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Short communication

DNA Barcoding of *Rocinela niponia* (Isopoda, Cymothooidea, Aegidae) from South Korea

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ABSTRACT

An aegid species, *Rocinela niponia* Richardson, 1909, is a Far Eastern species known from Korean and Japanese waters. In this study, mitochondrial cytochrome *c* oxidase subunit I (*COI*) sequences of *R. niponia* were determined based on four specimens collected from the subtidal zone of Chujado Island, South Korea. We compared DNA barcoding data of this species with its congeners. As a result, there was no intra-specific genetic distance between the four *COI* sequences of *R. niponia*. Inter-specific distances between *R. niponia* and other five aegid species ranged from 23.8% to 35.6%. Morphological diagnosis and images of *R. niponia* are also provided as a valuable contribution toward the identification of *Rocinela* species in further taxonomic and ecological studies.

Keywords: aegids, *COI*, DNA barcode, isopods, Korean waters

INTRODUCTION

The isopod genus *Rocinela* Leach, 1818 including 41 species is one of the species-richest genera in the family Aegidae White, 1850 (Bruce, 2009; Cardoso et al., 2017; Kim and Yoon, 2020). *Rocinela* species are regarded as micro-predators attaching temporarily and feeding on fish's blood or mucus (Bruce, 2009; Smit et al., 2019). They are peculiarly known as the very few isopods that can attack humans (Garzón-Ferreira, 1990; Bruce, 2009; Smit et al., 2019). This genus can be distinguished from other aegid genera by having a pleonite 1 not abruptly narrowed than pereonite 7 and a 3-articled maxillipedal palp (Bruce, 2009). In the *Rocinela* species, the shape of the frontal margin and frontal lamina of the cephalon and the armature of the pereopods and uropods are the most useful characters for distinguishing each other (Brusca and France, 1992). So far, only seven *Rocinela* species have been reported from the Far East, although this genus has been shown to have the highest diversity in the high latitude (Bruce, 2009; Cardoso et al., 2017; Kim and Yoon, 2020). Of them, *R. maculata* Schiœdte and Meinert, 1879 and *R. nipoina* Richardson, 1909 have been reported in Korean waters (Schiœdte and Meinert, 1879; Richardson, 1909; Kussakin, 1974; Kim and Yoon, 2020).

Richardson (1909) described *Rocinela niponia* based on a single female specimen from the vicinity of Sado Island, Japan. After a century, the occurrence of this species from Chujado Island located in the Jeju strait, South Korea close to the type locality has been reported by Kim and Yoon (2020). This species is distinguished from its congeners in that its large eyes are separated from each other and the pereopod 1 has propodal blade bearing 8–10 robust setae (Richardson, 1909; Bruce, 2009; Kim and Yoon, 2020). This species requires further study because the original description contains a few illustrations of the cephalon and pereopod 1 (Richardson, 1909; Bruce, 2009). The morphology of Korean materials showed a minor difference in the numbers of robust setae in the propodal bade of pereopod 1 from the original description (Kim and Yoon, 2020). Although a molecular approach is regarded as an alternative method to distinguish species and to suggest a phylogenetic relationship in many invertebrate taxa (Costa et al., 2007; Raupach et al., 2015), molecular data for isopods as well as *Rocinela* species are still limited. In this situation, here we presented the first cytochrome *c* oxidase subunit I (*COI*) sequence of the Korean *R. niponia* for molecular identification.

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Sediment samples were collected from the subtidal zone of Chujado Island (33°59′07″N, 126°19′12″E) by SCUBA diving and using a Smith-McIntyre grab. Collected isopod specimens were immediately fixed in 95% ethyl alcohol. To identify materials, morphological observation was carried out under a dissecting microscope (SMZ 1500; Nikon, Japan). If required, materials were dissected and observed under a compound microscope (BX50; Olympus, Japan). Photographs were taken with the help of a CCD camera (iCM 3.0; Bernardy, Canada) using an iSolution Lite software (IMT i-solution; Bernardy). Genomic DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. LCO 1490 and HCO 2198 primers were used to obtain *COI* sequences of *R. niponia* (Folmer et al., 1994). These *COI* sequences were deposited in GenBank under accession numbers OM179771−OM19774. The alignment with five aegid species [*Aega psora* (Linnaeus, 1758), *Alitropus typus* Milne Edwards, H., 1840, *R. angustata* Richardson, 1904, *R. tridens* Hatch, 1947, and *Syscenus infelix* Harger, 1880] was conducted using Geneious Prime v2021.2.2 (Biomatters, Auckland, New Zealand). Genetic distances measured by the Kimura-2-parameter model and Maximum likelihood tree were calculated by using MEGA v.6.06 (Tamura et al., 2013; Kumar et al., 2018). Three non-*Rocinela* species, *Ae. psora*, *Al. typus*, and *S. infelix*, were included as an outgroup. The voucher specimen (NIBRIV0000876683) of *R. niponia* was deposited at the National Institute of Biological Resources (NIBR), Incheon, South Korea.

RESULTS AND DISCUSSION

Four partial *COI* sequences (each 658 bp) from each individual of *R. niponia* were newly obtained from Korean materials. Genetic analysis between this species and five other aegids, *Ae. psora*, *Al. typus*, *R. angustata*, *R. tridens*, and *S. infelix*, available from GenBank was performed (Costa et al., 2007; Radulovici et al., 2009; Hata et al., 2017). The alignment length of these six species including eleven partial *COI* sequences was 583 bp in the genetic comparison. There were no intra-specific variations among Korean materials (Table 1). Comparing intra-specific variations with the previous studies on other Korean isopods (Song, 2020; Kim et al., 2021), this result shows a lower value. The inter-specific genetic distance among *R. niponia* and five aegid species ranged from 23.8% to 35.6% (Table 1). The genetic distance between three *Rocinela* species and two aegids ranged from 33.0% to 36.2%, while the distance among *Rocinela* species ranged from 22.5% to 28.5%. Moreover, Korean materials of *R. niponia* were much more closely clustered with two *Rocinela* species than other genera in our tree (Fig. 1). Taken all together, the

Table 1. Genetic distances (measured by K2P method) based on 658 bp-size *COI* sequences between *Rocinela niponia* Richardson, 1909 and four aegid species

No.	Species	Accession No.	1	2	3	4	5	6	7	8	9	10	11	Data source
1	*Rocinela niponia*	OM179771												Present study
2	*Rocinela niponia*	OM179772	0.000											Present study
3	*Rocinela niponia*	OM179773	0.000	0.000										Present study
4	*Rocinela niponia*	OM179774	0.000	0.000	0.000									Present study
5	*Rocinela angustata*	EF432739	0.239	0.239	0.239	0.239								Costa et al. (2007)
6	*Rocinela angustata*	MH242961	0.285	0.285	0.285	0.285	0.225							Unpublished
7	*Rocinela angustata*	MH242962	0.285	0.285	0.285	0.285	0.225	0.000						Unpublished
8	*Rocinela tridens*	MH242963	0.243	0.243	0.243	0.243	0.210	0.258	0.258					Unpublished
9	*Alitropas typus*	KT445864	0.350	0.350	0.350	0.350	0.334	0.351	0.351	0.352				Hata (2017)
10	*Syscenus infelix*	FJ581911	0.344	0.344	0.344	0.344	0.349	0.349	0.349	0.383	0.330			Radulovici et al. (2009)
11	*Aega psora*	FJ581463	0.356	0.356	0.356	0.356	0.323	0.351	0.351	0.338	0.362	0.332		Radulovici et al. (2009)

K2P, Kimura-2-parameter; COI, cytochrome *c* oxidase subunit I.

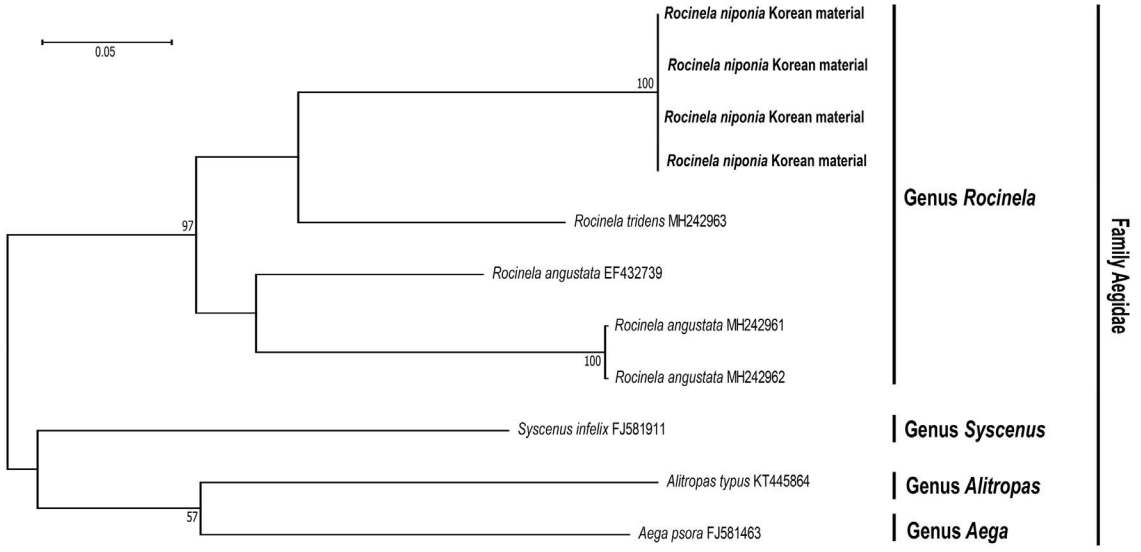

Fig. 1. A maximum likelihood tree of aegid species based on mitochondrial cytochrome *c* oxidase subunit I (*COI*) sequences. Korean materials of *Rocinela niponia* Richardson, 1909 are presented in bold font. Bootstrap values above 50% of 500 bootstrap replicates are given at each nod.

molecular analysis based on *COI* sequences distinguished each species and genera from others. These results also well corresponded with morphological taxonomy results (Bruce, 2009). This study would be helpful for distinguishing *R. niponia* from other aegid species. However, additional DNA information of more *Rocinela* species is needed for further studies. Additionally, the intra-specific genetic distance of *R. angustata* ranged from 0% to 22.5% based on three *CO1* sequences in this study. This high genetic variation probably means that specimens of *R. angustata* between Vancouver Island of Canada (accession No. EF432739) and San Juan Island of USA (accession Nos. MH242961, MH242962) could be noncongenetic, considering the intra-specific genetic distance of other isopods did not exceed 11% (Taiti et al., 2003; Song, 2020; Kim et al., 2021). So, further morphological and genetic studies on this species between these two different regions seem to be required.

Order Isopoda Latreille, 1817
Superfamily Cymothooidea Leach, 1814
Family Aegidae White, 1850
Genus *Rocinela* Leach, 1818

***Rocinela niponia* Richardson, 1909 (Fig. 2)**

Rocinela niponia Richardson, 1909: 83, figs. 9, 10; Kim and Yoon, 2020: 373, figs. 1–3.

Diagnosis. Body oval, flattened dorsoventrally; dorsal surface smooth. Cephalon triangular, ambiguously tri-sinuated posteriorly; rostrum rounded distally; eyes separated each other.

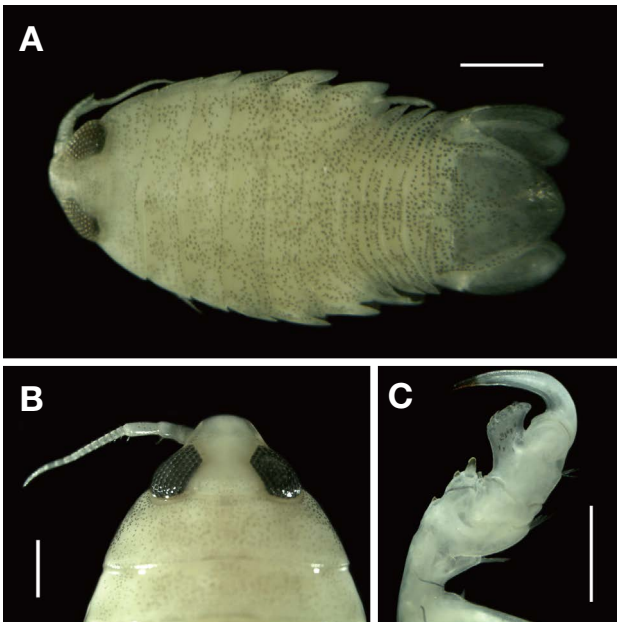

Fig. 2. Photographs of *Rocinela niponia* Richardson, 1909 from Chujado Island of South Korea. A, Habitus, dorsal view; B, Cephalon, dorsal view; C, Pereopod 1 excluding the basis, lateral view. Scale bars: A–C = 1 mm.

Pereon widest in pereonites 4–6; coxal plates of pereonites 2–4 rounded posteriorly, whereas coxal plates of pereonites 5–7 acute. Pleonites not abruptly narrow; pleonite 1 invisible dorsally; pleonite 5 surrounded by pleonite 4. Pleotelson shield-shaped, tapering posteriorly, with 1 pair of dorsal de-

pressions proximally; distal end rounded. Antenna reaching posterior margin of pereonite 2. Pereopods 1−3, ischium with 1 robust seta superodistally; merus with 4−5 robust setae interiorly; carpus with 1 robust seta inferiorly; propodus with blade on palm; propodal blade 0.7 times as long as wide, with 8−10 blunt robust setae distally. Uropod not over distal end of pleotelson.

Distribution. South Korea and Japan.

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CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article are reported.

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