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Systematic assessment of the Panopeidae and broader Eubrachyura (Decapoda: Brachyura) using mitochondrial genomics

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Abstract

This study provides a broad phylogenetic analysis for the Eubrachyura, with the inclusion of three new Panopeidae mitochondrial genomes: *Eurypanopeus depressus* (flatback mud crab) (15,854bp), *Panopeus herbstii* (Atlantic mud crab) (15,812bp) and *Rhithropanopeus harrisii* (Harris, or 'white-fingered' mud crab) (15,892bp). These new mitogenomes were analyzed alongside all available brachyuran mitochondrial genomes (n = 113), comprising 80 genera from 29 families, to provide an updated phylogenetic analysis of the infra-order Brachyura ("true crabs"). Our analyses support the subsection Potamoida within the Eubrachyura as the sister group to Thoracotremata. The family Panopeidae aligns with the family Xanthidae to form the Xanthoidea branch, which is supported by current morphological and genetic taxonomy. A unique gene arrangement termed 'XanGO' was identified for the panopeids and varies relative to other members of the subsection Heterotremata (within the Eubrachyura) via a transposition of the *trnV* gene. This gene arrangement is novel and is shared between several Xanthoidea species, including *Etisus anaglyptus* (hairy spooner crab), *Atergatis floridus* (brown egg crab), and *Atergatis integerrimus* (red egg crab), suggesting that it is a conserved gene arrangement within the Xanthoidea superfamily. Our study further reveals a need for taxonomic revision of some brachyuran groups, particularly the Sesarmidae. The inclusion of panopeid mitogenomes into the greater brachyuran phylogeny increases our understanding of crab evolution and higher level Eubrachyuran systematics.

Keywords

Xanthidae, *Panopeus*, *Eurypanopeus*, *Rhithropanopeus*, mud crab, marine, genomics

1. Introduction

Brachyura ("true" crabs) is the largest subgroup of the Decapoda (Crustacea). It is a ubiquitous group, whose members thrive in terrestrial and aquatic habitats but are particularly prevalent in marine environments (Tsang 2009; Jia et al. 2018; Tan et al. 2018; Ma et al. 2019; Tan et al. 2019). Marine Brachyura boast a broad range of morphological and ecological diversity, leading to a complex taxonomy (Yong-kun et al. 2014). Historically, brachyurans were divided into two sections: the Eubrachyura and the Podotremata, with the Eubrachyura being further divided into the subsections: Heterotremata and Thoracotremata (Guinot 2013). The sub-sectioning of these Eubrachyura is based almost entirely on typological morphology (particularly the genital openings) and has been subject to debate with regards to monophyly. Due to the morphological complexity of this group, genetic tools and analytical methods are typically used to resolve systematic discrepancies (Basso et al. 2017; Bai et al. 2018; Jia et al. 2018; Tan et al. 2018; Wang et al. 2018).

High throughput sequencing (HTS) has proven effective in advancing and resolving taxonomies (Tan et al. 2018). Early studies on brachyuran mitogenomics relied on long PCR and primer walking techniques to read and assemble the mitogenome (Yamauchi 2003; Miller 2005). The rapid sequencing and assembly of the mitochondrial genome (mitogenome) using HTS has proven to be a powerful tool for conducting phylogenetic studies of eukaryotes (Gan et al. 2018). The small size of mitogenomes (~ 14–16 kb), potential for high mutation rate, and a simple closed structure, make them an ideal marker for inferring an organism's mitogenetic phylogeny (Boore 1999). Brachyura has been classified into 93 families with over 7000 species (Ng 2008); for 111 species (representing 28 families) the complete mitogenomes are known (NCBI and Supplementary material 1). The Brachyuran genomes that have been sequenced thus far all are between 10-25 kb in length. Much of these data represent three brachyuran families [Portunidae (n = 15); Varunidae (n = 14); Sesarmidae (n = 10)]. The remaining 25 families have < 5 mitogenomes sequenced per family, many of them only having 1 (see Supplementary material). Recent publications using mitogenomics have challenged the validity of the morphologically founded Eubrachyuran subsections of Heterotremata and Thoracotremata. For example, freshwater crabs in the family Potamidae fall into the Heterotremata based on morphology but based on mitogenomics align with members of the Thoracotremata (Basso 2017; Bai et al. 2018; Tan et al. 2018).

Mitogenomes also offer insights into gene arrangement, which can have diagnostic properties at different systematic levels (Boore 1998; Boore 2000; Moret 2001; Perseke et al. 2008; Zhuang 2010; Babbucci 2014; Mindell 2016; Nakjima et al. 2016; Zhang 2020). Within the Malacostraca, mitogenome gene arrangements are conserved within certain groups (Shen 2011; Tan et al. 2019), which allows for simple comparisons at different taxonomic levels. Grouping the Crustacea with the Insecta to form the Pancrustacea has strong support based on the near-identical arrangement of the shared genes across taxa (Boore 1998). However, gene arrangement can vary greatly within crustacean orders. Specifically, in Brachyura many species share a gene arrangement that is thought to be ancestral to Brachyura (termed BraGO). However, recent research has shown that most groups deviate from this pattern forming new arrangements at the family and subfamily levels (Basso 2017; Tan et al. 2018; Wang et al. 2018; Wang 2020a; Wang 2020b). BraGO differs from the Pancrustacea genomic order (PanGO) by a transposition of the gene *trnH* from a location between the *nad5* and *nad4* genes to a location between the *trnD* and *trnF* genes (Basso 2017). To date, 20 different gene arrangements have been identified within the Brachyura (Basso 2017; Tan et al. 2018; Zhang 2020a), but many brachyuran groups remain un-sequenced and this syntenic diversity could be much higher.

An example of an understudied brachyuran group is the superfamily Xanthoidea (Brachyura), which boasts high diversity across the world's oceans (Karasawa 2006). Species within this superfamily share a high level of morphological similarity and are often poorly described both morphologically and genetically (Ng 2008; Thoma 2014). The number of families and subfamilies within the Xanthoidea has changed drastically in recent years (Lai 2011). Two common families, the Xanthidae and Panopeidae, share several morphological features that can lead to systematic confusion and difficulty in identifying them beyond the family level (Shih 2011). Both families are found in temperate and tropical shallow intertidal and subtidal zones, but xanthid crabs have a circumtropical distribution while panopeids are only found in the Americas, excluding global invasions (Thoma 2014). To date, there are only four mitogenomes available for the Xanthidae and none for the Panopeidae, whose systematics have primarily relied upon a select number of genes or morphological keys (Williams 1984; Schubart 2000). Studies using conventional PCR to amplify and sequence mitochondrial and nuclear markers revealed that the genera *Eurypanopeus* and *Panopeus* are polyphyletic (Schubart 2000). Similarly, studies on the panopeid genus *Hexapanopeus* using 12S and 16S genes as markers have also suggested that this genus is polyphyletic (Thoma 2009). Later studies using three mitochondrial markers (COI, 12S and 16S) and three nuclear markers [18S, enolase (ENO) and Histone H3 (H3)] revealed that Xanthoidea is monophyletic, but its two families are not and are in need of taxonomic revision (Thoma 2014).

In this study, we enhance understanding of brachyuran systematics by adding three complete mitogenomes for the Panopeidae: *Eurypanopeus depressus*, *Panopeus herbstii* and *Rhithropanopeus harrisii* from their native range along the Atlantic coast of North America. The genetic composition, genetic similarity and gene ar-

rangement of these three panopeid species are described relative to other brachyuran mitogenomes, allowing us to update the brachyuran mitogenomic phylogeny and explore brachyuran-wide classification. A new gene arrangement for the superfamily Xanthoidea is described as well as a renaming of previously reported gene arrangements suggested for other Brachyura.

2. Materials and methods

2.1. Specimen collection and dissection

Three species of panopeid mud crabs were collected for this study. First, an individual *Eurypanopeus depressus* was sampled on December 1, 2018 from Hoop Pole Creek, a polyhaline site located in Atlantic beach, North Carolina (NC), USA. The individual was hand-collected at low tide from an intertidal oyster reef and then brought back to the lab for dissection. Second, an individual *Panopeus herbstii* was sampled on August 12, 2019 from Middle Marsh (Beaufort, NC), another polyhaline site, using a passive sampler attached to a wooden stake that had been driven into the sediment. The sampler design is a small plastic milk crate (19×22×16 cm) filled with autoclaved oyster shell (Roche 2007). Third, an individual *Rhithropanopeus harrisii* was sampled on February 5, 2020 from Mallard Creek (Washington, NC), a mesohaline site, using the same passive sampling design as above, but this time attached to a small fishing dock. Crabs were brought back to the lab and anesthetized prior to dissection in a –20°C freezer. Dissections for all three species were carried out using a sterilized razor blade, and part of the hepatopancreas and gills were removed and placed into separate tubes for later DNA extraction.

2.2. DNA extraction, sequencing and assembly

The DNA extractions were conducted on the hepatopancreas and gill tissue of *E. depressus*, *P. herbstii*, and *R. harrisii* using a Zymo DNA extraction kit, according to manufacturer's protocols. The DNA samples were shipped on dry ice to Novogene, California, who conducted library preparation using the NEBNext Ultra DNA Library Prep Kit. The library was loaded on to a NovaSeq 6000 (Illumina) system using the 150 bp NovaSeq 600 SP reagent kit (300 cycles) for paired end metagenomic sequencing for each individual sample. The resulting data were delivered to the University of Florida for bioinformatic analysis. The data were quality checked and trimmed using Trimmomatic v.0.36 (Bolger 2014) using default parameters. The paired and unpaired reads were assembled using SPAdes v.3.13.0 (Bankevich et al. 2012) with default parameters and k-mer lengths: 21, 33, 55, 77 and 99. The resulting datasets provided a series of contigs that were compared to the NCBI nr database using BLASTx. The mitochondrial genomes of *E. depressus* (574.088X coverage), *P. herbstii* (122.084X coverage) and *R. harrisii* (400.520X coverage) were each identified and circularized. Confirmation of their sequence coverage was conducted using CLC genomics workbench v.12.

The circularized mitogenomes were annotated using MITOS (Bernt 2013). Using the MITOS output, the location of the cox1 gene was determined and the sequences were re-annotated with the cox1 gene at the start of the genome. The putative amino acid and rRNA sequences determined by MITOS were checked using BLASTn and BLASTp (Tables 1–3). The completed genomes were then annotated graphically using Circa (http://omgenomics.com/circa). The genomes are deposited in GenBank under accession numbers MN399962 (*E. depressus*), MT024989 (*R. harrisii*), and MT024990 (*P. herbstii*).

2.3. Phylogenetic and mitochondrial gene order assessment

There were 112 brachyuran mitogenomes (see Supplementary material 1) obtained from the GenBank database (NCBI) for phylogenetic comparison using the Brachyura taxonomic ID (txid6752) and filtering results to yield DNA sequences of 10,000–25,000 bp (search date: January 2020). The amino acid and nucleotide sequences were retrieved and annotated from these genomes (13 and 15 sequences, respectively) using the Mitophast pipeline (Tan et al. 2015) which downloads each gene in the mitochondrial genome as separate files. The amino acid and nucleotide sequences files were then aligned individually using MAFFT in Geneious (v10.0.2), trimmed to the smallest sequence and concatenated using Geneious. Phylogenetic analyses were conducted in IQtree (Trifinopoulos 2016), which computed the most appropriate evolutionary model (mtMet+F+I+G4) according to BIC for both the amino acid sequences and the nucleotide sequences. A maximum likelihood tree using the amino acid sequences was created using 1000 bootstrap replicates and an SH-aLRT branch test (Guindon 2010) over 3733 positions; the tree had a log score of –157295.9511. A maximum likelihood tree using the nucleotide sequences was created using 1000 bootstrap replicates and an SH-aLRT branch test over 13790 positions; the tree had a log score of –598390.2632. The resulting trees were annotated using FigTree (http://tree.bio.ed.ac.uk/software/figtree). Both trees were rooted with the mitogenomes of *Coenobita brevimanus* (KY352233), *C. rugosus* (KY352235) and *C. perlatus* (KY352234).

All previously reported gene orders for the Brachyura were annotated according to Basso et al. (2017) and Tan et al. (2018). Pairwise comparisons of the gene orders were performed using CREx software (Bernt et al. 2007) at common intervals. The nomenclature for the gene orders follows Basso et al. (2017). MITOS was used to determine the putative location of the control region (CoRe) for *E. depressus*, *P. herbstii*, *R. harrisii*, *Etisus*

anaglyptus, *Leptodius sanguineus*, *Atergatis floridus*, and *A. integerrimus* through manual examination of the start and stop codons of the open reading frames to look for intergenic spacers. The CoRe for the crabs in the family Potamidae were obtained from Genbank. The CoRes for *Echinoecus nipponicus* and *Pilumnus vespertilio* were determined using MITOS following the same method as for the Panopeidae.

3. Results

3.1. The mitochondrial genomes of panopeid crabs

The mitochondrial genomes of the panopeid crabs used in this study were closed circular molecules containing 13 protein coding genes, 22 tRNA genes, 2 rRNA genes, and a single control region (CoRe) (Fig. 1). The *E. depressus* mitogenome was 15,854 bp in length. The *P. herbstii* mitogenome was 15,812 bp in length. The *R. harrisii* mitogenome was 15,967 bp in length. As with most brachyurans, the *rrnL* (16S) and *rrnS* (12S) genes were located on the negative strand, as are the *nad5*, *nad4*, *nad4L*, *nad1* and 8 tRNA genes (Table 1).

The nucleotide composition of the complete *E. depressus* mitochondrial genome was as follows: A=5442 (34.32%), T=5509 (34.75%), G=1652 (10.42%), C=3251 (20.51%). The A+T and G+C contents were 69.07% and 30.93%, respectively. The protein coding regions include 7 NADH dehydrogenases (*nad1–nad6* and *nad4L*), three cytochrome c oxidases (*cox1–cox3*), 2 ATPases (*atp6* and *atp8*) and 1 cytochrome *b* (*cob*) and account for 10,838 bp of the mitogenome. The 22 rRNA genes present in the mitogenome range in size from 62 (*trnD*)–71 (*trnL1*) bp in length, and the ribosomal RNA genes *rrnL* (16S) and *rrnS* (12S) have a length of 1393 bp and 817 bp, respectively. The 13 protein coding genes and majority of the ncRNA sequences showed similarity among the panopeid crabs used in this study (Table 1).

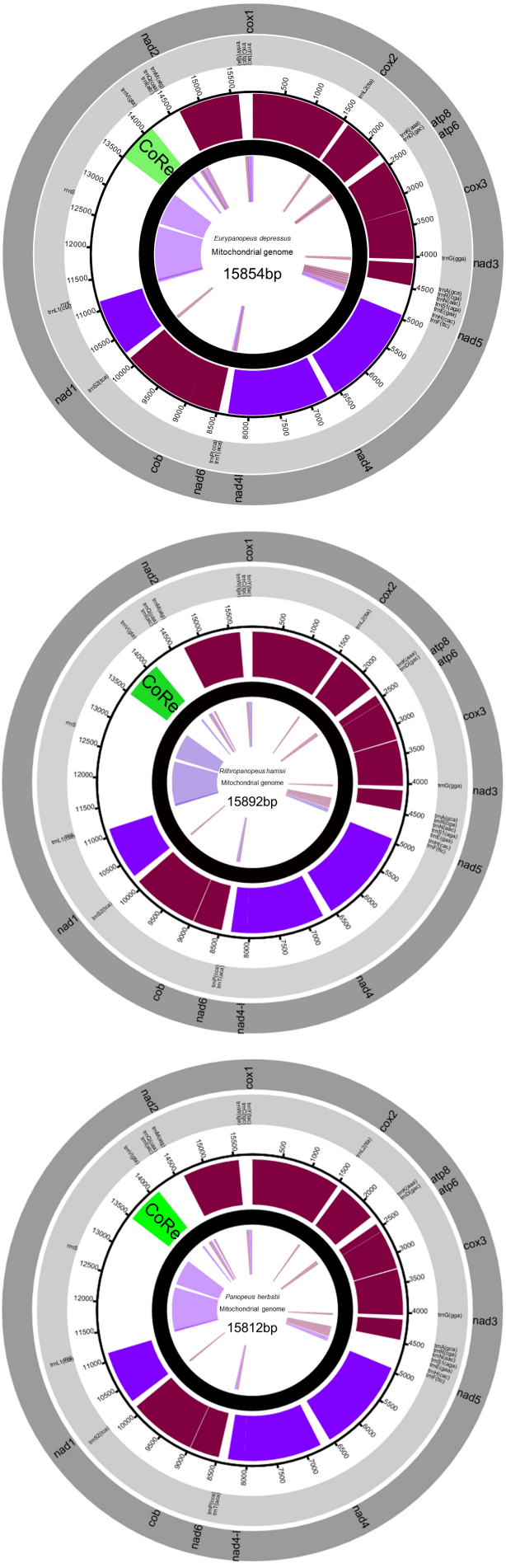


Figure 1. Annotated Circa plots for the circular mitochondrial genomes of Eurypanopeus depressus, Panopeus herbstii and Rithropanopeus harrisii. Each mitogenome is represented by a thick circular black line near the centre of the plot. Protein coding genes are on the outside of this line (negative = dark violet, positive = maroon). Non-coding RNA genes are on the inside of this line (negative = light violet, positive = light maroon). The genome sizes are written in the centre of each plot. The protein coding gene names are represented in the outer most circle (dark grey). The ncRNA gene names are listed in the second internal circle (light grey). The green rectangle labelled "CoRe" indicates the putative control region of the mitochondrial genomes. Figure 1 layout: Portrait. Associated with section 3.1

Table 1. Nucleotide and protein similarity data for the protein-coding and non-coding genes of the Eurypanopeus depressus mitochondrial genome. The data represented were acquired from BLASTn and BLASTp outputs via comparison against the complete non-redundant database. The accession number of the specific nucleotide or amino acid sequence are provided in addition to the species, if known, belonging to the sequence isolate. The similarity (%), coverage comparison (%) and e-value are all provided. MCG = mitochondrion, complete genome.

Genome	Start	End	Gene	Strand	Gene hit	Gene similarity (%)	Gene Coverage (%)	Gene e-value	Gene accession	Protein hit	Protein similarity	Protein cover	Protein -value	Protein accession
Eurypanopeus depressus mitochondrial genome	1	1515	cox1	+	*Rhithropanopeus harrisii* MCG	88.05	100	0.0	Present study	cytochrome c oxidase subunit I [*Rhithropanopeus harrisii*]	99.8	100	0.0	Present study
	1535	1598	trnL2(tta)	+	*Panopeus herbstii* MCG	92.42	100	5e-23	Present study	—	—	—	—	—
	1606	2277	cox2	+	*Rhithropanopeus harrisii* MCG	88.99	100	0.0	Present study	cytochrome c oxidase subunit II [*Panopeus herbstii*]	99.11	100	1e-170	Present study
	2291	2357	trnK(aaa)	+	*Panopeus herbstii* MCG	95.52	100	2e-27	Present study	—	—	—	—	—
	2358	2420	trnD(gac)	+	*Rhithropanopeus harrisii* MCG	100	100	3e-32	Present study	—	—	—	—	—
	2421	2573	atp8	+	-	—	—	—	—	ATP synthase F0 subunit 8 [*Panopeus herbstii*]	90.20	100	6e-18	Present study
	2576	3238	atp6	+	*Panopeus herbstii* MCG	87.80	100	0.0	Present study	ATP synthase F0 subunit 6 [*Panopeus herbstii*]	99.10	100	6e-158	Present study
	3256	4032	cox3	+	*Rhithropanopeus harrisii* MCG	89.83	100	0.0	Present study	cytochrome c oxidase subunit III [*Rhithropanopeus harrisii*]	99.61	100	0.0	Present study
	4038	4100	trnG(gga)	+	*Rhithropanopeus harrisii* MCG	98.41	100	1e-30	Present study	—	—	—	—	—
	4107	4448	nad3	+	-	—	—	—	—	NADH dehydrogenase subunit 3 [*Panopeus herbstii*]	96.49	99	1e-79	Present study
	4456	4518	trnA(gca)	+	*Panopeus herbstii* MCG	98.41	100	1e-28	—	—	—	—	—	—
	4519	4582	trnR(cga)	+	*Rhithropanopeus harrisii* MCG	98.44	100	4e-31	Present study	—	—	—	—	—
	4583	4649	trnN(aac)	+	-	—	—	—	—	—	—	—	—	—
	4652	4718	trnS1(aga)	+	*Rhithropanopeus harrisii* MCG	98.51	100	9e-31	Present study	—	—	—	—	—
	4721	4786	trnE(gaa)	+	*Rhithropanopeus harrisii* MCG	95.45	100	6e-29	Present study	—	—	—	—	—
	4808	4871	trnH(cac)	—	*Rhithropanopeus harrisii* MCG	95.31	100	9e-26	Present study	—	—	—	—	—
	4872	4935	trnF(ttc)	—	*Rhithropanopeus harrisii* MCG	95.31	100	9e-26	Present study	—	—	—	—	—
	4943	6574	nad5	—	*Rhithropanopeus harrisii* MCG	88.79	98	0.0	Present study	NADH dehydrogenase subunit 5 [*Rhithropanopeus harrisii*]	92.75	98	0.0	Present study
	6721	8046	nad4	—	*Rhithropanopeus harrisii* MCG	87.30	99	0.0	Present study	NADH dehydrogenase subunit 4 [*Panopeus herbstii*]	96.15	99	0.0	Present study
	8043	8318	nad4L	—	*Rhithropanopeus harrisii* MCG	91.21	98	1e-107	Present study	NADH dehydrogenase subunit 4L [*Panopeus herbstii*]	100.00	100	8e-66	Present study
	8345	8408	trnT(aca)	+	*Rhithropanopeus harrisii* MCG	98.39	95	2e-29	Present study	—	—	—	—	—
	8409	8473	trnP(cca)	—	*Rhithropanopeus harrisii* MCG	98.46	100	1e-31	Present study	—	—	—	—	—
	8476	8970	nad6	+	*Rhithropanopeus harrisii* MCG	85.51	98	4e-147	Present study	NADH dehydrogenase subunit 6 [*Rhithropanopeus harrisii*]	92.73	100	6e-90	Present study
	8982	10118	cob	+	*Rhithropanopeus harrisii* MCG	86.77	99	0.0	Present study	cytochrome b [*Panopeus herbstii*]	98.68	100	0.0	Present study

Genome	Start	End	Gene	Strand	Gene hit	Gene similarity (%)	Gene Coverage (%)	Gene e-value	Gene accession	Protein hit	Protein similarity	Protein cover	Protein -value	Protein accession
Eurypanopeus depressus mitochondrial genome	10117	10183	trnS2(tca)	+	*Echinoecus nipponicus* voucher MABIK CR00241788 MCG	95.85	97	2e-15	NC_039618.1	—	—	—	—	—
	10235	11134	nad1	—	*Rhithropanopeus harrisii* MCG	88.95	99	0.0	Present study	NADH dehydrogenase subunit 1 [*Rhithropanopeus harrisii*]	98.67	100	0.0	Present study
	11171	11242	trnL1(cta)	—	—	—	—	—	—	—	—	—	—	—
	11217	12610	rrnL	—	*Eurypanopeus depressus* voucher USNM 16S RNA gene	91.03	98	0.0	KT959469.1	—	—	—	—	—
	12705	13522	rrnS	—	*Eurypanopeus depressus* voucher ULLZ 3976 12S ribosomal RNA gene, partial sequence; mitochondrial	99.73	44	0.0	EU863325.2	—	—	—	—	—
	14140	14204	trnV(gta)	—	*Rhithropanopeus harrisii* MCG	95.45	100	3e-26	Present study	—	—	—	—	—
	14422	14489	trnI(atc)	+	*Panopeus herbstii* MCG	97.06	100	4e-31	Present study	—	—	—	—	—
	14487	14555	trnQ(caa)	—	*Rhithropanopeus harrisii* MCG	95.65	100	2e-30	Present study	—	—	—	—	—
	14579	14646	trnM(atg)	+	*Atergatis floridus* MCG	98.53	100	1e-22	NC_037201.1	—	—	—	—	—
	14659	15621	nad2	+	*Rhithropanopeus harrisii* MCG	82.18	100	0.0	Present study	NADH dehydrogenase subunit 2 [*Panopeus herbstii*]	90.62	99	0.0	Present study
	15656	15723	trnW(tga)	+	—	—	—	—	—	—	—	—	—	—
	15724	15787	trnC(tgc)	—	*Panopeus herbstii* MCG	96.88	100	2e-29	NC_037201.1	—	—	—	—	—
	15788	15852	trnY(tac)	—	*Etisus anaglyptus* MCG	90.77	100	4e-12	NC_042208.1	—	—	—	—	—

Table 2. Nucleotide and protein similarity data for the protein-coding and non-coding genes of the Panopeus herbstii mitochondrial genome. The data represented were acquired from BLASTn and BLASTp outputs via comparison against the complete non-redundant database. The accession number of the specific nucleotide or amino acid sequence are provided in addition to the species, if known, belonging to the sequence isolate. The similarity (%), coverage comparison (%) and e-value are all provided. MCG = mitochondrion, complete genome.

Genome	Start	End	Gene	Strand	Gene hit	Gene similarity (%)	Gene Coverage (%)	Gene e-value	Gene accession	Protein hit	Protein similarity	Protein cover	Protein e-value	Protein accession
Panopeus herbstii Mitochondrial Genome	1	1515	cox1	+	*Rhithropanopeus harrisii* MCG	87.52	100	0.0	Present study	cytochrome c oxidase subunit I [*Rhithropanopeus harrisii*]	100.00	100	0.0	Present study
	1535	1600	trnL2(tta)	+	*Eurypanopeus depressus* MCG	92.42	100	6e-23	Present study	—	—	—	—	—
	1607	2278	cox2	+	*Rhithropanopeus harrisii* MCG	88.24	100	0.0	Present study	cytochrome c oxidase subunit II [*Eurypanopeus depressus]*	99.11	100	1e-170	Present study
	2292	2358	trnK(aaa)	+	*Eurypanopeus depressus* MCG	95.52	100	2e-29	Present study	—	—	—	—	—
	2359	2421	trnD(gac)	+	*Rhithropanopeus harrisii* MCG	95.24	100	3e-27	Present study	—	—	—	—	—
	2422	2574	atp8	+	—	—	—	—	—	ATP synthase F0 subunit 8 [*Eurypanopeus depressus*]	90.20	100	6-e18	Present study
	2577	3239	atp6	+	*Eurypanopeus depressus* MCG	87.80	100	0.0	Present study	ATP synthase F0 subunit 6 [*Eurypanopeus depressus*]	99.10	100	6e-158	Present study
	3257	4033	cox3	+	*Rhithropanopeus harrisii* MCG	89.32	100	0.0	Present study	cytochrome c oxidase subunit III [*Eurypanopeus depressus*]	98.46	100	0.0	Present study
	4039	4102	trnG(gga)	+	*Rhithropanopeus harrisii* MCG	96.88	100	6e-29	Present study	—	—	—	—	—
	4109	4450	nad3	+	—	—	—	—	—	NADH dehydrogenase subunit 3 [*Eurypanopeus depressus*]	96.49	99	1e-79	Present study
	4458	4520	trnA(gca)	+	*Eurypanopeus depressus* MCG	98.41	100	1e-28	Present study	—	—	—	—	—
	4521	4583	trnR(cga)	+	*Rhithropanopeus harrisii* MCG	96.88	100	6e-29	Present study	—	—	—	—	—
	4584	4651	trnN(aac)	+	—	—	—	—	—	—	—	—	—	—
	4653	4719	trnS1(aga)	+	*Eurypanopeus depressus* MCG	97.01	100	4e-29	Present study	—	—	—	—	—
	4722	4785	trnE(gaa)	+	*Eurypanopeus depressus* MCG	95.45	100	2e-28	Present study	—	—	—	—	—
	4805	4868	trnH(cac)	—	*Rhithropanopeus harrisii* MCG	96.88	100	2e-27	Present study	—	—	—	—	—
	4869	4935	trnF(ttc)	—	—	—	—	—	—	—	—	—	—	—
	4943	6550	nad5	—	*Rhithropanopeus harrisii* MCG	87.93	99	0.0	Present study	NADH dehydrogenase subunit 5 [*Eurypanopeus depressus*]	93.64	99	0.0	Present study
	6725	8047	nad4	—	*Rhithropanopeus harrisii* MCG	85.54	99	0.0	Present study	NADH dehydrogenase subunit 4 [*Eurypanopeus depressus*]	96.15	99	0.0	Present study
	8044	8319	nad4L	—	*Rhithropanopeus harrisii* MCG	90.84	98	6e-106	Present study	NADH dehydrogenase subunit 4L [*Eurypanopeus depressus*]	100.00	100	8e-66	Present study
	8346	8409	trnT(aca)	+	—	—	—	—	—	—	—	—	—	—
	8410	8474	trnP(cca)	—	*Rhithropanopeus harrisii* MCG	98.46	100	1e-31	Present study	—	—	—	—	—
	8477	8974	nad6	+	*Eurypanopeus depressus* MCG	83.54	97	2e-130	Present study	NADH dehydrogenase subunit 6 [*Rhithropanopeus harrisii*]	91.52	99	7e-89	Present study

Genome	Start	End	Gene	Strand	Gene hit	Gene similarity (%)	Gene Coverage (%)	Gene e-value	Gene accession	Protein hit	Protein similarity	Protein cover	Protein e-value	Protein accession
Panopeus herbstii Mitochondrial Genome	8983	10119	cob	+	*Rhithropanopeus harrisii* MCG	87.13	99	0.0	Present study	cytochrome b [*Eurypanopeus depressus*]	98.68	100	0.0	Present study
	10118	10184	trnS2(tca)	+	*Rhithropanopeus harrisii* MCG	92.65	100	4e-26	Present study	—	—	—	—	—
	10230	11135	nad1	—	*Rhithropanopeus harrisii* MCG	89.40	98	0.0	Present study	NADH dehydrogenase subunit 1 [*Eurypanopeus depressus*]	97.00	99	0.0	Present study
	11171	11239	trnL1(cta)	—	—	—	—	—	—	—	—	—	—	—
	11194	12584	rrnL	—	*Panopeus herbstii* voucher USNM: 16S RNA gene, mitochondrial	100.00	37	0.0	KT959516.1	—	—	—	—	—
	12683	13502	rrnS	—	*Panopeus herbstii* voucher ULLZ 8457 12S ribosomal RNA gene, partial sequence; mitochondrial	99.46	44	0.0	EU863296	—	—	—	—	—
	14124	14190	trnV(gta)	—	—	—	—	—	—	—	—	—	—	—
	14357	14423	trnI(atc)	+	*Eurypanopeus depressus* MCG	97.06	100	4e-31	Present study	—	—	—	—	—
	14421	14489	trnQ(caa)	—	*Eurypanopeus depressus* MCG	95.65	100	2e-30	Present study	—	—	—	—	—
	14541	14609	trnM(atg)	+	*Etisus anaglyptus* MCG	98.55	100	9e-25	NC_042208	—	—	—	—	—
	14622	15581	nad2	+	—	—	—	—	—	NADH dehydrogenase subunit 2 [*Eurypanopeus depressus*]	90.62	100	0.0	Present study
	15619	15685	trnW(tga)	+	—	—	—	—	—	—	—	—	—	—
	15685	15748	trnC(tgc)	—	*Rhithropanopeus harrisii* MCG	98.44	100	4e-31	Present study	—	—	—	—	—
	15749	15812	trnY(tac)	—	*Rhithropanopeus harrisii* MCG	92.31	100	2e-24	Present study	—	—	—	—	—

Table 3. Nucleotide and protein similarity data for the protein-coding and non-coding genes of the Rhithropanopeus harrisii mitochondrial genome. The data represented were acquired from BLASTn and BLASTp outputs via comparison against the complete non-redundant database. The accession number of the specific nucleotide or amino acid sequence are provided in addition to the species, if known, belonging to the sequence isolate. The similarity (%), coverage comparison (%) and e-value are all provided. MCG = mitochondrion, complete genome.

Genome	Start	End	Gene	Strand	Gene hit	Gene similarity (%)	Gene Coverage (%)	Gene e-value	Gene accession	Protein hit	Protein similarity	Protein cover	Protein e-value	Protein accession
Rhithropanopeus harrisii Complete Mitochondrial Genome	1	1515	cox1	+	*Rhithropanopeus harrisii* mitochondrial partial COI gene for cytochrome oxidase subunit 1, isolate R617-8	99.39	65	0.0	LN810615	cytochrome c oxidase subunit I [*Panopeus herbstii*]	100.00	100	0.0	Present study
	1535	1599	trnL2(tta)	+	-	—	—	—	—	—	—	—	—	—
	1607	2278	cox2	+	*Eurypanopeus depressus* MCG	88.99	100	0.0	Present study	cytochrome c oxidase subunit II [*Panopeus herbstii*]	99.11	100	8-e170	Present study
	2292	2357	trnK(aaa)	+	*Panopeus herbstii* MCG	95.52	100	7e-29	Present study	—	—	—	—	—
	2358	2420	trnD(gac)	+	*Eurypanopeus depressus* MCG	100.00	100	3e-32	Present study	—	—	—	—	—
	2421	2573	atp8	+	-	—	—	—	—	ATP synthase F0 subunit 8 [*Panopeus herbstii*]	84.31	100	2e-16	Present study
	2576	3238	atp6	+	*Eurypanopeus depressus* MCG	88.54	100	0.0	Present study	ATP synthase F0 subunit 6 [*Eurypanopeus depressus*]	97.74	100	1e-156	Present study
	3256	4032	cox3	+	*Eurypanopeus depressus* MCG	89.86	100	0.0	Present study	cytochrome c oxidase subunit III [*Eurypanopeus depressus*]	99.61	100	0.0	Present study
	4038	4100	trnG(gga)	+	*Eurypanopeus depressus* MCG	98.41	100	1e-30	Present study	—	—	—	—	—
	4107	4448	nad3	+	-	—	—	—	—	NADH dehydrogenase subunit 3 [*Eurypanopeus depressus*]	94.74	100	9e-73	Present study
	4455	4517	trnA(gca)	+	*Eurypanopeus depressus* MCG	98.41	100	1e-28	Present study	—	—	—	—	—
	4518	4581	trnR(cga)	+	*Eurypanopeus depressus* MCG	98.44	100	4e-31	Present study	—	—	—	—	—
	4582	4648	trnN(aac)	+	-	—	—	—	—	—	—	—	—	—
	4651	4717	trnS1(aga)	+	*Eurypanopeus depressus* MCG	98.51	100	9e-31	Present study	—	—	—	—	—
	4721	4786	trnE(gaa)	+	*Eurypanopeus depressus* MCG	95.45	100	6e-29	Present study	—	—	—	—	—
	4803	4866	trnH(cac)	—	*Panopeus herbstii* MCG	96.88	100	2e-27	Present study	—	—	—	—	—
	4867	4930	trnF(ttc)	—	*Eurypanopeus depressus* MCG	95.31	100	8e-28	Present study	—	—	—	—	—
	4941	6554	nad5	—	*Eurypanopeus depressus* MCG	88.79	99	0.0	Present study	NADH dehydrogenase subunit 5 [*Eurpanopeus depressus*]	92.75	98	0.0	Present study
	6712	8037	nad4	—	*Eurypanopeus depressus* MCG	87.30	99	0.0	Present study	NADH dehydrogenase subunit 4 [*Eurypanopeus depressus*]	94.80	100	0.0	Present study
	8034	8309	nad4L	—	*Eurypanopeus depressus* MCG	91.21	98	1e-107	Present study	NADH dehydrogenase subunit 4L [*Eurypanopeus depressus*]	96.74	100	2e-64	Present study
	8336	8400	trnT(aca)	+	*Eurypanopeus depressus* MCG	98.39	95	2e-29	Present study	—	—	—	—	—
	8401	8465	trnP(cca)	—	*Panopeus herbstii* MCG	98.46	100	1e-31	Present study	—	—	—	—	—

Genome	Start	End	Gene	Strand	Gene hit	Gene similarity (%)	Gene Coverage (%)	Gene e-value	Gene accession	Protein hit	Protein similarity	Protein cover	Protein e-value	Protein accession
Rhithropanopeus harrisii Complete Mitochondrial Genome	8468	8962	nad6	+	*Eurypanopeus depressus* MCG	85.45	98	3e-148	Present study	NADH dehydrogenase subunit 6 [*Eurypanopeus depressus*]	92.73	100	6e-90	Present study
	8974	10107	cob	+	*Panopeus herbstii* MCG	87.13	100	0.0	Present study	cytochrome b [*Panopeus herbstii*]	98.68	100	0.0	Present study
	10109	10175	trnS2(tca)	+	*Panopeus herbstii* MCG	92.65	100	4e-24	Present study	—	—	—	—	—
	10224	11123	nad1	—	*Panopeus herbstii* MCG	89.40	99	0.0	Present study	NADH dehydrogenase subunit 1 [*Eurypanopeus depressus*]	98.67	100	0.0	Present study
	11160	11228	trnL1(cta)	—	-	—	—	—	—	—	—	—	—	—
	11184	12583	rrnL	—	*Rhithropanopeus harrisii* voucher USNM 12S ribosomal RNA gene, partial sequence; mitochondrial	100.00	37	0.0	KT959486.1	—	—	—	—	—
	12683	13499	rrnS	—	*Rhithropanopeus harrisii* voucher ULLZ 3995 12S ribosomal RNA gene, partial sequence; mitochondrial	98.90	44	0.0	EU863280	—	—	—	—	—
	14143	14208	trnV(gta)	—	*Eurypanopeus depressus* MCG	95.45	100	3e-26	Present study	—	—	—	—	—
	14430	14496	trnI(atc)	+	*Eurypanopeus depressus* MCG	97.06	100	4e-31	Present study	—	—	—	—	—
	14494	14562	trnQ(caa)	—	*Panopeus herbstii* MCG	92.75	100	3e-27	Present study	—	—	—	—	—
	14620	14687	trnM(atg)	+	*Panopeus herbstii* MCG	97.10	100	1e-29	Present study	—	—	—	—	—
	14700	15680	nad2	+	*Eurypanopeus depressus* MCG	82.16	98	0.0	Present study	NADH dehydrogenase subunit 2 [*Eurpanopeus depressus*]	91.28	98	2e-168	Present study
	15697	15764	trnW(tga)	+	-	—	—	—	—	—	—	—	—	—
	15764	15827	trnC(tgc)	—	*Panopeus herbstii* MCG	98.44	100	4e-31	Present study	—	—	—	—	—
	15828	15892	trnY(tac)	—	*Panopeus herbstii* MCG	92.31	100	2e-24	Present study	—	—	—	—	—

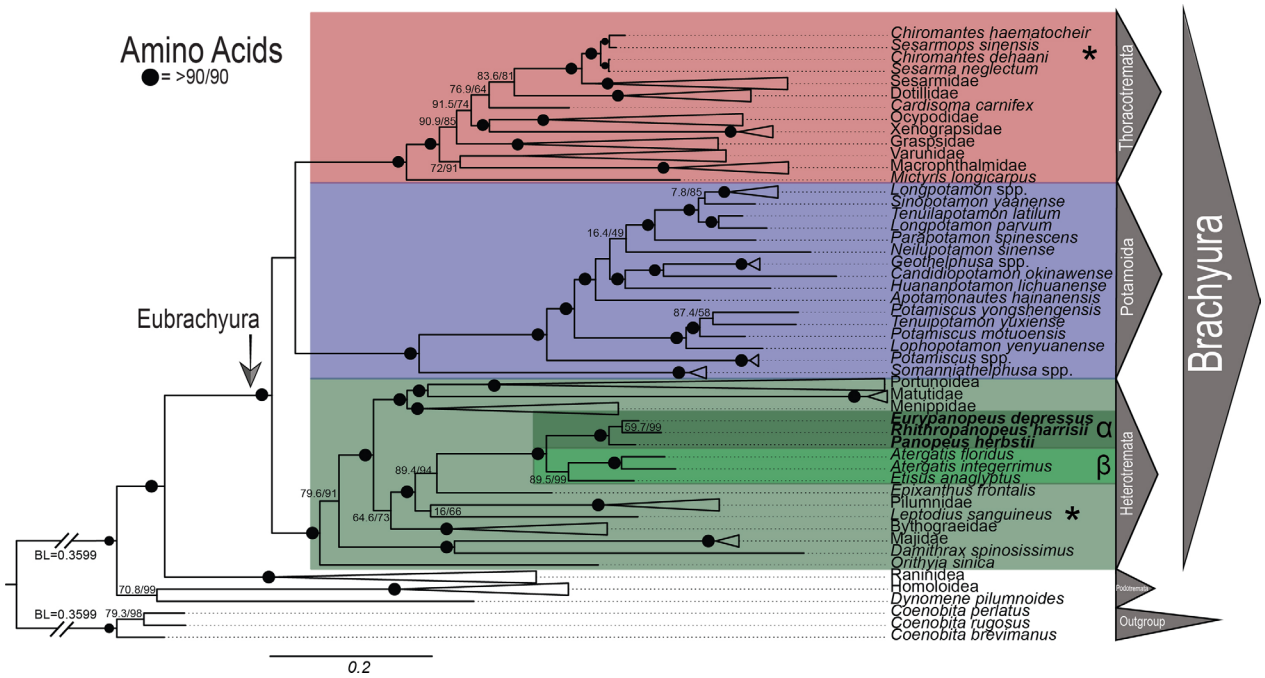


Figure 2. Maximum-likelihood phylogenetic relationships derived from 112 species of brachyuran crabs, using 13 concatenated amino acid sequences (*cox1–cox3*, *cob*, *atp6*, *atp8*, *nad1*, *nad5*, *nad4*, *nad4-L*). Some families have been collapsed for increased clarity (triangles). Black circles on nodes represent an SH-aLRT and bootstrap support of greater than 90/90. Stars (*) indicate areas on the tree with taxonomic conflicts related to previous literature. The symbol α indicates the family Xanthidae; β indicates the family Panopeidae. See Supplementary material 1 for a list of the species used and their accession numbers involved in this analysis.

The nucleotide composition of the complete *P. herbstii* mitochondrial genome was as follows: A=5520 (34.91%), T=5687 (35.97%), G=1627 (10.29%), C=2980 (18.85%). The A+T and the G+C contents were 70.87% and 29.13%, respectively. The protein coding region contains 7 NADH dehydrogenases (*nad1–nad6* and *nad4L*), three cytochrome c oxidases (*cox1–cox3*), 2 ATPases (*atp6* and *atp8*) and 1 cytochrome *b* (*cob*) and accounts for 10947 bp of the mitogenome of *P. herbstii*. The 22 rRNAs present in the mitogenome range in size from 63 (*trnD*, *trnA*, *trnR*) – 69 (*trnL1*, *trnQ*, *trnM*) bp in length, and the ribosomal RNA genes *rrnL* (16S) and *rrnS* (12S) have a length of 1392 bp and 820 bp, respectively. All 13 protein coding genes showed high similarity to the panopeid crabs used in this study. The ncRNAs all showed similarity to decapod crustaceans with the majority having high similarity with the Panopeidae (Table 2).

The nucleotide composition of the complete *R. harrisii* mitochondrial genome was as follows: A=5595 (34.21%), T=5873 (37.00%), G=1556 (9.82%), C=2866 (17.99%). The A+T and the G+C contents were 72.20% and 27.080%, respectively. The protein coding region contains 7 NADH dehydrogenases (*nad1–nad6* and *nad4L*), three cytochrome c oxidases (*cox1–cox3*), 2 ATPases (*atp6* and *atp8*) and 1 cytochrome *b* (*cob*) and account for 10,848 bp of the mitogenome. The 22 rRNAs present in the mitogenome range in size from 63 (*trnD*, *trnG*, *trnA*) – 69 (*trnL1*, *trnQ*) bp in length, and the ribosomal RNA genes *rrnL* (16S) and *rrnS* (12S) have a length of 1400 bp and 817 bp, respectively. The 13 protein coding genes showed high similarity with the panopeid crabs used in this study (Table 3).

3.2. Phylogenetics

To establish where the panopeid crabs align within the Eubrachyrua, amino acid and nucleotide sequences from 112 mitogenomes comprising 77 genera from 28 families of brachyuran crabs were used along with the three new mitogenomes (Fig. 2). Two sequences that are publicly available for brachyurans were not included in our analysis due to inconsistences with the sequences. (1) The protein sequences for *Gecarcoidea natalis* contained ambiguous amino acid identifications, resulting in poor alignment with other members within the superfamily Grapsoidea. (2) The protein sequences for *Pyrhila pisum* aligned poorly with other members of the Brachyura; however, there were no missing protein codes. When tested in BLASTp, the proteins for *P. pisum* yielded low identity with other brachyurans; < 60% identity in most cases.

Four distinct clades were identified (Fig. 2). One clade belongs to crabs in the subsection Heterotremata (n=40), a second belongs to crabs in the subsection Thoracotremata (n=44) and a third belongs to crabs in the section Podotremata (n=7). The fourth clade belongs to the 'Old World' freshwater crabs in the superfamilies Potamoidea and Gecarcinucoidea (n=20). This fourth clade forms a subsection termed Potamoida, a sister group to Thoracotremata. The split between the Heterotremata and the Potamoida/Thoracotremata clades is well supported using both amino acid sequences (Sh-aLRT/UFBoot: 100/100) as well as nucleotide sequences (Sh-aLRT/UFBoot:100/100). The Potamoida and Thoracotremata split is also well supported using both sequence types (amino

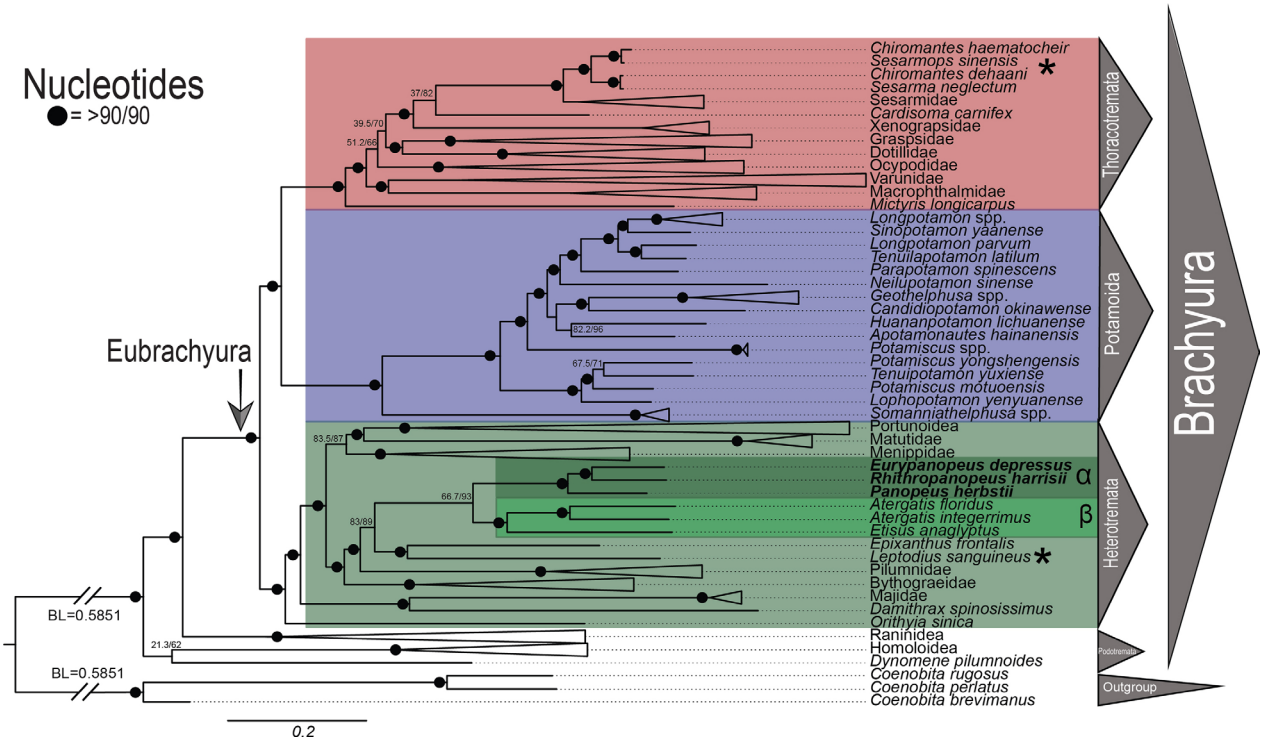


Figure 3. Maximum-likelihood phylogenetic relationships derived from 112 species of brachyuran crabs, using 15 concatenated nucleotide sequences (*cox1–cox3*, *cob*, *atp6*, *atp8*, *nad1*, *nad5*, *nad4*, *nad4-L*, *rrnL*, *rrnS*). Some families have been collapsed for increased clarity (triangles). Black circles on nodes represent an SH-aLRT and bootstrap support of greater than 90/90. Stars (*) indicate areas on the tree with taxonomic conflicts related to previous literature. The symbol α indicates the family Xanthidae; β indicates the family Panopeidae. See Supplementary material 1 for a list of the species used and their accession numbers involved in this analysis.

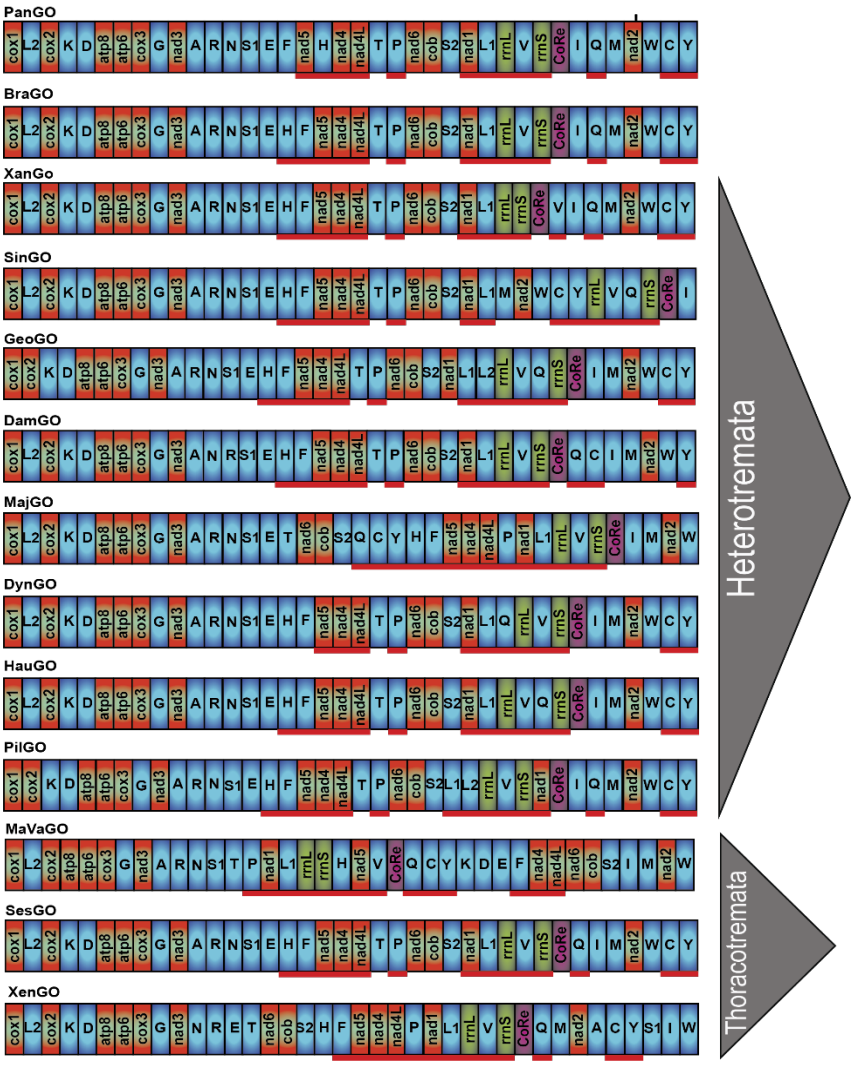
acids- Sh-aLRT/UFBoot: 98.9/99; nucleotides- Sh-aLRT/UFBoot: 92.9/98).

The panopeid crab species *E. depressus*, *P. herbstii* and *R. harrisii* formed a branch for the family Panopeidae (Fig. 2 and Fig. 3; "β") aligned alongside the xanthid branch to form the superfamily Xanthoidea (amino acids- Sh-aLRT/UFBoot: 100/100; nucleotides- Sh-aLRT/UFBoot: 100/100). The xanthid branch contains members of the Xanthidae family: *E. anaglyptus*, *A. floridus* and *A. integerrimus* (Fig. 2 and Fig. 3; "α"). When considering its amino acid sequences, the crab species *Epixanthus frontalis* from the family Oziidae aligns with the Xanthoidea superfamily with moderate support (Sh-aLRT/UFBoot: 89.4/94) (Fig. 2). The nucleotide sequences for *E. frontalis* show a similar pattern; however, *Leptodius sanguineus* is part of the branch with middling support (Sh-aLRT/UFBoot: 66.7/93) (Fig. 3). Based on amino acid comparison, *L. sanguineus* (considered a member of the Xanthidae) aligns between *E. frontalis* and members of the Pilumnidae, on a branch separate from other xanthid crabs (Sh-aLRT/UFBoot: 16/66) (Fig. 2). The amino acid phylogeny suggests that the hydrothermal vent crabs in the family Xenograpsidae align with the terrestrial crabs in the family Ocypodidae (Sh-aLRT/UFBoot: 83.2/72), yet the nucleotide sequences suggest that the xenograpids form their own branch alongside of the sesarmid crabs (Sh-aLRT/UFBoot: 100/99).

The family Sesarmidae (10 mitogenomes) appears to be polyphyletic. Rather than grouping together, the genus *Chiromantes* is split, where *C. dehaani* aligns with *Sesarma neglectum* (amino acids- Sh-aLRT/UFBoot: 99.5/100; nucleotides- Sh-aLRT/UFBoot: 100/100), and *C. haematocheir* aligns with *Sesarmops sinensis* (amino acids- Sh-aLRT/UFBoot: 99.7/100; nucleotides- Sh-aLRT/UFBoot: 100/100) (Fig. 2 and Fig. 3).

3.3. Gene arrangement among the Brachyura, incorporating the Panopeidae

The gene arrangements for the panopeid crabs *E. depressus*, *P. herbstii* and *R. harrisii* (Fig. 3) correspond in synteny to other sequenced xanthid species: *E. anaglyptus*, *A. floridus* and *A. integerrimus*. This gene arrangement differs from both the PanGO and BraGO, where the *rrnL* and *rrnS* are adjacent to each other and the *trnV* is transposed past the CoRe (Table 1). The gene order for the xanthid crab species *L. sanguineus* reported by Tan et al. (2018) is different from the other xanthids presented in this study, following the basic BraGO pattern rather than the shared pattern of the superfamily Xanthoidea. Based on the CREx test, the new gene arrangement XanGO shares 870 common intervals with PanGO and 988 common intervals with BraGO (Fig. 3), suggesting it to be a low-level rearrangement relative to the common gene arrangements. The new XanGO is most different to the MaVaGO, sharing only 80 common intervals.



	PanGo	BraGo	XanGo	MajGO	MaVaGO	SesGO	XenGo	SinGO	GeoGO	DamGO	DynGO	HuaGO	PotGO	PilGO	SomGO
PanGo	1326														
BraGo	1188	1326													
XanGo	1122	1256	1326												
MajGO	290	300	292	1326											
MaVaGO	122	118	124	156	1326										
SesGO	1122	1256	1188	296	124	1326									
XenGo	136	146	140	242	58	152	1326								
SinGO	680	782	776	276	116	786	132	1326							
GeoGO	600	690	670	232	116	708	102	644	1326						
DamGO	876	994	934	296	124	1064	150	766	654	1326					
DynGO	942	1064	1060	308	120	1124	136	782	690	886	1326				
HuaGO	1058	1188	1118	296	126	1256	144	792	728	1002	1188	1326			
PotGO	996	1122	1124	292	124	1190	140	782	690	944	1188	1188	1326		
PilGO	548	634	630	228	108	620	106	574	878	582	598	598	596	1326	
SomGO	194	194	186	240	74	190	134	148	126	288	202	190	186	122	1326

Figure 4. Gene orders (-GO) found among brachyuran crabs. Red boxes indicate protein coding genes. Blue boxes indicate tRNA's. Green boxes indicate rRNAs. Purple boxes indicate the control region (CoRe). The red lines along the bottom of the gene orders represents areas within the gene order that are located on the negative strand. Not shown are the 9 unique gene orders for the freshwater crabs (see Zhang et al. 2020). The CREx results are listed for the different gene orders. In the associated table, gene orders with high similarity (> 1000) have red boxes while those with low similarity (< 200) have blue boxes. Intermediate similarity remains white.

The mitogenomes of the crabs in the family Panopeidae all shared a ~600 bp long intergenic spacer between the *rrnS* and *trnV* ncRNA genes (*E. depressus*, 618 bp; *P. herbstii*, 622 bp; *R. harrisii*, 644 bp) representing the control region (CoRe) (Fig. 1). The CoRe in the panopeid mitogenomes are A + T skewed (78.40–80.22%) and contain the repeated motifs TA (125–107), AT (112–104), TAA (47–39), TTA (30–40), ATA (43–35) and TAT (41–37). The mitogenomes of the xanthid crabs used in this study also have similar sized intergenic spacers in this region, suggesting that this is the putative location of the CoRe for members of the superfamily Xanthoidea. The CoRe

nucleotide sequence for all species within Xanthoidea were isolated and run through BLASTn, resulting in a lack of any significant similarity, suggesting high mutability.

4. Discussion

This study provides the first mitochondrial genomes for three members of the Panopeidae and an updated concatenated mito-phylogenetic analysis for the Eubrachyura (excluding nuclear genetic data), informing upon the systematics of multiple families and higher taxonomic rankings. In addition, the mitochondrial genomes for members of the Panopeidae are identified with a consensus gene arrangement shared with other Xanthoidea (XanGO). These results advance our systematic understanding of the brachyurans through the exploration of mitochondrial genomics and gene synteny rearrangement events.

4.1. Xanthid systematics considering panopeid mitogenomic data

The mitogenomes of the panopeid crabs *E. depressus*, *P. herbstii* and *R. harrisii* support the position of the Panopeidae within the Heterotremata, helping to build/support the branch belonging to the superfamily Xanthoidea (Ng 2008). Along this branch, the Xanthidae and Panopeidae form sister groups, additionally supported by previous genetic data using five or less mitochondrial and nuclear genes (Thoma 2009; Lai 2011; Thoma 2014). The genera within these families have been historically identified as polyphyletic (Thoma 2009) and the limited number of mitogenomes available makes it difficult to determine their validity. We acknowledge that the families Xanthidae and Panopeidae both occur in two forms: sensu stricto and sensu lato (Ng 2008). There are 4 publicly available mitogenomes for the Xanthidae (GenBank) and we provide 3 additional mitogenomes for the Panopeidae. We have treated these families in their simple form due to the lack of genetic information to split them further. As more mitogenomes become available, the validity of the two forms should be revisited.

Several taxonomic conflicts appear when considering mitogenetics surrounding the Xanthoidea. First, based on morphology and limited mitogenome availability, the genus *Leptodius* is considered a member of the family Xanthidae. However, despite this genus having 12 separate species, only one mitogenome (the species *L. sanguineus*) is available for analysis. Previous studies showed that *L. sanguineus* aligns closely with other members of the Xanthidae (Sung 2016; Karagozlu 2018; Xie 2018; Ma 2019), but these studies use fewer brachyuran mitogenomes in their analysis prior to our study. When considering all mitogenomes available for the Brachyura in our investigation, *L. sanguineus* aligns more closely with the members of the family Oziidae, rather than the Xanthidae. This interesting observation merits further exploration.

4.2. Mitogenomic gene arrangements across the Brachyura

Gene arrangement changes were once thought to be rare (Boore 2000) but with greater availability of mitogenome sequencing, it appears that changes in gene arrangements can be common across groups. For example, gene order is conserved within Osteichthyes and some subgroups of Mammalia, while it varies strongly in e.g. Ctenophora (Arafat 2018), Mollusca (Guerra 2018), Hymenoptera (Dowton 1999) and Anomura (Tan et al. 2018). For Crustacea, some species within the Stomatopoda, Amphipoda and Dendrobranchiata still carry the PanGO ground pattern of Pancrustacea (Shen 2011), while no sequenced species within the Brachyura have retained this gene order. Studies on gene order rearrangement are ongoing with some hypothesizing that the evolution to living within harsh environments, such as the deep sea or hydrothermal vents, can lead to new gene synteny (Nakajima 2016; Gan 2018; Tan et al. 2019).

The brachyurans include several families found in the deep sea. Two of them are represented herein: Bythograeidae and Xenograpsidae. Bythograeidae possess the BraGO arrangement plesiomorphic for Brachyura, while Xenograpsidae have their own gene arrangement (XenGO). In contrast, the freshwater crab family Potamidae has 9 different gene arrangements (Zhang 2020). Brachyuran crabs represent both cases: the adaptation from a marine to a freshwater environment was likely harsh and may have resulted in several new gene arrangements, while in contrast, the evolution of crabs to the deep-sea benthos resulted in some retaining the ancestral gene order in the face of a new environmental extreme. Therefore, when considering crabs, living within harsh environments does not seem to be the only answer to gene arrangement plasticity, but perhaps requires consideration at the finer scale of environmental adaption. Similar findings have been reported by Tan et al. (2019) who found little evidence for linking gene order rearrangements with adaptations to extreme environments, concluding that these cues are poorly understood and merit a more detailed approach.

A comparison of the eubrachyuran subsections shows that Heterotremata has a higher diversity of gene arrangements than Thoracotremata. Both subsections share species whose gene arrangement follows the basic BraGO pattern. Aside from the BraGO, Thoracotremata only has 3 unique gene arrangements while Heterotremata has 8 unique gene arrangements (including the herein newly established XanGO). This does not include the gene arrangements for the freshwater crabs in the superfamilies Potamoidea and Gecarcinucoidea. The freshwater crabs have more unique gene arrangements than the known Heterotremata.

The panopeid crabs *E. depressus*, *P. herbstii* and *R. harrisii* all have the *trnV* gene transposed from between the *rrnL* and *rrnS* genes to a location past the CoRe.

This differs from the PanGO, BraGO, SesGO, XenGO, DamGO, MajGO and DynGO, which all have the *trnV* gene located between the *rrnL* and *rrnS* genes, with the CoRe following the *rrnS* gene. The xanthid crabs *E. anaglyptus*, *L. sanguineus*, *A. floridus* and *A. integerrimusi* all share the latter gene arrangement, suggesting that it might be a conserved arrangement within Xanthoidea and thus support our interpretation of the new Xanthoidea gene arrangement (XanGO). The intergenic spacer found between the *rrnS* and *trnV* genes in panopeids appears to be the putative location of the CoRe for these species and is shared with xanthid species, *E. anaglyptus*, *A. floridus* and *A. integerrimus*. All have similarly sized intergenic spacers (600–750 bp long) at this location, suggesting that this may be the location of the CoRe across Xanthoidea. Apart from *L. sanguineus*, the Xanthidae all follow the new gene arrangement XanGO. *Leptodius sanguineus* follows the plesiomorphic brachyuran gene arrangement BraGO and based on its amino acid sequences, it groups more closely with the family Pilumnidae than the members of the Xanthidae or the panopeids presented here; however, nodal support is low, meriting further study and sequencing of closer relatives. Higher nodal support is offered with the nucleotide tree, where *L. sanguineus* groups with *Epixanthus frontalis* from Oziidae rather than with the xanthids. Based on the molecular taxonomy and its gene arrangement, the placement of *L. sanguineus* within Xanthidae appears to be invalid and in need of revision, adding to our explanation above.

The mitogenome analysis we performed also supports the renaming of two gene arrangements and confirms the correct gene sequence for another. Two mitogenomes were available for the pilumnid crabs, *Echinoecus nipponicus* and *Pilumnus vespertilio*. They follow the gene arrangement reported by Tan et al. (2018) and differ from BraGO in having the *trnL* gene transposed from its location between the *cox1* and *cox2* genes to a location between the second *trnL* and *rrnL* genes. This gene arrangement was reported by Tan et al. (2018) as number 12, but we propose Pilumnidae gene order (PilGO) to follow the original gene nomenclature determined by Basso et al. (2017). Similarly, the gene arrangement reported as number 5 by Tan et al. (2018) we rename to the *Somanniathelphusa* gene order (SomGO). Basso et al. (2017) report the gene arrangement GeoGO as having the *trnL* gene between the *cox1* and *cox2* genes, but based on the gene arrangement listed in Genbank, this is nonconcurrent. The correct gene arrangement was reported by Tan et al. (2019) and is supported here with the addition of the mitogenome for *Geothelphusa* sp. (MG674171), where the *trnL* gene is located between *nad1* and the second *trnL* gene. This corrected nomenclature should be incorporated into further taxonomic assessments.

4.3. Conclusions

This study provides an updated mitophylogeny for the Brachyura, utilizing all available mitogenomes, along with the first mitogenomes for the Panopeidae, a highly abundant group of ecologically important estuarine crabs with a limited phylogenetic understanding. Our data support the subsection, Potamoida, within the Eubrachyura. The addition of *E. depressus*¸ *P. herbstii* and *R. harrisii* mitogenomes provides a greater phylogenetic understanding of a group that has been taxonomically challenging in the past. Moreover, the addition of mitogenomes from the Panopeidae further supports the split of the Xanthoidea into multiple families. The novel gene arrangement we describe within the Heterotremata, increases the total number of unique gene arrangements within this subsection to eight. Whilst our results clarify some phylogenetic relationships, they also highlight the need for further study of the genus *Leptodius* which appears to be incorrectly placed within the subfamily Xanthoidea. Greater sequencing efforts will provide more comparative data for these underrepresented crab groups, and should include the incorporation of nuclear genetic data where possible.

5. Author contributions

AMHB collected the crabs used in the study. JB performed the extraction and bioinformatic processing/assembly of the mitogenomes. LAJ and JB performed the phylogenetics and gene similarity assessments. Gene order analysis and annotation was performed by LAJ and JB. LAJ, AMHB, KAM, DCB and JB contributed to the writing of the manuscript.

6. Competing interests

The authors declare no competing interests.

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8. References

Arafat H, Alamaru A, Giss C, Huchon D (2018) Extensive mitochondrial gene rearrangements in Ctenophora: insights from benthic Platyctenida. BMC Ecology and Evolution 18(65): 1–11. https://doi.org/10.1186/s12862-018-1186-1

Babbucci M, Basso A, Scupola A, Patarnello T, Negrisolo E (2014) Is it an ant or a butterfly? Convergent evolution in the mitochondrial gene order of Hymenoptera and Lepidoptera. Genome Biology and Evolution 6(12): 3326–3343.

Bai J, Xu S, Nie Z, Wang Y, Zhu C, Wang Y, Min W, Cai Y, Zou J, Zhou X (2018) The complete mitochondrial genome of *Huananpotamon lichuanense* (Decapoda: Brachyura) with phylogenetic implications for freshwater crabs. Gene 646: 217–226.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski, AD, Pyshkin AV (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology 19(5): 455–477.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology 19(5): 455–477.

Basso A, Babbucci M, Pauletto M, Riginella E, Patarnello T, Negrisolo E (2017) The highly rearranged mitochondrial genomes of the crabs *Maja crispata* and *Maja squinado* (Majidae) and gene order evolution in Brachyura. Scientific Reports 7: 1–13. https://doi.org/10.1038/s41598-017-04168-9

Bernt M, Merkle D, Ramsch K, Fritzsch G, Perseke M, Bernhard D, Schlegel M, Stadler PF, Middendorf M (2007) CREx: inferring genomic rearrangements based on common intervals. Bioinformatics 23(21): 2957–2958.

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina Sequence Data. *Bioinformatics* 30(15): 2114–2120.

Boore JL, Brown WM (1998) Big trees from little genomes: mitochondrial gene order as a phylogenetic tool. Current Opinion in Genetics & Development 8(6): 668–674.

Boore JL, Lavrov DV, Brown WM (1998) Gene translocation links insects and crustaceans. Nature 392(6677): 667–668.

Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Research 27(8): 1767–1780.

Boore JL (2000) The duplication/random loss model for gene rearrangement exemplified by mitochondrial genomes of deuterostome animals. In: Sankoff D, Nadeau JH (eds) Comparative Genomics. Springer, Dordrecht, 133–147.

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF (2013) MITOS: improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69(2): 313–319.

Dowton M, Austin AD (1999) Evolutionary dynamics of a mitochondrial rearrangement" hot spot" in the Hymenoptera. Molecular Biology and Evolution 16(2): 298–309.

Gan H, Tan MH, Lee YP, Schultz MB, Horwitz P, Burnham Q, Austin CM (2018) More evolution underground: accelerated mitochondrial substitution rate in Australian burrowing freshwater crayfishes (Decapoda: Parastacidae). Molecular Phylogenetics and Evolution 118: 88–98.

Guerra D, Bouvet K, Breton S (2018) Mitochondrial gene order evolution in Mollusca: inference of the ancestral state from the mtDNA of *Chaetopleura apiculata* (Polyplacophora, Chaetopleuridae). Molecular Phylogenetics and Evolution 120: 233–239.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59(3): 307–321.

Guinot D, Tavares M, Castro P (2013) Significance of the sexual openings and supplementary structures on the phylogeny of brachyuran crabs (Crustacea, Decapoda, Brachyura), with new nomina for higher-ranked podotreme taxa. Zootaxa 3665(1): 1–414.

Jia XN, Xu SX, Bai J, Wang YF, Nie ZH, Zhu CC, Wang Y, Cai YX, Zou JX, Zhou XM (2018) The complete mitochondrial genome of *Somanniathelphusa boyangensis* and phylogenetic analysis of genus *Somanniathelphusa* (Crustacea: Decapoda: Parathelphusidae). PLoS ONE 13(2): e0192601. https://doi.org/10.1371/journal.pone.0192601

Karagozlu MZ, Dinh TD, Nguyen VQ, Kim CB (2018) Analysis of complete mitochondrial genome of *Etisus anaglyptus* (Arthropoda, Decapoda, Xanthidae) with phylogenetic consideration. Mitochondrial DNA Part B 3(1): 278–279.

Karasawa H, Schweitzer, CE (2006) A new classification of the Xanthoidea *sensu lato* (Crustacea: Decapoda: Brachyura) based on phylogenetic analysis and traditional systematics and evaluation of all fossil Xanthoidea *sensu lato*. Contributions to Zoology 75(01–02): 23–73.

Lai JC, Mendoza JCE, Guinot D, Clark PF, Ng PK (2011) Xanthidae MacLeay, 1838 (Decapoda: Brachyura: Xanthoidea) systematics: a multi-gene approach with support from adult and zoeal morphology. Zoologischer Anzeiger – A Journal of Comparative Zoology 250(4): 407–448.

Klaus S, Magalhães C, Salas-Gismondi R, Gross M, Antoine PO (2017) Palaeogene and Neogene brachyurans of the Amazon basin: a revised first appearance date for primary freshwater crabs (Brachyura, Trichodactylidae). Crustaceana 90(7–10): 953–967.

Ma KY, Qin J, Lin CW, Chan TY, Ng PK, Chu KH, Tsang LM (2019) Phylogenomic analyses of brachyuran crabs support early divergence of primary freshwater crabs. Molecular Phylogenetics and Evolution 135: 62–66.

Mindell DP, Sorenson MD, Dimche DE (1998) Multiple independent origins of mitochondrial gene order in birds. Proceedings of the National Academy of Sciences 95(18): 10693–10697.

Miller AD, Murphy NP, Burridge CP, Austin CM (2005) Complete mitochondrial DNA sequences of the decapod crustaceans *Pseudocarcinus gigas* (Menippidae) and *Macrobrachium rosenbergii* (Palaemonidae). Marine Biotechnology 7(4): 339–349.

Moret BM, Wang LS, Warnow T, Wyman SK (2001) New approaches for reconstructing phylogenies from gene order data. Bioinformatics 17(suppl_1): S165–S173.

Nakajima Y, Shinzato C, Khalturina M, Nakamura M, Watanabe H, Satoh N, Mitarai S (2016) The mitochondrial genome sequence of a deep-sea, hydrothermal vent limpet, *Lepetodrilus nux*, presents a novel vetigastropod gene arrangement. Marine Genomics 28: 121–126.

Perseke MM, Fritzsch G, Ramsch K, Bernt M, Merkle D, Middendorf M, Bernhard D, Stadler PF, Schlegel M (2008) Evolution of mitochondrial gene orders in echinoderms. Molecular Phylogenetics and Evolution 47(2): 855–864.

Roche DG, Torchin ME (2007) Established population of the North American Harris mud crab, *Rhithropanopeus harrisii* (Gould 1841) (Crustacea: Brachyura: Xanthidae) in the Panama Canal. Aquatic Invasions 2: 155–161.

Schubart CD, Neigel JE, Felder DL (2000) Molecular phylogeny of mud crabs (Brachyura: Panopeidae) from the northwestern Atlantic and the role of morphological stasis and convergence. Marine Biology 137(1): 11–18.

Shen X, Wang H, Wang M, Liu B (2011) The complete mitochondrial genome sequence of *Euphausia pacifica* (Malacostraca: Euphausiacea) reveals a novel gene order and unusual tandem repeats. Genome 54(11): 911–922.

Shih HT, Ng PK (2011) Diversity and biogeography of freshwater crabs (Crustacea: Brachyura: Potamidae, Gecarcinucidae) from East Asia. Systematics and Biodiversity 9(1): 1–16.

Sternberg RV (1997) Cladistics of the freshwater crab family Trichodactylidae (Crustacea: Decapoda): appraising the reappraisal. Journal of Comparative Biology 2(1): 49–62.

Sung JM, Lee J, Kim SG, Karagozlu MZ, Kim CB (2016) Complete mitochondrial genome of *Leptodius sanguineus* (Decapoda, Xanthidae). Mitochondrial DNA Part B 1: 500–501.

Tan MH, Gan HM, Schultz MB, Austin CM (2015) MitoPhAST, a new automated mitogenomic phylogeny tool in the post-genomic era with a case study of 89 decapod mitogenomes including eight new freshwater crayfish mitogenomes. Molecular Phylogenetics and Evolution 85: 180–188.

Tan MH, Gan HM, Lee YP, Linton S, Grandjean F, Bartholomei-Santos ML, Miller AD, Austin CM (2018) ORDER within the chaos: insights into phylogenetic relationships within the Anomura (Crustacea: Decapoda) from mitochondrial sequences and gene order rearrangements. Molecular Phylogenetics and Evolution 127: 320–331.

Tan MH, Gan HM, Lee YP, Bracken-Grissom H, Chan TY, Miller AD, Austin CM (2019) Comparative mitogenomics of the Decapoda reveals evolutionary heterogeneity in architecture and composition. Scientific Reports 9: 1–16. https://doi.org/10.1038/s41598-019-47145-0

Tan BP, Xin ZZ, Liu Y, Zhang DZ, Wang ZF, Zhang HB, Chai XY, Zhou CL, Liu QN (2017) The complete mitochondrial genome of *Sesarmops sinensis* reveals gene rearrangements and phylogenetic relationships in Brachyura. PLoS ONE 12(6): 1–16. https://doi.org/10.1371/journal.pone.0179800

Tan BP, Liu Y, Xin ZZ, Zhang DZ, Wang ZF, Zhu XY, Wang Y, Zhang HB, Zhou CL, Chai XY, Liu QN (2018) Characterisation of the complete mitochondrial genome of *Helice wuana* (Grapsoidea: Varunidae) and comparison with other brachyuran crabs. Genomics 110(4): 221–230.

Thoma BP, Schubart CD, Felder DL (2009) Molecular phylogeny of western Atlantic representatives of the genus *Hexapanopeus* (Decapoda: Brachyura: Panopeidae). In: Decapod Crustacean Phylogenetics. Crustacean Issues, CRC Press, Boca Raton, 551–565.

Thoma BP, Guinot D, Felder DL (2014) Evolutionary relationships among American mud crabs (Crustacea: Decapoda: Brachyura: Xanthoidea) inferred from nuclear and mitochondrial markers, with comments on adult morphology. Zoological Journal of the Linnean Society 170(1): 86–109.

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44(W1): 232–235.

Tsang LM, Schubart CD, Ahyong ST, Lai JC, Au EY, Chan TY, Ng PK, Chu KH (2014) Evolutionary history of true crabs (Crustacea: Decapoda: Brachyura) and the origin of freshwater crabs. Molecular Biology and Evolution 31(5): 1173–1187.

Wang Q, Tan D, Guo H, Wang J, Xu X, Wang Z (2020) Comparative mitochondrial genomic analysis of *Macrophthalmus pacificus* and insights into the phylogeny of the Ocypodoidea & Grapsoidea. Genomics 112(1): 82–91.

Wang Z, Wang Z, Shi X, Wu Q, Tao Y, Guo H, Ji C, Bai Y (2018) Complete mitochondrial genome of *Parasesarma affine* (Brachyura: Sesarmidae): Gene rearrangements in Sesarmidae and phylogenetic analysis of the Brachyura. International Journal of Biological Macromolecules 118: 31–40.

Wang Z, Shi X, Guo H, Tan D, Bai Y, Wang Z (2020) Characterization of the complete mitochondrial genome of *Uca lacteus* and comparison with other brachyuran crabs. Genomics 112(1): 10–19.

Williams AB (1984) Shrimps, lobsters, and crabs of the Atlantic coast of the eastern United States, Maine to Florida. Washington, DC: Smithsonian Institution Press 550.

Xie Z, Tan H, Lin F, Guan M, Waiho K, Fang S, Ikhwanuddin M, Fazhan H, Ma H (2018) Characterization of the complete mitochondrial genome of xanthid crab, *Atergatis integerrimus* from China (Decapoda: Brachyura) and its phylogenetic analysis. Mitochondrial DNA Part B 3(1): 397–398.

Yamauchi MM, Miya MU, Nishida M (2003) Complete mitochondrial DNA sequence of the swimming crab, *Portunus trituberculatus* (Crustacea: Decapoda: Brachyura). Gene 311: 129–135.

Yong-Kun J, Wang A, Lu XL, Song DH, Jin YH, Lu JJ, Sun HY (2014) Mitochondrial genomes of two Brachyuran crabs (Crustacea: Decapoda) and phylogenetic analysis. *Journal of Crustacean Biology* 34(4): 494–503.

Zhang Z, Xing Y, Cheng J, Pan D, Lv L, Cumberlidge N, Sun H (2020) Phylogenetic implications of mitogenome rearrangements in East Asian potamiscine freshwater crabs (Brachyura: Potamidae). Molecular Phylogenetics and Evolution 143: 106669.

Zhuang X, Cheng CHC (2010) ND6 Gene "Lost" and Found: Evolution of mitochondrial gene rearrangement in Antarctic notothenioids. Molecular Biology and Evolution 27: 1391–1403.

Supplementary material 1

Table S1

Authors: Jennings et al. (2021)
Data type: .docx
Explanation note: NCBI accession numbers for species used to conduct phylogenetic analysis.

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