The Coleopterists Bulletin 76(1): 61–69. 2022.

First Records of the Palearctic Species *Trechus obtusus* Erichson from the Appalachian Region of the Southeastern United States (Coleoptera: Carabidae: Trechinae: Trechini)

Curt W. Harden, Michael S. Caterino
Department of Plant and Environmental Sciences, Clemson University
277 Poole Agricultural Center, Clemson, SC 29634, USA
c_har@fastmail.com

and

Thomas E. Malabad
Virginia Department of Conservation and Recreation, Division of Natural Heritage
8 Radford Street, Suite 102A, Christiansburg, VA 24073, USA
tom.malabad@dcr.virginia.gov

Abstract

The Palearctic species *Trechus obtusus* Erichson is reported for the first time from eastern North America. Recent collections in Virginia and North Carolina (**new state records**) indicate the establishment of the species in the Appalachian region. DNA was extracted from six individuals, and the COI barcoding region was sequenced. All but one of the COI barcode haplotypes were identical to those of *T. obtusus* collected in western North America. No matches were found with available Palearctic sequences. Individuals of *T. obtusus* were collected from caves and forest leaf litter, both habitats used by native trechine species. Sampled Appalachian populations of *T. obtusus* appear to be small, with the exception of the population in Mebane Saltpeter Cave (Pulaski County, VA), which is large and included teneral individuals. Currently, there is no evidence that the species is displacing or otherwise negatively affecting native trechines. The species should be monitored carefully going forward, and targeted sampling of synanthropic habitats will be the best method for early detection.

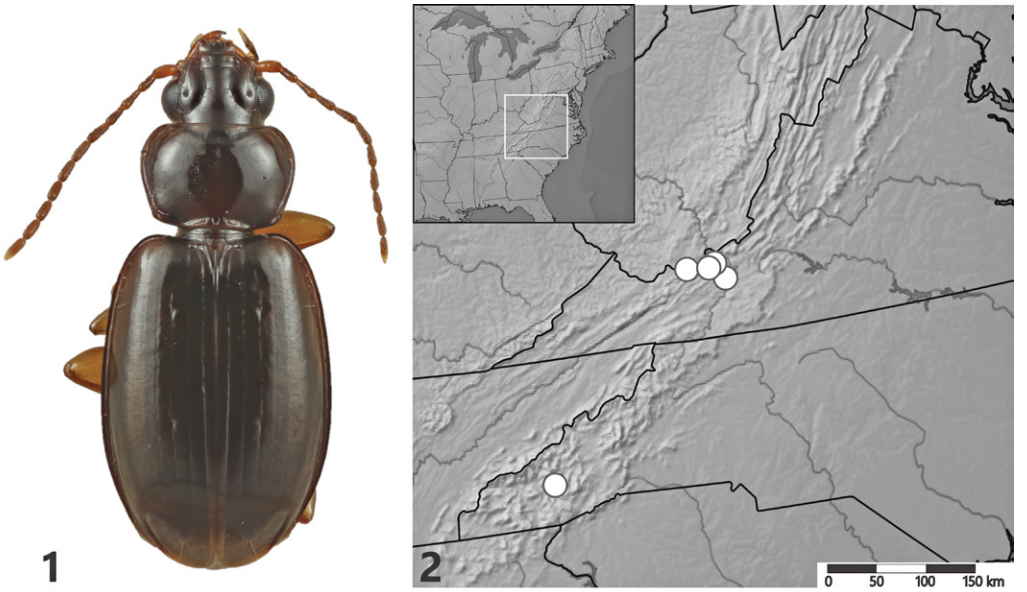
Keywords: adventive species, caves, DNA barcoding, range expansion

DOI.org/10.1649/0010-065X-76.1.61

Introduction

The native fauna of the tribe Trechini in the southeastern United States is the most diverse in North America, with 201 described species (Bousquet 2012). Much of this diversity is owed to the many flightless short-range endemics in isolated cave systems (*Pseudanophthalmus* Jeannel and related genera) and high mountain ranges in the southern Appalachians (*Trechus* Clairville, subgenus *Microtrechus* Jeannel). The only nonnative trechine reported in the literature from this region is *Trechus quadristriatus* (Schrank), from two sites in Virginia (Harden 2018). We here report the first records of *Trechus obtusus* Erichson in the region, which are also the first records for eastern North America.

Like *T. quadristriatus*, *T. obtusus* is native to the Palearctic region (Bousquet 2012). The two species, both members of the nominate subgenus, are closely related and challenging to separate using external characters, with the distance of the supraorbital setae from the inner margin of the eye being considered the most constant (see Boer 1965 for a review). Hirschfelder and Zucchi (1993) also found that the metaventrite in individuals of *T. obtusus* from Germany is shorter than the length of the mesocoxal cavity (versus longer in individuals of *T. quadristriatus*). This is true of the Appalachian specimens of *T. obtusus* as well, and might prove to be a reliable character to separate the two. The male genitalia are markedly different, and provide an unambiguous means for identification (Bousquet *et al.* 1984: figs. 4, 5). From the native southern Appalachian species of *Trechus* (*s. str.*), both species can be separated externally by their more elongate habitus, broad pronotum with obtusely rounded hind angles, and well-developed eyes (Fig. 1). Additionally, all specimens known so far from the Appalachian region possess fully-developed flight wings, which are lacking in native *Trechus* individuals. This might not remain a reliable character for separating the species, because changes in the wing condition of introduced populations of *T. obtusus* over time have been documented elsewhere (Liebherr and Krushelnycky 2007). Several subspecies of *T. obtusus* have been proposed for Palearctic



Figs. 1–2. *Trechus obtusus*. **1)** Dorsal habitus of male (length: 4 mm) from VA, Giles County, Flat Top Mountain, 37.24207, −80.86470, 28 June 2019, CUAC000134426; **2)** Collection localities in the Appalachian region.

populations, but assigning the Nearctic individuals to one of these has been problematic, since these categories are based largely on flight wing condition and geographic occurrence (Kavanaugh and Erwin 1985).

Both *T. obtusus* and *T. quadristriatus* have been established in the Nearctic region for several decades, but until now only *T. quadristriatus* was known from the eastern half of North America. *Trechus obtusus* has been established in western North America since the 1920s, and has been reported from British Columbia, Canada and from Washington, Oregon, California, Idaho, and Utah in the United States (Bousquet 2012). Kavanaugh and Erwin (1985) provided a review of western North American specimen data and reconstructed the history of the species' introduction and spread in the region. They concluded that the species was most likely being transported with nursery stock; all life stages of the species may be found in soil at the base of cultivated plants, and may therefore be easily moved in this manner. Liebherr and Takumi (2002) reported the establishment of *T. obtusus* on the island of Maui, Hawaii, and gave evidence in support of a western North American origin of this population. Evenhuis and Imada (2013) reported the species from the island of Hawaii.

While examining beetles collected from Virginia caves by staff of the Virginia Department of Conservation and Recreation's Division of Natural Heritage, author CWH identified several *T. obtusus*. Personal collecting in Virginia and searching in the collections of the Virginia Museum of Natural History (VMNH), Martinsville, Virginia and Clemson University Arthropod Collection (CUAC), Clemson, South Carolina produced several more records from Virginia and North Carolina (**new state records**, Fig. 2). The purpose of this paper is to report these records, the first from eastern North America. To corroborate the identity of these specimens and compare haplotypes with other populations, DNA sequences of the barcoding region of the COI mitochondrial gene were obtained from six specimens of *T. obtusus* and seven specimens of native Appalachian *Trechus* species.

Materials and Methods

The key and figures in Bousquet *et al.* (1984) were used to confirm the identity of specimens of *T. obtusus*. Males were available from each collecting event, and the genitalia of all were examined. When the distinctive apex of the median lobe was not protruding, dry-mounted male specimens were softened in hot water for 30 minutes and dissected with fine forceps and bent insect pins. Morphological characters were studied using a Leica M80 stereomicroscope at magnifications of 7.5–60×. After study, male genitalia were placed in glycerin microvials pinned beneath the specimens.

Specimens selected for DNA barcoding had been previously collected into either 95% or 100%

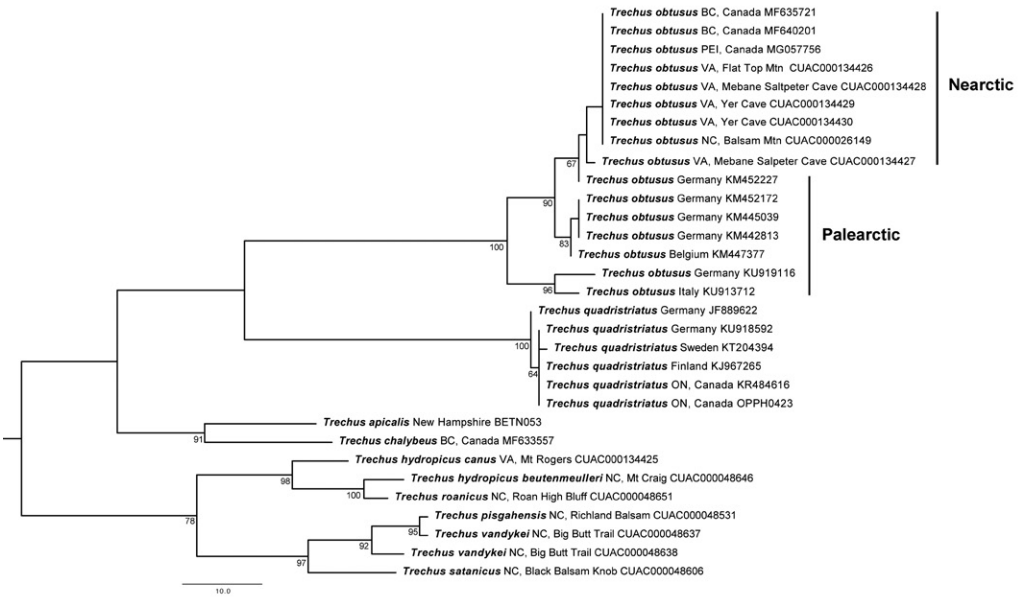


Fig. 3. One of two equally parsimonious trees from maximum parsimony analysis of the COI barcoding region in PAUP*, with Nearctic and Palearctic individuals of *Trechus obtusus* indicated. Bootstrap values over 50 are shown. Outgroups not shown.

ethanol and stored in −20 °C freezers. Before extracting, dorsal habitus images 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). Image stacking was done using Helicon Focus (www.heliconsoft.com). Specimens were prepared for whole-body extraction by separation at the prothorax-mesothorax junction and separation of the abdomen from the metathorax. Extractions were carried out using the Thermo Scientific Genejet Genomic DNA purification kit following the manufacturer's protocol. Extracted DNA is stored at −20 °C in the Caterino Arthropod Biodiversity Lab at Clemson University, Clemson, South Carolina.

For the six *T. obtusus* specimens and the specimen of *Trechus hydropicus canus* Barr, the barcoding region of the mitochondrial gene cytochrome oxidase subunit I was amplified using the primers LCO1490 (GGTCAACAAATCATAA AGATATTGG) and HCO2198 (TAAACTTCAG GGTGACCAAAAAATCA) in 25-µL PCR reactions containing 1.0 µL DNA template, 1.0 µL each primer, 2.5 µL dNTPs, 2.0 µL $MgCl_2$, 0.125 µL *Platinum Taq*, 2.5 µL buffer, and 14.875 µL H_2O. Reactions were carried out using an Eppendorf MasterCycler with the following settings: initial denaturation stage of 180 seconds at 95 °C followed by 35 cycles of a denaturation stage at 94 °C for 30 seconds, annealing stage at 45 °C for 30 seconds, and an extension stage at 72 °C for 45 seconds, with a final extension at 72 °C for 180 seconds. Six sequences from native Southern Appalachian *Trechus* species were obtained as part of a larger ongoing project documenting litter arthropod diversity at high elevations in the Southern Appalachians. This 421-bp segment was amplified using the primers BF2 (GCHCCHGAYAT RGCHTTYCC) and BR2 (TCDGGRTGNCC RAARAAYCA) (both from Elbrecht and Leese 2017), and the same PCR settings as above. PCR products were shipped to Psomagen, Inc. (Maryland, USA) for cleaning and sequencing. Forward and reverse sequences were assembled and trimmed using Geneious (ver. 8.1.8; Auckland, NZ). No insertions or deletions were present, so alignment with GenBank sequences was straightforward, and was performed using Mesquite Version 3.61 (Maddison and Maddison 2019).

Our dataset comprised 31 COI barcoding region sequences from European, and eastern and western US *Trechus,* in addition to three outgroup sequences from other tribes of Carabidae (Bembidiini, Pterostichini, and Harpalini). All sequences analyzed are listed in Table 1, with localities and accession numbers. This matrix was analyzed using parsimony in PAUP*. We ran a heuristic search of 1,000 replicates, and 1,000 bootstrap replicates to assess branch strength.

Table 1. Specimen data for COI barcoding region sequences used in maximum parsimony analysis.

Species	Locality	GenBank/ BOLD accession	Voucher number
Trechus obtusus	Canada: British Columbia, Gulf Islands National Park Reserve	MF635721	BIOUG22849-G04
Trechus obtusus	Canada: British Columbia, Gulf Islands National Park Reserve	MF640201	BIOUG22748-F01
Trechus obtusus	Canada: Prince Edward Island, Miscouche	MG057756	BIOUG25559-H01
Trechus obtusus	Germany: Saxony, Niederwiesa, Braunsdorf, Zschopau-Tal	KU919116	ZFMK-TIS-2508906
Trechus obtusus	Italy: Veneto, Auronzo-Somprade, Fiume Ansiei	KU913712	ZFMK-TIS-2512131
Trechus obtusus	Germany: North Rhine-Westphalia, Niederrhein	KM452227	BFB_Col_FK_8244
Trechus obtusus	Germany: Rhineland Palatinate, Noerdliche Oberrheinebene	KM452172	BFB_Col_FK_9881
Trechus obtusus	Belgium: Vlaams-Brabant, Vlanderen	KM447377	BFB_Col_FK_8703
Trechus obtusus	Germany: Rhineland Palatinate, Noerdliche Oberrheinebene	KM445039	GBOL_Col_FK_3373
Trechus obtusus	Germany: Rhineland Palatinate, Noerdliche Oberrheinebene	KM442813	GBOL_Col_FK_3374
Trechus obtusus	United States: Virginia, Giles Co., Flat Top Mountain.	OL806579	CUAC000134426
Trechus obtusus	United States: Virginia, Pulaski Co., Mebane Saltpeter Cave	OL806580	CUAC000134427
Trechus obtusus	United States: Virginia, Pulaski Co., Mebane Saltpeter Cave	OL806581	CUAC000134428
Trechus obtusus	United States: Virginia, Giles Co., Yer Cave	OL806582	CUAC000134429
Trechus obtusus	United States: Virginia, Giles Co., Yer Cave	OL806583	CUAC000134430
Trechus obtusus	United States: North Carolina, Jackson Co., Balsam Mountain Preserve	OL806584	CUAC000026149
Trechus quadristriatus	Germany	JF889622	BC_ZSM_COL_01235
Trechus quadristriatus	Germany: Rheinland-Pfalz, Altrip, Neuhofener Altrhein	KU918592	ZFMK-TIS-2505316
Trechus quadristriatus	Sweden: Uppland	KT204394	-
Trechus quadristriatus	Finland: Nylandia, Hanko, Kolaviken	KJ967265	ZMUO<FIN>:004670
Trechus quadristriatus	Canada: Ontario, Wellington County, Guelph	KR484616	BIOUG08803-G04
Trechus quadristriatus	Canada: Ontario, Grand Bend, Pinery Provincial Park	OPPHO423-17. COI-5P	BIOUG33535-A12
Trechus apicalis Motschulsky	United States: New Hampshire, Bartlett Experimental Forest	BETN053-18. COI-5P	NEON.BET.D01.001369
Trechus chalybeus Dejean	Canada: British Columbia, Glacier National Park	MF633557	BIOUG22339-H08

(*Continued*)

Table 1. (*Continued*)

Species	Locality	GenBank/ BOLD accession	Voucher number
Trechus hydropicus canus	United States: Virginia, Grayson Co., Mount Rogers	OL806585	CUAC000134425
Trechus hydropicus beutenmuelleri Jeannel	United States: North Carolina, Yancey County, Mt. Mitchell State Park, Mt. Craig near summit	OL813797	CUAC000048646
Trechus roanicus Barr	United States: North Carolina, Mitchell County, Roan High Bluff	OL813798	CUAC000048651
Trechus pisgahensis Barr	United States: North Carolina, Haywood County, Blue Ridge Parkway National Park, Richland Balsam Mt.	OL813799	CUAC000048531
Trechus vandykei	United States: North Carolina, Buncombe, Pisgah National Forest, Big Butt Trail	OL813800	CUAC000048637
Trechus vandykei	United States: North Carolina, Buncombe, Pisgah National Forest, Big Butt Trail	OL813801	CUAC000048638
Trechus satanicus Barr	United States: North Carolina, Haywood County, Pisgah National Forest, Black Balsam Knob	OL813802	CUAC000048606
Pterostichus trinarius (Casey)	United States: Virginia, Clarke Co., Casey Tree Farm	BETN1237-18. COI-5P	NEON.BET.D02.002071
Harpalus providens Casey	United States: Virginia, Warren Co., Smithsonian Conservation Biology Institute	BETN1299-18. COI-5P	NEON.BET.D02.002867
Bembidion pedicellatum LeConte	United States: Virginia, Clarke Co., Casey Tree Farm	BETN1231-18. COI-5P	NEON.BET.D02.002008

Results

Twenty-four individuals of *T. obtusus* from the southern Appalachian region were identified (Table 2). Fifteen of these were male and nine were female. All individuals possessed fully developed flight wings. One individual was collected from a Malaise trap set above a small stream, indicating flight ability. Several of the individuals from Mebane Saltpeter Cave were teneral. Sample sizes per site were low (one or two individuals) with the exception of Mebane Saltpeter Cave, where over 17 were collected and over 100 additional putative specimens were observed.

Three cases of syntopy with individuals of native trechine species were documented. On Flat Top Mountain in Giles County, Virginia, near Pearisburg, Winkler extraction of a sifted litter sample produced a single male *T. obtusus* and a short series of *T. hydropicus canus*. Berlese extraction of a sifted litter sample from Balsam Mountain Preserve in Jackson County, North Carolina produced a single male *T. obtusus* and numerous specimens of a *Microtrechus* species near *Trechus vandykei* (Jeannel). Both litter samples also contained typical numbers of other native flightless beetles found in deciduous forest litter habitats in the Southern Appalachians. In Yer Cave in Giles County, Virginia, a series of 15 *Pseudanophthalmus cf. egberti* Barr were collected on the same occasion as two individuals of *T. obtusus*. The *Pseudanophthalmus* occurred throughout the cave, but were most abundant along the main stream passage deep in the cave. Individuals of *T. obtusus* were found only within 500 ft of the entrance.

DNA barcodes of all North American (eastern and western) individuals of *T. obtusus* were identical except for one individual from Mebane Saltpeter Cave (Pulaski County, Virginia) that differs in two bases. These haplotypes are distinct from, but nested within, a paraphyletic group of European *T. obtusus* haplotypes available through GenBank (representing

Table 2. Locality data for *Trechus obtusus* specimens identified from the Appalachian region.

State	County	Location	Latitude	Longitude	Date	Count	Collection
NC	Jackson	Balsam Mountain Preserve; Malaise trap over Sugarloaf Creek	35.3914	−83.11030	16 September–13 October 2015	1 male	CUAC
NC	Jackson	Balsam Mountain Preserve; litter sample	35.3916	−83.10910	20 April 2015	1 male	CUAC
VA	Bland	Rt 598, 1 mi W of I-77; Winkler sample	37.2647	−81.13250	29 July 2015	1 male, 1 female	VMNH
VA	Giles	Yer Cave, 4.3 mi SW of Pearisburg	Concealed	Concealed	9 August 2019	1 male 1 female	CUAC
VA	Giles	Flat Top Mountain	37.24207	−80.8647	28 June 2019	1 male	CUAC
VA	Pulaski	Mebane Saltpeter Cave, 0.3 mi N of Dublin	Concealed	Concealed	3 October 2018	10 males, 7 females	CUAC (2), VMNH (15)

populations in Germany, Italy, and Belgium). Maximum parsimony analysis of our dataset produced two equally parsimonious trees, one of which is shown in Fig. 3.

Discussion

Our data from multiple localities indicate that *T. obtusus* is established in eastern North America, although the mode and origin of introduction are uncertain. The DNA barcode data suggest that the eastern populations were introduced from either the western North American population or from the same Palearctic source that established it. None of the Appalachian haplotypes matched Palearctic sequences available through GenBank and BOLD, so members of this source population are probably not represented on those databases. We consider an introduction from western North America more likely than a separate Palearctic introduction, given the inland localities of the Appalachian individuals studied.

In addition to the expansion of the known established range, the Appalachian discoveries of *T. obtusus* are surprising for their occurrence in caves and deep leaf litter, both of which are habitats required by the flightless native trechines in the region. Most species of *Pseudanophthalmus* and *Trechus* (*Microtrechus*) are short-range endemics, occurring in a single cave system or mountain chain (Barr 1985; Donabauer 2005a, b, 2009; Niemiller *et al.* 2017). While they are often abundant where they occur, their seemingly narrow microhabitat requirements make them vulnerable to extirpation or extinction due to ecological change, including introduction of nonnative species. The effect that phylogenetic proximity of introduced species has on their ability to compete with native members of a community is a popular and contested topic in invasion ecology ("Darwin's naturalization conundrum", see Diez *et al.* [2008]), but it may not be applicable to this scenario for two reasons: 1) within the tribe Trechini, *T. obtusus* is not particularly closely related to *Pseudanophthalmus* or to the native Appalachian *Trechus*; recent molecular phylogenies of trechines indicate that *Trechus* as currently defined is not monophyletic (Faille *et al.* 2011; Maddison *et al.* 2019), and 2) while singletons of *T. obtusus* have been collected from leaf litter in association with series of native *Trechus*, we believe that these have been serendipitous captures of individuals who strayed from nearby synanthropic habitats. Similarly, the occurrence of *T. obtusus* in Mebane Saltpeter Cave and Yer Cave is probably due to the proximity of disturbed hayfield habitats near the entrances to these caves. While native *Pseudanopthalmus* also occur in Yer Cave, they were more abundant than *T. obtusus* and were present in a wider range of habitats and depths within the cave.

The ecological impact of adventive carabid beetles is generally considered minimal, largely because they typically use only frequently disturbed habitats such as agricultural fields, where they are unlikely to directly compete with narrow habitat specialists (Larochelle and Larivière 2003). Contrastingly, LaBonte (2011) presented data on the exceptional case of *Nebria brevicollis* (Fabricius),

an introduced species that has spread rapidly and proved capable of thriving in nearly all available habitats in its established range in Oregon, including old-growth forests and pristine mountain balds, sometimes becoming the most abundant carabid encountered. This has apparently not been the case with western North American populations of *T. obtusus* (Sokolov and Kavanaugh 2014), but Liebherr and Krushelnycky (2007) presented data showing an increase in abundance of *T. obtusus* populations that share microhabitats with native flightless carabids in Hawaii. These authors also documented a rapid transformation to a brachypterous condition in these populations, as well as an apparent tolerance for an invasive ant species, both of which could give them a competitive advantage in this landscape.

In North America, *T. obtusus* has been reported as occurring in "Cultivated fields, wastelands, roadsides, gravel pits, city lawns, gardens, parks, orchards (*e.g.*, apple), and forest edges" (Larochelle and Larivière 2003). Our two Virginia litter samples were taken in close proximity to small roads within secondary deciduous forest. The Flat Top Mountain site had recently been burned, and so may qualify as a disturbed habitat. The North Carolina site is within a private residential community (Balsam Mountain Preserve) that contains extensive mature forest habitat as well as disturbed areas, including an 18-hole golf course. The *T. obtusus* specimens from Balsam Mountain Preserve were both taken from mature forest habitat near a residential home. In our two litter samples that contained both *T. obtusus* and native *Trechus* (Flat Top Mountain and Balsam Mountain Preserve), a single *T. obtusus* was collected among a larger series of the native species. Since 2015, author MSC and others at Clemson University (Clemson, South Carolina) have collected and processed approximately 350 litter samples from the southern Appalachian Mountains, and only one *T. obtusus* has been collected.

The largest population of *T. obtusus* in the Appalachian region was observed in Mebane Saltpeter Cave in Pulaski County, Virginia. The cave was mined for saltpeter during the American Revolutionary and Civil Wars, and some evidence of these operations is still visible today in the form of beams and tool marks. The entrance to the cave is located in a dissected complex of hayfields bordered by small patches of woods in close proximity to residential and commercial properties. A farm pond is located upstream of the cave entrance and in wet weather the cave receives water from the pond and surrounding surface area, introducing large quantities of organic matter such as grasses, sticks, and logs. On 3 October 2018, author TEM and Katarina Kosič Ficco (KKF) observed over 100 putative individuals of *T. obtusus* in this cave, in addition to the 17 specimens collected. These individuals were mostly concentrated in the lower back section of the cave, in areas with damper soil and decomposing organic material. The presence of several teneral individuals might mean that reproduction is taking place within the cave, but it is also possible that recently eclosed adults from the surface moved into the cave or were washed in. Four as yet undetermined staphylinid beetle species were collected in association. No native trechines have been collected in this cave.

Yer Cave in Giles County, Virginia is a complex vertical cave developed on multiple levels with an active stream in the lower portion and a surveyed length of 18,019 ft and a depth of 269 ft. The entrance is a narrow pit at the bottom of a sinkhole surrounded by hayfields and cattle pastures. On 9 August 2019 TEM and KKF collected two and observed several (< 10) putative individuals of *T. obtusus* within 500 ft of the entrance, among organic material washed in from the surface. The troglophilic staphylinid beetles *Aleochara lucifuga* (Casey), *Quedius alesi* Brunke, and *Aloconota* sp. were collected in association. On the same date, TEM and KKF also collected 15 native trechines of the genus *Pseudanophthalmus* from Yer Cave, and observed approximately 12 additional specimens. These represent the first collection of *Pseudanophthalmus* from this cave. *Pseudanophthalmus* occurred throughout the cave, in particular on mud banks adjacent to streams, but were most abundant in the lower level of the cave next to the larger main stream. TEM and KKF have documented *P. egberti* in the same drainage area within hydrologically related caves, and *Pseudanophthalmus quadratus* Barr, 1965 from caves in the neighboring drainage. Based on the location of the cave, *P. egberti* is most likely what is present in Yer Cave. Taxonomic work by Karen A. Ober (College of the Holy Cross, Worcester, Massachusetts) to confirm these beetles to the species level is underway.

These two cave occurrences of *T. obtusus*, especially the population in Mebane Saltpeter Cave, demonstrate that members of the species can reach relatively high abundances in cave habitats under certain conditions. The regular transport by water of organic material into the caves, as well as the proximity of open hay pastures, likely contribute to the species' occurrence in these caves. It would be worthwhile to carefully search surface habitats in close proximity to these caves to determine if *T. obtusus* is established and abundant outside the caves. Likewise, future efforts to survey for *T. obtusus* in the Southern Appalachian region should target synanthropic habitats such as highway margins, grassy lawns, compost sites, and open

habitats maintained for recreation and agriculture. While it will certainly be important to monitor the occurrence and abundance of *T. obtusus* in intact native montane forests, our data indicate that litter sifting is not an efficient collecting method for the species, and early detection efforts should be directed to the more suitable habitats listed above.

We note that one of the *T. obtusus* sequences obtained through BOLD (BIOUG25559-H01) is from a specimen collected on Prince Edward Island, Canada in 2015. We have not examined the specimen and cannot vouch for this record's accuracy, but if it is genuine then the species is likely much more widespread in eastern North America than our records indicate. Synanthropic habitats typically do not receive as much attention from carabid collectors as pristine habitats harboring interesting endemic species, so it is possible that dedicated efforts will discover *T. obtusus* to be quite common and widespread in eastern North America. Even the relatively massive and gaudily colored *Carabus auratus* Linnaeus has apparently escaped detection over large portions of its presumed introduced range in New England due to its preference for uncharismatic habitats such as residential lawns (Lewis *et al.* 2015).

Acknowledgments

We thank Managing Editor Matthew Gimmel and an anonymous reviewer for their comments and corrections that improved the quality of this manuscript. Dr. Shelley S. Langton-Myers and Dr. John C. Morse provided valuable specimens for this study. CWH's collecting trip to Flat Top Mountain in 2019 was facilitated and assisted by fellow staff of the Virginia Museum of Natural History: Dr. Kal Ivanov, Dr. Joe B. Kieper, and Benjamin R. Williams. Dr. Kal Ivanov also allowed access to the VMNH collection. Cave bioinventory surveys conducted by TEM and KKF were made possible by the generous support of the Cave Conservancy of the Virginias provided to the Virginia Department of Conservation and Recreation, Division of Natural Heritage. Some collections in North Carolina were made under permits from the North Carolina State Parks, and Blue Ridge Parkway National Park. Funding for DNA sequencing was provided by an E. W. King Memorial Grant Fund awarded to CWH in 2020, NSF grant DEB-1916263 to MSC, and USDA/NIFA project SC-1700596. This is Technical Contribution No. 7029 of the Clemson University Experiment Station.

References Cited

Barr, T. C. 1985. Pattern and process in speciation of trechine beetles in eastern North America (Coleoptera: Carabidae: Trechinae) [pp. 350–407]. *In*: Taxonomy, Phylogeny and Zoogeography of Beetles and Ants (G. E. Ball, editor). Dr. W. Junk Publishers, Dordrecht, 514 pp.

Boer, P. J. den. 1965. External characters of sibling species *Trechus obtusus* Er. and *T. quadristriatus* Schrk. (Coleoptera). Tijdschrift voor Entomologie 108: 219–239.

Bousquet, Y. 2012. Catalogue of Geadephaga (Coleoptera: Adephaga) of America, north of Mexico. ZooKeys 245: 1–1722.

Bousquet, Y., A. Smetana, and D. R. Maddison. 1984. *Trechus quadristriatus*, a Palearctic species introduced into North America (Coleoptera: Carabidae). The Canadian Entomologist 116: 215–220.

Diez, J. M., J. J. Sullivan, P. E. Hulme, G. Edwards, and R. P. Duncan. 2008. Darwin's naturalization conundrum: Dissecting taxonomic patterns of species invasion. Ecology Letters 11: 674–681.

Donabauer, M. 2005a. New species of the *Trechus* (*Microtrechus*) *uncifer*-group from the southern Appalachians (Coleoptera: Carabidae). Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen 57: 51–62.

Donabauer, M. 2005b. New species of the *Trechus* (*Microtrechus*) *nebulosus*-group from the southern Appalachians (Coleoptera: Carabidae). Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen 57: 65–92.

Donabauer, M. 2009. New species of the *Trechus* (*Microtrechus*) *vandykei*-group from the southern Appalachians (Coleoptera: Carabidae). Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen 60: 129–141.

Elbrecht, V., and F. Leese. 2017. Validation and development of COI metabarcoding primers for freshwater macroinvertebrate mioassessment. Frontiers in Environmental Science 5(11): 1–11. doi.org/10.3389/fenvs.2017.00011.

Evenhuis, N. L., and C. T. Imada. 2013. New records of nonindigenous Carabidae (Coleoptera) from the Big Island [pp. 57–58]. *In*: Records of the Hawaii Biological Survey for 2012 (N. L. Evenhuis and L.G. Eldgredge, editors). Bishop Museum Occasional Papers 114: 1–69.

Faille, A., A. Casale, and I. Ribera. 2011. Phylogenetic relationships of Western Mediterranean subterranean Trechini groundbeetles (Coleoptera: Carabidae). Zoologica Scripta 40: 282–295.

Harden, C. W. 2018. Eight ground beetles (Coleoptera: Carabidae) new to Virginia, with additional records for West Virginia and Maryland. Banistera 50: 15–20.

Hirschfelder, A., and H. Zucchi. 1993. On a new distinguishing feature of the species *Trechus obtusus* and *Trechus quadristriatus* (Coleoptera: Carabidae). Beiträge zur Entomologie 43: 375–378.

Kavanaugh, D. H., and T. L. Erwin. 1985. *Trechus obtusus* Erichson (Coleoptera: Carabidae), a European ground beetle, on the Pacific coast of North America: Its distribution, introduction, and spread. The Pan-Pacific Entomologist 61(2): 170–179.

LaBonte, J. L. 2011. *Nebria brevicollis* (Fabricius, 1792) in North America, benign or malign? (Coleoptera, Carabidae, Nebriini). ZooKeys 147: 497–543.

Larochelle, A., and M.-C. Larivière. 2003. A Natural History of the Ground Beetles (Coleoptera: Carabidae) of America North of Mexico. Pensoft Series Faunistica, No 27. Pensoft, Sofia-Moscow, 583 pp.

Lewis, J. H., R. P. Webster, and D. F. McAlpine. 2015. First occurrence in Canada of *Carabus auratus* L. (Coleoptera: Carabidae), an adventive ground beetle of European origin. The Coleopterists Bulletin 69(2): 264–266.

Liebherr, J. K., and P. D. Krushelnycky. 2007. Unfortunate encounters? Novel interactions of native *Mecyclothorax*, alien *Trechus obtusus* (Coleoptera: Carabidae), and Argentine ant (*Linepithema humile*, Hymenoptera: Formicidae) across a Hawaiian landscape. Journal of Insect Conservation 11: 61–73.

Liebherr, J. K., and R. Takumi. 2002. Introduction and distributional expansion of *Trechus obtusus* (Coleoptera: Carabidae) in Maui, Hawai'i. Pacific Science 56(4): 365–375.

Maddison, D. R., K. Kanda, O. F. Boyd, A. Faille, N. Porch, T. L. Erwin, and S. Roig-Junent. 2019. Phylogeny of the beetle supertribe Trechitae (Coleoptera: Carabidae): Unexpected clades, isolated lineages, and morphological convergence. Molecular Phylogenetics and Evolution 132: 151–176.

Maddison, W. P., and D. R. Maddison. 2019. Mesquite: A modular system for evolutionary analysis. Version 3.61. www.mesquiteproject.org.

Niemiller, M. L., K. S. Zigler, K. A. Ober, E. T. Carter, A. S. Engel, G. Moni, T. K. Philips, and C. D. R. Stephen. 2017. Rediscovery and conservation status of six short-range endemic *Pseudonophthalmus* cave beetles (Carabidae: Trechini). Insect Conservation and Diversity 10: 495–501.

Sokolov, I. M., and D. H. Kavanaugh. 2014. New records of adventive Carabidae (Coleoptera) found in California. The Pan-Pacific Entomologist 90(2): 66–71.

(Received 12 March 2021; accepted 12 January 2022. Publication date 18 March 2022.)