



ISSN 1175-5326 (print edition) ZOOTAXA ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5325.3.7 http://zoobank.org/urn:lsid:zoobank.org:pub:85D91AED-ED2F-4369-8A74-EB558CF7D440

A palearctic bark beetle, *Crypturgus hispidulus* Thomson (Coleoptera: Curculionidae: Scolytinae), new to North America discovered in New England, U.S.A.

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Abstract

Specimens of a bark beetle, *Crypturgus hispidulus* Thomson, 1870, were discovered in New England, U.S.A. This is the first record of this species established in North America. Misidentified specimen records were found from multiple states in different years, confirming establishment of the species in the Northeastern United States. The morphology is presented and a modified key to *Crypturgus* in North America is provided to facilitate species identification.

Key words: bark beetle, Scolytinae, adventive species, Picea, dichotomous key

Introduction

Bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are one of most frequently intercepted insect groups at ports of entry worldwide (Brockerhoff *et al.* 2006) due to their due to their small size, cryptic nature, and varied hosts. With global trade constantly increasing, it is not surprising that over half of the non-native scolytines in North America have become established, or were first discovered, within the past forty years (Haack & Rabaglia 2013), many of which are important economic forest pests (Smith & Hulcr 2015). Despite strict regulations and inspections at ports of entry, species continue to be intercepted and introduced in North America. We herein report another newly established non-native scolytine in North America, *Crypturgus hispidulus* Thomson, 1870.

Methods

While trapping and searching for spruce feeding *Pityopthorus* spp. on Peaks Island, Maine, U.S.A., some beating was conducted on foliage of recently windthrown *Picea rubens* in the vicinity of traps. A species of *Crypturgus* Erichson was collected that exhibited a combination of characters unlike any of those known to occur in North America. Using available keys to Palearctic species (Pfeffer 1995; Jordal & Knížek 2007) it was determined that the specimen was likely the transpalearctic *C. hispidulus*. Direct comparison with specimens of *C. hispidulus* from Austria, Poland, and Russia in different collections were also a perfect match. The specimen was preserved in 95% ethanol for later DNA sequencing. Checking available collections resulted in a total of 17 records of *C. hispidulus* from two different states dating as far back as 2006 (Fig. 1), indicating that *C. hispidulus* is well established in Maine and New Hampshire.

To verify the morphological determination, the specimen was analyzed genetically by sequencing the total DNA from the organism and comparing the assembled cytochrome c oxidase subunit 1 (COI) gene against the BOLD Identification System Database of COI barcodes (Ratnasingham & Hebert 2007). DNA was extracted from approximately half of one individual following the ThermoFisher Scientific MagMAX Microbiome Ultra Nucleic Acid Isolation protocol. Although not necessary for the COI gene species identification, we sequenced the

Accepted by S. Smith: 27 Jul. 2023; published: 9 Aug. 2023

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full genome for potential future studies. Illumina library preparation was completed following the dilute Nextera library preparation kit protocol. Sequencing was completed at the Hubbard Center for Genome Studies using a NovaSeq 6000 instrument to produce paired-end 250 bp reads. Raw sequencing data was demultiplexed using bcl2fastq v2.20.0.422 and adapters and low-quality bases were trimmed with Trimmomatic v0.32 with default settings (Bolger *et al.* 2014). Assembly of the nuclear and mitochondrial genomes was completed with the SPADES v3.13.0 assembler with default settings. The mitochondrial genome was identified from the assembly by performing a BLAST (Ye *et al.* 2007) of the assembled contiguous sequences against the NCBI nt database. The mitochondrial genome was annotated using the MITOS v2 webserver and the COI gene was uploaded to the BOLD web server for species identification. The complete mitochondrial genome and raw sequencing reads were submitted to the NCBI under the BioProject accession PRJNA966944.



FIGURE 1. Geographic distribution of *Crypturgus hispidulus* in Maine and New Hampshire, USA (black dots), with general distribution of *Picea rubens* in Northeastern United States and adjacent Canada (light green) and basal area of *Picea* spp. within northern New England and New York (dark green).

Results

A total of 54,394,356 paired-end reads were generated in this study. *De novo* genome assembly of the data produced a 16,332 bp contiguous sequence that best matched to publicly available insect mitochondrial genomes. Annotation of the mitochondrial contiguous sequence resulted in thirty-seven gene features: thirteen protein-encoding genes, twenty-two tRNAs, and two ribosomal subunits. The COI gene sequence matched with 100% identity to four specimens of *C. hispidulus* in BOLD (SCOL037-12, SCOL042-12, SCOL046-12, SCOL064-12). The next closest match is to *Crypturgus pusillus* (Gyllenhal, 1813) at 87.71% identity.

Specimens are deposited in the following collections:

CMNH—Carnegie Museum of Natural History, Pittsburgh, PA DFOC—US Forest Service Durham Field Office Collection, Durham, NH JPBC—Joshua P. Basham Collection, Atlanta, GA MFDC—Marc F. DiGirolomo Collection, Dover, NH MFS—Maine Forest Service Collection, Augusta, ME NMNH—National Museum of Natural History, Washington, DC

Crypturgus hispidulus Thomson, 1870

Figures 2A, 3A

Crypturgus hispidulus Thomson, 1870: 338. *Crypturgus maulei* Roubal, 1910: 203.

Diagnosis: *Crypturgus hispidulus* may be distinguished from other members of the genus by the combination of a subreticulate pronotum, long erect interstrial setae on the disc and sides of the elytra (which may be absent in particularly worn specimens), width of strial punctures and interstriae approximately equal, and females lacking a spongy patch on the elytral declivity. It is very similar to *C. pusillus* (Fig. 2B, 3B), which has a slightly shinier pronotum, nearly glabrous elytral disc, and smaller strial and interstrial punctures.



FIGURE 2. Dorsal habitus of Crypturgus spp. A Crypturgus hispidulus B Crypturgus pusillus. Scale bar: 0.5 mm.



FIGURE 3. Elytral disc of *Crypturgus* spp. A *Crypturgus hispidulus* B *Crypturgus pusillus*. Arrows indicate width of striae and interstriae.

Morphology: Length 1.1–1.3 mm, 2.7 times as long as wide; color dark brown. Frons convex; surface reticulate, punctures fine; vestiture sparse, inconspicuous. Pronotum 1.1 times as long as wide; widest slightly behind middle, sides moderately arcuate, converging equally anteriorly and posteriorly, anterior margin slightly more narrowly rounded than posterior margin; surface of disc subreticulate and shining, becoming more reticulate toward margins; punctures moderately coarse, deep, separated by 1–2x their diameter; vestiture hairlike setae originating from punctures, evenly distributed. Elytra 1.8 times as long as wide, 1.8 times as long as pronotum; sides varying from straight and parallel on more than basal two-thirds to slightly arcuate and wider on apical two-thirds, evenly rounded behind; striae not impressed, punctures subequal in width to interstriae, deep; interstriae shining, interstrial punctures on declivity smaller than on elytral disc. Elytral vestiture consisting of conspicuous, erect, uniseriate interstrial setae about as long as interstrial width, and finer strial setae subequal in length to interstrial setae but more recumbent. Females similar to males.

Distribution: Transpalearctic – **Asia:** China, Japan (Iturup, Kuril Islands, ownership disputed with Russia), North Korea, Russia, Turkey; **Europe:** Austria, Belarus, Bulgaria, Czechia, Denmark, Estonia, Finland, Germany, Hungary, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Russia, Slovakia, Slovenia, Sweden, Switzerland, Ukraine (Bright 2021; Jurc 2003; Krivolutskaya 1973, Kumbaşli *et al.* 2018).

New North American records: UNITED STATES: **MAINE**: Cumberland Co., Portland, Peak's Island, 43.65608°, - 70.19087°, 7 Jul 2022, M.F. DiGirolomo, beating foliage of recently windthrown *Picea rubens* (1, DFOC); Franklin Co., Farmington, 6 Jun 2006, funnel trap, PSB Survey (1, MFS); Kingfield, Mt. Abraham, 44.9774°, -70.3058°,

19–28 May 2021, F.G. Guarnieri, funnel trap, α -pinene (1, MFDC); 1–7 Jun 2021, F.G. Guarnieri, funnel trap, α-pinene (1, MFDC); Hancock Co., 3.2 km SSE Ellsworth, 44.52016°, -68.39542°, 85 m, 22 May-4 Jun 2013, S. Bonstedt, black cross-vane panel trap, BSF#51541 (2, CMNH 642637-38); 1.3 km NE Somesville, 44.36794°, -68.32085°, 17 m, 21 May-4 Jun 2013, S. Bonstedt, black cross-vane panel trap, BSF#51549 (1, CMNH 642639); 4.2 km SSE Southwest Harbor, 44.24409°, -68.30743°, 33 m, 3-19 Jun 2014, S. Bonstedt, black cross-vane panel trap, BSF#56605 (1, CMNH 639311); 19 Jun-1 Jul 2014, S. Bonstedt, black cross-vane panel trap, BSF#56606 (2, CMNH 639315&17); 0.8 km N Trenton, 44.44554°, -68.37176°, 25 m, 22 May–4 Jun 2013, S. Bonstedt, Lindgren funnel trap, BSF#51459 (1, CMNH 642640); 6.3 km SSE Winter Harbor, 44.34011°, -68.0611°, 19 m, 25 May-8 Jun 2016, G. Gamester, R. Nyce, black cross-vane panel trap, BSF#67506 (1, CMNH 701653); Penobscot Co., 4.8 km SE Hermon, 44.79078°, -68.85939°, 51 m, 15–24 May 2017, J. Nicolelli, Lindgren funnel trap, BSF#72896 (1, CMNH 710290); Piscataquis Co., T6 R10 WELS, Baxter State Park, 46.19475°, -69.06703°, 12 Jun 2014, M.F. DiGirolomo, modified funnel trap, EtOH, α-pinene, ipsenol, ipsdienol (1, MFS); Somerset Co., Bigelow Preserve, 45.1706°, -70.2526°, 14 May–2 Jun 2021, F.G. Guarnieri, funnel trap, α-pinene (1, JPBC); 2–10 Jun 2021, F.G. Guarnieri, funnel trap, α-pinene (1, JPBC); New HAMPSHIRE: Carroll Co., Bartlett Experimental Forest, 44.07414°, -71.29091°, 29 Jul 2020, K.J. Dodds, green funnel trap, syn-2,3-hexanediol, 3-hydroxyhexan-2-one, 3-hydroxyoctan-2-one, EtOH (1, DFOC); 17 May 2021, K.J. Dodds, green funnel trap, syn-2,3-hexanediol, 3-hydroxyhexan-2-one, 3-hydroxyoctan-2-one, EtOH (1, DFOC); Jun 2021-20 Apr 2022, K.J. Dodds, emergence chamber, Picea rubens (4, NMNH; 9, DFOC).

Biology and hosts. As with other members of the genus, *C. hispidulus* typically gain entry to the host cambium from existing galleries of other beetle species, and has been found associated with *Polygraphus poligraphus* L. and *Pityogenes chalcographus* (L.) (Nikulina *et al.* 2015). It has not been reported as a pest in any of the literature searched. Eurasian hosts include *Abies sibirica, Larix decidua, Picea abies, Picea ajanensis, Picea obovata, Picea jezoensis, Pinus brutia, Pinus koraiensis, Pinus mugo, Pinus nigra, Pinus pumila, Pinus rotundata, and Pinus sylvestris* (Stark 1952; Krivolutskaya 1973; Grüne 1979; Pfeffer 1995; Kumbaşli et al. 2018). It is common on fallen spruce trees (Nikulina *et al.* 2015). In North America it has been found emerging from *Picea rubens* logs, and beaten from the foliage of recently windthrown *P. rubens* trees.

To accommodate *C. hispidulus* in Wood's (1982) key to North American *Crypturgus*, couplet 1 may be modified as follows:

1.	Pronotal disc subreticulate, lines radiating from punctures only; female without spongy area on elytral declivity 1a
1'.	Pronotum uniformly, strongly reticulate; female with a spongy area on elytral declivity
1a(1).	Elytral disc and sides with interstrial rows of long, erect setae (Fig 2A); strial punctures about same width as interstriae (Fig.
	3A); 1.1–1.3 mm Crypturgus hispidulus
1a(1)'.	Elytral disc nearly glabrous (short, fine hairs may be present), fine setae restricted mostly to the elytral margins (Fig 2B); strial
	punctures about 0.5x width of interstriae (Fig 3B); 1.0–1.2 mm Crypturgus pusillus

Conclusion

Based on the biology of the genus, it is not predicted that *Crypturgus hispidulus* will be a pest of any real economic concern in North America. This report, however, does show how small and cryptic scolytines can become established and go undetected for long periods despite active surveillance and regular trapping. This has happened multiple times with morphologically similar species, such as with *Euwallacea interjectus* (Blandford) being mistaken for *Euwallacea validus* (Eichhoff) for 27 years (Cognato et al. 2015), and the complex of similar *Cyclorhipidion* spp. that took over 20 years to unravel and report (Smith & Cognato 2022). This recurrent theme highlights the need for identifiers and prescreeners to be extra attentive while processing samples, and to trust their instincts when something looks "a little off" from what they are used to seeing.

Acknowledgements

We would like to thank Peaks Island Land Trust for allowing access to collect on their preserves which resulted in

the initial specimen that triggered this study, as well as Robert Androw, Joshua Basham, Frank Guarnieri, Robert Rabaglia, and Thomas Schmeelk for providing loans of specimens to examine. We would also like to thank W. Kelley Thomas, Muriel Kelley, and Adane Nigatu from the Hubbard Center for Genome Studies at the University of New Hampshire. Computational work was supported by New Hampshire—INBRE through an Institutional Development Award (IDeA), P20GM103506, from the National Institute of General Medical Sciences of the NIH. Sequencing was funded through the USDA NIFA NH00694 award.

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