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Research article

urn:lsid:zoobank.org:pub:6A5B371D-2A28-43C1-A6C3-12C8E7016515

Phylogenetic position of genera *Acrostilicus* Hubbard and *Pachystilicus* Casey (Staphylinidae, Paederinae) and their redescription

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Abstract. *Acrostilicus* Hubbard, 1896 and *Pachystilicus* Casey, 1905 are North American genera known from only one and two species, respectively, and have never been a subject of a modern revision. In fact, *Acrostilicus* was not even properly described as its author provided only a sketchy diagnosis of the genus and species. Here, we provide a redescription of the genus *Acrostilicus* and its species and illustrate the habitus and male genital features. For the first time, we also redescribe *Pachystilicus* and its two species, and provide their differential diagnoses. Additionally, we tested the phylogenetic position of both genera. They were scored into a morphological matrix supplemented with molecular data and the analyses were run using Bayesian inference and maximum likelihood methods. A total of 119 morphological characters and 4859 bp of nuclear (28S, TP, Wg, CADA, CADC, ArgK) and mitochondrial (COI) sequences were analysed for 46 taxa. The results confirmed that both *Acrostilicus* and *Pachystilicus* are members of the subtribe Stilicina, but at the same time challenged the monophyly of the subtribe in its current composition. Additionally, we provided further evidence for non-monophyly of the subtribe Medonina and discussed the biology of *Acrostilicus* and *Pachystilicus*.

Keywords. Beetles, morphology, systematics, taxonomy, phylogeny, total-evidence analysis.

Żyła D., Tokareva A. & Koszela K. 2022. Phylogenetic position of genera *Acrostilicus* Hubbard and *Pachystilicus* Casey (Staphylinidae, Paederinae) and their redescription. *European Journal of Taxonomy* 819: 1–22.
<https://doi.org/10.5852/ejt.2022.819.1773>

Introduction

Paederinae Fleming, 1821 is one of the most species-rich subfamilies of Staphylinidae Latreille, 1802, with around 7600 described species in more than 225 genera (Newton 2018; Żyła *et al.* 2021). As such a large but still poorly known group, they face the problem of the taxonomic impediment. Although new species are described rather regularly, Paederinae are rarely the subject of modern taxonomic revisions. The vast majority of the known genera and species remains unrevised, and a large proportion of publications did not include photographs or drawings of habitus and genitalia with their descriptions, nor did they attempt to establish the phylogenetic placement of these taxa. Examples of taxa that were originally described more than a hundred years ago and have never been the subject of phylogenetic study are the two exclusively Nearctic genera *Acrostilicus* Hubbard, 1896 and *Pachystilicus* Casey, 1905. *Acrostilicus* was only very recently redescribed based on newly collected material (Brunke & Schnepf 2021) but *Pachystilicus* has never been studied using a modern taxonomic approach.

Acrostilicus are slender beetles with rather unusually long legs and were originally found in burrows made by the gopher tortoise, *Gopherus polyphemus* (Daudin, 1801), in Florida by Hubbard (1896). The description of the new genus and species, *Acrostilicus hospes* Hubbard, 1896, consisted of a single character stating that the labrum has only one tooth, and it was compared to *Stilicopsis* Sachse, 1852 (subtribe Stilicopsina Casey, 1905). Although Hubbard (1896), establishing the new genus and species name, provided a note that he would like to give a description for it, he never fulfilled his plans. Blackwelder (1952) mentioned the species in a catalogue of generic names and included the genus in his key to paederine genera (Blackwelder 1939), where he keyed it to a group of genera that are currently in the subtribe Stilicina Casey, 1905, not Stilicopsina. It was placed in a couplet with *Stiliderus* Motschulsky, 1858, and the presence of a single median tooth on the labrum was the separating character (more than one tooth in *Stiliderus*). The first more detailed morphological description for the genus and its habitus, together with characters in a key, were provided in Moore & Legner's (1979) illustrated guide to the genera of Staphylinidae of America north of Mexico excluding Aleocharinae Fleming, 1821, but Moore and Legner did not provide a species description or a formal diagnoses for the genus or its single species. However, they stated that the genus is distinguished from others by the narrow neck and a single tooth on the labrum and it was again keyed with the same group of genera in Stilicina. Newton *et al.* (2001) included *Acrostilicus* in the key to the Nearctic genera of Stilicina and commented on the poor taxonomic status of the genus, indicating the need for its re-evaluation. In addition to the single tooth on the labrum, Newton *et al.* (2001) also included one additional character to distinguish the genus, i.e., punctures of the head not very dense. Brunke & Buffam (2018) listed the species in their review of the Nearctic rove beetles specialised on the burrows and nests of vertebrates and mentioned its uncertain status. Recently, Brunke & Schnepf (2021) redescribed *Acrostilicus* based on the study of the type material and two recently collected specimens. They confirmed the placement of the genus in Stilicina based on shared morphological characters and tentatively considered the genus as valid pending more detailed phylogenetic study.

More widely distributed, from the Atlantic to Pacific coasts but still poorly collected (Casey 1905), *Pachystilicus* was described by Casey (1905) in his revision of North American Paederini Fleming, 1821. Casey (1905) redescribed and included the species *Stilicus quadriceps* LeConte, 1880 and *Stilicus hanhami* Wickham, 1898 in his new genus, and provided a key for the genus within the new group, Stilici, which refers to the modern subtribe Stilicina (Bouchard *et al.* 2011). It was placed in the couplet with the genus *Stilicus* Berthold, 1827 (currently *Rugilus* Leach, 1819), to which it was suspected to be closely allied, but differed in the more robust habitus, larger and quadrate head, deeply sinuate at the base, finer punctuation, and much shorter and thickened legs (Casey 1905). The first biological data and morphological illustrations of the genus were provided by Wickham (1898) for *Pachystilicus hanhami*, which was collected from ant nests in Manitoba, Canada. The type species of the genus was later fixed

as *Stilicus quadriceps* by Lucas (1920). Further, the genus was also included in Blackwelder's key (1939), again in a couplet with *Stilicus*. However, the characters separating them were different from those suggested by Casey (1905) and were as follows: head emarginate at the base, labrum with median teeth separated by twice their average width, notch rounded, pronotum punctured very differently from the head. Moore & Legner (1979) used the last character to separate *Pachystilicus* from *Rugilus* in their key and provided a habitus illustration and updated description for this genus. They also noted that both species were found with ants, which was the first such mention for *P. quadriceps* as LeConte (1880) in his original description wrote that all *Stilicus* species that he collected were found on the seashore under seaweed, cast up by the waves. This information was not confirmed later by Newton *et al.* (2001) who stated that the biology of *Pachystilicus quadriceps* was unknown, and only *P. hanhami* was confirmed to be found in ant nests. Newton *et al.* (2001) again keyed the genus together with *Rugilus* and used the same characters as Blackwelder (1939), adding Casey's (1905) "robust habitus" character. Frania (1986), in his study on several Stilicina genera, suggested that *Pachystilicus* is closely related to or congeneric with *Rugilus*. No further studies were undertaken on this genus or any of its species.

The uncertain taxonomic status of both genera, partly resulting from an unclear separation from the diverse genus *Rugilus*, motivated us to redescribe each species of *Pachystilicus* and supplement the recent redescription of *Acrostilicus*, as well as conducting a phylogenetic analysis to confirm their assignment to the subtribe Stilicina. In this study, we provide the habitus photographs and illustrations of the male genitalia of all three species, as well as the results of a total-evidence phylogenetic analysis, where both genera were included in the morphological partition of the data matrix.

Material and methods

Examination and deposition of taxa

Specimens of the redescribed taxa were studied using a Nikon SMZ1500 and an Olympus SZX9 stereo microscopes. All measurements are given in millimetres and were made with an ocular micrometer mounted on a stereoscopic microscope.

Abbreviations for measurements are as follows:

- EL = elytra length
- EW = elytra width
- HL = head length
- HW = head width
- PL = pronotum length
- PW = pronotum width

A Nikon DS-Fi1 camera was used for taking pictures of habitus, and photographs were stacked using Zerene Stacker software (ver. 1.04, Zerene Systems LLC, Richland, Washington State, USA, 2009). The pictures were further edited in Adobe Photoshop CS6, while illustrations were made in Adobe Illustrator CS6 (ver. 16.0.0, Adobe Systems Incorporated, San Jose, California, USA, 2007) based on a photograph and observations. Specimens were relaxed in warm water and dissected. Morphological terminology follows Bogri *et al.* (2020). Data from the specimen labels are cited verbatim (text between double quotes, ""), with original spelling retained. A slash '/' separates labels for each specimen. Additional morphological observations on North American Stilicina were made during the visits of the first and last authors to the respective collections, without dissecting the specimens. Whenever possible, the type specimens were studied for comparison. If no actual specimen was present in the collection, the original descriptions were used (Fall 1901; Casey 1905; Fall & Cockerell 1907). Information on the status and repositories of all specimens used for such comparison is given in Supp. file 1.

Material from the following collections was used in the current study and its currently deposited there:

AMNH	=	American Museum of Natural History, New York City, New York, USA (Lee Herman)
CNC	=	Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada (Adam J. Brunke)
MCZ	=	Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA (Crystal Maier)
NHM	=	Natural History Museum of London, London, United Kingdom (Maxwell V.L. Barclay)
NHMW	=	Natural History Museum of Vienna, Vienna, Austria (Harald Schillhammer)
NMNH	=	National Museum of Natural History (former USNM), Smithsonian Institution, Washington, USA (the late Terry Erwin, Charyn Micheli, Floyd Shockley)
SEMC	=	Snow Entomological Museum Collection, Biodiversity Institute, University of Kansas, Lawrence, Kansas, USA (Zach Falin)
TAMUIC	=	Texas A&M University Insect Collection, College Station, Texas, USA (John Oswald, Karen Wright)

Most of the specimens used for phylogenetic analysis were scored as in Żyła *et al.* (2021). Those that were newly scored into the matrix were relaxed in warm water and dissected. Information on the repositories of all taxa used for phylogenetic analysis is given in Supp. file 2.

Taxon sampling and outgroup for phylogenetic analyses

Representatives of all currently recognised tribes of Paederinae were included in the analysis. Since the main goal of our research was testing the phylogenetic position of *Acrostilicus* and *Pachystilicus*, we increased the number of representatives of the subtribe Stilicina, where they are traditionally classified. We also scored more representatives of the subtribe Medonina Casey, 1905 as it is potentially closely related to the genera under study (Żyła *et al.* 2021). In total, 46 taxa were included in the final combined dataset. Both molecular and morphological data were available for most representatives, except for 10 taxa for which only morphological characters were included in the matrix, including four Stilicina (*Acrostilicus hospes* Hubbard, 1896, *Megastilicus formicarius* Casey, 1889, *Pachystilicus hanhami* (Wickham, 1898), *Panscopaeus lithocharoides* (Sharp, 1889)); two representatives of Medonina (*Deroderus* Sharp, 1886, *Ecitocleptis* Borgmeier, 1949); and four genera classified as Lathrobiini Laporte, 1835 incertae sedis (*Micrillus* Raffray, 1873, *Mimophites* Fauvel, 1904, *Scymbalium* Erichson, 1839, and *Synecitonides* Reichensperger, 1936). We chose representatives of the subfamily Staphylininae Latreille, 1802 as the closest related outgroup and Tachyporinae MacLeay, 1825 as a more distantly related outgroup.

Morphological characters

We constructed the morphological matrix in Mesquite ver. 3.5 (Maddison & Maddison 2018) using 119 characters, which were primarily derived from the matrices of Bogri *et al.* (2020) and Żyła *et al.* (2021). Unknown character states were coded using ‘?’, while inapplicable states were marked as ‘-’. The list of characters is provided in Supp. file 3. The nexus file containing the character matrix is available as Supp. file 4 and in MorphoBank (project no 4067) under this permalink: <http://morphobank.org/permalink/?P4067>.

GenBank data

To construct our molecular matrix, we used seven gene fragments: the nuclear protein-encoding genes carbamoylphosphate synthetase (CADA and CADC), topoisomerase I (TP), arginine kinase (ArgK), and wingless (Wg), the mitochondrial protein-encoding cytochrome c oxidase I (COI), and the nuclear ribosomal 28S. The Genbank accession numbers of all sequences are given in Supp. file 2. All sequences

were already used in Żyła *et al.* (2021); thus, the amplification, sequencing, sequence editing and assembly protocols are described there.

Sequence alignment

Sequences were newly aligned in Geneious ver. 9.1.7 (Biomatters Ltd, Auckland, New Zealand, 2005) using the MAFFT plugin ver. 1.3.6, based on MAFFT (Kato *et al.* 2002). 28S was aligned using the E-INS-i algorithm of MAFFT, and ambiguously aligned regions were identified and removed with the server version of Gblocks (Talavera & Castresana 2007). We allowed gap positions within the final blocks and less strict flanking positions but did not allow many contiguous non-conserved positions. The resulting 28S alignment was 866 bp and had very few scattered and, usually, single-nucleotide gaps. Individual gene alignments were concatenated with the ‘concatenate’ function of Geneious. The concatenated sequence alignment is provided in Supp. file 5 in fasta format.

Data matrix and partitioning

Our combined matrix of molecular (4982 bp) and morphological (119 characters) data for the total number of taxa under study (46) was analysed using Bayesian inference (BI) and maximum likelihood (ML). For the molecular data matrix, the alignment was initially partitioned by gene and, for protein-encoding genes, by codon position. The optimal partitioning scheme and the corresponding models of nucleotide evolution were determined by PartitionFinder ver. 2.1.1 (Lanfear *et al.* 2016) using the Bayesian Information Criterion running on CIPRES Science Gateway ver. 3.3 (Miller *et al.* 2010). Models for MrBayes and IQ-TREE were considered, branch lengths were unlinked, and the search was set to the ‘greedy’ algorithm (Lanfear *et al.* 2012). The morphological data in the combined matrix were analysed as a single, separate partition using the maximum likelihood model for discrete morphological character data, under the assumption that only characters that varied among taxa were included (Mkv) (Lewis 2001).

Phylogenetic analysis

Bayesian analysis was performed using MrBayes ver. 3.2.6 (Ronquist *et al.* 2012) running on CIPRES. The analysis used four chains (one cold and three heated) and two runs of 30 million generations with default prior settings, except for the temperature, which was set to ‘temp = 0.08’ for better mixing. The analysis was conducted with a gamma distribution for the morphological partition, and autapomorphic characters were included. The third COI codon positions were excluded as in previous studies (e.g., Żyła *et al.* 2021). A script for the combined analysis in MrBayes is given in Supp. file 6 and is also available at https://github.com/DagmaraZyła/Acrostilicus_Pachystilicus.

The convergence of both runs was assessed in Tracer ver. 1.7.1 (Rambaut *et al.* 2018), as well as by the examination of Potential Scale Reduction Factor (PSRF) values and Average Standard Deviation of Split Frequencies in the MrBayes output.

Maximum likelihood (ML) analysis was performed using IQ-TREE ver. 2.0.7 (Minh *et al.* 2020) with the same set of partitions. Node support was evaluated by 1000 ultrafast bootstrap replicates (UFB) (Hoang *et al.* 2018) (command line: `iqtree2 -p scheme.nex -B 1000 -nt AUTO`).

Trees were examined in FigTree ver. 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) and later edited and annotated in Adobe Illustrator CS6. Clade support was estimated by BI posterior probability (PP) and ultrafast bootstrap approximation (UFB) in ML. Nodes with PP > 0.80 and UFB > 95 were considered well supported, nodes with PP = 0.70–0.79 and UFB = 80–94 were considered to be weakly supported and nodes with PP < 0.70 and UFB < 80 were considered unsupported.

Results

Phylogenetic analyses

PartitionFinder found the following five partitions: 1) 28S; 2) TP2, CADC2, CADA2, COI2, ArgK2, COI1, ArgK1, Wg2, Wg1, TP1, CADC1, CADA1; 3) Wg3, ArgK3, TP3; 4) CADC3, CADA3 and 5) COI3. In the case of MrBayes, GTR+I+G was found to be the best supported model for the first four partitions. For the last one containing the third codon positions of COI, the HKY+G model was selected as the best supported. For IQ-TREE, GTR+I+G was also found to be the best supported model for the first two partitions. For the third and fourth partitions, TVM+I+G and TRN+I+G were selected, respectively. For the fifth one, the HKY+G model was also found as the best supported. However, this partition was excluded in both analyses as it has been shown that it can potentially bias phylogenetic analyses (e.g., Swofford *et al.* 1996; Lin & Danforth 2004). Our independent Markov chains converged on the same stationary distribution as visualized in Tracer ver. 1.7.1, and both combined and individual traces were inspected. The effective sample size (ESS) values were greater than 200 for all parameters indicating good mixing of the chains. The tree topology presented in Fig. 1 is the 50% majority-rule consensus tree of BI analysis with support values of both BI and ML.

Both BI and ML analyses showed almost identical topologies (Fig. 1, Supp. file 7, respectively). The subfamily Paederinae was recovered as monophyletic with strong support (PP = 1, UFB = 100), as well as all currently recognised tribes of Paederinae, i.e., Paederini, Pinophilini Nordmann, 1837, and Lathrobiini (PP = 0.99, UFB = 100; PP = 1, UFB = 100; PP = 0.76, UFB = 93, respectively). The tribe Lathrobiini was found as sister to Pinophilini with strong support in BI (PP = 0.80) and weak support in ML (UFB = 85).

Several subtribes within the tribe Lathrobiini were resolved as non-monophyletic. The two genera *Micrillus* and *Scymbalium* were the first clade that branched off (PP = 0.97, UFB = 95) as a sister group to all other Lathrobiini (PP = 0.76, UFB = 93). Both these genera are currently included in Lathrobiini incertae sedis (Bogri *et al.* 2020). The second clade to branch off was also supported (PP = 0.99, UFB = 88) and consisted of *Dysanabatium* Bernhauer, 1915 and *Notobium* Solsky, 1864, currently classified in Lathrobiina and sister to the rest of Lathrobiini (PP = 0.99, UFB = 93). Next, in both analyses, the genus *Pseudolathra* Casey, 1905 (Lathrobiini incertae sedis after Żyła *et al.* 2021) and the subtribe Cylindroxystina Bierig, 1943 were resolved within the tribe Lathrobiini as sister to each other (PP = 1, UFB = 95). In the BI tree, this clade was recovered as sister (P = 0.93) to the well supported ‘true’ Lathrobiina (PP = 1, UFB = 100), which was comprised of four taxa, and altogether were recovered as sister (PP = 1) to the well-supported ‘Medonina and allied taxa’ clade (PP = 1, UFB = 100). In this part of the tree, the ML analysis resulted in a different topology, and the clade was resolved as sister directly to the ‘Medonina and allied taxa’ clade but with no support.

Within the ‘Medonina and allied taxa’, the first clade to branch off was well-supported (PP = 1, UFB = 100) and consisted of *Enallagium* Bernhauer, 1915 (Lathrobiina), an unidentified genus of Medonina from Far East Russia and *Scopaeus* Erichson, 1839 (*Scopaeina* Mulsant & Rey, 1878). The subtribe Medonina was resolved as not monophyletic and its members were recovered in a few positions on the tree. The genus *Pseudomedon* Mulsant & Rey, 1878 was resolved in an isolated position as sister (PP = 1, UFB = 100) to the remaining Lathrobiini recovered in three clades. The first one in BI contained the subtribe Echiasterina Casey, 1905 sister to Astenina Hatch, 1957 + Stilicopsina (PP = 1, UFB = 100), while in ML the clade of six Medonina taxa was recovered as two subclades (PP = 0.77, UFB = 76 and PP = 1, UFB = 100, respectively). The sister group relationships of these Medonina taxa were unresolved in BI, while recovered as sister to the rest of ‘Medonina and allied taxa’ clade in ML without support. The Stilicina genus *Panscopaeus* Sharp, 1889 was resolved in isolated positions: in the BI tree as sister to the clade containing three Medonina species + Lathrobiini incertae sedis and Stilicina (with

no support); or in the ML tree as sister (UFB = 99) to the clade containing the above-mentioned taxa and representatives of the subtribes Echiasterina, Astenina and Stilicopsina. The final clade consisted of Stilicina and several other taxa resolved inside the subtribe, thus rendering Stilicina as polyphyletic with no support. *Acrostilicus* and *Pachystilicus* were recovered inside a clade consisting of six Stilicina taxa (PP = 0.79, no support in ML). The position of *Pachystilicus* remained unresolved inside this clade, while *Acrostilicus* was resolved as sister to the genus *Rugilus* (PP = 0.78, no support in ML).

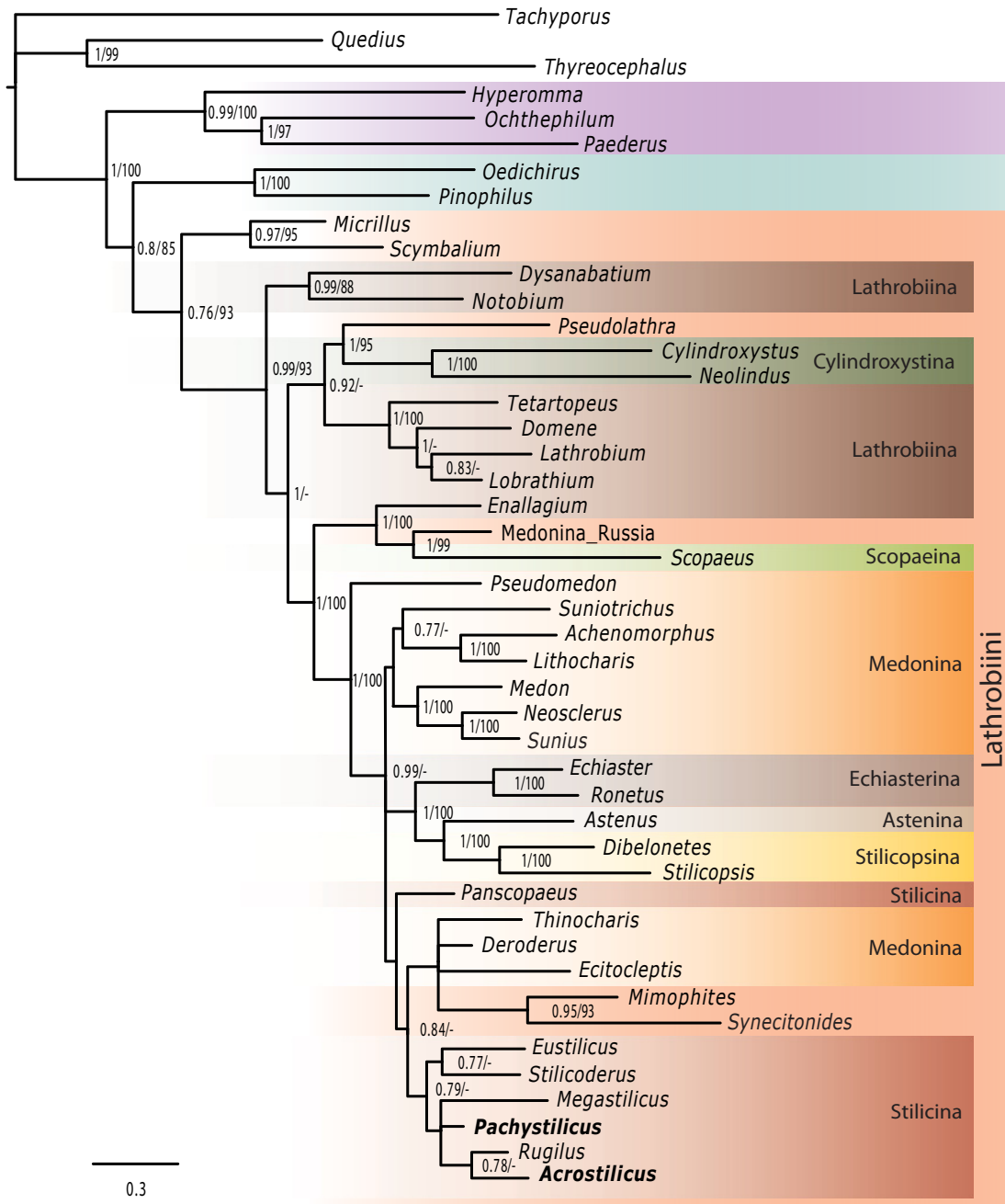


Fig. 1. 50% majority-rule consensus tree from a Bayesian analysis of combined molecular and morphological datasets. Posterior probabilities (PP) and UFB values >70 are shown near the corresponding nodes in PP/UFB format. A hyphen (-) refers to a lack of support in the result of certain analyses. Tribes and subtribes of Paederinae Fleming, 1821 are highlighted in colour.

Taxonomy

Class Insecta Linnaeus, 1758
Order Coleoptera Linnaeus, 1758
Family Staphylinidae Latreille, 1802
Subfamily Paederinae Fleming, 1821
Tribe Lathrobiini Laporte, 1835
Subtribe Stilicina Casey, 1905

Genus *Acrostilicus* Hubbard, 1896
Fig. 2

Acrostilicus Hubbard, 1896: 229 (original designation).

Acrostilicus – Blackwelder 1939: 107; 1952: 37 (notes). — Moore & Legner 1979: 11 (key), 100 (description). — Frania 1986: 368 (comment). — Poole & Gentili 1996: 370. — Newton *et al.* 2001: 327 (characters in key), 387 (comment on taxonomic status). — Brunke & Buffam 2018: 154 (note on biology). — Brunke & Schnepf 2021: 885 (redescription).

Type species

Acrostilicus hospes Hubbard, 1896.

Diagnosis

As in species redescription.

Redescription

As is species redescription.

Acrostilicus hospes Hubbard, 1896
Fig. 2

Acrostilicus hospes Hubbard, 1896: 229 (original description).

Acrostilicus hospes – Blackwelder 1939: 107, 117; 1952: 37 (notes). — Moore & Legner 1979: 101. — Frania 1986: 368. — Newton *et al.* 2001: 387 (comment on taxonomic status). — Brunke & Buffam 2018: 154 (note on biology). — Brunke & Schnepf 2021: 888 (redescription).

Diagnosis

The species and genus can be differentiated from all other Stilicina based on the combination of the following characters: the presence of a single median tooth on the labrum, mandibles symmetrical, posterior margin of head rounded, and legs elongated.

Type material

Lectotype (designated here)

USA • ♂; “Clearwater, 27.6 Fla / CollHubbard and Schwarz / Gopher / Cotype No 22508 U.S.N.M / *Acrostilicus hospes* Hubbard”; NMNH.

Paralectotypes

USA • 2 ♀♀; “Clearwater, 27.6 Fla / Coll Hubbard and Schwarz / Cotype No 22508 U.S.N.M; Funiak 4.7 Fla / Coll Hubbard and Schwarz / Cotype No 22508 U.S.N.M.”; NMNH.

Redescription

MEASUREMENTS. Medium sized (whole body length: 4.4–4.7 mm);

HABITUS. Slender, elongated, with long legs. Integument shining, with coarse, umbilicate sculpture. Head and pronotum differently punctured.

COLOURATION. Pale brown/sandy with darker head tip (Fig. 2A).

HEAD. Orbicular (HL: 1–1.2 mm; HW: 0.85–0.9 mm), widest in middle, wider and longer than pronotum, temples rounded and more than $\frac{1}{2}$ of head length, posterior margin rounded, not emarginate, disk rugose, integument smooth and shining. Eyes large, about $\frac{1}{4}$ of head length, $\frac{1}{2}$ of temple length, shifted anteriorly, slightly protruding, with setae between ommatidia. Antenna 11-segmented; antennomeres 1 and 2 with sparse pubescence; antennomeres 3 and 11 more densely pubescent, with tomentose pubescence. Antennomere 1 elongate, widest at tip; antennomeres 2–6 elongate; antennomere 7 weakly elongate; antennomeres 8–10 gradually becoming transverse; antennomere 11 elongate, $1.5\times$ as long as antennomere 10. Clypeal margin straight. Labrum large, transverse, twice as wide as long or wider, expanded, covering mandibles when closed, weakly sclerotised, with single short median tooth; six long, pale setae on anterior margin, evenly distributed from one edge to another, and multiple shorter setae, organised into two rows. Mandible without prosthema, with three teeth on both right and left mandible (largest one closest to base). Maxillary palpus 4-segmented; maxillary palpomere 1 short; maxillary palpomere 2 longer than wide, slightly expanded towards apex, with only few setae; maxillary palpomere 3 longer than palpomere 2 and as wide as it, slightly expanded towards apex with denser

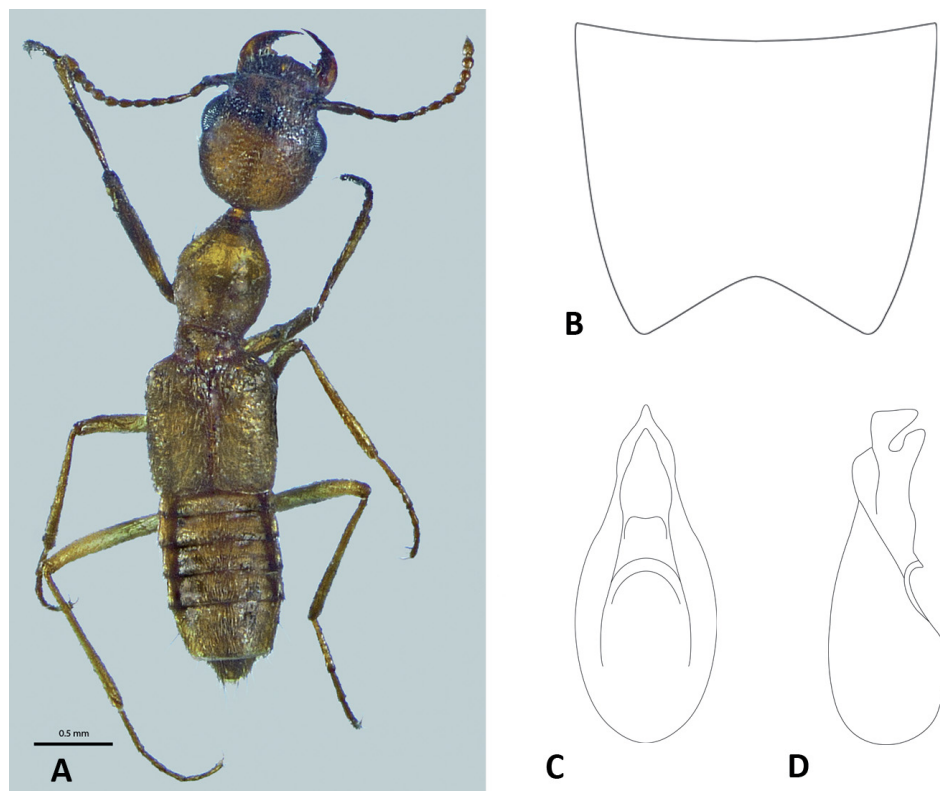


Fig. 2. *Acrostilicus hospes* Hubbard, 1896, habitus photograph and drawings of the apical abdominal sternite and genital structures. **A.** Habitus, lectotype, ♂ (NMNH). **B.** Male sternite VIII. **C.** Aedeagus, parameral view. **D.** Aedeagus, lateral view. Scale bar = 0.5 mm.

pubescence, vase-like; maxillary palpomere 4 small, acicular, glossy, and thin, equal to or shorter than width of palpomere 3. Labial palpus 3-segmented; labial palpomere 1 longer than wide; labial palpomere 2 more than twice as long as wide, wider than labial palpomere 1; labial palpomere 3 thin, much shorter and slightly narrower than labial palpomere 2. Mentum transverse, rectangular. Submentum with pair of setae on each side. Ligula entire, not bilobed, dorsal plate without setae. Gular sutures fully fused, not reaching posterior margin of head. Neck narrow, less than $\frac{1}{5}$ of head width.

THORAX. Pronotum longer than wide (PL: 0.8 mm; PW: 0.6–0.7 mm), with anterior angles obtuse, narrowed in front, widest in middle, disc shiny, coarsely and sparsely punctured, punctures sparser than on head, evenly distributed. Basisternum of prosternum long, with longitudinal carina, prolonged behind coxa and slightly expanded laterally but not connected to hypomerion, without macrosetae or microsculpture, but surface wrinkled. Furcasternum of prosternum longer than $\frac{1}{2}$ of basisternum length, triangular, acute, with sharp longitudinal carina and transversal carina. Hypomerion not delimited from pronotal disk by carina. Furcasternum of mesosternum with longitudinal carina, short, reaching $\frac{1}{3}$ of distance between coxae, triangular. Elytra quadrate, longer and wider than pronotum (EL: 0.95–1 mm; EW: 0.85–0.9 mm), without epipleural ridge, without row of setae on edge of posterior margin and without stiff upright bristles; humeral angle indistinct, rounded; surface shiny, covered with setae not organised in distinctive rows. Scutellum without ridges, moderate, impunctate. Hind wing fully developed, MP3 vein absent, veins MP4 and CuA fused with each other. Trochantins moderate sized, oval. Coxae large, exerted. Mesocoxa contiguous, ridge below coxal rests absent. Tibiae without spines or long bristles on outer edge. Protibia with two fully developed, longitudinally placed, comb-like rows of setae, and three associated macrosetae. Tarsi 5-segmented, with one pair of empodial setae on each tarsus, equal or slightly shorter than claws (not shorter than half). Protarsus with tarsomeres 1–4 not inflated, narrower or equal to meso- and metatarsomeres, with dense pale adhesive setae on ventral side. Protarsomere 1 shorter than protarsomere 2, protarsomere 3 equal to protarsomere 2, but longer than protarsomere 4, protarsomere 4 not bilobed, protarsomere 5 longest, equal to protarsomeres 1–2 combined. Mesotarsus with mesotarsomere 1 longer than mesotarsomere 2, mesotarsomere 3 shorter than mesotarsomere 2, but longer than mesotarsomere 4, mesotarsomere 4 not bilobed, mesotarsomere 5 equal to mesotarsomere 1. Metatarsi with metatarsomere 1 twice as long as metatarsomere 2 and longer than metatarsomere 5, metatarsomeres 2–4 decreasing in length, metatarsomere 4 similar as metatarsomere 3 or shortest, metatarsomere 5 equal to 3, shorter than metatarsomeres 2–4 combined.

ABDOMEN. Finely sparsely punctate, widest at tergite V. Tergites III–VI shallowly impressed at base, with fringe of setae on posterior margin. Tergites III–VII with pair of paratergites on each side. Tergite VIII with posterior margin rounded. Sternite III without keel between coxae. Sternite VII with straight apical margin. Female: posterior margin of sternite VIII straight. Male: sternite VIII with moderately shallow and broad median emargination of posterior margin; sides of emargination on sternum VIII rounded (Fig. 2B). Aedeagus with parameres reduced and fused to median lobe; ventral process slightly shorter than uneverted internal sac; in parameral view with apex of ventral process rounded, sides sinuate; dorsal plate rather large and relatively weakly sclerotised (Fig. 2C–D).

Distribution

The species has only been recorded from Florida (USA) (Brunke & Schnepf 2021).

Genus *Pachystilicus* Casey, 1905

Figs 3–4

Pachystilicus Casey, 1905: 226 (original description), 228 (comparison with *Megastilicus*).

Pachystilicus – Blackwelder 1939: 107; 1952: 285 (notes). — Moore & Legner 1979: 11 (key), 111 (description). — Newton *et al.* 2001: 327 (characters in key), 387 (comment on taxonomic status).

Type species

Pachystilicus quadriceps (LeConte, 1880).

Diagnosis

The genus can be recognised based on the following combination of features: body robust (usually slender in *Rugilus*), covered with fine, dense, golden setae (absent in *Acrostilicus*, *Eustilicus* Sharp, 1886 and *Megastilicus* Casey, 1889); antennal tomentose pubescence starting from antennomere 4; paired teeth on labrum (single in *Acrostilicus*); the base of head broadly emarginate, punctation of head clearly denser and coarser than that of pronotum, head without dense ground sculpture (often present in *Rugilus*); absence of scutellar ridges of mesoscutellum (one or two present in other North American Stilicina).

Redescription

HABITUS. Medium sized, robust, covered with fine golden setae, integument densely punctured. Head more densely punctured than pronotum, without short and stout bristles.

COLOURATION. Dark brown; head darker, legs and antennae reddish brown.

HEAD. Trapezoidal, widest in middle, wider and longer than pronotum, temples straight, hind angles rounded, posterior margin emarginate (sinuate), disc rugose, punctation dense, rather fine, interstices reduced to narrow ridges, without microsculpture. Eyes large, approximately $\frac{1}{3}$ of head length, slightly protruding, eyes with setae between ommatidia. Antenna 11-segmented, somewhat incrassate; antennomeres 4–11 with tomentose pubescence. Clypeal margin straight. Labrum transverse, twice as wide as long or wider, expanded, covering mandibles when closed, highly sclerotised in posterior part, less sclerotised in anterior part, anterior margin arcuate, with two long median teeth and four long setae (two per each side of teeth), incision between teeth rounded. Mandible without prosthema; maxillary palpus 4-segmented; maxillary palpomere 1 small; maxillary palpomere 2 longer than wide, widest near apex, with only few setae, with denser pubescence than maxillary palpomere 1; maxillary palpomere 3 almost twice as long as maxillary palpomere 2 and wider than it, slightly expanded, widest near apex, vase-like; maxillary palpomere 4 small, acicular, glossy and thin, shorter than wide, narrower and shorter than width of maxillary palpomere 3. Labial palpus 3-segmented, labial palpomere 1 slightly longer than wide, widest near apex; labial palpomere 2 about as wide as labial palpomere 1, longer than it, widest near apex; labial palpomere 3 shorter and distinctly narrower than labial palpomere 2, cylindrical. Mentum transverse, rectangular. Ligula entire, not bilobed, dorsal plate without setae. Gular sutures fully fused, not reaching posterior margin of head. Neck narrow, less than $\frac{1}{5}$ of head width.

THORAX. Pronotum wider than long, rhomboid, with anterior angles obtuse, narrower in anterior and posterior part, widest in middle, disk finely densely punctured, midline less punctured. Long black seta on each side of pronotum, in apical portion of widest part; shorter black seta in posterior portion of widest part. Superior marginal line deflexed, not meeting with inferior line. Basisternum of prosternum without macrosetae or microsculpture, but surface wrinkled, longitudinal carina present. Furcasternum of prosternum longer than $\frac{1}{2}$ of basisternum length, reaching farther than tip of postcoxal process, triangular, acute, with sharp longitudinal carina and transversal carina. Hypomeron not delimited from pronotal disc by carina. Furcasternum of mesosternum with longitudinal carina, short, reaching less than $\frac{1}{3}$ of distance between coxae, rectangular. Elytra quadrate, longer and wider than pronotum, with row of setae on edge of posterior margin, without epipleural ridge; humeral angle indistinct, rounded; surface glossy, interstices without microsculpture. Scutellum without ridges, integument reticulate, anterior margin rounded. Hind wing fully developed, with MP3 vein present. Legs, as rest of body, covered with shiny gold setae. Trochantins large, quadrate. Middle coxa contiguous, ridge below coxal

rests present. Tibiae without spines or long bristles on outer edge. Protibia with two fully developed, longitudinally placed, comb-like rows of setae, and three associated macrosetae. Tarsi 5-segmented, stout, with one pair of empodial setae on each tarsus, equal to or slightly shorter than claws. Protarsus narrowly dilated, protarsomere 1 slightly longer than protarsomere 5, protarsomeres 2–4 decreasing in length, protarsomeres 1–4 narrower or equal to meso- and metatarsomeres 1–4, with dense pale adhesive setae on ventral side, protarsomere 4 not bilobed. Mesotarsus with mesotarsomere 1 longer than mesotarsomere 2, mesotarsomere 4 similar to preceding one. Metatarsus with metatarsomere 1 longer than metatarsomere 2, metatarsomere 4 similar to preceding one, metatarsomere 5 equal to metatarsomere 1, longer than metatarsomere 4, but shorter than metatarsomeres 2–4 combined.

ABDOMEN. Finely pubescent, with fine and dense punctation, wider than elytra, widest at tergite V. Both ventral and dorsal sides with golden setae mixed with numerous longer black ones. Tergites III–VI impressed at base. Tergites III–VII with pair of paratergites on each side. Tergite VIII with posterior margin rounded. Sternite III without keel between coxae. Female: posterior margin of sternum VIII straight. Male: sternum VIII with moderately deep and broad median emargination of posterior margin; sides of emargination rounded (Figs 3B, 4C).

AEDEAGUS. With parameres reduced and fused to median lobe; ventral process stout, apically broadly truncate, slightly longer than uneverged internal sac; in parameral view ventral process bent and with apex acute (Figs 3C–D, 4D–E).

Pachystilicus quadriceps (LeConte, 1880)

Fig. 3

Stilicus quadriceps LeConte, 1880: 178 (original description, key, notes).

Pachystilicus quadriceps – Casey 1905: 227 (redescription). — Leng 1920: 104. — Lucas 1920: 475 (genotype fixed). — Blackwelder 1952: 285 (notes). — Moore & Legner 1979: 112. — Poole & Gentili 1996: 370. — Newton *et al.* 2001: 387 (comment on taxonomic status). — Bousquet *et al.* 2013: Dwc-A database.

Diagnosis

Differs from *P. hanhami* by longer and denser setae covering body, longitudinal carina of basisternum more elevated. Additionally, median emargination of posterior margin of sternite VIII is deeper and broader than in *P. hanhami*, depth of emargination of ca ¼ of sternite length.

Type material

Lectotype (designated here)

USA • ♂; “Cal. / ♂ / Type 6584 / *S. quadriceps* Lec. / J.L. LeConte Collection”; MCZ.

Additional studied material

CANADA – **British Columbia** • 1 spec.; “Mts. Between Hope and Okanagan B. C. Sept. 90 / F C Bowditch Coll. / *Stilicus quadriceps* Lec”; MCZ.

USA – **California** • 1 spec.; “Cala / Coll Hubbard and Schwarz / *quadriceps* Lec”; NMNH. – **Massachusetts** • 1 spec.; “Brookline Mass 4-14-88 / F. C. Bowditch Coll”; MCZ.

Redescription

MEASUREMENTS. Body length: 5 mm; forebody length: 2.75–2.90 mm.

COLOURATION. Dark brown to reddish brown; head darker, legs and antennae reddish brown (Fig. 3A).

HEAD. As in generic description (HL: 0.95–1.00 mm; HW: 1.0–1.1 mm), with antennomere 1 elongate, widest at tip; antennomeres 2–4 elongate; antennomere 5 weakly elongate; antennomeres 6–10 gradually becoming transverse; antennomere 11 elongate, 1.5× as long as antennomere 10.

THORAX. As in generic description (pronotal size: PL: 0.8–0.9 mm; PW: 0.8–0.9 mm; elytra size: EL: 1 mm; EW: 1.1–1.2 mm).

ABDOMEN. As in generic description; sternite VII with apical margin straight.

Distribution

The species has previously been recorded from the following US states: California, Massachusetts, and Missouri (Newton 2018). We newly report this species from the Canadian province of British Columbia.

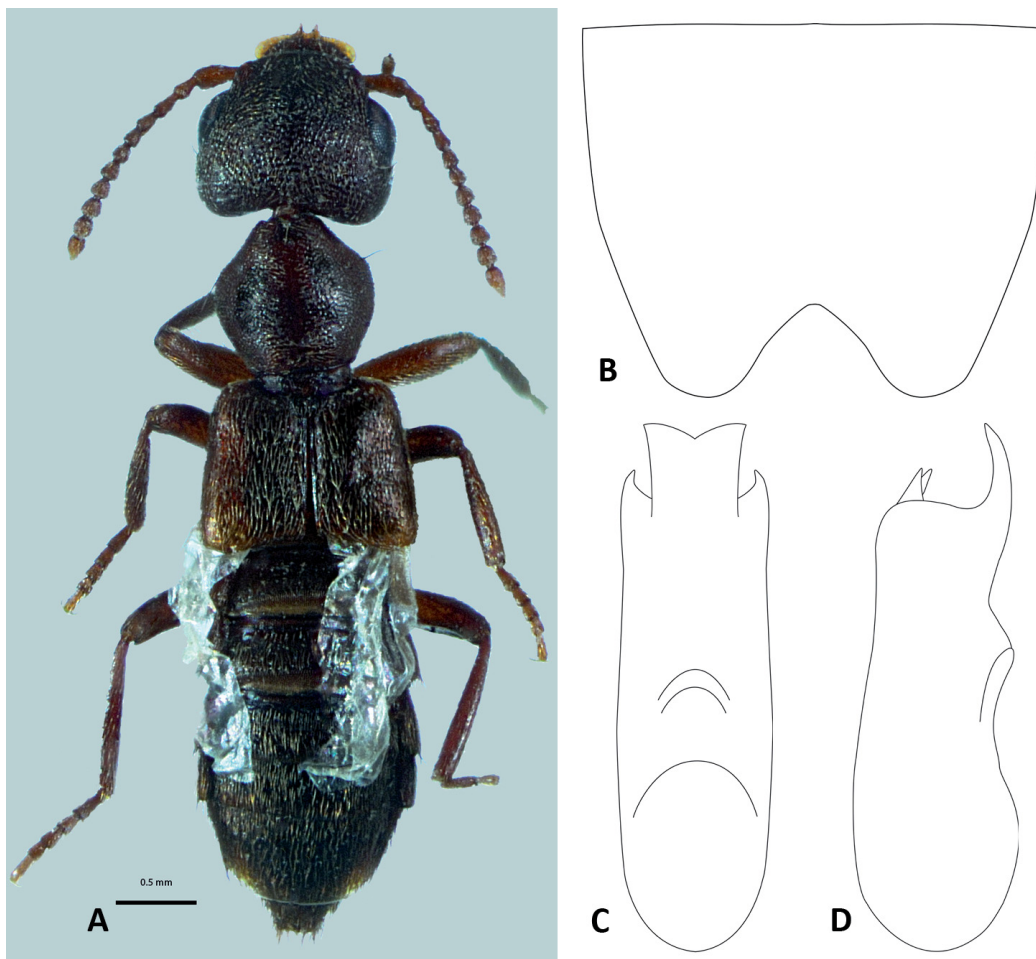


Fig. 3. *Pachystilicus quadriceps* (LeConte, 1880), habitus photograph and drawings of the apical abdominal sternite and genital structures. **A.** Habitus, lectotype, ♂ (MCZ). **B.** Male sternite VIII. **C.** Aedeagus, parameral view. **D.** Aedeagus, lateral view. Scale bar = 0.5 mm.

Pachystilicus hanhami (Wickham, 1898)

Fig. 4

Stilicus hanhami Wickham, 1898: 220 (original description, notes).

Pachystilicus hanhami – Casey 1905: 227 (redescription). — Blackwelder 1939: 107, 120; 1952: 285 (notes). — Poole & Gentili 1996: 370. — Webster 2016: 435. — Newton *et al.* 2001: 387 (comment on taxonomic status).

Diagnosis

Differs from *P. quadriceps* by shorter and slightly sparser setae covering body, longitudinal carina of basisternum less elevated. Additionally, the median emargination of posterior margin of male sternite VIII is shallower and narrower than in *P. quadriceps*, with the depth of emargination ca $1/5$ of sternite length.

Type material

Holotype

USA • ♂; “Winnipeg Man. Hanham / Wickham Collection 1933 / Type No 50215 U.S.N.M / *Stilicus hanhami* Wick.? Type of drawing [?] of description”; NMNH.

Additional studied material

CANADA – **Alberta** • 1 spec.; “[with ants] Banff. Alta. Aug. 20’15 Wheeler leg. / 00744589”; MCZ • 1 spec.; “Calgary, Hwy 4, 2.IV.1981, with ants [*Myrmica* sp.] under stone, B+J Carr”; MCZ • 1 spec.; “Calgary, Hwy 4, 7.VI.1964 with ants [*Myrmica* sp.] under stone, B+J Carr”; MCZ • 1 spec.; “Calgary, Hwy 4, 23.X.1955 with ants [*Myrmica* sp.] under stone, B+J Carr”; MCZ • 1 spec.; “Calgary, Hwy 4, 13.X.1955 with ants [*Myrmica* sp.] under stone, B+J Carr”; MCZ • 1 spec.; “Calgary, Hwy 4, 25.IV.1953 with ants [*Myrmica* sp.] under stone, B+J Carr”; MCZ • 1 spec.; “Calgary, Hwy 4, 8.V.1953 with ants [*Myrmica* sp.] under stone, B+J Carr”; MCZ • 1 spec.; “Calgary, Hwy 4, 24.III.1956 with ants [*Myrmica* sp.] under stone, B+J Carr”; MCZ • 1 spec.; “Banff, 17.IV.1955, with ants, B+J Carr”; MCZ • 1 spec.; “Coleman, 26.VI.1961, B+J Carr”; MCZ • 1 spec.; “Seebe, 14.X.1963, B+J Carr”; MCZ • 1 spec.; “Canmore, 3.IV.1953, B+J Carr”; MCZ • 1 spec.; “Canmore, 4.IV.1953, B+J Carr”; MCZ • 1 spec.; “Ghost Dam, 22.III.1953, B+J Carr”; CNC. – **Manitoba** • 1 spec.; “Winnipeg Man. Hanham / F. C. Bowditch Coll / *Stilicus hanhami* Wick / 00744583”; MCZ • 1 spec.; “Winnipeg Man. Hanham / *Stilicus hanhami* Wickh. / 00744584”; MCZ • 1 spec.; “Winnipeg Man. Hanham / ♀ / Fredrick Blanchard Collection / *Stilicus hanhami* Wickh. / 00744585”; MCZ • 1 spec.; “Winnipeg Man. Hanham / Wickham Collection 1933 / *Stilicus hanhami* Wickh.”; MCZ • 1 spec.; “Winnipeg Man./CASEY bequest 1925/*Pachystilicus hanhami* Wick.”; MCZ • 1 spec.; “Winnipeg Man. Hanham/ CASEY bequest 1925/CASEY determination *hanhami*-2”; NMNH.

USA – **Massachusetts** • 1 spec.; “Tyngs Mass. / ♂ / Fredrick Blanchard Collection / *Stilicus quadriceps* Lec. / 00744586”; MCZ • 1 spec.; “Under stones / C.A. Frost. IV Sherborn 17-10 Mass / *Stilicus ? apicalis* Csy / C.A. Frost Collection 196200744590”; MCZ • 1 spec.; “Frmghm (Framingham?) Mass Frost / H. C. Fall Collection / *Stilicus hanhami* / 00744532”; MCZ. – **New Hampshire** • 1 spec.; “Franconia N.H. / Liebeck Coll. / H. C. Fall Collection / 00744582”; MCZ • 1 spec.; “Franconia N.H. / with ants mas., blossom / Liebeck Coll. / H. C. Fall Collection / 00744581”; MCZ • 1 spec.; “Plainfield N.H. Oct. 9.27. / F.C. Bowditch Coll / *Stilicus* / 00744588”; MCZ • 1 spec.; “Plainfield N.H. Oct.9.27. / F.C. Bowditch Coll / *Stilicus* / 00744587”; MCZ. – **Oklahoma** • 1 spec.; “Oklahoma: Latimer Co., 5 Mi. W. Red oak, Oct. 1980 K. Stephen / *Pachystilicus quadriceps* Lec.’ 84 det. L. E. Watrous”; TAMUIC • 1 spec.; “Nev. Elko Co., Wildhorse Crossing St. Pk, R. Haswell 8.IX.15 / in ant nest under rock / *Pachystilicus* sp. det. R. Haswell 1922 / ex. Richard H. Haswell Collection, October 2008, TAMU Insect Collection”; TAMUIC.

Redescription

MEASUREMENTS. Body length: 4.5 mm; forebody length: 2.70–2.95 mm.

COLOURATION. Dark brown to yellow; head darker, legs and antennae reddish brown, elytra (except for base) light brown to yellow. Abdomen black, tip lighter in colour (Fig. 4A–B).

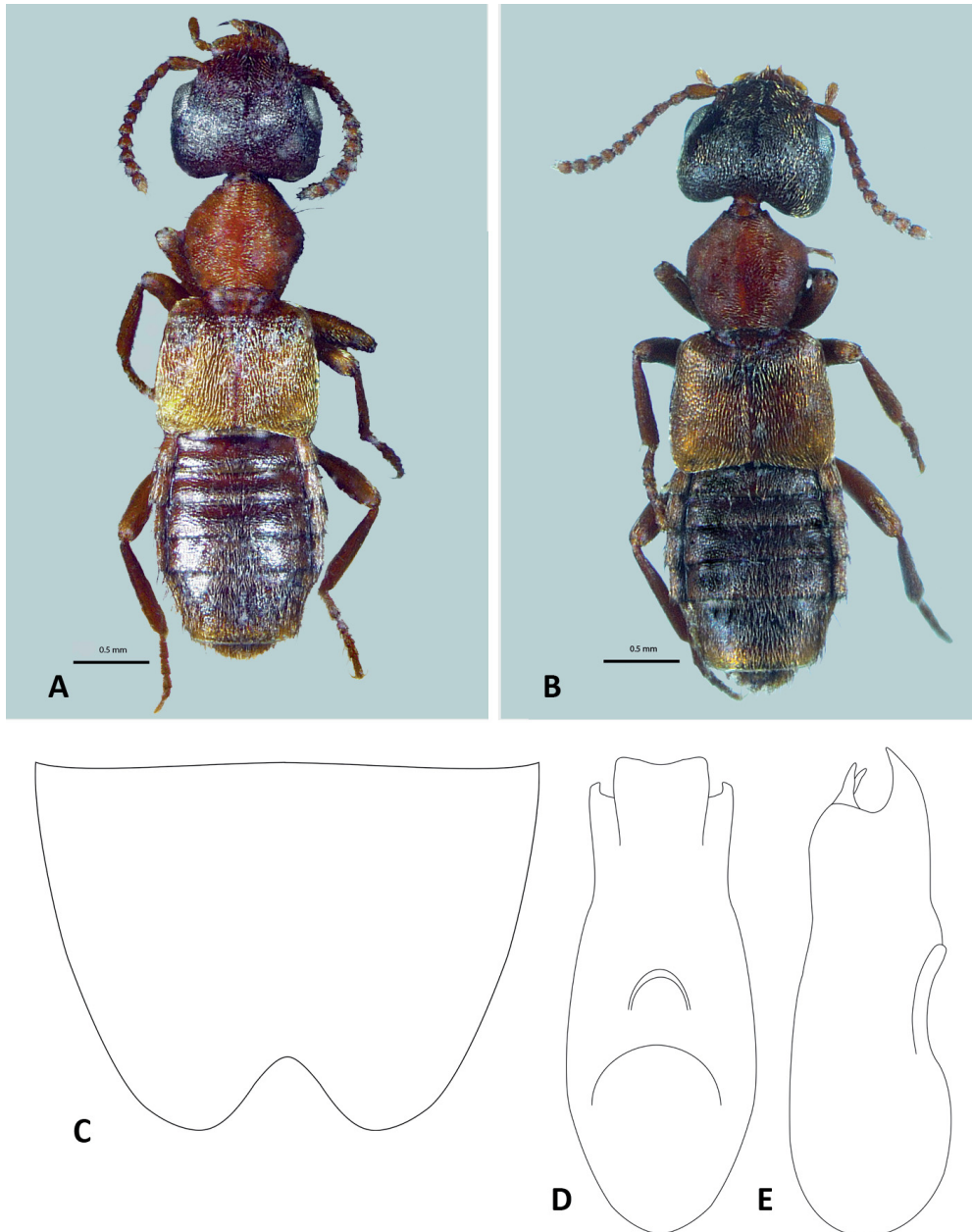


Fig. 4. *Pachystilicus hanhami* (Wickham, 1898), habitus photographs and drawings of the apical abdominal sternite and genital structures. **A.** Habitus, holotype, ♂ (NMNH). **B.** Habitus, additional male specimen with darker colouration (NMNH). **C.** Male sternite VIII. **D.** Aedeagus, parameral view. **E.** Aedeagus, lateral view. Scale bars = 0.5 mm.

HEAD. As in generic description (HL: 0.9–1.0 mm; HW: 1.0–1.1 mm), with antennomere 1 elongate, widest at tip; antennomeres 2–4 elongate; antennomere 5 weakly elongate; antennomeres 6–10 gradually becoming transverse; antennomere 11 elongate, 1.5× as long as antennomere 10. Mandible with three teeth on right and left mandible, but two outer teeth on right mandible much larger than others.

THORAX. As in generic description (pronotal size: PL: 0.8–0.9 mm; PW: 0.8–0.9 mm; elytra size: EL: 1.00–1.05 mm; EW: 1.2 mm).

ABDOMEN. As in generic description; sternite III with short medial longitudinal keel.

Distribution

The species has previously been recorded from the Canadian provinces of Alberta, British Columbia, Manitoba, New Brunswick, and Saskatchewan and the US state of Oregon (Newton 2018). New records include the following US states: Massachusetts, New Hampshire, and Oklahoma.

Discussion

Position of *Acrostilicus* and *Pachystilicus* in the phylogeny

Our total-evidence dataset combining molecular and morphological data allowed us to demonstrate the phylogenetic position of *Acrostilicus* and *Pachystilicus* for the first time and confirm their classification as subtribe Stilicina within the tribe Lathrobiini. The characters supporting their placement in Stilicina are as follows: small, acicular maxillary palpomere 4; expanded labrum, almost or completely covering mandibles when closed; gular sutures at least partially fused; narrow neck, equal to or less than $\frac{1}{5}$ of head width; prosternal apophysis present as clear invagination; and metatibia with apical ctenidium on one side only. *Acrostilicus* was resolved as sister to *Rugilus*, and this relationship is supported by several morphological characters: postoccipital suture not crossing gular sutures and indistinct at the base of gula; lack of ridge below coxal rests on mesosternum, lack of MP3 vein on the hind wing, and lack of a row of setae on the edge of elytral posterior margin. The position of *Pachystilicus* was unresolved within a group consisting of *Acrostilicus* + *Rugilus* and *Megastilicus*. The clade consisting of these four genera was supported by the lack of a marginal ridge on the elytral epipleuron. All four genera were resolved as sister to *Eustilicus* + *Stilicoderus* and together form the ‘core’ of subtribe Stilicina.

Generic status of *Acrostilicus* and *Pachystilicus*

As both genera are easy to diagnose and recognise, we confirm that they should be treated as separate genera in the subtribe Stilicina and not synonymised with any other genus. *Acrostilicus* is the only genus within the Nearctic representatives of the subtribe with a single median tooth on the labrum, and when combined with its slender habitus and prolonged legs, make it easy to identify. As for *Pachystilicus*, which has been suggested to be a synonym of *Rugilus*, the following characters separate the two genera in our morphological matrix: the presence of the tomentose pubescence on the antennomere 4 (absent in *Rugilus*), postoccipital suture that crosses gular sutures and continues through the base of gula (in *Rugilus*, the suture does not cross gular sutures and is indistinct at the base of gula), head longer than pronotum (head shorter or as long as pronotum in some *Rugilus*), presence of longitudinal median carina on basisternum (absent in some *Rugilus*), presence of ridge below mesosternal coxal rests (absent in *Rugilus*), absence of scutellar ridges of scutellum (one ridge present in *Rugilus*), presence of ridge below mesosternal coxal rests (absent in *Rugilus*), presence of the row of setae on the edge of the posterior margin of elytra (absent in *Rugilus*), and presence of MP3 vein on the hind wing (absent in *Rugilus*). Additional differences are the body shape (stout in *Pachystilicus*, usually rather slender in *Rugilus*), separation of labral teeth by twice their average width and rounded labral medial notch (although according to Frania (1986) present also in some *Rugilus* species), distinctive emargination of the head in *Pachystilicus* (in a majority of specimens), and differently punctured head and pronotum (very fine and

dense in *Pachystilicus*, while coarse and elongate in many *Rugilus*). It is, however, possible that among the mega-diverse genus *Rugilus* with 265 described species (Newton 2018), there might be species that possess some of the abovementioned characters. Nevertheless, the combination of features given in the *Pachystilicus* diagnosis is unique for the genus. Among North American Stilicina, *Pachystilicus* can be easily distinguished from the genera *Acrostilicus*, *Eustilicus* and *Megastilicus* by its rectangular head with straight temples, emargination at the head base, and dense, gold setation. All three genera have a (sub)orbicular head with temples rounded and without the emargination. Additionally, *Megastilicus* has its body covered with characteristic short, black, stout setae and the dorsal surface of the head, pronotum, and elytra are granulate (Żyła & Koszela 2021). The same combination of characters above distinguishes *Pachystilicus* from the following North American *Rugilus* species: *R. angularis* (Erichson, 1840), *R. angustatus* (Geoffroy, 1785), *R. apicalis* (Casey, 1905), *R. biarmatus* (LeConte, 1880), *R. dentatus* Say, 1831, *R. lacustrinus* (Casey, 1905), *R. occiduus* (Fall, 1901), *R. orbiculatus* (Paykull, 1789) (head more quadrate but converging in the posterior part), and *R. rudis* (LeConte, 1863). From *Rugilus* species with a more rectangular head, i.e., *R. ceylanensis* (Kraatz, 1859), *R. oblitus* (Fall, 1907), *R. opaculus* (LeConte, 1880), and *R. rufipes* Germar, 1836, *Pachystilicus* differs in the overall stout habitus, presence of head emargination, dense gold setation, and differently punctured head and pronotum.

As opposed to other Stilicina, both *Acrostilicus* and *Pachystilicus* do not have any scutellar ridges.

Non-monophyly of Stilicina and Medonina

Another genus present in the analysis and currently assigned to the subtribe, *Panscopaeus* Sharp, 1889, was not resolved as part of the ‘core’ Stilicina clade and renders Stilicina non-monophyletic. *Panscopaeus* is a small genus with seven described species distributed in the Indomalayan region (Assing 2011). It was originally described from a single species *Scopaeus lithocharoides* Sharp, 1874 and later considered as a subgenus of *Achenomorphus* Motschulsky, 1858 (Blackwelder 1952). Herman (2003) revalidated its status as a distinct genus and later it was placed in the subtribe Medonina (Smetana 2004). Assing (2011), in his revision of *Panscopaeus*, moved it to Stilicina, mostly based on the morphology of the mouthparts, some other external characters, and the male sexual characters. Characters that morphologically distinguish *Panscopaeus* from the rest of Stilicina are: smaller labrum, not fully covering mandibles from above (but it occurs at least in some *Rugilus* species as well), separated gular sutures, and the presence of both anterior and posterior ridges on mesoscutellum. In our BI tree, it was separated from the rest of Stilicina by a clade consisting of the three Medonina genera *Thinocharis* Kraatz, 1859, *Deroderus*, and *Ecitocleptis*, and highly modified ant inquilines *Mimophites* and *Synecitonides* (subtribe incertae sedis in Lathrobiini in both cases). As all these genera, except for *Thinocharis*, were included in the morphological dataset only, we treat the result with caution and do not make any taxonomic changes. Moreover, in our ML tree, *Panscopaeus* was resolved in a position even more distant from Stilicina. A possible explanation is that the current limit of Stilicina is too narrow and more genera are, in fact, part of this subtribe. Alternatively, it is also likely that we found the sister clade to a more restricted Stilicina. Future research, preferably also including molecular data when DNA grade samples become available, should investigate whether our results are due to sampling bias and/or lack of molecular data in the analysed dataset. This is the first time the medonine genera *Deroderus* and *Ecitocleptis* have been included in broader phylogenetic studies, and the non-monophyly of Medonina is confirmed once more (e.g., Żyła *et al.* 2019, 2021). The sister-group relationship of *Deroderus* and *Eustilicus*, suggested by Frania (1986), was not confirmed in our studies. Myrmecophilous *Mimophites* and *Synecitonides* have also never been included in a phylogenetic analysis, but it was suggested before that at least *Mimophites* could belong to Stilicina (Seevers 1965). Both genera were resolved together in a clade, which might be a result of their morphological modifications that could have evolved convergently. A test of this potential relationship would be possible with a molecular-based phylogenetic study.

Biology of *Acrostilicus* and *Pachystilicus*

One of the specialised habitats where Paederinae rove beetles may occur are the nests and burrows of various vertebrates. The genus *Acrostilicus* is an example of such a nidicolous rove beetle, which lives in the burrow of the gopher tortoise in Florida. The gopher tortoise is a burrowing reptile living in the dry and sandy longleaf pine ecosystem of the southeastern USA (Jones & Dorr 2004). Tortoise dung, which accumulates at the blind end of the burrow, provides a resource for many invertebrates, including beetles (Jackson & Milstrey 1989). So far, three species of Staphylinidae were reported from there, i.e., *Acrostilicus hospes*, *Philonthus gopheri* Hubbard, 1894, and *Philonthus testudo* Smetana, 1995 (the latter two belong to Staphylininae) (Brunke & Buffam 2018). All three nidicolous species are rather pale in colouration, which is consistent with the other invertebrates of this assemblage (Brunke & Buffam 2018). Another morphological modification of *Acrostilicus* may include elongated legs and an overall slender body. Brunke & Buffam (2018) hypothesised that all three Staphylinidae species could be members of a specialised dung community. It is possible that the distribution of *Acrostilicus hospes* is much wider and overlaps with the occurrence range of its tortoise host and/or that more species of *Acrostilicus* are awaiting discovery since only a few samples of rove beetles have been collected from these burrows.

Paederinae is one of a few subfamilies within Staphylinidae where myrmecophily has evolved in several different lineages. Many of them possess significant morphological modifications, e.g., in the body shape and appendages or developed special glands. None of these are obvious in *Pachystilicus*, which might mean that the beetles are not integrated into the host nest and live in their vicinity. However, many records of beetles from ants' nests were isolated events and most likely not indicative of myrmecophily (Parker 2016). This could be the case for *Pachystilicus hanhami*, where a direct interaction with ants has never been observed, but the labels indicate that specimens were collected both in the ant nest and outside, although the former situation occurred more often. None of the known specimens of *P. quadriceps* have labels that mention co-collection with ants.

Comparison with previous phylogenetic results

The tree topologies obtained as a result of BI (Fig. 1) and ML (Supp. file 7) analyses were consistent with the previous result of Żyła *et al.* (2021), the dataset of which served as a base for the current study. The most important differences concerned the relations of the Paederine tribes and the position of the *Pseudolathra* + *Cylindroxystina* clade. In Żyła *et al.* (2021), the tribe Pinophilini was resolved as sister to Lathrobiini + Paederini, but in our analyses only to Lathrobiini. In the second case, the *Pseudolathra* + *Cylindroxystina* clade was resolved as sister to 'Medonina and allied taxa' in Żyła *et al.* (2021), while we here obtained such a result only in our ML analysis. A different topology was recovered in the BI analysis, where this clade was resolved as sister to the 'true' Lathrobiina and they together formed the sister group to the 'Medonina and allied taxa' clade. The above differences are attributed to sparser taxon sampling in the present study for non-Lathrobiini. Within the 'Medonina and allied taxa' clade, topologies differed between BI and ML but Medonina, the largest subtribe within this lineage, was always recovered as non-monophyletic, consistent with other studies (Schomann & Solodovnikov 2017; Żyła *et al.* 2019, 2021; Bogri *et al.* 2020).

Acknowledgements

We are grateful to the curators and collection managers for access to the collections under their care and for all their help. We acknowledge Adam J. Brunke for kindly revising the language of the manuscript and checking several *Pachystilicus* specimens from the CNC collection. DŽ and KK thank Gregory Courtney for access to his laboratory and photography equipment at the Department of Entomology, Iowa State University. Dean Adams is acknowledged for providing DŽ with equipment that made this study possible. We are grateful for the comments of the two anonymous reviewers and the editor

that greatly improved the manuscript. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 797823 (postdoctoral fellowship of DŻ) and from the Polish National Science Centre, grant number 2019/35/B/NZ8/03431 (to DŻ). DŻ was additionally funded by the Ernst Mayr Travel Grant in Animal Systematics through the Harvard Museum of Comparative Zoology to visit the MCZ collection. We also thank the CIPRES Scientific Gateway, which provided access to computational resources.

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Manuscript received: 23 September 2021

Manuscript accepted: 7 March 2022

Published on: 9 May 2022

Topic editor: Tony Robillard

Section editor: Max Barclay

Desk editor: Pepe Fernández

Printed versions of all papers are also deposited in the libraries of the institutes that are members of the *EJT* consortium: Muséum national d'histoire naturelle, Paris, France; Meise Botanic Garden, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; Royal Belgian Institute of Natural Sciences, Brussels, Belgium; Natural History Museum of Denmark, Copenhagen, Denmark; Naturalis Biodiversity Center, Leiden, the Netherlands; Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain; Real Jardín Botánico de Madrid CSIC, Spain; Zoological Research Museum Alexander Koenig, Bonn, Germany; National Museum, Prague, Czech Republic.

Supplementary files

Supp. file 1. List of North American species of *Stilicina* Casey, 1905 compared with *Acrostilicus* Hubbard, 1896 and *Pachystilicus* Casey, 1905 with the status of studied specimens and their repositories. <https://doi.org/10.5852/ejt.2022.819.1773.6719>

Supp. file 2. GenBank accession numbers of all sequences. <https://doi.org/10.5852/ejt.2022.819.1773.6721>

Supp. file 3. List of morphological characters. <https://doi.org/10.5852/ejt.2022.819.1773.6723>

Supp. file 4. Matrix of morphological characters. <https://doi.org/10.5852/ejt.2022.819.1773.6725>

Supp. file 5. Concatenated sequence alignment. <https://doi.org/10.5852/ejt.2022.819.1773.6727>

Supp. file 6. MrBayes script. <https://doi.org/10.5852/ejt.2022.819.1773.6729>

Supp. file 7. The ML tree resulting from the IQ-TREE analysis of combined molecular and morphological datasets. Ultrafast bootstrap approximation values >80 are shown to the right of each corresponding node. <https://doi.org/10.5852/ejt.2022.819.1773.6731>