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Vertigo shimochii Kuroda & Amano 1960 synonymized with *Gastrocopta servilis* (Gould, 1843) based on conchological and DNA sequence data

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Kuroda (1960) noted that his new species, *Vertigo shimochii* Kuroda & Amano, 1960, was distinct from other members of the genus in Japan by possessing a "relatively large and long shell with deep suture and much inflated whorls" (p. 77). While shell shape was noted to be similar to a *Gastrocopta*, they assigned the species to *Vertigo* based on its "shell color and lamella characteristics" (p. 77). *Vertigo shimochii* has since been considered endemic to the southern parts of Japan (The Environment Agency 1988; Minato 1988; Azuma 1995; Biodiversity Center of Japan 2002), and of near threatened status in the Kagoshima Prefecture Red Data Book (Kagoshima Prefectural Government 2003).

However, careful examination of *V. shimochii* shell features suggests that this taxon may have been misclassified. The most striking evidence of this is the presence of a bifid parietal lamella, which is never present in *Vertigo* (Figure 1; Nekola & Coles 2010). Additionally, brown shells are not limited to *Vertigo*; species in *Gastrocopta* subgenus *Gastrocopta* also possess this trait. In fact, *V. shimochii* shells appear essentially identical to Caribbean material of *Gastrocopta servilis* (Gould, 1843), a well–known waif that has been commonly transported with horticultural plants across many Pacific archipelagos (Cowie 1998).

To resolve the taxonomic status of *V. shimochii*, we compared DNA sequences from the mitochondrial *cytochrome oxidase subunit 1* (*CO1*) and *16S ribosomal RNA* (*16S*) and *internal transcribed spacer–2* (*ITS–2*) of the nuclear *ribosomal RNA* gene complex from topotype material collected on Okinawa to other *Gastrocopta* and *Vertigo* species from across the northern hemisphere.

Methods

Twenty–one specimens, representing two *V. shimochii*, fourteen *Gastrocopta* and five *Vertigo* were chosen for analysis. *Vertigo japonica* and *G. armigerella* represent comparative material from the Japanese fauna. The remainder were sourced either from North America or Europe (Table 1). All specimens were either live, live–collected and preserved in ethanol, or mummified. Genomic DNA was extracted using the OmegaBioTek Mollusk DNA Extraction Kit. PCR amplification and sequencing of *CO1, 16S,* and *ITS–2* was accomplished using standard methods (Nekola *et al.* 2009). In addition, previously analyzed sequence data (*CO1, 16S, ITS–2*) from an extra twelve *Gastrocopta, Pupilla, Vallonia* and *Vertigo* specimens were retrieved from GeneBank (Table 1).

Primer ends were removed and all amplicons were aligned by eye. Mega 5.0 was used to construct both nearest–neighbor joining (NNJ) and maximum likelihood (ML) analyses for each gene. NNJ was based on Maximum Composite Distance (MCL) including transitions and transversions with pairwise gap deletion. 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 both cases support values were estimated from 1000 bootstrap replicates.

Results

All 32 *CO1* sequences were 655 bp long. The 31 *16S* sequences ranged from 447–456 bp in *Gastrocopta*, 443–446 in *Vertigo*, 450–453 in *Pupilla*, and was 443 for *Vallonia*. The *ITS–2* sequences ranged from 825–865 bp in *Gastrocopta*, 618–763 in *Vertigo*, and was 907 for *Pupilla*.

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FIGURE 1. Representative examples of white–shelled *Gastrocopta* (top row), brown–shelled *Gastrocopta* (middle row; all species members of the subgenus *Gastrocopta*), and *Vertigo* (bottom row), with location information for each. Accession numbers for lots from the Nekola collection are preceded by 'JCN'. **A.** ***Gastrocopta holzingeri***, Allison, Stone Co., Arkansas, 35°56′32″ N., 92°6′54″ W., JCN 14368. **B.** ***Gastrocopta tappaniana***, Faith Fen, Norman Co., Minnesota, 47°15′42″ N., 96°5′11″ W., JCN 6624. **C.** ***Gastrocopta armigerella***, Ohama Beach, Iki Island, Japan, 33°44′51″ N., 129°47′15″ E. **D.** ***Gastrocopta corticaria***, Canton Glade, Jones Co., Iowa, 42°10′46″ N., 90°59′52″ W., JCN 3743. **E.** ***Gastrocopta servilis***, Bartram–Carr Woods, Gainesville, Alachua Co., Florida, 29°38′37″ N., 82°20′44″ W. **F.** '***Vertigo schimochii***', Kunigami, Okinawa Island, Japan, 26°51′43″ N., 128°15′20″ E. **G.** ***Gastrocopta pellucida***, Charlotte Harbor, Charlotte Co., Florida, 26°57′13″ N., 82°3′42″ W., JCN 17447. **H.** ***Gastrocopta rogersensis***, Beams Cabin, Jones Co., Iowa, 42°8′32″ N., 91°20′44″ W., JCN 11465. **I.** ***Vertigo ventricosa***, Portage Lake, Aroostook Co., Maine, 46°47′6″ N., 68°32′27″ W., JCN 15915. **J.** ***Vertigo japonica***, Sarusawa, Ichinoseki–city, Iwate, Japan, 38°59′13″N., 141°15′18″ E. **K.** ***Vertigo gouldii***, Deer Creek, Fillmore Co., Minnesota, 43°43′56″ N., 92°20′39 W., JCN 14646. **L.** ***Vertigo concinnula***, Neutrioso South, Apache Co., Arizona, 33°54′14″ N., 109°9′43″ W., JCN 14007.

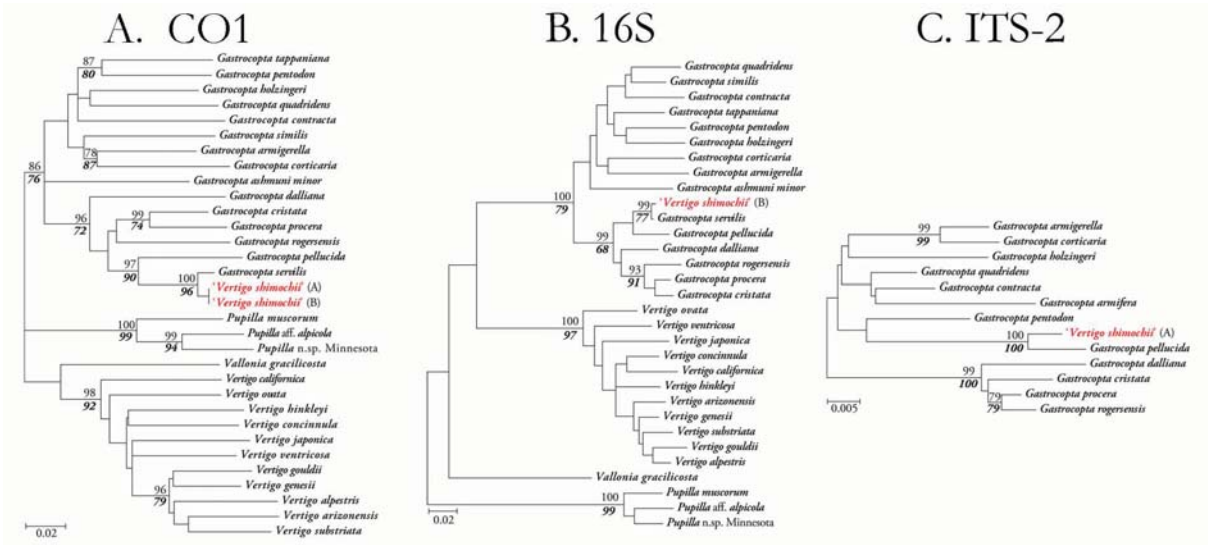

FIGURE 2. *Phylogram* showing the distribution of analyzed *Gastrocopta*, *Vertigo*, *Vallonia* and *Pupilla* species based on **A.** *CO1*; **B.** *16S*; and **C.** *ITS-2*. Nodes with support values of approximately 70 or more across both NNJ and ML have been labeled by two numbers to the left of each node. The upper (normal font) represents the NNJ support values while the lower (***bold italic font***) represents ML values. Bottom scale bar is presented in Maximum Composite Distance.

All NNJ and ML results for a given gene generated outputs with identical topologies for all highly supported nodes. As a result, only a single diagram is presented for each gene. The *CO1* (Figure 2A) and *16S* (Figure 3B) diagrams both demonstrate that '*V. shimochii*' is very similar to *G. servilis*. In *CO1* these two taxa possessed an MCL distance of only 0.009; in *16S* this distance was only 0.004. In *CO1*, the '*V. shimochii*'/*G. servilis* group was found to have a MCL distance of approximately 0.06 from its closest neighbor – *G. pellucida* – and 0.15 from *V. japonica*. In *16S* the '*V. shimochii*'/*G. servilis* group was found to have a MCL distance of approximately 0.05 from its closest neighbor – *G. pellucida* – and 3.8 from *V. japonica*.

Because the *16S* region evolves more slowly than *CO1* it should also provide better resolution for older evolutionary relationships. '*Vertigo shimochii*' not only resides within the highly supported group containing the genus *Gastrocopta*, but also within a highly supported subgroup that includes '*V. shimochii*' / *G. servilis* and *G. pellucida* (Pfeiffer, 1841)*, G. dalliana* (Sterki, 1898)*, G. rogersensis* Nekola & Coles, 2001*, G, procera* (Gould, 1840)*,* and *G. cristata* (Pilsbry & Vanatta, 1900). This group in essence corresponds with tropical and warm–temperate *Gastrocopta* (*Gastrocopta*). However, *G. dalliana* has previously been placed into subgenus *Immersidens* on the basis of angulo–parietal lamella architecture (Pilsbry 1948). Yet, it differs from most other subgenus *Immersidens* members by its cylindrical shell, a characteristic it shares with other subgenus *Gastrocopta* members. It should be noted that like *G. rogersensis*, *G. dalliana* has a channeled angulo–parietal lamella (Nekola & Coles 2001), implying that this character may not be useful in demarcating *Gastrocopta* subgenera.

The *ITS–2* sequence for '*V. shimochii*' could be aligned neither with *Vertigo* nor *Pupilla*, although it could be aligned with *Gastrocopta*. The *ITS–2 Gastrocopta* diagram (Figure 2C) demonstrates that '*V. shimochii*' and *G. pellucida* are both members of the same highly supported group. The MCL distance between these two taxa is 0.014.

Discussion.

These analyses demonstrate that '*V. shimochii*' represents a junior synonym of *G. servilis*, with both taxa possessing similar shells and an MCL distance of <0.01 in their DNA sequences. Both of these taxa also reside in a highly supported group that contains other members of *Gastrocopta* (*Gastrocopta*).

Gastrocopta servilis is a well–known exotic throughout the Pacific. In hindsight it is therefore not surprising that fossil or subfossil records for '*V. shimochii'* do not exist. While no populations were known until its initial discovery, 50 years later this species represents one of the most abundant microsnails on the Okinawa coast, with its range having expanded north into the Amami, Ogawawara, and Yaeyama Islands (Biodiversity Center of Japan 2002). Rather than representing a rare southern Japan endemic species of potential conservation importance, these populations simply represent a recent and continuing range expansion of a Caribbean exotic.

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

Taxon	Location	Latitude/Longitude	GENEBANK Accession number		
			CO1	*16S*	*ITS–2*
'*Vertigo shimochii*' Kuroda & Amano, 1960	Kunigami, Okinawa Island, Japan (A)	26.8620 N., 128.2556 E.	JN941048	JN941027	JN941015
'*Vertigo shimochii*' Kuroda & Amano, 1960	Kunigami, Okinawa Island, Japan (B)	26.8620 N., 128.2556 E.	JN941049		
Gastocopta armifera (Say, 1821)	USA				61228725
Gastrocopta armigerella (Reinhardt 1877)	Ohama Beach, Iki Island, Japan	34.7474 N., 129.7874 E.	JN941050	JN941028	JN941019
Gastrocopta ashmuni minor (Sterki, 1898)	Peloncillo Range, Hildago Co., New Mexico, USA	31.5207 N., 109.0060 W.	JN941051	JN941029	
Gastrocopta contracta (Say, 1822)	Wind Mountain, Otero Co., New Mexico, USA	32.0235 N., 105.5077 W.	JN941052	JN941030	JN941026
Gastrocopta corticaria (Say, 1816)	Brush Creek Canyon, Fayette Co., Iowa, USA	42.7796 N., 91.6890 W.	JN941053	JN941031	JN941020
Gastrocopta cristata (Pilsbry & Vanatta, 1900)	2621 McEarl Ave. SE, Albuquerque, New Mexico, USA	35.0727 N., 106.6161 W.	JN941054	JN941032	JN941017
Gastrocopta dalliana (Sterki, 1898)	Peloncillo Range, Hildago Co., New Mexico, USA	31.3526 N., 109.0315 W.	JN941055	JN941033	JN941024
Gastrocopta holzingeri (Sterki, 1889)	Allison, Stone Co., Arkansas, USA	35.9421 N., 92.1149 W.	JN941056	JN941034	JN941021
Gastrocopta pellucida (Pfeiffer, 1841)	Charlotte Harbor, Charlotte Co., Florida, USA	26.9536 N., 82.0617 W.	JN941057	JN941035	JN941016
Gastrocopta pentodon (Say, 1821)	Burnt Lands Alvar, Ottawa Division, Ontario, Canada	45.2546 N., 76.1505 W.	JN941058	JN941036	JN941014
Gastrocopta procera (Gould, 1840)	Root River, Preston, Fillmore Co., Minnesota, USA	43.7854 N., 92.0290 W.	JN941059	JN941037	JN941018
Gastrocopta quadridens (Pilsbry, 1899)	Rio Pueblo, Taos Co., New Mexico, USA	36.1628 N., 105.5755 W.	JN941060	JN941038	JN941025
Gastrocopta rogersensis Nekola & Coles, 2001	Norfolk, Baxter Co., Arkansas, USA	36.2237 N., 92.2813 W.	JN941061	JN941039	JN941022
Gastrocopta servilis (Gould, 1843)	Gainesville, Alachua Co., Florida, USA	29.6436 N., 82.3456 W.	JN941062	JN941040	
Gastrocopta similis (Sterki, 1909)	Hamilton Glade, Maquoketa Co., Iowa, USA	42.0731 N., 90.5691 W.	JN941063	JN941041	
Gastrocopta tappaniana (C.B. Adams, 1842)	Wesley School, Washington Co., Maine, USA	44.9274 N. 67.6590 W.	260875594	260875413	
Pupilla aff. *alpicola*	Happy Valley, North Slope Borough, Alaska, USA	69.3355 N., 148.7302 W.	260875598	260875414	
Pupilla muscorum Linne, 1758	Crawford Quarry, Cedar Rapids, Linn Co., Iowa USA	41.9866 N., 91.7400 W.	260875600	260875415	
Pupilla n.sp.	Lake Bemidji, Beltrami Co., Minnesota, USA	47.5328 N., 94.8247 W.	260875596	260875416	
Vallonia gracilicosta Reinhardt, 1883	Nenana North, Alaska, USA	64.5698 N., 149.0979 W.	260875592	260875412	
Vertigo alpestris Adler, 1838	Medvědický Hill, Milešov, Bohemia, Czech Republic	50.5279 N., 13.9314 W.	JN941064	JN941042	
Vertigo arizonensis (Pilsbry & Vanatta, 1900)	Nogal Canyon, Lincoln Co., New Mexico, USA	33.4987 N., 105.7839 W.	260875570	260875386	
Vertigo californica (Rowell, 1861)	Moss Landing Beach, Monterey Co., California, USA	36.8095 N., 121.7884 W.	260875455	JN941047	
Vertigo concinnula Cockerell, 1897	Neutriosa South, Apache Co., Arizona, USA	33.9039 N., 109.1619 W.	260875464	260875400	
Vertigo genesii (Gredler, 1856)	Kongsvoll, Norway	62.2672 N., 9.5855 E.	JN941065	JN941043	
Vertigo gouldii (A.Binney, 1843)	Deer Creek, Fillmore Co., Minnesota, USA	43.7322 N., 92.3443 W.	260875546	260875367	
Vertigo hinkleyi Pilsbry, 1921	Miller Canyon, Cochise Co., Arizona, USA	31.4105 N., 110.2824 W.	260875460	260875410	
Vertigo japonica Pilsbry et Hirase, 1904	Sarusawa, Ichinoseki–city, Iwate, Japan	38.9869 N., 141.2551 E.	JN941066	JN941044	
Vertigo ovata Say, 1822	Santo Domingo, Sandoval Co., New Mexico, USA	35.5356 N., 106.3519 W.	JN941067	JN941045	
Vertigo substriata (Jeffreys, 1833)	Liptovská Teplička, Slovakia	48.9632 N., 20.1044 E.	JN941068	JN941046	
Vertigo ventricosa (Morse, 1865)	Portage Lake, Aroostook Co., Maine, USA	46.7850 N., 68.5408 W.	260875590	260875411	

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