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RESEARCH PAPER

Yagder serratus, a new eyeless weevil from Mexico and the non-monophyly of Brachycerinae, the evolutionary twilight zone of true weevils (Coleoptera: Curculionidae)

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Abstract. We describe and illustrate a new eyeless weevil, *Yagder serratus* gen. & sp. nov., based on a single adult female collected by sifting forest leaf litter in Mexico. A phylogenetic analysis of 39 terminals and 2679 aligned positions from three DNA fragments places the new species into the subfamily Brachycerinae (as *incertae sedis*) and outside the highly diversified clade of 'higher' true weevils. Neither Brachycerinae, nor its tribe Raymondionymini traditionally uniting most eyeless weevils, are monophyletic unless the latter is limited to a Mediterranean core group. Both these taxa are taxonomic dumping-grounds likely containing species-poor sisters of species-rich clades. When resolved, the subfamily Brachycerinae will be likely split into two or more species-poor deeply-divergent subfamilies.

Key words. Coleoptera, DNA barcode, ITS2, 28S, phylogeny, forest litter, species discovery

Zoobank: http://zoobank.org/urn:lsid:zoobank.org:pub:B7C0C54B-7605-48C7-A568-7DD2FAB45D8A

Introduction

Large portions of the Tree of Life remain phylogenetically uncharted. This is particularly true for yet undescribed small-bodied dwellers of cryptic habitats in remote and/or biodiverse areas of the Globe. Discovery of such organisms is often akin to solving an equation with two unknowns: placing the new puzzling terminal into its unexplored phylogenetic neighbourhood. Inadequately known, likely deeply-divergent and species-poor clades are often artificially united into non-monophyletic 'basal' taxa. These assemblages of unrelated and unresolved clades are often referred to as 'twilight zones' (Lopardo & Hormiga 2015, Bai et al. 2016) or 'dumping grounds'. Such 'twilight zones' unite organisms lacking synapomorphies of the well-established and species-rich clades, thus simultaneously likely non-monophyletic. When relationships among constituents are finally resolved, new higher taxa are likely to emerge from these taxonomic dumping grounds. Prominent arthropod examples include pteromalid chalcid wasps (Heraty et al. 2013) and Endeostigmata mites (Klimov et al. 2018).

Our paper is pivoted on a discovery of a new weevil from Mexico (Figs 1–2) and its phylogenetic placement. Weevils (Curculionidae) are the globally distributed clade sister to the much smaller family Brentidae (Marvaldi et al. 2002, Haran et al. 2013, Gillett et al. 2014, Gunter et al. 2016, Shin et al. 2017). With >50,000 named species, Curculionidae is the second largest animal family, second only to rove-beetles (Staphylinidae). The basal-most dichotomies within the weevils are notoriously unresolved. This ambiguity is reflected in the continuous existence of the non-monophyletic subfamily Brachycerinae, which forms the twilight zone of true weevils. This artificial subfamily contains organisms most similar, or perhaps most closely related, to our new eyeless Mexican weevil, and therefore is highlighted, alas unresolved, in the present paper.

The hinge of our study is a single adult weevil (Figs 1–2) sampled in 2019 by sifting forest litter on a mountain ridge at 1370 m, some 5 km SW of the town of Hueytamalco, Puebla, SE Mexico (Fig. 3). The external morphology of this beetle and its DNA barcode (Hebert et al. 2003) were notably unlike most of what we have

seen in weevils globally. Specifically, the specimen was 3.8 mm in body length (without rostrum), slender and parallel-sided, with a straight and long rostrum, with head deeply retracted into pronotum, with deeply punctate and rugged dorsal surface of pronotum and elytra, and with sharp and large serrations along the elytral contour in dorsal view (Fig. 1). Most notably, the specimen was completely eyeless (Fig. 2D). Its overall appearance did not recall known eyeless species scattered among 'higher' weevils from Central America or from elsewhere (Osella 1979, Howden 1992, Morrone & Hlaváč 2018). The specimen might perhaps be best compared with some eyeless Brachycerinae, although it is at least twice greater in body length than their majority (Fig. 4). After preliminary morphological evaluation we concluded that this Mexican weevil is unlikely to belong to any subfamily of the higher Curculionidae, but likely to Brachycerinae.

This preliminary taxonomic solution was, however, unsatisfactory. Brachycerinae are notoriously non-monophyletic. As presently delimited (Oberprieler 2014), the subfamily is a classical wastebasket taxon accommodating all true weevils (= members of monophyletic Curculionidae) outside of the monophyletic (Davis 2017, Shin et al. 2017) and species-rich core of the family. This latter consists of two sister clades (Shin et al. 2017): one is formed by Dryophthorinae + Platypodinae (with or without monophyletic Bagoinae) and another a much greater monophylum of subfamilies, itself consisting of two sister clades: the CEGH clade and the CCCMS clade. Both CEGH and CCCMS clades (first defined by Gunter et al. 2016) are collectively referred to as 'higher' Curculionidae (Shin et al. 2017) and accommodate about four fifths of extant weevils. The CEGH clade consists of Cyclominae, Entiminae, Gonipterini, and Hyperini, while the larger CCCMS clade is formed by Curculioninae, Conoderinae, Cossoninae, Molytinae and Scolytinae (with at least some of these subfamilies non-monophyletic). This phylogenetic pattern consistently re-emerges in independent analyses (Haran et al. 2013, Gillett et al. 2014). All weevils not fitting into these major clades are taxonomically assigned to the family's twilight zone, the non-monophyletic subfamily Brachycerinae.

The exact number of unrelated clades of weevils currently assigned to Brachycerinae is impossible to estimate, with three (Shin et al. 2017) being likely the lowest number. Circumstantial evidence (Oberprieler 2014) suggests that this number might be higher, as more members of this subfamily gradually become available for phylogenetic analyses. Significantly for our purpose, Brachycerinae include a number of eyeless in deep soil living taxa, whose monophyly and sister-group relationships are far from certain. At this stage it became obvious to us that to place our eyeless Mexican weevil into the phylogenetic context, we must focus on Brachycerinae, particularly its eyeless members.

The concept of the subfamily Brachycerinae has varied widely and became stabilized (alas unavoidably temporarily) only recently (Oberprieler 2014). This subfamily formally consists of about 95 genera grouped in seven tribes (Oberprieler 2014): Brachycerini, Cryptolaryngini, Erirhinini (including the genus *Ocladius* Schönherr, 1825 and its relatives often treated separately from the rest of Erirhinini), Himasthlophallini, Tanysphyrini, Myrtonymini and Raymondionymini. The total number of Brachycerinae species is about 1,200 (Oberprieler et al. 2007) or 1,350 (Oberprieler 2014), which is merely 2–3% of the hyper-diverse Curculionidae. While most Brachycerinae are fully eyed and often volant, some are exclusively subterranean, eyeless, wingless, and are found in widely separated parts of the globe (Fig. 3). When eyeless, Brachycerinae are predominantly very small and slender beetles, averaging about 1–3 mm in body length (excluding rostrum and head capsule; as with our species, the latter is often deeply retracted into the pronotum and is not apparent when viewed from above; Fig. 4). Four of the seven tribes of Brachycerinae contain eyeless species, and three of them exclusively so. The tribe Erirhinini contains one such genus, *Absoloniella* Formánek, 1913 (Fig. 4), with five exceptionally poorly known Mediterranean species (Caldara & Colla 2018). The tribe Himasthlophallini consists of microphthalmic *Himasthlophallus flagellifer* Egorov & Zherikhin, 1991 (Fig. 4) from the Russian Far East (Fig. 3) with an eye formed of three ommatidia (Fig. 5F). The tribe Myrtonymini consists of two genera: *Myrtonymus* Kuschel, 1990 (Fig. 4) with six species in New Zealand, Australia and New Caledonia, as well as the monotypic *Hexonymus* Kuschel, 2014 from Australia (Fig. 3). Myrtonymini are the smallest weevils known, all seven species less than 1 mm in body length (excluding rostrum, Kuschel 2014). The genus *Absoloniella* and both aforementioned tribes are species-poor, geographically restricted and perhaps monophyletic. The last, the exclusively subterranean tribe of Brachycerinae, Raymondionymini, is, however, much larger in the number of species, widely distributed and questionably monophyletic.

Fifteen genera and 90 species were listed in Raymondionymini by Morrone & Hlaváč (2018). Most of their diversity centers on the circum-Mediterranean area (Fig. 3). In the area delimited by Belgium, the Caspian Sea, Algeria and Portugal the following nine endemic genera (60% of the tribe) and 74 species (82 %) are naturally distributed: *Alaocephala* Ganglbauer, 1906 (1 sp.), *Alaocyba* Perris, 1869 (10 spp., Fig. 4), *Coiffaitiella* Osella, 1977 (6 spp.), *Derosasius* Ganglbauer, 1906 (1 sp.), *Ferreria* Alonso-Zarazaga & Lyal, 1999 (2 spp.), *Raymondiellus* Ganglbauer, 1906 (15 spp., Fig. 4), *Raymondionymus* Wollaston, 1873 (27 spp.), *Tarattostichus* Ganglbauer, 1906 (2 spp.) and *Ubychia* Rost, 1893 (10 spp.). Mediterranean Raymondionymini include the type genus (*Ferreria*, replacement name for *Raymondionymus* Ganglbauer, 1906), have at least one potential morphological synapomorphy (6-segmented antennal funicle, Fig. 5A; perhaps also the peculiarly shaped tibiae, Fig. 5C) and likely form the monophyletic core of the tribe (Osella 1977, Grebennikov 2010). Monophyly of Raymondionymini as a whole is, however, threatened by inclusion of likely only distantly related non-Mediterranean members.

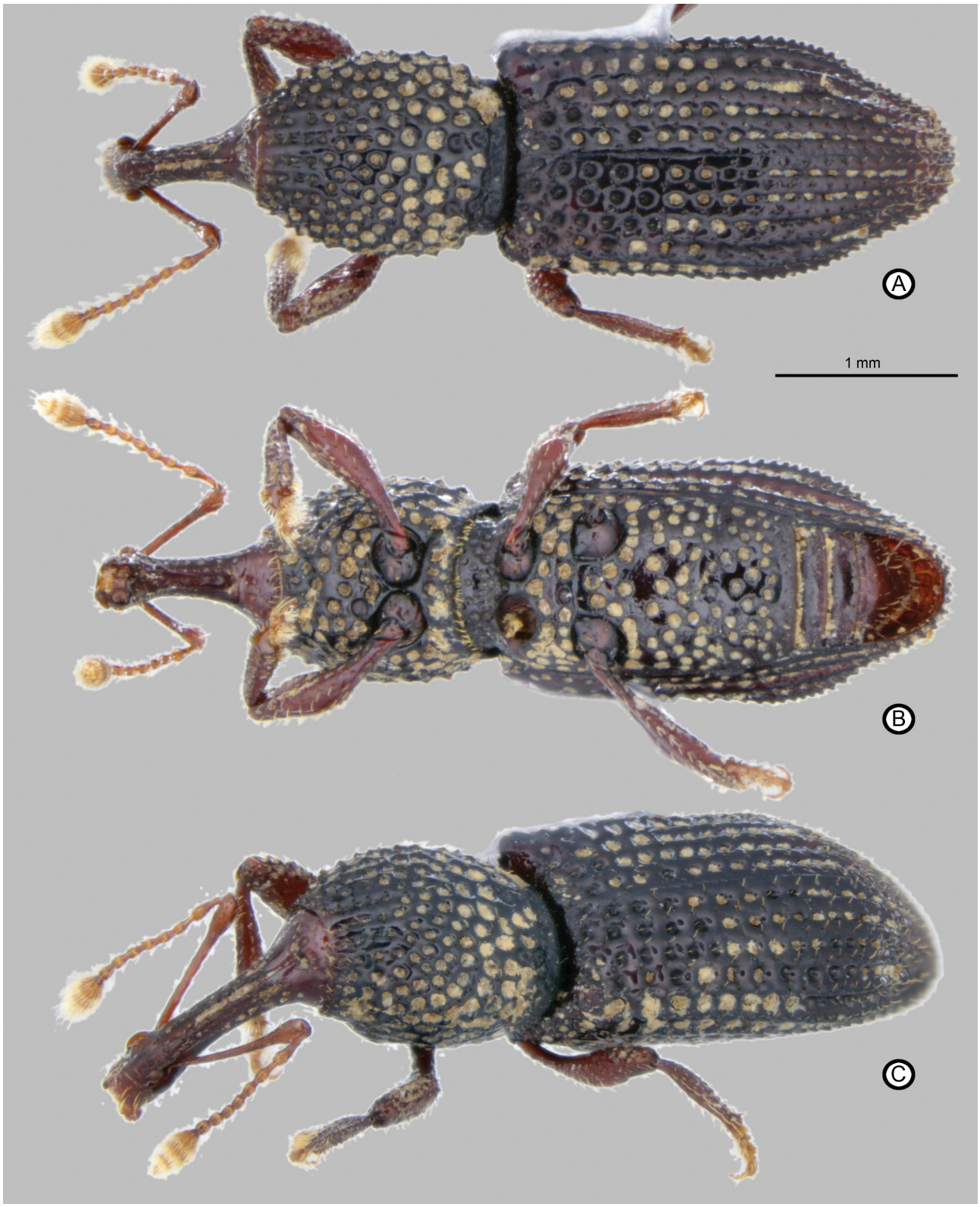

Fig. 1. *Yagder serratus* gen. & sp. nov., habitus, dorsal (A), ventral (B) and left fronto-lateral (C).

The non-Mediterranean remainder of Raymondionymini has a spotty global distribution. The state of California in the USA harbours three genera of Raymondionymini, two of them endemic to the state: *Gilbertiola* Osella, 1982 (2 spp.) and *Schizomicrus* Casey, 1905 (1 sp., Fig. 4). The third Californian genus, *Alaocybites* Gilbert, 1956 (with doubtful affinities to the tribe, Grebennikov 2010), has two species endemic to California, two others endemic to the Russian Far East (Fig. 4), plus an unnamed late Pliocene fossil from Alaska. Remaining Raymondionymini are known only from their type series. Mexico has the monotypic *Neoubychia* Gilbert & Howden, 1987 and

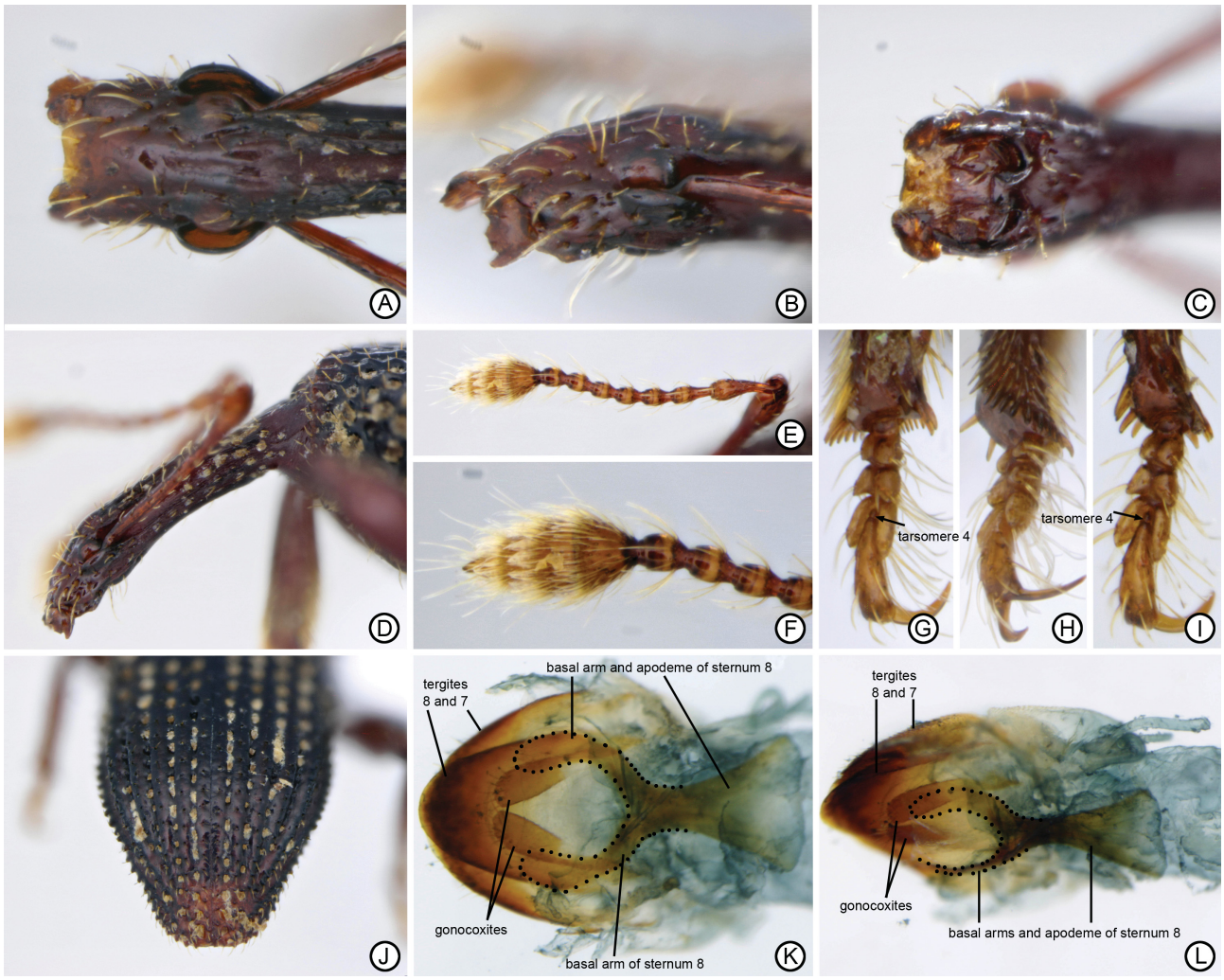

Fig. 2. *Yagder serratus* gen. & sp. nov., details. A–C – rostral apex, dorsal (A), left lateral (B), ventral (C); D – head, left lateral; E–F – left antenna, lateral (E), distal part magnified (F); G–I – tarsi, left front leg (G–H), left middle leg (I); J – elytral apex; K–L – dissected female abdominal apex, ventral (K) and right latero-ventral (L).

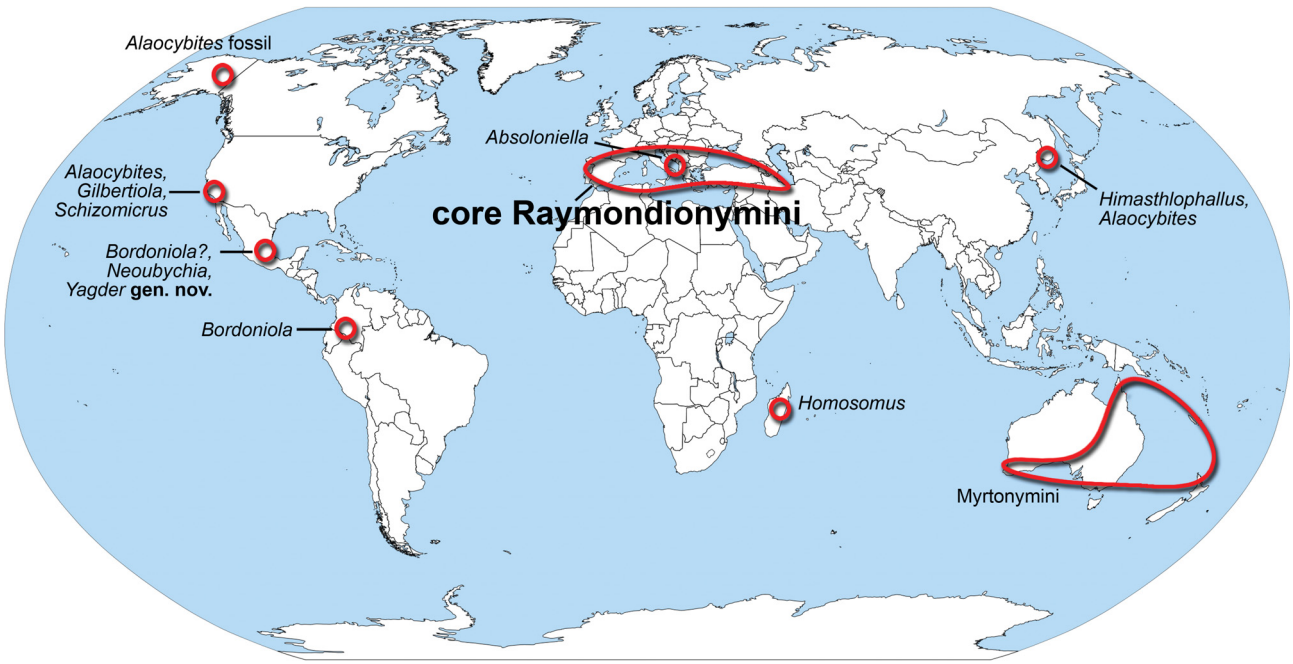

Fig. 3. Distribution of eyeless and nearly eyeless brachycerine weevils.

Ecuador and Venezuela support seven species of *Bordoniola* Osella, 1987 (Baviera et al. 2012). Madagascar has three species of *Homosomus* Richard, 1956 (Figs 4, 5B). Each of these small non-Mediterranean genera appear monophyletic. Most intriguingly, besides their overall body similarity likely brought about by their shared subterranean habits, no convincing evidence suggests that they are most closely related to each other and to the

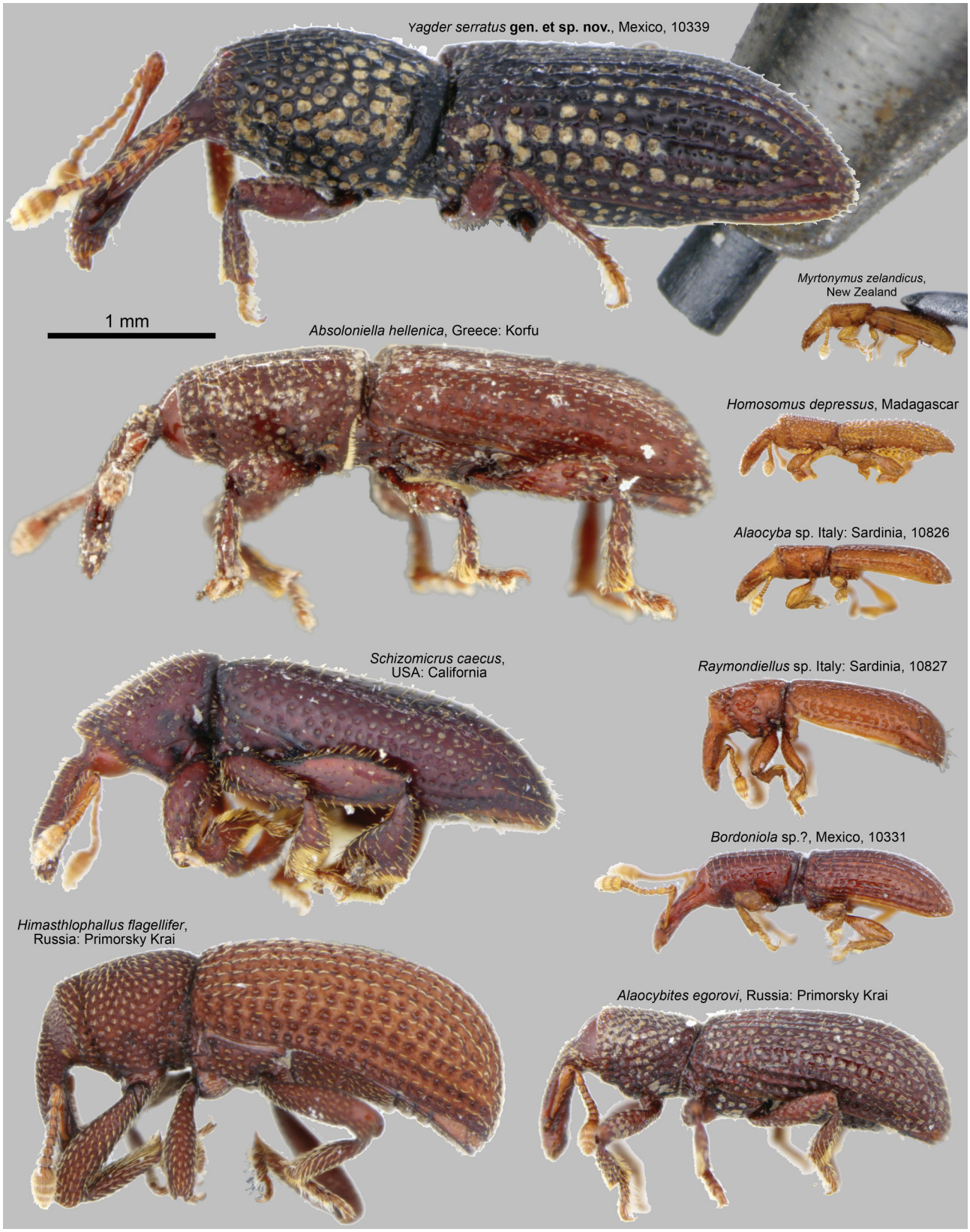

Fig. 4. Eyeless and nearly eyeless brachycerine weevils, habitus. A tip of a regular mechanical pencil with a 0.5 mm lead is added for size comparison. All images are to scale.

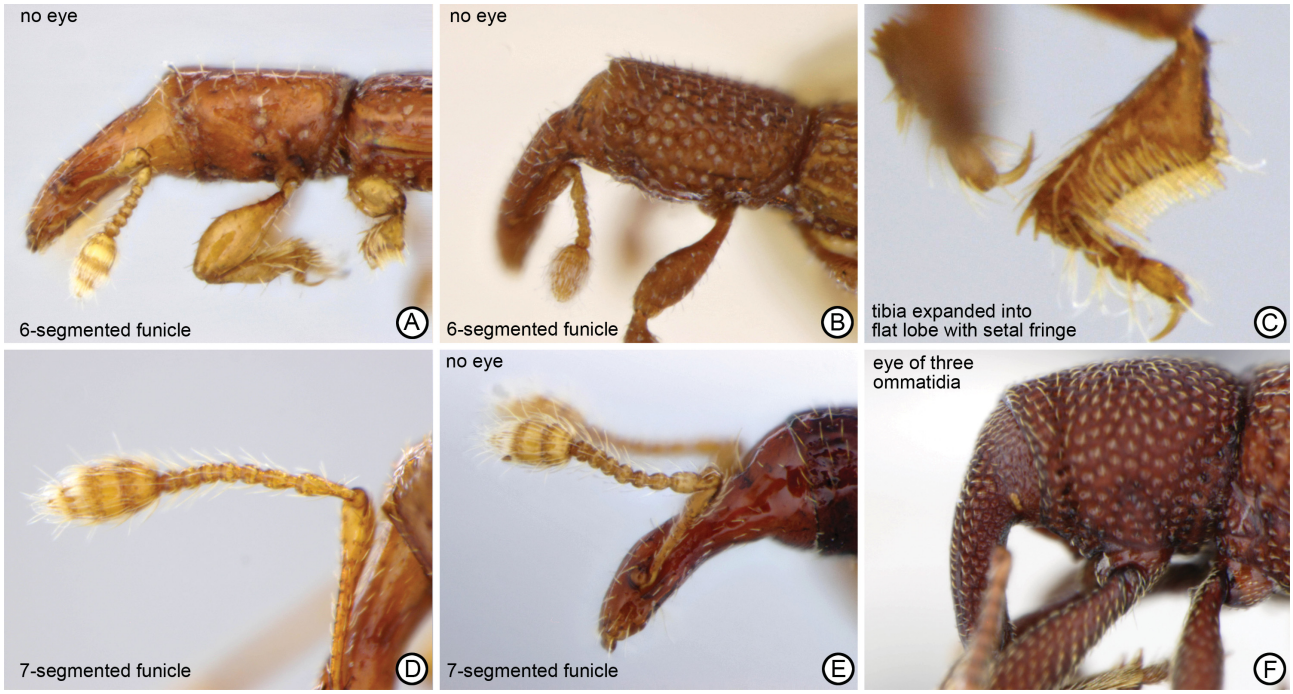

Fig. 5. Eyeless and nearly eyeless brachycerine weevils, details. A – *Alaocyba* sp. (10826), anterior body, left lateral; B – *Homosomus depressus* Richard, 1979, anterior body, left lateral; C – *Raymondiellus* sp. (10827), left middle leg, anterior; D – *Bordoniola* sp. (10330), left antenna, lateral; E – *Bordoniola* sp. (10331), head, left lateral; F – *Himasthlophallus flagellifer* Egorov & Zherikhin, 1991, anterior body, left lateral.

Mediterranean core of the tribe. These extremely small and cryptic organisms are rarely sampled and, therefore, are acutely understudied.

Rarity of DNA-grade specimens of these eyeless subterranean Brachycerinae conspicuously correlates with their absence in the majority of the recent weevil-wide phylogenetic analyses. Available phylogenetic evidence is, therefore, scarce and inconclusive. Among all of Brachycerinae, only Raymondionymini were a focus of an inconclusive morphology-based phylogenetic analysis (Grebennikov 2010). If the tribe is monophyletic, its sister (with or without Myrtonymini and/or *Alaocybites*) is equally obscure, and so are those of other eyeless Brachycerinae. The only two marginally relevant DNA-based attempts were all-weevil analyses recovering the morphologically aberrant *Schizomicrus* from California (which is, however, "...not possessing proper pedotectal genitalia..."; Gunter et al. 2016) either as sister to *Brachycerus* Olivier, 1789 (McKenna et al. 2009) or to *Ocladius* (Shin et al. 2017). Jordal et al. (2011) included *Himasthlophallus* in an outgroup when addressing wood boring weevils and the origin of subsociality. Although phylogenetically inconclusive with respect to eyeless Brachycerinae, these studies generated relevant genetic data on these rarely seen organisms (Table 1).

Having faced all these uncertainties, we designed two main objectives for our study. Firstly, we describe and introduce to science a new taxon for the blind Mexican weevil. Secondly, we document our attempts at placing this organism into the weevil phylogenetic framework, particularly with respect to the non-monophyletic Brachycerinae. In doing so, we find ourselves severely limited by the lack of any pre-existing phylogenetic hypothesis and even more so by the shortage of relevant DNA data, particularly on the difficult-to-find eyeless Brachycerinae. After having assessed our analytical limitations, we put to a formal test (Popper 1959) the following hypotheses:

1. The new eyeless Mexican weevil specimen taxonomically belongs to the non-monophyletic Brachycerinae (does not belong to any of the well-established clades of true weevils, particularly those known to contain eyeless species, such as Dryophthorinae, the CEGH clade and the CCCMS clade).
2. Available data are sufficient to place our new eyeless Mexican weevil in a well-supported sister relationship.
3. The Mediterranean core of the tribe Raymondionymini is monophyletic.
4. If so, then at least one non-Mediterranean member of Raymondionymini is/are sister to its Mediterranean core.
5. Eyeless Brachycerinae form a clade (which would imply a single and non-reversed eye reduction and disappearance).
6. None of the weevils currently assigned to Brachycerinae is nested within the main and well-supported weevil radiation delimited by as the least inclusive clade uniting any member of Dryophthorinae and any member of 'higher' Curculionidae (Shin et al. 2017).

Material and methods

Composition of the in- and outgroups. The ingroup of the herein implemented analysis was formed by the new Mexican weevil, plus 12 terminals of other Brachycerinae (Table 1, Fig. 6). Two tribes of eyeless (or nearly eyeless)

Table 1. DNA fragments and their GenBank accession numbers of 41 specimens used for an analysis assess phylogenetic placement of eyelesss *Yagder serratus* gen. & sp. nov. in true weevils (Curculionidae), specifically among non-monophyletic Brachycerinae. GenBank accession numbers in **bold** are those newly sequenced for this analysis; those in regular font are those from our previous studies; those in *italics* are GenBank sequences of other authors. Two chimera terminals were formed by sequences of different and conspecific specimens (*Tanysphyrus lemnae* (Paykull, 1792)) or different and congeneric species (*Lissorhoptrus* LeConte, 1876), thus the total terminal number is 39.

Subfamily	Code	Genus	Species	Voucher	Country	COI	ITS2	28S
Brentinae	Brent	*Cylas*	*formicarius*	BTOLDDM0533	?	*FJ867849.1*	none	*FJ867676.1*
Brachycerinae		*Yagder*	*serratus*	CNCCOLVG00010339	Mexico	**MW201355**	**MW201457**	**MW201468**
Brachycerinae	Brach_Raym1	*Schizomicrus*	*caecus*	BTOLDDM0509	USA	*FJ867824.1*	none	*FJ867709.1*
Brachycerinae	Brach_Raym2	*Bordoniola*		CNCCOLVG00010330	Mexico	**MW201357**	**MW201458**	**MW201470**
Brachycerinae	Brach_Raym3	*Bordoniola*		CNCCOLVG00010331	Mexico	**MW201361**	**MW201461**	**MW201474**
Brachycerinae	Brach_Raym4	*Bordoniola*		CNCCOLVG00010332	Mexico	**MW201360**	**MW201460**	**MW201473**
Brachycerinae	Brach_Raym5	*Alaocyba*		CNCCOLVG00010826	Italy	**MW201354**	**MW201455**	**MW201466**
Brachycerinae	Brach_Raym6	*Raymondiellus*		CNCCOLVG00010827	Italy	**MW201353**	**MW201454**	**MW201465**
Brachycerinae	Brach1	*Himasthlophallus*	*flagellifer*	ErHim01	Russia	*HQ883654.1*	none	*HQ883569.1*
Brachycerinae	Brach2	*Ocladius*		BTOLDDM0537	?	*FJ867815.1*	none	*FJ867696.1*
Brachycerinae	Brach3	*Lissorhoptrus*		BTOLDDM0503	?	none	none	*FJ867689.1*
Brachycerinae	Brach4	*Lissorhoptrus*	*kuscheli*	USNM:ENT:01453185	?	*MN344695.1*	none	none
Brachycerinae	Brach5	*Tanysphyrus*	*lemnae*	BTOLDDM0507	?	none	none	*FJ867720.1*
Brachycerinae	Brach6	*Tanysphyrus*	*lemnae*	GBOL_Col_FK_6085	?	*KM443047.1*	none	none
Brachycerinae	Brach7	*Notaris*	*scirpi*	CNCCOLVG00008489	Poland	KR736279	**MW201453**	**MW201464**
Brachycerinae	Brach8	*Tournotaris*	*bimaculata*	CNCCOLVG00008578	Poland	KR736283	**MW201456**	**MW201467**
Dryophthorinae	Dryop1	*Sphenophorus*	*parumpunctatus*	CNCCOLVG00000434	Morocco	HM417724	KY110320	KY110384
Dryophthorinae	Dryop2	*Sitophilus*	*zeamais*	CNCCOLVG00002735	China	KJ672255	MG968837	MG968894
Dryophthorinae	Dryop3	*Dryophthorus*	n/a	CNCCOLVG00003561	Tanzania	MG968913	MG968814	MG968871
Dryophthorinae	Dryop4	*Nephius*	*argus*	CNCCOLVG00004402	Vietnam	MH034380	MH034354	MH034411
Dryophthorinae	Dryop5	*Allaeotes*	*niger*	CNCCOLVG00009972	Cuba	MN621866	MN621859	MN621862
Entiminae	CEGH1	*Prothrombosternus*	*tarsalis*	CNCCOLVG00003280	Tanzania	KU748541	KY110337	KY110402
Entiminae	CEGH2	*Catapionus*	*fossulatus*	CNCCOLVG00007318	Russia	KU748528	KY110302	KY110364
Entiminae	CEGH3	*Graptus*	*triguttatus*	CNCCOLVG00008909	Czech Rep.	KY110616	KY110330	KY110395
Entiminae	CEGH4	*Nastus*		CNCCOLVG00009056	Kazakhstan	KY110618	KY110334	KY110399
Hyperinae	CEGH5	*Hypera*		CNCCOLVG00009750	Kazakhstan	**MW201362**	**MW201462**	**MW201475**
Entiminae	CEGH6	*Sitona*		CNCCOLVG00010325	Canada	**MW201359**	**MW201459**	**MW201472**
Cossoninae	CCCMS1	*Himatium*		CNCCOLVG00001678	Tanzania	JN265954	KY110323	KY110388
Molytinae	CCCMS2	*Aater*	*cangshanensis*	CNCCOLVG00002676	China	MG648761	MG648835	MG648747
Molytinae	CCCMS3	*Niphadomimus*	*maia*	CNCCOLVG00002731	China	KJ427744	KY110324	KY110389
Cossoninae	CCCMS4	*Carphonotus*	*testaceus*	CNCCOLVG00002970	Canada	KY110606	KY110309	KY110371
Molytinae	CCCMS5	*Devernodes*	*chthonia*	CNCCOLVG00004339	China	MH034400	MH034364	MH034421
Molytinae	CCCMS6	*Adexius*	*scrobipennis*	CNCCOLVG00005848	Poland	KJ445686	KY110305	KY110367
Molytinae	CCCMS7	*Euthycus*		CNCCOLVG00006683	Taiwan	KJ445702	KY110325	KY110390
Molytinae	CCCMS8	*Typoderus*	*antennarious*	CNCCOLVG00007166	Tanzania	KY250487	KY250484	KY250479
Molytinae	CCCMS9	*Cryptorhynchus*	*lapathi*	CNCCOLVG00007530	Russia	KY110605	KY110303	KY110365
Molytinae	CCCMS10	*Niphades*	*verrucosus*	CNCCOLVG00007531	Russia	KY110610	KY110314	KY110376
Molytinae	CCCMS11	*Lepyrus*	*palustris*	CNCCOLVG00008474	Poland	KX360483	KY110332	KY110397
Molytinae	CCCMS12	*Acicnemis*	*albofasciata*	CNCCOLVG00008936	Russia	KY110609	KY110312	KY110374
Molytinae	CCCMS13	*Paranchonus*	*verrucosus*	CNCCOLVG00009809	Guadeloupe	**MW201356**	none	**MW201469**
Molytinae	CCCMS14	*Dufauiella*	*heterorostris*	CNCCOLVG00009812	Guadeloupe	**MW201358**	none	MW201471

Table 2. DNA fragments used in phylogenetic analysis (total number of sequenced terminals, followed by minimal, maximal and aligned length of each fragment, and the first and the last position of each aligned fragment in the concatenated matrix).

Gene	#	min	max	aligned	positions
COI-5P	39	453	658	658	1 to 658
ITS2	31	363	636	1346	659 to 2004
28S	39	436	584	675	2005 to 2679

Brachycerinae were represented. The tribe Raymondionymini was represented by two European genera (*Alaocyba* and *Raymondiellus*), by Californian *Schizomicrus*, and by three terminals from Mexico (specimens 10330–2, Figs 4, 5D, E) tentatively assigned to the genus *Bordoniola*. The tribe Himasthlophallini was represented by its single species from the Russian Far East. Eyeless Mytronymini and Erirhinini (*Absoloniella*) were not represented due to the lack of DNA data. Eyed Brachycerinae were represented by five terminals; two of them were 'chimeras' (Table 1) composed from different DNA fragments of two closely related organisms (either conspecific, or congeneric). The outgroup was composed of 25 terminals (Table 1, Fig. 6) representing three remaining non-Brachycerinae clades of weevils: Dryophthorinae (5 terminals), the CEGH clade (6 terminals) and the CCCMS clade (14 terminals). To root the topology, we added a single representative of Brentidae, the sister family of Curculionidae.

DNA sequencing. To construct the matrix, the following three DNA fragments were used (Table 2, fragment

abbreviations are in brackets): mitochondrial cytochrome c oxidase I (COI); nuclear internal transcribed spacer 2 (ITS2) and nuclear 28S ribosomal DNA (28S). Three different sources of DNA data were used (Table 1): (I.) 32 newly sequenced fragments with their GenBank accession numbers MW201353–62 and MW201453–73, (II.) our previously released DNA data and (III.) 12 sequences generated by other authors and deposited in GenBank. The latter are those of McKenna et al. (2009), Hendrich et al. (2015) and Jordal et al. (2011). We sequenced DNA at the Canadian Centre for DNA Barcode using their standard protocols (CCDB, http://ccdb.ca/), while our primers are listed in Table 2 in Grebennikov (2017). All details pertaining to our lab work (such as DNA extraction, amplification, PCR protocols), as well as images of the original electropherograms, habitus images and locality data for all 32 specimens sequenced by us (Table 1, voucher codes starting with CNCCOLVG000) are available online in the Barcode of Life Database (BOLD, Ratnasingham & Herbert 2007) public dataset at dx.doi.org/10.5883/DS-VGDS16.

Sequence alignment and phylogenetic analysis. Alignment of all three DNA fragments was done separately using the online MAFFT Q-INS-i algorithm (Katoh et al. 2017; https://mafft.cbrc.jp/alignment/server/). We trimmed the extending 3′-end of one COI and of six 28S fragments sequenced by others (Table 1). No internal parts of DNA fragments were removed prior to the analysis, even if parts of the alignments consisted mainly of insertions/deletions (indels). Three aligned single-fragment datasets were concatenated using Mesquite 3.61 (Maddison & Maddison 2020) into a matrix of 2679 positions and containing 40 % of the completely undetermined characters (mainly due to ITS2 sequences absent for eights terminals and numerous indels in the remaining ITS2 sequences, Table 2). An unrooted topology was built using a Maximum Likelihood (ML) approach, as implemented in CIPRES Science Gateway online platform (Miller et al. 2010; http://www.phylo.org/, tool 'RAxML-HPC2 on XSEDE') and using RAxML 8 (Stamatakis 2014) algorithm. We applied CAT approximation to the widely used GTR + G nucleotide substitution model independently for each of three partitions. Support values were obtained based on 1000 bootstrap replicates (Stamatakis et al. 2008). The tree was visualized in FigTree v1.4.4. (Rambaut 2020).

Morphological methods. The single specimen of the new species (Figs 1–2) was first imaged *in toto* and then softened in warm water for dissection. Its abdomen was macerated in a warm water solution of potassium hydroxide and disarticulated to extract and illustrate internal structures (Figs 2K, L). Chlorazol Black was used to stain internal membranes in light blue (Figs 2K, L). Adult weevil morphological terms are those of Lyal (2020).

Results

The Maximum Likelihood analysis resulted in a moderately resolved tree (Fig. 6). The new eyeless Mexican weevil (marked with red arrow in Fig. 6) lacked a well-supported sister-group and was recovered outside the Dryophthorinae, CEGH or CCCMS clades. The clade of Dryophthorinae was taxonomically coherent and moderately supported (bootstrap 86%). The molytine genus *Devernodes* Grebennikov, 2018, taxonomically a member of the CCCMS clade, formed, however a weakly supported clade with two Brachycerinae genera; all three of them were in a weakly supported relationship with a weakly supported clade formed by the rest of the CCCMS clade and the CEGH clade. The CEGH clade and the CCCMS clade (without *Devernodes*) were both strongly supported (92% and 91%, respectively). Brachycerinae were non-monophyletic, forming the family's 'twilight zone'. Both European Raymondionymini formed a strongly supported clade (100%) distantly related to the two other tribe's members, the genera *Schizomicrus* and *Bordoniola* (Fig. 6); the latter two not forming a clade.

Yagder gen. nov.

Type species. *Yagder serratus* sp. nov., here designated.

Diagnosis. This genus can be recognized among all weevils (Curculionoidea, including true weevils Curculionidae) by the combination of the following characters: eye completely absent; body size larger, 3.8 mm in length (excluding rostrum and deeply inserted head), slender (ratio of length to maximal width 2.9) and parallel-sided in dorsal view; rostrum almost as long as pronotum, with deep median longitudinal furrow throughout greater portion of length both dorsally and ventrally; pronotum and elytra with large, deep round punctures, those on anterior portion of pronotum smaller, somewhat coalescent, forming striae; elytra with eight complete striae, five visible in dorsal view, strial punctures on elytra large basally, smaller towards elytral apex; tibial apices with short, stout spines, spines greatest in number and most closely spaced on front tibia; elytra with humeral angle sharp, produced anteriorly; female genitalia with gonocoxites flat, spade-like, lacking stylus, sternum 8 Y-shaped, with arms arcuate, apodeme short, broad, apically expanded.

Description. ***Body*** 3.8 mm in length (excluding rostrum and deeply inserted head), slender (ratio of length to maximal width 2.9) and parallel-sided in dorsal view; prothorax, elytra, meso- and metaventrites and two visible basal abdominal ventrites with deep round punctures, many of them accumulating fine soil particles; body and all appendages uniformly dark-brown; without dense pilosity; with short and sparse erect setae. ***Head capsule*** deeply retracted into pronotum, almost invisible in dorsal view; eye completely absent. ***Rostrum*** in lateral view about 0.9 times as long as pronotum in dorsal view; narrowest at mid-length; with deep longitudinal furrow along mid-line dorsally and ventrally; with antennae attached in apical third; with pterygia at points of antennal insertions exposed in dorsal view, scrobes shallow, not visible in dorsal view, directed posteriorly to middle of head. ***Antennae*** geniculate, with funicle and club consisting of seven and three antennomeres, respectively; scape about subequal in length to funicle. ***Prothorax*** without postocular lobes; procoxae subcontiguous; hypomeral lobes about 60% as long as

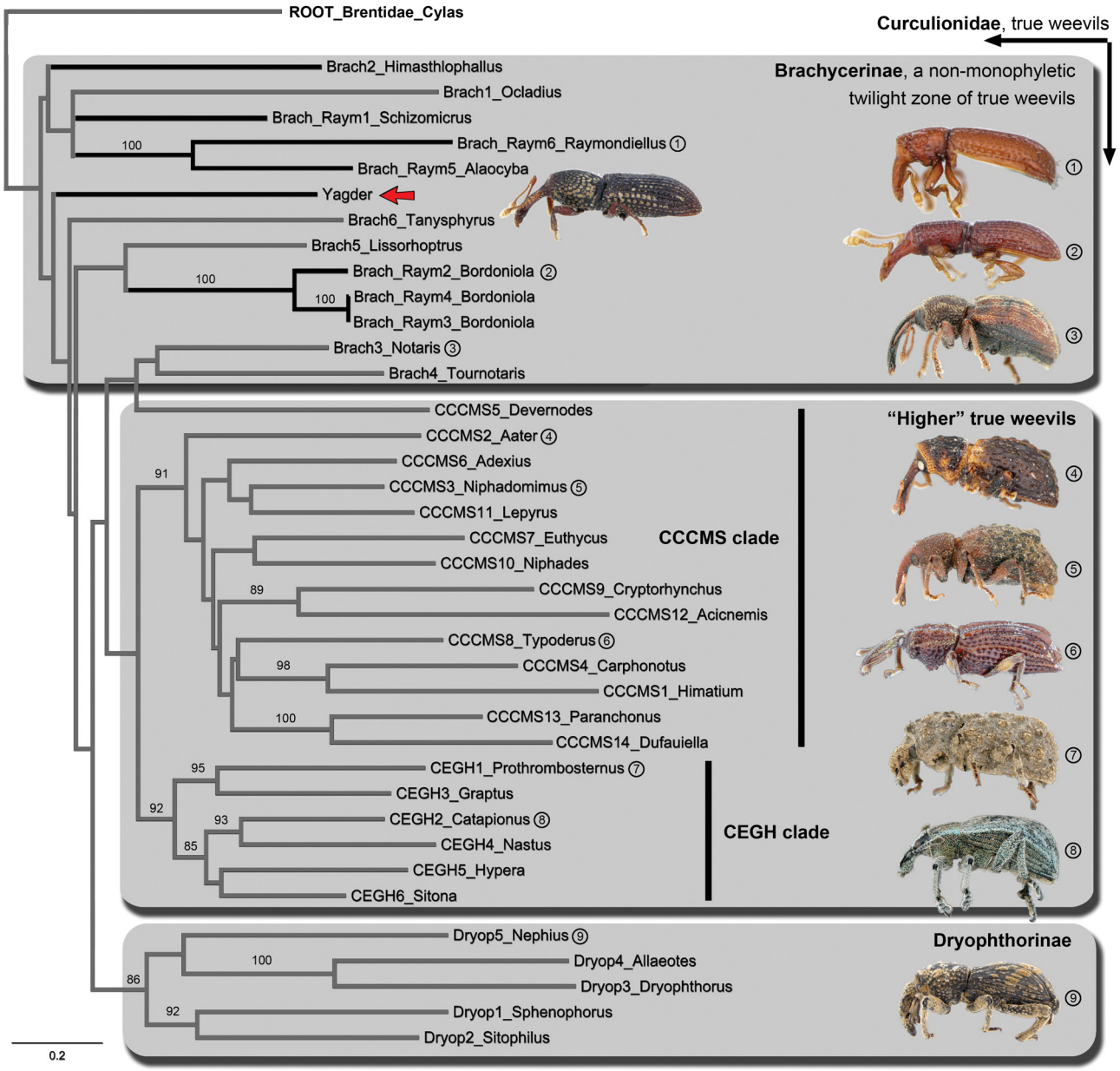

Fig. 6. Maximum Likelihood inference phylogram positioning *Yagder serratus* gen. & sp. within non-monophyletic Brachycerinae. Note non-monophyly of Raymondionymini. Black lines denote eyeless or nearly eyeless terminals and clades. Digits at internodes are bootstrap values >85%.

procoxal cavities; pronotum evenly rounded in dorsal view, without delimited disk, in anterior third with 5–6 fine striae each accommodating 3–5 smaller punctures arranged in longitudinally oriented lines, in posterior half with much larger punctures forming an irregular cross-pattern. ***Meso- and metathorax*** with minute scutellum visible externally; hind wings (if present) not examined; mesocoxae separated by about one-third of their individual diameter; metacoxae separated by about their individual diameter. ***Elytra*** with humeral angles sharp, slightly produced anteriorly; with eight complete striae, five visible in dorsal view, strial punctures very large in basal one-half of elytral length, decreasing in size in apical portion of length, those on striae 8 very small, shallow throughout length. ***Abdomen*** with ventrites I and II subequal in length laterally, densely deeply punctate, III and IV much shorter, combined length shorter than length of ventrite II, impunctate, ventrite V impunctate, about as long as III and IV combined. ***Legs*** without femoral teeth; tibial apices with only rows of short, stout spines, spines greatest in number and most closely spaced on front tibia (Figs. 2G–I), without tooth-like process potentially homologous to mucro, pre-mucro or uncus; femora without groove in basal half to receive tibiae; tibiae without flat lobes with setal fringes; tarsomere IV present, small and hidden between lobes of tarsomere III (Figs 2H, I); claws simple, large, widely divergent. ***Female genitalia*** with spiculum ventrale (= internal apodeme of sternum 8) consisting of two weakly sclerotized arcuate basal arms and short, broad, apically expanded apodeme of nearly equilateral triangular shape (Figs 2K, L); each gonocoxite (= part of sternum 9, "coxite-stylus") posteriorly rounded and setose, spade-like, well-sclerotized, lacking stylus

(Figs 2K, L); sclerotized spermatheca not detected. ***Male genitalia*** unknown.

Species composition and distribution. The genus is monotypic and its known distribution is limited to the type locality of its only species (see below).

Etymology. The generic name is a meaningless combination of letters; its gender is masculine.

Note. Males will be expected to have a pedotectal type of genitalia that is typical for Brachycerinae.

Yagder serratus sp. nov.

(Figs 1, 2, 4, 6)

Type locality. Mexico, Puebla, 5 km SW Hueytamalco, N 19.911° W 97.328°, 1370 m a.s.l.

Material examined. Holotype: female, 'MEXICO, Puebla, 19.911 -97.328, 1370m, 13.vi.2019, sift., J.Longino 10670', 'CNCCOL-VG00010339' (deposited in the Canadian Museum of Nature, Ottawa, Canada). The holotype is missing both hind legs (one used for sequencing), the right middle leg and right front tarsus.

Description. Body size large, 3.8 mm in length (excluding rostrum and deeply inserted head). Rostrum with pterygia very widely exposed in dorsal view (Fig. 2A), apical two antennomeres of funicle longer than wide (Fig. 1C). Pronotal and elytral contour in dorsal view with large saw-like serrations especially evident towards elytral apex (Fig. 2J). Abdomen with ventrites I and II densely deeply punctate. DNA: MW201355 (COI), MW201457 (ITS2) and MW201468 (28S).

Biology. A single female was sifted from leaf litter in primary mountain forest. Multiple samples taken at the same time and location by J. T. Longino and M. G. Branstetter failed to produce additional specimens.

Etymology. The species name is the Latin adjective meaning "serrated, toothed like a saw".

Distribution. This species is known only from the type locality.

Discussion

Our topology (Fig. 6), although based on only three DNA markers, agrees well with that of Shin et al. (2017) in all its most important aspects. The latter, however, was based on a >150 times greater dataset from 522 protein-coding genes. Our most significant deviation from Shin et al. (2017) and other comparably detailed DNA-based studies of weevil phylogeny (listed in Introduction) is the recovery of the Molytinae genus *Devernodes* outside the CCCMS clade and in a weakly supported and seemingly odd clade together with the Brachycerinae genera *Notaris* Germar, 1817 and *Tournotaris* Alonso-Zarazaga & Lyal, 1999 (Fig. 6). We treat this aberrant clade partly as a random artifact of our analysis, and partly as a reflection of the documented tendency of the genus *Devernodes* to form relationships with taxa outside of the CCCMS clade (Grebennikov 2018, Grebennikov & Anderson 2021). Most significantly, all 13 remaining CCCMS terminals formed a strongly supported clade, itself a sister (although weakly supported) to the strongly supported clade uniting all six CEGH terminals (Fig. 6). This combined CCCMC plus CEGH clade of 'higher' weevils corresponds to a more narrowly defined family Curculionidae sensu Thompson (1992) and is supported by the classical weevil morphological synapomorphy of the pedal type of male genitalia. Recovery in our analysis of the CCCMS + CEGH clade is consistent with earlier morphological and molecular results and, therefore, suggestive of the overall credibility of our topology (Fig. 6).

Disregarding the odd clade of *Devernodes* and two other genera (see above), interpretation of the herein presented results leads us to conclude that:

Prediction 1 (*Yagder serratus* gen. & sp. nov. belongs to a non-monophyletic Brachycerinae) is supported, because this beetle was recovered on our tree (Fig. 6) outside of the monophyletic core of the family Curculionidae formed by the least inclusive clade uniting any member of Dryophthorinae and any member of 'higher'Curculionidae (Shin et al. 2017).

Prediction 2 (available data are sufficient to place our new eyeless Mexican weevil in a well-supported sister relationships) is rejected, because the recovered sister-group placement of *Yagder* is weakly supported (Fig. 6) and is, therefore unreliable.

Prediction 3 (the Mediterranean core of the tribe Raymondionymini is monophyletic) is supported, because both representative terminals, the genera *Alaocyba* and *Raymondiellus*, formed a strongly supported (100%) clade (Fig. 6).

Prediction 4 (at least one non-Mediterranean member of Raymondionymini is sister to its Mediterranean core) cannot be adequately tested, because the monophyletic Mediterranean core of the tribe is a part of an unresolved and weakly supported polytomy including also *Schizomicrus* and *Ocladius* (Fig. 6).

Prediction 5 (eyeless Brachycerinae form a clade, implying a single and non-reversed eye reduction and disappearance) is rejected, because the trait of eyelessness (or microphthalmia, as with *Himasthlophallus*, Fig. 5C) is scattered among five not most closely related clades of Brachycerinae (Fig. 6).

Prediction 6 (none of weevils currently assigned to Brachycerinae is nested within the main weevil radiation delimited as the least inclusive clade uniting any member of Dryophthorinae and any member of 'higher'Curculionidae, Shin et al. 2017) is supported, on the same ground, as the Prediction 1 (above).

Summing up, our newly described eyeless weevil *Yagder serratus* gen. & sp. nov. from Mexico is a member of the twilight zone of true weevils (Curculionidae) taxonomically temporarily designated as the non-monophyletic subfamily Brachycerinae. This subfamily will probably be retained for as long as it takes to reliably resolve the branching pattern between two well-established dichotomies: one separating Curculionidae from its sister group (Brentidae), and another separating the clade of any Dryophthorinae plus any CCCMS/CEGH member from its presently unknown sister group. Once done, the subfamily Brachycerinae will likely be split into two or more species-poor early-divergent subfamilies, one of them potentially the sister to the remainder of all weevils.

Until then the artificial subfamily Brachycerinae might serve its present utilitarian purpose of temporary housing taxonomically and phylogenetically unresolved weevils not belonging to other better understood and species-rich clades. We, consequently, taxonomically designate the new genus *Yagder* as Brachycerinae *incertae sedis*, that is, not included in any tribe of Brachycerinae (at least one of them, Raymondionymini, comparably non-monophyletic).

Concluding remark

Numerous peculiarities of our discovery of *Yagder serratus* gen. & sp. nov. strongly recalls those of the mite *Proterorhagia oztotloica* Lindquist & Palacios-Vargas, 1991 (Lindquist & Palacios-Vargas 1991). In both cases a new species and a new genus were erected based on a single soil-inhabiting specimen from Mexico of strange morphology and unresolved phylogenetic position. In both cases the new organisms were assigned to a non-monophyletic twilight zone of much larger clades: Brachycerinae and Endeostigmata, respectively. The mite genus *Proterorhagia* is the type genus of the monotypic family Proterorhagiidae. This family is still known from a single specimen and is likely sister to the rest of Acariformes (Bolton et al. 2017), the latter a hyper-diverse clade of over 32,000 described species and perhaps half a million of undescribed ones. Does *Yagder serratus* gen. & sp. nov. occupy a similarly exalted place on the weevil Tree of Life worthy of a family-group name of its own? Only time will tell.

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