ma), according to the relaxed clock model implemented (see text for further details). Asterisks indicate species that underwent taxonomic changes following this study (see Discussion). Clades and lineages are discussed and coded with letters in Table 5. [Colour figure can be viewed at wileyonlinelibrary.com]

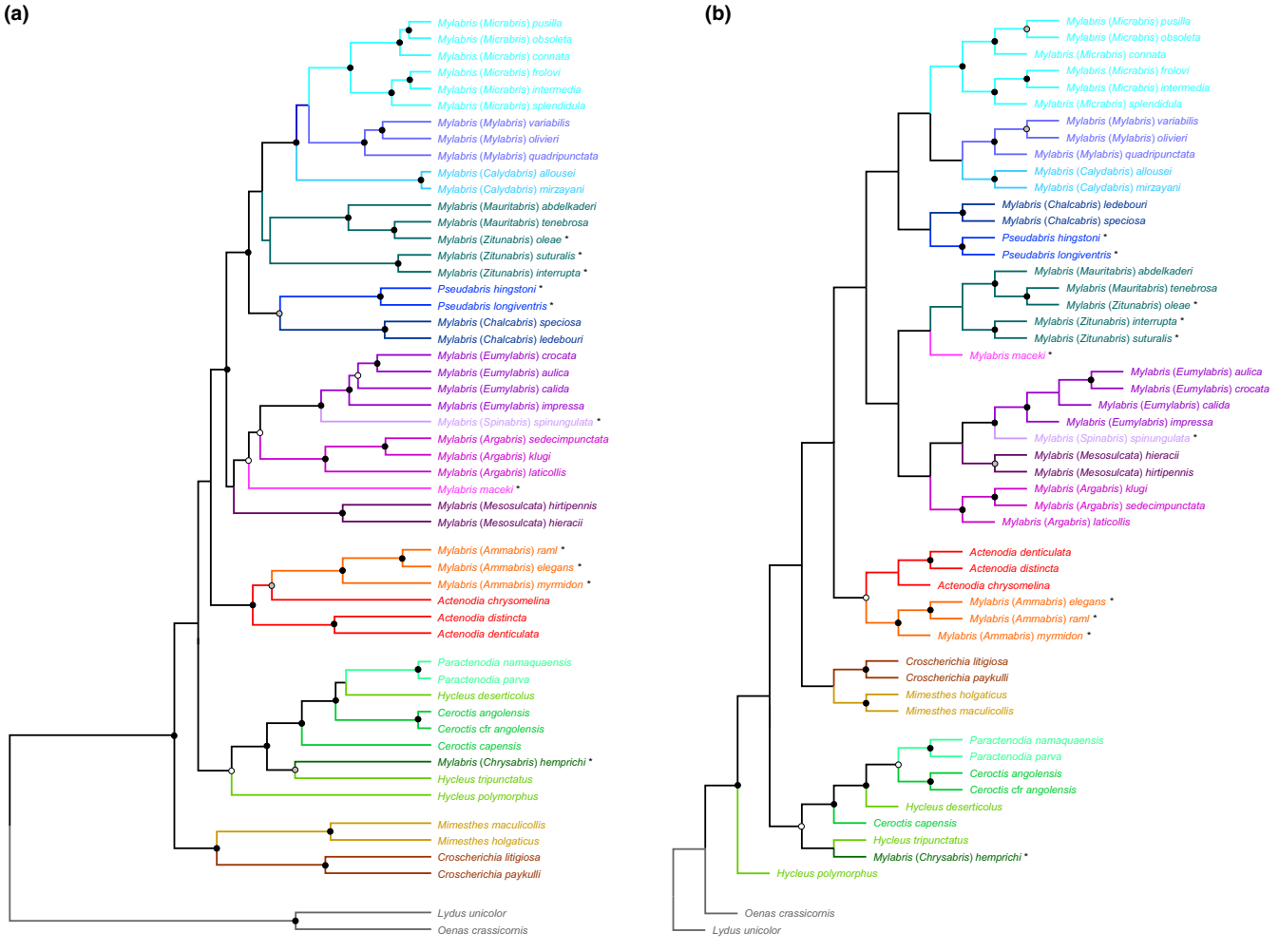

Fig. 3. Bayesian (a) and Maximum-parsimony (b) phylogenetic trees based on the total evidence approach (concatenated *cox1*, *16S*, *RpP0*, *CAD* and *ITS2* DNA sequence data). Black dots in correspondence of nodes represent very high node support (Bayesian posterior probability, BPP ≥ 0.98; bootstrap support, BS ≥ 90), grey dots represent high support (0.95 ≤ BPP < 0.98; 80 ≤ BS < 90), and white dots represent low support (0.90 ≤ BPP < 0.95; 50 < BS < 80). Asterisks indicate species that underwent taxonomic changes following this study (see Discussion). [Colour figure can be viewed at wileyonlinelibrary.com]

and the most parsimonious tree found by the extended implied weighting analysis rely on a few unsupported interclade relationships.

Description of new subgenera

Based on phylogenetic results, we describe two new subgenera of *Mylabris*: *Dvorabris* for *Mylabris maceki* and *Pardabris* for the species *M.* (*Zitunabris*) *suturalis* and *M.* (*Zitunabris*) *interrupta*. See the section '*Taxonomic and nomenclatural change*' for further discussion.

Mylabris (*Dvorabris*) Bologna **subgen.n**

Type species. *Croscherichia maceki* Dvořák, 1985, by present designation.

This species was transferred from the genus *Croscherichia* to *Mylabris* by Bologna and Coco (1991) and confirmed in *Mylabris* by Bologna and Pinto (2002).

Material examined. The species was described from E Iraq. We examined five paratypes housed at the Natural History Museum of Bratislava (Slovakia), two paratypes housed at the CB, Roma Tre University museum (Italy), and 11 additional specimens from NW Iran (new for this country): (i) Kordestan, 15 km N Kamyaran, 34°53′N-46°57′E, 1800 m a.s.l., 10.v.2008, G. Sama (2 exx. CB; 5 exx. Museo civico di Storia Naturale 'G. Doria', Genova, Italy); (ii) Kordestan, Divandarreh, Chehlchesme, 35°50′5N–46°33′05.7E, 2137 m a.s.l., vii.2014, S. Amjodi (4 exx. at CB and the Iranian Research Institute of Plant

Protection, Hayk Mirzayans Insect Museum, Teheran).

Diagnosis. A mylabrine taxon confused in the past with the genus *Croscherichia* because of hind outer metatibial spur spatulate, but clearly referable to the genus *Mylabris* based on the presence of wide anteriorly modified portion of mesosternum ('scutum'), and highly distinct mesosternal suture, both structures lacking in *Croscherichia*. The new subgenus is differentiated from all described subgenera of *Mylabris* by the unique spatulate metatibial outer spur, aedeagus with apical portion suddenly widened and subrectangular, male gonoforceps cylindrical but recurved at its apex and very elongate last antennomere, particularly in males.

Description. The description of the type species is very detailed (Dvořák, 1985) and greatly overlaps the subgeneric characteristic. We synthesize here the characters listed in this description adding some new distinctive features.

Middle sized (15–25 mm) (Fig. 4a,c), integuments black without metallic reflections and elytra uniformly brown-ochre, quite shiny; body setation entirely black; head and pronotum punctures distinct, denser and deeper on head; elytral surface with shiny but not raised longitudinal venations, and with dense but fine punctures. The sexual dimorphism concerns the shape of temples, antennae, forelegs and last abdominal ventrites.

Head subquadrate in male and more subtrapezoidal in female, temples slightly widened posteriorly to eyes; eye scarcely concave on fore margin near antennal base, not bulging and only slightly wider than temples; mandibles curved and almost completely covered by labrum, which is slightly sinuate on the fore margin. Male maxillary and labial palpomeres not distinctly modified. Antennae very elongate composed of 11 antennomeres: I elongate and widened apically, II short, III–X subcylindrical and slightly widened apically, III longer than IV, IV–X progressively slightly longer, XI *c*.1.5× length of X in female and *c*.2× length of X in male, apical third tapered and slender, particularly in male.

Pronotum about as wide as maximal width of the head, subtrapezoidal, slightly widening from base to the middle, distinctly narrowing in the fore half,

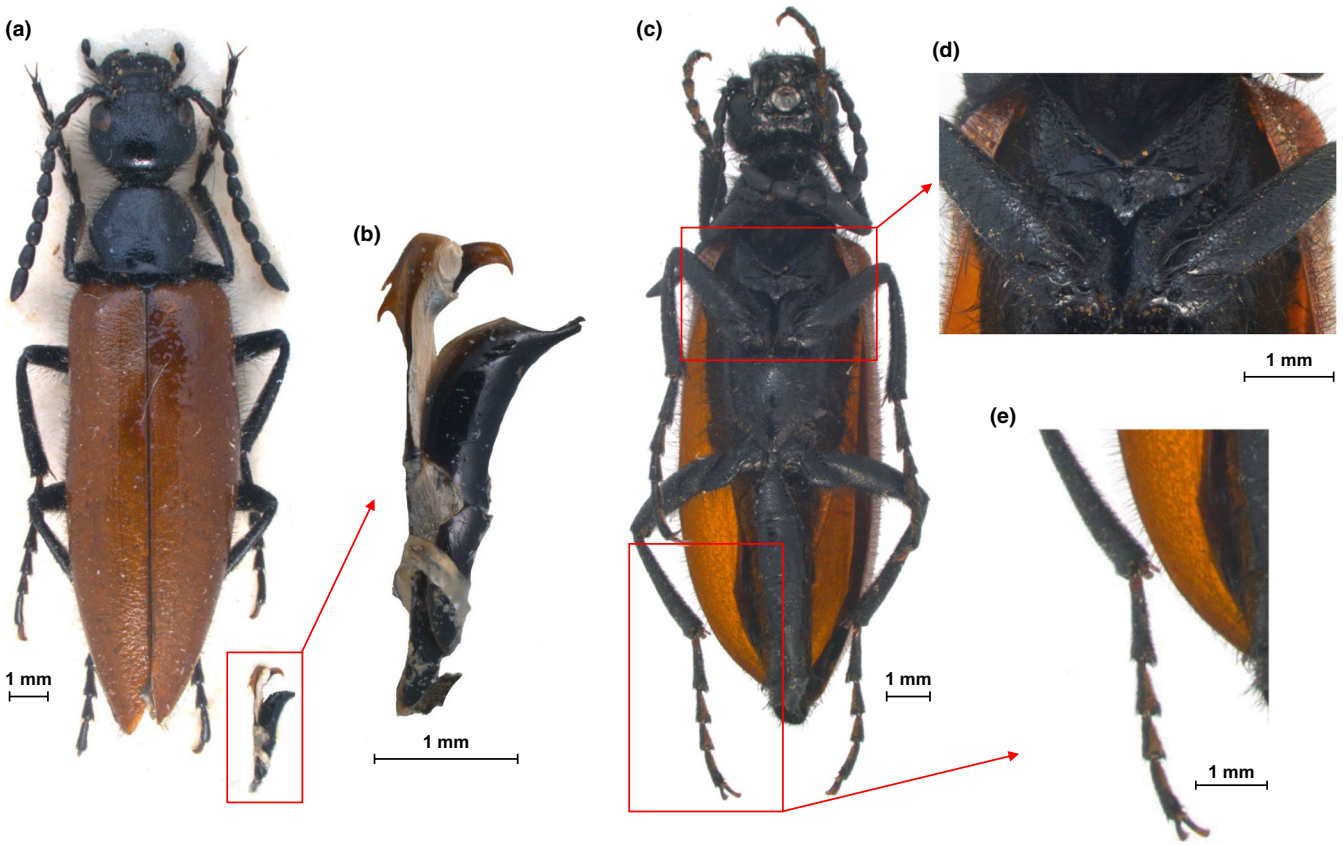

Fig. 4. *Mylabris* (*Dvorabris*) **subgen.n.**: habitus in dorsal (a) and ventral (c) view, aedeagus (b), mesosternal sclerites (d) and metatibial spurs (e). [Colour figure can be viewed at wileyonlinelibrary.com]

slightly longitudinal middle depression on the posterior half. Elytra unicolourous. Mesosternum with a modified fore portion ('scutum'), mesosternal suture highly distinct (Fig. 4d). External angle of protibiae in female pointed and longer than in male; female protibiae with long setae on external side mixed with regular setae, protarsi with elongate setae on both sides; pro- and mesotibial spurs similar and both slender and pointed, outer metatibial spur spatulate (Fig. 4e), *c*.2× as wide as the stick-like inner spur.

Posterior margin of last male ventrite slightly V-shaped, rounded in female. Male gonoforceps robust, cylindrical, abruptly recurved at apex (Fig. 4b); aedeagal stem narrow, apical portion suddenly widened, subrectangular, with two quite long hooks, similarly inclined, proximal one smaller and shorter than distal, endophallic hook large (Fig. 4b).

Etymology. The new subgenus is named after the crasis between part of the name of Miroslav Dvořák ('*Dvor-*') and of the genus *Mylabris* ('*abris*'). Dvořák was a Czech coleopterologist (1926–2008), who published some papers on Palaearctic and Oriental Meloidae and described the type species of this new subgenus, even if he referred it to another genus.

Remarks. The spatulate external metatibial spur is a character occurring in several blister beetles and produced taxonomic confusion in some genera because it was interpreted erroneously. In particular, within the tribe Mylabrini, it represents a synapomorphic condition of the genus *Croscherichia*, but it is also present in a few species of the genus *Hycleus*, specifically in the group of *H. pallipes*, which has been erroneously referred to *Croscherichia* (Pardo Alcaide, 1958; Bologna and Pinto, 2002; Bologna and Turco, 2007). The presence of this modified character also in the genus *Mylabris* represents a unique derived condition of the new subgenus (Bologna and Coco, 1991; Bologna and Pinto, 2002).

Male genitalia are very distinctive in Meloidae, particularly in the tribe Mylabrini and in the genus *Mylabris*. Both the very widened shape of aedeagal apex, and the recurved apical portion of the gonoforceps, represent unique derived conditions. The shape of the outer metatibial spur and of the aedeagus greatly distinguish *Dvorabris* from other *Mylabris* subgenera.

Phylogenetic relationships of this new subgenus within *Mylabris* are likely close to the subgenera *Mesosulcata, Argabris* and *Eumylabris,* but these are not well supported in our molecular analyses (Figs 2 and 3).

Distribution. *Mylabris* (*Dvorabris*) *maceki* was known until now only from the type locality, in northeastern Iraq (Kurdistan, Mishaw, 1750 m a.s.l.) (Dvořák, 1985). Data provided here indicate that the range of the species extends also to northwestern Iran.

Mylabris (*Pardabris*) Bologna **subgen.n**

Type species. *Mylabris syriaca* Klug, 1845, by present designation.

Material examined. We examined morphologically (CB): 6 exx. of *M. atrofasciata*, 6 exx. of *M. interrupta* Olivier, 1811, 21 exx. of *M. suturalis*, and 66 exx. of *M. syriaca* Klug, 1845, including some from Syria, the type locality.

Diagnosis. A mylabrine taxon confused in the past with the subgenera *Euzonabris, Eumylabris* and *Mauritabris* (see Discussion), very similar morphologically to this last subgenus. The distinction from *Mauritabris*, proposed by Pardo Alcaide (1969, as *Zitunabris*), is incorrect (see discussion) because the mesosternal distinctive features proposed are shared with *Mauritabris* (setation present or absent; fore suture more or less visible). The only clearly distinctive character of this subgenus seems to be the shape of antennomeres IV–X, which are distinctly perliform in *Pardabris*, whereas in *Mauritabris* they are slender and parallel in some species (*abdelkaderi, baulnyi, cyrenaica, damascena, filicornis, mateui, tenebrosa*), or subparallel and slightly widened apically in *oleae* (see Remarks as concerns the intraspecific variation in this species).

Description. Large sized (usually 20–30 mm), integuments black without metallic reflections, elytra brown-ochre with three transverse black fasciae, one anterior, one middle in position and one extended on the whole apex, sometimes coalescing, surface subopaque, body setation entirely black; head and pronotum punctures distinct, dense, deep and variolose or coalescing on pronotum; elytral surface with shiny longitudinal venation, surface densely subrugose. The sexual dimorphism concerns forelegs and last abdominal ventrite.

Head subtrapezoidal in both sexes, temples widened posteriorly to eyes; eye concave on fore margin near antennal base, not bulging mandibles curved and almost completely covered by labrum, which is slightly sinuate on fore margin. Male maxillary and labial palpomeres not distinctly modified. Antennae quite elongate, composed of 11 antennomeres: I elongate and widened apically, shorter than III, II very short and subglobose, III subcylindrical and slightly widened apically, III distinctly longer than IV, IV–X perliform, XI *c*.2× as long as X in both sexes, slightly more in male, apical third tapered and conical.

Pronotum about as wide as maximal width of head, pentagonal, distinctly widening from base to middle and narrowing in fore half, with one great and distinct transverse depression extended on the whole anterior third. Elytra bicolourous, brown-ochre with three black fasciae. Mesosternum with a fore modified portion ('scutum') small in size; mesosternal suture more or less distinct. External angle of female protibiae pointed and longer than in male; female protibiae with scattered and sparse slightly longer setae on external side, mixed with regular setae, protarsi with pad of setae, in male of some species with elongate setae on both sides; all tibial spurs similar and both slender and pointed, outer metatibial spur slightly thicker.

Posterior margin of last male ventrite slightly V-shaped rounded in female. Male gonoforceps robust, well-sclerotized, cylindrical, apical lobes short; aedeagus with two similar small hooks, far from apex and with the same inclination.

Etymology. The new subgenus is named after the crasis between Pardo, the first family name of Anselmo Pardo Alcaide (1913–1977) a Spanish specialist of Meloidae, who studied especially Mylabrini, and part of the name *Mylabris* ('*abris*').

Remarks. Mylabrine taxon confused by Kuzin (1954) with *Eumylabris* and described by Pardo Alcaide (1969) as *Zitunabris*. In this study, we show that *Zitunabris* is actually polyphyletic and the type species is here referred to *Mauritabris* (see below). Our molecular analyses confirm its relationships to the subgenus *Mauritabris*.

Distribution. The subgenus is distributed in the Near and Middle East; its presence in northern Africa must be confirmed after its revision.

Discussion

Phylogeny and classification of Mylabris *and Mylabrini*

Meloidae have a distinctive biology, because of their hypermetabolic development, the parasitoid habits of the larval phases and the production of cantharidin for defence and reproductive behaviour (Bologna, 1991; Bologna et al., 2010). The peculiar host–parasite and male–female specificities that exist in this group of insects imply that many morphological characteristics of both larvae and adults may be under severe adaptive pressure or under sexual selection. Moreover, groups such as Mylabrini, and in particular *Mylabris*, experienced an explosive species diversification with hundreds of species (Bologna, 1991, 2008). Therefore, morphological assessments of phylogenetic relationships between Mylabrini are particularly problematic because although some morphological characters may be evolutionarily convergent due to shared selective pressures between distantly related taxa, many others may be invariant because of the recent speciation within the group. The use of a multilocus molecular approach allowed estimation of a robust phylogeny of *Mylabris* and provided a guide for a cladistic assessment of both the classification and the morphological variation of Mylabrini.

The phylogeny we recovered is consistent across different datasets (3-, 4- and 5-gene datasets), methods (BI vs. MP) and approaches (species tree vs. total evidence). The great majority of the analysed species currently assigned to *Mylabris* form a monophyletic clade which received moderate to high support (Figs 2 and 3; BPP = 0.9 and 0.98, respectively). Species of the genus *Pseudabris* are clearly part of this clade, whereas *Mylabris hemprichi* and species of the subgenus *Ammabris* are not. Therefore, we can conclude that the genus *Mylabris*—as currently conceived (Bologna and Pinto, 2002; see Table 1) and given these two amendments—is a natural group of closely related species.

Most of the currently accepted subgenera of *Mylabris*, defined by morphology, represent well-supported monophyletic clades in the molecular phylogeny. *Micrabris*, *Mylabris*, *Calydabris*, *Chalcabris*, *Eumylabris*, *Argabris* and *Mesosulcata* received the maximum support in every phylogenetic analysis and form cohesive groups which are separated from one another by long branches in the trees. Also, *Ammabris* (raised to genus rank; see the Taxonomic section) is defined as a monophyletic group in the phylogenetic tree. Two exceptions regard the case discussed above of *M*. (*Chrysabris*) *hemprichi* (which is neither sister to *M*. (*Chrysabris*) *doriae* (Appendix S1) nor part of the *Mylabris* clade (Figs 2 and 3)) and *M*. (*Zitunabris*) *oleae*. *Mylabris oleae* is the type species of *Zitunabris* and is nested within the *Mauritabris* clade underlying a complex taxonomic situation (see the Taxonomic section), yet it represents a single phylogenetic exception to otherwise monophyletic clades of *Mauritabris* and *Zitunabris*. Thus, overall, the subgeneric designations in *Mylabris* based on morphology used in traditional taxonomy closely reflect phylogenetic clades as defined based on molecular sequence data.

Phylogenetic relationships among subgenera are not resolved with statistical support (Figs 2 and 3), likely because cladogenetic events behind their formation took place in a short timespan: according to our estimate between 15 and 12 Ma (Fig. 2). Rapid radiations such as this generate a phylogenetic pattern with short deep branches combined with long terminal branches (see Fig. 2), which is notoriously difficult to resolve in phylogenetic analysis (e.g. Cummins and McInerney,

2011; Mendes et al., 2016). Fossil evidence for *Mylabris* is not available. In fact, although three fossil records from European (Germany and France) Eocene, Oligocene and Miocene–Pliocene deposits have been referred to *Mylabris* (see Engel, 2005, for a synthesis), they cannot be assigned with confidence to any genus of Mylabrini (Bologna, personal observation). The oldest of these fossils is referred to the beginning of the Oligocene (33.9 Ma) or slightly earlier. This record fits well with our estimate of time for the cladogenesis of Mylabrini from other meloid lineages at about 32 Ma, thus providing some support for our molecular clock calibration.

The current taxonomy of Mylabrini mostly relies on Pardo Alcaide's classification, which shows significant differences with Kuzin's classification (Table 1). The main differences between the classification used by Kuzin and that assumed by Pardo Alcaide centre on four main issues: (i) Kuzin allocated in five subgenera of *Mylabris* (*Ceroctis*, *Decapotoma*, *Euzonabris, Sphenabris*, *Tigrabris*) species that Pardo Alcaide placed in two distinct genera (*Ceroctis* and *Gorrizia* = *Hycleus*); (ii) the subgenus *Mylabris* (*Eumylabris*) *sensu* Kuzin includes species that Pardo Alcaide placed in two distinct subgenera (*Mauritabris* and *Zitunabris*); (iii) the subgenus *Mylabris* (*Chalcabris*) *sensu* Kuzin includes some species that Pardo Alcaide placed in the subgenus *Mylabris* (*Micrabris*); and finally (iv) the species included within the genus *Coryna* by Kuzin were placed by Pardo Alcaide into two genera (*Rusadiria* = *Actenodia* and *Gorrizia* = *Hycleus*) (see Table 1 for further details).

Our phylogenetic reconstruction indicates that the taxa *Mylabris*, *Eumylabris*, *Chalcabris* and *Coryna* as defined by Kuzin are polyphyletic, whereas Pardo Alcaide's classification better reflects the evolutionary relationships of *Mylabris* and related Mylabrini. In particular: (i) taxa included in *Ceroctis* and *Gorrizia* = *Hycleus* are clearly distinct from *Mylabris* and not part of this genus; (ii) the subgenera *Eumylabris*, *Mauritabris* and *Zitunabris* form three independent lineages within *Mylabris* instead of a monophyletic clade; (iii) the subgenera *Chalcabris* and *Micrabris* as defined by Pardo Alcaide are monophyletic; and (iv) *Rusadiria* = *Actenodia* and *Gorrizia* = *Hycleus* belong to two independent lineages of Mylabrini and lumping them into *Coryna* would result in a polyphyletic genus.

It is undoubtedly not a coincidence that Pardo Alcaide's classification does consistently identify phylogenetic groups, whereas Kuzin's system does not. Both scientists used morphological features for grouping mylabrine species without building trees, because the quantitative phylogenetic methods used for analysing morphological characters were not yet available at their time. However, although Kuzin defined taxa based on the degree of overall similarity, the approach of Pardo Alcaide was explicitly based on a phylogenetic criterion for character evaluation, in which only those characteristics that were supposed to reflect a phylogenetic pattern were selected for the classification of species into (natural) groups (Pardo Alcaide, 1954: p. 58, note 3). Such an approach was based on a subjective judgment of the "phylogenetic significance" of characters as well as lacking a methodological framework for defining the relationship between features (character states) and phylogenetic pattern. However, the close match between the taxa defined by Pardo Alcaide and the monophyletic clades recovered in the species tree of Mylabrini suggests that, overall, he actually distinguished rather well between morphological similarities (symplesiomorphies and homoplasies) and derived characters underlying evolutionary relationships (synapomorphies). Using the molecular phylogeny of Mylabrini, we can further explore which morphological characters used in previous classifications and diagnoses represent synapomorphies that define mylabrine genera and subgenera (see Fig. 2 and Table 5).

Similarities and synapomorphies within Mylabris *and Mylabrini*

The first comprehensive taxonomy of Mylabrini was built by de Marseul (1872) using the number of antennomeres as *fundamentum divisionis* for delimiting genera. Meloidae usually show 11 antennomeres, whereas in Mylabrini we observed species with a reduced number of antennomeres (from ten to seven antennomeres). Kuzin (1954; see also Sumakov, 1915) largely followed the criterion used by Marseul, and based the classification of genera and subgenera mainly on the number and shape of antennomeres and additional ancillary characters, partially distinctive or common to other taxa. Accordingly, all species with 11 antennomeres were included in the genus *Mylabris*, with four exceptions: two subgenera of *Mylabris*, *Decapotoma* and *Chrysabris*, include species with 10 antennomeres; species with 11 antennomeres but distinctive spatulate metatibial spurs were assigned to the genus *Libycisca* (= *Croscherichia*); and those with 11 antennomeres but a distinctive shape of the last three were assigned to the genus *Lydoceras* as described by de Marseul (1870). The genus *Coryna* was defined by the presence of nine antennomeres and the genus *Actenodia* (*sensu* Kuzin, 1954 = *Paractenodia sensu* Bologna and Pinto, 2002) grouped species with seven antennomeres. Within *Mylabris*, Kuzin relied mostly on the shape of antennomeres to classify species into subgenera. Accordingly, *Ceroctis* was defined by the distinctive serrate shape of antennomeres, especially in males; *Tigrabris* by the last four antennomeres progressively widened; *Sphenabris* by narrowed

Table 5
Apomorphies and distinctive characters of taxa recovered as distinct clades or lineages in the phylogenetic tree of Mylabrini (see Fig. 2)

Phylogenetic clade/lineage	Taxon (updated taxonomy)	Synapomorphies/Apomorphies	Additional distinctive characters	References
A	*Mylabris (Micrabris)*	Mesosternal scutum wide, smooth and glabrous	Pronotum subglobose, distinctly convex dorsally; red spot on frons divided in two or absent; body (except elytra) black or metallic black-bluish slightly shining; small-sized species; male protibiae with a ventral plica usually visible	Pardo Alcaide (1954, 1969), Bologna (1991, 2008)
B	*Mylabris* (*Mylabris*)	Mesosternal scutum with distinct posterior tuft of long and robust setae; aedeagal hooks different in shape, the distal one usually subapical	Pronotum more or less distinctly depressed anteriorly; middle-sized species	Pardo Alcaide (1948, 1950), Bologna (1991), Pan and Bologna (2014)
C	*Mylabris* (*Calydabris*)	Ventral blade of claw fused at base to the dorsal one	Middle-sized species; often a tuft of regular setae at apex of mesosternal scutum	Kaszab (1960), Bologna and Pinto (2002), this paper
D	*Mylabris* (*Mauritabris*) + *Mylabris* (*Zitunabris*) *oleae* (*Mylabris* (*Mauritabris*))		Antennomeres slender and parallel or subparallel and slightly widened apically in *M. oleae;* large-sized species	Pardo Alcaide (1969), this paper
E	*Mylabris* (*Zitunabris*) pars (*Mylabris* (*Pardabris*))		Antennomeres IV–X distinctly perliform; large-sized species	Pardo Alcaide (1969) pars; this paper
F	*Pseudabris* (*Mylabris* (*Pseudabris*))	Mesosternum with a modified anterior area distinct from the remaining surface, including a longitudinal, furrowed carina, which is continuous anteriorly with the mesepisterna; elytra distinctly or slightly dehiscent posteriorly, with foveae on surface	Middle-sized species	Pan et al. (2014)
G	*Mylabris* (*Chalcabris*)	Male tarsomeres dorsally flattened; external setae of protibiae distinctly longer than internal	Pronotum convex dorsally but not distinctly subglobose; male protibiae without ventral plica; mesosternal scutum wide, slightly punctate with scattered long setae; body (including elytral spots) dark teal metallic black-bluish distinctly shining; middle-sized species	Kuzin (1954) pars; Pardo Alcaide (1954, 1969), Bologna, (2008)

Table 5
(Continued)

Phylogenetic clade/lineage	Taxon (updated taxonomy)	Synapomorphies/Apomorphies	Additional distinctive characters	References
H	*Mylabris* (*Eumylabris*) + *Mylabris* (*Spinabris*) (*Mylabris* (*Eumylabris*))	Dorsal blade of claw serrate or microcrenulate, ventral blade regular or reduced in length and width; mesosternal scutum large, nude, extended posteriorly, margin of mesepisterna posteriorly overimposed to scutum; pronotum with a middle lined depression; parameres straight and scarcely sclerotized	Last antennomeres asymmetric; large-sized species	Kuzin (1954), Pardo Alcaide (1969), Pan et al. (2010), this paper
I	*Mylabris* (*Argabris*)	Aedeagus distinctly curved at apex, both aedeagal hooks curved and greatly distinct in size and shape, the distal one smaller	Middle-sized species	Kuzin (1954)
J	*Mylabris maceki* (*Mylabris* (*Dvorabris*) *maceki*)	Metatibial external spur widened; apical portion of aedeagus suddenly widened and subrectangular, male gonoforceps cylindrical but directed backwards at apex	Middle-sized species	Dvořák (1985), this paper
K	*Mylabris* (*Mesosulcata*)	Mesosternum with two oblique furrow	Middle-sized species	Pardo Alcaide (1948), Pardo Alcaide (1954), Bologna (1991)
L	*Mylabris* (*Ammabris*) (*Ammabris*)	Mesosternum without scutum, laterally distinctly setated; pronotum distinctly narrowed and elongate in front with discal fovae and tubercles	Small- to middle-sized species	Kuzin (1954), Pardo Alcaide (1954), Ruiz and García-París (2008)
M	*Actenodia pars* (*Actenodia*)	Antennae apically clavate	Eight antennomeres (nine in *A. confluens*); antero-dorsal outline of eye near antennal base only slightly emarginate or straight	Pardo Alcaide (1954), Bologna et al. (2008b)
N	*Actenodia pars* (*Rusadiria*)	Antero-dorsal outline of eye near antennal base greatly emarginate; antennae apically clavated	Nine antennomeres (eight to nine in *R. septempunctata*, nine in *R. mateui*)	Pardo Alcaide (1954), Bologna et al. (2008a, b)
O	*Mimesthes*	Pronotum transverse; elytra truncate at apex; aedeagus with distal hook only; last antennomeres compressed		Bologna (2000)
P	*Croscherichia*	External metatibial spur widened; mesosternum without scutum; gonoforceps slender and straight		Pardo Alcaide (1948), Pardo Alcaide (1954), Bologna and Coco (1991)

Clades and lineages are coded with letters according to Fig. 2.

antennomere XI; *Euzonabris* by submoniliform antennomeres IV–IX; and *Mimesthes* by the last three antennomeres partially compressed. Other subgenera were instead defined mainly on the basis of body colour: *Glaucabris* (= *Mesosulcata*), green body and subrounded pronotum; *Chalcabris*, blue-green body and slightly widened apex of the antennae; *Chrysabris*, blue body and antennomeres distinctly widened at apex. Finally, *Eumylabris* was defined by Kuzin (1954) on the basis of the serrate ventral side of claws and features of the pronotum such as a longitudinal narrow line, whereas for *Ammabris* he listed a number of characters which are in common with other subgenera.

Taxonomic characters used by Kuzin are shared across different phylogenetic lineages of Mylabrini (Figs 2 and 3). Eleven antennomeres occur in most species of the *Mylabris* clade but also in all species of the clades of *Ceroctis* and *Ammabris*, as well as in most *Hycleus* and *Croscherichia*. Some species of the current *Actenodia* were lumped by Kuzin into either *Paractenodia* or *Hycleus* depending on whether they have seven or nine antennomeres, respectively; however, *Actenodia* belong to a distinct lineage in the phylogenetic tree with respect to *Paractenodia* and *Hycleus*. Overall, in the *Hycleus* lineage we found species with seven, eight, nine, ten or 11 antennomeres and in the species *Hycleus rouxi* (Laporte, 1840) we even observed individuals with either nine or ten antennomeres (Pardo Alcaide, 1958). This clearly indicates that the number of antennomeres, used by Marseul and Kuzin to define genera, is subject to convergent evolution (see also Pardo Alcaide, 1958; Bologna, 1978, 1991; Bologna and Pinto, 2002). For the same reason, relying mostly on the shape of antennomeres or on body colour led Kuzin to include distantly related species in polyphyletic taxa. For example, body colouration is a character with continuous variation from green to blue across many taxa. The green-blue colour is a feature that evolved independently in many lineages of Mylabrini as it occurs in several subgenera of *Mylabris* such as *Glaucabris (=Mesosulcata), Chalcabris, Micrabris* (e.g. *M. splendidula* (Pallas, 1781) and *M. frolovi* Fischer von Waldheim, 1823) and the nominotypical subgenus (e.g. *M. batnensis* de Marseul, 1870), but also in the *Hycleus* lineage (e.g. *M. hemprichii*, *H. viridimetallicus* (Pic, 1913), *H. viridescens* (Pic, 1908)). Likewise, the last antennomeres have a (sub-)clavate shape in *Actenodia*, *Paractenodia* and some *Hycleus* (e.g. in the *amoenus* and *zavattarii* groups), and a narrowed shape in *Lydoceras* as well as in *Hycleus* (in the *sexmaculatus* group), whereas submoniliform antennomeres are present in distinct clades of *Mylabris* such as *Eumylabris*, *Mauritabris* and *Zitunabris* (see Taxonomic paragraph). Thus, overall the taxonomic partitions defined by the number and shape of antennomeres and body colour are confounded by convergent evolution across genera and subgenera, as these characters represent morphological similarities that evolved independently in several clades of Mylabrini (see also Bologna and Pinto, 2002).

In contrast, Pardo Alcaide mainly considered characters of the mesosternal sclerites and male genitalia as a source of diagnostic characters for delimiting genera and subgenera. Overall, these features prove to be diagnostic for identifying phylogenetic groups as many of them represent synapomorphies of these groups as summarized in Table 5. First, he suggested two apomorphies, occurring only in some Mylabrini previously included in *Mylabris* and *Coryna*, to describe the genus *Gorrizia* (=*Hycleus*): the mesepisterna rebordered and with a lateral concave furrow; and both aedeagal hooks apical in position (see also Bologna and Pinto, 2002). A similar condition is observed in *Ceroctis* and *Paractenodia*, which were considered distinct genera by Pardo Alcaide (1961) and Bologna and Pinto (2002) based on weak antennal characters (serrate shape of antennomeres of *Ceroctis* and seven antennomeres in *Paractenodia*), but that are likely part of the genus *Hycleus* as suggested by molecular data (Bologna et al., 2008a; this study). The genus *Croscherichia* (=*Libycisca*) is characterized by the synapomorphic condition of the external metatibial spur which is spatulate (see Bologna and Coco, 1991; Bologna and Pinto, 2002). The genus *Rusadiria* (=current *Actenodia*) is correctly distinguished from *Hycleus* by Pardo Alcaide on the basis of the similar shape of mesosternal parts, without rebordered mesepisterna, aedeagal hooks positioned far from apex and distinctly clubbed antennae. Actually, molecular data suggest that species showing these features belong to two distinct lineages that may well be two distinct genera—*Actenodia* and *Rusadiria* (see the Taxonomic section). Bologna et al. (2008b) indicate two synapomorphies of the *Rusadiria* lineage: antero-dorsal outline of eye near antennal base distinctly emarginated in *Rusadiria* (only slightly emarginated or straight in *Actenodia*); larvae with numerous setae on claws in *Rusadiria* (claws with two setae in *Actenodia*).

Within the genus *Mylabris*, the subgenus *Mesosulcata* (=*Glaucabris*) is distinguished by Pardo Alcaide (1950) based on the synapomorphic condition of the mesosternum with two oblique furrows. The mesosternal scutum wide, smooth and glabrous is found by Pardo Alcaide as apomorphy of the subgenus *Micrabris*: (Pardo Alcaide, 1950, 1954, 1969; Bologna, 1991). Several *Micrabris* species, some of which were previously included in *Chalcabris* by Kuzin, also have male protibiae with a lateral keel (Pardo Alcaide, 1969). The subgenus *Chalcabris*, so amended, remains defined by nonexclusive characters as described by Kuzin (blue-green body and the slightly widened apex of the

antennae). We found two new synapomorphies for *Chalcabris*: males with protarsomeres dorsally flattened (cylindrical in *Micrabris*) and with setae on outer side of protibiae longer than on inner side (similar in length in *Micrabris*). The serrate condition of the ventral side of claws (listed by Kuzin, 1954 and well-defined by Pardo Alcaide, 1969) represents a clear synapomorphy of the subgenus *Eumylabris* and its sister lineage *Spinabris* as well as the shape of the mesosternal scutum not separate anteriorly by a suture in the middle, the scarcely sclerotized parameres and the pronotum with a middle lined depression (Pardo Alcaide, 1969). Pardo Alcaide (1969) correctly filtered out from *Eumylabris* some species with distinctive antennomeres (asymmetric and approximately as long as wide) and parameres (sclerotized and forming short lobes at apex) that were correctly allocated into two new subgenera, *Mauritabris* and *Zitunabris* (Pardo Alcaide, 1969). However, the characters proposed to distinguish these taxa, such as the presence/absence of fore mesosternal suture and the arcuate shape of this sternite, are not exclusive of *Mauritabris* and *Zitunabris*. Therefore, a morphological and taxonomic revision of these taxa is necessary (see Taxonomic section). Likewise, the condition of the mesosternum without fore scutum and laterally distinctly setated, suggested as distinctive of *Ammabris* (Pardo Alcaide, 1954), is also present in the genus *Croscherichia* and in some species of *Hycleus*.

Mesosternal and male genitalia features have been also useful synapomorphies in two taxa included in this study that were not considered by Pardo Alcaide. The single aedeagal hook far from apex, the transverse shape of pronotum and the truncated elytral apex are synapomorphies of *Mimesthes* (Bologna, 2000). In *Pseudabris*, the mesosternum is characterized by a modified anterior area including a longitudinal, furrowed carina (Pan et al., 2013). *Pseudabris,* endemic to the Tibetan plateau, also shows a distinctive morphology compared to other Mylabrini by its shortened and apically dehiscent elytra and the scattered and irregular foveae or depressions on the surface (Pan et al., 2013). Brachyptery in this taxon is likely associated with reduced flight activity and ground-dwelling habits in harsh cold and windy high-altitude environments (Mani, 1968; Strathdee and Bale, 1998; Hodkinson, 2005).

Finally, the monophyly and distinctive apomorphies of certain other taxa remain to be assessed. These include groups that were either not analysed by Pardo Alcaide or not thoroughly tested in this study (*Lydoceras*, *Semenovilia*, *Xanthabris, Mylabris* (*Chrysabris*), *Mylabris* (*Lachnabris*), *Mylabris* (*Monabris*)). On the one hand, preliminary *cox1* data support the inclusion of *Mylabris* (*Lachnabris*) *mannerheimii* within the *Mylabris* clade and the distinction of *Lydoceras lictor* and *Xanthabris baluchistanica* from other Mylabrini genera, in agreement with current taxonomy (Appendix S1). On the other, the subgenus *Chrysabris*, as originally defined by Kuzin, is polyphyletic considering that the species *M. hemprichi*, which is distinguished by the unique special brush setation on the black elytral spots (Pardo Alcaide, 1954), belongs to the *Hycleus* lineage according to molecular data. However, although we are unable to point out morphological characters in common between *M. hemprichi* and other members of the lineage of *Hycleus*, analyses of additional species of *Chrysabris* are necessary to assess its monophyly and identify its distinctive apomorphic features.

Taxonomic and nomenclatural changes

The multilocus approach and the comprehensive taxon sampling used in this study produced a robust phylogeny of *Mylabris* beetles and related Mylabrini providing a broad perspective on their systematics. Overall, phylogenetic results support the monophyly of the genus *Mylabris* and of most subgenera; however, some of the current taxa do not reflect monophyletic groups and require systematic changes in order to produce a cladistic classification. Here, we integrate these phylogenetic insights into an updated taxonomy of *Mylabris*.

The distinction between *Mylabris* and *Pseudabris* is not supported by molecular data. The species *P. hingstoni* and *P. longiventris* form a monophyletic unit within the *Mylabris* clade in all phylogenetic analyses. Therefore, *Pseudabris* is here considered as a subgenus of *Mylabris*: *Mylabris* (*Pseudabris*) **new status**. *Pseudabris* species show a highly modified elytral and wing morphology compared to other *Mylabris*, but this character is likely the result of adaptation to high-altitude habitats as discussed above.

Mylabris maceki represents a distinct lineage within *Mylabris* with unclear relationships with other subgenera. This species is assigned to *Dvorabris* new subgenus described in the Results section.

The monotypic *Spinabris* is closely related to *Eumylabris* (Figs 2 and 3). The presumed distinctive morphological feature of *M.* (*Spinabris*) *spinungulata*—that is, the ventral blade of claws greatly reduced in width—seems actually an extreme condition along a continuum of reduction in length and width of the ventral blade of claws which is observed also in *Eumylabris* in the species *M. schrenkii* Gebler, 1845 and *M. klapperichi* Kaszab, 1958. Therefore, molecular and morphological data do not support the taxonomic distinction of *Spinabris*: *Mylabris* (*Spinabris*) Pan et al. (2010) = *Mylabris* (*Eumylabris*) Kuzin (1954) **new synonymy**.

Chalcabris Kuzin (1954) is most likely a valid subgenus, but only in the emended definition of Pardo Alcaide (1969), who transferred to the subgenus *Micrabris* some species including *frolovi*, *intermedia* and *splendidula*. The assignment of these latter species to *Micrabris* is well supported by our phylogeny. The assignment to *Chalcabris* of *Mylabris cyanevaria* is confirmed by our *cox1* data, whereas the inclusion of *Mylabris marginata* is not (Appendix S1) and requires further investigation, which should also include the type species of *Chalcabris*, *M. festiva* (Pallas, 1773).

The subgenera *Mauritabris* and *Zitunabris* are not monophyletic in any phylogenetic tree due to the sister-group relationship between *M.* (*Mauritabris*) *tenebrosa* and *M.* (*Zitunabris*) *oleae*, which are nested within the *Mauritabris* clade. The type species of *Mauritabris* is *M. tenebrosa,* and the type species of *Zitunabris* is *M. oleae*. Because *Mauritabris* and *Zitunabris* have been created in the same paper (Pardo Alcaide, 1969), under art. 24.2 of the ICZN, we here give *Mauritabris* precedence over *Zitunabris* and therefore consider *Mylabris* (*Zitunabris*) Pardo Alcaide, 1969 as a synonym of *Mylabris* (*Mauritabris*) **syn.n.** As a consequence, we establish the following new combination: *Mylabris* (*Mauritabris*) *oleae* **comb.n.** For the clade formed by *M.* (*Zitunabris*) *suturalis* and *M.* (*Zitunabris*) *interrupta,* we propose the name *Pardabris* **subgen.n.** This subgenus, described in the Results section, will likely include also the Asiatic species *atrofasciata* Pic, 1921 and *syriaca* Klug, 1845 s.l., referred to *M.* (*Zitunabris*) by Pardo Alcaide (1969), whereas the Maghrebian species *baulnyi* de Marseul, 1870 and the East Mediterranean *M. filicornis* de Marseul, 1870 are morphologically close to *oleae* and would be assigned to *Mauritabris*. The diagnostic characters of *Mauritabris* are discussed below in the description of the new subgenus *Pardabris*.

The Saharo-Iranian species *Mylabris* (*Chrysabris*) *hemprichi* belongs to the *Hycleus* lineage and is therefore moved from *Mylabris* to *Hycleus* as *Hycleus hemprichi* **comb.n.** This species shows a distinct morphology compared to other *Chrysabris* species while sharing only the blue metallic colouration with them (e.g. Kuzin, 1954; Bologna, unpublished). We also analysed the *cox-1* sequences of *M.* (*Chrysabris*) *doriai* de Marseul, 1870 (which is morphologically close to *M. trifascis* (Pallas, 1773), the type species of *Chrysabris*) and confirmed its inclusion within the *Mylabris* clade (Appendix S1). This supports *Chrysabris* as subgenus of *Mylabris*, emended by the exclusion of *hemprichi*, whereas molecular data from the remaining seven Turano-Iranian species currently included in *Chrysabris* will be necessary to confirm their taxonomic status.

The three species of the subgenus *Ammabris* (*M. elegans,* the type species of the subgenus, *M. myrmidon* and *M. raml*) form a well-supported monophyletic clade which has a close relationship to species of the genus *Actenodia* rather than *Mylabris*. This taxon is therefore removed from *Mylabris* and raised to genus level: *Ammabris* **stat.n.** *Ammabris* is a morphologically heterogeneous group of 14 species, and it may need a systematic revision (Ruiz and García-París, 2008).

Finally, we discussed some Mylabrini genera that are likely not monophyletic, but their taxonomic status is maintained pending further research. The Afrotropical species *Actenodia chrysomelina* shows a closer relationship to *Ammabris* species rather than to the clade formed by the Palaearctic species *Actenodia distincta* and *Actenodia denticulata*, although this relationship is not supported in all phylogenetic analyses (BPP = 0.79 or 0.96 in Bayesian trees of Figs 2 and 3, respectively). A previous phylogenetic study already pointed out the morphological and molecular distinction between Palaearctic and Afrotropical species within *Actenodia* (Bologna et al., 2008b), but was unable to test the monophyly of this genus due to the lack of additional taxa of Mylabrini in the in-group set. Here, we show that *Actenodia* may not be monophyletic. The type species of the genus *Actenodia* is the southern African species *A. guttata* Laporte, 1840; consequently, in case future studies will prove the polyphyly of this genus, the name *Actenodia* should be maintained for the related Afrotropical species such as *chrysomelina* (Bologna et al., 2008b). For the Palaearctic species, such as *distincta* and *denticulata*, the genus name *Rusadiria* Pardo Alcaide, 1952 would be available. The type species of *Rusadiria* is *Mylabris billbergi* Gyllenhal, 1817, which is closely related to *distincta* and *denticulata* based on morphological and molecular data (Bologna et al., 2008b).

Species of the genera *Paractenodia, Ceroctis* and *Hycleus* (including *H. hemprichi*) form a well-supported monophyletic clade; however, *Hycleus* is paraphyletic relative to *Paractenodia* and *Ceroctis*, and the latter is polyphyletic. The inclusion of *Paractenodia* and *Ceroctis* within the genus *Hycleus* was already suggested based on molecular data (Bologna et al., 2008a) and shared morphological features of the mesosternum, aedeagus and larvae characters (Bologna, unpublished). *Hycleus* is the most speciose genus of Meloidae (*c.* 450 species), whereas about 60 species are altogether referred to *Ceroctis* and *Paractenodia* (Bologna and Pinto, 2001). This study indicates that a taxonomic revision is necessary for *Hycleus* and related genera; however, additional taxa need to be sequenced to complement morphological data and provide a comprehensive assessment of their phylogenetic and systematic relationships.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Bayesian tree based on *cox1* DNA sequence data of 108 taxa of Mylabrini and three Lyttini (*Lydus unicolor* 2996, *Lydus unicolor* 2976 and *Oenas crassicornis* 318b). The tree is obtained in BEAST using the multispecies coalescent model. Bayesian posterior probability values of nodal support are shown above he branches; tick lines identify supported branches. Asterisks indicate species for which only the *cox1* sequence was available and that were not used in multilocus phylogenetic analyses.