



## Note

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

Hiroshi KAJIHARA<sup>1,\*</sup>, Ayuta KATO<sup>2</sup>, Takafumi NAKANO<sup>3</sup>, Yi-Te LAI<sup>4</sup>

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

(Manuscript received 14 October 2019; Accepted 22 December 2019; Online published 15 January 2020)

**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'-CAAAATAGGATCCCCTCCTC-3'), was designed at Primer3 website (<http://bioinfo.ut.ee/primer3-0.4.0/primer3/>) (Koressaar and Remm, 2007; Untergasser *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



**Fig. 1.** **A:** Scenery of the sampling site, a branch of the Zhen-Ping River beside Tian-Shun Temple in Nantun, Taichung, Taiwan; specimens were collected near the shore, not in the middle of the brook. **B, C:** *Apatronemertes albimaculosa* Wilfert & Gibson, 1974, living state; in B, the worm is turning its head around, which is mostly underneath its succeeding trunk. **D:** *A. albimaculosa*, preserved specimen, ASIZ01000026, showing the position of the mouth and the cerebral organ canal opening; arrowheads pointing rudimentary lateral cephalic slit. Scale bar: D = 1 mm.

been deposited in the Biodiversity Research Museum, Academia Sinica, (ASIZ), Taipei, Taiwan, with catalogue numbers ASIZ01000026–01000028. Newly determined sequences have been deposited in DDBJ/EMBL/GenBank.

## 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



**Table 1.** 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 heteronemertean *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.

Species	Accession number	Country of origin	Nucleotide position			Amino-acid position
			574	575	576	192
<i>Apatronemertes albimaculosa</i>	HQ848584	Spain	T	T	G	L
<i>Apatronemertes albimaculosa</i>	LC095809	Japan	T	T	G	L
<i>Apatronemertes albimaculosa</i>	MH128987	Panama	T	T	T	F
<i>Apatronemertes albimaculosa</i>	MH128988	Panama	T	T	T	F
<i>Apatronemertes albimaculosa</i>	MH128989	Panama	T	T	–	?
<i>Apatronemertes albimaculosa</i>	LC506017	Taiwan	T	T	G	L
<i>Apatronemertes albimaculosa</i>	LC506018	Taiwan	T	T	G	L
<i>Iwatanemertes piperata</i>	KF719984	—	T	T	G	L
<i>Lineus viridis</i>	FJ839919	—	T	T	A	L

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. COI sequences were determined from the two by using newly designed specific primers; LC506017 from ASIZ01000026, LC506018 from ASIZ01000028. These two sequences were completely identical to each other, as well as to the ones from Spain (Andrade *et al.*, 2012) and Japan (Kajihara *et al.*, 2016) in terms of the determined 575-bp region. Comparison with the three sequences from Panama (Kvist *et al.*, 2018) could not be straightforward because two of them (MH128988, MH128989) contained a few degenerate sites and one (MH128989) was shorter than the others. The two of the longer sequences from Panama (MH128987, MH128988) differed from the ones from Taiwan, Japan, and Spain by having thymine, instead of guanine, at the 576th site in the entire 1,531-bp COI gene, making the 192nd amino-acid residue phenylalanine, instead of leucine (Table 1); the uncorrected *p*-distance between the Taiwanese and Panamanian sequences was minimally 0.17%.

## 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 *Yininemertes 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 nemertean. 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 heteronemertean are currently known: *Amniclineus zhujiangensis* 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 Bogor, 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.

## ACKNOWLEDGMENTS

This study was partially supported by Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research (grant number 17K07520) for HK. HK thanks Mr Daisuke Aoki (Hokkaido University) for ornithological literature. We are grateful to two anonymous referees and Dr Wen-Jer Wu for reviewing our manuscript.

## LITERATURE CITED

- Andrade, S. C. S., M. Strand, M. Schwartz, H.-X. Chen, H. Kajihara, J. von Döhren, S.-C. Sun, J. Junoy, M. Thiel, J. L. Norenburg, J. M. Turbeville, G. Giribet and P. Sundberg. 2012. Disentangling ribbon worm relationships: multi-locus analysis supports traditional classification of the phylum Nemertea. *Cladistics* **28**(2): 141–159.
- Beauchamp, P. de. 1928. Une hétéronémerte d'eau douce à Buitenzorg. *Bull. Soc. Zool. Fr.* **53**: 62–67.
- Beauchamp, P. de. 1929. Triclares terricoles, triclares paludicoles, némertien. *Treubia* **10**: 405–430.
- Boere, G. and D. A. Stroud. 2006. The flyway concept: what it is and what it isn't. In: Boere, G. *et al.* (eds.), *Waterbirds Around the World*. 40–47. The Stationery Office, Edinburgh, UK.
- Boom, R., C. J. Sol, M. M. Salimans, C. L. Jansen, P. M. Wertheim-van Dillen and J. van der Noordaa. 1990. Rapid and simple method for purification of nucleic acids. *J. Clin. Microbiol.* **28**: 495–503.
- Chernyshev, A. V. 2014. Nemertean biodiversity in the Sea of Japan and adjacent areas. In: Sun, S. *et al.* (eds.), *Marine Biodiversity and Ecosystem Dynamics of the Northwest Pacific Ocean*. 119–135. Science Press, Beijing, China.
- Corrêa, D. D. 1963. Nemerteans from Curaçao. *Stud. Fauna Curaçao Other Caribb. Isl.* **17**: 41–56.
- Du Bois-Reymond Marcus, E. 1948. An Amazonian heteronemertine. *Bol. Fac. Filos. Ciênc. Let. Univ. São Paulo* **13**: 93–109.
- Ehrenberg, C.G. 1831. Phytozoa turbellaria Africana et Asiatica in Phytozoorum Tabula IV et V delineata. In: Hemprich F. G. and C. G. Ehrenberg (eds.), *Symbolae physicae, seu icones et descriptiones corporum naturalium novorum aut minus cognitorum quae ex itineribus per Libyam, Aegyptium, Nubiam, Dongalam, Syriam, Arabiam et Habessiniam, pars zoologica II, animalia evertibrata exclusis insectis*. 53–67, pls IV–V. Officina Academica, Berolina [Berlin], Germany. [Plates published in 1828, text in 1831]
- Folmer, O., M. Black, W. Hoeh, R. Lutz and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **3**: 294–299.
- Gibson, R. 1995. Nemertean genera and species of the world: an annotated checklist of original names and description citations, synonyms, current taxonomic status, habitats and recorded zoogeographic distribution. *J. Nat. Hist.* **29**(2): 271–562.
- Gibson, R. and S. Qi. 1991. A new freshwater heteronemertean from the Zhujian (Pearl River), People's Republic of China. *Hydrobiologia* **220**(2): 167–178.



- Hookabe, N., M. L. Schwartz, H. Kajihara and J. L. Norenburg.** 2019. Molecular systematics of the heteronemertean genus *Dushia* (Nemertea, Pilidiophora), with descriptions of *D. wijnhoffae* sp. nov. and *D. nigra* species complex comb. nov. *Zootaxa* **4691(4)**: 333–358.
- Iwata, F.** 1970. On the brackish water nemerteans from Japan, provided with special circulatory and nephridial organs useful for osmoregulation. *Zool. Anz.* **184**: 133–154.
- Kajihara, H.** 2004. Usamaro Takakura (1867–1944), Japanese pioneer nemertean researcher. *Arch. Nat. Hist.* **31(2)**: 208–213.
- Kajihara, H., A. V. Chernyshev, S.-C. Sun, P. Sundberg, P. F. B. and Crandall.** 2008. Checklist of nemertean genera and species published between 1995 and 2007. *Spec. Divers.* **13(4)**: 245–274.
- Kajihara, H., M. Takibata and M. J. Grygier.** 2016. Occurrence and molecular barcode of the freshwater heteronemertean *Apatronemertes albimaculosa* (Nemertea: Pilidiophora) from Japan. *Spec. Divers.* **21(2)**: 105–110.
- Koressaar, T. and M. Remm.** 2007. Enhancements and modifications of primer design program Primer3. *Bioinformatics* **23(10)**: 1289–1291.
- Kumar, S., G. Stecher and K. Tamura.** 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33(7)**: 1870–1874
- Kvist, S., D. de Carle, A. Cornejo and A. Ocegüera-Figueroa.** 2018. Biological introductions or native ranges: two curious cases of new distributional records in the Panama Canal. *BioInvasions Rec.* **7**: 237–244.
- Müller, O. F.** 1774. *Vermivm terrestrium et fluviatilium, seu animalium infusorium, helminthicorum, et testaceorum, non marinorum, succincta historia. Voluminis Imi Pars Altera* [Volume 1, Part 2]. Heineck et Faber, Havniae et Lipsiae [locative case of Havnian and Lipsia, Copenhagen and Leipzig, respectively]. 72 pp.
- Newton, I.** 2008. *The Migration Ecology of Birds*, Elsevier, Amsterdam, The Netherland. viii + 976 pp.
- Park, T., S. H. Lee, S.-C. Sun and H. Kajihara.** 2019. Morphological and molecular study on *Yininemertes pratensis* (Nemertea, Pilidiophora, Heteronemertea) from the Han River Estuary, South Korea, and its phylogenetic position within the family Lineidae. *ZooKeys* **852**: 31–51.
- Podsiadlowski, L., A. Braband, T. H. Struck, J. von Döhren and T. Bartolomaeus.** 2009. Phylogeny and mitochondrial gene order variation in Lophotrochozoa in the light of new mitogenomic data from Nemertea. *BMC Genomics* **10(1)**: 364.
- Razjigaeva, N. G., L. A. Ganzey, T. A. Grebennikova, N. I. Belyanina, E. D. Ivanova, A. M. Lebedev, F. E. Maksimov and V. Yu. Kuznetsov.** 2015. Environmental changes at final warming of Middle Pleistocene (MIS 7) in South Kurils. *Quat. Int.* **355**: 90–100.
- Senz, W.** 1993. Nemertinen europäischer Küstenbereiche (nebst ergänzenden Angaben zur Anatomie von *Apatronemertes albimaculosa* Wilfert & Gibson, 1974). *Ann. Nathist. Mus. Wien Ser. B Bot. Zool.* **94/95**: 47–145.
- Shen, C.-Y., W.-Y. Sun and S.-C. Sun.** 2015. The complete mitochondrial genome of *Iwatanemertes piperata* (Nemertea: Heteronemertea). *Mitochondrial DNA* **26(6)**: 846–847.
- Smith, D. G.** 2001. *Pennak's Freshwater Invertebrates of the United States*, 4th ed., Porifera to Crustacea, John Wiley & Sons, New York, USA. x + 641 pp.
- Stimpson, W.** 1855. Descriptions of some of the new marine Invertebrata from the Chinese and Japanese seas. *Proc. Acad. Nat. Sci. Phila.* **7**: 375–384.
- Sun, S.-C. and J.-R. Lu.** 1998. A new genus and species of heteronemertean from the Changjiang (Yangtze) River Estuary. *Hydrobiologia* **367(1/3)**: 175–187.
- Sundberg, P. and R. Gibson.** 2008. Global diversity of nemerteans (Nemertea) in freshwater. *Hydrobiologia* **595(1)**: 61–66.
- Takakura, U.** 1932. Tansui himomushi no hanashi [On the freshwater nemerteans]. *Hakubutsugaku Zasshi* **30(4)**: 4–6. [In Japanese]
- Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B. C. Faircloth, M. Remm and S. G. Rozen.** 2012. Primer3-new capabilities and interfaces. *Nucleic Acids Res.* **40(15)**: e115.
- Viana, D. S., L. Santamaría and J. Figuerola.** 2016. Migratory birds as global dispersal vectors. *Trends Ecol. Evol.* **31(10)**: 763–775.
- Wilfert, M. and R. Gibson.** 1974. A new genus of hermaphroditic freshwater heteronemertean (Nemertinea). *Z. Morphol. Tiere* **79(2)**: 87–112.
- Yamaoka, T.** 1939. Two nemerteans from Formosa. *Annot. Zool. Jpn.* **18**: 283–289.