Topotype-based DNA Barcode of the Parasitic Isopod *Pseudione nephropsi* (Bopyridae), with a Supplementary Morphological Description

Keiichi Kakui^{1,4}, Michitaka Shimomura², Shoichi Kimura³, and Taeko Kimura³

¹ Faculty of Science, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan

E-mail: keiichikakui@gmail.com

² Seto Marine Biological Laboratory, Kyoto University, Nishimuro, Shirahama, Wakayama 649-2211, Japan

³ Faculty of Bioresources, Mie University, Tsu, Mie 514-8507, Japan

⁴ Corresponding author

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In 2017, we collected a pair (male and female) of topotypic individuals of the parasitic isopod *Pseudione nephropsi* Shiino, 1951 from a single nephropid lobster, *Metanephrops japonicus* (Tapparone-Canefri, 1873), at 310–298 m depth off the eastern coast of the Kii Peninsula, Japan. Based on these specimens, we provide a supplementary description of the male and female morphology, and present mitochondrial cytochrome *c* oxidase subunit I (COI) DNA barcode sequences. The sequence length amplified with primer pair LCO-1490/HCO-2198 was 658 nt, and there were four synonymous nucleotide substitutions (0.6% *p*-distance) between the two sequences.

Key Words: Crustacea, DNA barcoding, Japan, Isopoda, Nephropidae, North Pacific, parasite.

Introduction

DNA barcoding (Hebert *et al.* 2003) is a powerful tool for taxonomy. It enables us to test conspecificity among disjunct populations within widely distributed nominal species (*e.g.*, Tomioka *et al.* 2016), to test whether two or more taxa showing morphological differences are conspecific (*e.g.*, Cheang *et al.* 2013), and to link conspecific larvae, juveniles, males, and females in species with large ontogenetic change or sexual polymorphism (*e.g.*, Ota *et al.* 2012). In addition, DNA sequence data are increasingly being used as the only diagnostic character in species descriptions (*e.g.*, Nakano *et al.* 2017, 2018), especially for groups having few morphological characters with which to distinguish species. However, in order to use DNA barcoding for species identification or description, a database of sequences is necessary from previously described taxa in the target group.

The genus *Pseudione* Kossmann, 1881 in the isopod family Bopyridae Rafinesque, 1815 presently comprises 47 species (Boyko *et al.* 2008 onwards), all of which live as parasites in the gill chamber of astacidean, axiidean, gebiidean, anomuran, or caridean decapods (Boyko *et al.* 2017). While these isopods show relatively few morphological modifications related to their parasitic mode of life (Williams and Boyko 2012), male and female *Pseudione* appear simpler in morphology than free-living taxa; for example, some appendages are lacking or reduced (*e.g.*, uropods and pleopods in males), and there are fewer setae on the pereopods. The reduced number of morphological characters for discriminating species in *Pseudione* can lead to difficulties in species identification using morphology.

Pseudione nephropsi Shiino, 1951 is one of four *Pseudione* species parasitic on clawed lobsters (Nephropidae, Astacidea) (Horch *et al.* 2018). This species was originally described from specimens parasitic on the nephropid lobster *Metanephrops japonicus* (Tapparone-Canefri, 1873) collected from deep water off the coast of Owase in central Japan (Kumano Sea, North Pacific) (Shiino 1951). Markham (1999) later reported this species from a different host, *Metanephrops velutinus* Chan and Yu, 1991, collected from 401–393 m depth off the eastern coast of Palau Jamdena, Indonesia, in the Arafura Sea (7°45'S 132°42'E). The disjunct distribution of these two host species (*M. japonicus* in Japan; *M. velutinus* from the Philippines to Western Australia; Chan and Yu 1991) and slight morphological differences between the Japanese and Indonesian isopod specimens (Markham 1999) raise the possibility that the *Pseudione* specimens reported by Markham (1999) may not be *P. nephropsi*. DNA barcoding would be a good way to confirm or reject conspecificity, but no nucleotide sequences for *P. nephropsi* had been deposited in public databases (DDBJ 2018).

During a cruise of the TR/V *Seisui-maru* (Mie University, Japan) in 2017, a male-female pair of *P. nephropsi* parasitizing an individual of *M. japonicus* was collected off the coast of Owase, the type locality for *P. nephropsi*. Here we describe or redescribe the morphology of the two sexes, which was incompletely described by Shiino (1951), and report cytochrome *c* oxidase subunit I (COI) DNA barcode sequences to aid future DNA barcoding in species of *Pseudione*.

Materials and Methods

An individual of *Metanephrops japonicus* infested by bopyrid isopods was obtained from the Kumano Sea (34°07.753'N, 136°37.934'E to 34°07.983'N, 136°37.809'E) with a biological dredge at 310–298 m depth on 9 November 2017. The living animals were photographed, and then two branchiae of the host and the whole bodies of the isopods were fixed in 70% ethanol and preserved in 80% ethanol. The methods for dissection, preparation of slides, light microscopy, and drawing were as described by Kakui and Angsupanich (2012); after observation, dissected appendages were preserved in 80% ethanol. The morphological terminology herein follows Markham (1985, 1988). The bopyrids studied were deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Japan (female, ICHUM 5834; male, ICHUM 5835).

Total DNA was extracted from one branchia (*M. japonicus*), left pereopod 4 (female *P. nephropsi*), or right pereopod 6 (male *P. nephropsi*) by using a NucleoSpin Tissue XS kit (TaKaRa Bio, Japan); after extraction, the exoskeletons of the pereopods were recovered and preserved in 80% ethanol. Part of the COI gene was amplified by PCR using primers LCO-1490 and HCO-2198 (Folmer *et al.* 1994). PCR amplification conditions with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio) were 94°C for 1 min; 35 cycles of 98°C for 10 sec, 42°C for 30 sec, and 72°C for 50 sec; and 72°C for 2 min. Methods for sequencing and sequence assembly were as described by Tomioka *et al.* (2016). The sequences obtained from *M. japonicus* and the male and

female *P. nephropsi* individuals were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ), under accession numbers LC476592, LC476590, and LC476591, respectively. The nucleotide and protein sequences in the INSD most similar to our sequences were determined by BLAST searches (Altschul *et al.* 1990), using blastn (nucleotide sequences) and blastp (protein sequences) under both the default setting and with the 'Organism' option set to 'Bopyridae'.

Results and Discussion

Our male and female *Pseudione nephropsi* specimens were collected from the left gill chamber of the host (Fig. 1A). The male clung to the ventral surface in the region of female pleon surrounded by pleopods when alive (Fig. 1C). Based on our specimens, several morphological characters are described or redescribed as follows.

Female (Figs 1, 2A–H, 3A–D). Creamy white when alive (Fig. 1B, C). Body length 15.5 mm, maximum width 9.5 mm at pereomere 4; pereon weakly dextral (17°). Head with slight anterior depression, lacking eyes (Figs 1B, 2A); frontal lamina short, narrow. Barbula with elongate, angled outer projection and five small inner projections (Fig. 2B). Antenna 1 with three articles (Fig. 3A). Antenna 2 with eight articles (Fig. 3B, b1); combined length of articles 6–8 *ca.* 0.3 times length of article 5. Posterolateral point of oostegite 1 rounded at tip (Fig. 2C, D). Anterolateral region of pereomeres 5–7 not projecting laterally. Pereopod 1 (Figs 2E, 3C) with basis bearing dorsoproximal serration; car-

Fig. 1. *Pseudione nephropsi* Shiino, 1951 parasitizing *Metanephrops japonicus* (Tapparone-Canefri, 1873), fresh specimens. A, *M. japonicus* infested by bopyrids; B, C, female *P. nephropsi* extracted from host, dorsal (B) and ventral (C) views, with attached male (arrow). Scales: 50 mm (A), 5 mm (B, C).

pus with ventrodistal setal cluster. Pereopod 7 (Figs 2F, 3D) with basis bearing several dorsal serrate tubercles; merus and carpus fused; carpus with ventrodistal setal cluster. Five

pairs of biramous pleopods, ventrally tuberculate (Fig. 2G). Pleomere 6 with tiny dorsodistal anal cone (Fig. 2H, arrow). Uniramous uropods crenulate; right uropod longer than left

Fig. 2. *Pseudione nephropsis* Shiino, 1951, fixed specimens. A–H, female; I–O, male. A, head, dorsal view; B, left barbula, ventral view; C, D, left oostegite 1, ventral (C) and dorsal (D) views; E, F, left pereopods 1 and 7, respectively; G, tubercles on right pleopods, ventral view; H, posterior region of body, dorsal view; I, body, dorsal view; J, head, dorsal view; K, L, left pereopods 1 and 7, respectively; M, N, pleon, ventral (M) and slightly-left, ventral (N) views; O, pleomere 6, dorsal view. Abbreviations: *pl1*, pleopod 1; *pl1e*, exopodite of pleopod 1; *ur*, uropod. Arrows, anal cone. Scales: 1 mm (A–I), 0.5 mm (M, N), 0.1 mm (J–L, O).

Fig. 3. *Pseudione nephropsi* Shiino, 1951. A–D, female; E, F, male. A, left antenna 1, ventral view; B, left antenna 2, ventral view, with most portion of basal segment (*bs*) omitted; b1, distal region of left antenna 2, ventral view, with most setae omitted; C, E, left pereopod 1; D, F, left pereopod 7. Scales: 0.1 mm (A, B, E, F), 0.05 mm (b1), 0.5 mm (C, D).

(Fig. 2H).

Male (Figs 1, 2I–O, 3E, F). Creamy white when alive (Fig. 1C). Body length 6.2 mm, maximum width 1.3 mm at pereomere 4 (Fig. 2I). Articulations between cephalon, pereomeres 1–7, and pleomeres 1–4 distinct; those between pleomeres 4–6 indistinct. Width of pereomeres 1–7 remaining nearly constant; pleomeres successively narrower toward posterior end. Head suboval in dorsal view, lacking eyes (Figs 1C, 2J). Pereopod 1 (Figs 2K, 3E) with merus and carpus fused; carpus with ventrodistal setal cluster; ventroproximal region of propodus serrate, with two spiniform setae. Pereopod 7 (Figs 2L, 3F) with merus and carpus not fused; carpus bearing ventrodistal setal cluster; ventrodistal region of propodus serrate, with two spiniform setae. Pleomeres without midventral processes. Pleopods 1–5 appear as low, ventrolateral hemispherical swellings, slightly yellowish in fixed specimen (Fig. 2M, N). Pleomere 6 bilobed, with tiny dorsodistal anal cone (Fig. 2O, arrow).

Our specimens agreed very well morphologically with the types of Shiino (1951). Our female similarly has the ventral tubercles on the pleopods, a round-tipped posterolateral

point on oostegite 1, and the anterolateral regions of pereomeres 5–7 not projecting laterally. Our male similarly has a bilobed pleomere 6 and round pereopods 1–5. Shiino (1951: p. 34 and fig. 6E) found “a structure which is believed to be a lateral plate on the pleotelson of the female though only on one side”, but our specimen lacks this structure (Fig. 2H). The structure observed by Shiino (1951) might represent an abnormal condition. Shiino (1951) also observed an articulation between pleomeres 4 and 5 in male, whereas we did not. Our male (Fig. 2M, N) has an acute furrow between pleomeres 4 and 5, and Shiino (1951) possibly misidentified this as an articulation.

The partial COI sequences we determined were 658 nt long and can be translated into 219 amino acids. There were four synonymous nucleotide substitutions (0.6% *p*-distance) between two sequences. Table 1 lists the top five max-score nucleotide/protein sequences detected by blastn and blastp for our sequences. The blastn results show that *p*-distances among confamilial sequences can reach 26%, and also can be greater than the distance between *P. nephropsi* and taxa in different suborders (e.g., *Haloniscus* sp.), imply-

Table 1. Top five max-score nucleotide/protein sequences determined by BLAST searches for our *Pseudione nephropsi* sequences. IS, identity score; QC, query cover; MS, max score; Ac#, accession number.

Taxon	IS (%)	QC (%)	MS	Ac#	Reference
Blastn (default setting)					
Isopoda sp.	81	81	435	MG936523	Unpublished
Bopyridae sp.	78	93	404	MG936175	Unpublished
<i>Haloniscus</i> sp.	79	84	385	KT236034	Murphy <i>et al.</i> (2015)
<i>Haloniscus</i> sp.	78	84	379	KT236035	Murphy <i>et al.</i> (2015)
Isopoda sp.	79	84	375	HM466415	Plaisance <i>et al.</i> (2011)
Blastn ("Organism"=Bopyridae)					
<i>Athelges paguri</i> (Rathke, 1843)*	79	96	467**	KT208746**	Raupach <i>et al.</i> (2015)
<i>Gyge ovalis</i> (Shiino, 1939)	76	96	337	NC_037467	Yu <i>et al.</i> (2018)
<i>Probopyrus pandalicola</i> (Packard, 1879)	75	94	316	MH087672	Unpublished
<i>Bopyroides hippolytes</i> (Krøyer, 1838)	75	84	260	DQ889082	Costa <i>et al.</i> (2007)
<i>Orthione griffenis</i> Markham, 2004	74	80	214	KP412462	Unpublished
Blastp (default setting)					
<i>Athelges paguri</i>	93	100	337	ALH17253	Raupach <i>et al.</i> (2015)
<i>Gyge ovalis</i>	94	100	334	YP_009477596	Yu <i>et al.</i> (2018)
Decapoda sp.	89	100	327	AWU57895	Pearman <i>et al.</i> (2018)
Malacostraca environmental sample	93	97	324	AKA20637	Unpublished
<i>Probopyrus pandalicola</i>	89	100	323	AWM96874	Unpublished
Blastp ("Organism"=Bopyridae)					
<i>Athelges paguri</i>	93	100	337	ALH17253	Raupach <i>et al.</i> (2015)
<i>Gyge ovalis</i>	94	100	334	YP_009477596	Yu <i>et al.</i> (2018)
<i>Probopyrus pandalicola</i>	89	100	323	AWM96874	Unpublished
Bopyridae sp.	95	94	315	AXR87084	Unpublished
<i>Bopyroides hippolytes</i>	82	95	280	ABK05946	Costa <i>et al.</i> (2007)

*, the IS and QC of three sequences in Raupach *et al.* (2015) were identical, and only one sequence is presented here; **, sequence with highest max score.

ing nucleotide substitution saturation among bopyrid genera in the COI region sequenced. Blastp detected sequences from confamilial taxa in the default setting, indicating bopyrid protein sequences may be relatively well conserved. The decapod sequence (Decapoda sp.; accession number AWU57895) must have been misidentified or mislabeled, or represent a contaminant.

Here we determined partial COI sequences for *P. nephropsi* based on topotypic specimens. This is the first COI DNA barcode sequence for any species in *Pseudione*, and will be available to eventually test the identity of the Indonesian *P. nephropsi* reported by Markham (1999).

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