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ORIGINAL ARTICLE

Mitogenomic evidence reveals convergent evolution of minute eyeless soil-dwelling weevils in Mesoamerica and the Mediterranean (Coleoptera: Curculionidae)

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Key words. Mitogenomics, Guatemala, Mexico, Raymondionyminae, new genera, new species

Abstract. We describe and illustrate two new species of minute, eyeless, soil-dwelling weevils, each representing a new monotypic genus: *Kisin akabali* Barrios-Izás & Morrone, gen. et sp. n., from Guatemala, and *Xadarhinus dofoi* Barrios-Izás & Morrone, gen. et sp. n., from Mexico. Mitogenomic phylogenetic analyses recover these genera, together with the similarly eyeless *Yagder* serratus from Mexico and two additional undescribed Mexican specimens, as a deeply divergent lineage outside the clade of the "higher" Curculionidae. Despite their striking morphological resemblance to the minute, flightless, and eyeless Mediterranean Raymondionyminae, this newly recognized Mesoamerican lineage is phylogenetically distant from them, representing a remarkable example of convergent evolution. No available family-group name can be confidently assigned to this lineage. Pending a more comprehensive phylogenetic analysis of Curculionoidea, we therefore treat it as Curculionidae *incertae sedis*.

ZooBank Article Registration: https://zoobank.org/urn:lsid:zoobank.org:pub:6348625C-8374-47B6-9CC8-73954253B85A

1. INTRODUCTION

Convergent evolution, i.e., the independent acquisition of similar traits in distantly related lineages adapting to comparable environments, poses a well-known challenge for morphology-based phylogenetic classification. This is particularly true for edaphic animals, which frequently evolve eyelessness, small body size, and cryptic coloration. Notable examples include (i) fossorial mammals, such as moles, golden moles, marsupial moles, naked mole-rats, and African mole-rats; (ii) "worm"-like limbless animals such as earth worms, caecilians, nematodes, amphisbaenians, legless lizards, and 'Scolecophidia' snakes, the latter itself a paraphyletic assemblage of distantly related blind and small-bodied forms (Miralles et al., 2018); and (iii) many soil- and litter-dwelling arthropods, such as eyeless weevils forming the focus of the present paper.

Raymondionyminae have fewer than one hundred described species and are variably ranked as a subfamily or a tribe (Grebennikov & Anderson, 2021). Historically, they accommodated small, eyeless soil- and litter-dwelling weevils from across the globe with gonatocerous antennae and pedotectal penis (Alonso-Zarazaga, 2007). These soil-dwelling beetles are underrepresented in entomological collections and require specific sampling methods adapted to target leaf litter, humus layers, and deep soil. The only morphology-based phylogenetic study failed to resolve their relationships (Grebennikov, 2010), and molecular studies were hindered by the scarcity of DNA-grade specimens (e.g., Shin et al., 2018, Grebennikov & Anderson, 2021). Recently, a mitochondrial phylogenomic study based on a limited, largely European sampling demonstrated that the Mediterranean genera, which include the type genus, form a monophyletic lineage and are the sister group to the remaining 50,000+ species of true weevils (Andújar et al., 2024). The phylogenetic placement of non-European

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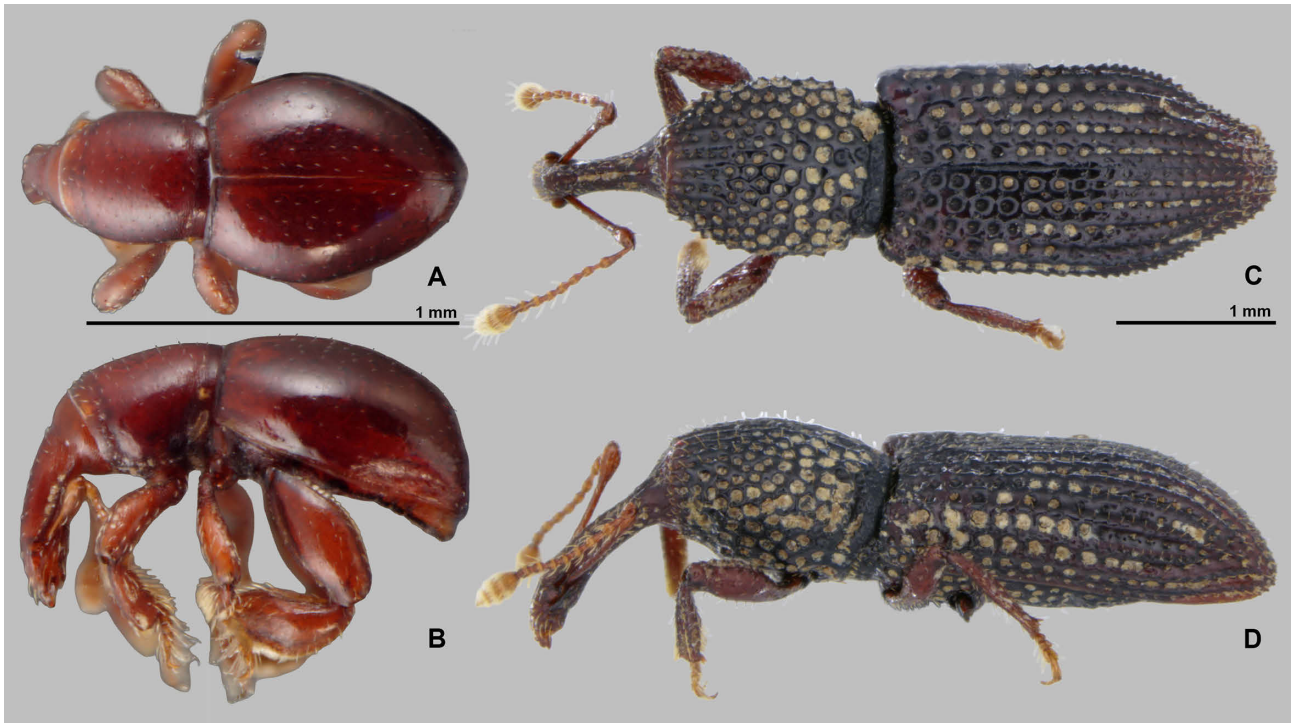


Fig. 1. Edaphic Mesoamerican weevils: *Neoubychia mexicana* (A, B); *Yagder serratus* (C, D).

Raymondionyminae, however, remains unresolved, and these taxa continue to be treated as incertae sedis.

Mesoamerica (i.e., Central America and Mexico) is home to the raymondionymine genus *Neoubychia* Gilbert & Howden, 1987 (Fig. 1A, B). The similarly eyeless and wingless genus *Yagder* Grebennikov & Anderson, 2021 (Fig. 1C, D) was not assigned to Raymondionyminae (then treated as a tribe of Brachycerinae), but instead placed in Brachycerinae incertae sedis, because Raymondionymini were not recovered as a clade. Both genera are monotypic and known solely from the type series of their respective type species, and both were collected from forest litter in Mexico. Recent fieldwork in Guatemala and Mexico by the authors of this study resulted in the discovery of two taxonomically distinct small, eyeless weevils that cannot be assigned to any previously known genera. Their discovery triggered the present study and provided difficult-to-obtain DNA-grade specimens of non-European Raymondionyminae.

Here, we sequenced mitogenomes of (i) representative specimens of two new, morphologically distinctive lineages collected by us in Guatemala and Mexico, (ii) the holotype and only known specimen of *Yagder serratus* from Mexico, and (iii) two unnamed, morphologically similar specimens from Mexico. Our main objectives are (i) to describe and formally introduce two new monotypic genera, each represented by a newly discovered eyeless species, (ii) to establish their phylogenetic placement within the broader phylogenetic framework of weevils based on complete mitogenome sequencing and analyses, and (iii) to test the monophyly of Raymondionyminae, particularly with respect to the non-European taxa.

2. MATERIAL AND METHODS

2.1. Specimen availability

Ten adult specimens of the new Guatemalan species and two adult specimens of the new Mexican species were collected by us from forest litter using a hand-held sifter and subsequently extracted in suspended Winkler funnels. Specimens were preserved in 96% ethanol and stored after field work in a –20°C freezer for DNA preservation. All 12 specimens were non-destructively DNA extracted, subsequently dry mounted, and morphologically studied for their formal description as two new monotypic genera (see below). In addition, we sequenced the holotype of *Yagder serratus* and two unnamed eyeless Mexican specimens, all of them sharing the morphological characteristic of Raymondionyminae.

2.2. Taxonomic study

Specimen measurements were taken using images obtained by a Nikon digital camera DXM1200F. Body length was measured along the midline between the anterior margin of the pronotum and the elytral apices. Pronotum length was measured along the midline between the anterior margin to the posterior edge of the pronotal disk, excluding the lower portion of the latter that is dorsally visible only in extended specimens but is normally covered by the bases of the elytra. Morphological terms are those of Lyal (2024). All specimens of both newly described species are deposited in the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Canada (CNC).

2.3. DNA extraction, barcoding and mitogenome sequencing

DNA extraction was performed from whole specimens using a non-destructive protocol with the Omega Mag-Bind® Blood and Tissue DNA Kit (Omega Bio-tek) on a KingFisher robotic system (Thermo Fisher Scientific Inc.). PCR amplification targeted the 5′ end of the COI gene (the standard DNA barcode region for Metazoa; Hebert et al., 2003) using degenerate Folmer barcode prim-

Table 1. Nine specimens of eyeless edaphic Mesoamerican Curculionidae newly sequenced for complete mitochondrial genomes. Their COI sequences, together with habitus images and locality data, are publicly available in the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007) dataset DS-VGDS30 (dx.doi.org/10.5883/DS-VGDS30).

Voucher Code	NCBI Accession	Country	Species
CNCCOLVG00010330	QB020480	Mexico	no name
CNCCOLVG00010331	QB020481	Mexico	no name
CNCCOLVG00010339	QB020482	Mexico	*Yagder serratus*
GR0253	QB020483	Guatemala	*Kisin akabali*
GR0255	QB020484	Guatemala	*Kisin akabali*
GR0269	QB020485	Mexico	*Xadarhinus dofoi*
GR0270	QB020486	Mexico	*Xadarhinus dofoi*
GR0272	QB020487	Guatemala	*Kisin akabali*
GR0273	QB020488	Guatemala	*Kisin akabali*

ers (FoldF: 5′-TCNACNAAYCAYAARRAYATYGG-3′; FoldR: 5′-TANACYTCNGGRTGNCCRAARAAYCA-3′; Folmer et al., 1994; Yu et al., 2012). PCR conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 48°C for 30 s, and 72°C for 3 min, with a final extension at 72°C for 10 min and a final hold at 10°C. PCR products were Sanger-sequenced using ABI technology at Macrogen (Spain).

Mitogenome sequencing and assembly followed the mitochondrial metagenomics approach (Dettai et al., 2012; Andújar et al., 2015; Crampton-Platt et al., 2015), in which complete mitochondrial genomes are assembled from shotgun sequencing of DNA mixtures from multiple specimens. Nine specimens were selected for de novo sequencing of complete mitogenomes (Table 1). The double-stranded DNA concentration of each raw DNA extract was measured using a Qubit 2.0 Fluorometer (Life Technologies Corp., Carlsbad, CA, USA). Four TruSeq Nano DNA libraries were subsequently constructed from four equimolar DNA pools, each containing one or more target specimens from this study together with several additional Coleoptera representatives from distantly related lineages. Sequencing was performed on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) using paired-end reads (2 × 300 bp; 700–900 bp insert size), with an expected coverage of approximately 1% of a MiSeq run per specimen. Illumina reads were processed with Trimmomatic v0.30 (Bolger et al., 2014) to remove sequencing adapters. Reads were subsequently filtered using BLAST v2.2.27 (Altschul et al., 1990) against a reference database comprising 2,344 mitochondrial genomes longer than 5,000 bp retrieved from the NCBI nucleotide database (accessed 5 November 2020). Mitochondrial reads were assembled using RAY v2.3.1 (Boisvert et al., 2012; parameters: –K 61, –minimum-seed-length 100, –minimum-contig-length 1000), SPAdes v3.14 (Prjibelski et al., 2020; parameters: –k 21, 33, 55, 77, 99, 127), and IDBA v1.1.3 (Peng et al., 2012; parameters: –maxk 300, –mink 50). Contigs obtained from the three assemblers were combined and reassembled in Geneious using the de novo assembly function. The resulting assemblies showed extensive overlap among methods, reducing the likelihood of chimeric mitogenome reconstruction.

Obtained mitogenomes were annotated using MITOS (Bernt et al., 2013), followed by manual inspection and editing in Geneious. All recovered mitogenomes exhibited the typical coleopteran mitochondrial gene order. Taxonomic identity of the assembled mitogenomes was confirmed by comparison with COI barcode sequences obtained from the same specimens using PCR and Sanger sequencing (see above).

2.4. Dataset assembly and phylogenetic analyses

To assemble a mitogenome dataset capable of elucidating the phylogenetic position of the eyeless Mesoamerican Curculionidae, we added the nine newly generated complete mitogenomes (including the ingroup formed by six mitogenomes from our target Guatemalan and Mexican species, plus two mitogenomes of an unnamed species from Mexico and the mitogenome of *Yagder serratus*, Table 1) to the 60 mitogenomes used by Andújar et al. (2024) in their analysis of Mediterranean Raymondionyminae. We did not use the mitogenome of the bark beetle genus *Scolytus* Geoffroy, 1762, accession number MK636869, because for unknown reasons it consistently clustered outside of Scolytinae (Andújar et al., 2024) and was potentially destabilizing the topology. In addition to the ingroup, the 69-terminal dataset contained: (i) the potential nearest outgroup consisting of Mediterranean Raymondionyminae (9 mitogenomes) and Brachycerinae (5); (ii) more distant curculionid outgroups represented by Dryophthorinae (5 mitogenomes), Platypodinae (3), Bagoinae (1), "higher" weevils of the CEGH (9) and CCCMS (19) clades; and (iii) non-Curculionidae outgroups included to place Curculionidae within Curculionoidea and containing representatives of Brentidae (3 mitogenomes; the putative sister group of Curculionidae), Attelabidae (3), Anthribidae (3), and Nemonychidae: Cimberidinae (1; often treated as a separate family). All topologies were rooted with the Anthribidae + Nemonychidae clade, consistent with current hypotheses on the earliest divergence within weevils (Oberprieler et al., 2007).

The assembled dataset comprised 69 annotated mitogenomes. Individual gene datasets were extracted in Geneious for the 13

Table 2. Results of eight phylogenetic analyses of 69 Curculionoidea mitogenomes performed to infer the phylogenetic position of eyeless edaphic Mesoamerican weevils. The first four columns summarize analytical parameters (data type [DNA or amino acids], number of genes, software, partitioning scheme, and model of sequence evolution), followed by a reference to the resulting topology and statistical support values for eleven selected clades. Clade abbreviations: MR – Mediterranean Raymondionyminae; C-MR – Curculionidae excluding Mediterranean Raymondionyminae; MA – Mesoamerican clade; MA+(E+L), Mesoamerican clade sister to Lissorhoptrus and Echinocnemus; D – Dryophthorinae; P – Platypodinae; D+P – Dryophthorinae + Platypodinae; CEGH – clade comprising Cyclominae, Entiminae, Gonipterini, and Hyperinae; CCCMS – clade comprising Conoderinae, Cossoninae, Curculioninae, Molytinae, and Scolytinae; CEGH+CCCMS – "higher" weevil clade; B+(CEGH+CCCMS) – Bagoinae + "higher" weevil clade.

Dataset	Software	Partitions	Model	Tree	MR	C-MR	MA	MA+(E+L)	D	P	D+P	CEGH	CCCMS	CEGH+ CCCCMS	B+(CEGH+ CCCMS)
DNA15	PhyloBayes	n.a.	GTR-CAT	Fig. S1	1	1	1	none	none	1	1	1	1	1	0.99
DNA13	PhyloBayes	n.a.	GTR-CAT	Fig. S2	1	0.9	1	none	1	1	1	1	1	1	0.99
AA13	PhyloBayes	n.a.	GTR-CAT	Fig. S3	1	0.8	1	0.6	1	1	1	1	1	0.77	1
DNA15	RAxML	gene & codon	GTRCAT/Γ	Fig. S4	100	81	91	69	80	100	none	100	100	83	43
DNA15	RAxML	gene	GTRCAT/Γ	Fig. S5	100	75	93	65	84	100	none	100	100	83	42
DNA13	RAxML	gene & codon	GTRCAT/Γ	Fig. S6	100	84	87	69	76	100	none	100	100	84	48
DNA13	RAxML	gene	GTRCAT/Γ	Fig. S7	100	68	93	75	72	100	none	100	100	81	49
AA13	RAxML	gene	PROTGTRCAT/Γ	Fig. S8	100	96	98	82	85	100	none	100	100	57	94



Fig. 2. *Kisin akabali* gen. et sp. n. A–G, K: specimen GR0273, paratype, male; H–J: specimen GR0255, holotype, female. Habitus in dorsal (A, H), lateral (B, I), and ventral (C, J) view; D–G male genitalia in dorsal (D), ventral (E), right lateral (F), and right dorso-lateral (G) view; K: right antenna.

protein-coding genes (PCGs) and two ribosomal genes, then independently trimmed and aligned using the FFT-NS-2 algorithm of MAFFT (Katoh et al., 2002). The resulting alignments were concatenated, yielding three datasets: (i) a 15-gene nucleotide dataset of 13,140 bp (DNA15); (ii) a 13-PCG nucleotide dataset of 11,045 bp (DNA13); and (iii) an amino acid dataset derived from the 13 PCGs using the invertebrate mitochondrial genetic code, comprising 3,669 amino acid positions (AA13). These datasets were used for Bayesian inference (BI) and maximum likelihood (ML) phylogenetic analyses. Bayesian analyses were performed in PhyloBayes (Lartillot & Philippe, 2004) using two independent chains under the GTR-CAT model for 5,000 generations. Consensus trees were generated for each dataset by combining the posterior trees from both chains after discarding the first 2,000 generations as burn-in. Maximum likelihood analyses were conducted in RAxML v8 (Stamatakis, 2014) using gene partitions and, for nucleotide datasets, additional codon-position partitions. Each partition was analyzed under an independent GTR model, using the CAT approximation during tree searches and GAMMA for final optimization. The best-scoring ML tree was selected among 100 independent searches using the original alignment and randomized parsimony starting trees. Node support was assessed using 500 bootstrap replicates (Felsenstein, 1985). In total, eight phylogenetic analyses were performed (Table 2). Resulting topologies were visualized using FigTree (Rambaut, 2012).

3. RESULTS AND DISCUSSION

3.1. New taxonomic acts

Kisin Barrios-Izás and Morrone, gen. n.

Fig. 2

ZooBank taxon LSID:
ADB66C5A-8B61-4263-A2D3-D488A218A609

Type species. *Kisin akabali* Barrios-Izás and Morrone, sp. n., here designated.

Diagnosis. Among all eyeless Mesoamerican weevils smaller than 4 mm and lacking a short and sharp tooth-like process on the tibial apex (potentially homologous to mucro/uncus), specimens of the new genus most closely resemble those of the genus *Xadarhinus* Barrios-Izás & Morrone, gen. n.; see the identification table below for distinguishing characters. Species of the genus *Bordoniola* Osella, 1987, endemic to Venezuela and Ecuador, differ in having a smaller body not exceeding 1.5 mm in length (excluding the rostrum and retracted head), in possessing pronotal and elytral setae longer than the distance between adjacent setae, and in having an abrupt transition between the antennal club and funicle.

Description. Body length 2.06–3.06 mm; maximum body width 0.77–1.09 mm; pronotal width to length ratio 0.77–0.88; elytral width to length ratio 0.60–0.67 (n = 10); slender and parallel-sided in dorsal view; prothorax, elytra, meso- and metaventrites, and two visible basal abdominal ventrites with deep round punctures often 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, barely visible in dorsal view; eye completely absent. **Rostrum** in lateral view about 0.75 times as long as pronotum in dorsal view; in lateral view straight and parallel-sided; without long and deep longitudinal groove along mid-line dorsally; antennae attached in apical third; pterygia at points of antennal insertions partly exposed in dorsal or frontal view; scrobes shallow, anteriorly partly visible in dorsal or frontal view. **Antennae** geniculate, funicle and club consisting of seven and three antennomeres, respectively; scape about subequal in length to funicle; antennal club evenly joining funicle; antennomeres of funicle weakly conical or cylindrical (their outlines appear, therefore, trapezoid or rectangular), widely joined to each other with more than half of their maximum width. **Prothorax** without postocular lobes; procoxae separated by about quarter of coxal diameter; hypomeral lobes subequal in length to diameter of procoxal cavities; pronotum evenly rounded in dorsal view, without delimited disk, evenly curved in lateral view; dorsal punctures irregularly placed and forming on each side not more than one innermost longitudinal row, therefore pronotum has no appearance of longitudinal striation. **Meso- and metathorax** with scutellum not 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, evenly curved dorsally (in lateral view); with eight complete striae. **Abdomen** with ventrites 1 and 2 subequal in length laterally, densely deeply punctate; ventrites 3 and 4 much shorter, combined length shorter than length of ventrite 2, impunctate; ventrite 5 about as long as 3 and 4 combined. **Legs** without femoral teeth; tibial apices with rows of short, stout spines, without tooth-like process potentially homologous to mucro or uncus; femora without groove in basal half to receive tibiae; tibiae without flat lobes with setal fringes; tarsomere 4 not detected; claws simple. **Male genitalia** with penis of pedal type (= tectum not distinguishable); ring of tegmen open dorsally (= lacking complete ring sclerotization); parameroid lobes absent; endophallus with two small irregularly-shaped sclerites; dorsal part of penis without two small transverse sclerites; tegminal apodeme longer than penis apodemes; tegmen posteriorly with large bilobed rectangular ventral sclerite. **Female genitalia** not studied.

Species composition and distribution. The genus is monotypic and is known from two localities in Guatemala, approximately 135 km apart. Although the two populations show some mitochondrial divergence, they form a clade that we treat as single, relatively widely distributed and genetically polymorphic species, rather than recognizing separate species from each locality.

Etymology. The generic name is a masculine noun, referring to the god of the underworld in K'iche' Mayan mythology.

Kisin akabali Barrios-Izás and Morrone, sp. n.

Fig. 2

ZooBank taxon LSID:
A29B5FE6-8CE3-476F-ABAA-68F7D234FF7D

Type locality. Sierra de las Minas, El Progreso, Guatemala, 15.0835°N, 89.9440°W.

Description. Because the genus is monotypic, the diagnostic characters of the species are included in the generic description.

Type material. Holotype (female): "GUATEMALA, S. de. L. Minas, 15.0835 –89.9440, 2574 m, 15.v.2022. siftGT04, Grebennikov", "Grebennikov GR0255". Paratypes: 3 exs., same locality, but "GR0256", "GR0257" and "GR0258", respectively; 2 exs., "GUATEMALA, Nebaj, 15.3718 –91.1319, 2598 m, 8.v.2022, siftGT01, Grebennikov", "Grebennikov GR0253" and "Grebennikov GR0254", respectively; 2 exs., "GUATEMALA: Quiché, Nebaj, 19.ix.2019, 15.36843 –91.1145, 2660 m, Bos. Nuboso, Cernido. Hojarasca, Col. M Barrios MBI584", "Grebennikov GR0271" and "Grebennikov GR0272", respectively; 2 exs., "GUATEMALA: El Progreso, San Augustín Ac., S. Minas, 12.ii.2022, 15.082397 –89.951773, 2539 m., Bos. Nuboso. Cernido Hojarasca, Col. M Barrios MBI 005-2022", "Grebennikov GR0273" and "Grebennikov GR0274", respectively. All specimens are deposited in CNC.

Distribution. This species is known from two localities in Guatemala, about 135 km apart.

Biology. All specimens of the new species were sifted from leaf litter in a deciduous forest.

Etymology. This species name commemorates Humberto Ak'abal, a K'iche' Maya poet.

Xadarhinus Barrios-Izás and Morrone, gen. n.

Fig. 3

ZooBank taxon LSID
DD72BF59-8A20-4B21-A500-52AF84137F63

Type species. *Xadarhinus dofoi* Barrios-Izás and Morrone, sp. n., here designated.

Diagnosis. Among all eyeless Mesoamerican weevils smaller than 4 mm and lacking a tooth-like process on the

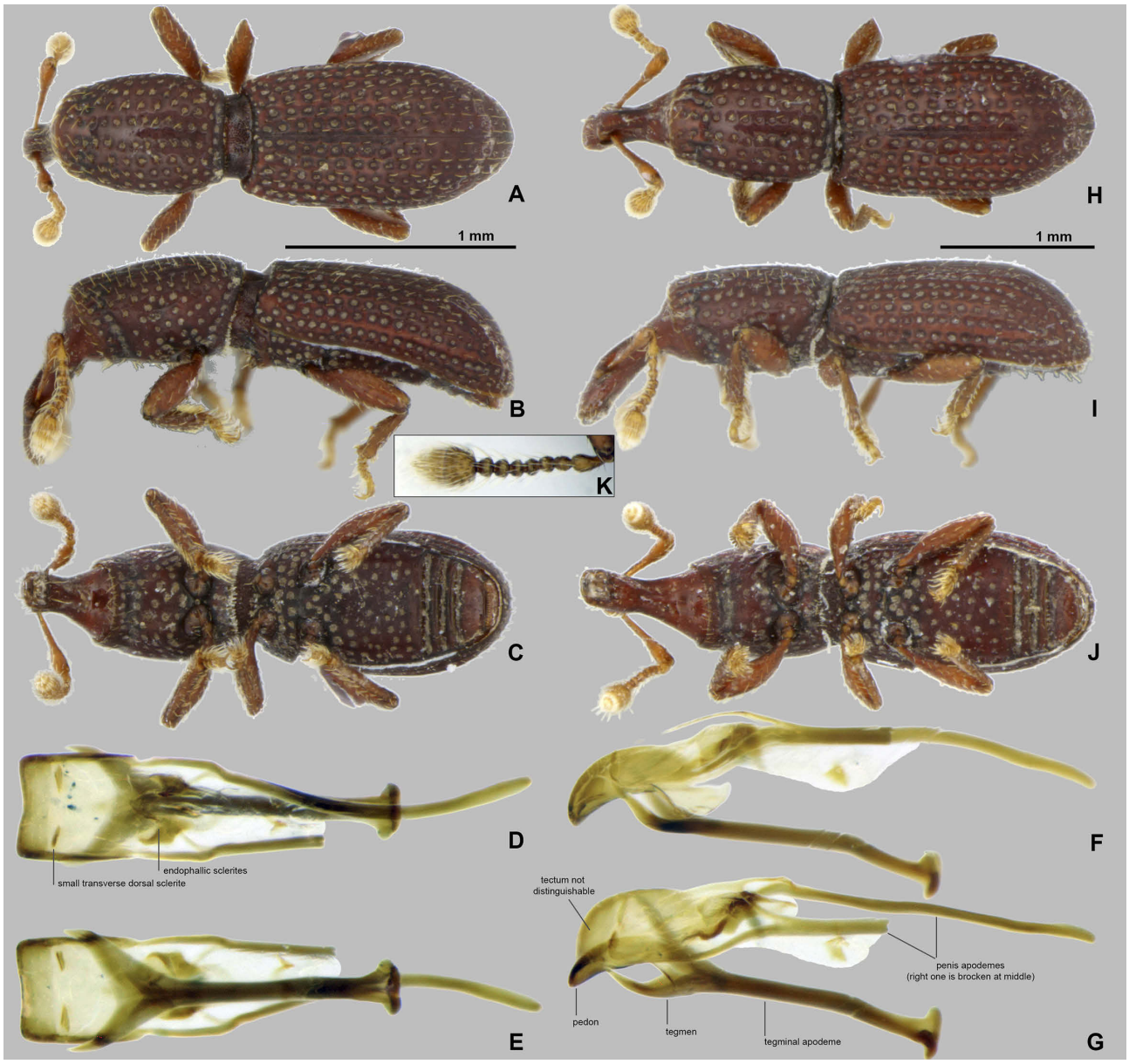


Fig. 3. *Xadarhinus dofoi* gen. et sp. n. A–G, K: specimen GR0270, holotype, male; H–J: specimen GR0269, paratype, female. Habitus in dorsal (A, H), lateral (B, I), and ventral (C, J) view; D-G male genitalia in dorsal (D), ventral (E), right lateral (F), and right dorso-lateral (G) view; K: right antenna.

tarsus (potentially homologous to the mucro, pre-mucro, or uncus), specimens of the new genus most closely resemble those of *Kisin* Barrios-Izás & Morrone, gen. n.; see the identification table below for distinguishing characters. Species of the genus *Bordoniola* differ in having a smaller body not exceeding 1.5 mm (excluding the rostrum and retracted head); in possessing pronotal and elytral setae longer than the distance between adjacent setae; and in having pronotal punctures arranged irregularly rather than in longitudinal rows, so the pronotum lacks the appearance of shallow longitudinal striation.

Description. Similar to the genus *Kisin* Barrios-Izás & Morrone, gen. n., except the following: body length 1.83–2.00 mm; maximum body width 0.62–0.64 mm; pronotal width to length ratio 0.73–0.75; elytral width to length ratio 0.56–0.57 (n = 2). Antennal club abruptly joining funicle; antennomeres of funicle nearly spherical (their outlines appear, therefore, as circles), narrowly joined to each other at not more than half of their maximum width; punctures on pronotum more regularly placed and forming on each side at least three innermost longitudinal rows, therefore pronotum has appearance of shallow longitudinal striation; dorsal part of penis with two transverse sclerites; tegminal apodeme shorter than penis apodemes; tegmen posteriorly without large bilobed rectangular ventral sclerite.

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

Etymology. The generic name is a masculine noun, formed by combining *xada* (meaning "blind" in the Otomi language) with *rhinos*, a root commonly used in Curculionidae.

Xadarhinus dofoi Barrios-Izás and Morrone, sp. n.

Fig. 3

ZooBank taxon LSID
29C2A740-08BD-401D-8B0D-794B82A27418

Type locality. Mexico, Oaxaca, 17.451029°N, 96.509923°W.

Description. Because the genus is monotypic, the diagnostic characters of the species are included in the generic description.

Type material. Holotype (male): "MEXICO: Oaxaca, San Juan Atepec, 10.vi.2018, 17.451029 –96.509923, 2998 m., Bos. Abietes + Pinus, Cernido Hojarasca, Col. M Barrios MBI798", "Grebennikov GR0270". Paratype (female): same locality, but "GR0269". Both specimens are deposited in CNC.

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

Biology. Specimens of the new species were sifted from leaf litter in an *Abies*-*Pinus* cloud forest.

Etymology. The species name is a noun in apposition referring to the small body size (*dofoi* means "dwarf" in the Otomi language).

3.2. Identification key to eyeless edaphic Mesoamerican weevils

1 Distal end of tibia without a short and sharp tooth-like process (= mucro/uncus) 2
– Distal end of tibia with a short and sharp tooth-like process (= mucro/uncus) (see Grebennikov & Anderson, 2022) .... Molytinae: Lymantini: Caecossonina
2 Larger, distance between anterior margin of pronotum and elytral apices more than 3.5 mm; contour of elytra (when viewed from above) notably serrate (its single species *Y. serratus* Grebennikov & Anderson, 2021 is known from the female holotype collected in Mexico: Puebla) .... *Yagder* Grebennikov & Anderson, 2021
– Smaller, distance between anterior margin of pronotum and elytral apices less than 2.5 mm; contour of elytra (when viewed from above) not serrate 3
3 Each eye represented by a single ommatidium (fig. 9I in Grebennikov, 2010); pronotum and elytra smooth, without punctures or striae; hind legs notably flattened, with hind tibia 0.5 times as wide as long (its single species *N. mexicana* Gilbert & Howden, 1987 is known only from the type series collected in Mexico: Hidalgo, Oaxaca, and San Luis Potosí)..... ..... *Neoubychia* Gilbert & Howden, 1987
– Eyes entirely absent (= not a single ommatidium); pronotum and elytra with punctures and striae; hind legs unmodified, with hind tibia not more than 0.3 times as wide as long 4
4 Larger, distance between anterior margin of pronotum and elytral apices more than 2.05 mm; antennal club evenly joining funicle (Fig. 2K); antennomeres of funicle weakly conical or cylindrical (their outlines appear, therefore, trapezoid or rectangular), widely joined to each other with more than half of their maximum width (Fig. 2K); punctures on pronotal disk irregularly placed and forming not more than one innermost longitudinal rows on each side, therefore pronotum has no appearance of longitudinal striation (Figs 2A, H); dorsal pronotal and elytral contour in lateral view each distinctly curved (Figs 2B, I) (its single species *K. akabali* Barrios-Izás and Morrone sp. n. is known from 10 specimens collected in Guatemala: El Progreso and Quiché) ....*Kisin* Barrios-Izás and Morrone, gen. n.
– Smaller, distance between anterior margin of pronotum and elytral apices less than 2.05 mm; antennal club abruptly joining funicle (Fig. 3K); antennomeres of funicle nearly spherical (their outlines appear, therefore, as circles), narrowly joined to each other with less than half of their maximum width (Fig. 3K); punctures on pronotal disk more regularly placed and forming at least three innermost longitudinal rows on each side, therefore pronotum has appearance of shallow longitudinal striation (Figs 3A, H); dorsal pronotal and elytral contour in lateral view each straight (Figs 3B, I) (its single species *X. dofoi* Barrios-Izás and Morrone sp. n. is known from two specimens collected in Mexico: Oaxaca)..... .....*Xadarhinus* Barrios-Izás and Morrone, gen. n.

3.3 Phylogenetic placement of minute and eyeless Mesoamerican soil-dwelling weevils

Nine specimens, including six belonging to both newly described taxa, have been here de novo sequenced for the complete mitochondrial genome (Table 1).

The eight phylogenetic analyses (Table 2; Supplementary Figs S1–S8) yielded highly congruent trees, recovering basal weevil divergences consistent with previous studies (Shin et al., 2018; Li et al., 2023; Andújar et al., 2024). Mediterranean Raymondionyminae were consistently recovered as the sister group to the remaining monophyletic Curculionidae (Fig. 4). All nine newly sequenced specimens of eyeless Mesoamerican weevils formed a clade branching from the non-Raymondionyminae rest of the family either alone or as sister to the clade formed by the genera *Lissorhoptrus* LeConte, 1876 and *Echinocnemus* Schoenherr, 1843 (Fig. 4). No clade composed wholly (or even predominantly) of the Mesoamerican and Mediterranean eyeless weevils was recovered. This topology strongly suggests a new remarkable case of morphological convergence between American and Palaearctic clades of minute soil-dwelling weevils, comparable to the classic vertebrate examples in which superficially similar Old and New World vultures (Seibold & Helbig, 1995) and porcupines (Huchon & Douzery, 2001) represent non-monophyletic groups. Internal relationships within the Mesoamerican clade were poorly resolved, although a weakly supported subclade comprising *Yagder* + *Xadarhinus* gen. n. was recovered in all but one analysis. It is primarily for this reason that the two newly described species, despite their external similarity, are assigned to different genera.

Morphological evidence supporting the monophyly and early-divergence of the Mesoamerican lineage is currently lacking, except for the remarkable and seemingly controversial observation concerning the structure of the male genitalia. Curiously, no tectum could be distinguished in their penises (Figs 2G, 3G), a condition representing the "advanced" pedal type of male genitalia (Alonso-Zarazaga, 2007), as opposed to the "primitive" pedotectal type, which possesses both a pedon and tectum (character 99 in Marvaldi et al., 2002). This is counterintuitive because the pedal penis is considered the principal morphological synapomorphy defining the "higher" true weevils. These comprise two species-rich lineages containing together the majority of curculionid diversity (Fig. 4): the CEGH clade (Cyclominae, Entiminae, Gonipterini, and Hyperinae)

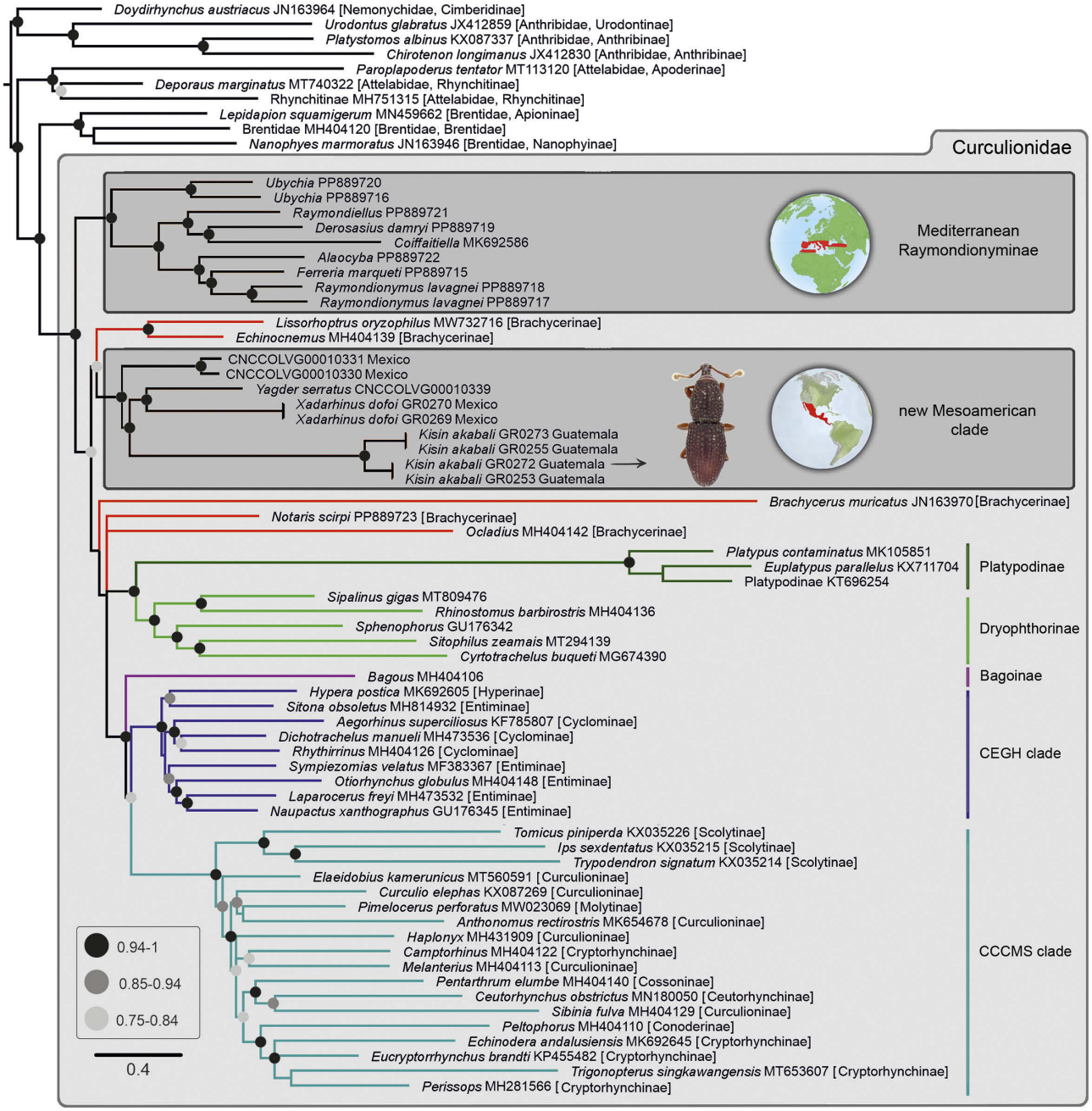


Fig. 4. Bayesian majority-rule consensus tree of Curculionoidea inferred from the AA13 dataset (amino acid sequences of the 13 mitochondrial protein-coding genes; 3,669 amino acid positions; invertebrate mitochondrial genetic code) using PhyloBayes. Analyses were performed under the GTR + CAT model with two independent Markov chains run for 5,000 generations, with the first 2,000 generations discarded as burn-in. Posterior probabilities are indicated at the nodes.

and the CCCMS clade (Conoderinae, Cossoninae, Curculioninae, Molytinae, and Scolytinae). Could the absence of the tectum in the Mesoamerican lineage represent an independent loss relative to the condition observed in the "higher" weevils and, therefore, be a synapomorphy of the members of the Mesoamerican clade? Notably, the same condition has also been documented in the genus *Alaocybites* Gilbert, 1956 (three species: one from California and two from eastern Russia), a similarly eyeless and potentially related edaphic weevil with uncertain phylogenetic placement (Grebennikov, 2010).

We are unable to confidently assign the newly detected clade of eyeless Mesoamerican weevils to any currently recognized subfamily or tribe of Curculionidae. Assignment to either Erirhininae or Erirhinini is not justified. The former subfamily Erirhininae was substantially reduced in both composition and rank by Oberprieler (2014), who treated it as the tribe Erirhinini within Brachycerinae. However, the current circumscription of Erirhinini has not, to our knowledge, been subjected to a phylogenetic test, and its monophyly remains unverified. In our analyses, the tribe Erirhinini is represented by its type genus *Notaris* Germar, 1817 (the current name for the lineage historically

associated with *Erirhinus* Schoenherr, 1825), which is not recovered as sister to the newly detected Mesoamerican clade. Thus, neither the obsolete concept of Erirhininae nor the currently recognized Erirhinini provides a satisfactory placement for the Mesoamerican lineage.

Two alternative taxonomic options warrant consideration. The first is assignment to Brachycerinae: Tanysphirini (= Stenopelmini). This tribe includes *Lissorhoptrus*, which is represented in our dataset and is often recovered (Table 2) as belonging to a lineage weakly supported as sister to our target clade. Nevertheless, Tanysphirini, if monophyletic, include predominantly eyed and volant aquatic weevils ecologically and biologically highly divergent from the soil-inhabiting Mesoamerican lineage.

The second possibility is application of the tribal name Himasthlophallini, which, when used, includes only the monotypic edaphic genus *Himasthlophallus* Egorov & Zherikhin, 1991, from the Russian Far East. Ecologically, this genus is more comparable to our lineage because of its soil-associated lifestyle; however, it was not included in our analyses, and its phylogenetic relationship to the Mesoamerican clade remains unknown.

Given the position of the newly detected Mesoamerican clade outside all currently recognized suprageneric clades of true weevils, and the non-monophyly of Brachycerinae (Fig. 4), the erection of a new subfamily might appear to be a justified taxonomic action. Nevertheless, we refrain from proposing such a change for several reasons. First, our taxon sampling remains incomplete. It lacks several geographically proximate and morphologically similar, small-bodied eyeless genera, including *Bordoniola* and *Neoubychia*, as well as three genera recorded from California: *Gilbertiola* Osella, 1982 (2 spp.), *Schizomicrus* Casey, 1905 (1 sp.), and *Alaocybites*. The phylogenetic affinities of these genera remain unknown; they may or may not be closely related to the Mesoamerican clade identified here or to the Mediterranean clade of Raymondionyminae sensu stricto. Second, our phylogenetic inference is based exclusively on mitochondrial data, representing a single maternally inherited, non-recombining locus. More robust conclusions regarding the evolutionary placement of this lineage will require broader analyses incorporating nuclear markers. Finally, the Mesoamerican clade, as currently delimited, lacks any mapped morphological or other synapomorphies. Consequently, its members remain difficult to distinguish morphologically from other distantly related and similarly small, eyeless, soil-dwelling weevils, such as Raymondionyminae. Given the phylogenetic and nomenclatural uncertainties, and pending the availability of more comprehensive integrative evidence, we adopt the most conservative approach and treat this lineage as Curculionidae incertae sedis.

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