

# DNA Barcoding for the Hydrothermal Vent Crab *Austinograea* Species (Crustacea: Bythograeidae) from the North Fiji Basin, Southwestern Pacific Ocean

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## ABSTRACT

The brachyuran crab Bythograeidae Williams, 1980 is common in hydrothermal vent fields worldwide and has recorded to sixteen species of six genera. In this study, we firstly determined the cytochrome *c* oxidase subunit 1 (*COI*) DNA barcodes for the fifth species of *Austinograea*, *A. hourdezi*, from hydrothermal vent regions of the North Fiji Basin in southwestern Pacific Ocean. All *COI* DNA barcodes of *A. hourdezi* were identical. The interspecies variations of three bythograeid genera were 10.9–13.3% for *Austinograea*, 6.6–15.7% for *Bythograea*, and 9.7% for *Gandalfus*. These results would be helpful to understand taxonomy of brachyuran crabs living in hydrothermal vent fields using *COI* DNA barcodes.

**Keywords:** Bythograeidae, *Austinograea hourdezi*, mitochondrial gene, *COI* DNA barcoding, 16S rRNA

## INTRODUCTION

The brachyuran crab Bythograeidae Williams, 1980 is common in hydrothermal vent fields worldwide and has recorded to sixteen species of six genera (Guinot and Segonzac, 2018). For DNA-based species identification, it is generally considered a suitable DNA barcode when sequence variation among species is 10 times greater than those within species (Hebert et al., 2003; Matzen da Silva et al., 2011). Based on these criteria, the mitochondrial cytochrome *c* oxidase subunit 1 (*COI*) DNA barcodes for bythograeid crabs were confirmed worth supporting the traditional taxonomy and utility recognizing the existence of sibling (or cryptic) species (Mateos et al., 2012; Kim et al., 2013; Guinot and Segonzac, 2018).

*Austinograea hourdezi* Guinot & Segonzac, 2018 is found across wide hydrothermal vent areas in the southwestern Pacific Ocean (Guinot, 1989; Guinot and Segonzac, 2018) (Fig. 1A). It is distinguished from its relatives with spots on outer surface of chelae in males, patches of setae on inner surface

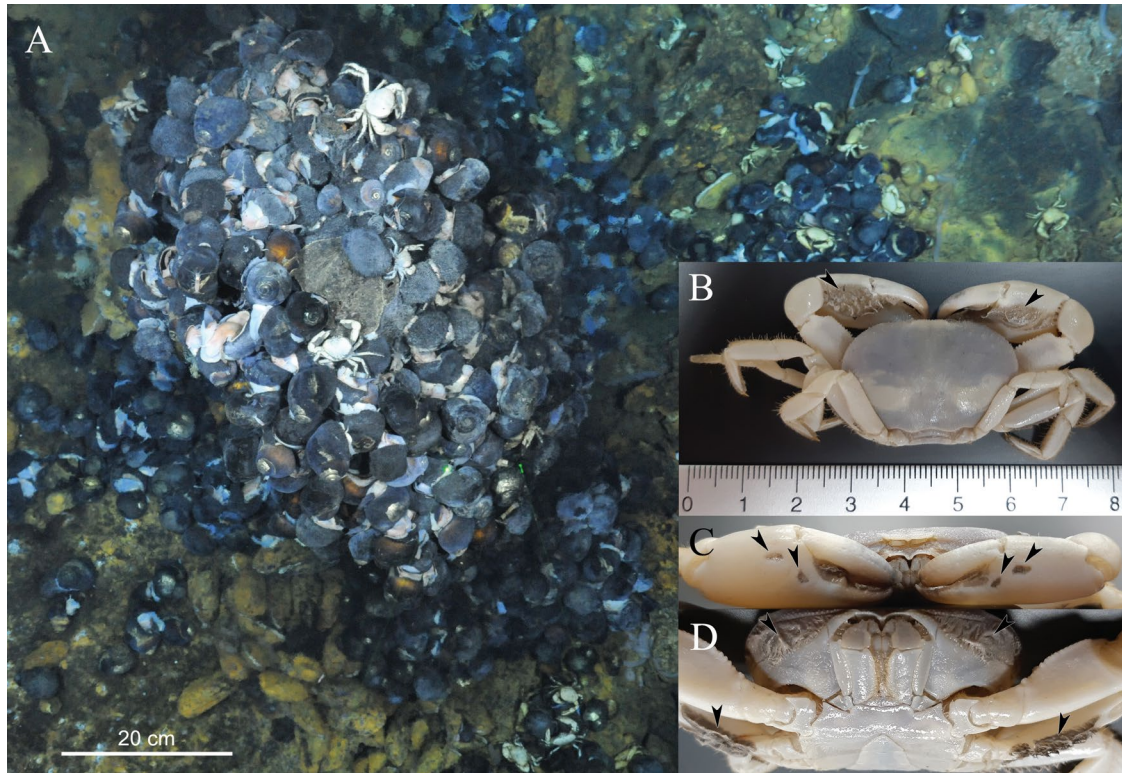
of chelae, subhepatic region and meri, and ratio between the 1st and 2nd pair of gonopods (Guinot and Segonzac, 2018) (Fig. 1B–D). In this study, we determined firstly the *COI* DNA barcodes of *A. hourdezi* collected from the hydrothermal vents of the North Fiji Basin in southwestern Pacific Ocean. Additionally, we obtained mitochondrial 16S rDNA sequences to compare the 16S rDNA sequence of *A. hourdezi* retrieved from GenBank.

## RESULTS AND DISCUSSION

In November 2016, six *A. hourdezi* individuals were collected from three hydrothermal vent regions (1 ind. from 18°49'S, 173°30'E, 2,719 m depth; 3 inds. from 18°51'S, 173°30'E, 2,721 m depth; 2 inds. from 19°03'S, 173°29'E, 2,717 m depth) (Fig. 1A) in the North Fiji Basin. Morphological identification, genomic DNA extraction, sequencing, sequence annotation, and nucleotide diversity analyses followed the methods of Guinot and Segonzac (2018) and Kim

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**Fig. 1.** Photograph of the hydrothermal vent chimney in the North Fiji Basin of the Southwestern Pacific Ocean where *Austinograea hourdezi* Guinot & Segonzac, 2018 was collected. A, Overview of sampling site and the surrounding community of species including bythograeid crabs *Austinograea* spp., alvinocaridid shrimp species, provannid snails *Alviniconcha bouchetti* Johnson et al., 2015 and *A. kojimai* Johnson et al., 2015, mytilid mussel *Bathymodiolus* sp., chiridotid sea cucumber *Chiridota* sp.; B–D, Diagnostic keys of *A. hourdezi* based on Guinot and Segonzac (2018). Arrowheads indicate patches of setae on inner surface of chelae (B), spots on outer surface of chelae in males (C), and patches of setae on subhepatic region and meri (D). Scale bar=20 cm.

**Table 1.** Sequence divergence among the partial sequences of mitochondrial 16S rDNA and cytochrome c oxidase subunit 1 (CO1) genes from Bythograeidae. Nucleotide sequence variations (%) were calculated using the p-distance method in Mega 10.0.5. Upper right is for 16S and lower left for CO1. The bold letters indicate interspecies variation within the genera *Austinograea*, *Bythograea* and *Gandalfus*

	1 (7 <sup>a</sup> , 0.22 <sup>b</sup> )	2	3	4	5	6	7	8	9	10	11	12	13	14
1. <i>Austinograea hourdezi</i> (6 <sup>a</sup> , 0.00 <sup>b</sup> )	–	<b>7.4</b>	<b>6.1</b>	<b>6.3</b>	10.4	14.9	15.1	14.4	14.5	15.3	9.3	9.8	9.9	13.2
2. <i>Austinograea alayseae</i>	<b>10.9</b>	–	<b>5.6</b>	<b>5.8</b>	12.2	15.6	14.4	15.1	14.1	15.1	10.2	9.0	9.4	13.6
3. <i>Austinograea rodriguezensis</i>	<b>11.7</b>	<b>11.1</b>	–	<b>4.5</b>	10.0	13.1	13.1	13.6	13.0	13.8	7.3	7.3	7.4	11.6
4. <i>Austinograea williamsi</i>	<b>11.1</b>	<b>10.3</b>	<b>13.3</b>	–	10.0	13.3	13.3	13.0	13.2	14.0	8.6	8.6	8.1	12.9
5. <i>Allograea tomentosa</i>	15.9	15.7	13.3	15.9	–	13.0	13.0	13.5	13.7	13.2	7.3	9.6	8.8	11.6
6. <i>Bythograea galapagensis</i>	17.7	15.7	16.5	17.1	15.1	–	<b>7.3</b>	<b>5.7</b>	<b>4.7</b>	<b>7.1</b>	11.3	12.2	11.9	12.4
7. <i>Bythograea laubieri</i>	16.3	15.3	15.5	16.1	14.9	<b>13.9</b>	–	<b>7.1</b>	<b>8.8</b>	<b>0.7</b>	11.7	11.7	12.1	13.0
8. <i>Bythograea microps</i>	17.5	18.5	18.3	18.7	17.9	<b>15.5</b>	<b>15.7</b>	–	<b>8.6</b>	<b>7.3</b>	11.9	13.8	12.6	13.5
9. <i>Bythograea thermydron</i>	19.3	17.3	19.7	17.9	18.3	<b>9.3</b>	<b>14.1</b>	<b>15.1</b>	–	<b>9.2</b>	12.1	11.3	11.3	12.5
10. <i>Bythograea vrijenhoeki</i>	16.1	15.7	16.5	16.3	15.7	<b>15.1</b>	<b>6.6</b>	<b>15.1</b>	<b>14.5</b>	–	11.9	12.0	12.3	13.2
11. <i>Cyanograea praedator</i>	17.3	17.3	15.5	18.3	12.3	14.1	15.9	18.1	15.5	16.7	–	9.6	8.8	9.1
12. <i>Gandalfus puia</i>	13.1	14.1	14.7	14.9	12.7	16.7	14.1	16.3	16.7	13.9	14.5	–	<b>5.2</b>	9.8
13. <i>Gandalfus yunohana</i>	15.9	14.9	15.7	14.3	13.5	16.7	16.1	18.3	17.9	16.3	15.9	<b>9.7</b>	–	9.2
14. <i>Segonzacia mesatlantica</i>	16.5	15.9	15.1	15.9	14.3	15.5	14.9	15.7	18.5	15.5	13.5	14.3	14.9	–

The accession No. of bythograeid crabs are *A. hourdezi*, JQ407451, MK382751–MK382753, MK359095–MK359097, MK359098–MK359103; *A. alayseae*, KC851803 (mitogenome); *A. rodriguezensis*, JQ407452 and JQ407476; *A. williamsi*, JQ407448 and JQ407474; *A. tomentosa*, JQ407446 and JQ407472; *B. galapagensis*, JQ407444 and JQ407470; *B. laubieri*, JQ407442 and JQ407468; *B. microps*, JQ407441 and JQ407467; *B. thermydron*, JQ407440 and JQ407466; *B. vrijenhoeki*, JQ407443 and JQ407469; *C. praedator*, JQ407445 and JQ407471; *G. puia*, NC\_027414 (mitogenome); *G. yunohana*, NC\_013713 (mitogenome); *S. mesatlantica*, JQ407447 and JQ407473.

<sup>a</sup>Number of individuals.

<sup>b</sup>Intraspecies variation (%).

et al. (2013). All specimens and genomic DNA extracts of *A. hourdezi* are deposited in Korea Research Institute of Bioscience and Biotechnology.

The newly obtained sequences for *A. hourdezi* were registered to GenBank (accession No. MK382751–MK382753, MK359095–MK359097 for 16S rDNA; MK359098–MK359103 for *COI*) (Table 1). The lengths of alignments were 446 bp for 16S rDNA and 503 bp for *COI*. All *COI* sequences were identical, while 16S rDNA sequences detected a variable nucleotide site in the single individual (MK382752). Interspecies variations of 16S rDNA and *COI* within the genus *Austinograea* were 4.5–7.4% and 10.3–13.3%, respectively. In the case of the genus *Bythograea*, interspecies variations were 0.7–9.2% for 16S rDNA and 6.6–15.7% for *COI*. Two *Gandalfus* species showed the variations of 5.2% for 16S rDNA and 9.7% for *COI*. The sequence diversity of *COI* was two times more than that of 16S rDNA. Meanwhile, the sequence variation of 16S rDNA between *B. laubieri* and *B. vrijenhoeki* was only 0.7%, which is considered to represent the most recently derived lineages or cryptic species.

Recently, *A. aff. williamsi* collected in the southwestern Pacific Ocean was revised as the fifth species of *Austinograea*, *A. hourdezi*, by Guinot and Segonzac (2018). As of 8 Jan. 2019, GenBank contains the sequences of mitochondrial 16S rDNA and cytochrome b, and nuclear 28S rDNA from one single individual of *A. aff. williamsi* (JQ407451, JQ407421, and JQ407435), but no sequence for *A. hourdezi*. These results would be helpful to understand taxonomy of brachyuran crabs living in hydrothermal vent fields using *COI* DNA barcodes.

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## REFERENCES

- Guinot D, 1989. *Austinograea alayseae* sp. nov., Crabe hydrothermal découvert dans le bassin de Lau, Pacifique sud occidental (Crustacea: Decapoda: Brachyura). Bulletin du Muséum National d’Histoire Naturelle, 11:879-903.
- Guinot D, Segonzac M, 2018. A review of the brachyuran deep-sea vent community of the western Pacific, with two new species of *Austinograea* Hessler & Martin, 1989 (Crustacea, Decapoda, Brachyura, Bythograeidae) from the Lau and North Fiji Back-Arc Basins. Zoosystema, 40:75-107.
- Hebert PDN, Cywinska A, Ball SL, deWaard JR, 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270:313-321. <https://doi.org/10.1098/rspb.2002.2218>
- Kim SJ, Lee KY, Ju SJ, 2013. Nuclear mitochondrial pseudogenes in *Austinograea alayseae* hydrothermal vent crabs (Crustacea: Bythograeidae): effects on DNA barcoding. Molecular Ecology Resources, 13:781-787. <https://doi.org/10.1111/1755-0998.12119>
- Mateos M, Hurtado LA, Santamaria CA, Leignel V, Guinot D, 2012. Molecular systematics of the deep-sea hydrothermal vent endemic brachyuran family Bythograeidae: a comparison of three Bayesian species tree methods. PLoS ONE, 7:e32066. <https://doi.org/10.1371/journal.pone.0032066>
- Matzen da Silva J, Creer S, dos Santos A, Costa AC, Cunha MR, Costa FO, Carvalho GR, 2011. Systematic and evolutionary insights derived from mtDNA *COI* barcode diversity in the Decapoda (Crustacea: Malacostraca). PLoS ONE, 6:e19449. <https://doi.org/10.1371/journal.pone.0019449>

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