EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
Eur. J. Entomol. **115**: 437–444, 2018
doi: 10.14411/eje.2018.044
ORIGINAL ARTICLE

Dryophthorinae weevils (Coleoptera: Curculionidae) of the forest floor in Southeast Asia: Three-marker analysis reveals monophyly of Asian Stromboscerini and new identity of rediscovered *Tasactes*

Vasily V. GREBENNIKOV

Canadian Food Inspection Agency, 960 Carling Ave., Ottawa, ON, K1A 0Y9, Canada;
e-mail: vasily.grebennikov@inspection.gc.ca

Key words. Curculionidae, Dryophthorinae, *Tasactes*, COI, ITS2, 28S, phylogeny, clogging taxonomy, China, Nepal, Myanmar

Abstract. The nominal genus *Tasactes* Faust, 1894, consisting of two originally included nominal species from Myanmar, is rediscovered for the first time since being erected. Adult weevils herein assigned to the taxonomically re-defined *Tasactes* were abundant in forest floor litter at five localities in China (Yunnan and Sichuan), plus one specimen is available from Shaanxi and three from Nepal. Phylogenetic analysis of a 2,275 bp matrix concatenated from one mitochondrial (COI) and two nuclear markers (ITS2 and 28S) revealed that the monophyletic *Tasactes* consists of eight evolutionary significant terminal clades, either allopatric (three) or sympatric (two on Cang Shan in Yunnan and three on Mount Emei in Sichuan). The genus *Tasactes* is nested within the monophyletic Stromboscerini, while the tribe is sister to monophyletic *Dryophthorus*. The two morphological diagnostic characters of *Tasactes,* which are unique within the tribe, are the transversely truncated antennal club and conically projecting velvety apex of the club. So defined, *Tasactes* renders the genus *Orthosinus* paraphyletic. Considering the taxonomic neglect and uncertainties surrounding nominal Stromboscerini, all herein reported members of this tribe, including the *Tasactes*, are not assigned to Linnaean species. This paper illustrates the "clogging taxonomy" phenomenon, in which obscure historical names render taxonomic assignment of newly sampled specimens precarious. All the data used herein (localities, sequences, specimen images) are available online in public datasets dx.doi.org/10.5883/DS-TASACT1 and dx.doi.org/10.5883/DS-TASACT2.

INTRODUCTION

This paper was motivated by the discovery of numerous minute and slow-moving Stromboscerini weevils (Fig. 1A) recorded (Figs 1B–E) when forest leaf litter in Southwest China and nearby areas was sifted (Fig. 5 and Supplementary Table S1). The original plan of producing an integrated phylogeny using DNA, morphology and other data sources was abandoned once the grossly unsatisfactory state of Stromboscerini taxonomy became apparent (Grebennikov, 2018). The nominal tribe lacks an underlying phylogenetic hypothesis and is likely to be multiply non-monophyletic (Grebennikov, 2018). The type genus from Madagascar was thought to be unrelated to the rest of the tribe distributed mainly in Southeast Asia (Grebennikov, 2018), potentially leaving all but the type genus without a tribal assignment. Furthermore, doubts remain about the monophyly of all non-monotypic Recent Stromboscerini genera. The widely accepted and century old synonymy of the type species of the genus *Xerodermus* is doubted (Grebennikov, 2018), which threatens the taxonomic identity of the genus. It became evident that any research on the numerous newly sampled Stromboscerini would be haunted by the phenomenon of "clogging taxonomy" (Grebennikov, 2016), in which poorly understood historical names render the usage of the Linnaean nomenclature very unreliable.

The nominal genus *Tasactes* was chosen as the focus of this paper because its phylogenetic identity as a clade detected using a DNA-based phylogenetic analysis could be clearly linked with a nominal genus through a pivotal combination of diagnostic morphological characters. The history of human encounter with these weevils, herein referred to as the genus *Tasactes*, is exceedingly short. Johannes Faust (1894) established the genus for two new species, *T. carinulatus* Faust, 1894 (Fig. 10A in Grebennikov, 2018) and *T. interruptus* Faust, 1894 (Fig. 10B in Grebennikov, 2018). Both type series were collected by Leonardo Fea in March 1887 on "Mt. Mooleyit" (likely Mount Mulayit Taung at 16°11´N and 98°31´E of the Dawna Range) in southern Myanmar (Fig. 5). Although all three names were later mentioned in various catalogues (i.e. Alonso-Zarazaga & Lyal, 1999), the type species of *Tasactes* remains undesignated and no specimens of the genus, other than the type series, are reported.

This paper has two main goals, both aimed at carrying out the first phylogenetic analysis of the tribe Stromboscerini. The first goal is to test the implied monophyly of the

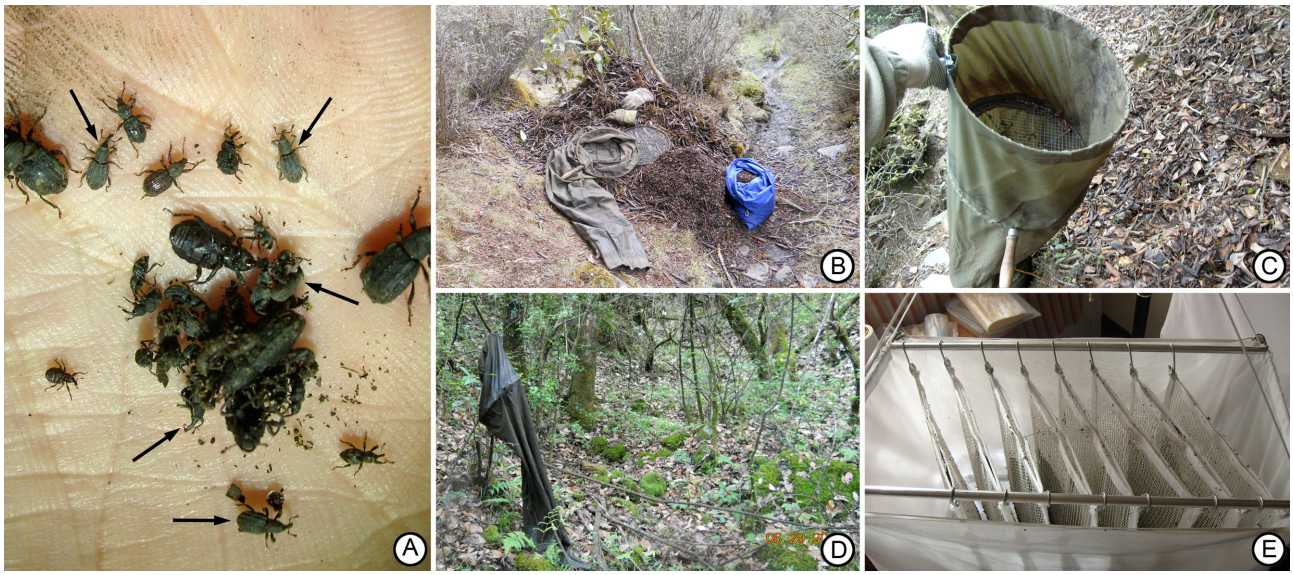

Fig. 1. Stromboscerini weevils. A – weevils extracted from a sample of typical forest floor litter collected on Mount Emei, Sichuan, China; arrows indicate Stromboscerini; B – sample of litter in a bag prior to sifting through a 12 × 12 mm mesh and then through a 7 × 7 mm mesh; C – sifter with 12 × 12 mm mesh; D – forest floor litter habitat of Stromboscerini weevils; E – Winkler funnel with litter suspended in mesh bags.

tribe, and if monophyletic, then to hypothesise its sister-group and shed light on its internal phylogenetic structure. The second goal is to anchor one of the inadequately known historical genus-group names (*Tasactes*) to a newly detected clade. Overall, this paper was designed as another example (Riedel, 2017) of the work required on taxonomically and phylogenetically orphaned groups, when newly hypothesized clades may for the first time be linked with old and inadequately known nominal taxa.

MATERIAL AND METHODS

Specimen sampling and handling

A total of 145 sifted samples of forest litter were collected in mainland China (122), Vietnam (7) and Taiwan (16); their two-letter two-digit codes are explained in Supplementary Table S1. Litter was sifted using a hand-held sifter (Figs 1B–D) first through 12 × 12 mm mesh, then through an insert (Fig. 1B) with a finer 7 × 7 mm mesh. Live specimens (Fig. 1A) were extracted using suspended Winkler funnels (Fig. 1E) and preserved in 96% ethanol. A few specimens were opportunistically collected by means other than litter sifting (mainly by hand), while specimens from Nepal (clade E in Fig. 3) were received from Joachim Schmidt; these specimens do not have litter sample codes (Table 2) and are denoted in Fig. 3 by country names. All the specimens have a label with the code CNCCOLVG0000XXXX serving as a Sample ID in the Barcode of Life Database (= BOLD, Ratnasingham & Hebert, 2007, www.bold.org), while the last four digits serve as unique specimenidentifiers (Figs 2–4 and Table 2). All the specimens studied were adults and are deposited in the Canadian National Collection of Insects, Arachnids and Nematodes in Ottawa, Canada (CNC).

Specimen selection for DNA barcoding and Neighbour Joining (NJ) clustering

Sixty specimens of Dryophthorinae (36 of *Tasactes* and 15 of four other Stromboscerini genera) were DNA barcoded (= sequencing of 658 bp of COI-5', Hebert et al., 2003a, b). All laboratory work (including DNA extraction, purification, PCR and bidirectional sequencing) was performed in a commercial laboratory "Canadian Center for DNA Barcode" (CCDB, http://www.ccdb.ca/) at the University of Guelph, Ontario, Canada, following the standard laboratory protocol (Ivanova et al., 2006, 2014). A cocktail of two primer pairs was used to amplify the DNA barcoding fragment (Supplementary Table S2). Obtained sequences, electropherograms, gel images, specimen data and specimen images (Supplementary Fig. S1) can be seen online in the public BOLD dataset available at dx.doi.org/10.5883/DS-TASACT1. Alignment of these sequences was easy as they contained no insertions/deletions (= indels), while their NJ clustering was performed using the online BOLD engine.

Three-marker matrix formation and Maximum Likelihood (ML) analysis

A subset containing 45 Dryophthorinae terminals was selected and sequenced for two additional nuclear DNA markers, ITS2 and 28S (Table 1), using the primers in Supplementary Table S2. A total of 21 *Tasactes* and 15 other Stromboscerini terminals were selected (Table 2) to best represent clusters recovered in the NJ analysis (Supplementary Fig. S2). To place Stromboscerini in a wider phylogenetic context and test its taxonomically-implied monophyly, nine non-Stromboscerini terminals were incorporated in the analysis, including two terminals representing the likely closely related genus *Dryophthorus* Germar, 1824 (Gunter et al., 2016, Fig. 2 and Table 2). In the absence of a phylogenetic hypothesis for Stromboscerini and Dryophthorinae (and to avoid an excessive number of indels in noncoding and fast evolving ITS2 when a distant organism is added to the matrix), no non-Dryophthorinae terminals were added to root the topology. All reported and newly generated DNA sequences, as well as their electropherograms, gel images, specimen data and specimen images can

Table 1. DNA fragments used in analyses.

Gene	#	min	max	aligned	positions
CO1–5'	45	576	658	658	1 to 658
ITS2	45	334	678	998	659 to 1656
28S	43	329	580	619	1657 to 2275

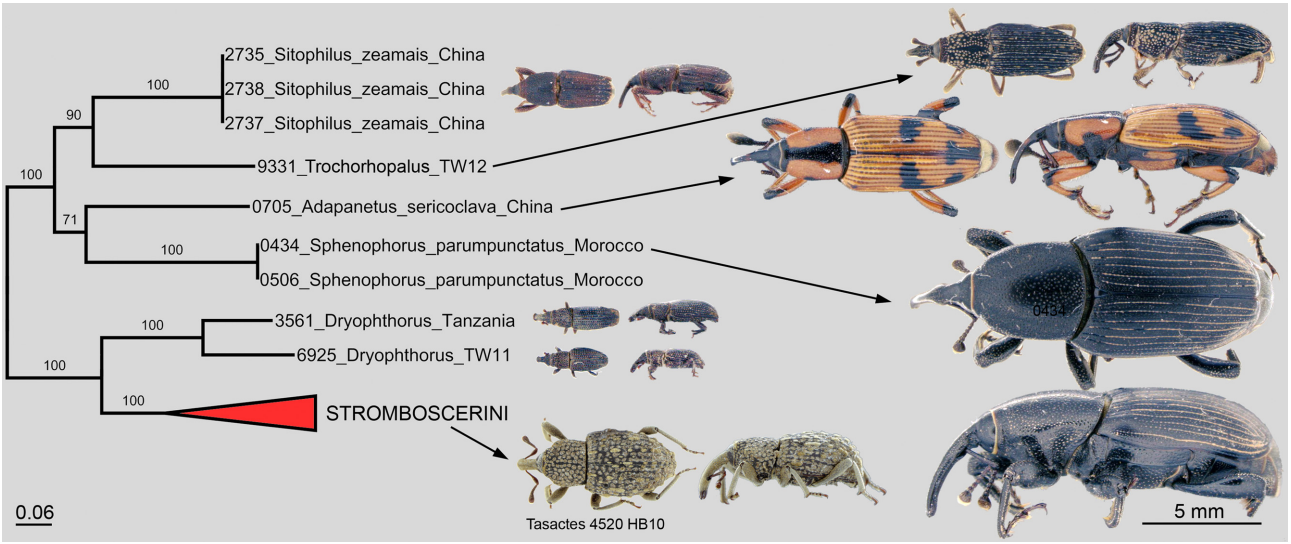

Fig. 2. Unrooted Maximum Likelihood inference phylogram of Dryophthorinae weevils based on the analysis of the concatenated 2,275 bp matrix. Digits at internodes are bootstrap values. Images are to scale and illustrate corresponding tree terminals.

Table 2. GenBank accession numbers of Dryophthorinae weevils; for sample code see Table S1.

Specimen	Species	Country	Locality	Sample	CO1	ITS2	28S
0434	*Sphenophorus parumpunctatus*	Morocco	Tiznit	n/a	HM417724	KY110320	KY110384
0506	*Sphenophorus parumpunctatus*	Morocco	Tiznit	n/a	HM417726	MG968848	MG968904
0705	*Adapanetus sericoclava*	China	Emei Shan	n/a	HQ987004	MG968817	MG968874
0766	*Tasactes*	China	Cang Shan	CN02	HQ987041	MG968836	MG968893
0790	*Tasactes*	China	Cang Shan	CN04	HQ987059	MG968821	MG968878
0876	*Tasactes*	China	Cang Shan	CN16	HQ987108	MG968834	MG968891
1058	*Orthosinus*	China	Gaoligong Shan	GL15	HQ986782	MG968833	MG968890
1060	*Orthosinus*	China	Gaoligong Shan	GL15	HQ986783	MG968822	MG968879
1061	*Orthosinus*	China	Gaoligong Shan	GL15	HQ986784	MG968813	none
1092	*Tasactes*	China	Emei Shan	EM13	HQ986795	MG968839	MG968896
1133	*Tasactes*	China	Emei Shan	EM15	HQ986820	MG968853	MG968909
1166	*Tasactes*	China	Emei Shan	EM17	HQ986846	MG968827	MG968884
1171	*Tasactes*	China	Emei Shan	EM17	HQ986851	MG968824	MG968881
1215	*Tasactes*	China	Emei Shan	EM18	HQ986875	MG968854	MG968910
2283	*Tetrasynommatus*	China	Emei Shan	EM24	MG968932	MG968838	MG968895
2325	*Tetrasynommatus*	China	Emei Shan	EM25	MG968938	MG968844	MG968900
2373	*Tasactes*	China	Emei Shan	EM27	MG968919	MG968820	MG968877
2405	*Tasactes*	China	Gongga Shan	GN03	MG968925	MG968828	MG968885
2519	*Tasactes*	China	Gongga Shan	GN10	MG968916	MG968818	MG968875
2554	*Tasactes*	China	Gongga Shan	GN15	MG968927	MG968830	MG968887
2690	*Tasactes*	China	Cang Shan	CN19	MG968943	MG968847	MG968903
2705	*Tasactes*	China	Cang Shan	CN20	MG968946	MG968850	MG968906
2735	*Sitophilus zeamais*	China	Gongga Shan	n/a	KJ672255	MG968837	MG968894
2737	*Sitophilus zeamais*	China	Gongga Shan	n/a	MG968933	MG968840	MG968897
2738	*Sitophilus zeamais*	China	Gongga Shan	n/a	MG968936	MG968842	MG968899
3561	*Dryophthorus*	China	Gongga Shan	n/a	MG968913	MG968814	MG968871
4120	*Orthosinus*	China	Cang Shan	CN06	MG968917	MG968819	MG968876
4177	*Tasactes*	China	Emei Shan	EM13	MG968923	MG968825	MG968882
4187	*Tasactes*	China	Emei Shan	EM13	MG968911	MG968811	MG968869
4310	*Orthosinus*	Vietnam	Tam Dao	TD02	MG968924	MG968826	MG968883
4330	*Allaeotes*	Vietnam	Tam Dao	TD02	MG968948	MG968852	MG968908
4331	*Allaeotes*	Vietnam	Tam Dao	TD02	MG968930	MG968835	MG968892
4334	*Orthosinus*	Vietnam	Tam Dao	TD02	MG968929	MG968832	MG968889
4346	*Orthosinus*	Vietnam	Tam Dao	TD03	MG968940	MG968846	MG968902
4401	*Allaeotes*	Vietnam	Tam Dao	TD06	MG968939	MG968845	MG968901
4520	*Tasactes*	China	Haba Shan	HB10	MG968928	MG968831	MG968888
4570	*Tasactes*	China	Haba Shan	HB17	MG968937	MG968843	none
6925	*Dryophthorus*	Taiwan	Lija Road	TW11	MG968914	MG968815	MG968872
6967	*Dexipeus*	Taiwan	Lija Road	TW14	MG968915	MG968816	MG968873
6974	*Dexipeus*	Taiwan	Lija Road	TW15	MG968935	MG968841	MG968898
6980	*Dexipeus*	Taiwan	Lija Road	TW16	MG968926	MG968829	MG968886
7781	*Tasactes*	Nepal	Barun Valley	n/a	MG968947	MG968851	MG968907
7782	*Tasactes*	Nepal	Barun Valley	n/a	MG968921	MG968823	MG968880
7783	*Tasactes*	Nepal	Barun Valley	n/a	MG968945	MG968849	MG968905
9331	*Trochorhopalus*	Taiwan	Lija Road	TW12	MG968912	MG968812	MG968870

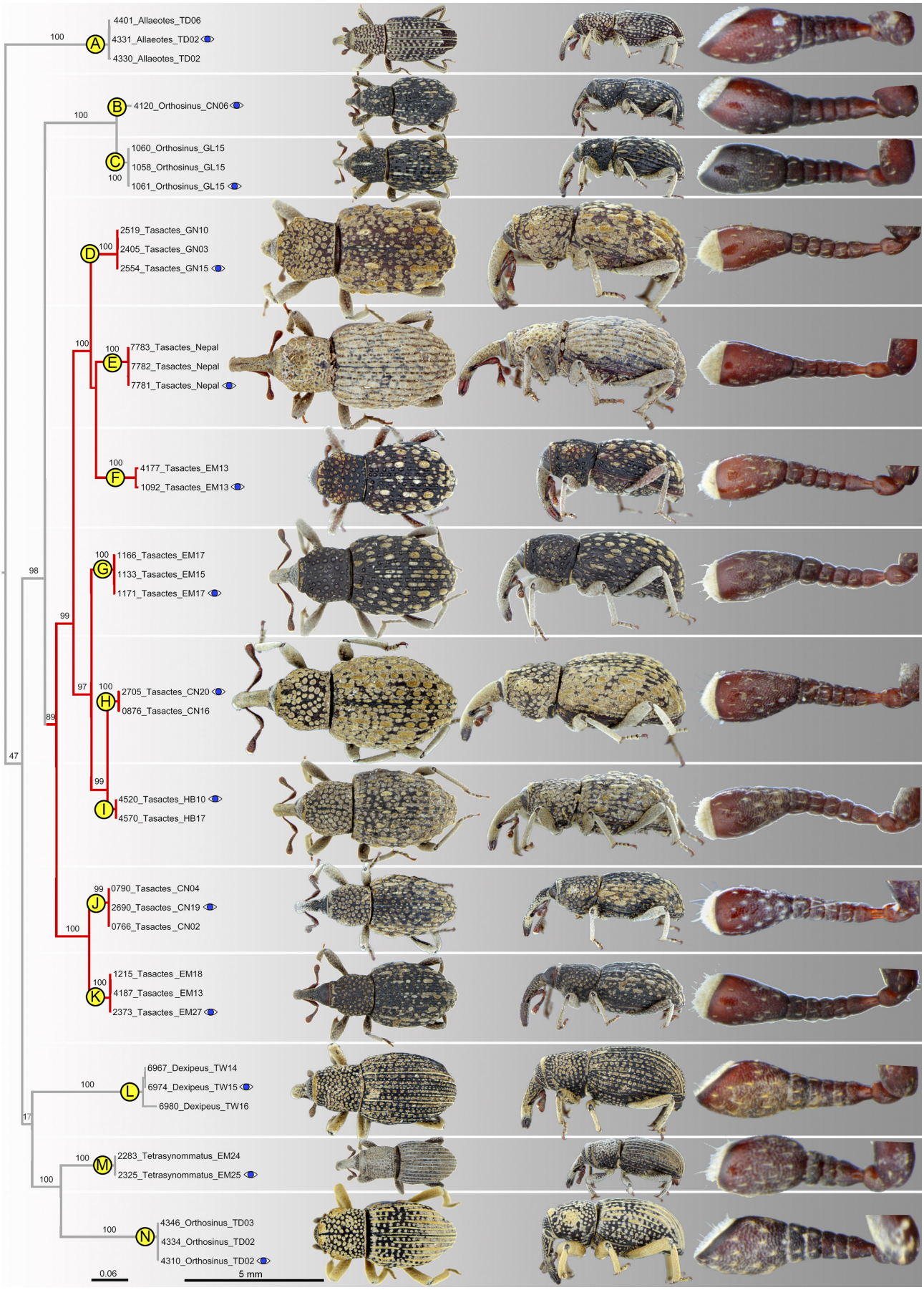

Fig. 3. Rooted Maximum Likelihood inference phylogram of monophyletic Asian Stromboscerini based on the analysis of the concatenated 2,275 bp matrix. Digits at internodes are bootstrap values. Fourteen evolutionary significant terminal clades are labelled A–N; eight of them belonging to the monophyletic genus *Tasactes* are in red. Blue eye signs indicate illustrated terminals; habitus images are to scale.

Fig. 4. Stromboscerini weevils. A–N – heads, left lateral view illustrating fourteen evolutionary significant terminal clades as in Fig. 3; O–P – antennae of syntypes of both nominal *Tasactes* species.

be seen online in the public BOLD dataset DS-TASACT2 available at dx.doi.org/10.5883/DS-TASACT2.

Alignment of the ITS2 and 28S sequences was done using the MAFFT 7 online platform (Katoh et al., 2002, Katoh & Toh, 2008a) with the Q-INS-i algorithm (Katoh & Toh, 2008b) utilising the information on the secondary structure. No parts of the three alignments were excluded from the analysis. Three aligned single-fragment datasets were concatenated using Mesquite 3.11 (Maddison & Maddison, 2011). Analysis of the 2,275 bp concatenated dataset was done using the ML phylogenetic method and the RAxML 7.2.7 (Stamatakis et al., 2008) algorithm on a computer cluster at the Cyberinfrastructure for Phylogenetic Research (CIPRES) (Miller et al., 2010) with 100 non-parametric bootstrap (Felsenstein, 1985) replicates. The topologies were visualized using FigTree v1.4 (Rambaut, 2014).

Matching names and clades, specimen visualization and the limitations of this study

To link the Stromboscerini clades with five nominal genera, primary types of all Recent valid genera of the tribe were studied (Grebennikov, 2018), including the syntypes of both nominal *Tasactes* species stored in the "Senckenberg Naturhistorische Sammlungen Dresden" in Dresden, Germany. Morphological characters given in the key to Stromboscerini genera (Morimoto, 1985) were also used. To visualize the adult morphology of Stromboscerini and link it with the DNA topology, habitus and antennae of specimens of all 14 evolutionary significant terminal clades (= ESTC, = candidate species) were imaged and superimposed on the phylogenetic tree (Figs 2, 3). Since sexual dimorphism has never been reported or noted in the Stromboscerini, no attempt was made to sex specimens. Lacking comparative data on the genitalia, no attempt was made to use this source of information. No new taxonomic acts (such as the description of new taxa or designation of the type species of *Tasactes*) are herein performed, since such actions would require an effort far exceeding the scope of this paper, which aims to provide the very first phylogenetic glimpse of the old and poorly known nominal taxa associated with the vaguely defined family-group name "Stromboscerini".

RESULTS

The ML tree of 45 terminals analyzed using the concatenated 2,275 bp matrix recovered the monophyletic Stromboscerini as a sister to the monophyletic *Dryophthorus*; all three clades have 100% bootstrap support (Fig. 2). All 36 Stromboscerini terminals are grouped in 14 ESTC (clades A–N in Fig. 3), each with high bootstrap support (99–100%); eight of these clades (D–K in Fig. 3, in red) form the monophyletic *Tasactes* with bootstrap support of 89%. Relationships among eight *Tasactes* clades are well-resolved with high bootstrap support (97–100%),

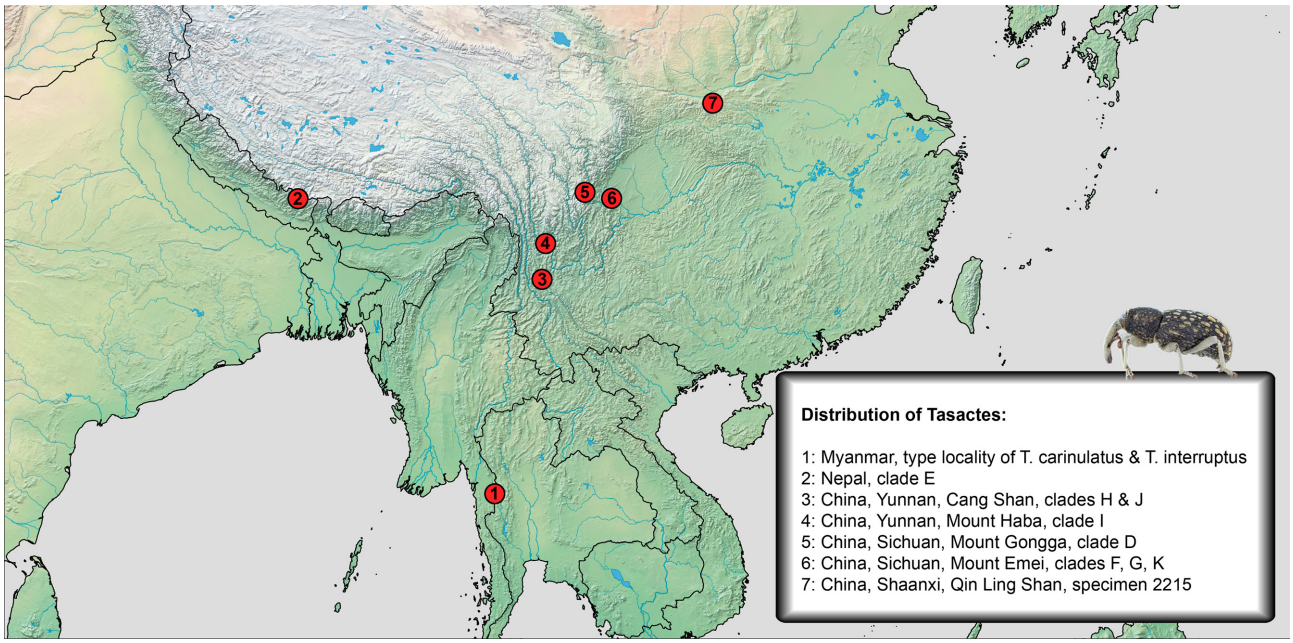

Fig. 5. Distribution of *Tasactes*. Map was prepared using online software SimpleMappr (Shorthouse, 2010); the northeastern record is based on a single specimen that was not included in the concatenated analysis (Fig. 3), but DNA barcoded and clusters with other *Tasactes* as a sister to clade G (Fig. S2).

and clade J+K is a sister to the rest of the genus (clades D–I), which itself is made up of two clades: D+(E+F) and G+(H+I). *Orthosinus* Motschulsky, 1863, the only genus besides *Tasactes* represented in the analysis by more than one ESTC, is non-monophyletic, with one of its two clades (clade B+C) being a sister to *Tasactes*, while clade N is a sister to *Tetrasynommatus* Morimoto, 1985 (Fig. 3).

DISCUSSION

Monophyly and sister group of Stromboscerini

Recovery of the strongly supported clade formed by reciprocally monophyletic *Dryophthorus* and Stromboscerini is one of the two most significant results of this study. Even though this relationship was recently, and for the first time hypothesized (Gunter et al., 2016) in a study also using three DNA markers (28S, 16S and COI), the reported clade's statistical support was notably lower (83–85%). Even more significantly, in Gunter et al. (2016) the tribe Stromboscerini is represented by a single terminal of *Dryophthoroides* Roelofs, 1879, thus leaving this tribe's monophyly untested. The herein reported analysis utilizing representatives of five nominal Stromboscerini genera is the first adequately designed and successful attempt to test tribe monophyly. Both "aberrant" genera of Stromboscerini (*Nephius* Pascoe, 1885 and *Stromboscerus* Schoenherr, 1838) are uniquely characterized by having ocular lobes on the anterior edge of the prothorax (Morimoto, 1985: 74) and thought to be unrelated to the rest of the tribe (Grebennikov, 2018), were not included in the present analysis, thus postponing the decisive test of the tribe's monophyly (and of its name, since the latter is the type genus).

Monophyly of *Tasactes*

The second most significant result is that all morphological diagnostic characters of nominal *Tasactes* (Morimoto 1985: 74: elongate and ventrally non-contiguous eyes, 6-segmented antennal funicle, transversely truncated antennal club and conically projecting velvet apex to the club) occur in the specimens forming clade D–K (Fig. 3). Most significantly, all specimens in this clade clearly display both club characters (Figs 4O, P), which among all Stromboscerini are unique to this genus. Remarkably, both club characters are not mentioned by Johannes Faust in the original generic description, but were first reported nearly a century later by Morimoto (1978, 1985). It might also be noted that four antennomers immediately proximal to the club in all specimens herein attributed to *Tasactes* are nearly subquadrate, while in all other herein examined Stromboscerini they are about 1.5 times as wide as long (Fig. 3). This unique morphological match, as well as the relative similarity in habitus (Fig. 10 in Grebennikov, 2018) and geographical distribution (Fig. 5) of the type specimens of both nominal *Tasactes* species and those forming clade D–K (Fig. 2), are the decisive factors supporting the linkage between the genus-group name and the herein so named clade. It should be added that within the herein re-defined genus *Tasactes*, clade D–I might be separated from clade J–K by two diagnostic characters of the former: larger body size (Fig. 3) and evenly bent rostrum (Fig. 4).

Flight ability of Stromboscerini

An inability to fly is a widespread phenomenon in Pterygota and weevils (Arzanov & Grebennikov, 2017). Even though the flight ability of Stromboscerini was not studied and the herein analyzed specimens were not systematically

examined for the presence of full-sized hind wings, members of *Allaeotes* Pascoe, 1885 differ from the rest of the tribe by having non-effaced elytral shoulders (= humeri present, Fig. 3) and being trapped by flight intercept traps (specimens from Vietnam provided by Adam Brunke, not included in the present analysis), both of which indicate that members of this genus can fly. The sister-group relationship between the supposedly volant genus *Allaeotes* (clade A in Fig. 3) and the supposedly flightless rest of the tribe (clade B–N in Fig. 3) indicates that flight capacity in Stromboscerini was irreversibly lost by the most recent common ancestor of clade B–N.

Elusive identity of *Orthosinus* and *Xerodermus*

The recovered Stromboscerini topology (Fig. 2) indicate that the genus *Orthosinus* currently containing seven nominal species from Sri Lanka, India, Myanmar, China, Indonesia and Japan (Grebennikov, 2018) is not monophyletic. Not only is it represented by two clades (clade B–C and clade N in Fig. 3) that are distinctly different in eye shape (Figs 4B, C, N), but there are numerous additional specimens attributable to this genus according to the key to genera (Morimoto, 1985), which were not included in the present analysis. The mystery is compounded by the existence of the nominal genus *Xerodermus* Lacordaire, 1866, with four nominal species in Sri Lanka and India (Grebennikov, 2018). This nominal genus was a synonym of *Orthosinus* when the most recent key to the tribe's genera was published (Morimoto, 1985), but was then resurrected as a valid genus (Alonso-Zarazaga & Lyal, 2002), even though the taxonomic identity of its type species is doubtful (Grebennikov, 2018). Both genera seem to represent the tribe's "garbage bin", accommodating all species not assignable to other easier-to-recognize nominal genera characterized, likely, by apomorphic character states (such as 4- or 5-segmented antennal funicle or by exceedingly narrow vertical eyes). Identifying and determining the phylogenetic limits of these two relatively large nominal genera is, therefore the main challenge to matching the taxonomy and phylogeny of the tribe.

ACKNOWLEDGEMENTS. J. Schmidt (Admannshagen, Germany) collected and made available specimens of *Tasactes* from Nepal (clade E in Fig. 3). A. Brunke (Ottawa, Canada) provided specimens of *Allaeotes* from FIT samples collected by him in Vietnam. M. Alonso-Zarazaga (Madrid, Spain) identified the specimens of *Adapanetus* Guenther, 1936 and *Trochorhopalus* Kirsch, 1877 used in this analysis (Fig. 2). K.-D. Klass and O. Jäger (both Dresden, Germany) facilitated visits to Senckenberg Naturhistorische Sammlungen Dresden to study the type series of both nominal species of *Tasactes*. M. Srour (Dresden, Germany) took images of the antennal clubs of *Tasactes* (Figs 4O, P). A. Slater (Ottawa, Canada) critically read an earlier version of the manuscript prior to its submission, while L. Chamorro (Washington, D.C., USA) reviewed the MS.

REFERENCES

Alonso-Zarazaga M.A. & Lyal C.H.C. 1999: *A World Catalogue of Families and Genera of Curculionoidea (Insecta Coleoptera) (excepting Scolytidae and Platypodidae).* Entomopraxis, Barcelona, 315 pp.

Alonso-Zarazaga M.A. & Lyal C.H.C. 2002: Addenda and corrigenda to '*A World Catalogue of Families and Genera of Curculionoidea (Insecta: Coleoptera)*'. — *Zootaxa* **63**: 1–37.

Arzanov Y.G. & Grebennikov V.V. 2017: Cleonini (Coleoptera: Curculionidae: Lixinae) are monophyletic and flightless: tribe overview, rampant adult homoplasy and illustrated global diversity. — *Zootaxa* **4329**: 1–63.

Faust J. 1894: Viaggio di Leonardo Fea in Birmania e regioni vicine. LX. Curculionidae. — *Ann. Mus. Genova Stor. Nat. Genova* **34**: 153–370.

Felsenstein J. 1985: Phylogenies and the comparative method. — *Am. Nat.* **125**: 1–15.

Grebennikov V.V. 2016: Flightless *Catapionus* (Coleoptera: Curculionidae: Entiminae) in Southwest China survive the Holocene trapped on mountaintops: new species, unknown phylogeny and clogging taxonomy. — *Zootaxa* **4205**: 243–254.

Grebennikov V.V. 2018: Dryophthorinae weevils (Coleoptera: Curculionidae) of the forest floor in Southeast Asia: illustrated overview of nominal Stromboscerini genera. — *Zootaxa* **4418**: 121–135.

Gunter N.L., Oberprieler R.G. & Cameron S.L. 2016: Molecular phylogenetics of Australian weevils (Coleoptera: Curculionoidea): exploring relationships in a hyperdiverse lineage through comparison of independent analyses. — *Austral Entomol.* **55**: 217–233.

Hebert P.D.N., Cywinska A., Ball S.L. & DeWaard J.R. 2003a: Biological identifications through DNA barcodes. — *Proc. R. Soc. Lond. (B)* **270**: 313–321.

Hebert P.D.N., Ratnasingham S. & DeWaard J.R. 2003b: Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. — *Proc. R. Soc. Lond. (B)* **270**: 96–99.

Ivanova N.V., DeWaard J.R. & Hebert P.D.N. 2006: An inexpensive, automation-friendly protocol for recovering high-quality DNA. — *Mol. Ecol. Notes* **6**: 998–1002.

Ivanova N.V., DeWaard J.R., Hajibabaei M. & Hebert P.D.N. 2014: *Protocols for High-volume DNA Barcode Analysis.* URL: https://www.researchgate.net/profile/Paul_Hebert2/publication/238509959_Protocols_for_High-Volume_DNA_Barcode_Analysis/links/53d169190cf220632f393902/Protocols-for-High-Volume-DNA-Barcode-Analysis.pdf?origin=publication_detail

Katoh K. & Toh H. 2008a: Recent developments in the MAFFT multiple sequence alignment program. — *Briefings Bioinform.* **9**: 286–298.

Katoh K. & Toh H. 2008b: Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. — *BMC Bioinform.* **9**: 212, 13 pp.

Katoh K., Misawa K., Kuma K. & Miyata T. 2002: MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. — *Nucl. Acids Res.* **30**: 3059–3066.

Maddison W.P. & Maddison D.R. 2001: *Mesquite: A Modular System for Evolutionary Analysis. Ver. 3.04*. URL: https://www.mesquiteproject.org/

Miller M.A., Pfeiffer W. & Schwartz T. 2010: Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA.* pp. 1–8.

Morimoto K. 1978: Check-list of the family Rhynchophoridae (Coleoptera) of Japan, with descriptions of a new genus and five new species. — *Esakia* **12**: 103–118.

Morimoto K. 1985: Supplement to the check-list of the family Rhynchophoridae (Coleoptera) of Japan, with descriptions of a new genus and four new species. — *Esakia* **23**: 67–76.

Rambaut A. 2014: *FigTree, Ver. 1.4*. URL: http://tree.bio.ed.ac.uk/software/figtree/ (last accessed 12 Mar. 2014).

Ratnasingham S. & Hebert P.D.N. 2007: BOLD: the barcode of life data system. — *Mol. Ecol. Notes* **7**: 355–364.

Riedel A. 2017: The weevil genera *Nyphaeba* Pascoe and *Pantiala* Pascoe and the problems of an unstable nomenclature in orphaned taxa. — *Zootaxa* **4244**: 377–389.

Shorthouse D.P. 2010: *SimpleMappr, an Online Tool to Produce Publication-Quality Point Maps*. URL: http://www.simplemappr.net (last accessed 12 Mar. 2014).

Stamatakis A., Hoover P. & Rougemont J. 2008: A rapid bootstrap algorithm for the RAxML web servers. — *Syst. Biol.* **57**: 758–771.

Received March 12, 2018; revised and accepted July 19, 2018
Published online August 24, 2018

Supplementary file: http://www.eje.cz/2018/044/S01.pdf.

Table S1. List of the localities sampled.

Table S2. List of the primers used.

Fig. S1. Images of 60 DNA barcoded Dryophthorinae.

Fig. S2. Neighbour joining clustering of 60 DNA barcoded Dryophthorinae performed using the online BOLD tree-building engine and Kimura 2 parameter.