The African endemic species "*Nychiodes*" *tyttha* Prout, 1915 (Lepidoptera, Geometridae, Ennominae) belongs to the genus *Aphilopota* Warren, 1899

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Abstract. An extensive examination of the external and internal morphological characters of the genus *Nychiodes* shows that "*Nychiodes*" *tyttha* Prout, 1915 is incorrectly placed in this genus. The systematic position of this species was investigated by using a multigene analysis, including one mitochondrial and up to nine protein-coding nuclear gene regions, and morphological characters. These results support a re-classification of this species as *Aphilopota tyttha*, **comb. nov.** A re-description supported by illustrations of morphological characters for *A. tyttha* is provided.

Introduction

Prout (1915) described an African geometrid species *tyttha* and placed it in the genus *Nychiodes* Lederer, 1853. In his description, he mentioned the much smaller size and slight differences in venation of *N. tyttha* from other *Nychiodes* species (Prout 1915). Since size can be influenced by various parameters (e.g., the amount of available nutrition), more informative are characters such as differences in venation, widely used in Geometridae for differential diagnoses of genera (Hausmann 2001; Awmack and Leather 2002; Wanke et al. 2020).

Recently, the genus *Nychiodes* has undergone intensive integrative taxonomic revisions (Müller et al. 2019; Wanke et al. 2020). The genus contains 25 species, distributed from western Europe and North Africa to Iran, Afghanistan and Pakistan. *Nychiodes tyttha* has remained as the only species outside the mentioned distribution range, occurring in central and southern Africa (Janse 1932). The results of our investigation of morphological characters strongly support *N. tyttha* being excluded from the genus *Nychiodes* (Wanke et al. 2020). However, a suitable genus for this species could not be found until now in the absence of data allowing a molecular analysis. For this study, we aimed

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to extract DNA to clarify the systematic position of *N. tyttha*. This allowed a multi-gene molecular phylogenetic analysis to be conducted together with an examination of morphological characters.

Material and methods

Specimens used in this study are deposited the following collections (acronyms after Evenhuis 2007):

NHMUK Natural History Museum, London, United Kingdom;
HSS Private Collection of Hermann Staude, South Africa;
SMNS Staatliches Museum für Naturkunde Stuttgart, Germany;
ZSM (SNSB) Zoologische Staatssammlung München (Staatliche Naturwissenschaftliche Sammlungen Bayerns), Germany.

Morphological examination

For the documentation of external characters, a Visionary Digital photography system (LK Imaging System, Dun. Inc., equipped with a Canon EOS 5DSR camera), an Olympus E3 digital camera, as well as a Leica digital microscope (Z16 APO) were used. Standard techniques were followed for the preparation of genitalia (e.g. Robinson 1976) and evertion of the vesica took place following the method described by Sihvonen (2001). Finally, genitalia were embedded in Euparal as permanent slides and photographed with a Keyence VHX-5000.

Molecular data generation

Extraction of DNA and amplification of the "DNA barcode" fragment (658 base-pairs of the 5' terminus) of the mitochondrial Cytochrome-C Oxidase I of the holotype of *Nychiodes tyttha*, was carried out at the Canadian Centre for DNA barcoding (CCDB, Guelph), in the framework of the Lepidoptera Campaign of the international Barcode of Life program (iBOL; www.lepbarcoding.org), using a protocol for old museum specimens based on Next-Generation-Sequencing (Hausmann et al. 2016; Prosser et al. 2016). Extraction and amplification of non-type specimens were also carried out at the Canadian Centre for DNA barcoding (CCDB, Guelph) using standard protocols (e.g., Ivanova et al. 2006). *Nychiodes tyttha* specimens used for analysis of the "barcode" fragment and metadata are available on BOLD. Sample ID numbers are: BC ZSM Lep 106645 (holotype); BC ZSM Lep 13914; BC ZSM Lep 98802. As the holotype's DNA was extracted in Canada no extract was left after DNA barcoding for genomic DNA analysis. Sample BC ZSM Lep 98802 was repatriated from Guelph and amplification of further genes was done at the molecular laboratory in Finnish Museum of Natural History "Luomus", (Helsinki) using the DNeasy Blood and Tissue kit (Qiagen), following the manufacturer's protocol. DNA amplification and sequencing were carried out following protocols proposed by (Wahlberg and Wheat 2008; Wahlberg et al. 2016). One mitochondrial (cytochrome oxidase subunit I, COI) and up to ten protein-coding nuclear gene regions, Arginine Kinase (ArgK), carbamolyphosphate synthetase (CAD), sarco/endoplasmic reticulum calcium ATPase (Ca-ATPase), Elongation factor 1 alpha (EF-1α), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), isocitrate dehydrogenase (IDH), cytosolic malate dehydrogenase (MDH), sorting nexin-9-like (Nex9), ribosomal Protein (RpS5), and wingless (wgl), were sequenced for phylogenetic analyses.

Multiple sequences were aligned using Muscle algorithms as implemented in MEGA11 (Tamura et al. 2021) for each gene including other sequences of Boarmiini (see Appendix 1, Fig. A1) retrieved from the local VoSeq database (Peña and Malm 2012). For the phylogenetic hypothesis of Boarmiini,

a total of 300 taxa, from Murillo-Ramos et al. (2019) were incorporated into our dataset, of which two geometroid species (Sematuridae: *Mania lunus* (Linnaeus, 1758) and Uraniidae: *Urania leilus* (Linnaeus, 1758)) served as outgroups. The newly produced DNA sequences through this study were managed with the VoSeq database. The final dataset included a total length of 7662 bp including gaps, and missing data made up 34% of the final data matrix. The sequences described here are accessible via GenBank with the following accession numbers: ON980557–ON980558; ON982490–ON982496. All GenBank accession numbers of the 300 taxa are provided in the Suppl. material 1.

DNA Barcoding analyses

Three different analyses where performed. First, COI fragments of "*Nychiodes*" *tyttha* (sequences of holotype and two non-type specimens) were compared to available sequences in the Barcode of Life Datasystems (BOLD) identification engine to search for the genetically nearest neighbor. Second, a neighbor-joining tree (K2P on BOLD) was constructed with the sequence of the holotype of "*Nychiodes*" *tyttha* and 99 samples suggested by BOLD as related taxa to find the genetically nearest neighbor. Finally, the minimum p-distance of "*Nychiodes*" *tyttha* from *Nychiodes dalmatina* was calculated, to calculate the distance to the genus *Nychiodes*.

Phylogenetic analysis

The molecular data set partitioned by gene and codon position was analysed using maximum likelihood as implemented in IQ-TREE 2.1.3 (Minh et al. 2020). Best-fitting substitution models were selected by ModelFinder (Kalyaanamoorthy et al. 2017) with "-m MFP+MERGE" option. The best-fit models were chosen as follows: GTR+F+I+G4 for ArgK, COI, Nex9, and wingless; TIMe+I+G4 for Ca-ATPase; TIM2+F+I+G4 for CAD and IDH; SYM+I+G4 for EF-1α, GAPDH, MDH, and RpS5. The phylogenetic analysis was carried out with "-spp" option (edge proportional) that allows each partition to have its own evolutionary rate. We evaluated the node supports with ultrafast bootstrap approximations (UFBoot2) and the SH-like approximate likelihood ratio test (Guindon et al. 2010; Hoang et al. 2018) using the "-B 1000 -alrt 1000" option. To reduce the risk of overestimating branch supports in ultrafast bootstrap approximation analysis, we used the "-bnni" option, which optimizes each bootstrap tree using a hill-climbing nearest-neighbour-interchange (NNI) search. The resulting tree was rooted and visualized in FigTree v1.4.2 (Rambaut 2015).

Results

The comparison of the COI fragments only of "*Nychiodes*" *tyttha* (holotype and two non-type specimens) with data from the BOLD database, suggested that the genetically nearest neighbors are in the genera *Jankowskia* Oberthür, 1884, *Tephronia* Hübner, 1825 and *Peribatodes* Wehrli, 1943 (genetic distances of 6.4–7.9%). When a neighbor-joining tree (K2P on BOLD) was constructed using the holotype DNA barcode sequence with the 99 nearest samples provided by BOLD, an Australian species, *Aeolochroma* sp. ANIC1 (BOLD:AAV4042), which is 8.33% divergent by p-distance, separated "*N.*" *tyttha* from the above and other genera. Sequences from the other two specimens of "*N.*" *tyttha* (BC ZSM Lep 106645, BC ZSM Lep 98802) were 1.23–1.39% divergent. By contrast, the minimum p-distance (COI, K2P, BOLD gap analysis) from *Nychiodes dalmatina* is 10.4%.

Additionally, five out of the eleven target genes of a single non-type specimen of "*Nychiodes*" *tyttha* were successfully amplified and sequenced (COI-1,COI-2, wgl, Ca-ATPase, Nex9). In the multigene phylogenetic analysis "*Nychiodes*" *tyttha* clustered as sister to other species of *Aphilopota*

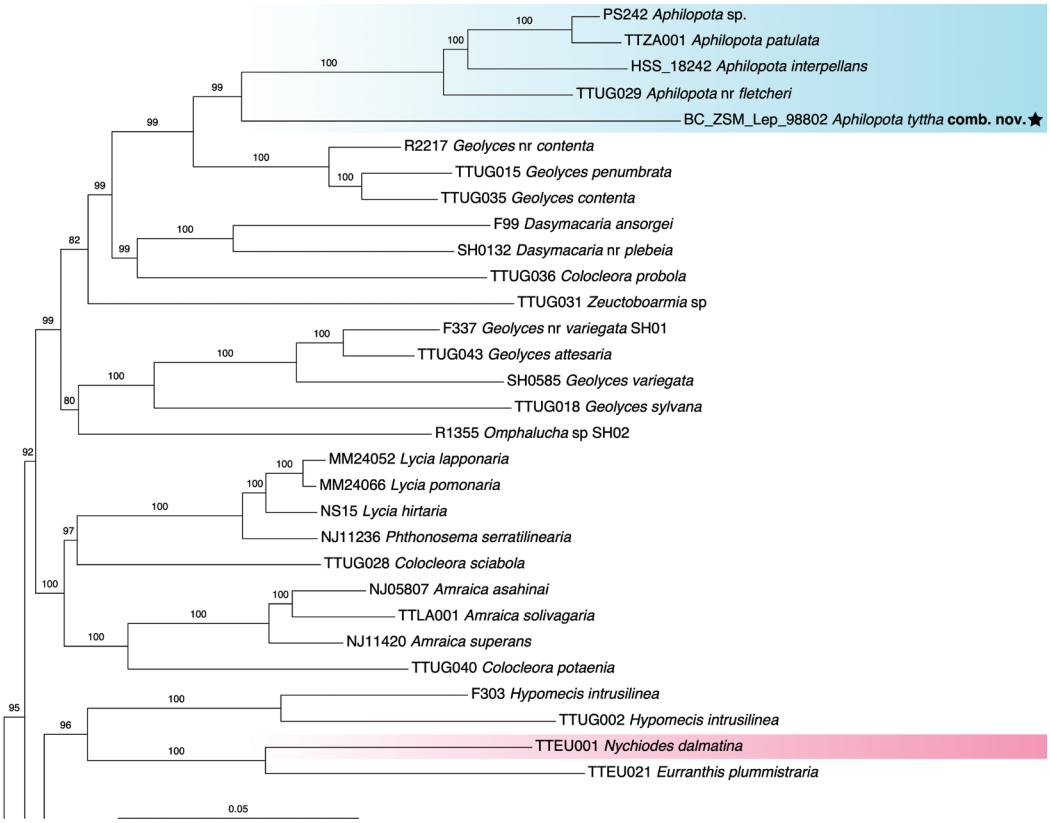


Figure 1. Phylogenetic position of *Aphilopota tyttha*, comb. nov. (marked with a star) within the tribe Boarmiini, supporting the tentative combination in genus *Aphilopota*. The numbers above the branches are the bootstrap values of the maximum likelihood IQ-TREE analysis. The complete tree is shown in Appendix 1, Fig. A1.

Warren, 1899 (Fig. 1, Appendix 1, Fig. A1). Moreover, the results of our morphological examination served as an additional line of evidence and revealed that "*Nychiodes*" *tyttha* has the diagnostic generic characters of *Aphilopota*, supporting its affiliation to this genus (for detailed comparison see the taxonomy part). The species is re-described in the taxonomic part of the discussion.

Discussion

Systematics

The results of our multi-gene molecular phylogenetic analysis show that "*Nychiodes*" *tyttha* groups as sister to *Aphilopota* (UFB = 97%). The phylogenetic analysis would allow us either to classify "*N.*" *tyttha* in a monotypic genus as sister to *Aphilopota*, or to combine it with other *Aphilopota*. The classification as sister to *Aphilopota* may not hold when more species of this genus are added to the dataset. Currently the genus *Aphilopota* consists of 44 species, distributed exclusively in Africa and Madagascar (Scoble 1999; Scoble and Hausmann 2007), but the genus urgently needs taxonomic revision. The detailed morphological investigation of the taxon *tyttha* in

the framework of the present study supports its combination with *Aphilopota*. Consequently, we transfer "*N.*" *tyttha* to the genus *Aphilopota*.

In the following diagnosis (see taxonomy part), the morphological characters of *Aphilopota tyttha* comb. nov. are compared to the type species of the genera *Aphilopota* (*A. interpellans* (Butler, 1875)) and *Nychiodes* (*N. obscuraria* (Villers, 1789)), which support the new combination.

Taxonomy

Aphilopota tyttha (Prout, 1915), comb. nov.

Figs 2–11, 17, 18, 21

Nychiodes tyttha Prout, 1915. Novitates zoologicae: a journal of zoology in connection with the Tring Museum, 22, 363. Holotype ♂ (Eritrea, Caraiai). Deposited in NHMUK (examined).

Type material examined. Holotype, ♂, Eritrea, Caraiai, 21.xi.1905, N. Beccari, Geometridae genitalia slide No. 4976, Rothschild Bequest B.M. 1939-1, NHMUK010920109, DNA barcode sample ID BC ZSM Lep 106645, DNA barcode process ID GWOTZ396-19, BIN BOLD:AAW8833 [579 bp]; in NHMUK

Additional material examined. 1♀, Eritrea, Caraiai, 21.xi.1905, N. Beccari, Geometridae genitalia slide No. 4977, NHMUK014173598; 1 ♂, Kenya, Kitale, 14.9.[19]25, leg. G.W. Jeffery, NHMUK010920119; 1 ♂, [Namibia], Sissekab, N.W. of Otavi, 1300 m, leg. K. Jordan, 11.xi.1933, NHMUK010920120; all in NHMUK. 1 ♂, South Africa, Mkuze Chaos, 27°39.490'S, 032°00'E, 28.xii.1990, 220 m, leg. H.S. Staude; in HSS. 1 ♂, Ethiopia, Bahar Dar, iii.1969, Lichtfang, leg. Schäuffele, g.prep. 0732/2020 D. Wanke; in SMNS. 1 ♂, Äthiopien [Ethiopia], Awassa, Awassa Lake, Reg. Bale, 07°02.886'N, 038°27.491'E, 23.–24.v.1999, leg. R. Beck & M. Hiermeier; 1 ♂, S. Ethiopia – SN, Arba Minch, Nechisar NP, 2.75 km SW headquarter, 1170 m (lux), 06°00'13.6"N, 37°33'23,4"E, 22.ii.2012, leg. Hacker & Schreier; all in ZSM.

Remark. The genus *Aphilopota* needs taxonomic revision, based on a broad integrative taxonomic approach. Therefore, a comparison with other species of this genus, except of the type species *A. interpellans*, is not possible and also not necessary here.

Diagnosis. In *A. tyttha* labial palpi thin, about two thirds of the diameter of the eye (labial palpi thick, about one diameter of the eye in *A. interpellans* and *N. obscuraria*) (Fig. 2). Proboscis reduced (similar in *A. interpellans* and *N. obscuraria*) (Fig. 2). In the forewing venation of *A. tyttha* R1 arising from the cell, not reaching costa, R2 fused with R1 (similar in *A. interpellans*; arising from the cell, R1 and R2 share a common stalk in *N. obscuraria*) (Fig. 4). In the male genitalia (Figs 17–20) of *A. tyttha* valva thin, without any ampulla or harpe (similar in *A. interpellans*; valva equipped with the two main processes ampulla superior and ampulla inferior in *N. obscuraria*). Juxta of *A. tyttha* forked, large and straight, reaching up to the gnathos (juxta forked, large and tip thick, reaching far beyond the gnathos, apex bent in *A. interpellans*; juxta anchor-shaped in *N. obscuraria*). Aedeagus in *A. tyttha* thin tapered, with one long and sclerotized cornutus (aedeagus funnel-shaped, without strong cornutus in *A. interpellans*; thickness of aedeagus variable with one sclerotized cornutus variable in length in *N. obscuraria*). In female genitalia (Figs 21–24) of *A. tyttha* ovipositor telescopic and strongly elongated (similar in *A. interpellans*; not elongated in *N. obscuraria*). Corpus bursae of *A. tyttha* arched, tube-like elongated (arched, long in *A. interpellans*; round membranous in *N. obscuraria*). Signum absent in *A. tyttha* (similar in *A. interpellans*; signum stellate in *N. obscuraria*).

Tribal assignment. According to the molecular phylogenetic analysis (Fig. 1, Appendix 1, Fig. A1) and morphology (Figs 2–24), *Aphilopota tyttha* is classified in Boarmiini.

Figure 2. Head structures of male specimens of **A, B.** *Aphilopota tyttha* (Prout), comb. nov., **C, D.** *Aphilopota interpellans* (Butler), and **E, F.** *Nychiodes obscuraria* (Villers). **A, C, E.** Head in ventral view; **B, D, F.** Lateral view.

Re-description. Wingspan ♂ 21–25 mm, ♀ 28 mm, average length of forewing 11.2 mm (n = 7). Antennae bipectinate in both sexes. Frons weakly convex, just reaching over the eyes, densely scaled. Labial palpi thin, about two third of the diameter of the eye. Proboscis reduced, represented by barely visible rudimentary slats (Fig. 2A). Chaetosemata as two small patches, each located between the eye margin and the antennal base (Fig. 2B). Foreleg epiphysis approximately as long as tibia. Base of the epiphysis starting after one fourth of tibia. Mesotibia with one pair of spurs, hindtibia with two pairs of spurs (Fig. 3). Head, thorax and abdomen concolorous with wings.

Ground colour of wings beige brown, transverse lines present in dark brown to black. Terminal line continuous, concolorous with transverse lines. In forewing antemedial line curved towards termen. Postmedial line curved between R5/M1 and M2. Medial area with more darker scales

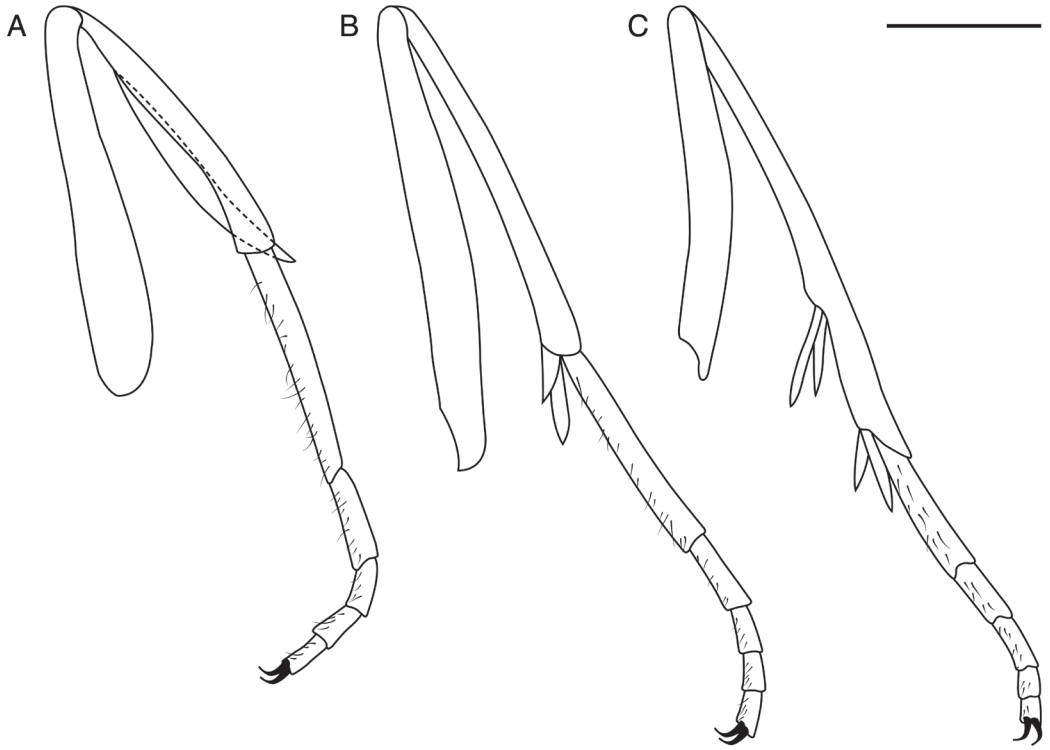


Figure 3. Drawings of the legs of *Aphilopota tyttha* (Prout) comb. nov. **A.** Foreleg; **B.** Midleg; **C.** Hindleg.

intermixed. In hindwing antemedial line curved towards termen on M1. Discal spots only present on underside (Figs 5–11).

In forewing, vein R1 arising from the cell, not reaching costa, R2 merged with R1, R3–5 with a common stalk arising from the cell. In hindwing Sc+R1 strongly curved in basal area, approximating to the cell, M2 absent, A3 and A1+2 originating separately (Fig. 4).

In male genitalia uncus strongly sclerotized, short, basally broad and triangular, apically pointed. Gnathos well developed and strongly sclerotized, triangular. Costa of valva sclerotized, valva thin, without any ampulla or harpe. Juxta forked and big, reaching up to gnathos. Saccus tapering. Aedeagus thin tapered, carrying one long and sclerotized cornutus. Cornutus almost same length as aedeagus (Figs 17–18).

Female genitalia thin and long, with strongly elongated ovipositor. Apophyses posteriores very long, apophyses anteriores 1/3 length of apophyses posteriors. Antrum sclerotized. Ductus bursae short, bend. Corpus bursae tube-like, elongated. Signum absent (Fig. 21).

Phenology. Adults observed from November to May.

Biology. Unknown.

Habitat. Investigated specimens collected at elevations from 220 to 1300 m in dry savanna ecoregions.

Distribution. In East Africa (Eritrea, Ethiopia, Kenya), south-western Africa (Namibia) and South Africa (Janse 1932).

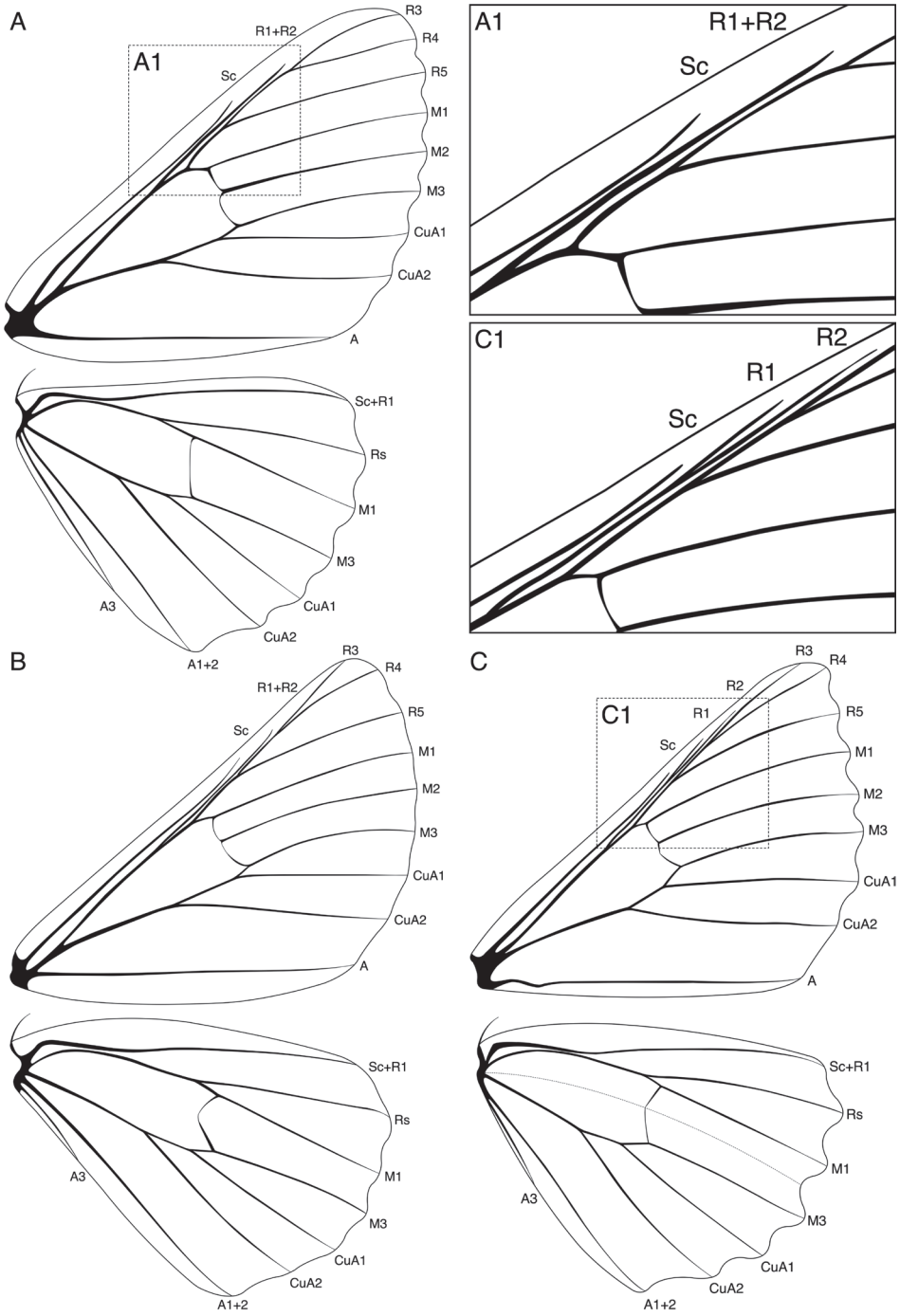
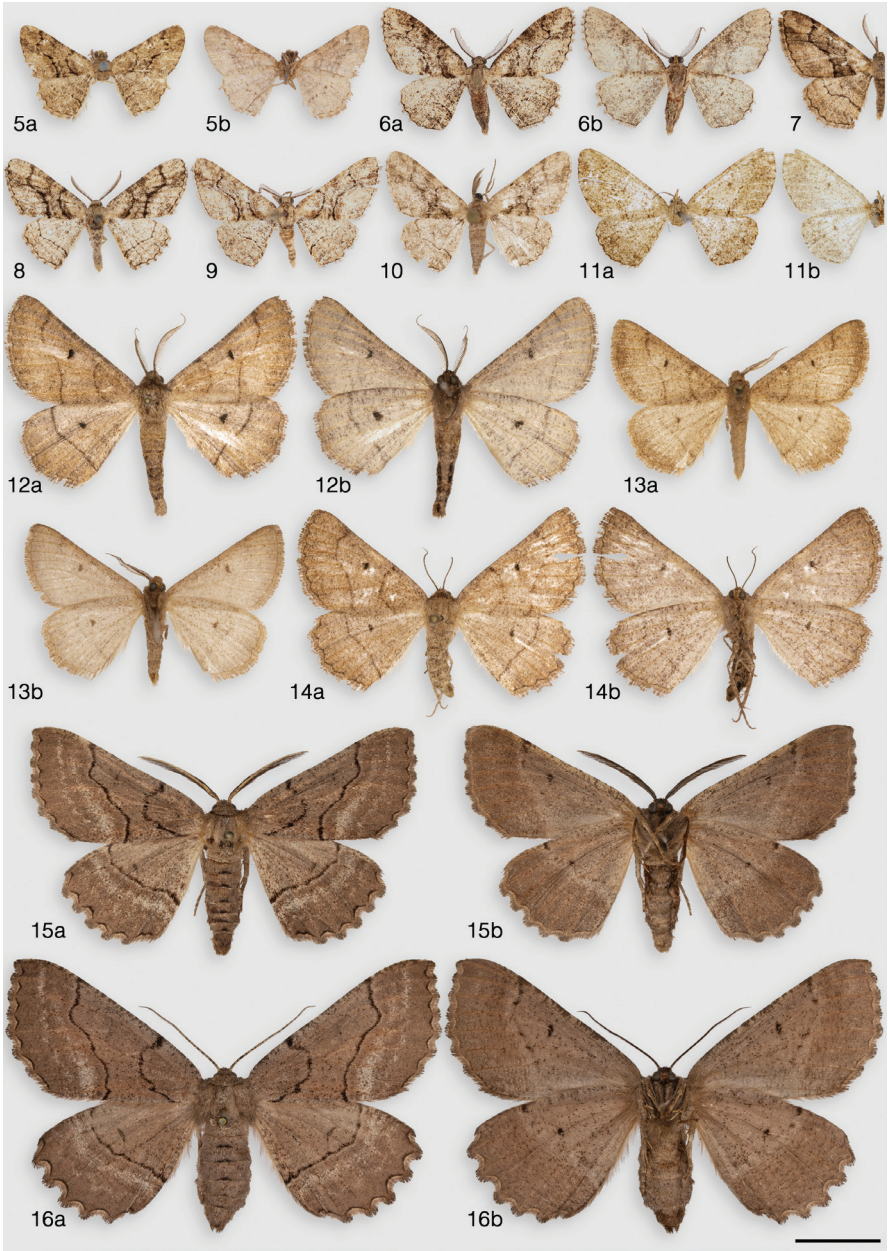
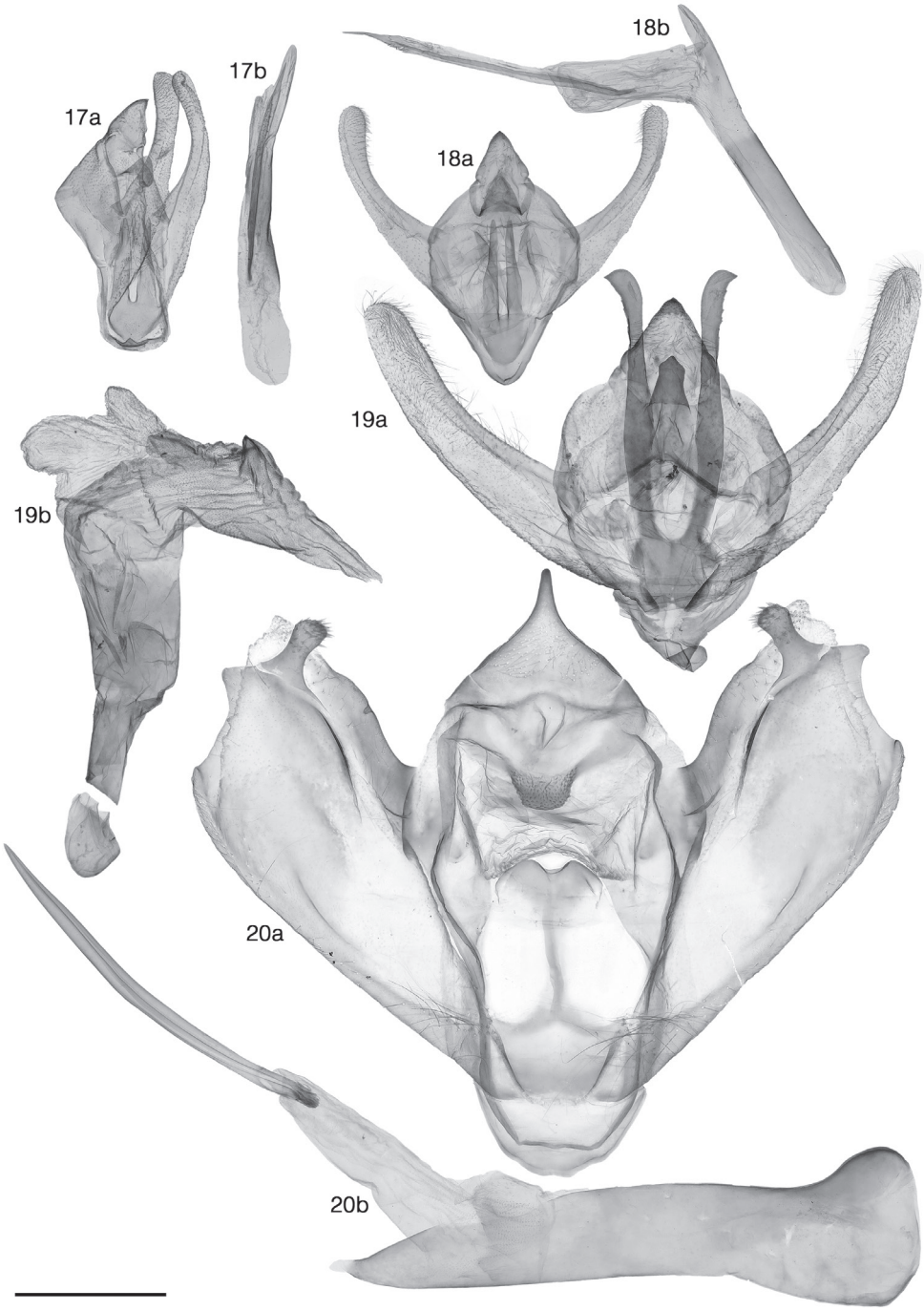


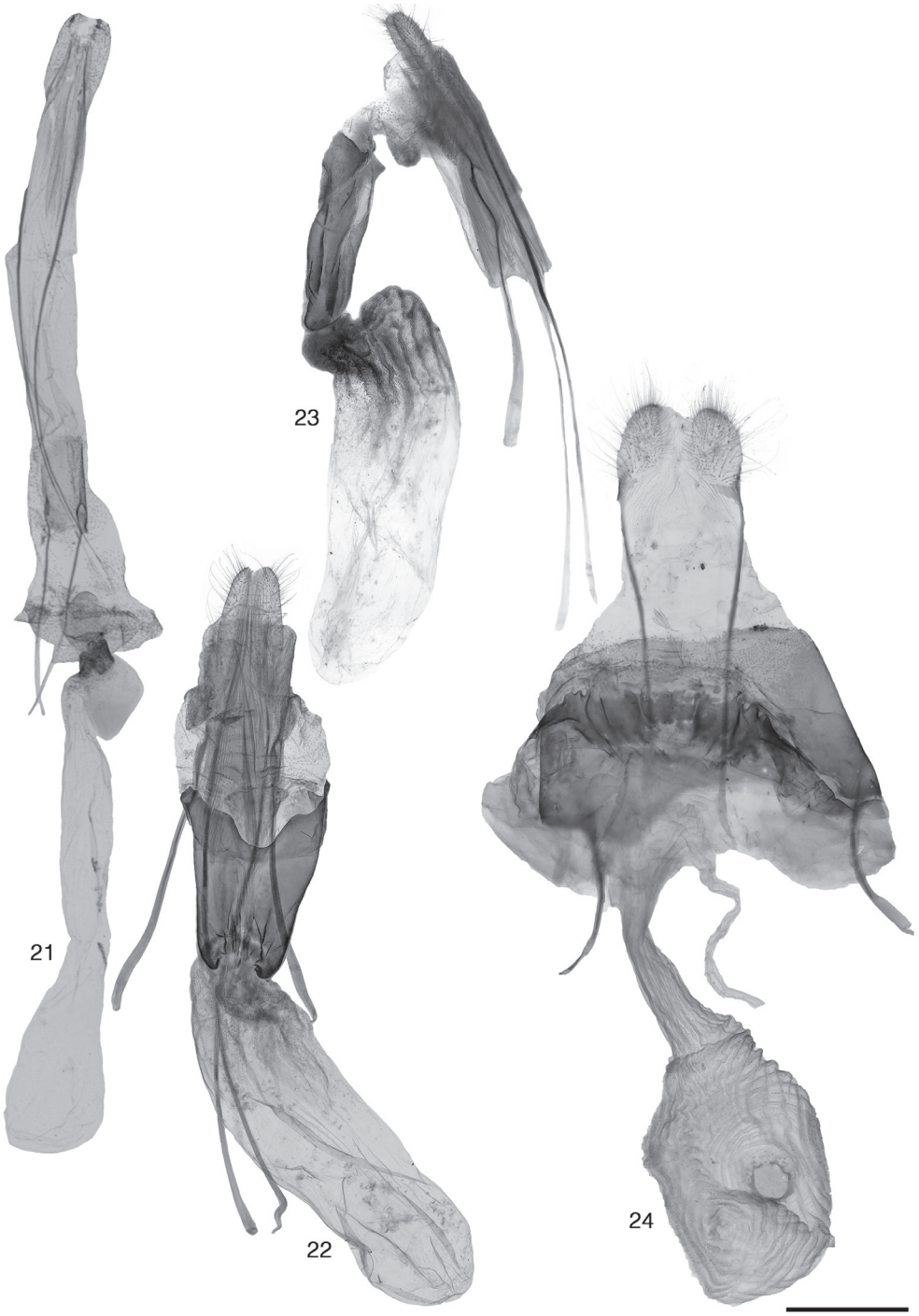
Figure 4. Wing venation drawings of male specimens of **A.** *Aphilopota tyttha* (Prout) comb. nov.; **B.** *Aphilopota interpellans* (Butler), and **C.** *Nychiodes obscuraria* (Villers). In the forewing of *A. tyttha* and *A. interpellans* vein R2 is fused with R1 (rectangle A1), vein R1 and R2 share a common stalk in *N. obscuraria* (rectangle C1). Remark: as in the genus *Nychiodes*, the veins R1 and R2 are on a common stalk. This suggests that the veins R1 + R2 are fused to one vein in the genus *Aphilopota*; therefore, here we name this vein R1+R2.



Figures 5–16. Wing pattern of *Aphilopota tyttha* (Prout), comb. nov., *Aphilopota interpellans* (Butler) and *Nychiodes obscuraria* (Villers). **5–11.** *A. tyttha* (**5.** Holotype, Eritrea, Caraiai, g.prep. 4976, NHMUK010920109; **6.** Ethiopia, Awassa, Awassa Lake; **7.** Kenya, Kitale, NHMUK010920119; **8.** [Namibia], Sissekab, N.W. of Otavi, NHMUK010920120; **9.** Ethiopia, Bahar Dar, g.prep. 0732/2020 D. Wanke; **10.** South Africa, Mkuze Chaos; **11.** Eritrea, Caraiai, NHMUK014173598); **12–14.** *A. interpellans* (**12.** South Africa, KwaZuluNatal, Vryheid, g.prep. 1314/2022 D. Wanke; **13.** [South Africa], Transvaal, Bords du Limpopo, g.prep. 1316/2022 D. Wanke; **14.** South Africa, KwaZuluNatal, Vryheid, g.prep. 1315/2022 D. Wanke); **15–16.** *N. obscuraria* (**15.** Spain, Albarracin, g.prep. 2096/2017 H. Rajaei; **16.** Spain, Albarracin, g.prep. 2097/2017 H. Rajaei); a = upperside; b = underside. Scale bar: 1 cm.



Figures 17–20. Male genitalia of *Aphilopota tyttha* (Prout), comb. nov., *Aphilopota interpellans* (Butler) and *Nychiodes obscuraria* (Villers). **17–18.** *A. tyttha* (**17.** Holotype, Eritrea, Caraiai, g.prep. 4976, NHMUK010920109; **18.** Ethiopia, Bahar Dar, g.prep. 0732/2020 D. Wanke); **19.** *A. interpellans* (South Africa, KwaZuluNatal, Vryheid, g.prep. 1314/2022 D. Wanke); **20.** *N. obscuraria* (Spain, Albarracin, g.prep. 2096/2017 H. Rajaei). a = genitalia capsule; b = aedeagus. Scale bar: 1 mm.



Figures 21–24. Female genitalia of *Aphilopota tyttha* (Prout), comb. nov., *Aphilopota interpellans* (Butler) and *Nychiodes obscuraria* (Villers). **21.** *A. tyttha* (Eritrea, Caraiai, g.prep. 4977, NHMUK014173598); **22–23.** *A. interpellans* (South Africa, KwaZuluNatal, Vryheid, g.prep. 1315/2022 D. Wanke; **22.** Ventral view; **23.** Lateral view); **24.** *N. obscuraria* (Spain, Albarracin, g.prep. 2097/2017 H. Rajaei). Scale bar: 1 mm.

Acknowledgements

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Appendix 1

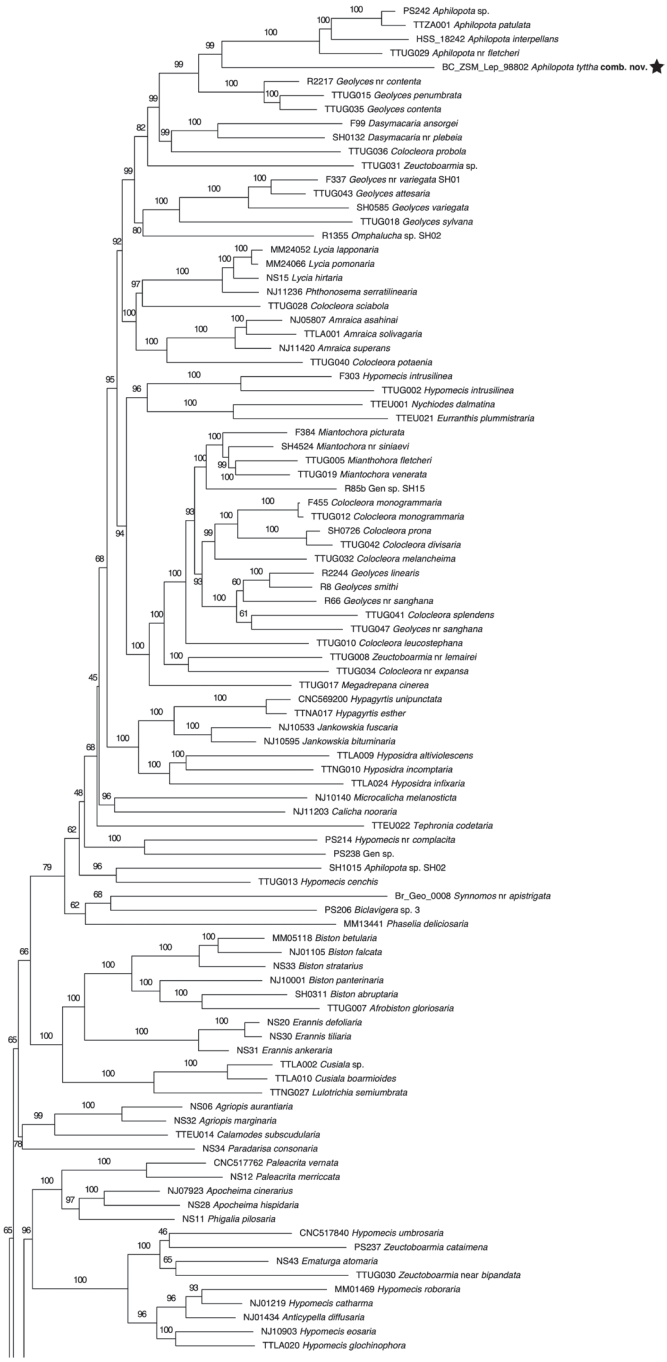

Figure A1. Complete phylogenetic analysis from IQ-TREE, showing the phylogenetic position of *Aphilopota tyttha* comb. nov. (marked with a star) within the tribe Boarmiini. Support values are indicated above the branch. Node confidence values were estimated based on 1000 ultrafast bootstrap replicates.

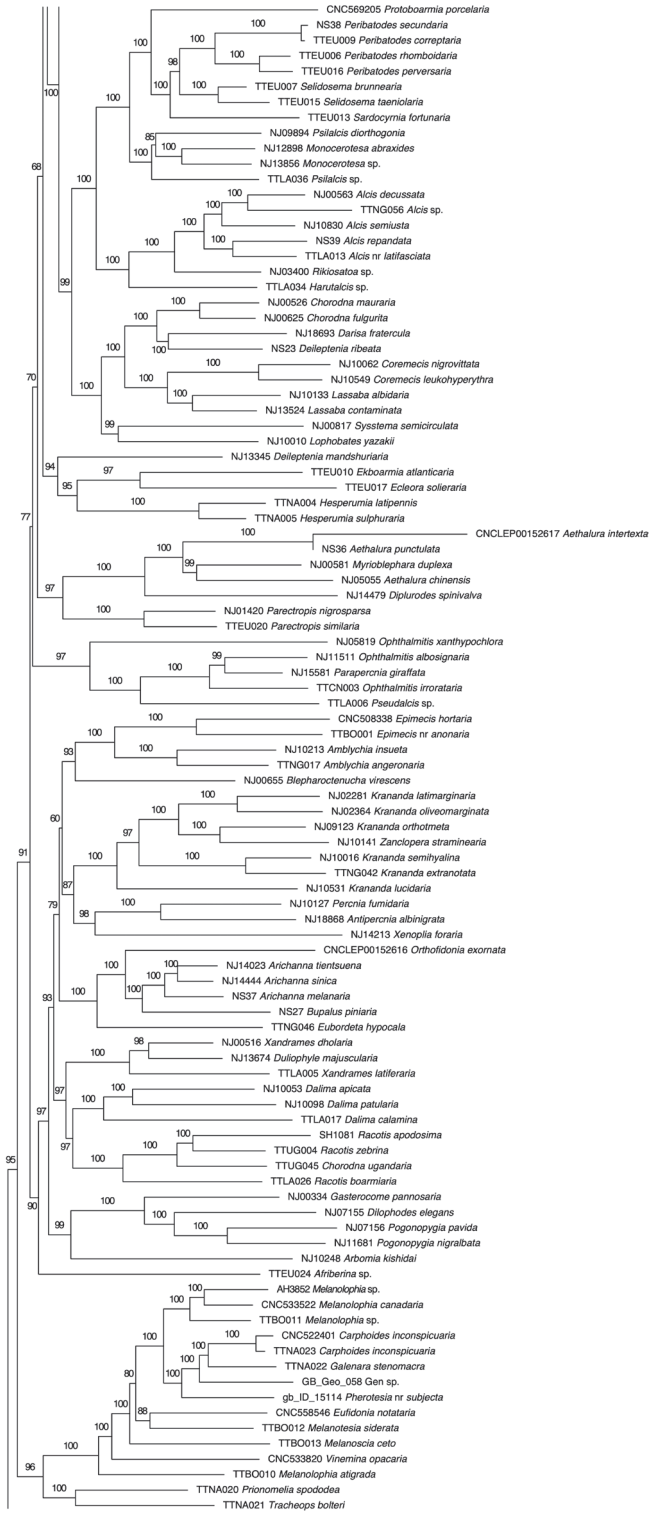

Figure A1. Continued.

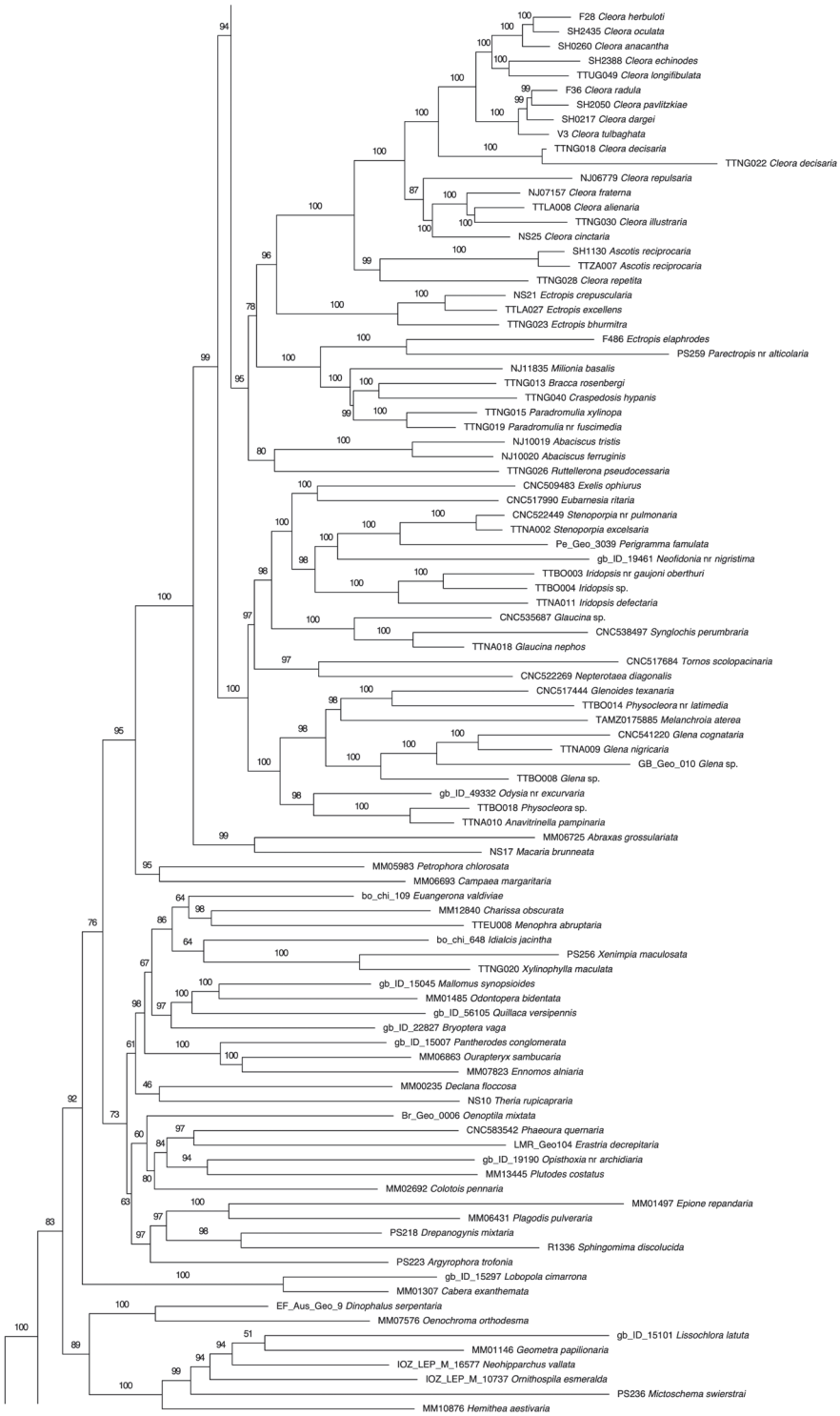

Figure A1. Continued.

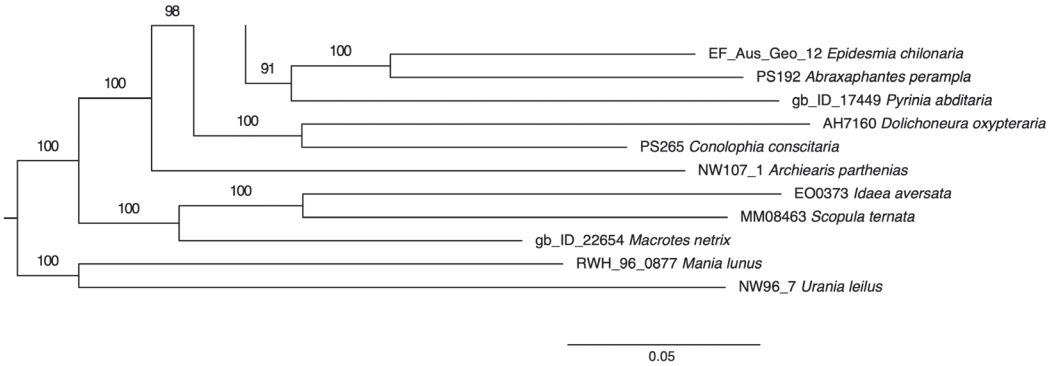

Figure A1. Continued.

Supplementary material 1

Taxa used in this study

Authors: Dominic Wanke, Axel Hausmann, David C. Lees, Kyung Min Lee, Geoff Martin, Pasi Sihvonen, Hermann Staude, Hossein Rajaei

Data type: table (excel file).

Explanation note: Taxa used in this study, with identification, process code, and GenBank accession numbers for each gene. Data from Murillo-Ramos et al. 2019 & Wanke et al. (current paper).

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Link: https://doi.org/10.3897/nl.46.94940.suppl1

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