

Vertigo marciae (Gastropoda: Vertiginidae), a new land snail from Jamaica

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ABSTRACT

Vertigo marciae, a new species of gastropod mollusk (Pupilloidea: Vertiginidae), is described from Jamaica. This species is known in the Recent fauna only from John Crow Peak in the Blue Mountains, but also occurs as a Pleistocene fossil at Red Hills Road Cave. *Vertigo marciae* has been confused with *Vertigo gouldii*, but differs by its smaller shell size, lack of distinct shell striation, lack of an angular lamella, and presence of a flared aperture base. DNA sequence analyses document that *V. marciae* possesses unique mtDNA and nDNA sequences and is most closely allied with *Vertigo alabamensis*, *Vertigo hebaridi*, and *Vertigo oscariana*. This group of species comprises a highly supported clade whose members are limited either to the Caribbean or the southeastern USA.

Additional Keywords: *Vertigo*, biogeography, southeastern USA, Caribbean, DNA sequence analysis

INTRODUCTION

In their synopsis of the Jamaican land snail fauna, Rosenberg and Muratov (2006) reported three species from the genus *Vertigo*, all of which possess ranges extending into the eastern half of North America (Nekola and Coles, 2010): *V. gouldii*, *V. milium*, and *V. ovata*. However, images of putative Jamaican ‘*V. gouldii*’ on the Discover Life website (<http://pick4.pick.uga.edu/mp/20q?search=Vertigo+gouldii&guide=1>) illustrate a shell quite unlike *V. gouldii* from eastern North America (Nekola and Coles, 2010) in its light-yellow shell color, lack of sharp shell striation, and absence of a basal lamella. Observation of the three known Jamaican lots for this entity in the Academy of Natural Sciences of Philadelphia (ANSP) collections confirmed these differences. Although they looked most like *V. hebaridi* Vanatta, 1912 of the Florida Keys, these lots clearly differed from that species by their taller shell, reduced striation, and absence of an angular lamella. Thus, rather than representing a population of *V. gouldii* isolated by 2000 km from its nearest neighbors (Nekola

and Coles, 2010), these specimens appear to represent an undescribed new species. Based on shell descriptions, reports of *Vertigo gouldii* from the Pleistocene Red Hills Road Cave deposits of Jamaica (Paul and Donovan, 2005) also appeared to represent this new species. This conclusion was subsequently confirmed via observation of digital images.

We therefore investigated this putative new species via analyses of both shell morphology and DNA sequence data, and are now in the unusual situation of describing and reporting a new species simultaneously from both living and fossil material, while also being able to report on its phylogenetic relationships.

MATERIALS AND METHODS

Field Collection: The three known Recent lots of the putative new Jamaican species (ANSP 402244, 403039, and 403040) were collected on John Crow Peak in the Blue Mountains of eastern Jamaica on May 22, 1999, during the Jamaican Biotic Survey. Collecting methods were detailed in Rosenberg and Muratov (2006), and included drying of soil litter samples over a Berlese Funnel. This encouraged snails to enter aestivation, allowing them to mummify upon death. For this reason, it was possible to successfully extract, amplify, and sequence selected amplicons from both their mitochondrial and nuclear genomes (Nekola et al., 2009).

Shell Measurements: Measurements were determined in 0.1 mm increments for adult shells with aperture facing up, using a dissecting microscope with a calibrated ocular micrometer. Height and width were measured as the dimensions of a bounding box with long axis parallel to the shell axis and sides tangent to the tip of the protoconch, the base of the lip, the right-most margin of the aperture and the left-most margin of the body whorl.

Imaging: Shells were imaged at 20× magnification using a digital camera attached to a stereomicroscope.

Approximately 14 separate 1388×1040 pixel images were made of each specimen with the image focal lengths positioned at 75 µm increments from the front to back of the shell. CombineZ5 freeware (<http://www.hadleyweb.pwp.blueyonder.co.uk/CZ5/combinez5.htm>) was used to assemble a final image from the focused parts of each separate image. Assembled images were imported into Adobe Photoshop, where brightness and contrast were optimized and the background made uniformly black. These images were then compiled into a single plate.

DNA Sequence Analysis: Mitochondrial cytochrome b (*CytB*), 16S ribosomal RNA (*16S*) and the internal transcribed spacers 1 (*ITS-1*) and 2 (*ITS-2*) of the nuclear ribosomal RNA gene were investigated to test the distinctiveness of the putative new Jamaican species and to resolve its nearest evolutionary neighbors. The mitochondrial cytochrome oxidase subunit 1 (*COI*) was not analyzed as we were unable to amplify this gene in the Jamaican species.

Sixteen specimens were chosen for analysis (Table 1). This set includes not only two specimens of the Jamaican species but also *Vertigo alabamensis*, *V. gouldii*, *V. hebaradi*, and *V. oscariana*. We attempted to maximally spread these individuals across the known geographic range of each species (see Nekola and Coles, 2010). Because *V. conecuhensis* is likely a simple shell form of *V. alabamensis* (Nekola and Coles, 2010),

only a single individual of this taxon was included. Topotype or near-topotype material was selected for *V. conecuhensis* (Pond Creek, Covington Co., Alabama, about 50 km from the type locality in Evergreen, Alabama) and *V. hebaradi* (Long Key, Florida which is the type locality). Previously analyzed *16S* sequence data from three *Vertigo gouldii* specimens analyzed by Nekola et al. (2009) were retrieved from GeneBank (Table 1). *Vertigo pusilla*, the type species of the genus, was also included, as were *Gastrocopta cristata* and *Pupilla muscorum* for outgroup comparisons.

Genomic DNA was extracted from live, ethanol-preserved, or mummified material using the OmegaBioTek Mollusk DNA Extraction Kit. Because shell destruction was required, all shells were imaged prior to extraction using the methods detailed above. PCR amplification and sequencing of *CytB*, *16S*, *ITS-1*, and *ITS-2* were accomplished using standard methods (Nekola et al., 2009). Resultant traces were examined, primer ends removed, and aligned by eye. Because of their lower evolutionary rates, the nDNA *ITS-1* and *ITS-2* regions were concatenated to provide a roughly similar number of nucleotide differences to either of the mtDNA amplicons. Mega 5.0 (Tamura et al., 2011) was used to calculate the average number of nucleotide differences in the *CytB*, *16S*, and concatenated *ITS-1* + *ITS-2* regions between all putative species-level taxa. Substitutions included both transitions and transversions with pairwise gap deletion.

Table 1. Specimen information for material used in DNA sequence analysis.

Location	Taxon	Latitude/Longitude	GENBANK Accession number			
			<i>CytB</i>	<i>16S</i>	<i>ITS-1</i>	<i>ITS-2</i>
<i>Vertigo marciae</i> new species						
John Crow Peak, St. Andrew Parish, Jamaica		18.1132 N., 76.6685 W.	KF214513	KF214503		
John Crow Peak, St. Andrew Parish, Jamaica		18.1132 N., 76.6685 W.	KF214514	KF214502	KF214489	KF214477
<i>Vertigo alabamensis</i> Clapp, 1915						
Johnson Mill Bay, Bladen Co., North Carolina, USA		34.7125 N., 78.5261 W.	KF214515	KF214501	KF214490	KF214478
Wolf Trap Bay, Leon Co., Florida, USA		30.3680 N., 84.5700 W.	KF214516	KF214500	KF214491	KF214479
<i>Vertigo conecuhensis</i> Clapp, 1915						
Pond Creek, Covington Co., Alabama		31.1036 N., 86.5343 W.	KF214517	KF214499	KF214492	KF214480
<i>Vertigo gouldii</i> (A. Binney, 1843)						
Brush Creek Canyon, Fayette Co., Iowa, USA		42.7796 N., 91.6890 W.	KF214508	GQ921506	KF214484	KF214472
Panther Creek, Searcy Co., Arkansas, USA		36.0858 N., 92.5649 W.	KF214509	GQ921507	KF214485	KF214473
11-Point River, Oregon Co., Missouri, USA		36.7931 N., 91.3334 W.	KF214510	KF214506	KF214486	KF214474
<i>Vertigo hebaradi</i> Vanatta, 1912						
Long Key, Monroe County, Florida, USA		24.8146 N., 80.8211 W.	KF214511	KF214505	KF214487	KF214475
Elliott Key, Miami-Dade County, Florida, USA		25.4553 N., 80.1925 W.	KF214512	KF214504	KF214488	KF214476
<i>Vertigo oscariana</i> (Sterki, 1890)						
Blanchard Springs, Stone Co., Arkansas, USA		35.9582 N., 92.1778 W.	KF214518	KF214498	KF214493	KF214481
Wadboo Creek, Berkeley Co., South Carolina, USA		33.1971 N., 79.9461 W.	KF214519	KF214497	KF214494	KF214482
<i>Vertigo pusilla</i> Müller, 1774						
Podyji National Park, Moravia, Czech Republic		48.8586 N., 15.8960 E.	KF214520	KF214496	KF214495	KF214483
<i>Gastrocopta cristata</i> (Pilsbry and Vanatta, 1900)						
Albuquerque, Bernalillo Co., New Mexico, USA		35.0727 N., 106.6160 W.	KF214522	JN941032		
<i>Pupilla muscorum</i> (Linnaeus, 1758)						
Masaryk University, Brno, Moravia, Czech Republic		49.2509 N., 16.5738 E.	KF214521	KF214507		

Mega 5.0 was used to conduct nearest-neighbor joining (NNJ), maximum parsimony (MP), and maximum likelihood (ML) trees for the three focal regions. NNJ was based on Maximum Composite Distance including transitions and transversions with pairwise gap deletion. MP used the close neighbor interchange search option with the random addition of 10 replicate trees. ML used all sites and was based on the Tamura-Nei substitution model, a five-category Gamma Distribution for substitution rates, and the Nearest Neighbor Interchange ML heuristic method. In all cases support values were estimated from 1000 bootstrap replicates. Additionally, Bayesian trees were generated using MrBayes 3.1 (Huelsenbeck and Ronquist, 2001) using a GTR substitution model assuming gamma-shaped rate variation over 1,000,000 generations with a sampling frequency of once each 1000 generations.

Nomenclature: Taxonomic names and concepts follow Nekola and Coles (2010). Apertural lamellae and fold nomenclature follows that of Pilsbry (1948: 869, fig. 469), i.e., parietal “teeth” are referred to as “folds” and all other “teeth” are termed “lamellae”, whatever their form. Also, we follow Pilsbry (1948) by referring to the raised riblets on the surface of *Vertigo* shells as “striae.”

SYSTEMATICS

Class Gastropoda
Subclass Pulmonata
Order Stylommatophora
Family Vertiginidae

Genus *Vertigo* Müller, 1773

Vertigo marciae new species
(Figures 1–6; Tables 1, 2)

GenBank Accessions KF214477, KF214489, KF214502, KF214503, KF214513, KF214514

Diagnosis: Minute shell reminiscent of *Vertigo hebardii* but taller, less striate, and lacking angular lamella; shell surface smooth and glossy with indistinct wrinkles; aperture flared toward bottom; four lamellae/folds present, including parietal, columellar, and two palatals.

Description: Shell 1.4–1.6 mm tall × 0.8–1.0 mm wide (holotype 1.5 × 0.8 mm), columnar-ovoid to ovoid, approximately 4–4.5 whorls, with moderately shallow suture and domed apex. Translucent, pale yellow-brown color. Body whorl approximately 60% of total height (Figures 1–3, 5, 6). Protoconch and neanic whorls smooth (Figure 4), with subsequent whorls having irregular, infrequent, and weak wrinkles. Immediately behind aperture sculpture takes the form of irregular low striae (Figure 2). Aperture flared on bottom, making it taller than wide and approximately 1/3 of shell height. Lip unthickened and slightly reflexed, sinulus moderate-weak, sometimes expressed as simple flatten-

ing of palatal wall. Basally aperture abruptly inflates to form rounded swelling but not crest (Figure 4). Umbilicus closed (Figure 3). Aperture with four lamellae/folds: parietal lamella strong, slightly sinuous (Figures 1, 2, 5, 6); columellar lamella downward-sloping, peg-shaped; two palatal folds with lower being approximately twice as long as upper and extending approximately 0.2 whorls into body whorl, lower slightly more immersed than upper, both highest at mid length (Figures 1, 2, 5, 6). Apertural end of lower palatal fold coincides with abrupt inflation of basal aperture. Externally shell only slightly impressed over palatal folds (Figure 4).

Type Material: Holotype (Figures 1–4), ANSP 450580, from type locality, May 22, 1999; paratypes (Figures 5–6), ANSP 402244, 8 shells and Institute of Jamaica, 2 shells, from type locality; ANSP 403039, Jamaica, St. Andrew Parish, John Crow Peak, Blue Mountains, elfin forest with bamboo near summit, 18°05'45" N, 76°40'08.4" W, altitude 1755 m, May 22, 1999; sta. JBS4a, 16 shells; ANSP 403040, Jamaica, St. Andrew Parish, Vinegar Hill Trail near head of Clyde River, 18°05' N, 76°39'18" W, altitude 1520 m, May 22, 1999, sta. JBS 5, 3 shells.

Type Locality: Jamaica, St. Andrew Parish, John Crow Peak, Blue Mountains; litter sample collected at base of limestone cap, 18°5'50" N, 76°40'5" W, altitude 1550 m, May 22, 1999, sta. JBS4c.

Other Material Examined: Digital images of two specimens from Red Hills Road Cave, Jamaica (Paul and Donovan, 2005), Paul collection.

Etymology: The specific name *marciae* refers to Dr. Marcia Mundle, then of the Jamaica Conservation and Development Trust. Dr. Mundle arranged for the vehicle used and park ranger guide that accompanied the expedition to the type location, and was present when the species was first collected.

RESULTS AND DISCUSSION

Variation: *Vertigo marciae* is rather constant in general appearance in terms of its shape, color, sculpture, and development of the apertural lamellae. While some variation in size (especially height) was noted, this was minor—only 16% difference between the largest and smallest shells was observed.

Comparison with Other *Vertigo* Species: *Vertigo marciae* differs from all other *Vertigo* species by its small (<1.7 mm in height) yellow shell with indistinct striae/wrinkles, flared aperture base, and lack of angular and basal lamellae. It is closest in appearance to *V. hebardii* of the Florida Keys (Figure 7), with which it shares a small yellow shell, the lack of a basal lamella, and preference for accumulations of tropical forest leaf litter. However, it differs from this species in its taller shell with less distinct striae and absence of an angular lamella. It is also reminiscent of *Vertigo marki* Gulick,



Figures 1–12. Stereomicroscope images of *Vertigo marciae* and related taxa. **1–4.** *Vertigo marciae*, holotype, Jamaica, St. Andrew Parish, John Crow Peak, Blue Mountains, 18°5'45" N, 76°40'8" W to 18°5'50" N, 76°40'5" W, ANSP 450580. **1.** Apertural view. **2.** Profile. **3.** Umbilical view showing parietal and upper palatal lamellae. **4.** Apical view showing protochonch and the basal apertural dilation. **5.** *Vertigo marciae*, ANSP 402244, Paratype, second specimen from the type locality, exhibiting a shorter shell. **6.** *Vertigo marciae*, ANSP 402244, Paratype, third specimen from the type locality, exhibiting a slightly more worn shell. **7.** *Vertigo hebardei*, Long Key, Monroe County, Florida, 24°48'52" N, 80°49'14" W, JCN 17479. **8.** *Vertigo gouldii* (small southern form), Tellico Gorge, Monroe Co., Tennessee, 35°19'49" N, 84°10'59" W, BFC 1332. **9.** *Vertigo alabamensis*, Lanier Quarry, Pender Co., North Carolina, 34°37'49" N, 77°40'27" W, JCN 10781. **10.** *Vertigo "conecuhensis,"* Pond Creek seep, Covington Co., Alabama, 31°6'12" N, 86°32'3" W, JCN 12364. **11.** *Vertigo oscariana*, Wadboo Creek, Berkeley Co., South Carolina, 33°11'50" N, 79°56'46" W, JCN 10908. **12.** *Vertigo gouldii* (normal form), Deer Creek, Fillmore Co., Minnesota, 43°43'56" N, 92°20'39" W, JCN 14646.

1904 from Bermuda, with which it shares a smooth, yellowish shell that lack both basal and angular lamellae (Pilsbry, 1919). However, *V. marciae* differs from this species by its smaller shell height and lack of a callus on the palatal wall of the aperture. *Vertigo oscariana* from the southeastern USA (Figure 11) also has a small, smooth yellow shell without a basal lamella, but this species also lacks an upper palatal fold, has a callus on the outer apertural margin, and a shell that is widest in the middle, tapering both to base and apex. *Vertigo alabamensis* of the southeastern North American coastal plain (Figures 9, 10) also has a yellowish shell lacking striae, but this species is larger (generally ≥ 1.8 mm tall), possessing both angular and basal lamellae, a strong crest on the apertural margin, and a strong sinulus on the palatal wall of the aperture.

Rosenberg and Muratov (2006) identified *Vertigo marciae* as *Vertigo gouldii* (Figures 8, 12) because of its general shell shape and placement of the parietal lamella and palatal folds, and also because *V. gouldii* was reported from Jamaica by Pilsbry and Cooke (1919: 99). However, *V. gouldii* has a larger shell of brown color, has a duller shell luster from the presence of abundant strong but irregular striae, and always possesses a basal lamella. Specimens documenting the Pilsbry and Cooke record have not been traced, and are apparently based on personal communication between Victor Sterki and Pilsbry. Bequaert and Miller (1973: 95) rejected Antillean records of *V. gouldii*.

While *Vertigo milium*—which also occurs in Jamacian tropical forest leaf litter—possesses a shell < 1.7 mm tall; this species is easily distinguished from *V. marciae* in its dark red-brown color, presence of both angular and basal lamellae, and its long, curved lower palatal fold which deeply enters the shell.

Geographic Distribution and Ecology: *Vertigo marciae* is currently known in the Recent fauna only from the crest of John Crow Peak and its immediate vicinity in the Blue Mountains of eastern Jamaica at elevations of 1520–1755 m. John Crow Peak is capped by an isolated limestone outlier known to have distinctive plant communities, including endemic species (Grossman et al., 1993). (Note that John Crow Peak is not the same location as the John Crow Mountains, the easternmost range of Jamaica.) *Vertigo marciae* is found mainly on or adjacent to limestone boulders and outcrops in tropical as well as scrub forest with bamboo, but has also been found at one site (JBS 5) that lacks exposed limestone.

As a Pleistocene fossil, it is known only from Red Hills Road Cave, which is about 21 km west of Rosenberg and Muratov's sites on John Crow Peak and 1000 m lower in elevation (520 m, Paul and Donovan, 2005). *Vertigo marciae* joins the two *Radiodiscus* species and the *Punctum* reported by Paul and Donovan (2005) on the basis of personal communication from Rosenberg as species known in Jamaica only from John Crow Peak and from the Red Hills Road Cave. Paul and Donovan interpreted the faunal changes from the Red Hills Road Cave fauna to the recent fauna around the cave as suggesting drying of the climate since the Pleistocene. As *Vertigo marciae* was found nowhere else in Jamaica among the hundreds of sites sampled by Rosenberg and Muratov, its current occurrence might represent a relict distribution.

Although it must currently be considered a Jamaican endemic, the general lack of local endemism in the genus *Vertigo* (Nekola, 2009) suggests the possibility that it may occur elsewhere in the adjacent Caribbean, especially on carbonate substrates in mid- to high-elevation montane forest.

Table 2. Average number of base-pair differences between all pairwise combinations of *Vertigo marciae* and related species.

A. <i>CytB</i> amplicon (355 base pairs)					
<i>V. oscariana</i>	53.1				
<i>V. alabamensis</i>	65.6	54.3			
<i>V. hebaridi</i>	62.3	52.0	8.3		
<i>V. marciae</i>	63.8	46.5	38.7	36.0	
	<i>V. gouldii</i>	<i>V. oscariana</i>	<i>V. alabamensis</i>	<i>V. hebaridi</i>	
B. <i>16S</i> amplicon (443–447 base pairs)					
<i>V. oscariana</i>	40.9				
<i>V. alabamensis</i>	37.3	44.5			
<i>V. hebaridi</i>	35.8	44.5	4.0		
<i>V. marciae</i>	42.5	42.0	18.0	18.0	
	<i>V. gouldii</i>	<i>V. oscariana</i>	<i>V. alabamensis</i>	<i>V. hebaridi</i>	
C. Concatenated <i>ITS-1</i> and <i>ITS-2</i> amplicons (1274–1284 base pairs)					
<i>V. oscariana</i>	34.8				
<i>V. alabamensis</i>	32.3	20.5			
<i>V. hebaridi</i>	34.3	22.5	5.0		
<i>V. marciae</i>	37.3	23.5	9.0	12.0	
	<i>V. gouldii</i>	<i>V. oscariana</i>	<i>V. alabamensis</i>	<i>V. hebaridi</i>	

Genetic Distinctness and Phylogenetic Relationships:

DNA sequence analysis clearly demonstrates that *V. marciae* is distinct at the species level. It possesses an average of 36 and 39 base pair differences with *V. hebardii* and *V. alabamensis*, respectively, in the 355 bp *CytB* amplicon (Table 2). This equates to a 10–11% variation across the entire amplicon. In addition, it possessed an average of 46.5 base pair differences in *CytB* (13%) with *V. oscariana* and 63.8 differences (18%) with *V. gouldii*. In the more slowly evolving 443 to 447 bp *16S* region *V. marciae* differed by 18 bases (4.0%) from *V. hebardii* and *V. alabamensis*, 42 bases (9.4%) from *V. oscariana*, and 42.5 bases (9.6%) from *V. gouldii*. In the concatenated 1274–1284 bp *ITS-1* + *ITS-2* nDNA amplicon, *V. marciae* possessed an average of 9 base-pair (0.7%) differences with *V. alabamensis*, 12 (0.9%) with *V. hebardii*, 23.5 (1.8%) with *V. oscariana*, and 37.3 (2.9%) with *V. gouldii*. These levels of difference were considerably larger than those exhibited between the conchologically distinct *V. alabamensis* and *V. hebardii* (Figures 7, 9, 10), which amounted to an average difference of 8.3 bases (2.3%) in *CytB*,

4.0 (0.9%) in *16S*, and 5.0 (0.4%) in concatenated *ITS-1* and *ITS-2*.

The topologies across phylogenetic reconstructions were largely compatible, with the same highly supported nodes being identified in all cases (Figures 13–15). These demonstrate that *V. marciae* is clearly a member of the genus *Vertigo*, being within the same highly supported clade in *CytB* and *16S* that includes *V. pusilla*, the type species of the genus. While it was not possible to root the concatenated *ITS-1* + *ITS-2* tree because of profound differences with both *Gastrocopta cristata* and *Pupilla muscorum* sequences, *V. marciae* was easily aligned with all *Vertigo* sequences.

The phylogenetic reconstructions also demonstrate that *V. marciae* is a member of a highly supported clade that includes both *V. alabamensis* and *V. hebardii*. The existence of a single ancestor to all of these species implies long distance dispersal from the southeastern United States to Jamaica, perhaps with migrating birds as a vector. This is not an unreasonable scenario, given that much longer feats of long distance dispersal via migrating birds have been documented in the eastern Atlantic (Gittenberger et al., 2006).

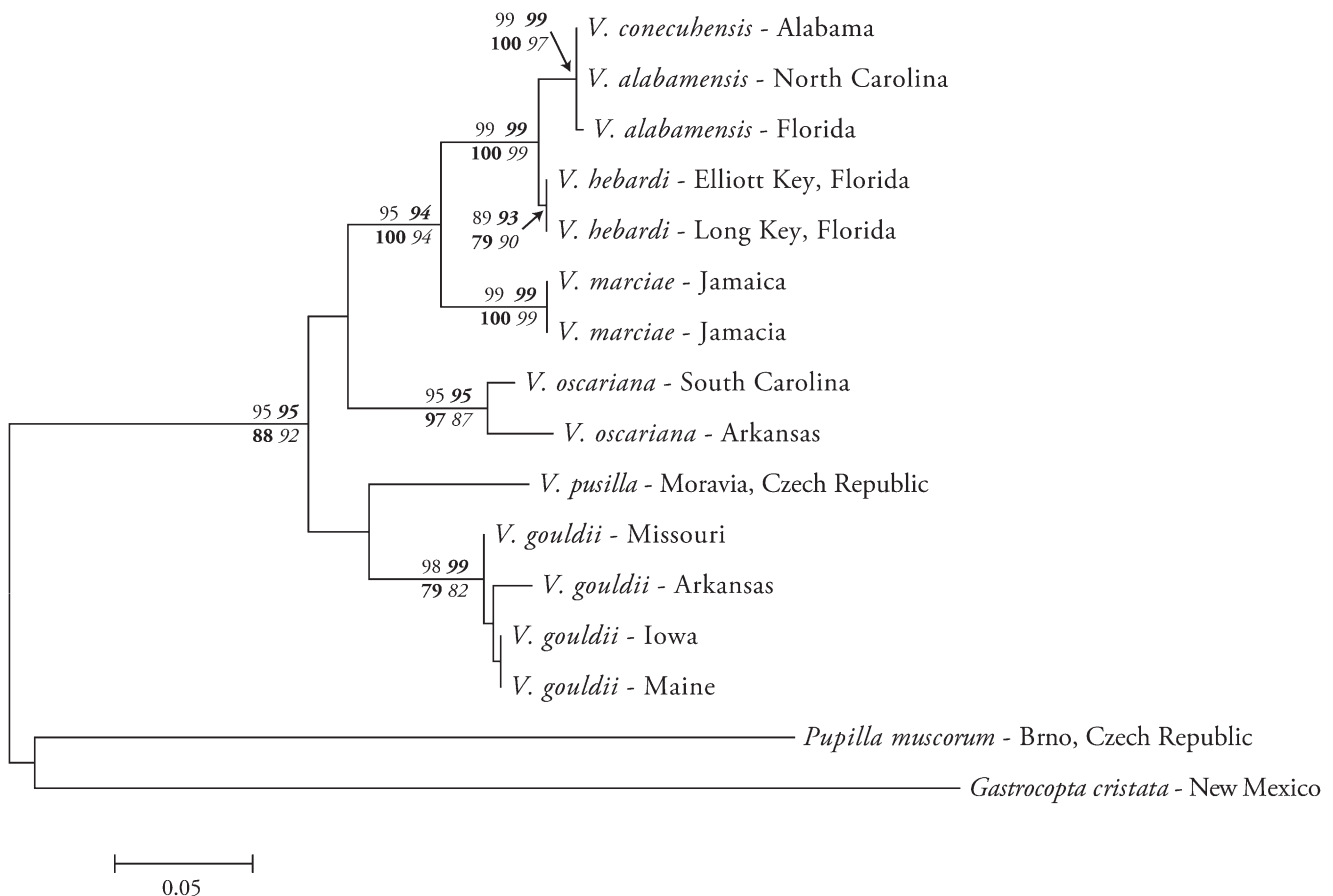


Figure 13. The phylogenetic relationships of *Vertigo marciae* as reconstructed by maximum-likelihood analysis from the *CytB* amplicon. Nodes with strong to moderate support across all four phylogenetic reconstruction methods have been labeled to the left of that node by four support values: The upper left (normal font) is for nearest neighbor joining. The upper right (**bold italic font**) is for maximum parsimony. The lower left (**bold font**) is for Bayesian. The lower right (*italic font*) is for maximum likelihood.

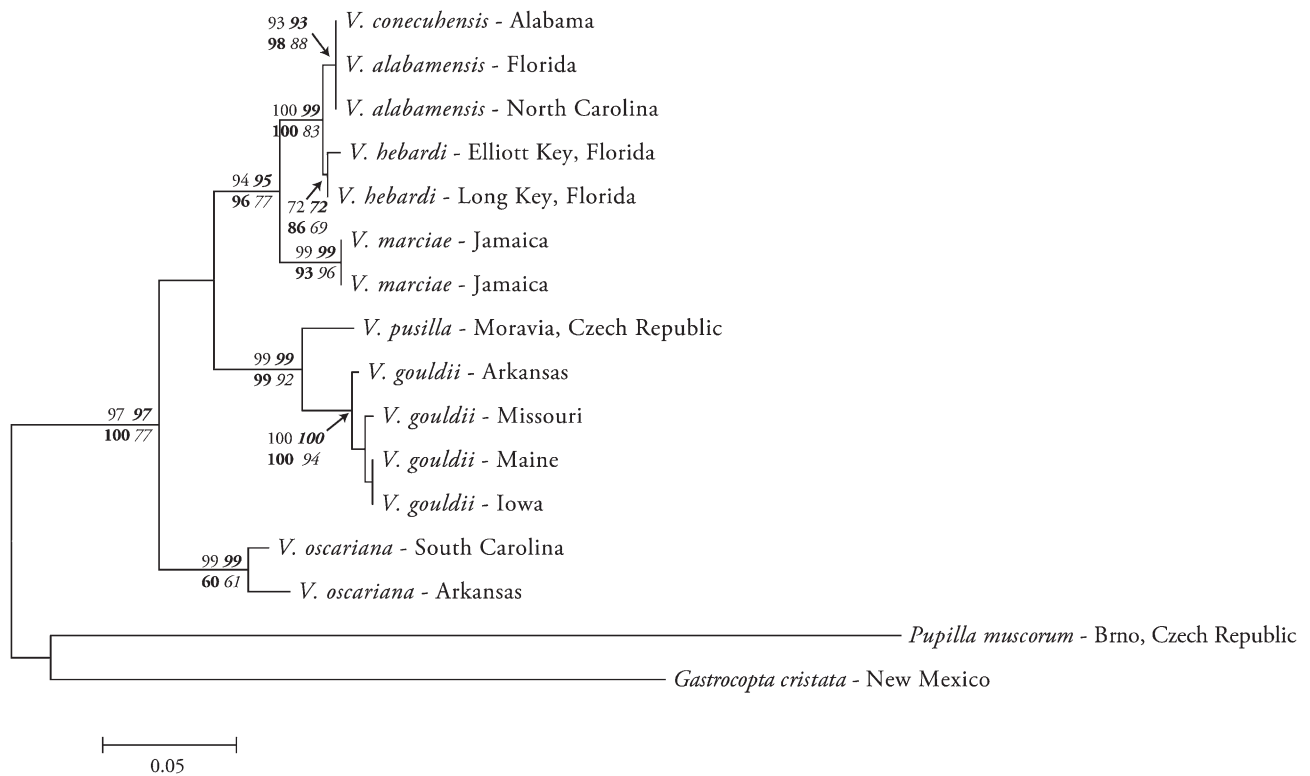


Figure 14. The phylogenetic relationships of *Vertigo marciae* as reconstructed by maximum-likelihood analysis from the 16S amplicon. Nodes with strong to moderate support across all four phylogenetic reconstruction methods have been labeled to the left of that node by four support values: The upper left (normal font) is for nearest neighbor joining. The upper right (**bold italic font**) is for maximum parsimony. The lower left (**bold font**) is for Bayesian. The lower right (*italic font*) is for maximum likelihood.

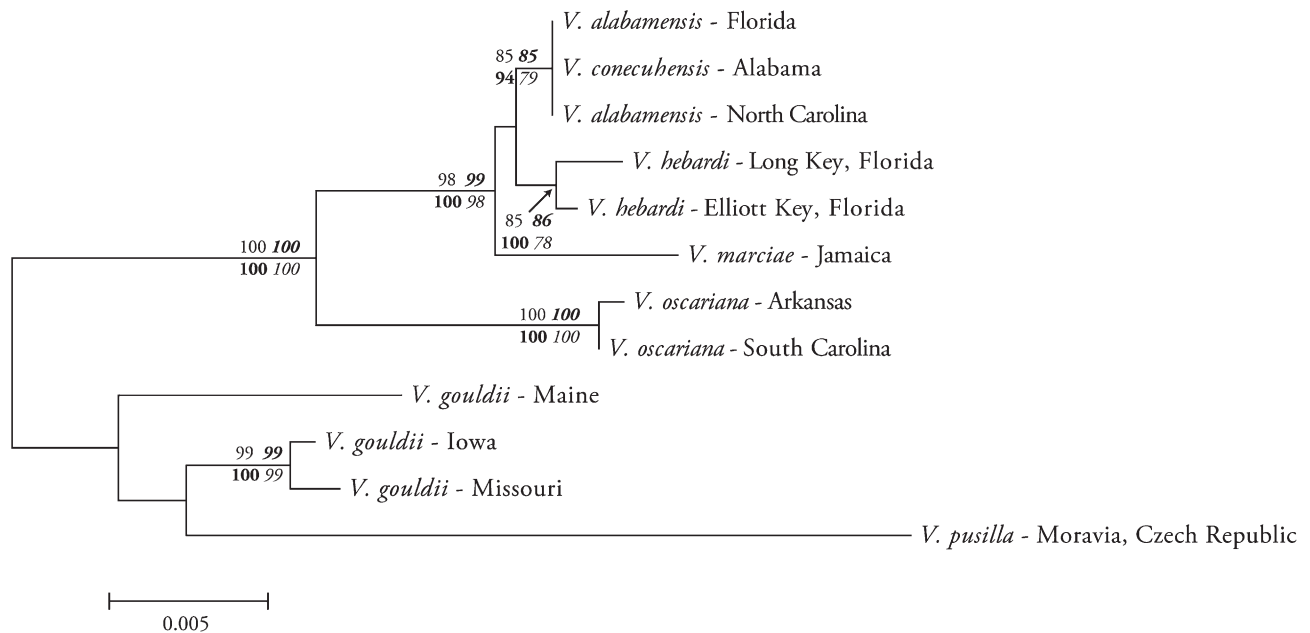


Figure 15. The phylogenetic relationships of *Vertigo marciae* as reconstructed by maximum-likelihood analysis from the concatenation of the ITS-1 and ITS-2 amplicons. Nodes with strong to moderate support across all four phylogenetic reconstruction methods have been labeled to the left of that node by four support values: The upper left (normal font) is for nearest neighbor joining. The upper right (**bold italic font**) is for maximum parsimony. The lower left (**bold font**) is for Bayesian. The lower right (*italic font*) is for maximum likelihood.

While resolution of deeper nodes within the genus *Vertigo* was not possible in the *CytB* and *16S* mtDNA amplicons (Figures 13, 14), presumably due to base-pair saturation, the concatenated *ITS-1*+*ITS-2* data (Figure 15) demonstrated a very highly supported node connecting the *V. marciae* / *V. hebardii* / *V. alabamensis* clade to *V. oscariana*. This radiation includes some of the most distinct members of the genus (Pilsbry, 1948). Additional field work across the Caribbean—especially in Cuba, Hispaniola, Puerto Rico, and Bermuda—will be required to determine the actual number of species contained within this group.

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John Slapcinsky of the Florida Museum of Natural History provided ethanol-preserved tissue samples of *Vertigo hebardii*, while Michal Horsak of Masaryk University in Brno, the Czech Republic, provided live samples for *Vertigo pusilla* and *Pupilla muscorum*. *CytB* and *16S* sequences for *V. pusilla*, *P. muscorum*, and *Gastrocopta cristata* were retrieved from full mitochondrial genomic sequences provided by Jason Marquardt and Ulfar Bergthorsson of the University of New Mexico. Christopher Paul, University of Bristol, provided digital images of the Red Hills Road Cave fossils. Field work which established the persistence of *Vertigo hebardii* in the Florida Keys was supported by The Bailey-Matthews Shell Museum through an R.T. Abbott Visiting Curatorship to JCN. Additional funding for PCR and sequencing analysis was provided by Michal Horsak. The field work which resulted in the Recent samples of the new species being collected was supported by NSF Grant DEB-9870233 to Gary Rosenberg.

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