Zootaxa 3838 (2): 151–173
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3838.2.1
http://zoobank.org/urn:lsid:zoobank.org:pub:62251D9C-65DD-4A4A-8AB9-B885A018D4BD

DNA barcode and phylogeography of six new high altitude wingless *Niphadomimus* (Coleoptera: Curculionidae: Molytinae) from Southwest China

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Abstract

The genus *Niphadomimus* Zherikhin, 1987 is taxonomically revised herein. In addition to the two recorded Nepalese species, *N. nigriventris* Zherikhin and *N. niger* Zherikhin, known only from the holotypes, two additional specimens of *N. nigriventris* are reported and six new species from China represented by 96 specimens are described and illustrated. These are: *N. alcyone* **sp. n.** (Sichuan), *N. celaeno* **sp. n.** (Yunnan), *N. electra* **sp. n.** (Yunnan), *N. maia* **sp. n.** (Yunnan), *N. merope* **sp. n.** (Shaanxi) and *N. sterope* **sp. n.** (Sichuan). All known *Niphadomimus* species are apterous inhabitants of the leaf litter in the upper *Rhododendron*-dominated forest zone between 2000 and 4114 m. Phylogenetic analyses using DNA barcodes of six new species and representatives of 13 other Molytinae genera with available DNA data (A.) corroborates *Niphadomimus* monophyly; (B.) strongly argues for the sister-group relationship between *N. merope* **sp. n.** from the Qinling Mt. Range and the rest of the species distributed in the Hengduan mountains; (C.) in two among four analyses weakly relates the genus with the East Palaearctic *Leiosoma.* The tribe Typoderini could not be shown as monophyletic, which may be due to insufficient signal content of the cox1 marker at the tribal level. The detected phylogeographic pattern of *Niphadomimus* is compared with that of similarly distributed or closely related clades. Temporal DNA analysis estimates the *N. merope* **sp. n.** split at 6–11 MY, while the diversification of the Hengduan clade dates between 5.5 MY and 3.6 MY, i.e. well before the onset of the Quaternary climate fluctuations.

Key words: *Niphadomimus*, CO1, DNA barcode, phylogeography, weevils

Introduction

The weevil subfamily Molytinae contains several thousand species arranged in approximately 430 genera (Alonso-Zarazaga & Lyal 1999) attributed to 57 currently recognized tribes or subtribes (Bouchard *et al.* 2011; Alonso-Zarazaga 2013). All Molytinae species with adequately known biology feed nearly exclusively on live or dead plant tissue both as larvae and adults. The subfamily contains many economically important taxa, including biological control agents against invasive plants, such as the European weevil *Hylobius transversovittatus* Goeze which was introduced in 1992 to the USA and Canada to control Purple loosestrife (*Lythrum salicaria*, see McAvoy & Kok 2002). More important, however, is the role of Molytinae as both forestry and agricultural pests. Best known among them are the two predominantly Holarctic genera *Hylobius* Germar and *Pissodes* Germar, each with a few dozen species, some of which are known as notorious pests of conifers (Lei Guilin *et al.* 2003; Långström & Day 2004). Species of the latter genus are capable of transcontinental travel and establishment, as demonstrated for the North American *Pissodes nemorensis* Germar that had significantly damaged pine plantations in South Africa (Gebeyehu & Wingfield 2003). The wood-associated insects such as Molytinae are the most common among those species that are unintentionally transported with untreated low-grade wood used as dunnage for the international maritime trade. Indeed, specimens of both *Hylobius* and *Pissodes* are the most commonly intercepted wood-associated non-Scolytinae weevils with 181 and 280 interceptions, respectively, recorded at United States ports of entry between 1985 and 2000 (together with species of five other Molytinae genera: *Conotrachelus* Dejean, *Heilipus* Germar, *Marshallius* Kuschel, *Niphades* Pascoe and *Rhyssomatus* Schoenherr; see Haack 2006). Live larvae and adults of the Molytinae genus *Pimelocerus* Lacordaire, native to Asia Pacific and

Accepted by R. Anderson: 9 Jun.. 2014; published: 17 Jul. 2014

known to develop in ash trees (*Fraxinus griffithii*, see Kojima & Morimoto 2012) as well as being a major pest of Japanese star anis (*Illicium anisatum*; see Wakayama *et al.* 2010), were intercepted in Vancouver in 2012 on a transpacific shipment (unpublished data). Other examples of human-assisted spread of Molytinae plant pests include *Aclees* Schoenherr species, also native to Asia Pacific where they are known to damage cedar plantations (Thu *et al.* 2010), which have been accidentally introduced and established as a significant pest in the Italian fig nurseries (Ciampolini *et al.* 2005). Overall it is fair to say that among all the hyperdiverse weevils, with >60,000 named species (Oberprieler *et al.* 2007), in their economic significance Molytinae are second only to the true bark beetles (Scolytinae).

The economic significance of molytine weevils notwithstanding, they remain remarkably poorly known. Among 470 Molytinae US interceptions reported by Haack (2006), only 28 have been identified to species level (6%). Also, these numbers may acutely under represent the real difficulty in species diagnostics, since at least some Molytinae adults and perhaps nearly all larvae could have been reported as unidentified Curculionoidea, or even Coleoptera. This is not surprising, since the bulk of Molytinae taxa have never been subjected to a modern taxonomic treatment, and phylogenetic and/or DNA-based studies (e.g., Zhang *et al.* 2007; Downie *et al.* 2008; Meregalli *et al.* 2013) are scarce. The majority of molytine tribes and genera continue to exist as inherited from historic authors when new taxa were freely introduced with very little, if any, phylogenetic awareness. The majority of Molytinae genera fall into this ill-fated category of scientifically neglected orphans, of which the genus *Niphadomimus* Zherikhin, 1987, the subject of the present paper, is a typical example.

The weevil genus *Niphadomimus* was established for two wingless species with a prosternal excavation, each known only from the holotype collected in the mountains of eastern Nepal at elevations between 1800 m and 2850 m. The genus was originally assigned to wingless Molytinae: Molytini in the vicinity of Aminyopini and later to Typoderina of Molytini (Alonso-Zarazaga & Lyal 1999) which has recently acquired tribal status (Alonso-Zarazaga 2013). The type species was noteworthy by having peculiar elytral tubercles, giving it a somewhat misshaped and triangular appearance (Fig. 7). For years no additional specimens came to sight, until a search through unidentified weevils collected by Aleš Smetana in Nepal revealed two specimens markedly resembling the *Niphadomimus* type species. In the years 2010–2012 dozens of newly collected specimens seemingly closely related to those assigned to *Niphadomimus* were discovered in the mountains of Southwest China at altitudes mostly above 3500 m. The influx of new material triggered the taxonomic revision of the genus, the first goal of the present paper. The second goal it to use the newly obtained sequences of the DNA barcoding fragment of the CO1 mtDNA gene available for all newly discovered Chinese *Niphadomimus* species in: (1.) testing the generic monophyly; (2.) placing it phylogenetically among a handful of other Molytinae with available comparative data; (3.) detecting phylogenetic affinities among *Niphadomimus* species, (4.) interpreting the revealed phylo- and/or biogeographic structure, (5.) dating the speciation events and (6.) comparing the *Niphadomimus* phylogeographic interpretations with those of similarly distributed and/or related clades. Overall this project intends to shed light on species diversity and associated biological, distributional, morphological and temporal information pertaining to the so far neglected molytine *Niphadomimus* previously represented by only two historical specimens.

Material and methods

Museum abbreviations, followed by the name of the curator:

CMN	Canadian Museum of Nature, Ottawa, Canada (R.S. Anderson, F. Genier);
CNC	Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Canada (P. Bouchard);
IZCAS	Institute of Zoology, Chinese Academy of Science, Beijing, P.R. China (R. Zhang);
MTD	Senckenberg Naturhistorische Sammlungen, Dresden, Germany (K.-D. Klass, O. Jäger);
SMNS	Staatliches Museum für Naturkunde, Stuttgart, Germany (W. Schawaller);
ZIN	Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia (B.A. Korotyaev).

This study is based on the examination of 99 *Niphadomimus* adults representing all species and specimens presently known, with the exception of *N. niger* Zherikhin, 1987, known only from the holotype. The majority of the studied specimens have a unique specimen identifier label pinned underneath with the code

"CNCCOLVG0000XXXX", while only the last four digits in the format #XXXX are cited below. Specimen labels are cited verbatim in quotation marks with the back slash \ separating different labels. Square brackets [] when citing labels indicate comments; [p] means "printed". Spelling of Zherikhin's surname, even if spelt as "Zherichin" in his *Niphadomimus* 1987 paper, consistently follows the alternative spelling adopted in Alonso-Zarazaga & Lyal (1999).

Specimen collecting, dissection and DNA extraction. All Chinese DNA-suitable and freshly collected specimens were discovered by sifting forest leaf litter with a sifter (Fig. 15), followed by a passive specimen extraction using Winkler funnels. Only adults were detected, while eggs, larvae and pupae are completely unknown. Specimens were preserved in 96% ethanol and whenever possible, kept in a freezer. A leg was cut off for DNA extraction following the routine DNA barcoding process (Hebert *et al.* 2003) and sent to the laboratory. All DNA extractions were done at the DNA barcoding facilities at the University of Guelph, Canada. Information on 63 voucher specimens included in the DNA analysis with their digital images and all relevant data such as primers and original chromatograms were deposited in the Barcode Of Life Database in a publicly accessible dataset *Niphadomimus*, doi: dx.doi.org/10.5883/DS-NIPF.

The voucher specimens used for DNA were dry mounted on pins for morphological study and long term storage. Dry specimens were removed from pins and softened in water for about 30 minutes. A thin pin was carefully inserted in the joint between the prothorax and mesothorax breaking the specimen into two parts. Both parts were incubated at 55 C for approximately 6 hours in proteinase K and ATL buffer to facilitate lysis of internal tissue, which is a more gentile approach compared to the commonly practiced KOH maceration (A. Riedel, pers. comm.). The abdominal ventrites 3–5 were then gently tilted ventrad from under the elytral apices and sharp eye surgical forceps were inserted in the small abdominal opening to cut morphological sternite 8 from visible ventrite 5 (=sternite 7), together with the membrane lateral to ventrite 5 on both sides. Sternite 8 was then pulled out by forceps with the genitalia attached to the membranes of sternites 8/9. This dissection can damage the specimen by wedging and displacing the fused elytra from their rigid attachment to the meso- and metathorax, which can result in the complete disarticulation of the former. The genital structures were carefully cleaned of the remaining tissue, gently stained with Chlorazol Black, placed in glycerol, digitally imaged, and mounted in a drop of Canada balsam on an insect plate below the specimen for further storage. Both parts of the specimen body (head with prothorax and the rest of the body) were spread on an insect mounting plate (not a point), glued together and to the plate to restore the habitus if possible. Dissecting genitalia of wingless weevils with a compact body and merged elytra required significant prior practise to avoid damage to specimens.

Species description, identification and contribution of the male genitalia characters. In describing species, the minimalistic approach of Riedel *et al.* (2013a, 2013b) was followed by providing a unique diagnostic combination of: (a.) short verbal description, (b.) holotype habitus image, (c.) holotype aedeagus image, whenever possible, (d.) holotype DNA barcode sequence and (e.) the type locality. The length of the body was measured in dorsal aspect from the elytral apex to the front of the pronotum. The main deviation from the Riedel *et al.* (2013b) format is that by dealing with a much lesser number of species, it was feasible to provide more than one image of the holotype habitus and aedeagus. An identification key to species is not provided, since practical identification of predominantly allopatric *Niphadomimus* species should be done by using geographical data. Besides, offering a key to species when likely less than 25% of the real number of species is known appears premature. Instead the reader should refer to illustrations which are ample to permit quick species recognition, aided by other data (geography, description, DNA barcode sequence). Male genitalia, commonly of much informative value for the species-level diagnostics in insects and specifically in weevils (Anderson 2010), do not appear to be similarly important in *Niphadomimus*. Due to limitations imposed by material availability, one male specimen representing each of five species was dissected to make interspecific comparisons. Detected differences were minor and since numbers of available specimens made documentation of intraspecific variation difficult to determine, male genitalia structures are not considered important for species recognition. Male genitalia are, however, illustrated for the five *Niphadomimus* species for which males were available in case that further material demonstrates diagnostic utility of this classical character system.

First DNA analysis: monophyly, internal relationships and phylogenetic placement of *Niphadomimus*. The first DNA analysis was designed to test the monophyly of the genus and to attempt to place the genus phylogenetically among the few other Molytinae, for which comparative data were available. Taxonomic neglect of Molytinae on the world scale and the lack of adequate pre-existing phylogenetic hypotheses introduced

significant uncertainties in the choice of outgroup taxa and, specifically, on how to root the topology. Current taxonomic placement of the genus in the tribe Typoderini and implicitly suggested relationships with other tribe's members was done without any supporting analysis, and the monophyly of the tribe has never been tested. The same might be partially true for the entire subfamily Molytinae, which in spite of innovative efforts to delimit it as a natural group (Kuschel 1987), still remains a highly polyphyletic taxon (McKenna *et al.* 2009). Facing such uncertainties, the first analysis was designed to include the widest possible range of Old World Molytinae genera in the hope that at least one of them might cluster as a sister to *Niphadomimus* and thus provide the much needed outgroup for sorting out species relationships within the genus. The matrix was constructed to include 17 sequences of all six new *Niphadomimus* species, plus an additional 43 sequences representing 15 species of 13 Molytinae genera (including three genera of Typoderini: *Anchonidium* Bedel, *Aparopion* Hampe, *Typoderus* Marshall), plus three *Graptus circassicus* Solari (Entiminae) sequences to root the trees. The total matrix consisted of 63 terminals and 658 positions, and with the total of 10 ambiguous positions (0.024%). All sequences are new and their GenBank accession numbers are HM386450, HM417677, HM417678, HM417679, HQ986888, HQ986889, HQ987002, HQ987003, HQ987110, KJ427730–KJ427749 and KJ445682–KJ445715.

The MEGA5 software (Tamura *et al.* 2011) was used to calculate pairwise distances, search for the best DNA substitution model and to run three (of the four) topology reconstructions (Neighbor-Joining using uncorrected p-distances: NJ; Maximum Parsimony: MP; Maximum Likelihood: ML; each with 1000 bootstrapping). MrBayes 3.2.2. software (Ronquist *et al.* 2012) was used for Bayesian Inference (BI) analysis with the default parameters and by using 7M generations and the commands "lset nst=6 rates=invgamma". For both ML and BI the best substitution model used was GTR+G+I (see Results). The resulting four consensus topologies (one from each of NJ, MP, ML, BI) were exported in FigTree v1.4 (Rambaut 2014) and visualized for comparison among themselves. The phylogram from the BI analysis (and the ultrameric time estimation tree from the second analysis, see below) was then exported as a Windows Enhanced Metafile (.emf) to CorelDraw and later to Photoshop to prepare them for publication as Figs. 12 and 13, respectively.

Second DNA analysis: temporal phylogeography of *Niphadomimus*. The second analysis attempted to date the *Niphadomimus* evolutionary events by converting mtDNA distances into absolute time. To do so, the original 63 terminal matrix from the first analysis was reduced in size to include only 17 *Niphadomimus* sequences, which were then subjected to the best nucleotide substitution model search (see above) and the matrix was then analysed with the BEAST v1.8.0 software package (Drummond *et al.* 2012).

In the absence of fossil or applicable geological evidences to date the *Niphadomimus* evolutionary events, the only informative data of the temporal aspect for genus evolution were the differences in the available mtDNA sequences accumulated since the existence of their last shared ancestor. Conversion of the DNA differences into absolute time is made on the basis of a set of assumptions, the most critical being the mutation rate, which varies among clades as well as among genes. At present two papers reported their attempt to measure this rate for the CO1 mtDNA gene in Polyphaga: Papadopoulo *et al.* (2010) gave a 0.018 for Tenebrionidae and Ribera *et al.* (2010) gave a 0.02 for Leiodidae: Leptodirini. Both results, however, pertain to the pat-jerry fragment of the CO1 mtDNA gene, which is the non-overlapping 3' end of the same gene. The rate of mutation for the former region seems some 20% higher than those of the latter, since Andújar *et al.* (2012) working with the more distantly related *Carabus* Linnaeus beetles (Adephaga) reported 0.0145 for the pat-jerry and 0.0113 for the barcode fragment. These minute rate differences, however, appear trivial when compared with other sources of uncertainty depicted by the 95% confidence intervals of most of the published trees (see, for example, Fig. 13) and, therefore, the rate of 0.018 was implemented, as reported by Papadopoulo *et al.* (2010) for Tenebrionidae, a group more closely related to *Niphadomimus* than Leiodidae.

Other assumptions were made while converting sequence distances to absolute age: (a.) disregarding the results of the first analysis, no internal monophyletic groups enforced; (b.) substitution model GTR+G, see Results; (c.) base frequencies: estimated; (d.) number of gamma categories: 4; (e.) clock: strict. The analysis ran for 10 M generations with all other parameters set up at default, and the outputted log file visualised in Tracer v1.6 (Rambaut 2014). The tree file output after the BEAST analysis was further analysed in TreeAnnotator from the same BEAST v1.8.0 software package and then saved as a nexus tree file to be opened in FigTree and eventually converted into Fig. 13 (following the steps described for the first analysis; see above). The chronostratigraphic timing is that of Cohen *et al.* (2013) with the Pliocene-Pleistocene boundary set at 2.588 MY.

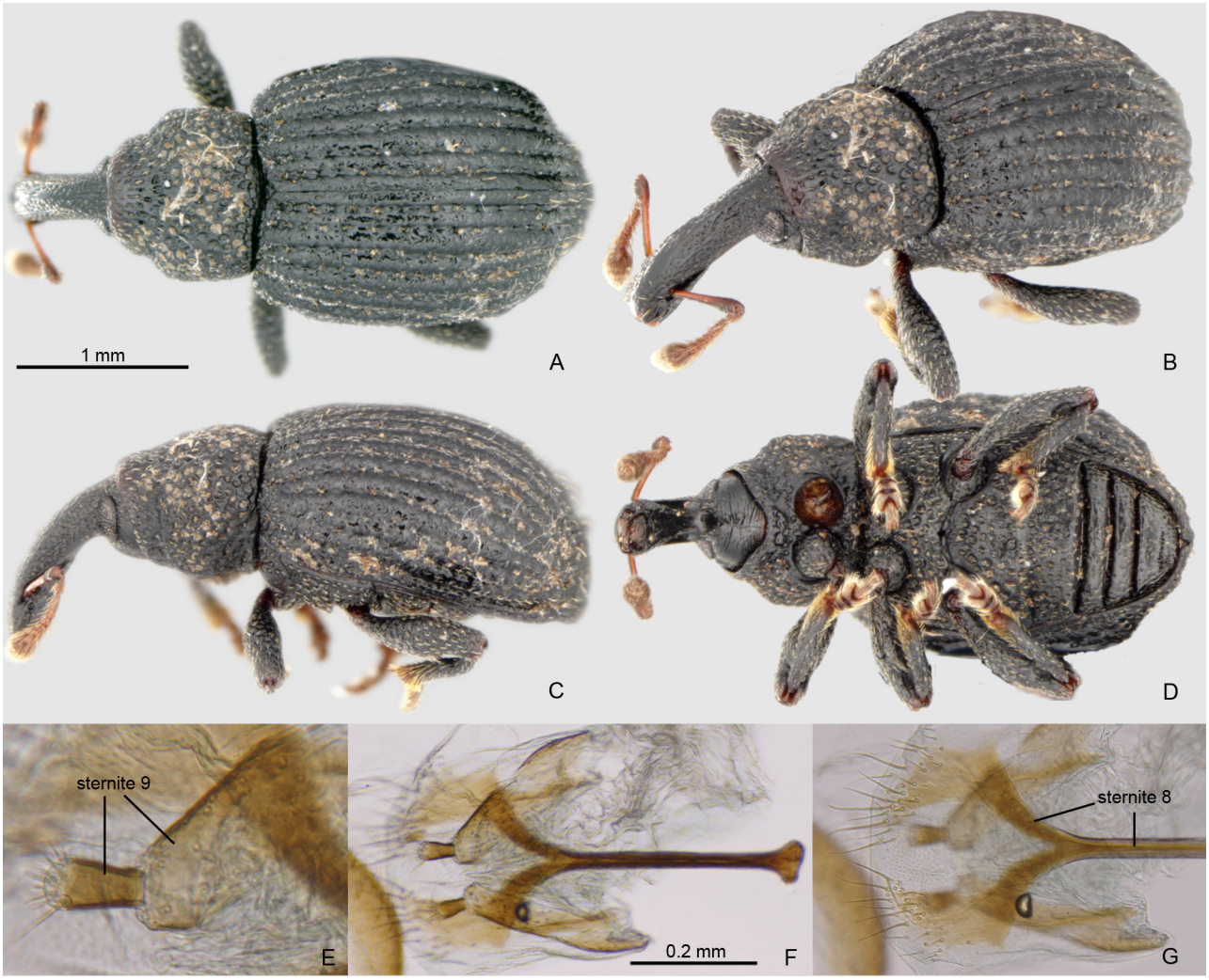

FIGURE 1. *Niphadomimus alcyone* **sp. n.**, holotype, female, #2493. A-D: habitus; E: apical part of basal hemisternite 9 ("coxite") and much smaller apical cylindrical hemisternite 9 (="stylus"); F: sternites 8 and IX; G: sternite 8 (sternite 9 in the background).

Taxonomy

Niphadomimus Zherikhin, 1987

Zherikhin, 1987: 15 (species included: *nigriventris*, *niger*). Type species: *Niphadomimus nigriventris* Zherikhin, 1987 by original designation.

Diagnosis. Adults of the genus *Niphadomimus* can be immediately recognized from other wingless Asian weevils by the following unique combination of characters: (1.) rostrum longer than wide; (2.) prosternum anterior of procoxae with a shallow and broad depression (Fig. 11); (3.) mesoventrum at middle anterad of mesocoxae with distinct and sizable projection pointed ventrad, vertically sloped anteriorly and oblique posteriorly (Fig. 11). In its natural habitat *Niphadomimus* co-occurs with similarly wingless *Colobodes* Schoenherr and *Niphadonyx* Schenkling. Adults of these two genera are most easily separated from those of *Niphadomimus* by either widely separate procoxae and or by the claws having a sizable inner lobe (Meregalli 2013, fig. 2M), respectively.

The original generic description by Zherikhin appears adequate, even though it did not refer to the internal structures, such as the proventiculus, or male and female genitalia. These and a few other characters are mentioned below in an attempt to elaborate the original Zherikhin description, which should also be consulted.

Description. Fully apterous genus of Molytinae with elytra fused to each other and to metathorax. Length 2.45–6.27 mm. Colour dark, from reddish-brown to almost black; legs and elytra with bluish or reddish hue,

FIGURE 2. *Niphadomimus alcyone* **sp. n.**, paratype, female, #4429. A–D: habitus; E: spermatheca.

sometimes partly bicoloured (Fig. 9A–B), never metallic or shiny. Antennal funicle with seven antennomeres. Procoxal cavities adjacent to each other (Fig. 11); prosternum anterad of procoxal cavities with depression at middle (Fig. 11), this depression normally in form of wide longitudinal groove marked by ridge on each side (Fig. 11H). Ventral surface of thorax and abdomen with numerous punctures (Fig. 11). Mesoventrum at middle anterad of mesocoxae with distinct and sizable projection pointed ventrad, vertically sloped anteriorly and oblique posteriorly (Fig. 11). Entire length of meso-metaventral suture between metacoxae formed by deep narrow transverse groove (Fig. 11) often separated at middle by septum into two equal parts (Fig. 11I). All femora with single variously developed femoral tooth at distal third. Scutellum with minute externally visible part. Elytra with nine punctate striae, odd interstriae with our without tubercles, some of which might be markedly enlarged (Fig. 4A–C). Proventriculus sclerotized and easily distinguishable (Fig. 8E). **Male genitalia.** Male abdominal sclerite 9 variously asymmetrically V-shaped (Fig. 3E), with long apodeme (= "spiculum gastrale"); sternite 8 consisting of two hemisternites (Fig. 3E). Aedeagus symmetrical, longer than wide, almost parallel sided and evenly rounded, without internal sclerites and with partly sclerotized walls on endophallus proximad to it (Fig. 3F–H); aedeagal apodemes about 1.5x as long as aedeagus; tegmen fully closed dorsally, with two asetose and weakly sclerotized parameral lobes each longer than wide. **Female genitalia.** Sternite 8 Y-shaped with setose apical lobes (Fig. 1G) and long apodeme; sternite 9 consisting of two hemisternites each formed by larger basal piece (= "coxite") and smaller apical piece (= "stylus") (Fig. 1E, F); spermatheca present (Fig. 2E).

***Niphadomimus alcyone* sp. n.**
Figs. 1, 2, 11A–B.

Diagnostic description. Holotype, female (Figs. 1, 11A). Genbank accession: KJ427731. Length: 2.78 mm. Color black; prosternal depression not delimited on each side by longitudinal keel; femoral tooth higher than its width at base and markedly developed (Fig. 2B); elytral interstriae not tuberculate.

Intraspecific variation. Length 2.45–2.78 mm.

Material examined. Holotype female (IZCAS): #2439, "P.R. CHINA, Sichuan, NE slope Gongga Shan, N29°53'23" E102°01'31", 08.vi.2011, 3886m, sift13, V.Grebennikov". Paratype (CNC), female (Figs. 2, 11B), #4429, "CHINA, Sichuan, 23km E Songpan, N32°37'57" E103°49'20", 27.v.2012, 3761m, sifting 12, V. Grebennikov".

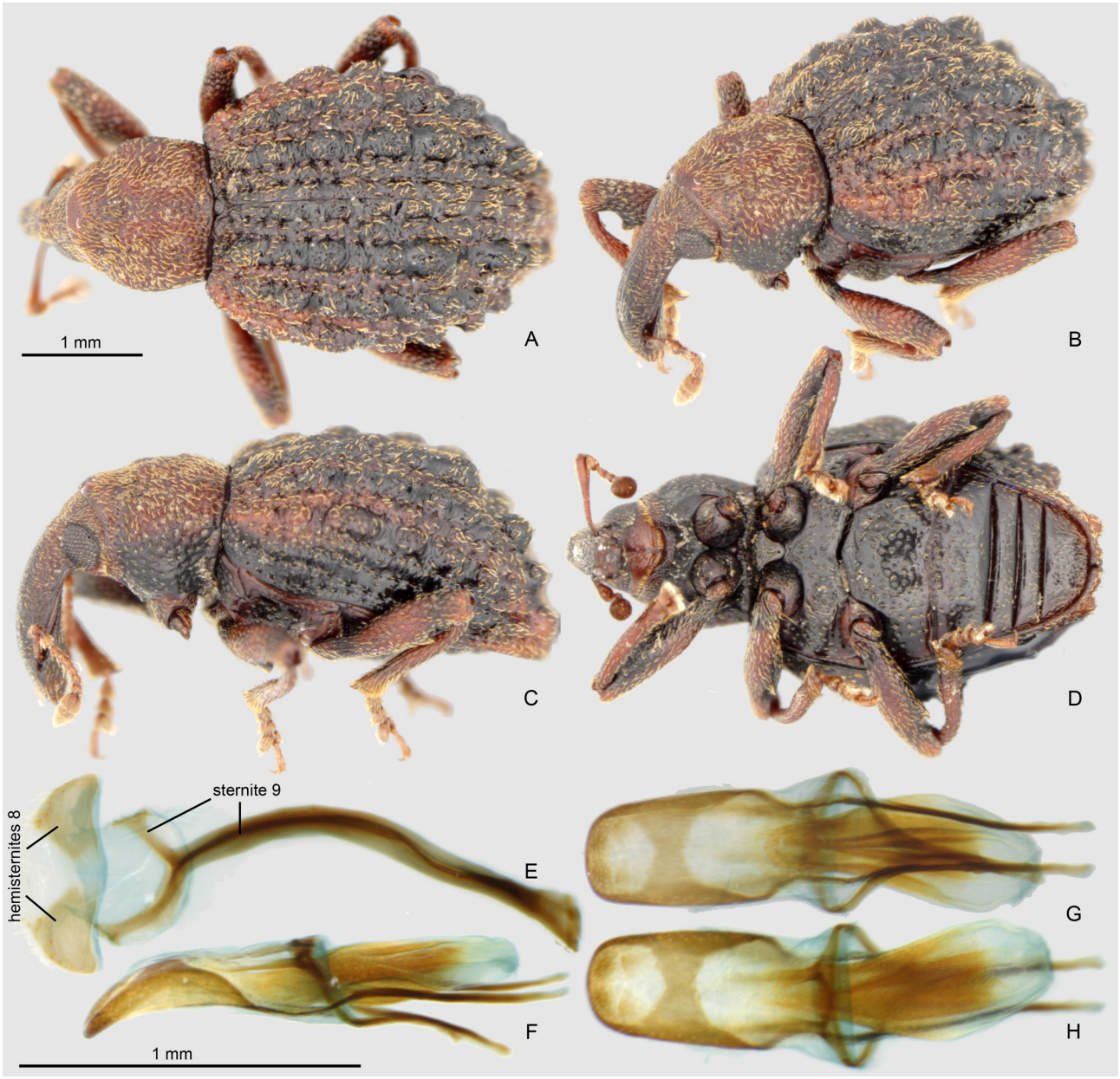

FIGURE 3. *Niphadomimus celaeno* **sp. n.**, holotype, male, #2476. A–D: habitus; E: sternites 8 and 9; F–H: aedeagus and tegmen, lateral (F), dorsal (G) and ventral (H).

Distribution. Gongga Shan and Songpan regions in Sichuan, China; in the Gongga Shan area sympatrically with *N. celaeno* sp. n. Elevation: 3761–3886 m.

Etymology. The species epithet is a Latinized Greek mythical name of Alcyone, one of the Pleiades, mother of Hyrieus, Hyperenor and Aethusa by Poseidon; noun in apposition.

Niphadomimus celaeno sp. n.

Figs. 3, 11C.

Diagnostic description. Holotype, male (Figs. 3, 11C). Genbank accession: KJ427748. Length: 4.17 mm. Color dark reddish; prosternal depression delimited on each side by longitudinal keel; femoral tooth not higher than its width at base and moderately developed; elytral interstriae evenly and pronouncedly tuberculate.

Material examined. Holotype male (IZCAS): #2476, "P.R. CHINA, Sichuan, NE slope Gongga Shan, N29°52'10" E102°02'01", 12.vi.2011, 3620m, sift16, V.Grebennikov".

Distribution. Gongga Shan region in Sichuan, China; sympatrically with *N. alcyone* **sp. n.** Elevation: 3620 m.

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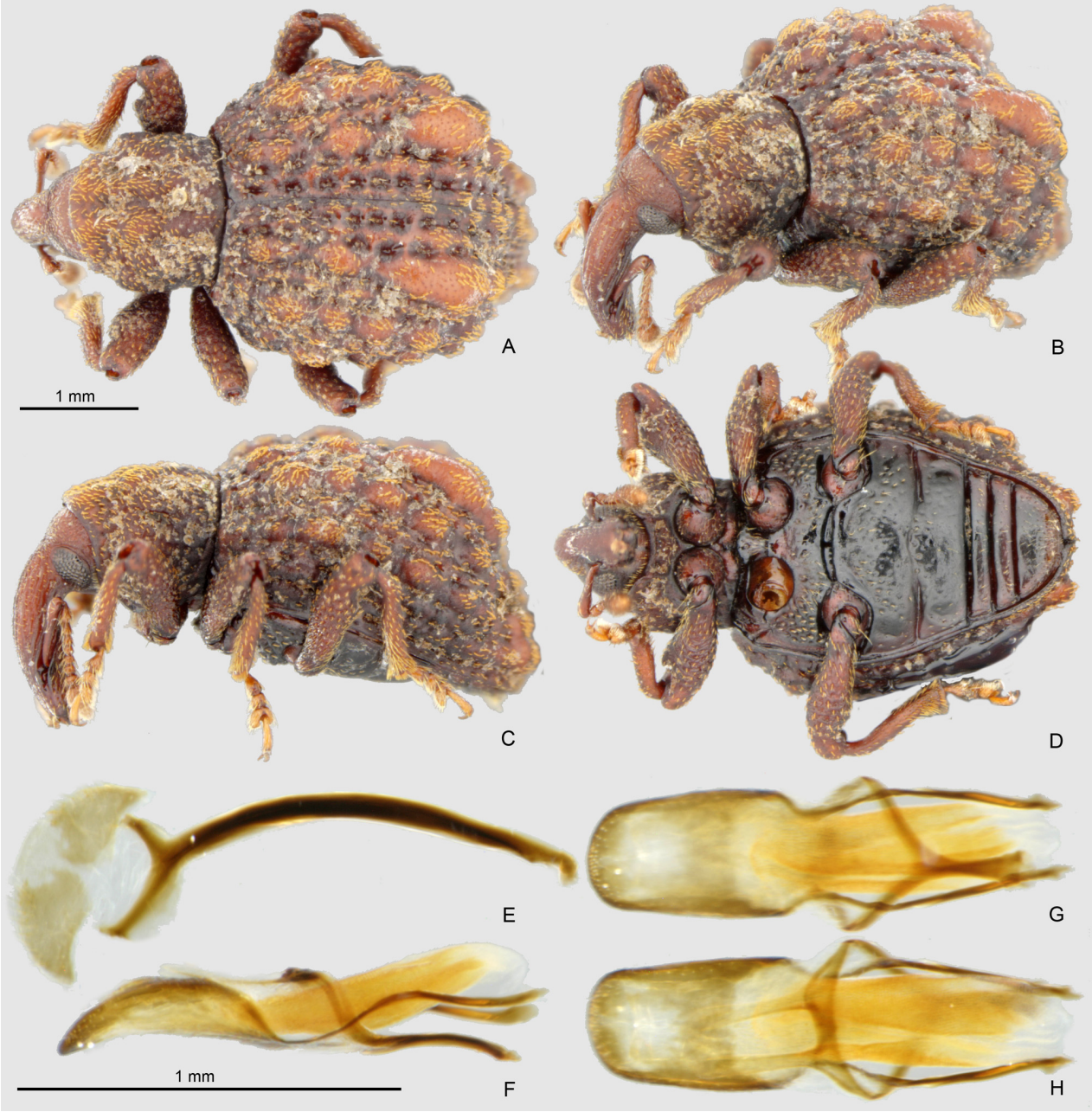

FIGURE 4. *Niphadomimus electra* **sp. n.**, holotype, male, #2727. A–D: habitus; E: sternites 8 and 9; F–H: aedeagus and tegmen, lateral (F), dorsal (G) and ventral (H).

Etymology. The species epithet is a Latinized Greek mythical name of Celaeno, one of the Pleiades, mother of Lycus and Eurypylus by Poseidon; noun in apposition.

***Niphadomimus electra* sp. n.**
Figs. 4, 11D.

Diagnostic description. Holotype, male (Fig. 4). Genbank accession: KJ427746. Length: 3.76 mm. Color dark reddish; prosternal depression delimited on each side by longitudinal keel; femoral tooth not higher than its width at base and weakly developed; elytral interstriae unevenly and pronouncedly tuberculate with those on interstria 3 markedly enlarged (Fig. 4A).

Intraspecific variation. Length 3.18–4.64 mm.

FIGURE 5. *Niphadomimus maia* **sp. n.**, holotype, male, #2730. A–D: habitus; E: sternites 8 and 9; F–H: aedeagus and tegmen, lateral (F), dorsal (G) and ventral (H).

Material examined. Holotype male (IZCAS): #2727, "P.R. CHINA, Yunnan, Cang Shan at Dali, N25°40'12" E100°06'10", 05.vii.2011, 3740m, sift37, V.Grebennikov". Paratypes (CNC, CMN, IZCAS, MTD, ZIN): female, #0879, "P.R. CHINA, Yunnan, E slope Cangshan at Dali, N25°40'07.6 E100°06'12.9, 19.v.2010, 3887m, sifting18, V.Grebennikov"; 50 exx, #2707–09 and 47 exx not numbered, "P.R. CHINA, Yunnan, Cang Shan at Dali, N25°40'07" E100°06'14", 04.vii.2011, 3890m, sift35, V.Grebennikov"; 29 exx, #2728, #2729 and 27 exx not numbered, same data as holotype.

Distribution. Eastern slope on Cang Shan range above the city of Dali, Yunnan, China; sympatrically with *N. maia* **sp. n.** Elevation: 3740–3890 m.

Etymology. The species epithet is a Latinized Greek mythical name of Electra, one of the Pleiades, mother of Dardanus and Iasion by Zeus; noun in apposition.

FIGURE 6. *Niphadomimus merope* **sp. n.**, holotype, female #2201. A–D: habitus; E: spermatheca; F: sternite 8; G: sternite 9.

***Niphadomimus maia* sp. n.**
Figs. 5, 11E.

Diagnostic description. Holotype, male (Figs. 5, 11E). Genbank accession: KJ427733. Length: 5.00 mm. Color black; prosternal depression delimited on each side by longitudinal keel; femoral tooth not higher than its width at base and weakly developed; elytral interstriae evenly and weakly tuberculate.

Intraspecific variation. Length 5.00–6.27 mm.

Material examined. Holotype male (IZCAS): #2730, "P.R. CHINA, Yunnan, Cang Shan at Dali, N25°40'12" E100°06'10", 05.vii.2011, 3740m, sift37, V.Grebennikov". Paratypes (CNC, IZCAS, MTD): 8 exx, #2731–33 and 5 exx not numbered, same data as holotype.

Distribution. Eastern slope on Cang Shan range above the city of Dali, Yunnan, China; sympatrically with *N. electra* sp. n. Elevation: 3740 m.

Etymology. The species epithet is a Latinized Greek mythical name of Maia, eldest of the seven Pleiades, mother of Hermes by Zeus; noun in apposition.

***Niphadomimus merope* sp. n.**
Figs. 6, 11F.

Diagnostic description. Holotype, female (Figs. 6, 11F). Genbank accession: KJ427741. Length: 5.54 mm. Color

black; prosternal depression delimited on each side by longitudinal keel; femoral tooth not higher than its width at base and moderately developed; elytral interstriae evenly and weakly tuberculate.

Material examined. Holotype female (IZCAS): #2201, "P.R. CHINA, Shaanxi, S slope Qin Ling Shan, N33°51'40" E108°59'27", 15.v.2011, 2000–2600m, sift01, V.Grebennikov".

Distribution. Southern slope of Qinling Shan in Shaanxi, China. Elevation: 2000–2600 m.

Etymology. The species epithet is a Latinized Greek mythical name of Merope, youngest of the seven Pleiades who married Sisyphus, a mortal; noun in apposition.

FIGURE 7. *Niphadomimus nigriventris*, holotype. A–B: habitus; C: pronotum. Specimen not measured when imaged; according to the original description body length without rostrum 5.0 mm (Zherikhin 1987: 17).

Niphadomimus niger Zherikhin, 1987

niger Zherikhin, 1987 (*Niphadomimus*)
Zherikhin, 1987: 18 (description)

Type locality. Nepal, Ramechap [*sic*] Dist., between Jiri and Shivalaya, 2500–1800 m.

Type specimens. Holotype (not studied) originally cited as male and not dissected (SMNS). Described from the holotype.

Material examined. None.

Diagnostic description. As noted in the original description, this species is black in colour, subglobular in shape, and has non-tuberculate elytra, which are not markedly narrowed in their lateral outline before apices.

Distribution. Ramechhap District in Eastern Nepal. Elevation: 1800–2500 m.

Niphadomimus nigriventris Zherikhin, 1987

Figs. 7–9, 11G–H.

nigriventris Zherikhin, 1987 (*Niphadomimus*)
Zherikhin, 1987: 17 (description)

Type locality. Nepal, Terhathum Dist., Tinjura Dara, 2450–2850 m, mixed broad leafved [*sic*] forest.

Type specimens. Holotype (Fig. 7) originally cited as female and not dissected (SMNS), label data not recorded when the specimen was studied in 2008. Described from the holotype.

Other material examined. Male (Figs. 8, 11G) #0796 (CMN) "Nepal, Khandbari District, "Bakan" W of Tashigaon, 3250m 4.iv.1982, A.&Z.Smetana [p]"; female (Figs. 9, 11H) #4697 (CMN) "Nepal, Nuwakot District, between Ghopte and Thare Pati, 3220m, 23.iv.1985, A.Smetana [p]".

Diagnostic description. Color dark reddish; prosternal depression delimited on each side by longitudinal keel; femoral tooth not higher than its width at base and weakly developed; elytral interstriae unevenly and pronouncedly tuberculate with those on interstria 3 markedly enlarged (Figs. 7A–B).

Intraspecific variation. Length: 4.04–4.55 mm.

Distribution. Nuwakot, Sankhuwasabha and Terhathum districts in Eastern Nepal. Elevation: 2450–3250 m.

***Niphadomimus sterope* sp. n.**
Figs. 10, 11I.

Diagnostic description. Holotype, male (Figs. 10, 11I). Genbank accession: KJ427737. Length: 3.69 mm. Color black with reddish hue; prosternal depression delimited on each side by longitudinal keel; femoral tooth not higher than its width at base and moderately developed; elytral interstriae evenly and pronouncedly tuberculate.

Intraspecific variation. Length 3.55–3.69 mm.

Material examined. Holotype (Figs. 10, 11I) male (IZCAS): #4475, "CHINA, Yunnan, Haba Shan, N27°20'58" E100°05'58", 19.vi.2012, 4114m, sift24, V. Grebennikov". Paratype (CNC): 1 ex, #4480, "CHINA, Yunnan, Haba Shan, N27°21'01" E100°05'44", 21.vi.2012, 4072m, sift26, V. Grebennikov".

Distribution. Mount Haba in Yunnan, China. Elevation: 4072–4114 m.

Etymology. The species epithet is a Latinized Greek mythical name of Sterope, one of the Pleiades, mother of Oenomaus by Ares; noun in apposition.

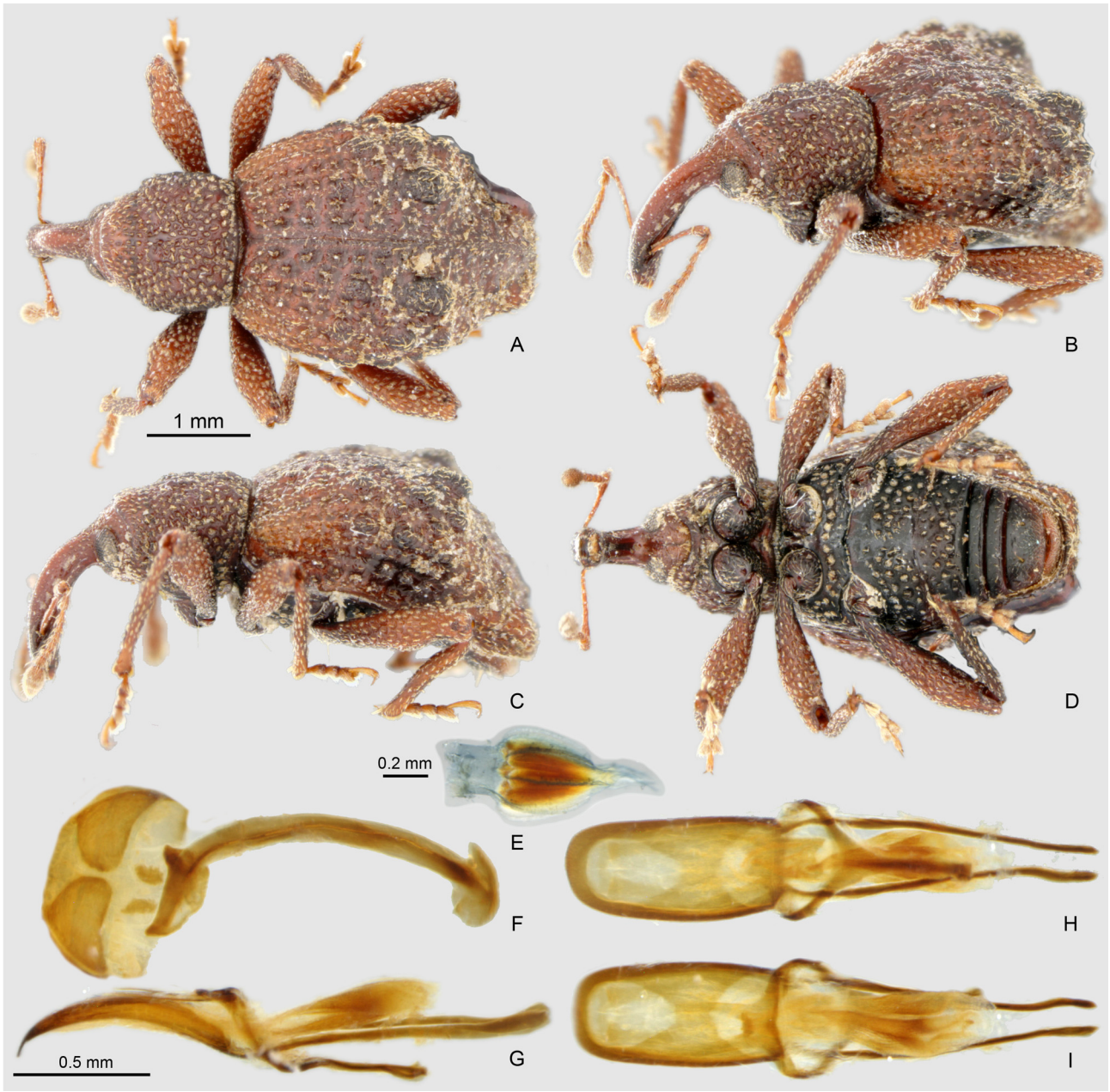

FIGURE 8. *Niphadomimus nigriventris*, male, #0796. A–D: habitus; E: proventriculus; F: sternites 8 and 9; G–I: aedeagus and tegmen, lateral (G), dorsal (H) and ventral (I).

Results of the DNA analyses

All 17 *Niphadomimus* specimens submitted over the course of three years to DNA barcoding gave full length fragment of 658 bp. Neither *Niphadomimus*, nor others among the 63 analysed weevil sequences gave indications of pseudogenes, even though their presence might have been undetected. The uncorrected interspecific p-distances for *Niphadomimus* species were 9.27% (minimum), 11.68% (mean) and 6.72% (maximum). The model search for the first (*Niphadomimus* monophyly, species relationships and generic phylogenetic placement) and second (temporal phylogeography) analyses found two best models, one specific to each dataset: (1.) the generalised time-reversible with gamma distributed rate heterogeny and inferred proportion of invariable sites (GTR+G+I), (2.) and (GTR+G), respectively.

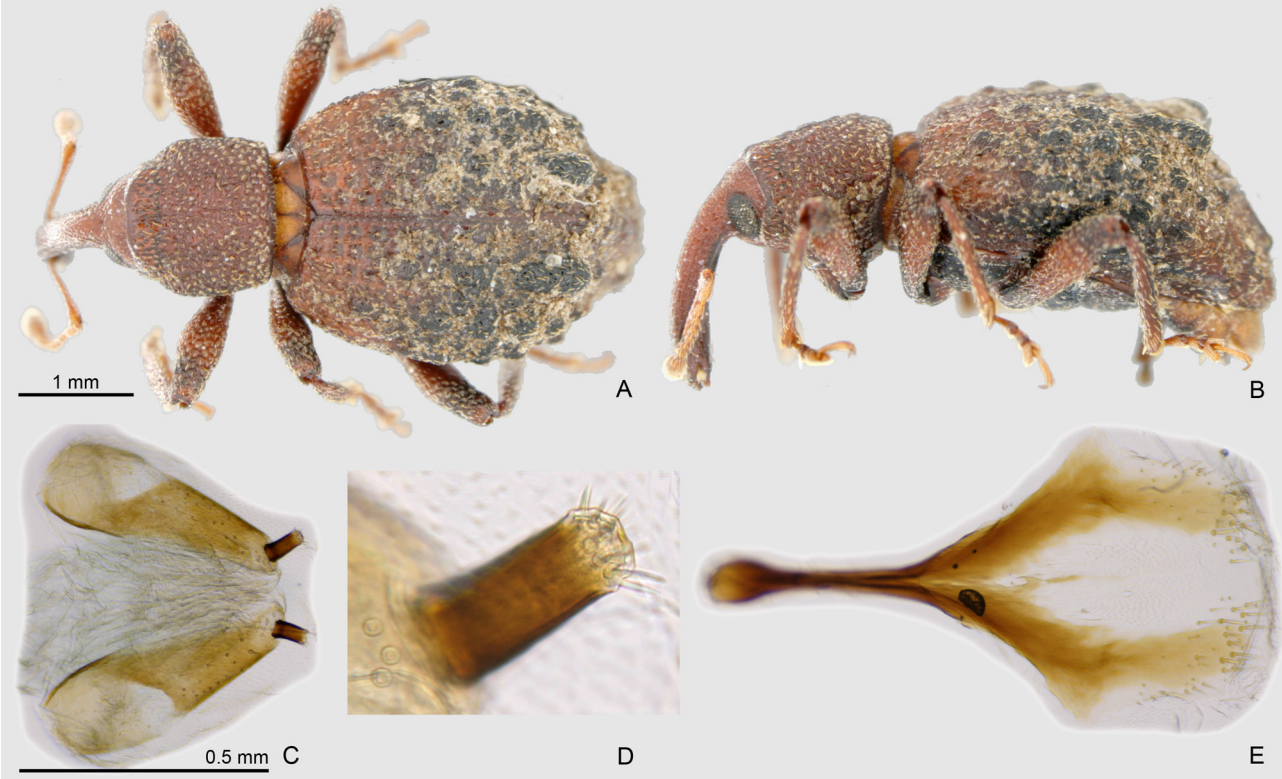

FIGURE 9. *Niphadomimus nigriventris*, female, #4697. A–B: habitus; C: sternite 9; D: apical hemisternite 9 (= "stylus"); E: sternite 8.

Phylogenetic analysis of the *Niphadomimus* dataset resulted in four consensus trees. The single most parsimonious tree (not shown) was 1,561 steps long, with the consistency index of 0.331824 and the retention index of 0.782389. The Maximum Likelihood method recovered the best tree (not shown) with the highest log likelihood of –7110.0359. The Bayesian Inference analysis (Fig. 12) had the lowest standard deviation of split frequencies of 0.004843. These four consensus trees each representing a different analytical method consistently recovered the following clades: (a.) all Molytinae species represented by more than one haplotype; (b.) the genus *Niphadomimus* exclusive of *N. merope* sp. n.; (c.) *Ectatorhinus* Lacordaire + *Typoderus* and (d.) *Hylobius* + *Euthycus* Pascoe (Fig. 12 and Table 1). The genus *Niphadomimus* was recovered as a clade in NJ, ML and BI, while in MP it formed a clade with *Leiosoma* Stephens (Table 1); the latter clade was also recovered in ML. Support statistics for the seven most concisely recovered clades are recorded in Table 1.

Temporal analysis in BEAST estimated that the split between the eastern most *N. merope* **sp. n.** from the Qinling Mt. Range and the rest of the genus from the Hengduan mountains had occurred around late Miocene (ca. 8.53 MY, Fig. 13). The diversification of the Hengduan clade was dated ca. 5.48 MY, and the speciation events took place between this date and the mid-Pliocene at about 3.64 MY (Fig. 13).

Discussion

Monophyly and relationships of *Niphadomimus*

Before monophyly of the genus can be discussed, six limitations of the material, data and knowledge have to be highlighted. First, the DNA analysis was lacking data on *N. nigriventris*, the type species, so the application of the generic name to the Chinese species might be questionable. Second, the only known specimen of the somewhat aberrant *N. niger* was not studied. Third, the rooting of the DNA topologies of the Chinese *Niphadomimus* species was done without the benefit of having a strong pre-existing hypothesis on the nearest relatives of the genus. Fourth, the other 13 Molytinae genera involved in the analysis represent about 3% of the subfamily genus-level diversity and, therefore, none of them might be a part of the *Niphadomimus* sister group. Fifth, the *Niphadomimus* morphological data were only compared and not analysed according to the strict phylogenetic practices, as discussed by Cassis and Schuh (2010). Sixth, only relatively short 658 nucleotid sequences from the single and relatively fast evolving maternally inherited CO1 gene were used, thus blurring both older and more recent evolutionary events.

FIGURE 10. *Niphadomimus sterope* **sp. n.**, holotype, male, #4475. A–D: habitus; E: sternite 9; F–H: aedeagus and tegmen, lateral (F), dorsal (G) and ventral (H).

These six limitations can hardly be avoided at present and should be acknowledged before addressing the issue of the generic monophyly. With the above in mind, the genus has been demonstrated as monophyletic based on its consistent recovery as a moderately supported clade in three of four DNA-based analyses (NJ, ML, BI). When it was not recovered as a clade (MP), it has been found paraphyletic with respect to *Leiosoma*, the most likely candidate for the *Niphadomimus* sister group (Table 1). Further indirect evidence in support of the generic monophyly comes from the inductive reasoning of supposing that at least some among the *Niphadomimus* diagnostic characters are synapomorphic. These shared characters are biological (flightless species inhabiting leaf litter of high altitude, predominantly *Rhododendron* shrubs and trees), geographical (all species are known from a relatively restricted region of the Eastern Himalaya, Hengduan and Qinling mountains at the south-eastern fringes of the Tibetan Plateau), or morphological (numerous external and male genital similarities, see Generic Diagnosis). Summing up, our results strongly suggest that the genus *Niphadomimus* is indeed monophyletic.

FIGURE 11. *Niphadomimus* spp., pro, meso- and metathorax, ventral (A–G, I) and left ventro-lateral (H). A: *Niphadomimus alcyone* **sp. n.**, holotype, female, #2493; B: *N. alcyone* **sp. n.**, paratype, female, #4429; C: *N. celaeno* **sp. n.**, holotype, male, #2476; D: *N electra* **sp. n.**, paratype, female, #0879; E: *N. maia* **sp. n.**, holotype, male, #2730; F: *N. merope* **sp. n.**, holotype, female #2201; G: *N. nigriventris*, male, #0796; H: *N. nigriventris*, female, #4697; I: *N. sterope* **sp. n.**, holotype, male, #4475.

Unlike its monophyly, the phylogenetic affinities of Niphadomimus remain highly obscure. The same, however, is true for the vast majority of the weevil groups, if indeed monophyletic, presently classified as Molytinae genera or tribes (and when leaving aside numerous unrecognized objective and subjective generic synonyms; unpublished data). The present inclusion of Niphadomimus in the tribe Typoderini is likely not informative, since (A.) the tribe was not recovered as monophyletic and (B.) even in its most restricted sense when represented only by the type genus, Niphadomimus is unlikely to form the sister-group to it. Indeed, four Typoderini genera included in the DNA analysis (Niphadomimus, Aparopion, Anchonidium and the type genus Typoderus) do not group consistently (Fig. 12). Instead, in half of the analyses (MP, ML; Table 1) Niphadomimus formed a weakly supported clade with the West Palaearctic Leiosoma, the type genus of the subtribe Leiosomatina. The latter includes two more genera (Alonso-Zarazaga & Lyal 1999): Lyperobius Pascoe with more than a dozen species from New Zealand and the nearby islands, as well as the monotypic Pterotomus Quedendeldt from Angola.

The subtribe has never been a subject of phylogenetic analysis and, like Typoderini, is not recovered as monophyletic. Morphological characters alone do not offer suggestions of the Niphadomimus sister group, except perhaps the prosternal excavation resembling a similar character found in the sympatrically distributed Aminyopini. No more can be presently said about Niphadomimus affinities without adding confusion to the already highly disordered generic grouping inside Molytinae, the latter likely being a highly polyphyletic assemblage of unrelated lineages (McKenna et al. 2009).

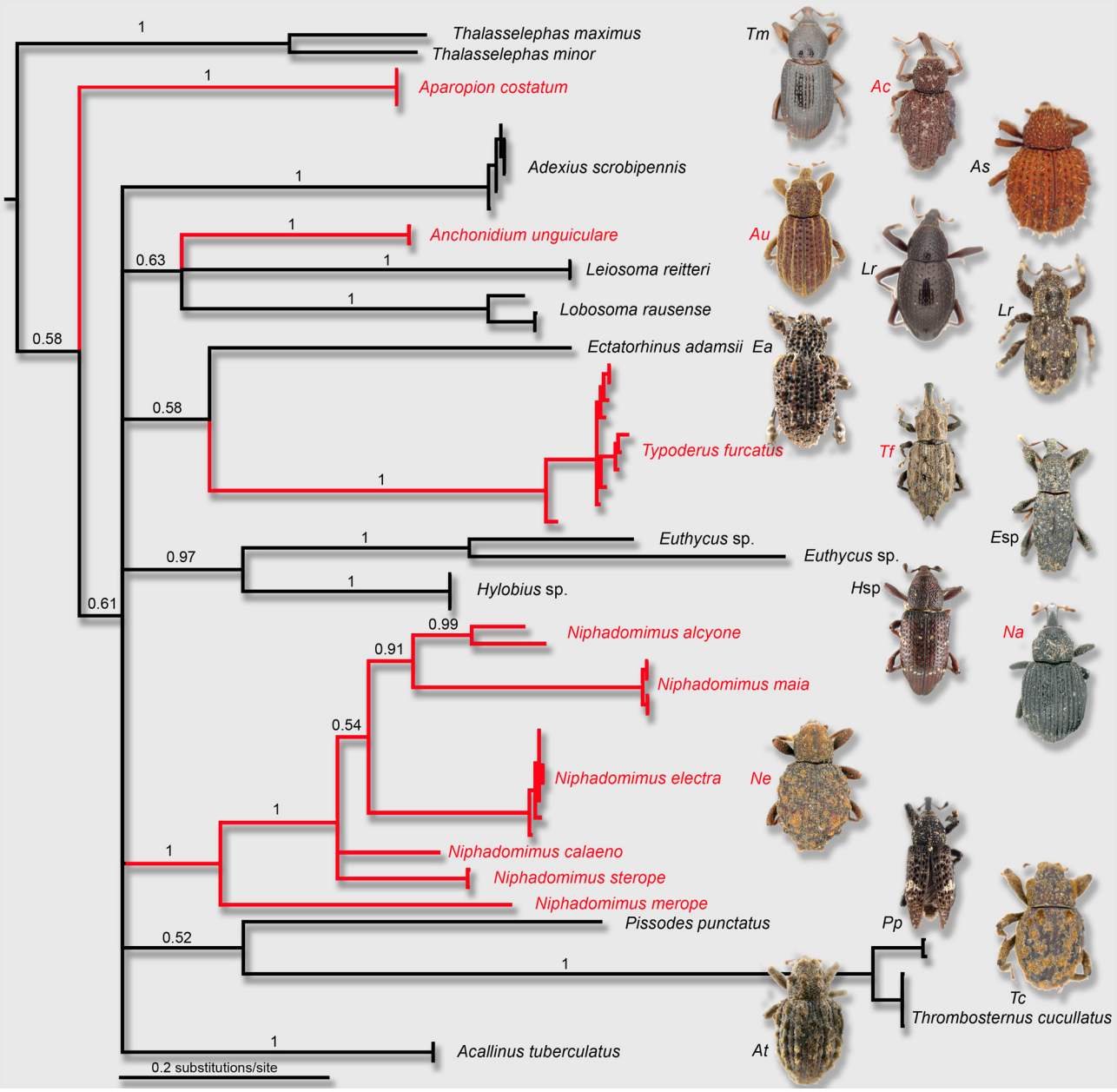

FIGURE 12. Bayesian inference phylogram positioning monophyletic *Niphadomimus* among other analysed Molytinae genera using the 658bp of the DNA barcoding CO1 gene fragment. *Niphadomimus* and the three other genera currently assigned to Typoderini are in red. Values at nodes are posterior probabilities; clades supported with less than 0.5 posterior probabilities are ignored and collapsed. The tree is rooted on *Graptus circassicus* (Entiminae; not shown). Habitus images are denoted by abbreviated genus and species letters on the same level with the terminal and are not to scale.

Niphadomimus species delimitation, generic diversity and distribution

The availability of DNA data with their superior lineage resolution power, as compared to morphological characters only, tends to intensify, rather than to clarify, the long-recognised uncertainty on species limits (Darwin 1859: 44). When delimiting species among the well-resolved and closely related lineages, it becomes a necessity to make an unambiguous reference as to which species concept has been used (Funk & Omland 2003). In the present work the "phylogenetic species concept" of Mishler & Theriot (2000) is followed, though it likely just one of the aspects of the more inclusive "unified" species concept of De Queiroz (2007). For most of the analysed *Niphadomimus* it is irrelevant which species concept is used, except for two specimens #2439 and #4429 presently recognised as the only two known representatives of *N. alcyone* **sp. n.** Under a different "species concept" it could have been possible, or perhaps necessary, to designate each of them as a separate species, since the specimens are morphologically dissimilar (compare Figs. 1 and 2), have uncorrected genetic p-distance of 5.94% (Fig. 12) and are allopatric (Fig. 14). Moreover, both specimens exhibit uncorrected DNA p-distance of 6.24% and each qualifies for a separate Barcode Index Numbers, recently suggested as a rough species approximation (Ratnasingham & Herbert 2013). Adopting the "phylogenetic species concept" of Mishler & Theriot (2000) permits grouping both specimens into the same nominal species, which can be tested again later, when more specimens of this clade become available for analysis.

TABLE 1. Seven most consistently recovered groups of *Niphadomimus* and Molytinae and their statistical support on four consensus trees from the first analysis, each representing one of the following methods: Neighbor-Joining (NJ; a not phylogenetic approach and provided here only for comparative purposes), Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI). Support values are percentages of 1000 bootstraps (NJ, MP, ML; clades supported with less than 10% are ignored and collapsed) or posterior probabilities (BI; clades supported with less than 50% posterior probabilities are ignored and collapsed), multiplied by 100. "None" indicates the group was not recovered.

	specimens	species	NJ	MP	ML	BI
Niphadomimus	17	6	63	none	66	66
Niphadomimus calaeno & *N. sterope*	3	2	72	77	62	none
Niphadomimus except *N. merope*	16	5	89	75	66	100
Niphadomimus + *Leiosoma*	19	7	none	26	31	none
Ectatorhinus + *Typoderus*	11	2	29	31	17	58
Hylobius + *Euthycus*	5	3	50	59	44	97
Pissodes + *Thrombosternus*	7	2	25	none	28	52

It is highly likely that even after the present study, the genus *Niphadomimus* will for years or decades remain an inadequately known group of "rare" and undersampled organisms. Such is the fate for many, if not the majority of, invertebrate lineages, also quite often irrespective of their economic significance. Consider that among 278 *Agrilus* (Coleoptera: Buprestidae) species-group taxa native to East Asia, 74 (26.6%) were new to science (Jendek & Grebennikov 2011), while this genus harbours *Agrilus planipennis* Fairmaire, the infamous Emerald Ash Borer, the agent of "the most costly biological invasion by an exotic forest insect to date" (Herms & McCullough 2014). Therefore, and similarly to any poorly known invertebrate group, the true number of *Niphadomimus* species is likely in time, if not in order of magnitude, greater than the present count of eight. This estimation is made based on the following five observations: (1.) the present paper quadruples the number of species from two to eight and increases the number of known specimens from two to exactly 100; (2.) except for *N. electra* sp. n. and *N. maia* sp. n., all other species are known by three or less specimens; (3.) with the exceptions *N. alcyone* sp. n. (but see its species concept above) and perhaps *N. nigriventris*, all *Niphadomimus* species are known only from the type localities; (4.) one of two species sampled in relatively great numbers, *N. maia* sp. n., was detected in a single sample among three taken in relative proximity to each other; (5.) in two of five sampled areas (Chinese localities, Fig. 14) two sympatrically occurring *Niphadomimus* species were detected. Such elevated species number estimations were even exceeded for the Middle American litter inhabiting weevil genus *Theognete* Champion, which in a single publication (Anderson 2010) multiplied its numbers from two syntypes to 94 species, and still counting (R.S. Anderson, pers. comm.). All these facts strongly suggest that *Niphadomimus* species are normally

restricted in their distribution to a single high-altitude area, not necessarily allopatric, rarely sampled even when targeted for and, therefore, numerous new species can be expected to inhabit other suitable habitats along the great wrinkled arc formed by the south-eastern edge of the Tibetan Plateau (Fig. 14) and stretching for at least 2500 km from central Nepal to Qinling Mt. Range in Shaanxi, China.

Niphadomimus temporal phylogeography

The most significant phylogeographic result is the detection of the relatively robust *Niphadomimus* tree with the sister group relations between the Qinling species *N. merope* **sp. n.** and the rest of the genus. Similarly to the genus *Niphadomimus*, numerous other Animalia clades are restricted in their distribution to the highlands of the south-eastern edges of the Tibetan Plateau (Fig. 14, Favre et al. 2014), i.e. the eastern Himalayas (Nepal, Sikkim, Bhutan and Arunachal Pradesh), the Hengduan mountains (northern Myanmar and the adjacent parts of SW China) and the Qinling mountain range (Shaanxi). The Red Panda (*Ailurus fulgens* Cuvier) presently ranges longitudinally through Sichuan, Yunnan, northern Myanmar, Bhutan, Sikkim and Nepal and unlike *Niphadomimus* it seems to be a recent inhabitant resulting from a post-glacial range expansion by this presumably highly mobile mammalian species (Le *et al.* 2005). The presumably less mobile Asian shrew-like moles (*Uropsilus* Milne-Edwards) have a distribution more closely matching that of *Niphadomimus* and exhibit a robust phylogeographic structure with their single sampled Qinling population nested deeply inside the Hengduan clade (Wan *et al.* 2013). The latter is not

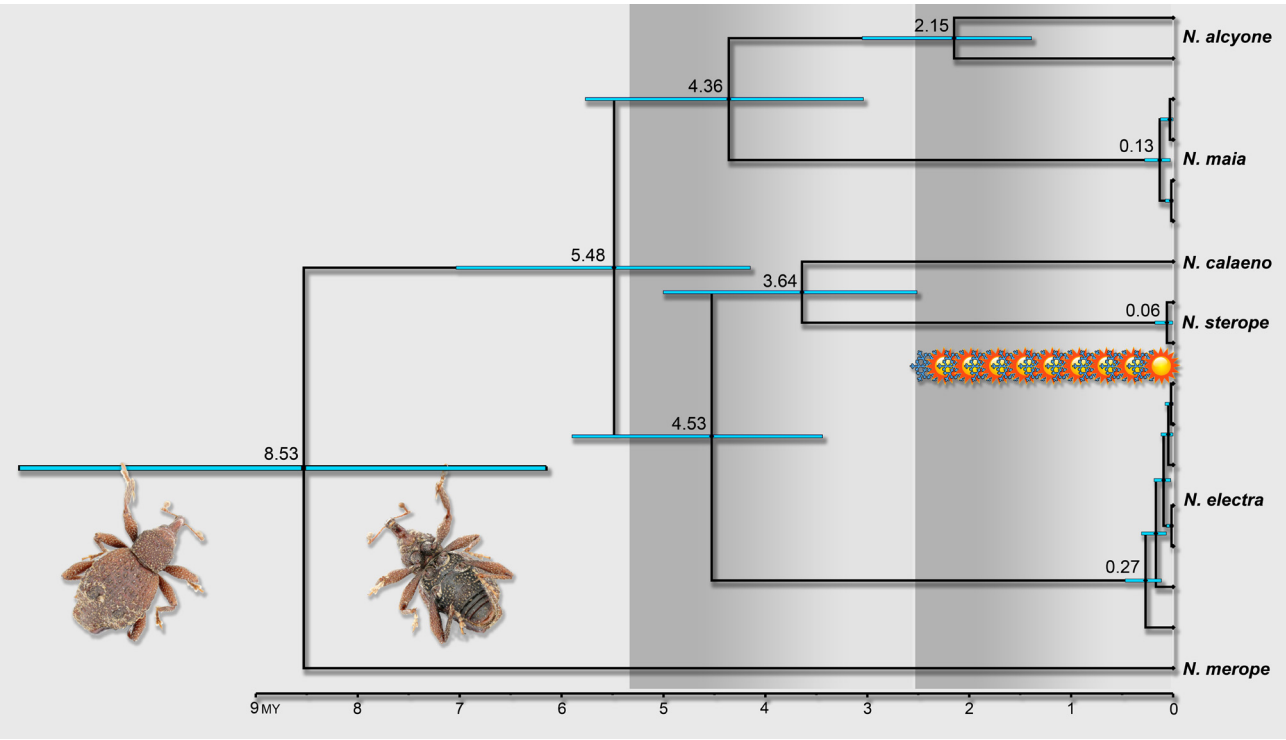

FIGURE 13. Ultrametic time tree of the Chinese species of the genus *Niphadomimus* using BEAST software to analyse 658bp of the "barcoding" mtDNA calibrated at a rate of 0.018 substitutions/site/MY. Numbers at nodes and on the scale below are million years before present (MY). Node bars represent a 95% confidence interval of the age estimate. Epoch dating after Cohen *et al.* (2013). Alternating snowflake and sun symbols denote Pleistocene climatic fluctuations.

similar to the *Niphadomimus* pattern, where the Qinling species *N. merope* **sp. n.** forms the sister group to the rest from Hengduan (Fig. 14). The comparison of the *Niphadomimus* temporal phylogeography with the aforementioned examples should be done with caution, since they also rely on an *a priori* DNA substitution rate taken as the main dating source, which introduces a significant element of circular logic.

Only a few works on the low dispersing wingless weevils distributed in temperate mountains are detailed enough to permit adequate comparison with the reported *Niphadomimus* phylogeographic results. Among them, Meregalli *et al.* (2013) offers the best reference point being the most comparable in size and nature to the DNA

data, as well as dealing with a genus of high altitude wingless weevils distributed along the southern edge of the last glacial maximum in South Europe. The authors utilized 775 nt of 18 CO1 haplotypes representing seven *Dichotrachelus* Stierlin species sampled on the southern slopes of the Alps. They reported the mean interspecies *p*-distance in the range of 11.0–16.7%, which by using the 2.1% MY^{-1} sequence divergence rate dated the speciation events between 6.5 MY and 3.3 MY, i.e. from later Miocene to late Pliocene. They concluded that instead of being the main force behind the *Dichotrachelus* phylogeographic structure, the Quaternary glaciation cycles are primary responsible for the highly fragmented present day high-altitude species distribution. For the most part the conclusions on *Dichotrachelus* phylogeography match those for *Niphadomimus*, and conceivably suggest a common pattern, even though both studies used a markedly different sequence divergence rate for the same CO1 gene (2.1% MY^{-1} in Meregalli *et al.* 2013, as compare to 3.6% MY^{-1} adopted here).

Regardless of the uncertainties about the exact order of the bifurcation points in the (*Niphadomimus* except *N. merope* **sp. n.**) clade (compare Figs. 12 and 13), temporal analysis places all events leading to the origin of all presently recognised *Niphadomimus* species well before Pleistocene (the last dichotomy between *N. celaeno* **sp. n.** and *N. strerope* **sp. n.** taking place in mid Pliocene at about 3.64 MY; Fig. 13). These results add to the growing body of evidence denying the Pleistocene repeated glaciation and aridification effects of being the most important stimulus to the present day speciation. The mtDNA distance-based interpretation of *Niphadomimus* species diversification agrees well with the similar conclusions reached based on fossil records for the North American amphibians and reptiles (Holman 1995: 28) and mammals (Barnosky 2005). These similarities further corroborate the hypothesis that the Quaternary climatic changes do not represent the main driving force of the presently observed species diversity (Rull 2008). These *Niphadomimus* temporal results, however, should be taken only tentatively, since the generic tree (Fig. 13) is likely lacking many more extant branches for numerous hypothetically undetected species. If true, they will be likely inserted among those already known and some of them originating more recently, than what is presently hypothesised.

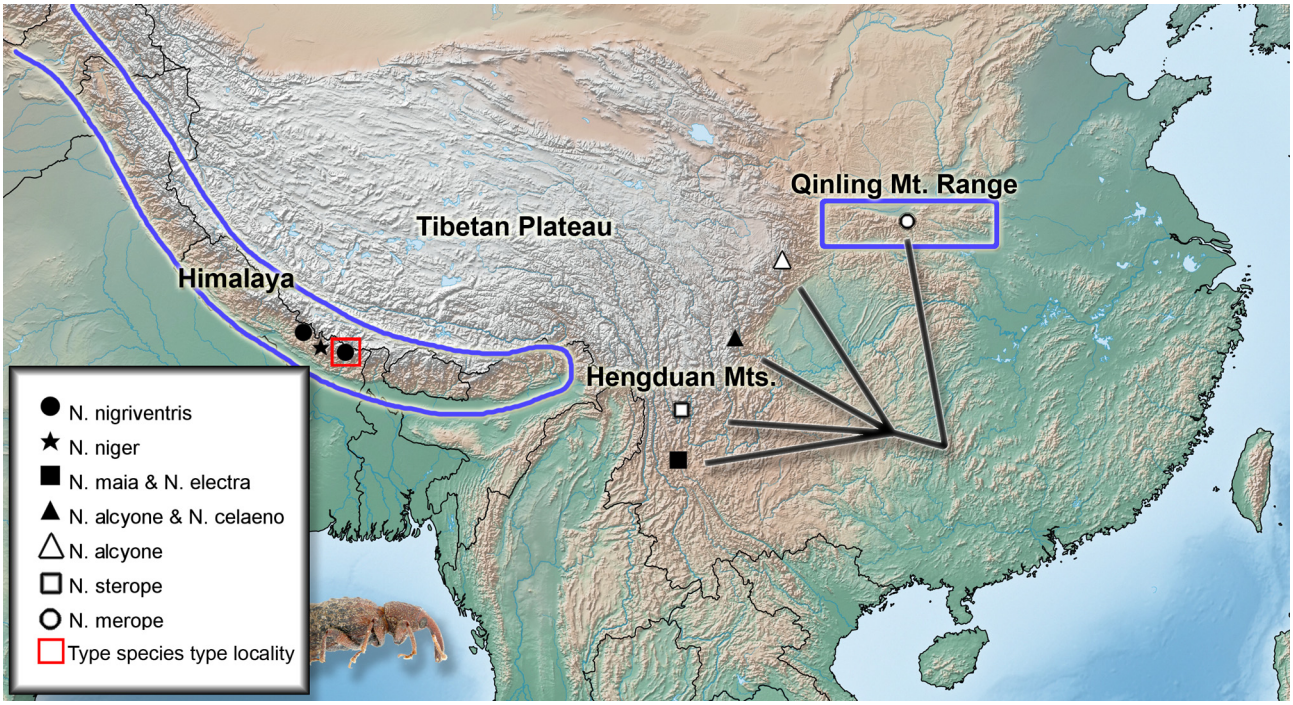

FIGURE 14. Known distribution of the genus *Niphadomimus* in Nepal (two species) and China (six species). The overlaying topology illustrates sister-group relations between the easternmost *N. merope* **sp. n.** from the Qinling Mt. Range and the unresolved rest of the genus. Base map generated using the on-line SimpleMappr tool (Shorthouse 2010).

Biology of *Niphadomimus* weevils

No immature stages or a host plants are known for *Niphadomimus* and, therefore, all biological data is derived from what can be interpreted from the adult collecting circumstances. The genus, as presently known, consists of exclusively wingless and relatively high-altitude species, as least some of which are bisexual. All *Niphadomimus* specimens for which collecting information could be assessed (all, except Zherikhin's two holotypes), have been collected by sifting forest litter in the *Rhododendron*-dominated forests or shrubs. None have been taken by opportunistically sampling under rocks in the alpine zone. The latter habitat is frequented by many other wingless high altitude weevils presumably for day time shelter. These latter species are normally not detected by sifting and are frequently found a relatively large distance away from the nearest woody vegetation, suggesting their independence from the latter resource. These considerations suggest that the species of *Niphadomimus* are not truly alpine species but rather inhabitants of the upper forest zone partly or fully dependant on wooded vegetation in their development and could not be expected to be found far from the food source.

FIGURE 15. Habitat and the type locality of *Niphadomimus electra* **sp. n.** and *N. maia* **sp. n.** on the eastern slope of Cang Shan in south-eastern Yunnan, China, above the town of old Dali at 3,740m (noted on labels as sifting #37 taken on 5.vii.2011). This is the only locality where two *Niphadomimus* species have been seen together in the same sample. This is also the exact locality for *N. maia* **sp. n.** known from nine specimens from a single sample. Note the presence of conifers (in the background) in the predominantly broadleaved high altitude forest dominated by tall *Rhododendron* spp. overhanging the trail. The sifter with the half-full bag containing 39 live specimens of both *Niphadomimus* species, including both holotypes, is in the foreground. The darker sifted litter, appearing disturbed, is immediately to the left of the trail. This sample, together with another taken nearby on the previous day (siftings #35 and #36 in 2011) are by far the richest for *Niphadomimus* providing 89 of the 100 known specimens.

Another biological peculiarity of *Niphadomimus* weevils is their remarkable rarity in sifted samples, coupled with occasional abundance. The great majority (70–90%) of samples taken in the seemingly appropriate *Rhododendron*-dominated habitats in the localities where *Niphadomimus* is known to occur failed to record the genus (total number of samples not shown). When detected, the genus would normally be represented by a single specimen of a single species discovered after a few days of intense sampling. Consider that 11 among 14 *Niphadomimus* collecting events pertained indeed to singletons (all four specimens from Nepal, plus seven Chinese records, including the 2010 record of *N. electra* **sp. n.**). Only two samples from the Cang Shan Mountain Range taken within two days of 4–5.vii.2011 resulted in long series of *N. electra* **sp. n.**, and, most uncommonly, one of them additionally included all nine presently known specimens of *N. maia* **sp. n.** (Fig. 15). It is extraordinary that both the exceptionally rich 2011 sites are located within one kilometre from each other, were equally diligently sampled a year earlier (18–19.v.2010) and provided only a singleton of *N. electra* **sp. n.**, the first representative of the genus ever recorded in China. The latter results suggest strong and presently unexplainable temporal fluctuations of adult specimen density.

Subfamily placement of Molytinae

An important work by Lyal (2014) was published after the manuscript of the present paper has already been completed. In this work the tribes of Molytinae were significantly reorganized and many genera reshuffled, as compared to the earlier standard works of reference (Alonso-Zarazaga & Lyal 1999, Bouchard *et al*. 2011; Alonso-Zarazaga 2013). The most noticeable novelty, however, was the incorporation of the entire former "subfamily Cryptorhynchinae" into Molytinae, the latter presently containing 37 tribes plus ca. 28 genera *incertae sedis* (Lyal 2014: 530, 531). This taxonomic change is not implemented in the present paper for purely technical reasons. Lyal (2104) should be consulted for the most recent in-depth treatment of what is herein considered as the "subfamily Molytinae"; however, changes therein will not affect the results and conclusions of this paper.

Acknowledgements

Curators of four collections mentioned above variously helped in accessing *Niphadomimus* and other specimens under their care. Massimo Meregalli (Torino, Italy) permitted photographing the holotype of *N. nigriventris*, then (2008) on loan to him from SMNS. Aleš Smetana (Ottawa, Canada) collected the only two known non-holotype specimens of *N. nigriventris*. David Langor (Edmonton, Canada), Lutz Behne (Müncheberg, Germany) and Genrikh Ed. Davidian (St. Petersburg, Russia) identified the sequenced specimens of *Pissodes punctatus*, *Anchonidium unguiculare* and *Graptus circassicus*, respectively. Marek Wanat (Wroclaw, Poland) provided and identified the sequenced specimens of *Adexius scrobipennis*. Alexander Riedel (Karlsruhe, Germany) advised on specimen dissection techniques and, together with Matthew Van Dam (Berkeley, USA), reviewed the manuscript. Ignacio Ribera (Barcelona, Spain) advised on implementation of DNA analytical methods; he and Karen McLachlan Hamilton (Ottawa, Canada) critically read the early drafts of this paper.

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