Arianta | Volume 10 | 26–38 | Vienna, March 2023

Update on the phylogeny of the land snail genus *Montenegrina* Boettger, 1877 (Mollusca: Gastropoda: Clausiliidae)

Katharina Mason[1,2], Willy De Mattia[2,3], Zoltán Fehér[4], Elisabeth Haring[2,3]

[1]*3rd Zoological Department, Natural History Museum, Burgring 7, 1010 Vienna, Austria*
[2]*Department of Evolutionary Biology, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria*
[3]*Central Research Laboratories, Natural History Museum, Burgring 7, 1010 Vienna, Austria*
[4]*WWF Hungary, Álmos vezér útja 69/A, 1141 Budapest, Hungary*

Correspondence: katharina.mason@nhm-wien.ac.at

Abstract: The door snail genus *Montenegrina* (subfamily Alopiinae) is comprised of rock-dwelling species specialised on limestone, distributed in the western parts of the Balkan Peninsula. In previous years, several studies on the genus dealt with genetic and morphological diversity, phylogeography and taxonomy of the taxa cumulating finally in the latest revision of the whole genus, which currently consists of 110 taxa assigned to 69 species. Here we present the phylogenetic trees of *Montenegrina* resulting from the integrative work published in 2020 (Mason et al. 2020; De Mattia et al. 2020) and following the last revision. Thus, the trees depict the current taxonomy of *Montenegrina*, which is very useful for future studies. Furthermore, remaining paraphylies and open questions, visualized by the updated trees are listed.

Key Words: taxonomy, phylogenetic trees, mitochondrial DNA sequences, paraphyly, rock-dwelling snails

Zusammenfassung: *Montenegrina* ist eine felsenbewohnende Gattung der Schließmundschnecken (Unterfamilie Allopiinae), deren Arten in der westlichen Balkanregion obligat auf Kalkstein leben. Mehrere Studien der letzten Jahre beschäftigten sich mit genetischer und morphologischer Diversität, Phylogeographie und Taxonomie der Taxa, was schließlich zur aktuellen Revision der Gattung führte, die nun 110 Taxa mit 69 Arten enthält. Hier präsentieren wir die phylogenetischen Bäume der Gattung, die aus integrativen Studien aus dem Jahr 2020 (Mason et al. 2020; De Mattia et al. 2020) resultierten. Daher veranschaulichen die hier gezeigten Bäume die aktuelle Taxonomie der Gattung, was für zukünftige Studien sehr hilfreich ist. Weiters werden verbliebende Paraphylien und offene Fragen zusammengestellt.

Schlüsselwörter: Taxonomie, phylogenetische Bäume, mitochondrielle DNA-Sequenzen, Paraphylie, felsenbewohnende Schnecken

Introduction

Rock-dwelling door snails of the genus *Montenegrina* Boettger, 1877 (subfamily Alopiinae) are distributed in the western parts of the Balkan Peninsula (Montenegro, Kosovo, North Macedonia, Albania and Greece). The species are limited to limestone and occur from the southernmost parts of the Dinaric Mountains to the northern part of the Pindos Mountains. As in many other rock-dwelling gastropods, populations are patchily distributed in limestone habitats. Sometimes, taxa occur on single or a few localities, and thus, many narrow-ranged endemic taxa are found within *Montenegrina*. According to the assumption of Gittenberger (1991, 2004), evolution of highly diversified and species-rich rock-dwelling gastropods may be best explained by non-adaptive radiation rather than adaptation to different ecological niches; a hypothesis that was supported by Fehér et al. (2018) based on a huge data set on species occurrence. Until the time of that analysis, taxonomy of *Montenegrina* was solely based on shell morphology, and it remained unknown whether the morphologically differentiated and geographically separated populations represent phylogenetic clades.

The first attempt for a phylogenetic analysis of the genus *Montenegrina* – at that time 29 species and 106 subspecies were known – was done by Mason et al. (2020) including a broad geographic sample, covering the whole distribution range of the genus (28 species; 104 subspecies; 823 individuals). The phylogenetic tree resulting from the analysis of three mitochondrial marker sequences showed 13 clades, most of them divided into several subclades representing the various taxa. This data was used to test whether there was agreement with the current taxonomy. Furthermore, histone gene sequences were analysed; yet, it turned out that they did not provide informative trees and thus they could not contribute

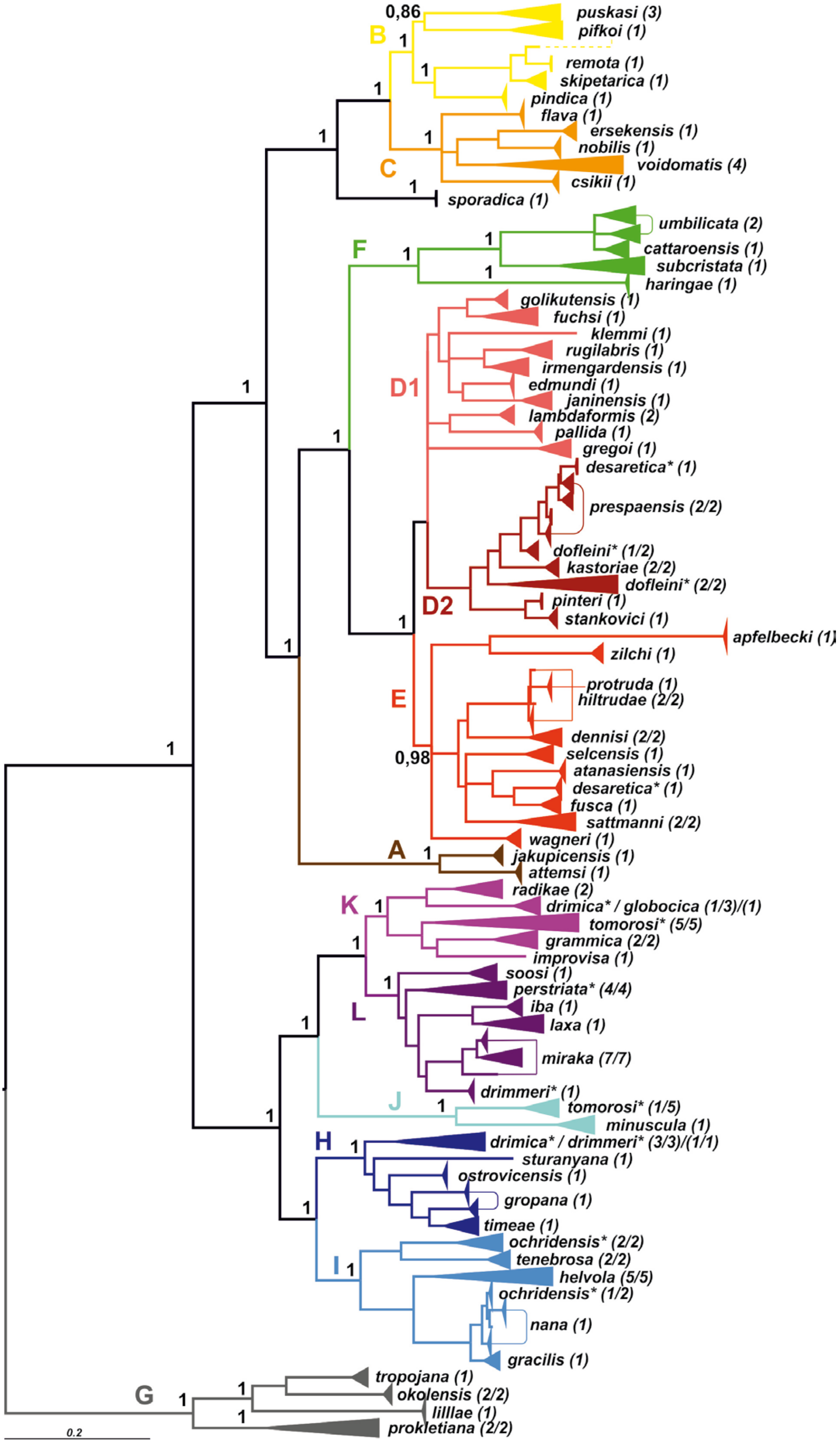

Fig. 1: Bayesian Inference (BI) tree based on the concatenated mt data set (*COI*, *12S*, *16S*) rooted with clade G. Colours correspond to those in the distribution maps in Mason et al. (2020). PP values (>0.80) of major nodes are indicated. Asterisks: species occurring in more than one clade. Data of specimens (geographic origin, taxonomy) are found in Mason et al. (2020), Table S1.

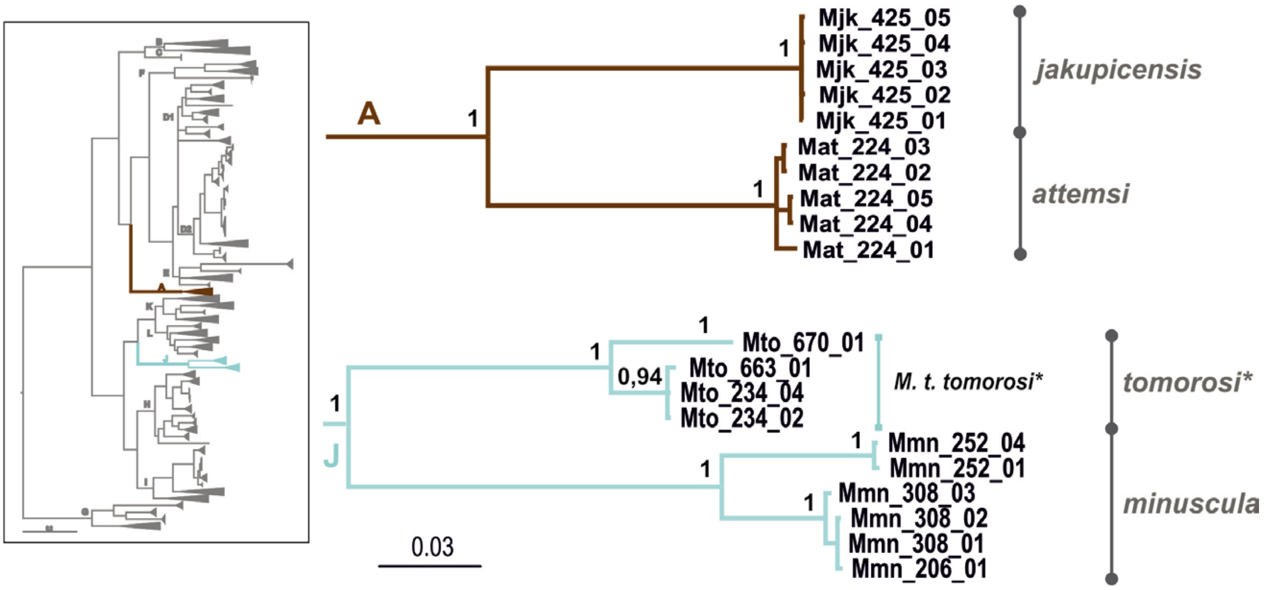

Fig. 2: BI tree of Clades A + J based on the concatenated mt data set (*COI*, *12S*, *16S*). Clade A rooted with Clade F, Clade J rooted with Clades H + I (not shown). PP values (>0.80) of major nodes are indicated. Asterisks: taxa occurring in more than one clade. Data of specimens (geographic origin, taxonomy) are found in Mason et al. (2020), Table S1.

to resolving the phylogeny of *Montenegrina*. In a parallel study by De Mattia et al. (2020), the genus was investigated regarding the genital anatomical features. For the first time, genital anatomy was described and depicted in detail for *Montenegrina*. The results of both studies led – in an integrative approach – to the revision of the genus. Some of the paraphylies appearing in the phylogenetic trees could be resolved by taxonomic revisions through the change of status and/or new combinations of certain taxa. Furthermore, some incongruencies were best explained by introgression of mitochondrial DNA, even between distant clades.

These two comprehensive analyses, the phylogenetic study of Mason et al. (2020) as well as the anatomical analysis (De Mattia et al. 2020), led to a detailed description of the taxa and resulted in a new nomenclatural arrangement. In De Mattia et al. (2020: table 2) the recapitulatory checklist of the nomenclatural changes of the *Montenegrina* taxa in comparison to that of Fehér & Szekeres (2016) was presented. However, the trees shown by Mason et al. (2020) were based on a pre-study situation following the nomenclature and systematics provided by Fehér & Szekeres (2016). Therefore, a clear and up-to-date overview on the genus *Montenegrina* was missing. For any future work on the genus, it would be necessary to relate to trees that represent the current taxonomy of *Montenegrina*. For that reason, we present here the phylogenetic trees of *Montenegrina* resulting from the integrative work published in 2020 (Mason et al. 2020, De Mattia et al. 2020). Furthermore, we point out several open questions, that are visualized now with the updated trees based on mitochondrial marker sequences.

Results and discussion

The trees were constructed with the Bayesian inference method (BI) and are based on concatenated *mt* data sets consisting of partial sequences of three mitochondrial genes: cytochrome c oxidase subunit 1 gene (*CO1*), 12S rRNA gene (*12S*) and 16S rRNA gene (*16S*). For further details see Mason et al. (2020). Here we present them with the new taxonomical assignments as proposed in De Mattia et al. (2020).

According to De Mattia et al. (2020) the genus *Montenegrina* comprises now 110 taxa with 69 species (108 taxa, 68 species represented in the tree). Fig. 1 shows an overview tree of *Montenegrina* summarising the clades, which are subsequently shown in detail in the following figures. The same letter code as in all previous work (Fehér et al. 2018; Mason et al. 2020, De Mattia et al. 2020) was used for the 13 clades (letters A–L, D subdivided into D1 and D2) to make comparisons with earlier work easier. Only *M. sporadica* was not assigned to one specific clade (see below). The number of species within clades varies from 2–11. Fourteen species still appear paraphyletic.

Clade A

One former subspecies of *M. attemsi* (*M. attemsi jakupicensis*) was raised to species level: *M. jakupicensis* (Fig. 2).

Clade J

No changes have been made. For completeness the tree is shown here, too (Fig. 2).

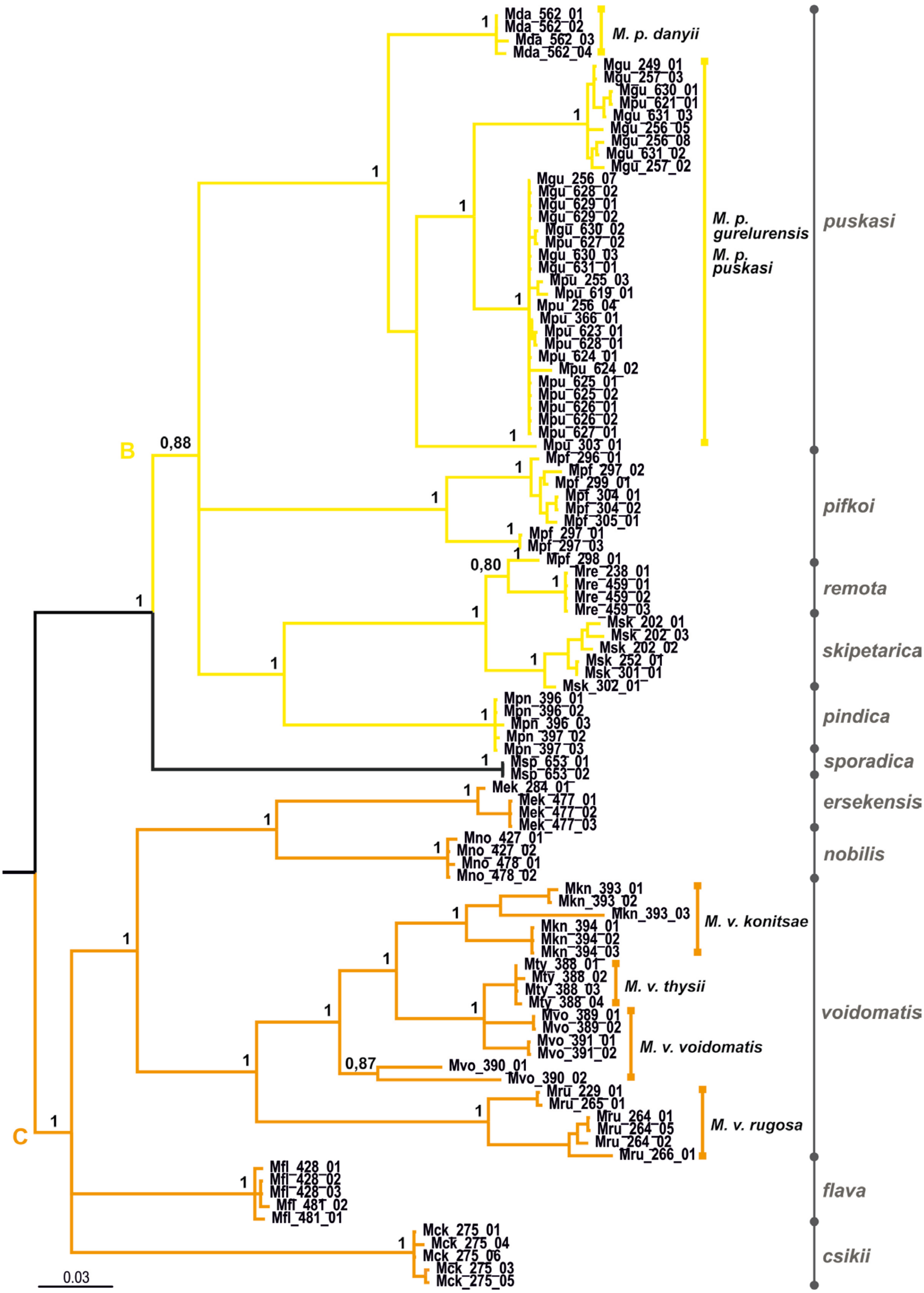

Fig. 3: BI tree of Clades B + C as well as *M. s. sporadica* based on the concatenated mt data set (*COI*, *12S*, *16S*, midpoint rooted). PP values (>0.80) of major nodes are indicated. Data of specimens (geographic origin, taxonomy) are found in Mason et al. (2020), Table S1.

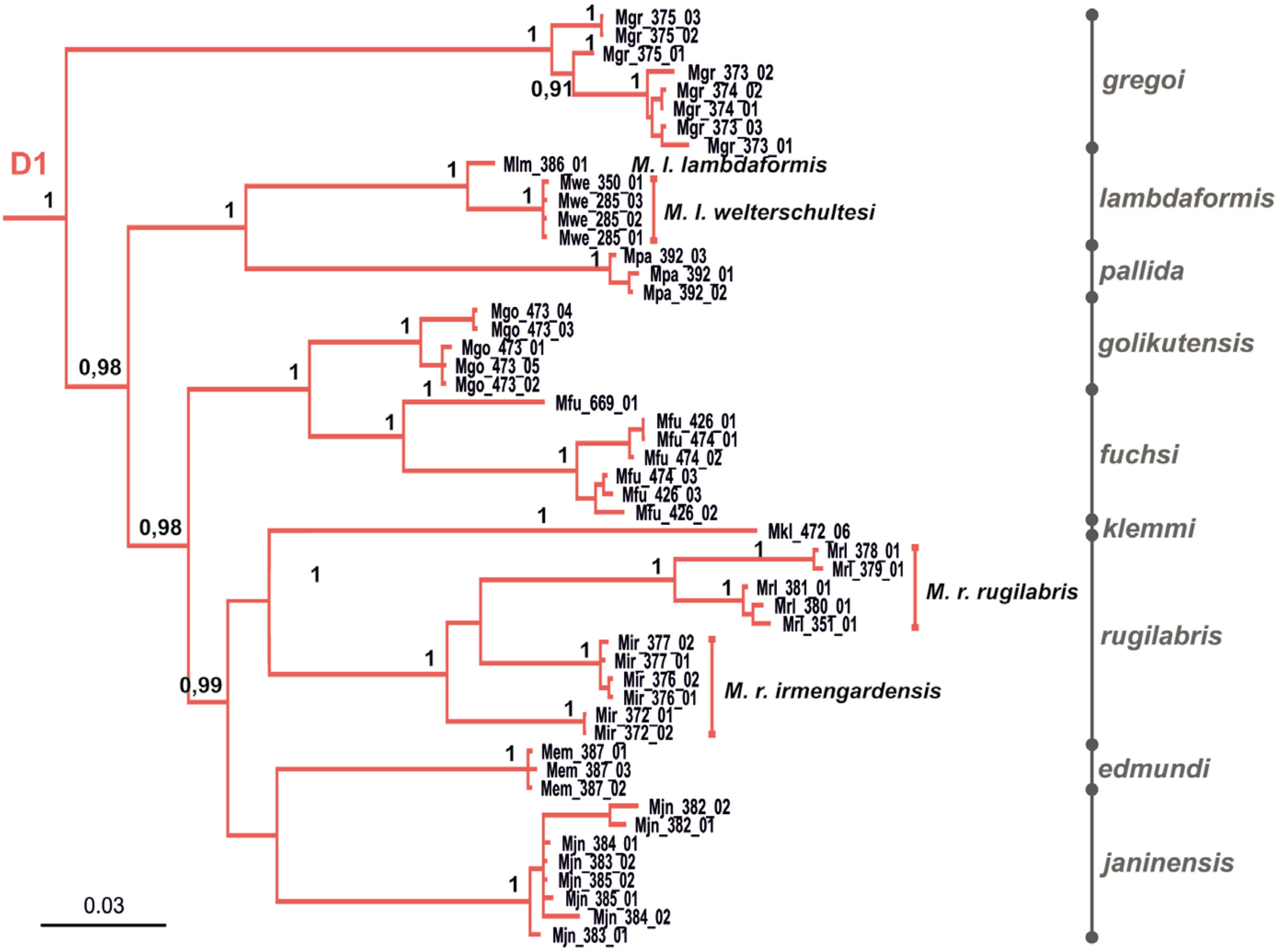

Fig. 4: BI tree of Clade D1 based on the concatenated mt data set (*COI*, *12S*, *16S*), rooted with Clade D2 (not shown). PP values (>0.80) of major nodes are indicated. Data of specimens (geographic origin, taxonomy) are found in Mason et al. (2020), Table S1.

Clades B & C

Clades B and C formerly contained only one species (*M. skipetarica*), which was geographically divided. Samples from the northern parts of Albania fell into Clade B and samples from the southern part into Clade C. As most of the subspecies were raised to species level, both clades B and C now contain five species each (Fig. 3). In clade B, nearly all taxa are monophyletic, except *M. pifkoi* where one individual (Mpf_298_01) stands out as it appears next to the *M. remota* individuals. It was assigned to *M. pifkoi* based on shell morphology, while, unfortunately, no individual of this population was available for anatomical investigations. To resolve this taxonomic problem further investigation is needed. The two subspecies, *M. puskasi puskasi* and *M. p. gurelurensis* are not monophyletic, they can be distinguished by shell morphology (ribbing). In clade C, all taxa are monophyletic, except *M. voidomatis voidomatis* where one population (Mvo_390_01) appears quite distant from the other *M. v. voidomatis,* despite they are morphologically very similar. This population deserves a more comprehensive analysis based on more individuals.

The nominal subspecies *M. sporadica* is placed as sister group to Clade B. This taxon represents a quite distinct lineage which was not included in Clade B and, therefore, had not been designated with a letter. The other individuals of "*M. sporadica tropojana*" appeared in Clade G (Mason et al. 2020). Since the latter population was raised to species level and now is assigned to *M. tropojana*, *M. sporadica* is now monophyletic. Yet, this lineage is composed of only two individuals in the present data set, indicating that further research is needed.

Clade D1

All taxa in this clade are monophyletic (Fig. 4). Within *M. fuchsi* one individual (Mfu_669_01) is quite distinct from the other *M. fuchsi* specimens, which also reflects its geographic distance.

Clade D2

In this clade only three out of the six species are monophyletic (*M. kastoriae, M. pinteri, M. stankovici*; Fig. 5). The problems behind the paraphyletic taxa have been al-

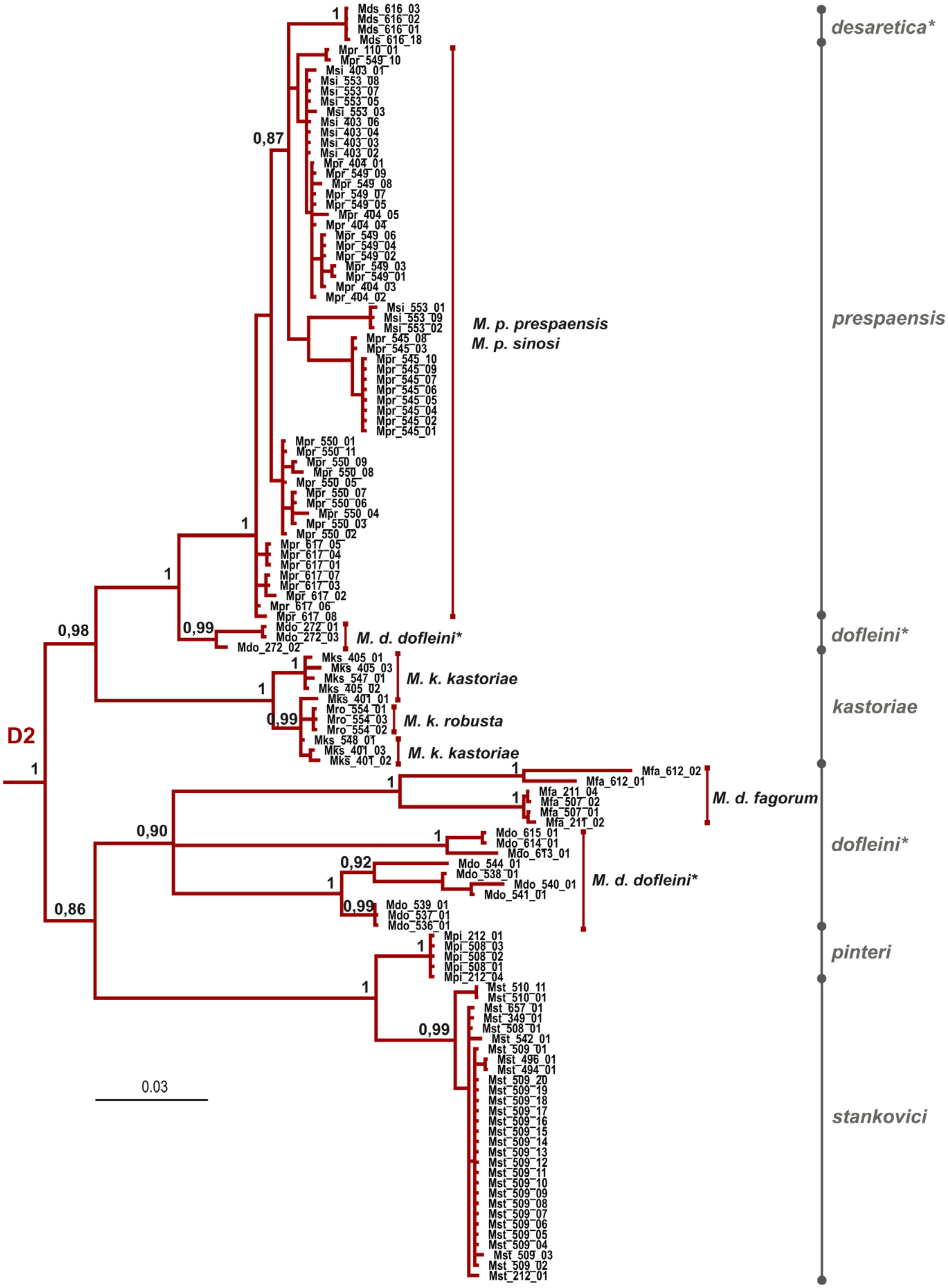

Fig. 5: BI tree of Clade D2 based on the concatenated mt data set (*COI*, *12S*, *16S*), rooted with Clade D1 (not shown). PP values (>0.80) of major nodes are indicated. Asterisks: taxa occurring in more than one clade. Data of specimens (geographic origin, taxonomy) are found in Mason et al. (2020), Table S1.

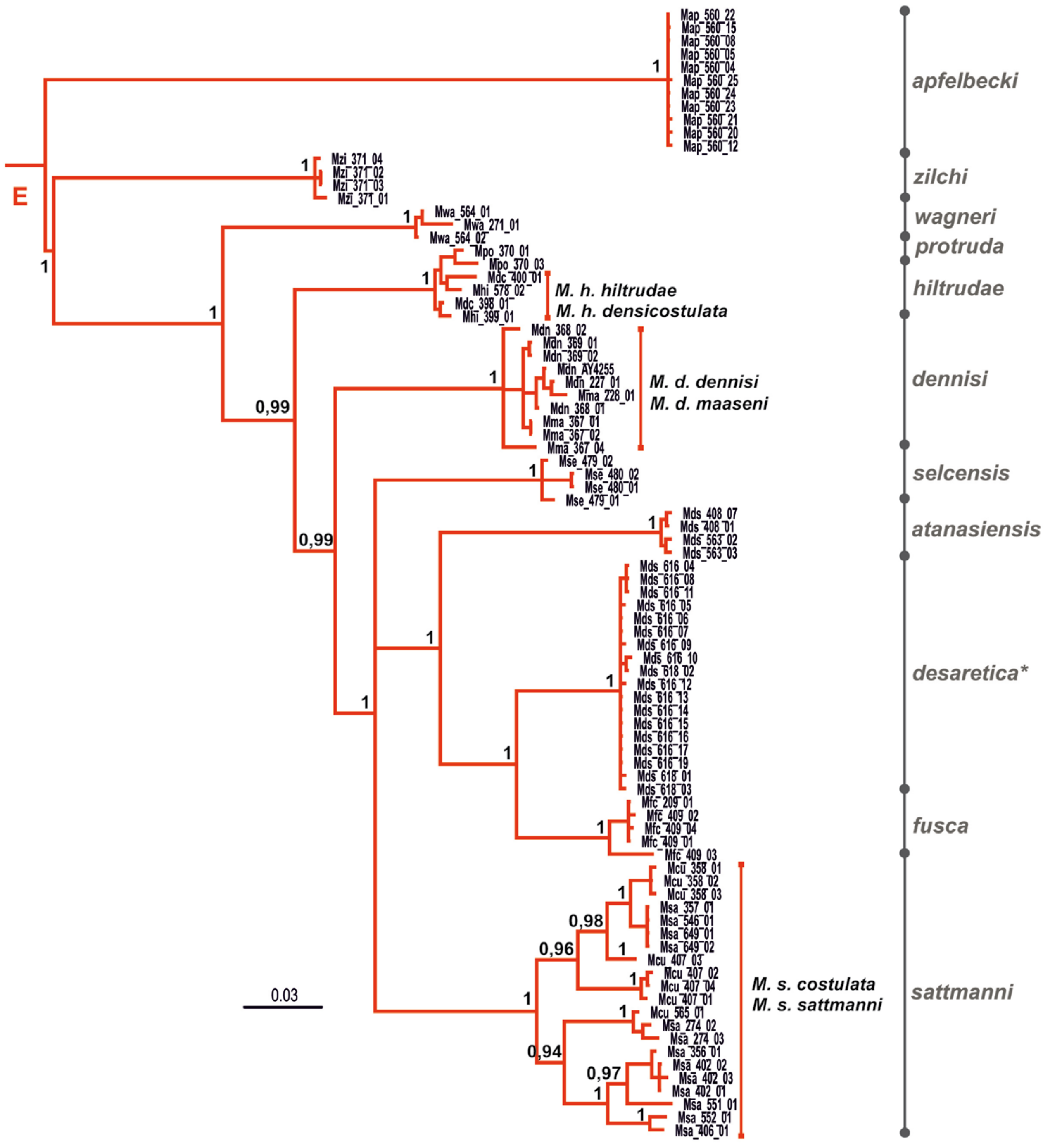

Fig. 6: BI tree of Clade E based on the concatenated mt data set (*COI*, *12S*, *16S*, midpoint rooted). PP values (>0.80) of major nodes are indicated. Asterisk: species occurring in more than one clade. Data of specimens (geographic origin, taxonomy) are found in Mason et al. (2020), Table S1.

ready described in detail in Mason et al. (2020) and De Mattia et al. (2020). This species complex deserves thorough investigation, optimally at the genomic level, and might turn out as an interesting case study of re-merging of formerly diverged taxa.

Clade E

This clade was highly affected by taxonomical changes, as six subspecies previously belonging to *M. hiltrudae*, were raised to species level (*M. protruda, M. dennisi, M. selcensis, M. desaretica, M. fusca, M. sattmanni*), two subspecies have changed combination (*M. dennisi maaseni, M. sattmanni costulata*) and one species was newly described (*M. atanasiensis;* Fig. 6). Two out of the 11 species in this clade are still paraphyletic (*M. hiltrudae* clade E; *M. desaretica* clades E and D2). Details about the phylogenetic problems within this clade have already been discussed in Mason et al. (2020) and De Mattia et al. (2020).

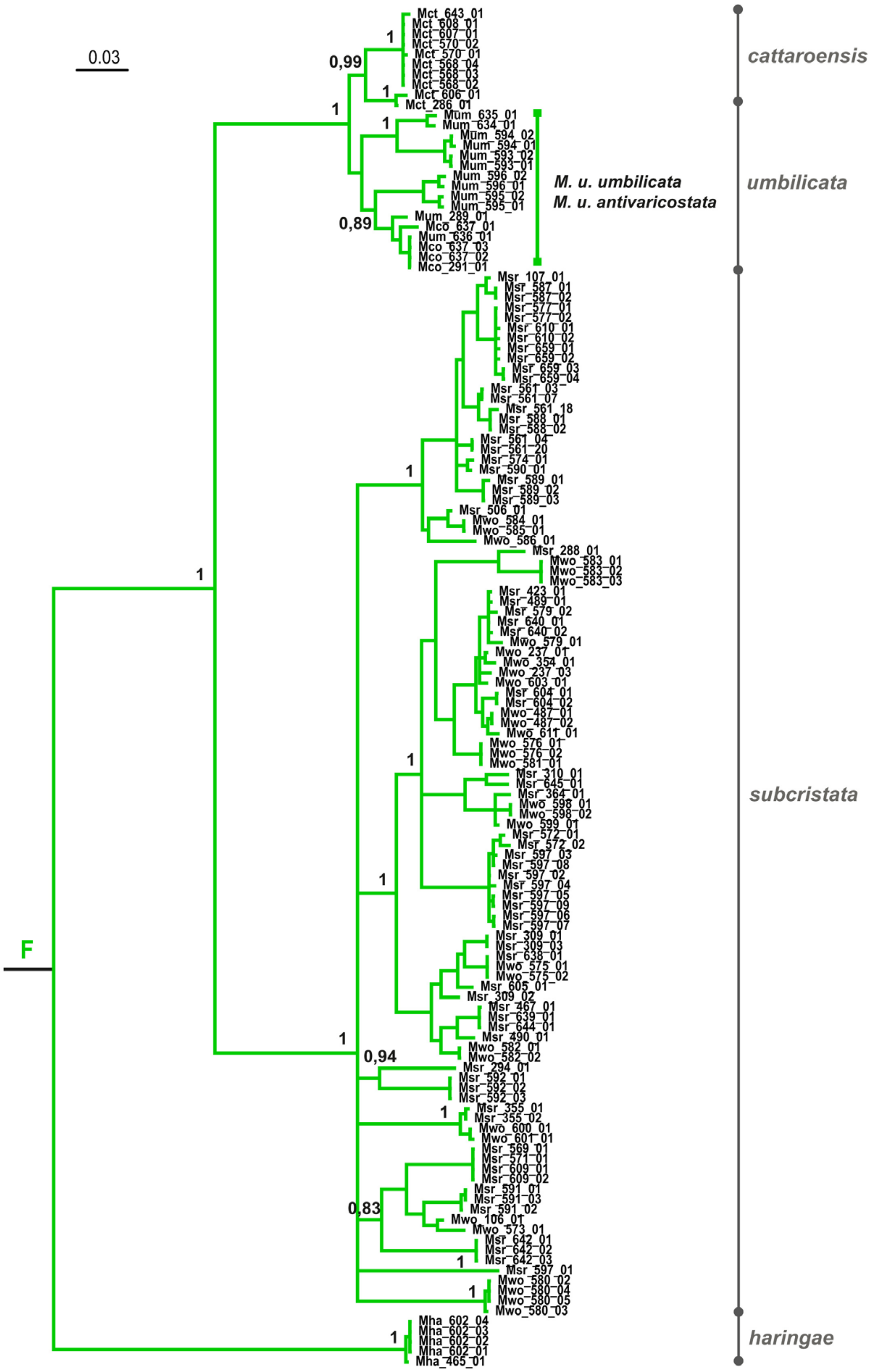

Fig. 7: BI tree of Clade F based on the concatenated mt data set (*COI*, *12S*, *16S*), rooted with Clade A (not shown). PP values (>0.80) of major nodes are indicated. Data of specimens (geographic origin, taxonomy) are found in Mason et al. (2020), Table S1.

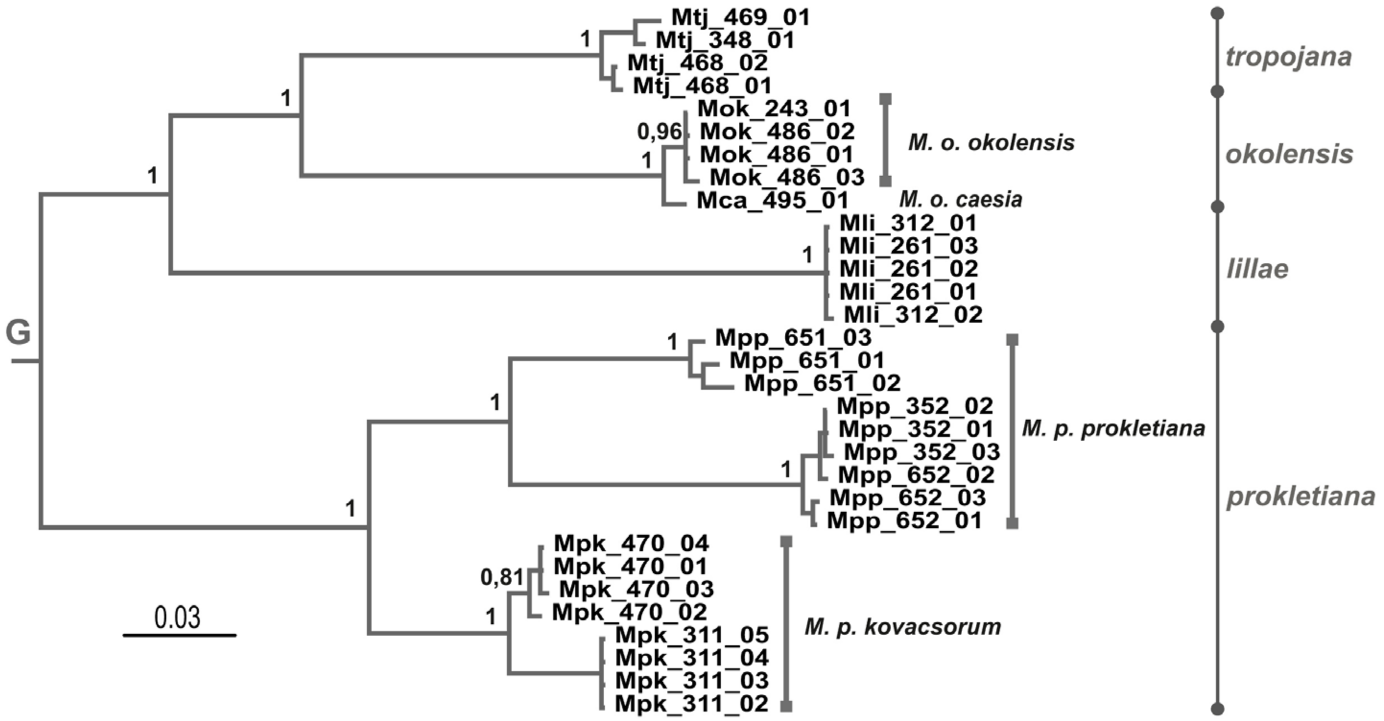

Fig. 8: BI tree of Clade G based on the concatenated mt data set (*COI*, *12S*, *16S*, midpoint rooted). PP values (>0.80) of major nodes are indicated. Data of specimens (geographic origin, taxonomy) are found in Mason et al. (2020), Table S1.

Clade F

Only two changes have been made in clade F: *M. cattaroensis umbilicata* has been raised to species level (*M. umbilicata*) and another subspecies *M. cattaroensis antivaricostata* was assigned to this species (now *M. umbilicata umbilicata* and *M. umbilicata antivaricostata*; Fig. 7).

Clade G

Within Clade G, only *M. tropojana* was raised to species level (formerly, *M. sporadica tropojana*, see comments to Clade B & C). The status of all other taxa remained unchanged (Fig. 8).

Clade H

A major subclade of Clade H is formed by the new species *M. drimica* (three subspecies), which was formerly a subspecies of *M. perstriata* (sensu Fehér & Szekeres, 2016; Fig. 9). The latter was polyphyletic in the *mt* analysis of Mason et al. (2020), as it was scattered across four clades. By De Mattia et al. (2020) most taxa have changed combination and/or status and *M. perstriata* is now found in clade L with five subspecies (see below).

In Clade H, the outstanding position of one *M. drimmeri* specimen (Mde_204_01) within *M. d. drimica* (taxonomic assignment supported by genital anatomy), remains enigmatic.

Finally, two of the subspecies of *M. sturanyana* had been raised to species level: *M. ostrovicensis, M. gropana*, while *M. timeae* remained unchanged.

Clade I

In Clade I, the former *M. perstriata ochridensis*, and *M. p. tenebrosa* (sensu Fehér & Szekeres, 2016) were raised to species level (now *M. ochridensis* and *M. tenebrosa*; Fig. 9). The former *M. perstriata* subspecies *callistoma* was assigned to *M. ochridensis* as well. Yet, *M. o. callistoma* still remains paraphyletic: The population Mcs_441, clearly assigned to *M. ochridensis* by shell morphology, is positioned within *M. nana*. It showed a very similar genital setup as *M. nana* and also occurs geographically close to the *M. nana* populations. The position in the tree is, therefore, most likely due to hybridization.

Montenegrina helvola ssp. remained unchanged. *Montenegrina nana* formerly comprised three subspecies (*M. n. nana, M. n. barinai, M. n. gracilis*). Now *M. n. gracilis* was raised to species level (now *M. gracilis*) and *M. n. barinai* was assigned to this species (now *M. g. barinai*).

Clade K

This clade formerly contained *M. perstriata* with three subspecies, which were revised according to the anatomical results: *M. p. radikae* was raised to species level (now *M. radikae*) and one population described as a new subspecies: *M. radikae paparistoae* (Fig. 10). The former *M. p. drimica* populations of Clade K were newly assigned to

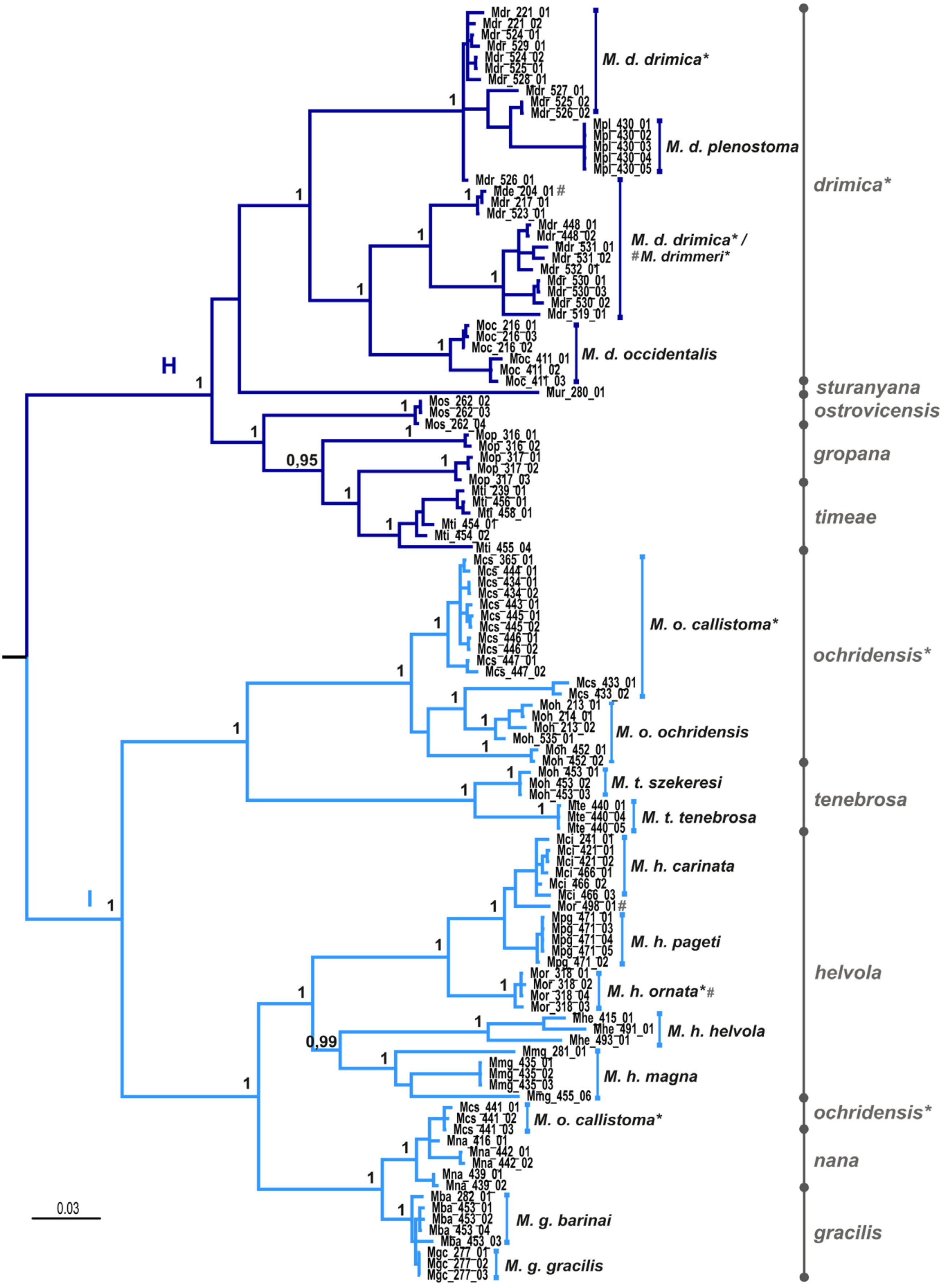

Fig. 9: BI tree of Clades H + I based on the concatenated mt data set (*COI*, *12S*, *16S*, midpoint rooted). PP values (>0.80) of major nodes are indicated. Asterisks: taxa occurring in more than one clade. #: single sequences within other (sub) species. Data of specimens (geographic origin, taxonomy) are found in Mason et al. (2020), Table S1.

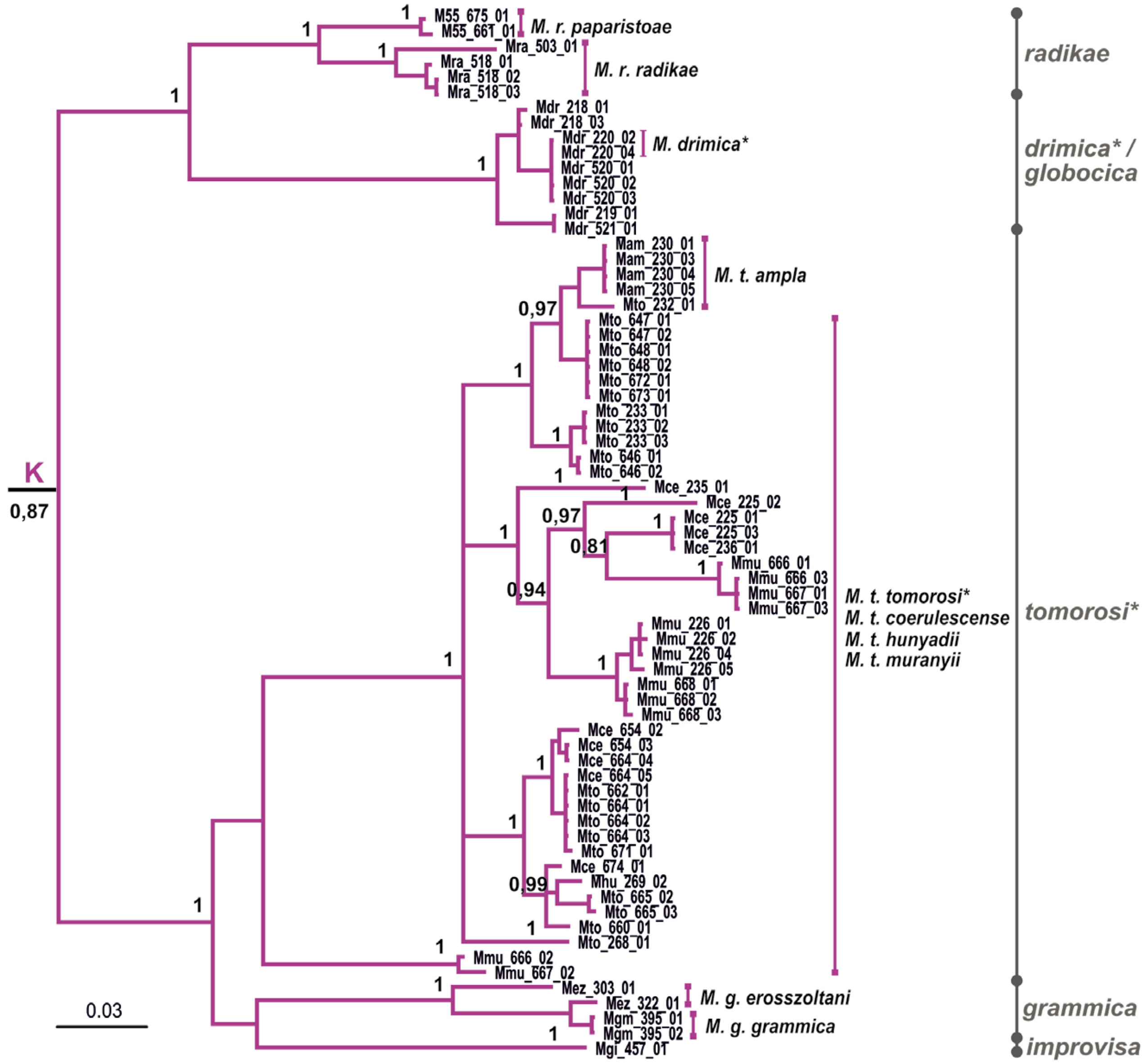

Fig. 10: BI tree of Clade K based on the concatenated mt data set (*COI*, *12S*, *16S*, rooted with Clade J). PP values (>0.80) of major nodes are indicated. Asterisks: taxa occurring in more than one clade. Data of specimens (geographic origin, taxonomy) are found in Mason et al. (2020), Table S1.

M. globocica. Yet, the complicated taxonomy of *M. p. drimica* (sensu Fehér & Szekeres, 2016) was not completely resolved. In Mason et al. (2020) this taxon was located in both Clades H and K. Even after the split into the two distantly related species *M. drimica* (Clade H) and *M. globocica* (Clade K), one population (Mdr_220) remained as *M. drimica* within *M. globocica* (Fig. 10).

Montenegrina t. tomorosi remained paraphyletic (see clade J) and thus problematic. Inside the *M. tomorosi*-complex, *M. fuchsi muranyii* was newly assigned to *M. tomorosi* (as *M. tomorosi muranyii*), leaving *M. fuchsi* now monophyletic in clade D1. *Montenegrina grammica improvisa* was raised to species level (now *M. improvisa*).

Clade L

Three of the six present species in Clade L remained unchanged (*M. soosi, M. perstriata, M. drimmeri*; Fig. 11). As already mentioned above, *M. perstriata*, which was divided over four clades before, remains only in this clade and is now monophyletic.

Due to differences in shell morphology and anatomical features, *M. iba* is now classified as valid species, yet leaving *M. laxa* paraphyletic. The big cluster of paraphyletic subspecies of *M. laxa* was assigned to *M. miraka*, which is now a monophyletic species comprising seven subspecies (*M. m. miraka, M. m. kontschani, M. m. errans, M. m. disjuncta, M. m. deli, M. m. dedovi, M. m. lakmosensis*).

Concluding remarks

The graphical overview provided here, combining the work of De Mattia et al. (2020) and Mason et al. (2020) should illustrate the present status of the various taxa and point out which questions are left behind. Each of the still

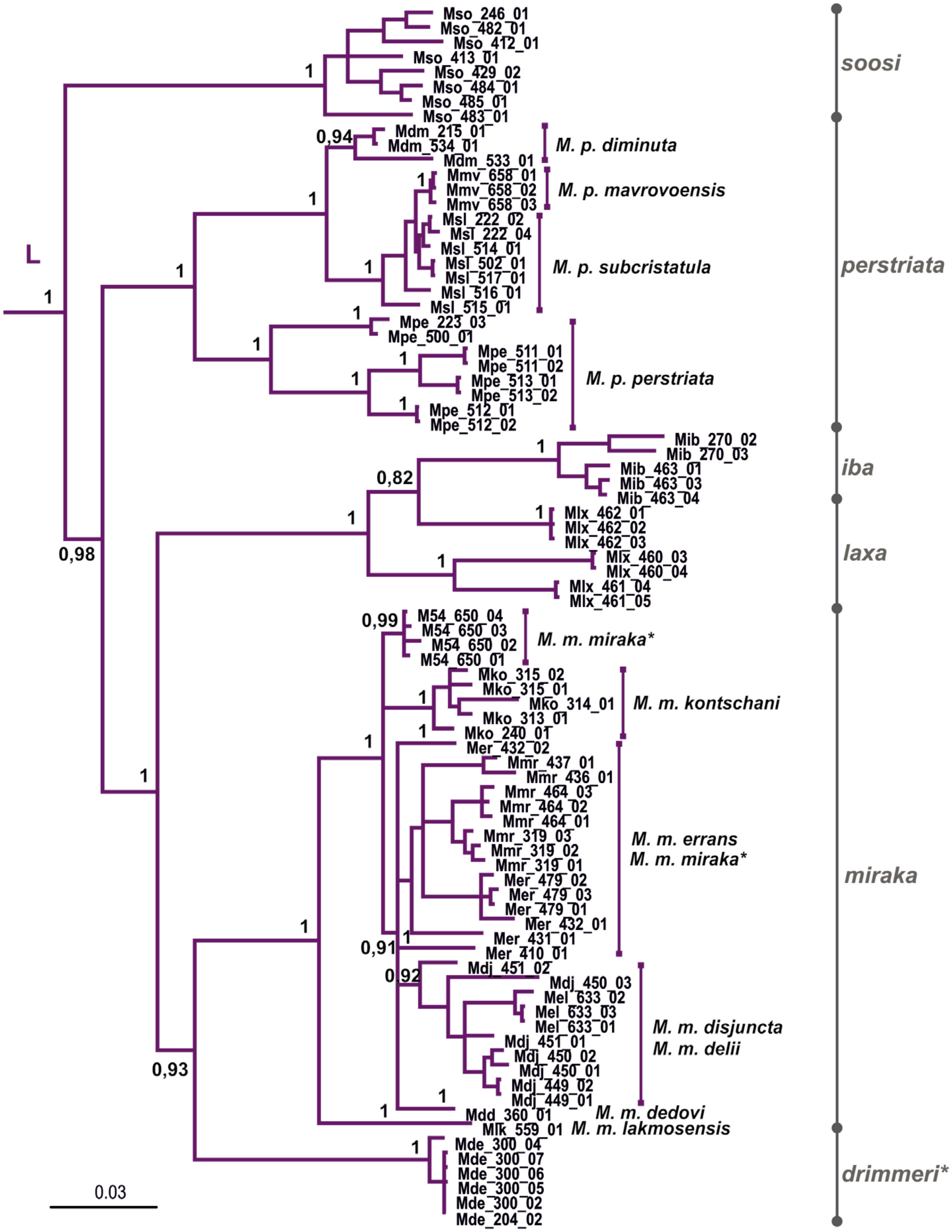

Fig. 11: BI tree of Clade L based on the concatenated mt data set (*COI*, *12S*, *16S*, rooted with Clade J). PP values (>0.80) of major nodes are indicated. Asterisks: taxa occurring in more than one clade. Data of specimens (geographic origin, taxonomy) are found in Mason et al. (2020), Table S1.

unclear relationships in this huge genus deserves further investigation. Isolation, micro-endemism, rapid differentiation of genital anatomical features, together with sporadic introgression are the factors making the systematics of *Montenegrina* such an arduous endeavour.

Acknowledgements

The *Montenegrina* project (2014–2018), which was the basis for the present paper, was funded by the Austrian Research Fund (FWF; project P 26581-B25). We are grateful to many colleagues for all the help while collecting the material as well as for so many discussions throughout the long-lasting project on *Montenegrina*.

References

De Mattia, W., Fehér, Z., Mason, K. & Haring, E. (2020): An integrative approach to the taxonomy and systematics within the genus *Montenegrina* Boettger, 1877 (Mollusca, Gastropoda, Clausiliidae). Journal of Zoological Systematics and Evolutionary Research 58: 691–808. https://doi.org/10.1111/jzs.12407

Fehér, Z. & Szekeres, M. (2016): Taxonomic revision of the rock-dwelling door snail genus *Montenegrina* Boettger, 1877. ZooKeys, 599, 1–137. https://doi.org/10.3897/zookeys.599.8168

Fehér, Z., Mason, K., Szekeres, M., Haring, E., Bamberger, S., Páll-Gergely, B. & Sólymos, P. (2018): Range-constrained co-occurrence simulation reveals little niche partitioning among rock-dwelling *Montenegrina* land snails (Gastropoda: Clausiliidae). Journal of Biogeography, 45(6), 1444–1457. https://doi.org/10.1111/jbi.13220

Gittenberger, E. (1991): What about non-adaptive radiation? Biological Journal of the Linnean Society, 43(4), 263–272. https://doi.org/10.1111/j.1095-8312.1991.tb00598.x

Gittenberger, E. (2004): Radiation and adaptation, evolutionary biology and semantics. Organisms, Diversity & Evolution, 4, 135–136. https://doi.org/10.1016/j.ode.2004.04.002

Mason, K., Fehér, Z., Bamberger, S., Reier, S., Szekeres, M., Sattmann, H., Kruckenhauser, L., De Mattia, W. & Haring, E. (2020): New insights into and limitations of the molecular phylogeny in the taxon-rich land snail genus *Montenegrina* (Mollusca: Gastropoda: Clausiliidae). Journal of Zoological Systematics and Evolutionary Research 58(3): 662–690. https://doi.org/10.1111/jzs.12413

Zeitschrift/Journal: Arianta

Jahr/Year: 2023

Band/Volume: 10

Autor(en)/Author(s): Mason Katharina, De Mattia Willy, Feher Zoltan, Haring Elisabeth

Artikel/Article: Update on the phylogeny of the land snail genus Montenegrina Boettger, 1877 (Mollusca: Gastropoda: Clausiliidae) 26-38