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Occurrence of the freshwater heteronemertean *Apatronemertes albimaculosa* (Nemertea: Pilidiophora) in Taiwan

Hiroshi KAJIHARA¹,* Ayuta KATO², Takafumi NAKANO³, Yi-Te LAI⁴

1. Faculty of Science, Hokkaido University, Kita-ku, N10 W8, Sapporo 060-0810, Japan.
2. School of Science, Hokkaido University, Kita-ku, N10 W8, Sapporo 060-0810, Japan.
3. Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.
4. Department of Life Science, National Taiwan University, Roosevelt Road 1, Section 4, Taipei 106, Taiwan.

*Corresponding author’s tel: +81-11-706-2755; Email: kajihara@eis.hokudai.ac.jp

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ABSTRACT: We report the freshwater heteronemertean *Apatronemertes albimaculosa* Wilfert & Gibson, 1974 for the first time from Taiwan based on specimens collected under stones and rocks on gravelly bottom near the shore of a brook in Nantun District, Taichung City. Species identification was corroborated by mitochondrial cytochrome c oxidase subunit I gene sequences. The species had been reported from aquaria with tropical freshwater plants in Germany, Austria, USA, Spain, and Japan, before it was discovered from wild environment in Panama. Our report represents the second instance of field-caught *A. albimaculosa* in the world.

KEY WORDS: COI, cox1, DNA barcoding, freshwater invertebrates, Heteronemertea, Lineidae, Taiwan, trans-Pacific distribution.

INTRODUCTION

Nemerteans, or ribbon worms, are mainly marine, benthic invertebrates, comprising the phylum Nemertea. About 1,300 species of nemerteans have been described from the world (Gibson, 1995; Kajihara et al., 2008), of which 22 are known from freshwater habitats (Sundberg and Gibson, 2008). One of the freshwater forms, *Apatronemertes albimaculosa* Wilfert & Gibson, 1974, was originally described from among the aquarium plant *Vallisneria spiralis* L. in Düsseldorf, Germany (Wilfert and Gibson, 1974). The species had subsequently been reported from freshwater aquaria in Austria (Senz, 1993), USA (Smith, 2001), Spain (Andrade et al., 2012), and Japan (Kajihara et al., 2016), before it was found in the field from submerged logs and rocks in a small pond near Lago Miraflores, Panama Canal (Kvist et al., 2018). Taiwanese nemerteans have been scarcely documented. Based on marine intertidal material from Su’ao, Yamaoka (1939) reported *Baseodiscus hemprichii* (Ehrenberg, 1831) and *Micrura formosana* Yamaoka, 1939; the latter is likely related closely to, if not synonymous with, *Meckelia nigra* Stimpson, 1855, and should be placed in the genus *Dushia* Corrêa, 1963 (Hookabe et al., 2019). Earlier, Takakura (1932) reported a freshwater heteronemertean from Taiwan, putatively around Taipei, although it was not identified to species (Kajihara, 2004).

In this brief note, we report the occurrence of *A. albimaculosa* for the first time from Taiwan, which is substantiated by the second wild-caught material in the world.

MATERIAL AND METHODS

Three specimens of freshwater nemerteans were collected by Yi-Te Lai on 23 September 2018 from under stones and rocks on gravelly bottom near the shore of a brook (a branch of the Zhen-Ping River, Fig. 1A) at 24.1274°N, 120.6218°E, beside Tian-Shun Temple in Nantun, Taichung, Taiwan. They were fixed in ethanol. Total DNA was extracted from the posterior end of the body from each specimen using a silica-based method (Boom et al., 1990). For PCR amplification and sequencing of the mitochondrial cytochrome c oxidase subunit I (COI) gene, a specific primer pair, Apa_COI_F (5′-TTGGGTATTTGATCTGGTCTTG-3′) and Apa_COI_R (5′-CAAATAGGATCCCCCTCCTC-3′), was designed at Primer3 website (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) (Koressaar and Remm, 2007; Unterberger et al., 2012) based on the following sequences available in public databases: HQ848584 (Andrade et al., 2012); LC095809 (Kajihara et al., 2016); MH128987, MH128988, MH128989 (Kvist et al., 2018). PCR amplification was done by 94°C for 5 min; 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1.5 min; and 72°C for 7 min. Direct sequencing was carried out with a BigDye Terminator Kit ver. 3.1 and a 3730 Genetic Analyzer (Life Technologies, California, USA). Sequence comparison was made by using MEGA ver. 7 (Kumar et al., 2016) against two heteronemertean mitochondrial genomes from *Iwatanemertes piperata* (Stimpson, 1855) (Shen et al., 2015) and *Lineus viridis* (Müller, 1774) (Podsiadlowski et al., 2009). Voucher specimens have
RESULTS

In the living state, the body is uniformly reddish, with yellowish-white gonads seen through the integument and arranged on both sides of the intestine (Fig. 1B, C). Fixed
specimens ranged 12–35 mm in length and 1–2 mm in width (Fig. 1D), and were uniformly whitish orange-brown in color. The cephalic slits are extremely shallow and rudimentary (Fig. 1D). The openings of the cerebral organ canals were more or less distinct, situated at a level slightly anterior to the mouth (Fig. 1D).

PCR amplification and sequencing using the primer pair LCO1490/HCO2198 (Folmer et al., 1994) resulted in poor chromatogram quality (data not shown), implying base substitution(s) at the primer site(s) and/or contamination of other organism(s). PCR was successful for only two of the three specimens, probably due to DNA degradation in the other specimen during storage.

Comparison of nucleotides and translated amino acids at the site where these were different between the seven partial COI sequences (575 bp) of *Apatronemertes albimaculosa* Wilfert & Gibson, 1974 known from Spain (Andrade et al., 2012), Japan (Kajihara et al., 2016), Panama (Kvist et al., 2018), and Taiwan (present study). The positions indicate those within the entire cytochrome c oxidase subunit I gene (1,531 bp) when aligned along with the two complete mitochondrial genomes of the lineid heteronemerteans *Iwatanemertes piperata* (Stimpson, 1855) (Shen et al., 2015) and *Lineus viridis* (Müller, 1774) (Podsiadlowski et al., 2009). The 576th nucleotide position corresponds to the 500th site in the partial LC506017 and LC506018.

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession number</th>
<th>Country of origin</th>
<th>Nucleotide position</th>
<th>Amino-acid position</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Apatronemertes albimaculosa</em></td>
<td>HQ848584</td>
<td>Spain</td>
<td>T T G</td>
<td>L</td>
</tr>
<tr>
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<td>LC095809</td>
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<td>MH128987</td>
<td>Panama</td>
<td>T T T</td>
<td>F</td>
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<tr>
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<td>F</td>
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<tr>
<td><em>Apatronemertes albimaculosa</em></td>
<td>MH128989</td>
<td>Panama</td>
<td>T T –</td>
<td>?</td>
</tr>
<tr>
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<td>T T G</td>
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<tr>
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<tr>
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<td>—</td>
<td>T T G</td>
<td>L</td>
</tr>
<tr>
<td><em>Lineus viridis</em></td>
<td>FJ839919</td>
<td>—</td>
<td>T T A</td>
<td>L</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Our findings of *A. albimaculosa* in Taiwan lend support for the idea that the place of natural origin of the species would be somewhere in Asia (Kajihara et al., 2016). The species was originally described from an aquarium tank (Wilfert and Gibson, 1974), and its native distribution has been still unknown (Kajihara et al., 2016; Kvist et al., 2018). Even so, there are some indirect evidences that are consistent with - if not in favor of - the Asian-origin hypothesis.

Phylogenetically, *A. albimaculosa* is sister to another brackish/freshwater species *Thininemertes pratensis* (Sun & Lu, 1998), which has so far been known from the Yangtze River Estuary in China (Sun and Lu, 1998) and the Han River Estuary in Korea (Park et al., 2019). This sister-taxon relation, however, does not automatically mean that the natural distribution of *A. albimaculosa* should be also close to China and Korea, because the branches from the node between the two species on the phylogenetic tree were rather deep (Park et al., 2019); moreover, nothing is known as to nucleotide substitution rates among nemerteans. Still, this evolutionary relation does not contradict with the view that *A. albimaculosa* has phylogeographically originated in Asia.

Takakura’s (1932) unidentified freshwater heteronemertean from Taiwan likely represented *A. albimaculosa*, although verification of this is impossible because Takakura’s voucher material is not extant (Kajihara, 2004). If Takakura’s (1932) species was indeed *A. albimaculosa*, the present study would have been the second record of the species from Taiwan after 87 years, suggesting a stable population of the species in Taiwan, and possibly in more inclusive East/Southeast Asia as well.

Apart from *A. albimaculosa* and *Y. pratensis*, four species in four genera of brackish/freshwater heteronemerteans are currently known: *Ammiclineus zhuijiangensis* Gibson & Qi, 1991 from the Pearl River Estuary in China (Gibson and Qi, 1991); *Hinumanemertes kikuchii* Iwata, 1970 from Lake Hinuma, Japan (Iwata, 1970) and Primorsky Krai, Russia (Chernyshev, 2014); *Planolineus exsul* Beauchamp, 1928 from a botanical garden in Bogol, Indonesia (Beauchamp, 1928, 1929); and *Siolineus turbidus* Du Bois-Reymond Marcus, 1948 from the
Tapajós, a major tributary of the Amazon River in Brazil (Du Bois-Reymond Marcus, 1948). Of these, P. exsul has not been described well enough to be decisively distinguished from A. albimaculosa. We refrain from going into morphological detail because taxonomic debunking is not the scope of this article, but A. albimaculosa was alleged to differ from P. exsul only by trivial and/or ambiguous differences; the possibility cannot be ruled out that P. exsul is actually synonymous with A. albimaculosa. If this was the case, this freshwater heteronemertean would have been distributed in East/Southeast Asia for more than 90 years.

An alternative idea is that A. albimaculosa has been originally distributed in the tropical/subtropical regions on both sides of the Pacific, as Kvist et al.’s (2018) report from Panama suggests. In addition, as is the case with P. exsul, the purported distinction between the Brazilian S. turbidus and the aquarium-derived A. albimaculosa - put forward when the latter was established (Wilfert and Gibson, 1974) - is not fully substantiable, suggesting the conspecificity between the two. If true, this species would have been known from the American continent for more than 70 years.

As to the occurrence of A. albimaculosa in a wild environment in Panama, Kvist et al. (2018) speculated that the species might have been transported with ballast water into the Panama Canal, whilst at the same time pointed out the possibility that Panama might be within the native range of the species. If the latter was the case, the observed 1-base difference within a 575-bp partial COI region (0.17%) between the Taiwanese and Panamanian specimens might represent an intraspecific population genetic structure. Assuming such trans-Pacific gene flow, migratory birds might be potential vectors (Viana et al., 2016). However, waterfowl-mediated intercontinental dispersal of freshwater invertebrates would be quite infrequent, because migratory-birds’ flyways are generally latitudinal, rather than longitudinal (Newton, 2008). Although the East Asian Oceanic Flyway (or whatever flyway that covers Taiwan) and the Trans-American Flyway (or whatever flyway that covers Panama) of migratory waterbirds may overlap on Alaska (Boere and Stroud, 2006), such a pathway via the North America would be rare, if not impossible, because exposure to temperature below 10°C for more than about a week seems to be fatal to A. albimaculosa (Kajihara et al., 2016). Therefore, the transportation might have happened when the climate was warmer than the present, e.g., during the Middle Pleistocene, when the summer surface seawater temperature in the Southern Kurile is estimated to have been 6–8°C above that of today (Razjigaeva et al., 2015).

Another alternative to explain the occurrence of A. albimaculosa in Taiwan and Panama is human-activity-mediated distribution expansion, as Kvist et al. (2018) argued. In that case, detailed population genetic studies based on extensive sampling from both sides of the Pacific would be necessary to pin down the natural place of origin of the species.

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**LITERATURE CITED**


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2020

Kajihara et al.: *Apatronemertes albimaculosa* from Taiwan