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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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