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A New, Flightless Species of *Medon* (Coleoptera: Staphylinidae: Paederinae) from High Appalachia, with Intraspecific Phylogeographic Analysis and Description of its Associated Larva

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Abstract

Medon icarus Caterino, **new species** (Staphylinidae: Paederinae) is described, restricted largely to the highest elevations of the southern Appalachian Mountains, USA. This flightless species occurs across several high ranges in the region, and analysis of COI sequences from known populations reveals deep genetic divergences among them. Insufficient morphological differentiation has been observed to subdivide them, but the possibility remains that this represents a cryptic species complex, with from 5–12 genetically but otherwise indistinguishable members. This is the only member of this mainly western Nearctic and Palearctic genus in the southeastern US, though it appears to be closely related to the northeastern winged species *Medon americanus* Casey. Description of DNA-associated larval specimens, along with adult morphological and molecular information, will help determine its position among global Medonina diversity.

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Introduction

The highest elevations of the southern Appalachian Mountains host a distinctive biota. Above 1,500 masl the typical southeastern deciduous forests give way, in most places, to a conifer forest dominated by Fraser fir (*Abies fraseri* (Pursh) Poir.) and red spruce (*Picea rubra* Sarg.) (both Pinaceae). This forest bears resemblance to, and shares some species with, boreal forests far to the north. However, many taxa live in these southern high forests that are found nowhere else in the world, including a number of lineages that have radiated into diverse species complexes, such as *Trechus* Clairville ground beetles (Carabidae; Barr 1979), *Geostiba* Thomson rove beetles (Staphylinidae; Gusarov 2002), and *Appadelopsis* Gnaspini fungus beetles (Leiodidae; Peck 1973, 1978), among others. While this endemic diversity has drawn some attention from entomologists, the arthropod fauna of high Appalachia is far from fully known, with numerous arthropod species having been described over the past several years (*e.g.*, Draney *et al.* 2019; Hedin and Dellinger 2005; Hedin and Milne 2023; Marek 2010; Marek *et al.* 2018; Park *et al.* 2010; Sierwald *et al.* 2019; Smolis and Bernard 2017) including many Coleoptera (Caterino 2022; Caterino and Vásquez-Vélez 2017, 2022; Ferro 2010; Owens and Carlton 2016; Sokolov *et al.* 2004).

In this paper I describe a new species of Staphylinidae in the Paederinae genus *Medon* Stephens known only from the higher elevations of the southern Appalachians. The assignment of the species to *Medon* might initially be surprising, as nearly all of the native North American diversity of this Holarctic genus is found in the western US (Newton *et al.* 2000). The few eastern species that do exist all occur to the north and northeast—none have been reported from the montane southeast. The generic taxonomy of Medonina, in general, is very inadequately resolved (Assing 2015), despite many years of work on the Palearctic fauna by Volker Assing (Assing 2004, 2006, 2018, 2021). Circumscriptions of American genera and species have received little attention since early descriptions by Casey (1905) and some rearrangements by Blackwelder (1939), and "definitions" of the various genera still rely on variable and questionable characters such as neck width, labral dentition, degree of gular suture separation, and tarsomere length. It is evident that considerable revision in light of phylogeny will still be necessary. Nevertheless, this new species shares significant characters with Palearctic and other US representatives of the genus, most particularly in the "palisade setae", combs of thick, stiff setae, on male abdominal sternite VII. These are conspicuous in the type species, *Medon castaneus* Gravenshorst (= *Medon ruddii* Stephens), and seem a reliable basis for a putative assignment to the group.

At the same time, this new species presents numerous differences from many known *Medon* due primarily to its flightlessness. Thus, it exhibits short, weakly conjoined elytra, reduced eyes, and general facies rather different from typical members of the genus. Its flightlessness, in combination with occurrence primarily in isolated patches of spruce-fir forest, have also resulted in considerable isolation and genetic (COI) divergence among known populations. This initially suggested the potential for this "species" being a cryptic species complex. However, all of these populations are more or less uniform in external and male genitalic morphology. So, while I explore the intraspecific diversity and relationships across the range of this new species, the possibility that multiple species are involved is left for future exploration using a broader selection of genes. A broader survey of all litter-associated arthropods in spruce-fir forests also revealed several larval specimens of this new species, unambiguously associated by DNA sequences. As a result of this, I also describe the larva and compare it to other known larvae of Medonina.

Materials and Methods

All specimens came from recent collections made as part of a study of high Appalachian litter arthropod communities. Nearly all specimens were collected by sifting leaf litter, at sites ranging in elevation from 1,300–2,000 m (~4,500–6,600 ft). Most sites above 1,500 m were from coniferous litter under a red spruce-Fraser fir canopy. At the lower end of this elevational range specimens were also associated with a wider variety of vegetation types, including shrubby "bald" vegetation and deciduous litters of oak, maple, and birch, as well as *Rhododendron* L. (Ericaceae). Litter was sifted through an 8-mm mesh to the soil surface over approximately one square meter. Each sifted sample amounted to approximately 6 L, and precise GPS coordinates were captured for each sample. Samples were extracted in the lab using Berlese-Tullgren funnels until thoroughly dry, approximately 12 h per batch. Specimens were collected directly into 100% ethanol, and moved to −20 °C storage after each subsample was complete. Most specimens are deposited in the Clemson University Arthropod Collection, Clemson, SC, USA (**CUAC**), although some will also be deposited in the National Museum of Natural History, Washington, DC, USA (**USNM**), the Field Museum of Natural History, Chicago, IL, USA (**FMNH**), the American Museum of Natural History, New York, NY, USA (**AMNH**), the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, ON, Canada (**CNCI**), and the Great Smoky Mountains National Park Collection, Gatlinburg, TN, USA (**GSNP**), detailed below.

Morphology was examined using a Leica stereomicroscope, with temporary, glycerin-mount slides of mouthparts, genitalia, and larvae examined using a Nikon compound microscope. Numerous specimens were cleared in conjunction with DNA extractions (detailed further below). The aedeagus was extracted through the abdominal apex following enzymatic tissue digestion. Photographs were taken using Visionary Digital's Passport II imaging system (based on a Canon 6D SLR with 65-mm MP-E 1–5× macro lens). Drawings were penciled by hand, and digitized on a drawing pad into Adobe Illustrator. Larval descriptions were based on five specimens, all cleared using proteinase K and tissue digestion buffers, all vouchered in the CUAC.

Measurements were taken using a Leica M125 calibrated eyepiece micrometer. Head length (HL) was measured from the anterior clypeal margin to the upper anterior edge of the neck constriction (ignoring the neck); head width (HW) was measured at the widest point, near the posterior angles; pronotal length (PnL) was measured along the midline; pronotal width (PnW) was the maximum width, near the anterior corners; elytral length (EL) was measured along the suture from the base of the scutellum to the apex of the suture; elytral width (EW) was the maximum width of the conjoined elytra, near the apices; total abdomen length (AL) was measured in a straight line from the base of tergite III (the first wholly visible) to the apex of tergite VIII (ignoring telescopy and/or curvature); total length (TL) was calculated as head length + pronotum length + elytral length + abdomen length.

About 40 specimens from 23 sampling localities were processed through an NGS barcoding pipeline. Each was imaged, subdivided or punctured to permit tissue digestion, and placed in a separate well in a 96-well plate. Images of morphospecies are archived on the Caterino Lab Flickr page (https://flickr.com/search/?user_id=183480085%40N02&text=medon), identifiable by morphospecies code (site.visit.###, as given in Supplemental File 1). Tissues were digested with lysis buffer and proteinase K (Omega BioTek, Norcross, GA), then the liquid fraction was removed to a new plate. The liquid fraction of the digested tissue mixture was extracted using Omega BioTek's MagBind HDQ Blood and Tissue kit on a Hamilton Microlab Star automated liquid handling system, eluting with 150 µL elution buffer. Remains of extracted specimens were recombined with any non-extracted body parts, labeled, and assigned unique CUAC (Clemson University Arthropod Collection) identifiers.

These analyses included sequences from two sequencing approaches. Mini-barcodes of 421 bp were amplified from the mitochondrial COI gene using the primers BF2-BR2 (GCHCCHGAYATRGCHTTYCC and TCDGGRTGNCCRAARAAYCA; Elbrecht and Leese 2017), corresponding to the downstream two-thirds of the standard barcoding region. Each PCR product was tagged with a unique combination of forward and reverse 9-bp indexes (Meier *et al.* 2016), synthesized as part of the primer by Eurofins Genomics (Louisville, KY). All PCRs were conducted in 12.5-µL volumes (5.6 µL water, 1.25 µL Taq buffer, 1.25 µL dNTP mix [2.5 mM each], 0.4 µL MgCl [50 mM], 1.5 µL each primer, 0.05 µL Platinum Taq polymerase, 1 µL DNA template, with a 95 °C initial denaturation for 5 min, followed by 35 cycles of 94 °C (30 sec), 50 °C (30 sec), 72 °C (30 sec), and a 5-min 72 °C final extension on an Eppendorf Gradient Mastercycler, then sequenced on either Illumina or Nanopore MinION platforms.

For Illumina library preparation, PCR products were combined and purified using Omega Bio-Tek's Mag-Bind Total Pure NGS Kit, in a ratio of 0.7:1 (enriching for fragments > 300 bp). Illumina adapters and sequencing primers were ligated to PCR products using New England BioLab's Blunt/TA Ligase Master Mix. The amplicon + adapter library was again purified using Mag-Bind Total Pure NGS, and subsequently quantified using a Qubit fluorometer. These final libraries were sequenced on an Illumina MiSeq using a v.3 2×300 paired-end kit. For sequences generated on the Nanopore MinION, libraries were prepared using the ligation sequencing kit LSK-112 (Oxford Nanopore Technologies, Oxford, UK), and loaded onto a v10.4 flowcell.

Illumina reads were processed with bbtools software package (https://jgi.doe.gov/data-and-tools/bbtools; v38.87) (Bushnell *et al.* 2017) to merge paired read ends, remove PhiX reads, trim adapters, filter reads for the correct size, remove reads with quality score < 30, cluster sequences by similarity allowing five mismatches (~1%), and generate a final matrix in FASTA format. Nanopore reads were basecalled using the "super-accurate" algorithm of Guppy (v6.1.2), then demultiplexed using ONTbarcoder v0.1.9 (Srivathsan *et al.* 2021), with minimum coverage set at five. FASTA files from all sequencing runs were combined and aligned with the online version of Mafft v7 (Katoh *et al.* 2017) using the auto strategy. All barcode sequences have been deposited in GenBank, with accession numbers listed in Supplemental File 1.

As a preliminary assessment of possible cryptic diversification, COI sequences were analyzed using ASAP (Assemble Species by Automatic Partitioning; Puillandre *et al.* 2021). Kimura 2-parameter distances were used and the top three delimitations were saved, though only the best (lowest ASAP) score results are reported here. Relationships among the sampled populations were analyzed with W-IQ-Tree (Trifinopoulos *et al.* 2016) under maximum likelihood criteria, using its automated model selection to choose GTR + gamma, with empirical base frequencies. Branch support was estimated using ultrafast bootstrapping. A variety of outgroups with available barcode-region sequences were included to root the species tree, including representatives of other Medonina genera (*Pseudomedon* Mulsant and Rey, *Hypomedon* Mulsant and Rey, *Sunius* Stephens, and *Achenomorphus* Motschulsky), the eastern US *Medon americanus* Casey, several Palearctic members of *Medon*, members of a few other tribes of Paederinae (*Astenus* Dejean and *Lathrobium* Gravenhorst), and rooted ultimately by *Pseudopsis* Newman (Staphylinidae: Pseudopsinae). These represent a mix of our own sequences (given in Supplemental File 1) and sequences previously available from GenBank and the Barcoding of Life (BOLD) database.

Results

Phylogeny

Most sampled populations of *Medon icarus*, **new species** were resolved in a single well-supported clade (Fig. 1), although a northeastern lineage was resolved as sister group to *M. americanus.* This non-monophyly of the new species is probably attributable to poor resolving power of COI for deeper divergences. The highly consistent morphology in *M. icarus* populations and significant differences from *M. americanus* (detailed in diagnosis, below), support its recognition. Divergences among populations of *M. icarus* were extremely high, reaching 17% uncorrected p-distance between the most divergent northeastern (Big Bald and Grandfather Mountain) populations and others. The frequency histogram of intraspecific Kimura 2-parameter distances (Fig. 2) reveals the bulk of intra-individual differences to exceed an inferred "barcoding gap" of about 5% corrected divergence. ASAP suggests as many as 8–12 distinct species from these data (putative "species" assignments are shown with sequenced specimens in Supplemental File 1). At present, inadequate morphological divergence has been detected to support such a scenario, but it may guide future work on the population genetics of the group, and the potential non-monophyly of the species also suggests the possibility of further subdivision. Similarly, outgroup relationships made little sense, with members of Medonina and Lathrobiina intermingled. This small fragment of COI is clearly inadequate to resolve deeper, intergeneric relationships, and they are not addressed further.

Relationships among the populations are well-resolved and highly supported. Firstly, a distinct north-south break is observed, with a northern clade of populations from Big Bald, Grandfather Mountain, and the Black and Roan Mountains (those allegedly closer to *M. americanus*), and all populations from southwest of the Asheville Basin comprising a second clade (Fig. 3). In the northern clade, populations from four distinct ranges are grouped into two tight clades, with little differentiation between the Black Mountains and Big Bald populations on the one hand, and between the Roan

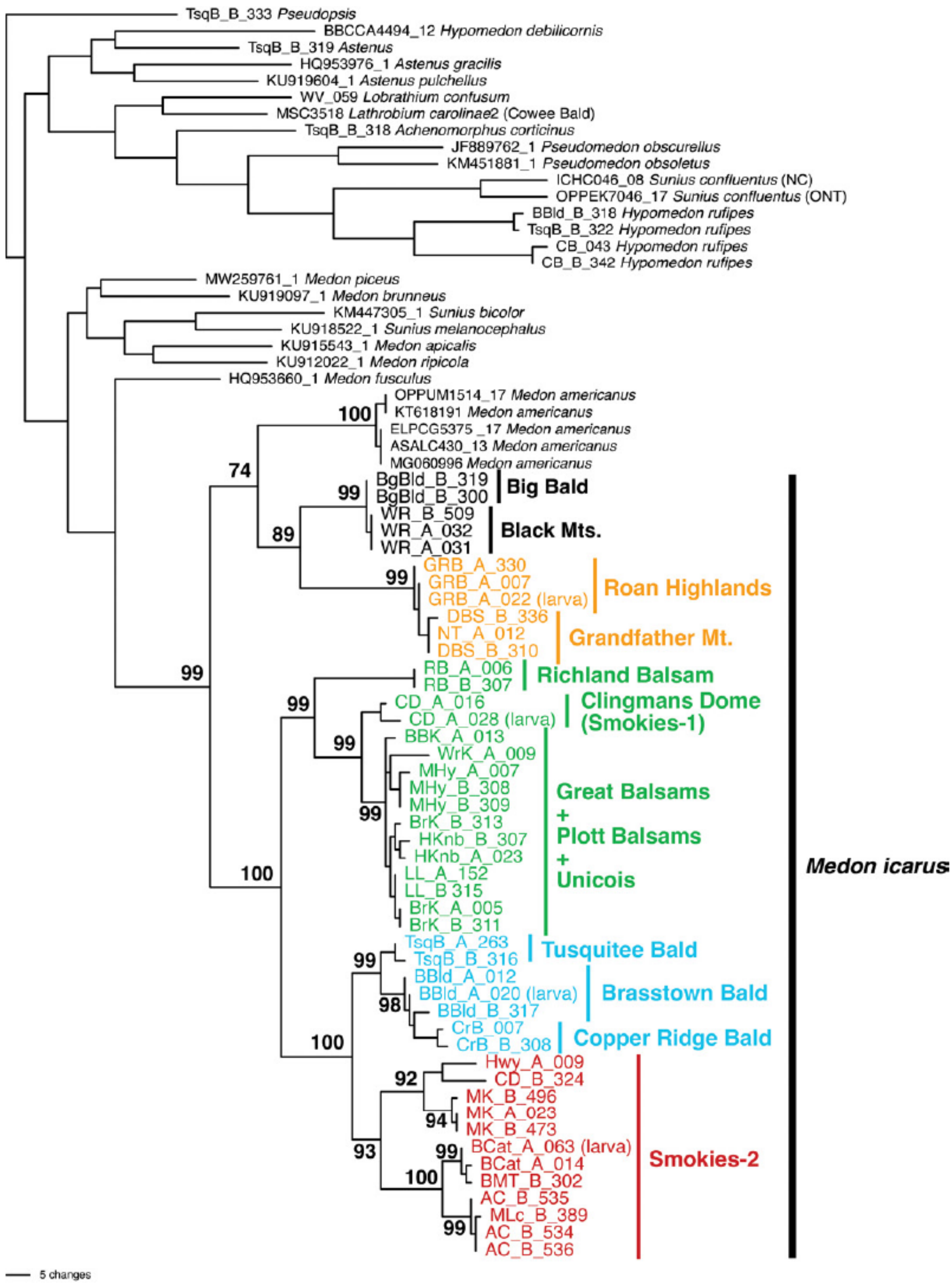

Fig. 1. IQ-tree topology with ultrafast bootstrap values. Colors match colors of symbols on the map in Fig. 3.

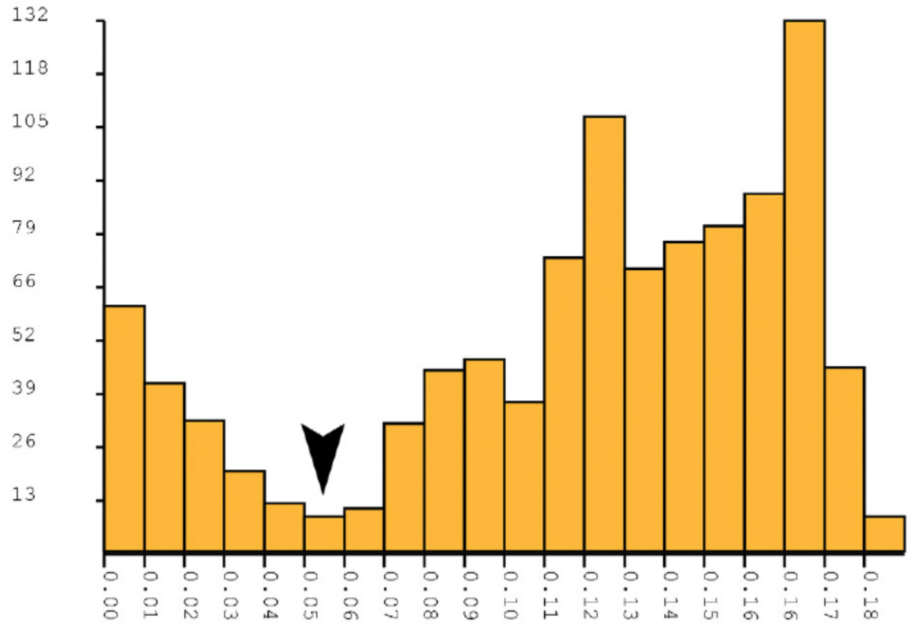

Fig. 2. Frequency histogram of intra-individual Kimura 2-parameter distances, with the "barcoding gap" as inferred by ASAP indicated by the black arrow.

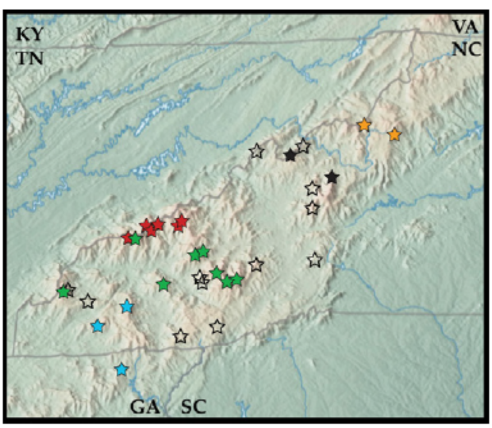

Fig. 3. Map of all known collecting localities for *Medon icarus*, **new species**. Colored symbols refer to colors on cladogram in Fig. 1. Unfilled symbols indicate localities that are not represented by DNA sequence data.

Highlands and Grandfather Mountain populations on the other.

Relationships among southern populations are more complicated. One large clade comprises some individuals from the Great Smoky Mountains (Clingmans Dome), the Plott Balsams (Lyn Lowry, Waterrock Knob, and Browning Knob), the Great Balsams (Mount Hardy, Black Balsam Knob, and Richland Balsam), and Huckleberry Knob in the Unicoi Mountains, far to the southwest of the others. Another rather shallow clade includes populations from Tusquitee Bald and Copper Ridge Bald in the Central Nantahalas, as well as Brasstown Bald, GA, directly to the south. This is sister to another clade comprising only Great Smoky Mountains populations, including Mount Kephart, Mount LeConte, Big Cataloochee, and (again) Clingmans Dome, as well as a number of slightly lower-elevation localities near each of them.

Taxonomy

Subfamily Paederinae Fleming, 1821
Tribe Lathrobiini Laporte, 1835
Subtribe Medonina Casey, 1905
Genus *Medon* Stephens, 1833

***Medon icarus* Caterino, new species**
zoobank.org/urn:lsid:zoobank.org:act:
0E4008C1-B4CB-4401-A1B8-71F03A423F7A
(Figs. 4–8)

Type Specimens. Holotype male: "USA: TN: Sevier Co., 35.6529°N,83.4378°W, SmokyMts NP, 6467', Mt LeConte, vi.25.2019 M.Caterino&M. Ferro, sifted conifer litter" / "Clemson ENT CUAC000079305" / "Caterino DNA voucher, Ext. MSC-3486, Morphosp.MLc.012"; deposited in USNM. **Paratypes** (38; USNM, CUAC, AMNH,

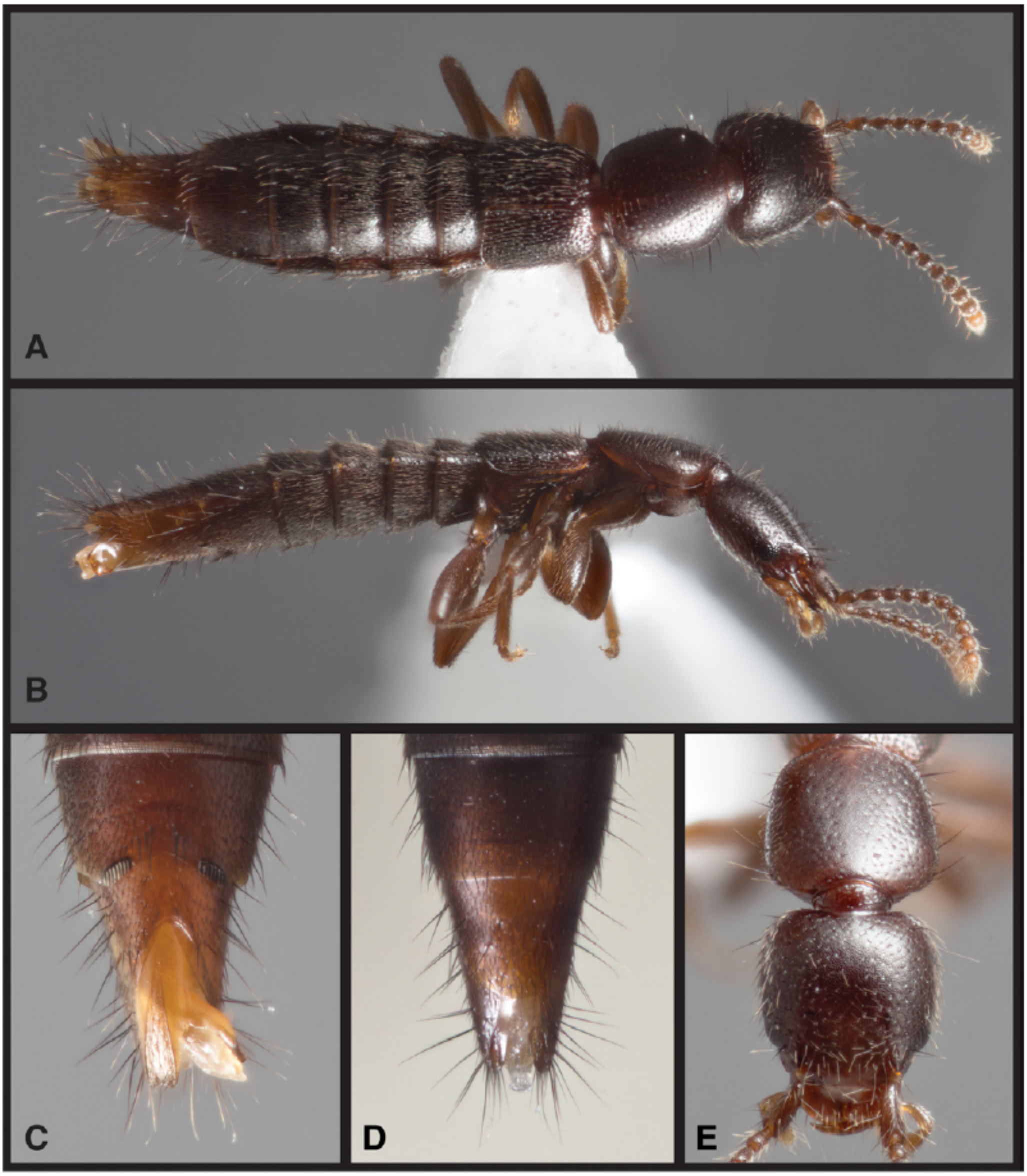

Fig. 4. Habitus and detail of external morphology of adult *Medon icarus*, **new species**. A) Dorsal view, B) Lateral view, C) Ventral view of apex of male abdomen, showing combs of ventrite VII, D) Ventral view of apex of female abdomen, E) Dorsal view of head and prothorax.

CNCI, FMNH): USA: TN: Sevier Co., Great Smoky Mountains National Park, Mt. LeConte, 35.6538, −83.4373, 1,999 m, 25-Jun-2019 (CUAC000176446, CUAC000176447, CUAC000176448, CUAC000176449, CUAC000176450, CUAC000176451, CUAC000176452, CUAC000176453, CUAC000176454, CUAC000176455); USA: TN: Sevier Co., Great Smoky Mountains National Park, Mt. LeConte, 35.6542, −83.4368, 2,006 m, 28-Sep-2021 (CUAC000159987); USA: TN: Sevier Co., Great Smoky Mountains National Park, Mt. Kephart, 35.6311, −83.3895, 1,885 m, 4-Jun-2018 (CUAC000079123); USA: TN: Sevier Co., Great Smoky Mountains National Park, Mt. Kephart, 35.6311, −83.3893, 1,886 m, 14-Sep-2021 (CUAC000156758, CUAC000176435, CUAC000176436); USA: TN: Sevier Co., Great Smoky Mountains National Park, Mt. Kephart, 35.631, −83.3895, 1,894 m,

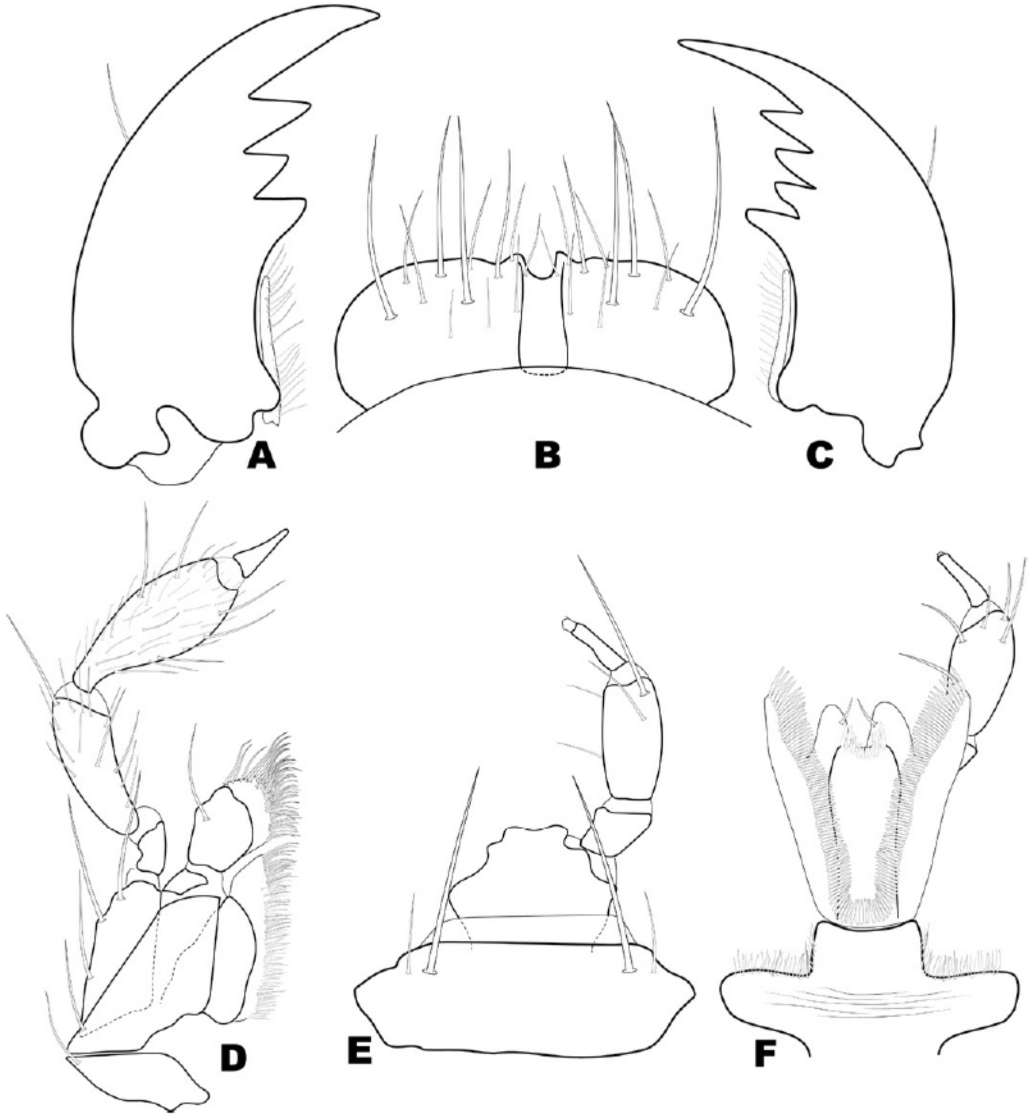

Fig. 5. Adult mouthparts of *Medon icarus*, **new species**. A) Left mandible, dorsal view, B) Labrum, dorsal view, C) Right mandible, dorsal view, D) Right maxilla, ventral view, E) Labium, ventral view, right palp omitted, F) Hypopharynx/ligula of labium, internal view.

14-Sep-2021 (CUAC000156780); USA: TN: Sevier Co., Great Smoky Mountains National Park, Off Hwy 441, 35.6237, −83.4163, 1,394 m, 12-Mar-2020 (CUAC000110840); USA: NC: Swain Co., Great Smoky Mountains National Park, Clingmans Dome, 35.5597, −83.4992, 1,909 m, 14-Sep-2021 (CUAC000156742); USA: NC: Haywood Co., Great Smoky Mountains National Park, Big Cataloochee Mt., 35.6686, −83.1749, 1,745 m, 14-Jul-2020 (CUAC000137686); USA: NC: Haywood Co., Great Smoky Mountains National Park, Big Cataloochee Mt., 35.6724, −83.1761, 1,868 m, 14-Jul-2020 (CUAC000137730); USA: NC: Haywood Co., Great Smoky Mountains National Park, Big Cataloochee Mt., 35.6722, −83.1756, 1,876 m, 5-Nov-2020 (CUAC000135165, CUAC000139637); USA: NC: Haywood Co., Great Smoky Mountains National Park, Big Cataloochee Mt., 35.6727, −83.1762, 1,862 m, 5-Nov-2020 (CUAC000176419, CUAC000176420, CUAC000176421, CUAC-000176422, CUAC000176423); USA: NC: Haywood Co., Great Smoky Mountains National Park,

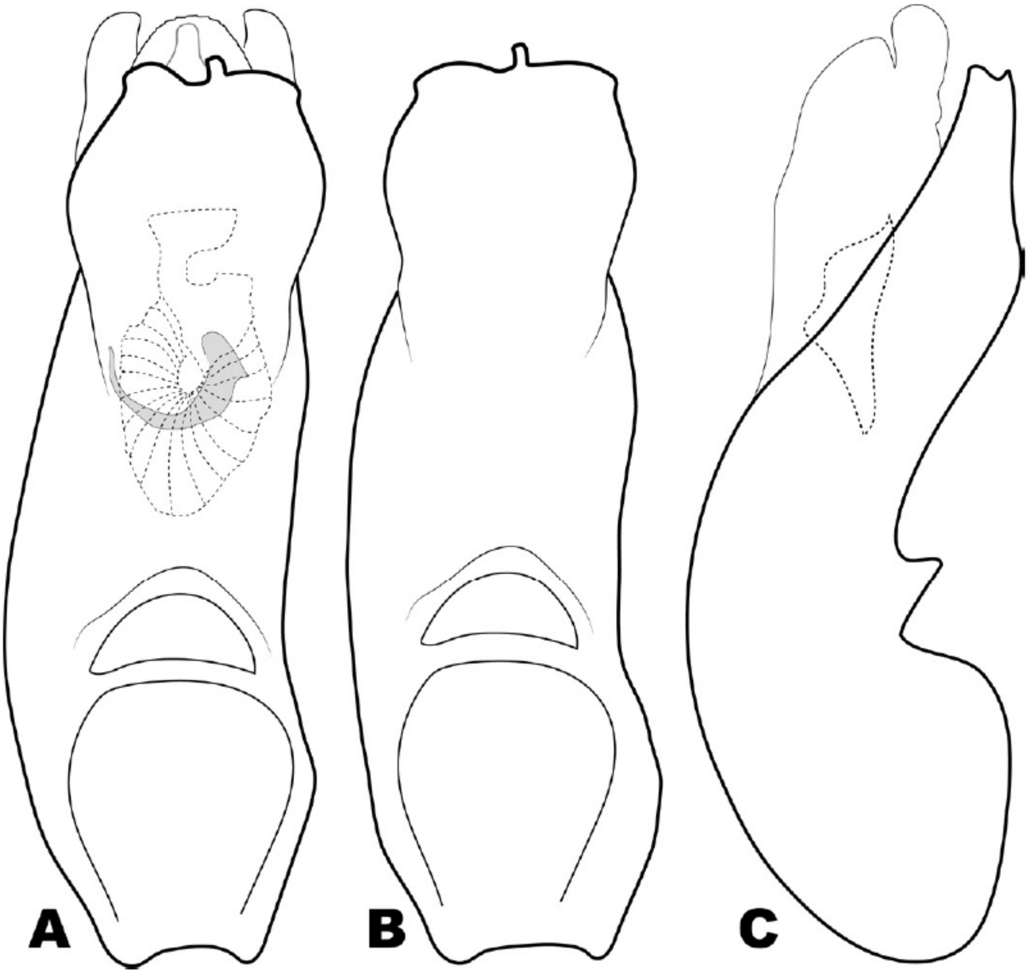

Fig. 6. Aedeagus of *Medon icarus*, **new species**. A) Ventral view of "typical" form, from Clingmans Dome, NC, B) Ventral view of slight variant, from Grassy Ridge Bald, NC, C) Lateral view of "typical" specimen.

Balsam Mt. Tr., 35.6425, −83.2007, 1,575 m, 5-Nov-2020 (CUAC000139752); USA: NC: Haywood Co., Great Smoky Mountains National Park, Balsam Mt. Tr., 35.6453, −83.2025, 1,550 m, 5-Nov-2020 (CUAC000135195); USA: TN: Sevier Co., Great Smoky Mountains National Park, Alum Cave Trail, 35.6427, −83.4426, 1,581 m, 28-Sep-2021 (CUAC000160050, CUAC000160051, CUAC000160052); USA: TN: Sevier Co., Great Smoky Mountains National Park, Alum Cave Trail, 35.6425, −83.4427, 1,584 m, 25-Jun-2019 (CUAC000176424, CUAC000176425, CUAC000176426, CUAC000176427, CUAC000176428, CUAC000176429).

Other Material. NC: Jackson Co., Blue Ridge Pkwy National Park, Richland Balsam Mt., 35.3676, −82.9902, 1,950 m, 8-May-2018; NC: Jackson Co., Blue Ridge Pkwy National Park, Richland Balsam Mt., 35.3627, −82.9885, 1,890 m, 11-Sep-2019; NC: Haywood Co., Pisgah NF, Black Balsam Knob, 35.3258, −82.8777, 1,851 m, 8-May-2018; NC: Haywood Co., Pisgah NF, Black Balsam Knob, 35.3289, −82.8745, 1,839 m, 20-Oct-2020; NC: Jackson Co., Blue Ridge Pkwy National Park, Browning Knob, 35.463, −83.131, 1,871 m, 22-Sep-2020; NC: Graham Co., Nantahala NF, Huckleberry Knob, 35.324, −83.9939, 1,662 m, 4-May-2020; NC: Graham Co., Nantahala NF, Huckleberry Knob, 35.3235, −83.994, 1,680 m, 13-Oct-2020; NC: Graham Co., Nantahala NF, Cherohala Skyway, 35.3171, −83.9833, 1,433 m, 4-May-2020; NC: Jackson Co., Blue Ridge Pkwy National Park, Mt. Lyn Lowry, 35.4645, −83.1131, 1,858 m, 15-Jun-2021; NC: Jackson Co., Blue Ridge Pkwy National

Fig. 7. Lateral habitus views of 1st instar larva of *Medon icarus*, **new species**. A) Pre-extracted, intact larva, B) Cleared larva, following proteinase digestion.

Park, Mt. Lyn Lowry, 35.464, −83.11, 1,891 m, 22-Sep-2020; NC: Haywood Co., Blue Ridge Pkwy National Park, Mt. Hardy, 35.3027, −82.9278, 1,865 m, 14-Apr-2020; NC: Haywood Co., Blue Ridge Pkwy National Park, Mt. Hardy, 35.3036, −82.9269, 1,857 m, 8-Sep-2020; NC: Transylvania Co., Blue Ridge Parkway NP, 35.3042, −82.8885, 1,690 m, 4-Apr-2015; NC: Haywood Co., Blue Ridge Pkwy National Park, Mt. Hardy, 35.3036, −82.9269, 1,857 m, 8-Sep-2020; NC: Haywood Co., Pisgah NF, Mt. Pisgah, 35.4254, −82.7564, 1,726 m, 13-Sep-2022; NC: Jackson Co., Blue Ridge Pkwy National Park, Waterrock Knob, 35.4641, −83.1379, 1,913 m, 29-May-2018; NC: Swain Co., Great Smoky Mountains National Park, Clingmans Dome, 35.5613, −83.5006, 1,940 m, 4-Jun-2018; GA: Towns Co., Chattahoochee NF, Brasstown Bald, 34.8763, −83.8107, 1,385 m, 2-Jul-2020; GA: Towns Co., Chattahoochee NF, Brasstown Bald, 34.8782, −83.8108, 1,346 m, 17-Nov-2020; NC: Macon Co., Nantahala NF, Copper Ridge Bald, 35.2357, −83.5602, 1,568 m, 9-Jul-2019; NC: Macon Co., Nantahala NF, Copper Ridge Bald, 35.2376, −83.5594, 1,545 m, 15-Sep-2020; NC: Graham Co., Joyce Kilmer Forest, jct. Indian & Santeetlah Cks., 35.34501, −83.967, 855 m, 24-Jun-2015; NC: Graham Co., Joyce Kilmer Forest, jct. Indian & Santeetlah Cks., 35.3426, −83.966, 865 m, 24-Jun-2015; NC: Clay Co., Nantahala NF, Tusquitee Bald, 35.1415, −83.7273, 1,582 m, 6-Jul-2021; NC: Clay Co., Nantahala NF, Tusquitee Bald, 35.1419, −83.7269, 1,606 m, 6-Jul-2021; NC: Clay Co., Nantahala NF, Tusquitee Bald, 35.1419, −83.7178, 1,532 m, 1-Sep-2020; NC: Graham Co., Nantahala NF, Teyahalee Bald, 35.2585, −83.7959, 1,597 m, 9-Jul-2019; NC: Macon, Nantahala NF, Jones Gap, 35.0746, −83.2355, 1,042 m, 16-Jul-2015; NC: Caldwell Co., Grandfather Mountain State Park, Daniel Boone Scout Tr., 36.111, −81.7964, 1,558 m, 6-Oct-2020; NC: Mitchell Co., Pisgah NF, Grassy Ridge Bald, 36.1002, −82.0822, 1,865 m, 15-Aug-2018; NC: Mitchell Co., Pisgah NF, Grassy Ridge

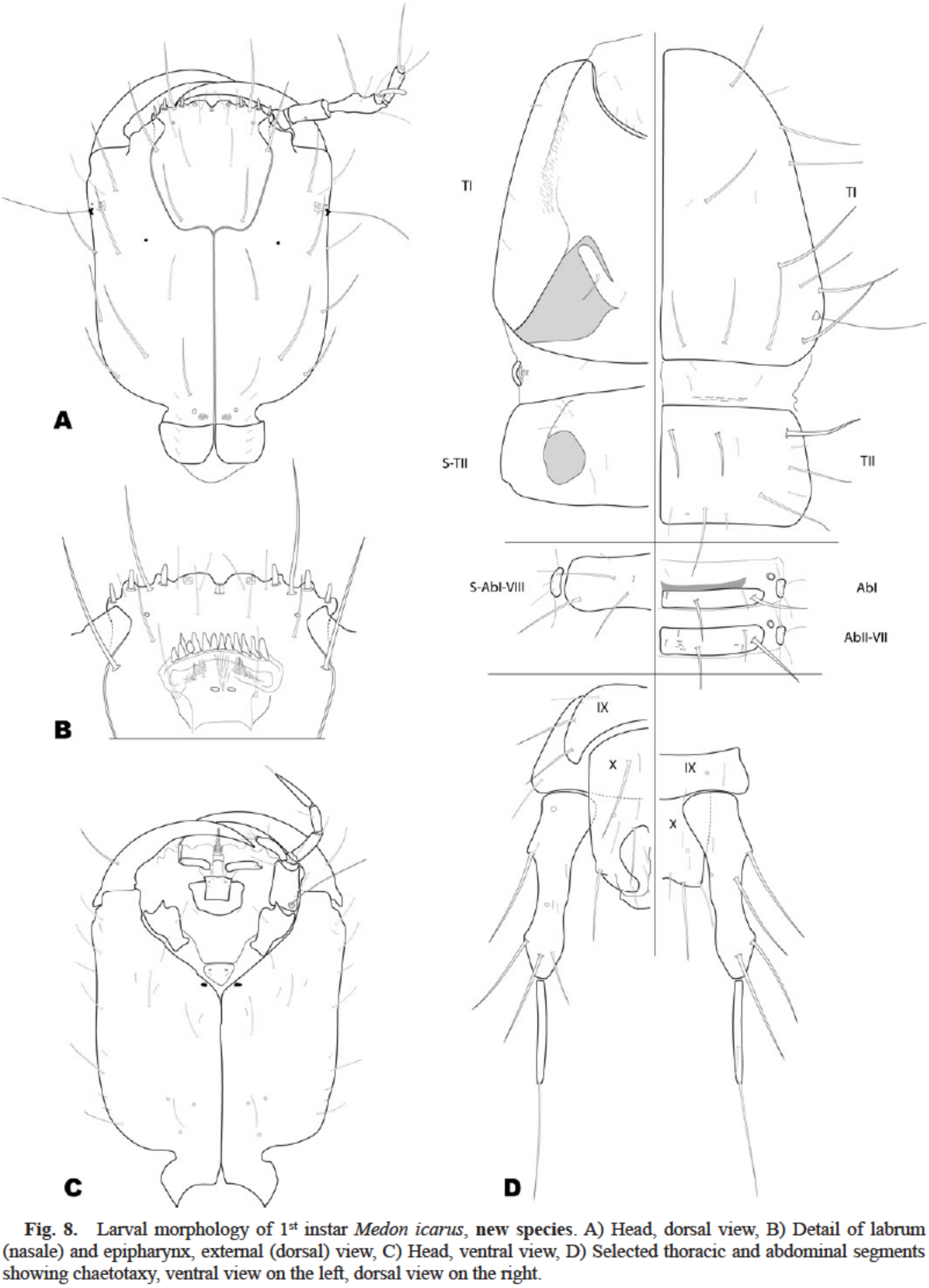

Fig. 8. Larval morphology of 1st instar *Medon icarus*, **new species**. A) Head, dorsal view, B) Detail of labrum (nasale) and epipharynx, external (dorsal) view, C) Head, ventral view, D) Selected thoracic and abdominal segments showing chaetotaxy, ventral view on the left, dorsal view on the right.

Bald, 36.1038, −82.0809, 1,854 m, 8-Jun-2020; NC: Yancey Co., Pisgah NF, Big Bald, 35.9893, −82.4903, 1,651 m, 5-Aug-2020; TN: Unicoi Co., Cherokee NF, Big Bald, 35.99038, −82.49269, 1,606 m, 25-May-2021; NC: Yancey Co., Pisgah NF, Woody Ridge Tr., 35.8448, −82.2377, 1,616 m, 15-Jun-2020; NC: Yancey Co., Pisgah NF, Woody Ridge Tr., 35.844, −82.2347, 1,550 m, 19-Oct-2021; NC: Yancey Co., Pisgah NF, Woody Ridge Tr., 35.8456, −82.2321, 1,397 m, 19-Oct-2021; NC: Jackson Co., Balsam Mt. Preserve, 35.3703, −83.1216, 1,369 m, 15-Jun-2015; NC: Jackson Co., Balsam Mt. Preserve, 35.3699, −83.1216, 1,391 m, 7-Feb-2015; NC: Jackson Co., Balsam Mt. Preserve, 35.3751, −83.0981, 1,671 m, 15-Jun-2015; NC: Jackson Co., Balsam Mt. Preserve, 35.3879, −83.1005, 1,211 m, 16-Jun-2015; NC: Jackson Co., Balsam Mt. Preserve, 35.3753, −83.0982, 1,671 m, 7-Feb-2015; NC: Jackson Co., Balsam Mt. Preserve, 35.3916, −83.1091, 1,007 m, 20-Jun-2015; NC: Buncombe Co., Pisgah NF, Big Butt Trail, 35.8017, −82.3401, 1,626 m, 26-Jul-2015; NC: Buncombe Co., Pisgah NF, Big Butt Trail, 35.8042, −82.3449, 1,488 m, 26-Jul-2015; NC: Buncombe Co., Pisgah NF, Big Butt Trail, 35.7853, −82.3458, 1,798 m, 26-Jul-2015; NC: Buncombe Co., Pisgah NF, Big Butt Trail, 35.7926, −82.3435, 1,709 m, 19-Mar-2016; NC: Buncombe Co., Pisgah NF, Craggy Dome, 35.7061, −82.3666, 1,838 m, 13-Sep-2022; NC: Buncombe Co., Pisgah NF, Craggy Dome, 35.7077, −82.3657, 1,761 m, 13-Sep-2022; NC: Yancey Co., Pisgah NF, Devils Gap, 36.037, −82.431, 1,036 m, 24-Jul-2015; NC: Yancey Co., Pisgah NF, Devils Gap, 36.0442, −82.4279, 1,140 m, 24-Jul-2015.

Description. Total body length: 2.6–3.4 mm; HL 0.51–0.59 mm (avg. 0.55), HW 0.55–0.63 mm (avg. 0.58); PnL 0.53–0.61 mm (avg. 0.56); PnW 0.55–0.59 mm (avg. 0.57); EL 0.41–0.51 mm (avg. 0.48); EW 0.57–0.61 mm (avg. 0.59); AL 1.47–2.25 mm (avg. 1.70); $n = 4$ ♂ + 4 ♀. Coloration (Figs. 4A, B): body darkly rufescent, setose on most surfaces, with longer setae projecting from sides of head, pronotum, and abdomen, particularly posteriorly; apical antennomeres and palpomeres slightly lighter. Head (Fig. 4E) subquadrate, widest to rear, sides weakly convex, posterior angles distinctly rounded, dorsum slightly flattened, more convex ventrally; neck slightly more than one-third maximum head width; gular sutures separated throughout, minimum width of gula just behind middle, approximately one-tenth maximum head width, widening anteriorly and posteriorly; head with conspicuous reticulate microsculpture throughout; dorsal setae of head mostly inclinate toward midline, laterally becoming proclinate, with larger macrosetae projecting near posterior corners and above mandible insertions; a few anteriorly projecting and two cruciate setae on epistoma. Eye small, occupying about one-fourth of lateral side of head, comprising about 30 ommatidia. Antenna rather short, scape shorter than antennomeres 2–4 combined, antennomeres 2–7 slightly longer than wide, gradually widening to penultimate 3 antennomeres being as wide as long, apical antennomeres longer, subacute, all antennomeres setose.

Epistoma transverse, elevated slightly above labrum; labrum (Fig. 5B) short, rounded at sides, thickened at middle, with abrupt but shallow median emargination, with distinct anterior teeth at each side of it, anterior margin otherwise weakly sinuous; mandibles (Figs. 5A, C) strong, each with fimbriate basal prostheca about one-third mandible length; left mandible with strong apical tooth and three median teeth; right mandible with strong apical tooth and four median teeth. Labium (Fig. 5E) with broadly hexagonal mentum, ligula broadened to bases of palpomeres, its inner surface (prementum, Fig. 5F) with continuous, U-shaped comb of stiff setae, apical lobes of prementum extending to apex of basal labial palpomere; inner apex of ligula with apical brush of stout setae, and a pair of slightly longer, fleshy lateral lobes; labial palpomere II twice as long as basal palpomere, bearing long subapical seta on ventrolateral surface, terminal labial palpomere thin, about two-thirds as long as penultimate. Maxillary stipes (Fig. 5D) with one strong seta at outer basal corner, galea and lacinia simply brush-like, without distinct teeth; palpifer with three strong setae along outer margin; basal maxillary palpomere short, curved, palpomere II longer, widening to apex, palpomere III (penultimate) slightly longer than 2, swollen to about 1.5× width of palpomere II, apical palpomere thin, digitiform, just over one-fourth length of palpomere III.

Pronotum (Fig. 4E) about as long as wide, widening from rounded basal corners to front, maximum width near anterior margin; disc with distinct, continuous lateral (and hypomeral) margin, including along anterior, cervical margin; dorsal setae of disc inclinate toward middle, each set in a broad, shallow puncture, impunctate along a narrow median band; lateral margins with 5 prominent projecting setae; disc with densely reticulate microsculpture. Elytra (Fig. 4A) short, midline length about two-thirds combined width, densely setose, all setae posteriorly directed, disc lacking distinct microsculpture; flight wings absent. Abdominal sclerites densely and finely setose, faintly shining.

Protibia slender, with continuous comb of setae along inner margin, margin disrupted in basal half by antennal cleaner; male protarsomeres 1–4 short, widened, with dense, flattened setae beneath; meso- and metatarsi with basal tarsomere only slightly longer than tarsomeres 2–4, apical tarsomere about as long as tarsomeres 3 and 4 combined.

Male sternite VII (Fig. 4C) with a comb of ~8 short, thick palisade setae on each side of shallow median emargination, with sparse, irregular row of longer setae basad the emargination; male sternite VIII more deeply emarginate, with few stronger setae lining emargination; male tergite IX narrowly but deeply emarginate, apices on either side obliquely truncate; male sternite IX elongate, setose, weakly emarginate at apex; female ventrites unmodified. Aedeagus (Fig. 6) with broad basal foramen, sinuously parallel-sided to near the apex, ventral plate flat, widening to subtruncate apex, apical corners weakly acute, slightly recurved, apex with shallow off-center emargination with single, small, blunt median tooth, only very minor variation in shape observed among sites (*e.g.*, Fig. 6B); internal sac (Fig. 6A) with only obscure internal structure, with a weakly coiled basal pouch, an internal "hook" sclerite, and a tri-lobed apex, the median portion with a moderately well-sclerotized inner rod beneath a dimpled, membranous dome.

Adult Diagnosis. Within its limited range, there are very few other Paederinae that could be confused for *M. icarus*. The closest in morphology and range is *Hypomedon rufipes* (Casey, 1905), which is similar in size, coloration, and is also flightless, with short elytra and reduced eyes (and which may, in fact, represent a complex of similar species; unpublished data). Generally, *H. rufipes* occurs at lower elevations than *M. icarus*. However, the two do appear to co-occur at some middle-elevation sites, with *H. rufipes* known up to around 1,300 m, and *M. icarus* as low as ~900 m—both are found in the old-growth Joyce Kilmer Memorial Forest in far western North Carolina, for example (though not yet in any of the same exact samples). Males of the two are easy to distinguish, as *H. rufipes* lacks any palisade setae on sternite VII, though sternite VIII is similarly emarginate. The aedeagus of the two species is completely different, with that of *H. rufipes* elongate, tapered to near the apex, then with a knobbed, blade-like tip. In both sexes the basal metatarsomeres of *H. rufipes* are longer than those of *M. icarus*, nearly as long as metatarsomeres 2 and 3 combined, and the gular sutures are more widely separated at the base, most narrowly separated well forward of the middle of the bottom of the head. Generally, the body coloration of *H. rufipes* is lighter, more rufescent, though both species are somewhat variable. At higher elevations *M. icarus* is found in company with some flightless *Lathrobium* species, but these are significantly larger in body size, and none have the specialized setae of sternite VII.

Medon americanus was described from the eastern US (type locality "Pennsylvania"), but is so far only known from there and northward into Canada, so does not overlap at all in range. It is a somewhat larger, fully winged species with longer elytra, a more strongly quadrate head, a more distinctly toothed labrum, and distinctly transverse waves of microsculpture on the head and pronotum (minutely reticulate in *M. icarus*). Its male genitalia are very similar to those of *M. icarus*, and given the variation in the latter, the two probably cannot be reliably distinguished on that basis. Male sternite VII of *M. americanus* may have more palisade setae (10 vs. 8 in *M. icarus*) although samples have been inadequate to assess the consistency of that difference.

The Palearctic *Medon fusculus* (Mannerheim), *Medon apicalis* (Kraatz), and *Medon ripicola* (Kraatz) have all been reported in North America. but are apparently so far restricted to eastern Canada (Brunke and Marshall 2011; Pentinsaari *et al.* 2019), so would not be encountered in this range. Males of two of these, *M. ripicola* and *M. fusculus*, do have palisade setae on sternite VII, but all three are fully winged and easily distinguished on that basis. In addition, the introduced species all have coarse, umbilicate punctures on the head and pronotum, whereas those of *M. icarus* and *M. americanus* are finer and not umbilicate (A. Brunke, personal communication).

Larva. 1st instar very lightly sclerotized (Fig. 7A), head light brown, legs and sclerites of body light gray, translucent; 2nd instar with head and pronotal sclerite distinctly darker, other dorsal sclerites light brownish gray; head flattened, tapered anteriad (in lateral view), quadrate, sides weakly narrowed just before middle, with setae and pores as shown in Fig. 8; neck less than half as wide as head, bearing patches of egg-bursting setae (Fig. 8A) on either side, and a basal collar; 6 stemmata present behind mandibular insertions, a dorso-ventral arc of 4 in front of an obliquely longitudinal pair; posteriodorsad of stemmata a small pyramidal sensorium present bearing a long trichobothrium, with a dense cluster of minute teeth above; nasale weakly arcuate (Fig. 8B), with 4 blunt teeth on each side, the outer 3 with short, wide basal seta, the innermost tooth broader, short, with circular patch of minute sensory setae near its outer base, the two median teeth separated by a shallow emargination; median emargination with two small, approximate setae beneath, only barely projecting into base of emargination.

Mandibles (Figs. 8A, C) long, falcate, smooth (not serrulate) along inner margin, bearing a single seta near the base on the outer ventral edge. Antenna (Fig. 8A) with sensory appendage on inner margin of antennomere 3 nearly as long as terminal antennomere, characteristically curved laterad. Maxilla (Fig. 8C) with cardo short, widened from base to apex, apical margin with strongly projecting tooth overlapping base of stipes; stipes cylindrical, with conspicuous basolateral trichobothrium; mala digitiform, weakly curved, about as long as maxillary palpomere I; palpomere II slightly shorter than I or

III; palpomere III bearing long terminal sensilla. Apotome of gula weakly separated from largely membranous submentum, bearing 2 small pores. Labium with mentum subquadrate, weakly widened at apex; ligula with wide basal sclerite, with elongate, bulbiform, membranous apex densely fimbriate; labial palpomere I twice as long and twice as wide as palpomere II; palpomere II bearing long sensilla at apex, appearing as a small third segment. Epipharynx (Fig. 8B) without lateral denticles; buccal opening with prominent row of teeth along anterior margin, 5 larger median teeth flanked and partially overlapped by shorter lateral ones; posteriad buccal opening are smaller lateral combs of fine denticles on either side of a sparser longitudinal field of fine, long cuticular processes.

Dorsal sclerites (Fig. 8D) TI–III with ecdysial lines along midline of body; prothorax narrowed to front, with prominent setae along posterior and lateral margins; TI with prominent median discal seta, prominent setae along posterior and lateral margins, and a trichobothrium near the posterolateral corner on a pyramidal base; thoracic tergites II and III similar in size and chaetotaxy, with one median and two lateral dominant setae, as in Fig. 8D; series of very fine cuticular combs present in membrane between terga I and II and between TIII and AbI; tergites and ventrites of AbI–VIII divided along midline, with two small pleural sclerites per segment on each side; AbI with transverse, sclerotized carina along anterior margin of tergite; with similar combs on the median and lateral areas of main dorsal sclerites AbII–VII. Prosternum with dense field of minute setae along lower hypopleural margin; thoracic ventrites with few fine setae, particularly in precoxal area, ventrites I–VIII similar in chaetotaxy, as in Fig 8D; basal segment of urogomphus about 1.5× as long as terminal segment, with prominent lateral setal bases; terminal segment of urogomphus slender, with one short dorsal seta and a single long terminal seta.

Larval Remarks. The larva of *M. icarus* conforms to diagnostic characteristics of Paederinae previously cited (Kasule 1970; Newton 1990; Staniec *et al.* 2022), having three characteristic paired trichobothria on the basal corner of the stipes, on the side of head behind the ocelli, and at the posterolateral corner of the pronotum. It further conforms with diagnoses of Lathrobiini and Medonina presented in Staniec *et al.* (2022), having a relatively large maxillary mala, a bulbiform and fimbriate labial ligula, lack of lateral epipharyngeal denticles, and lacking a median tooth on the nasale. Some of these characters conflict with those described for *M.* "nr. *johni*" in Frania (1986), which is described as having both mesal and lateral epipharyngeal denticles. Frania did not illustrate these for *Medon* specifically, however, so some ambiguity in just what was meant remains. The *M. icarus* larva also does not key directly to *Medon* in Staniec *et al.* (2022), as there is only a single row of teeth along the anterior margin of the buccal cavity. With only two larvae described from a diverse, Holarctic, and questionably monophyletic genus such as *Medon*, little more can be said about generic characterization.

Diagnosis from other co-occurring Paederinae larvae is complicated by generally poor documentation. Newton (1990) illustrated the larval head of *Sunius confluentus* (Say), which is conceivably sympatric with *M. icarus* in some lower elevation localities. They are similar in gross structure, but the *Sunius* mandibles are illustrated as finely serrulate (which is also the case in *Pseudomedon obsoletus* (Nordmann); Staniec *et al.* 2022), whereas the inner margin is smooth in *M. icarus*.

Etymology. The name "*icarus*" calls to mind the proud Greek boy who flew too high and lost his wings, appropriate for this high-elevation, flightless (but proud) beetle. It is to be treated as a noun in apposition.

Discussion

Deep divergences among the populations of *M. icarus* fit the emerging model for flightless arthropods in high Appalachia (Dukes *et al.* 2022; Garrick *et al.* 2017, 2018; Hedin *et al.* 2015; Newton *et al.* 2020), unsurprising for such an ancient, topographically and climatologically complex region. The "true" species diversity for many such lineages remains unclear, as many exhibit extreme genetic divergence, like seen here, in concert with limited or no clear morphological divergence (*e.g.*, Caterino and Langton-Myers 2019; Hedin *et al.* 2015; Newton *et al.* 2020; Thomas and Hedin 2008). Whether these meet biological species criteria, with disjunct and divergent populations remaining reproductively compatible, is only speculative in most cases. Regardless, most lineages qualify as evolutionarily significant units, likely to harbor unique adaptive traits that mirror neutral genetic divergence.

The particular relationships observed among populations of *M. icarus* reflect some common biogeographic patterns. The primary separation of northern and southern lineages across the Asheville Depression is nearly universal in phylogeographic studies in the region, though the putative age of this disjunction has varied, from post-Pleistocene in the range of thousands of years (*e.g.*, Browne and Ferree 2007), to late Pliocene, 5–6 million years ago (Caterino and Langton-Myers 2018, 2019). Divergences in *M. icarus* of > 15% would also put them at the older end of this range. Isolation of a group of populations south of the Little Tennessee River (our Brasstown Bald, Copper Ridge Bald, and Tusquitee Bald populations) is also frequently observed (Hedin and McCormack

2017; Thomas and Hedin 2008). Close relationships between populations of some of the northern pairs of populations (Black Mts. + Big Bald, 25 km across a 700-m valley; Roan Highlands + Grandfather Mt., 24 km across an 1,100-m valley) do not have obvious precedent in the literature. In *Microhexura montivaga* Crosby and Bishop (Araneae: Microhexuridae), populations from these ranges are all on independent, deeply divergent branches, and the sister area of each is different from those of *M. icarus* (Hedin *et al.* 2015). Here, each of these closely related populations would seem to reflect broader historical populations that became separated relatively recently, likely with the most recent retreat of montane habitats to these peaks. Yet all of them remain each other's closest relative at a deeper level.

Intermingling of lineages from the Smokies and Plott Balsams, as we see, is relatively common (*e.g.*, in *Microhexura* Crosby and Bishop: Hedin *et al.* 2015; *Fumontana deprehendor* Shear [Opiliones: Triaenonychidae]: Thomas and Hedin 2008; *Euconnus falcatus* Caterino [Staphylinidae: Scydmaeninae]: Caterino 2022), and it is evident that high-elevation lineages have not perceived significant biogeographic boundaries between these two ranges in recent times. The inclusion of individuals from the Great Balsams (Mount Hardy and Black Balsam Knob) is less common (though see *Desmognathus wrighti* King [Urodela: Plethodontidae]; Crespi *et al.* 2003). But a close relationship of these to populations in the far western Unicoi Mts. (Huckleberry Knob) is without precedent. This location is not only distant in gross terms (50 km minimum, to Clingmans Dome) from any related populations, but spans the Little Tennessee River, which has served as an effective barrier not only for other organisms, but for other lineages of *M. icarus*. We would expect this population to be much more closely related to proximate ones from Tusquitee Bald, Copper Ridge Bald, and north Georgia. That they are not suggests that there are multiple old lineages that have sorted among these ranges, and likely experienced differential extinction, leaving idiosyncratic patterns.

The fact that two highly divergent lineages appear to be sympatric at Clingmans Dome, one from the preceding lineage and one from a Smokies-exclusive lineage, is particularly interesting, and supportive of the cryptic differentiation hypothesis. However, this, again, requires testing with additional markers. With only COI available, it is difficult to say whether this represents reproductively isolated lineages or simply deep intrapopulational haplotype lineages that have come back together at this spot. That this only seems to pertain to one of the Smokies populations, that there are not intermingled representatives of other clades more broadly within that region, is peculiar, and further supports the distinct cryptic lineage hypothesis. In addition to more markers, sequencing additional individuals from populations in the area of overlap would also help to resolve these and other outstanding biogeographic questions.

Part of the biogeographic story of *M. icarus* is its apparent isolation from any ancestral congeners. Based not only on available sequences, but on male genitalic morphology, the closest relative of *M. icarus* would appear to be *M. americanus*. The latter occurring in cooler, higher-latitude localities to the northeast would fit the prevailing model of high Appalachian endemics becoming isolated in relictual cool areas in the south while ancestors retreated further north during post-Pleistocene warming. However, the ages of isolation of *M. icarus* populations seem too old to be consistent with such an origin, and deeper biogeographic events should be considered. Available sequences for the genus, however, remain sparse, with none available for the diverse western North American fauna, and relatively few for that of the western Palearctic. Grossly similar male secondary sexual characters can be seen in either area (Caterino, personal observation; Assing 2004). A number of Palearctic species are flightless, or wing-polymorphic (Assing 2004, 2006), an intriguing commonality, although a number of these are troglomorphic, or fully subterranean, while *M. icarus* remains active in surface litters. Given the unclear, and avowedly confused, relationships between *Medon* and *Sunius* (Assing 2004), further clarity of the relationships of *M. icarus* will have to await a broader global analysis of these (and likely other Medonina) genera.

That such a widespread, locally abundant species has escaped detection until now may seem surprising on the surface. But it is unfortunately indicative of the limited attention small members of the litter arthropod fauna have received, not just in Appalachia, but globally. Many more beetles, and many other arthropods, await proper scientific attention in these diverse habitats.

Supplemental Data (Available Online)

Supplemental File 1. Excel spreadsheet containing specimen-level data for all material of *M. icarus*, **new species** examined, as well as for outgroups used in phylogenetic analyses. Columns include taxonomic information, GenBank accession numbers, type status, localities, dates, project-specific morphospecies codes (searchable on Flickr for photographs of vouchers) and Caterino Lab DNA extraction codes (MSC_NNNN), hypotheses of subdivision of *M. icarus* by algorithmic species delimitation ("ASAP species"), life stage, and repository.

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