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Genomic survey sequencing and complete mitochondrial genome of the elkhorn coral crab *Domecia acanthophora* (Desbonne in Desbonne & Schramm, 1867) (Decapoda: Brachyura: Domeciidae)

Henrique Bravo^{1,}, J. Antonio Baeza^{2,3,4} and Sancia E.T. van der Meij^{1,5,}

¹Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen, The Netherlands

²Department of Biological Sciences, Clemson University, Clemson, SC 29634, USA

³Smithsonian Marine Station at Fort Pierce, Florida, USA

⁴Universidad Católica del Norte, Coquimbo, Chile

⁵Marine Biodiversity Group, Naturalis Biodiversity Center, Leiden, The Netherlands

Correspondence: H. Bravo; e-mail: h.bravo.go@gmail.com

ABSTRACT

The elkhorn coral crab *Domecia acanthophora* inhabits shallow-water coral reefs in the Western Atlantic. The species has a wide distribution and, although primarily associated with endangered *Acropora* corals, has been recorded from a myriad of hosts. Here we conducted the first genomic survey and complete mitochondrial assemblage and characterisation of any species of Domeciidae, as well as the first species within Trapezioidea. The estimated size of the nuclear genome ranged from 0.64 Gbp to 1.76 Gbp, revealing a small genome. Repetitive elements of the genome were estimated here at 66.4% and 74%, respectively, with the majority of the repetitive elements consisting of LINE, LTR, and satellite DNA. The assembled A-T rich mitochondrial genome consisted of 15,568 bp in length, with 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes and two ribosomal RNA (rRNA) genes. A 619 bp long non-coding region was identified as the supposed D-loop/control region, containing eight microsatellites. The 22 tRNA genes, ranging from 65 to 71 bp in length, displayed a typical "cloverleaf" secondary structure, with the exception of *tRNA-Ser1* which lacked part of the DHU arm and *tRNA-Asp* displayed a deletion of the TYC loop but not the arm. Two transposition events of two tRNA genes were also found when comparing the gene order of *D. acanthophora* to that of the brachyuran basic gene order, which had not been reported before. Despite belonging to a widely distributed, well-known superfamily of coral-associated crabs, the Trapezioidea, very little was known about this species from a genetics perspective, which is remedied here by providing a new genomic resource for *D. acanthophora*.

KEY WORDS: Crustacea, gene order, genome skimming, mitogenome, Trapezioidea

INTRODUCTION

The family Domeciidae (superfamily Trapezioidea) is composed of coral-associated crabs with a circumtropical distribution. This family consists of seven recent species across four genera, and a further five fossil species (Castro *et al.*, 2004; DecaNet, 2023). Domeciids live in symbiosis with soft and stony corals, but despite their wide distribution on coral reefs, relatively little is known about these crabs (Patton, 1967; Lai *et al.*, 2009; Castro, 2015).

Fossil species of *Cherusius* Low & Ng, 2012 (= *Jonesius* Sankarankutty, 1962) have been retrieved from coral-rich rocks, extending the geological record of Domeciidae from the Oligocene to the Recent (Schweitzer, 2005). This implies that members of the family had already adapted to living in association with corals in the Oligocene (33.9–23 mya). The nature of their relationship with corals is unresolved, with the various domeciids having been described as "commensals", "obligate associates", or "facultative or obligate symbionts", highlighting the uncertainty of the true nature of the associations (Castro, 2015). Domeciids have also been observed on dead corals, and on non-coral substrates (i.e., sponges and hydrozoans) (Castro, 2015; van der Meij *et al.*, 2022). Based on the morphology of the mouthparts and stomach contents, Patton (1967) suggested that *Domecia acanthophora* (Desbonne in Desbonne & Schramm, 1867) might feed on food taken from the water column and possibly coral mucus. The

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species, however, lacks feeding setae on their ambulatory legs, found in most other trapezioids and adapted for coral-mucus feeding (Castro, 2015). Heterochely, differences in the size and shape of chelae, is observed among domeciids (Castro *et al.*, 2004), but it is unclear if this is related to feeding and/or predator activities.

The Atlantic representatives of Domeciidae include Domecia acanthophora and D. africana Holthuis, Edwards & Lubbock, 1980; however, there are some doubts about the status of the latter species (van der Meij et al., 2022). Domecia acanthophora has a Western Atlantic distribution, and predominantly associates with Acropora Oken, 1815 corals, and to a lesser extent with calcified hydrozoans belonging to Millepora Linnaeus, 1758. Moreover, there are various records of *D. acanthophora* associated with other corals and even sponges (Patton, 1967; van der Meij et al., 2022). The critically endangered coral A. palmata (Lamarck, 1816) appears to be particularly important for ovigerous females (Grajal & Laughlin, 1984). Domecia acanthophora is so far the only species of Domeciidae known to induce dwellings in some of its hosts (Castro, 1976), particularly in Acropora and Millepora, where structural deformations in the shape of crevices, folds or spaces between branches can be easily observed (Patton, 1967; van der Meij et al., 2022). The Indo-West Pacific D. glabra Alcock, 1899 and D. hispida Eydoux & Souleyet, 1842 inhabit Acroporidae and Pocilloporidae corals, but in contrast to D. acanthophora, they reside between the branches of their coral host, where they occur in "pairing mates" (Barry, 1965; Garth, 1984).

Given their specialised life history as obligate coral-dwelling crabs and the sparse amount of (genetic) information available for domeciid crabs, we thought it opportune to increase the amount of genetic information available for the Western Atlantic *D. acanthophora.* We aimed to assemble and characterise in detail the mitochondrial genome of *D. acanthophora*, and provide genomic resources: a genome size estimation and an inquiry into the repetitive elements found in the genome of this species.

Mitochondrial genomes, given their low rate of recombination, fast substitution rate. and maternal inheritance (Brown *et al.*, 1979), have the potential to shed light on the evolutionary history of species and the phylogenetic relationships between groups of closely related species (Tan *et al.*, 2019). There has been some debate around the monophyly of families within Trapezioidea (Lai *et al.*, 2009), so genetic resources such as the one presented here, when complemented with similar resources for other species, could help validate (or resolve) some of the findings regarding the phylogenetic relationships amongst trapezioid crabs (Castro *et al.*, 2004) and their co-evolutionary history with their coral hosts (Schweitzer, 2005), for example.

The remainder of the genomic resources presented in this study, whilst they can also be used towards a greater understanding of the evolution of the species and Domeciidae, will help gain more knowledge regarding the biology of the species and consequently, towards its conservation.

MATERIAL & METHODS

Sampling and sequencing

A specimen of *D. acanthophora* was collected from *Millepora alcicornis* Linnaeus, 1758 at Anse à Jacques reef, Guadeloupe (French Lesser Antilles) (16°12′29.4″N, 61°25′22.1″W) on 26 May 2021, and preserved in 70% ethanol. The collected

specimen was sent in 70% ethanol to BGI (BGI Tech Solutions, Hong Kong) for DNA extraction following standard manufacturer's protocol and sequenced in a DNBseq system (MGI, Hong Kong) using a short-insert library in a 2×150 cycle. A total of 115,489,693 pairs of paired-end (PE) reads were generated and used in the subsequent analyses (accession number of raw reads on NCBI's Sequence Read Archive (SRA) SRR25583094).

Genome size estimation

A quality control step was done with fastp (v.0.23.2) (Chen *et al.*, 2018) using the default parameters to remove low-quality sequences (Phred scores < 20), leaving a total of 230,979,370 high-quality PE reads. A further cleaning step was performed with Kraken2 (Wood *et al.*, 2019) using the Standard database in order to remove contaminants, leaving 214,212,636 PE reads that were free from archaea, bacteria, plasmid, viral, and human reads. These reads were then used for the estimation of genome size by counting k-mers (N = 21) with Jellyfish (Marçais & Kingsford, 2011) using a hash size of 10 billion and a k-mer frequency of 100,000. All other parameters were run with default settings. The generated histogram was then used to process the k-mer frequency distribution with GenomeScope (v.2.0) (Ranallo-Benavidez *et al.*, 2020) and RESPECT (v.1.0) (Sarmashghi *et al.*, 2021) in order to estimate genome size.

Repetitive elements in the nuclear genome

We identified, characterised, and quantified repetitive elements in the genome of *D. acanthophora* using RepeatExplorer (v.2.3.8.1) (Novák *et al.*, 2020). After an initial assessment of the extent of overlap between PE reads, we found a large proportion (60%) of reads that overlapped. Following the protocol of Baeza *et al.* (2022a), we discarded the overlapping reads and ran the remainder of the pipeline with non-overlapping reads (N = 85,049,506PE reads) in order to have a higher efficiency in the discovery and retrieval of repetitive elements. Given the high number of reads, RepeatExplorer capped the maximum number of non-overlapping PE reads for the clustering analysis at (N = 2,350,546), but all other parameters were run with default values.

Mitochondrial genome assembly and annotation

We assembled the mitochondrial genome *de novo* using GetOrganelle (v.1.7.6.1) (Jin *et al.*, 2020) and NOVOPlasty (v.4.3.1.) (Dierckxsens *et al.*, 2017) for double validation. The assemblies used a seed of the cox1 gene of *D. acanthophora* retrieved from GenBank (OM669766).

The assembled genome was annotated using MITOS (Bernt *et al.*, 2013) and MITOS2 (Donath *et al.*, 2019) for cross-validation. Start and stop codon corrections were done manually, with the use of MEGA X (Kumar *et al.*, 2018).

Mitochondrial genome characterisation

Nucleotide composition of the entire mitochondrial genome was estimated with MEGA X. Codon usage, after the aforementioned manual curation, was estimated with the Sequence Manipulation Suite (SMS) using the mitochondrial invertebrate settings (Stothard, 2000). Relative synonymous codon usage (RSCU) and amino acid composition of the concatenated protein-coding genes were estimated and visualised using the EZcodon tool (Cucini *et al.*, 2021). The tRNA genes were identified with MiTFi (Jühling *et al.*, 2012), implemented within the MITOS web server. The secondary structures of each individual tRNA gene were visualised with Forna (Kerpedjiev *et al.*, 2015).

The non-coding region identified as the supposed D-loop/ control region was analysed in detail, with the calculation of nucleotide composition, the detection of microsatellite sequences using the Microsatellite Repeats Finder web server (Bikandi *et al.*, 2004) and detection of tandem repeat sequences (Benson, 1999). The prediction of the lowest free energy secondary structure of the control region using the RNAfold Secondary Structure web server (Lorenz *et al.*, 2011) was also estimated.

Mitochondrial gene order analysis

The Mitochondrial Gene Order (MGO) analysis of the mitochondrial genome of our study was compared to that of the brachyuran basic (mitochondrial) gene order, which is the most common gene order within Brachyura (Basso *et al.*, 2017; Wang *et al.*, 2021). Given the lack of mitochondrial genomes of other Trapezioidea, no further comparisons were conducted. The analysis was performed with CREx (Bernt *et al.*, 2007).

RESULTS

Genome size estimation

The estimated haploid genome size of *D. acanthophora* using a k-mer approach ranged from 637,752,110 bp (0.64 Gbp, with GenomeScope) to 1,762,907,104 bp (1.76 Gbp, with RESPECT). The estimated unique genome content was, however, relatively consistent in both methods, ranging from 33.6% with GenomeScope to 26% with RESPECT, indicating a high percentage of repetitive elements in the genome of *D. acanthophora*.

Repetitive elements in the nuclear genome

The analysis of repetitive elements in the genome of *D. acanthophora* revealed a total of 1,557,397 reads (out of the 2,350,546 that were analysed) that were contained in 154,830 clusters. Out of these clusters, 321 were identified as top clusters (i.e., a cluster than contains 0.01% or more of the total reads, or > 235 reads in this case), encompassing 45% of all reads (i.e., N = 1,063,454).

A large proportion of reads in these top clusters (56%, N =596,412) were, however, reported as unclassified, with only 134 top clusters being annotated by RepeatExplorer. The most abundant repetitive elements that were identified during the analysis were: Class I: long interspersed nuclear elements (LINE; N = 78 clusters, 306,699 reads), Class I: long tandem repeats (LTR) Ty3-gypsy retrotransposons (N = 21 clusters, 44,448 reads), Class I: LTR Bel-Pao retrotransposons (N = 5 clusters, 9,972 reads), Class I: DIRS (N = 7 clusters, 10,582 reads), unclassified Class I retrotransposons (N = 3 clusters, 4,651 reads) and Satellite DNA (N = 10 clusters, 65,758 reads). Class I: LTR transposons (795 reads), Class I: LTR Ty1 copia transposons (1810 reads), Class I: Penelope retrotransposons (625 reads) and Class II: Subclass II Helitron (1053 reads) were all classified with a single cluster each. There were also four clusters identified as 45S rDNA, (16,815 reads) and two clusters (3,834 reads) were classified as mitochondrial DNA (Fig. 1).

Mitochondrial genome

Both *de novo* assemblies generated an identical circular sequence with 15,568 bp (coverage of 1530× for NOVOPlasty and 415× for GetOrganelle; GenBank accession number OR253028). The mitochondrial genome encodes for 37 genes altogether, with 22 transfer RNA genes (tRNA), 13 protein-coding genes (PCGs), and two ribosomal RNA genes (rRNA). We also identified a non-coding region of 619 bp that is assumed to correspond to the D-loop/control region.

The majority of the *D. acanthophora* genes were encoded on the heavy strand, with only four PCGs (in order from 5' to 3': *nad5, nad4, nad4l,* and *nad1*), seven tRNA genes (*tRNA-His, tRNA-Phe, tRNA-Pro, tRNA-Val, tRNA-Gln, tRNA-Cys,* and *tRNA-Tyr*) and the two rRNA genes encoded on the light strand (Fig. 2, Table 1).

The nucleotide composition of the entire mitochondrial genome of *D. acanthophora* revealed an overall A+T bias (70.3%; base composition: A = 31.7%, T = 38.6%, C = 18.0%, and G = 11.7%).

There were a total of 11,121 codons present in the PCGs of *D. acanthophora* (Supplementary material Fig. S1). ATG was the start codon most widely used (five PCGs: *cox1, cox2, cox3, nad4l, cob*), followed by ATT (three PCGs: *atp6*, atp8, nad6), then ATA



Figure 1. Repetitive elements in the genome of *Domecia acanthophora*. Each bar corresponds to a different type of annotated cluster. Numbers between parentheses in the legend represent the total number of annotated clusters in that category.

(two PCGs: *nad4*, *nad1*), and finally ATC (*nad3*), GTG (*nad5*), and TTG (*nad2*), all with a single occurrence. TAA was the most frequently used stop codon occurring on seven PCGs, followed by TAG with one occurrence (*nad2*) and five PCGs displayed a truncated stop codon T (*cox1*, *cox2*, *cox3*, *nad3*, and *cob*; Table 1). The most commonly used codons were TTA (Leu) used 293 times (51%), TTT (Phe) used 274 times (79%) and ATT (Ile) used 254 times (86%). The least used codons, with the exception of stop codons, were CGC and CGG (both Arg) used one (2%) and four times (7%) respectively; and GCG (Ala) used five times (3%).

The majority of tRNA genes display the typical "cloverleaf" structure, with the exception of *tRNA-Ser1*, which lacks part of the dihydrouridine (DHU) arm, whilst *tRNA-Asp* displays a deletion of the thymine pseudouracil cytosine (T Ψ C) loop (Fig. 3).

Both rRNA genes of *D. acanthophora* are located in the light strand. The *16S* rRNA gene is 1317 bp long, located between *tRNA-Val* and *12S*, and the *12S* gene is 823 bp long and located between *16S* and the control region (CR). The AT-content for *16S* is 75% and 73.1% for *12S*.

The 619 bp long region assumed to be the CR is located between the *12S* rRNA and the *tRNA-Ile* genes, starting at position 13,519 and ending at position 14,137. This region was AT rich with the following base composition: A = 34.7%, T = 38.1%, C = 16.2%, and G = 11%. Eight microsatellites were found in the CR and they consisted of multiple AT, AA, TA, TC, and TT dinucleotides and one AAA trinucleotide (Supplementary material Table S2). No tandem repeats were found within the CR. The RNAfold tool calculated 20 possible secondary structures (Gibbs free energy (Δ G) ranged from -77.0 to -76.1 kcal mol⁻¹), with all of them revealing the presence of hairpin structures along most of this region.



Figure 2. Visualisation of assembled mitochondrial genome of Domecia acanthophora. Photo by Yun Scholten.

Name	Туре	Start	Stop	Strand	Length	Start	Stop	Anticodon	ovl/nc
cox1	PCG	1	1534	+	1534	ATG	T(AA)		20
tRNA-L1 (Leu1)	tRNA	1555	1619	+	65			CTA	12
tRNA-L2 (Leu2)	tRNA	1632	1697	+	66			TTA	7
cox2	PCG	1705	2389	+	685	ATG	T(AA)		0
tRNA-K (Lys)	tRNA	2390	2455	+	66			AAA	18
tRNA-D (Asp)	tRNA	2474	2539	+	66			GAC	0
atp8	PCG	2540	2698	+	159	ATT	TAA		-7
atp6	PCG	2692	3360	+	669	ATT	TAA		3
cox3	PCG	3364	4153	+	790	ATG	T(AA)		0
tRNA-G (Gly)	tRNA	4154	4219	+	66			GGA	0
nad3	PCG	4220	4568	+	349	ATC	T(AA)		3
tRNA-A (Ala)	tRNA	4572	4637	+	66			GCA	-1
tRNA-R (Arg)	tRNA	4637	4704	+	68			CGA	2
tRNA-N (Asn)	tRNA	4707	4776	+	70			AAC	-1
tRNA-S1 (Ser1)	tRNA	4776	4844	+	69			AGA	0
tRNA-E (Glu)	tRNA	4845	4911	+	67			GAA	-2
tRNA-H (His)	tRNA	4910	4976	-	67			CAC	5
tRNA-F (Phe)	tRNA	4982	5048	-	67			TTC	5
nad5	PCG	5054	6781	-	1728	GTG	TAA		38
nad4	PCG	6820	8127	-	1308	ATA	TAA		18
nad4l	PCG	8148	8447	-	300	ATG	TAA		1
tRNA-T (Thr)	tRNA	8449	8513	+	65			ACA	0
tRNA-P (Pro)	tRNA	8514	8580	-	67			CCA	2
nad6	PCG	8583	9089	+	507	ATT	TAA		-1
cob	PCG	9089	10223	+	1135	ATG	T(AA)		7
tRNA-S2 (Ser2)	tRNA	10231	10299	+	69			TCA	34
nad1	PCG	10334	11266	-	933	ATA	TAA		43
tRNA-V (Val)	tRNA	11310	11380	-	71			GTA	-2
rrnL	rRNA	11379	12695	-	1317				0
rrnS	rRNA	12696	13518	-	823				0
CR/D-loop		13519	14137		619				0
tRNA-I (Ile)	tRNA	14138	14208	+	71			ATC	2
tRNA-Q (Gln)	tRNA	14211	14279	-	69			CAA	19
tRNA-M (Met)	tRNA	14299	14365	+	67			ATG	0
nad2	PCG	14366	15379	+	1014	TTG	TAG		-2
tRNA-W (Trp)	tRNA	15378	15443	+	66			TGA	-8
tRNA-C (Cys)	tRNA	15436	15500	-	65			TGC	0
tRNA-Y (Tyr)	tRNA	15501	15568	-	68			TAC	0

Table 1. Arrangement and annotation of the mitochondrial genome of *Domecia acanthophora*. Abbreviations: ovl, overlaps; nc, non-coding (i.e., intergenic spacers); "+", heavy strand; "-", light strand.

The MGO analysis also revealed the transposition of the tRNA genes *Leu1* (L1) and *Val* (V), when compared to the brachyuran basic gene order (Fig. 4).

DISCUSSION

Crustaceans have one of the most variable genome sizes when compared to other phyla (Jeffery & Gregory, 2014). This is hypothesised to be related to the eco-physiological and life-history traits of the species in question. The genome size of *D. acanthophora* that we calculated, despite the wide range between 0.64 Gbp and 1.76 Gbp, is on the small end of the spectrum, but in line with that of many other families in Brachyura, such as Cancridae, Gecarcinidae, Leucosiidae, Menippidae, and Plagusiidae, which all have small genomes (i.e., < 2 Gbp). Smaller genomes in particular appear to be associated with species that have an indirect or extended development, live in shallow waters or have a certain degree of air-breathing mechanisms (Iannucci *et al.*, 2022). This is in agreement with the life-history of *D. acanthophora*, which lives in shallow-water environments and has an indirect development (Patton, 1967; Alves *et al.*, 2016).

The supposed repetitiveness of the nuclear genome, estimated here at 66.4% and 74%, is higher than the few studies that have assembled complete genomes of other crab species (Zhao *et al.*, 2019, 2021; Tang *et al.*, 2020; Cui *et al.*, 2021; Lv *et al.*, 2022). Given the small number of assembled genomes, it is hard to





Figure 4. Mitochondrial gene order (MGO) of *Domecia acanthophora* compared to that of the brachyuran basic gene order, with the two transposition events highlighted.

make further inferences into the composition of the genome, but the nature of the repetitive elements, albeit still preliminary, is in agreement with that of other studies (Baeza, 2021; Zhao *et al.*, 2021; Baeza *et al.*, 2022b; Lv *et al.*, 2022), with LINE, LTR, and satellite DNA accounting for the majority of the repeat sequences in this study.

The mitochondrial genome of *D. acanthophora* is the first of 58 species in the superfamily Trapezioidea (which includes the non-fossil Domeciidae, Tetraliidae, and Trapeziidae) to have a full mitochondrial genome assembled and annotated. Based on a two-marker study by Lai *et al.* (2009), Domeciidae is closest to Tetraliidae, a result that was corroborated by a study on the first zoea of *D. glabra* (Clark & Ng, 2010). A description of the first zoeal stages of *D. acanthophora* revealed several autapomorphies for this species (Alves *et al.*, 2016), and further larval description of the several description de

tions are needed to be of use in study on trapezioid systematics. Because of the novelty of this mitochondrial genome for Trapezioidea, inferences with closely related species were not possible. There were still, however, noteworthy insights found when compared to other brachyurans.

The assembled mitochondrial genome had a length of 15,568 bp and the typical metazoan composition of 37 genes, with 22 tRNA, 13 PCGs and two rRNA genes. The nucleotide composition of the entire mitochondrial genome was A+T biased (70.3%), which is comparable to that of other brachyuran families such as Grapsoidea, Pilumnidae, Portunidae, Sesarmidae, or Varunidae (Ki *et al.*, 2009, Tang *et al.*, 2017; Duan *et al.*, 2022) and even other decapods such as hermit crabs (Colín *et al.*, 2023), lobsters (Baeza *et al.*, 2022b), or shrimps (Tan *et al.*, 2017). An A+T bias is the norm not only in the mitochondrial

genomes of most crustaceans, but also of most animal species, and a G+C bias is considered a rare occurrence (see Tan *et al.*, 2019 for some examples in crustaceans). The reasons for this G+C bias remains poorly understood, with multiple reasons being suspected for the processes that promote this GC enrichment (Smith, 2012).

All but two genes displayed a "cloverleaf" structure in the tRNA genes. The *tRNA-Ser1* lacked part of the DHU arm. It is common for metazoans and brachyurans to display an anomaly in this gene (Wolstenholme, 1992; Yamauchi *et al.*, 2003; Tang *et al.*, 2017; Baeza *et al.*, 2022b; Duan *et al.*, 2022; Rodriguez-Pilco *et al.*, 2022). The *tRNA-Asp* displayed a deletion of the T Υ C loop. Truncated tRNA genes are not uncommon in metazoans, and whilst the arms of tRNA genes are known ribosome recognition sites, these truncated genes might have developed other means of recognition and binding (Watanabe *et al.*, 2014).

Over 20 different MGOs have been identified in Brachyura, with the brachyuran basic gene order being the most common out of all of them (Basso *et al.*, 2017), and the one used in our comparative analysis. Arrangement events evolving tRNA genes is also the most frequent cause of unique MGOs, with those involving the rearrangement of PCGs and rRNA genes being less frequent (Jia *et al.*, 2018). The transposition of the two tRNA genes that resulted in the MGO we report has so far, to the authors' knowledge, not been reported for other crustaceans, revealing a new MGO pattern, which could be indicative of the evolutionary history of this group of crabs.

The present study is the first to perform a nuclear genome enquiry and assembly and characterisation of the mitochondrial genome of *Domecia acanthophora*, the first species within Trapezioidea for which this data is available. Thus, with the increase in genomic studies of this nature, it will be possible to shed light on the evolution of these coral-associated crabs and on the phylogenetic relationship with other families of Brachyura.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Figure. Relative synonymous codon usage (RSCU) in *Domecia acanthophora*.

S2 Table. Microsatellites detected in the control region in the mitochondrial genome of *Domecia acanthophora*.

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