ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

Anim. Syst. Evol. Divers. Vol. 36, No. 2: 182-184, April 2020
https://doi.org/10.5635/ASED.2020.36.2.013

Short communication

DNA Barcoding of *Isaacsicalanus paucisetus* (Copepoda: Calanoida: Spinocalanidae) from the Hydrothermal Vent in the North Fiji Basin, Southwestern Pacific Ocean

Chailinn Park[1], Won-Kyung Lee[2], Se-Joo Kim[2], Se-Jong Ju[1,*]

[1]Global Ocean Research Center, Korea Institute of Ocean Science and Technology, Busan 49111, Korea
[2]Genome Editing Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Korea

ABSTRACT

Isaacsicalanus paucisetus Fleminger, 1983, a monotypic species of the family Spinocalanidae Vervoort, 1951, was first reported from a hydrothermal vent field in the East Pacific Rise off the mouth of the Gulf of California. The mitochondrial cytochrome oxidase I (*mtCOI*) DNA barcodes are considered a useful tool to assist traditional taxonomy and species discrimination in calanoid copepods. However, the *mtCOI* DNA barcodes of *I. paucisetus* have not been reported due to the species rarity and the difficulty of sampling. In this study, we firstly determined the *mtCOI* DNA barcodes of the *I. paucisetus* newly collected from a hydrothermal vent in the North Fiji Basin of the southwestern Pacific. All *mtCOI* DNA barcodes of *I. paucisetus* were identical and intraspecies variations of spinocalanid species were 0.0−3.0%. Interspecies and intergeneric variations were 13.4−25.2% and 16.7−24.1%, respectively. The DNA barcodes of *I. paucisetus* obtained in the present study would be helpful for understanding taxonomic relationships of widespread spinocalanid species.

Keywords: hydrothermal vent endemic calanoid, spinocalanid copepod, southwestern Pacific, DNA barcodes, *mtCOI*

INTRODUCTION

The family Spinocalanidae Vervoort, 1951, living from meso- to benthopelagic depths of all oceans, is comprised of 48 species belonging to 12 genera (Walter and Boxshall, 2020). Its members are omnivores so they play an important role in the cycling of organic matters (Bode et al., 2017). Among them, two monotypic genera, *Methanocalanus* Ivanenko, Defaye & Cuoc, 2007 and *Isaacsicalanus* Fleminger, 1983, were found in the hydrothermal vents of the southeastern Atlantic Ocean and eastern Pacific Ocean, respectively (Fleminger, 1983; Ivanenko et al., 2007).

In calanoid copepods, the mitochondrial cytochrome oxidase I (*mtCOI*) DNA barcodes are considered a useful tool to assist traditional taxonomy and species discrimination (Bucklin et al., 2003; Blanco-Bercial et al., 2014). However, although *mtCOI* DNA barcodes of 24 spinocalanid species belonging to six genera are registered in GenBank as of 21 Feb 2020, no *mtCOI* DNA barcodes of hydrothermal vent species have been reported yet.

As the habitat range of Spinocalanidae expanded to the hydrothermal vent, interest in seeking its phylogenetic relationships has increased (Fleminger, 1983; Bode et al., 2017). As mentioned above, DNA barcoding would be helpful for understanding the taxonomic relationships of planktonic assemblages (Bucklin et al., 1999), especially in widespread spinocalanid species. In this study, we firstly determined the *mtCOI* DNA barcodes of *I. paucisetus* from the hydrothermal vent that would help in understanding the taxonomic relationships of the family Spinocalanidae.

Specimens are collected from the diffusing vent (16°59′S, 173°54′E) in the North Fiji Basin of the southwestern Pacific Ocean (Fig. 1A, B) using a remotely operated vehicle 'ROPOS' (Canadian Scientific Submersible Facility, Cana-

*To whom correspondence should be addressed
Tel: 82-51-664-3456
E-mail: sjju@kiost.ac.kr

eISSN 2234-8190

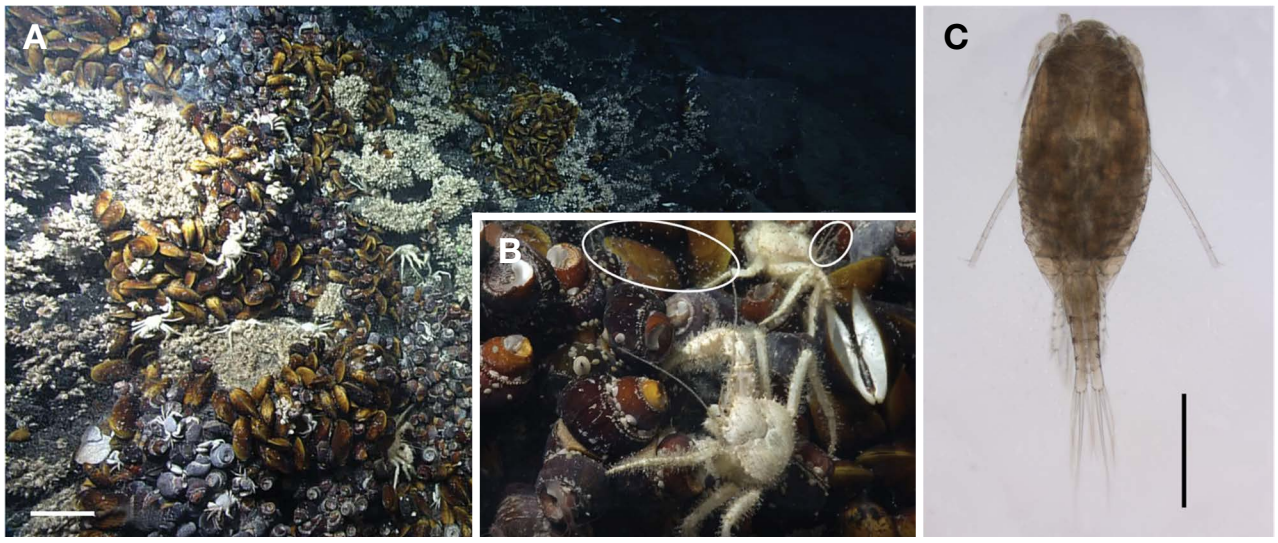

Fig. 1. Photographs of the hydrothermal diffusing vent (16°59′S, 173°54′E) in the North Fiji Basin in southwestern Pacific Ocean where *I. paucisetus* Fleminger, 1983 was collected. A, Overview of sampling site; B, Close-up of sampling site. White circles indicate the swarms of *I. paucisetus* found right above the vent; C, *I. paucisetus* was taken with a digital microscope camera (Olympus DP26, Japan) mounted on a microscope (Olympus BX53, Japan). Scale bars: A = 20 cm, C = 500 μm.

Table 1. Pairwise distances (p-distance) among the partial sequences of *mtCOI* gene from 10 spinocalanid species

Species (nos. of individuals, intraspecies variation [%])	Distances (%)									
	1	2	3	4	5	6	7	8	9	10
1. *Isaacsicalanus paucisetus* (11, 0.0)										
2. *Foxtonia barbatula* (2, 0.0)	20.0									
3. *Mimocalanus crassus* (3, 1.0)	20.1	15.1								
4. *Mimocalanus cultrifer* (3, 3.0)	17.9	17.7	**16.3**							
5. *Mimocalanus heronae* (3, 0.0)	19.0	17.4	**15.4**	**13.4**						
6. *Monacilla typica* (3, 0.0)	22.2	21.6	18.1	18.7	19.5					
7. *Mospicalanus* cf. *schielae* (3, 0.0)	20.7	20.9	19.9	17.8	20.0	19.0				
8. *Spinocalanus aspinosus* (3, 0.0)	23.9	24.6	23.1	23.2	24.5	22.3	21.4			
9. *Spinocalanus brevicaudatus* (3, 2.0)	25.9	21.9	23.0	20.5	21.4	22.0	20.0	**20.3**		
10. *Spinocalanus magnus* (3, 0.0)	22.5	17.9	19.2	18.5	19.5	19.1	16.8	**25.2**	**20.6**	

The bold letters indicate interspecies variation within the genera.
mtCOI, mitochondrial cytochrome oxidase I.
The accession numbers of spinocalanid species are *I. paucisetus*, MT093559–MT093569; *F. barbatula*, HQ150081, KC287644; *M. crassus*, KU247717–KU247719; *M. cultrifer*, KU247724–KU247726, *M. heronae*, KU247735–KU247737; *M. typica*, KU247696–KU247698; *M.* cf. *schielae*, KU247776–KU247778; *S. aspinosus*, KU247834–KU247836; *S. brevicaudatus*, KU247704–KU247706; *S. magnus*, KU247750–KU247752.

da) in December, 2016. They were morphologically identified under a dissecting microscope (Leica M125, Germany). For *mtCOI* DNA barcoding, genomic DNA extraction, sequencing, and nucleotide diversity analyses were carried out following the method of Baek et al. (2016).

RESULTS AND DISCUSSION

Copepod specimens from the North Fiji Basin were identified as *Isaacsicalanus paucisetus* (Fig. 1C) by the following morphological characteristics: prosome produced into acute point posteriorly, a short and lobate rostrum without bifurcation, basis of maxilliped armed with 2 setae, second exopodal segment of leg 1 without outer distal spine, leg 2 with 2-segmented endopod, and leg 3 with 3-segmented endopod (Boxshall and Halsey, 2004). In the present study, *I. paucisetus* was firstly reported from the hydrothermal vent in the southwestern Pacific Ocean.

The 11 new sequences of *mtCOI* DNA barcodes from the

North Fiji population of *I. paucisetus* are registered to GenBank (accession nos. MT093559–MT093569). All sequences were identical and 659 bp in length with overall G+C content of 38.4%. In spinocalanids, the range of intraspecies variation was 0.0–3.0%, which the maximum variation appeared in *Mimocalanus cultrifer* Farran, 1908 (Table 1). Interspecific and intergeneric variations were 13.4–25.2% and 16.7–24.1%, respectively. All values corresponded well to those of previous studies (Bucklin et al., 2003; Bode et al., 2017).

Based on the morphological characteristics, we recognized no difference between two allopatric populations of *I. paucisetus*, the type specimens found in the eastern Pacific Ocean vs. ours collected in the southwestern Pacific Ocean (Fleminger, 1983; Boxshall and Halsey, 2004). However, we could not compare *mtCOI* DNA barcodes between two populations because sequences of the eastern Pacific population were not available from open-access sequence databases.

According to the previous study based on DNA barcoding, it is assumed that there are more sibling and cryptic species existing in spinocalanid species than expected (Bode et al., 2017). To assign the taxonomic status of allopatric populations of *I. paucisetus*, further research should be considered, including morphological revision, biogeographic analysis of additional populations, and life-history attributes, as well as genome-based population studies.

ORCID

Chailinn Park: https://orcid.org/0000-0003-2477-5129
Won-Kyung Lee: https://orcid.org/0000-0001-7283-298X
Se-Joo Kim: https://orcid.org/0000-0003-1653-072X
Se-Jong Ju: https://orcid.org/0000-0003-3479-6652

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

The authors thank two anonymous reviewers for their helpful comments and suggestions. This work was a part of the R&D project 'Exploration for Seafloor Hydrothermal Deposits and Fe-Mn Crusts in the Southwestern Pacific and the Indian Ocean (19992001)' funded by the Ministry of Oceans and Fisheries, Korea and the Korea Research Institute of Bioscience and Biotechnology (KRIBB) Research Initiative Program.

REFERENCES

Baek SY, Jang KH, Choi EH, Ryu SH, Kim SK, Lee JH, Lim YJ, Lee J, Jun J, Kwak M, Lee YS, Hwang JS, Maran BAV, Chang CY, Kim IH, Hwang UW, 2016. DNA barcoding of metazoan zooplankton copepods from South Korea. PLoS One, 11:e0157307. https://doi.org/10.1371/journal.pone.0157307

Blanco-Bercial L, Cornils A, Copley N, Bucklin A, 2014. DNA barcoding of marine copepods: assessment of analytical approaches to species identification. PLoS Currents Tree of Life, 6. https://doi.org/10.1371/currents.tol.cdf8b74881f87e3b01d56b43791626d2

Bode M, Laakmann S, Kaiser P, Hagen W, Auel H, Cornils A, 2017. Unravelling diversity of deep-sea copepods using integrated morphological and molecular techniques. Journal of Plankton Research, 39:600-617. https://doi.org/10.1093/plankt/fbx031

Boxshall GA, Halsey SH, 2004. An introduction to copepod diversity. The Ray Society, London, pp. 1-966.

Bucklin A, Frost B, Bradford-Grieve J, Allen L, Copley N, 2003. Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. Marine Biology, 142:333-343. https://doi.org/10.1007/s00227-002-0943-1

Bucklin A, Guarnieri M, Hill RS, Bentley AM, Kaartvedt S, 1999. Taxonomic and systematic assessment of planktonic copepods using mitochondrial COI sequence variation and competitive, species-specific PCR. Hydrobiologia, 401:239-254. https://doi.org/10.1007/978-94-011-4201-4_18

Fleminger A, 1983. Description and phylogeny of *Isaacsicalanus paucisetus*, n. gen., n. sp., (Copepoda: Calanoida: Spinocalanidae) from an East Pacific hydrothermal vent site (21°N). Proceedings of the Biological Society of Washington, 96:605-622.

Ivanenko VN, Defaye D, Cuoc C, 2007. A new calanoid copepod (Spinocalanidae) swarming at a cold seep site on the Gabon continental margin (Southeast Atlantic). Cahiers de Biologie Marine, 48:37-54.

Walter TC, Boxshall G, 2020. World of Copepods database. Spinocalanidae (Vervoort, 1951). Accessed 13 Apr 2020, <http://www.marinespecies.org/aphia.php?p=taxdetails&id=104104>.

Received March 10, 2020
Revised April 14, 2020
Accepted April 14, 2020