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Commentary: Integrative Taxonomy Reveals Freshwater Shrimp Diversity (Decapoda: Atyidae: *Neocaridina*) from Kyushu and Southern Honshu of Japan, with a Discussion on Introduced Species

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Shih et al. (2024) reported on the detection of *Neocaridina* species in Japan and their morphological characteristics in *Zoological Studies*. Eleven taxa were identified based on mitochondrial DNA (mtDNA) analysis and morphological examination. Among these, they identified two taxa that formed sister groups: *N. denticulata* and *N. davidi*, which are primarily found in Japan and China. In this commentary, I argue that both species are actually *N. davidi*. This conclusion was previously drawn by Onuki and Fuke (2022) based on their examination of genome-wide SNPs, mtDNA, and morphological data. The doubts raised about this identification represent a serious issue in terms of conservation, as *N. denticulata* is a native species, whereas *N. davidi* is considered an invasive alien species in Japan. Two likely reasons for this misidentification are the oversight of previous studies and the inability to account for the effects of interspecific and intraspecific hybridization. Inaccurate or unsubstantiated identifications pose significant challenges to taxonomy and conservation, underscoring the need for research grounded in reliable methods and well-characterized specimens.

Key words: Commentary, DNA barcoding, Invasive species, *Neocaridina*, Mitochondrial DNA

Shih et al. (2024) detected 11 taxa of *Neocaridina* species in Japan, based on mitochondrial DNA (mtDNA) analysis and morphological examination. Of these, the two sister taxa found mainly in Japan and China were identified as *N. denticulata* and *N. davidi*, respectively (denoted by double quotations below). Correct identification of both of these species is extremely important in Japan because *N. denticulata* is a native species that requires protection, whereas *N. davidi* is an invasive alien species, whose spread needs to be controlled. However, Shih et al. (2024) reached a different conclusion than those of previous studies regarding the identification of these two lineages. Onuki and Fuke (2022) identified *N. denticulata* and *N. davidi* (containing two lineages) in the Lake Biwa Basin based on genome-wide polymorphisms, mtDNA, and quantitative morphological analyses. Shih et al. (2024) did not compare their results with those of Onuki and Fuke (2022). Therefore, I have decided to show the differences in their identifications in figure 1. The taxa identified as "*N. denticulate*" and "*N. davidi*" by Shih et al. (2024) were both identified as *N. davidi* Clade B by Onuki and Fuke (2022) (as SNP cluster 3; mtDNA clade 2; mtDNA haplotype name: dvB-). The taxon identified as *N. davidi* Clade A near Lake Biwa by Onuki and Fuke (2022) was treated as *N.* aff. *Palmata*, while *N.*

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denticulata was treated as *N.* aff. *denticulata* by Shih et al. (2024). This difference in interpretation is important for conservation. In the following section, I examine the validity of Shih et al.'s (2024) identification.

Several previous studies have supported the notion that the two species, "*N. denticulata*" and "*N. davidi*," represent the same taxon. Cui et al. (2023) investigated the population structure of *N. davidi* (as *N. denticulata sinensis*) in the Baiyangdian drainage area of China, and Zhou et al. (2021) conducted DNA barcoding of freshwater shrimp species on Hainan Island, China. They detected the two mtDNA lineages (Clade A and Clade B, including "*N. denticulata*" and "*N. davidi*" in Shih et al. (2024)) sympatrically. These two lineages have also been found in Japan, and nuclear genome-wide analysis has provided evidence of undifferentiation between lineages (Onuki and Fuke 2022). Chinese *Neocaridina* species are imported to Japan from various parts of China as pets and also as fishing bait (Niwa 2010). The sympatric occurrence of both lineages in their native habitats and their ability to interbreed suggest that they belong to the same species.

Second, I highlight the limitations of mtDNA markers and the importance of nuclear markers.

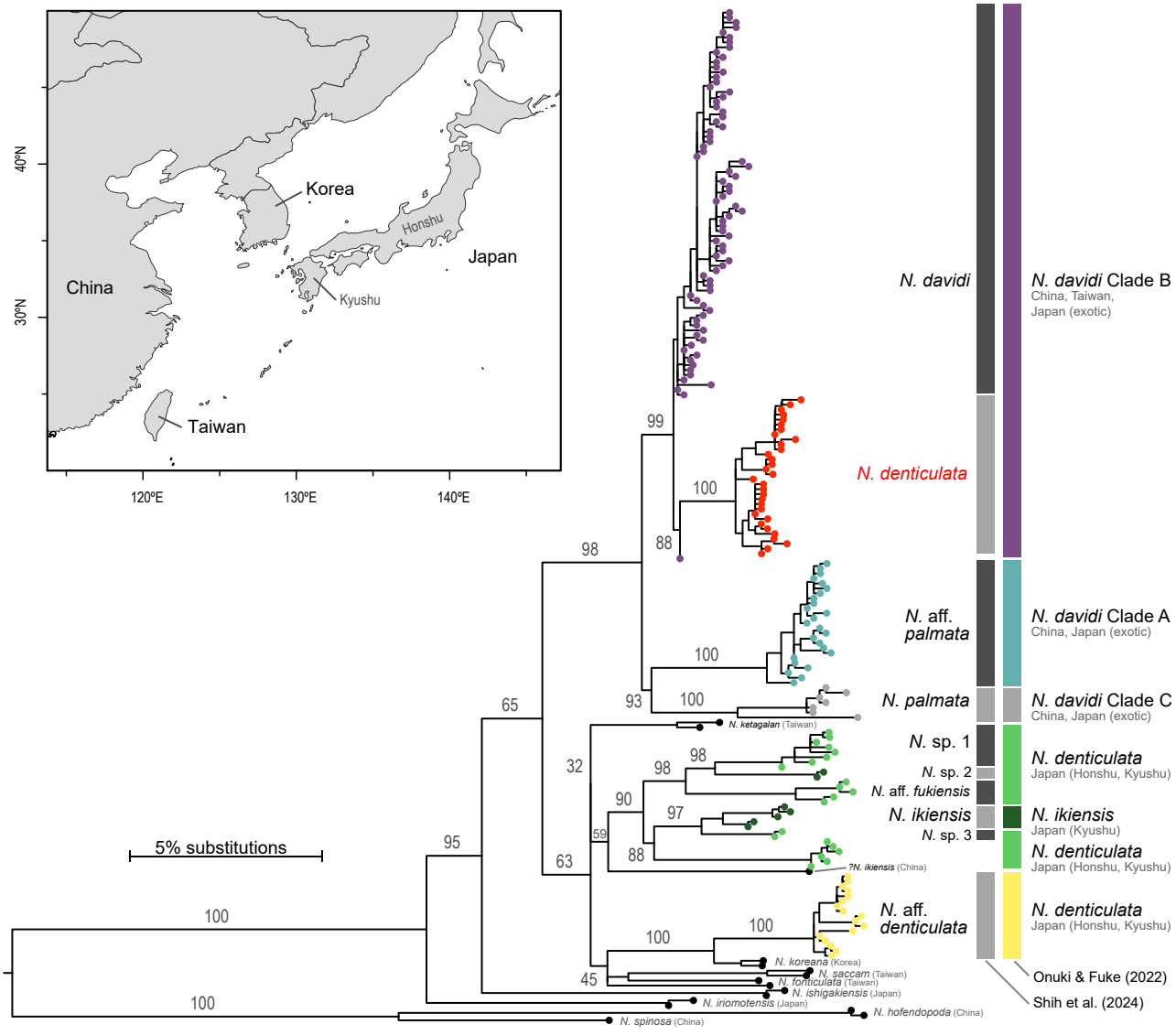

Fig. 1. Phylogenetic tree of *Neocaridina* species based on the mtDNA *COI* region (644 bp) and comparison of species recognition in Shih et al. (2024) and Onuki and Fuke (2022). As a dataset, all *Neocaridina* spp. available in the International Nucleotide Sequence Database were downloaded and aligned using MAFFT (Katoh and Standley 2013) after removing identical sequences using vsearch 2.23.0 (Rognes et al. 2016). Phylogenetic tree estimation was based on the maximum likelihood method using IQ-TREE 2.2.2.6 (Minh et al. 2020) with the codon partitioning model selected by ModelFinder based on BIC (Kalyaanamoorthy et al. 2017). The reliability of each branch was assessed using the Ultrafast bootstrap method (Hoang et al. 2018).

Maternally inherited and non-recombining mtDNA lineages do not necessarily correspond to species or population lineages (Ballard and Whitlock 2004), and a distinct lineage in the mtDNA of *N. davidi* may not represent the inclusion of multiple species. Despite strong mtDNA-based evidence that the taxon "*N. denticulata*" in Shih et al. (2024) is *N. davidi*, the individual in the sketch certainly appears to meet the characteristics of *N. denticulata*. However, the authors did not consider possible hybridization or intraspecific variations even though "*N. denticulata*" were obtained sympatrically with other *Neocaridina* shrimps, including undescribed species (Shih et al. 2024). It is necessary to confirm that the individuals considered for morphology are pure strains. In this context, the authors should carefully consider the results of a morphometric analysis by Onuki and Fuke (2022), which was conducted in conjunction with a genome-wide analysis. As demonstrated by Onuki and Fuke (2022), *Neocaridina* shrimps exhibit a wide range of intraspecific variation and overlap in discriminatory traits between species, even after excluding the effects of interspecific hybridization. Future studies should quantitatively assess trait values and carefully identify them to account for variation and phenotypic plasticity. Descriptions (illustrations) of arbitrarily selected single or very few individuals do not necessarily represent species characteristics.

Neocaridina denticulata is difficult to identify due to the loss of type specimens (Yamaguchi 1993) and the insufficient detailed morphological information in the original description (De Haan 1844). Furthermore, the only available information regarding the type locality is that it is in Japan: this makes it difficult to refer to topotypes. Onuki and Fuke (2022) reported *N. denticulata* in Lake Biwa Basin, Japan. They identified the species by comparing specimens and morphological descriptions obtained before the invasion of any alien species into Lake Biwa (Kemp 1918). These results were also supported genetically; the haplotype of *N. denticulata* is found only in Japan. In contrast, Shih et al. (2017 2024) based their identification on their own definition of *N. denticulata*, which was not morphologically characterized, and treated individuals that did not fit this definition as different species. A serious problem is that their definitions are based on the specimens that have not been confirmed to be pure strains without interspecific hybrids. Ideally, the identification of *N. denticulata* should be based on a neotype (not yet designated), and provisional identification should be performed by comparison with reliable historical records. Furthermore, the division of several intraspecific lineages of *N. denticulata* into several undescribed species without any basis is problematic (*Neocaridina* spp. 1, 2, and 3). *Neocaridina denticulata* contains multiple genetically divergent lineages (Fuke et al. 2021), but the mtDNA results alone are insufficient evidence that they are actually separate species. To find the diagnostic morphological features of *N. denticulata* and putative undescribed species by examining current specimens, a combination of genome-wide DNA and quantitative morphological analyses is important.

Research on *Neocaridina* species often faces difficulties owing to taxonomic confusion. However, the accumulation of precise basic research based on accurate identification will eliminate such difficulties and contribute to the further development of invasion ecology and biodiversity management. Dr. Shih's research group certainly has the skills and enthusiasm to contribute to this, and I hope they will progress their research after resolving the issues raised in this study.

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Availability of data and materials: The datasets used in this study (https://doi.org/10.6084/m9.figshare.26028799.v1) and the phylogenetic tree with accession numbers (https://doi.org/10.6084/m9.figshare.26028763.v1) are available on Figshare.

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