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Research paper

Mitochondrial genomes of the land hermit crab *Coenobita clypeatus* (Anomura: Paguroidea) and the mole crab *Emerita talpoida* (Anomura: Hippoidea) with insights into phylogenetic relationships in the Anomura (Crustacea: Decapoda)

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ABSTRACT

The infraorder Anomura is a species-rich clade of decapod crustaceans recognized by its remarkable disparity in terms of morphology, anatomy, ecology, physiology, and behavior. This study assembled and characterized the complete mitochondrial genomes of two anomuran species, the hermit crab *Coenobita clypeatus* and the mole crab *Emerita talpoida*. The AT-rich mitochondrial genomes of *C. clypeatus* and *E. talpoida* are 16,469 bp and 15,810 bp long, respectively, and are composed of 13 protein-coding genes (PCGs), two ribosomal RNA genes, and 22 transfer RNA genes. A 1,390 bp and 553 bp long intergenic space is assumed to be the D-loop in *C. clypeatus* and *E. talpoida*, respectively. Mitochondrial synteny in *C. clypeatus* is identical to that reported in other congeneric hermit crabs while synteny in *E. talpoida* is identical to that described for the confamilial mole crab *Stemonopa insignis*. No major differences occur between the studied species and their respective congeneric / cofamilial species in terms of nucleotide composition and codon usage profiles of PCGs. Selective pressure analysis in PCGs, rarely conducted in anomuran crabs, indicate that all these mitochondrial PCGs experience purifying selection and that this purifying selection is stronger in some (i.e., *cox* family genes and *cob*) compared to other PCGs (e.g., *atp8*). Most of the tRNA genes exhibited a typical 'cloverleaf' secondary structure with few exceptions in the two studied species. In *C. clypeatus*, *tRNA-Ser1* lacks the thymine pseudouracil cytosine (TΨC) loop while *tRNA-Phe* and *tRNA-Tyr* each exhibit a deletion of the dihydroxyuridine (DHU) loop but not the arm. In turn, in *E. talpoida*, *tRNA-Phe* and *tRNA-Arg* exhibit a deletion of the DHU loop but not the arm while *tRNA-Ser1* lacks the TΨC arm. A phylogenomic analysis based on translated PCGs confirms the monophyly of the infraorder Anomura and retrieves most/all relationships at the superfamily and family level previously reported for anomurans. The

Abbreviations: CR, control region; CSB, conserved sequence block; ETAS, extended terminal associated sequences; atp6, ATP synthase subunit 6; atp8, ATP synthase subunit 8; cob, cytochrome b apoenzyme; cox1, cytochrome oxidase subunit I; cox2, cytochrome oxidase subunit II; cox3, cytochrome oxidase subunit III; nad1, NADH dehydrogenase subunit 1; nad2, NADH dehydrogenase subunit 2; nad3, NADH dehydrogenase subunit 3; nad4, NADH dehydrogenase subunit 4; nad4l, NADH dehydrogenase subunit 4L; nad5, NADH dehydrogenase subunit 5; nad6, NADH dehydrogenase subunit 6; rrnL, large ribosomal subunit RNA; rrnS, small ribosomal subunit RNA; trnA, Transfer RNA specifying Alanine; trnC, Transfer RNA specifying Cysteine; trnD, Transfer RNA specifying Aspartic acid; trnE, Transfer RNA specifying Glutamic acid; trnF, Transfer RNA specifying Phenylalanine; trnG, Transfer RNA specifying Glycine; trnH, Transfer RNA specifying Histidine; trnI, Transfer RNA specifying Isoleucine; trnK, Transfer RNA specifying Lysine; trnL1, Transfer RNA specifying Leucine 1; trnL2, Transfer RNA specifying Leucine 2; trnM, Transfer RNA specifying Methionine; trnN, Transfer RNA specifying Asparagine; trnP, Transfer RNA specifying Proline; trnQ, Transfer RNA specifying Glutamine; trnR, Transfer RNA specifying Arginine; trnS1, Transfer RNA specifying Serine 1; trnS2, Transfer RNA specifying Serine 2; trnT, Transfer RNA specifying Tryptophan; trnV, Transfer RNA specifying Valine; trnY, Transfer RNA specifying Tyrosine.

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analysis supports the monophyletic status of the families Albuneidae, Lithodidae, Coenobitidae, and Porcellanidae. In turn, the superfamily Paguroidea, and the families Paguridae and Diogenidae are polyphyletic.

1. Introduction

Anomuran crabs (Infraorder Anomura) are a species-rich clade of crustaceans within the Order Decapoda (Ahyong et al., 2009; De Grave et al., 2009; Lozano-Fernandez et al., 2019) with remarkable disparity in terms of morphology, anatomy, ecology, physiology, and behavior (De Grave et al., 2009; Bracken-Grissom et al., 2013; Lozano-Fernandez et al., 2019). They include representatives such as the coconut, king, mole, hairy stone, hermit, and porcelain crabs as well as the squat lobsters. During the last decade, anomuran crabs have been used as model systems in evolutionary biology. For example, the porcelain crab *Petrolisthes violaceus* has been used to test the adaptive value of active parental care (i.e. embryo grooming) (Förster and Baeza, 2001), the 'yeti crab' *Kiwa puravida* has been used to understand the evolution of dense body setation and its role in the "culturing" of chemosynthetic bacteria as a food source at hydrothermal vents (Goffredi et al., 2008; Thatje et al., 2015), and the coconut crab *Birgus latro* has been used to explore genomic adaptations to terrestrial life (Veldsman et al., 2020, 2021). Furthermore, some species are heavily fished for human consumption by industrial fleets in both low (the squat lobster *Pleuroncodes monodon* in Costa Rica (Wehrtmann and Acuña, 2011) and high latitudes (e.g., the red king crab *Paralithodes camtschaticus* in Alaska - Dvoretsky and Dvoretsky, 2018; the yellow squat lobster *Cervimunida johni* in Chile - Wolff and Aroca, 1995). Lastly, some species are invasive (Wassick et al., 2017) or targeted by fisheries supplying the ornamental aquarium trade industry (Baeza et al., 2013).

Although anomuran crabs are used as model systems in evolutionary and ecological studies and hold tremendous economic value, only a few genomic resources exist (Tan et al., 2018; Lozano-Fernandez et al., 2019; Wolfe et al., 2019, and references therein). For instance, as it pertains to mitochondrial genomes, the National Center for Biotechnology Information (NCBI) nucleotide database (GenBank) lists only 46 curated mitochondrial genomes [2 of them incomplete] belonging to anomuran crabs (consulted 05-04-2022). The development of genomic resources across anomurans, including mitochondrial genomes, is increasingly important as it will promote the continued understanding of their remarkable evolutionary history and innovations.

Among anomuran crabs, the terrestrial hermit crab *Coenobita clypeatus* is the only crab in the genus that inhabits the western North Atlantic (Walker, 1994). It is distributed from the east coast of Florida, along the Gulf of Mexico and Central America, to the coast of Venezuela (Provenzano, 1959; Walker, 1994; Lewis and Rotjan, 2009; Copeland, 2020). It is found on all Caribbean islands, from the Florida Keys to Trinidad, with Bermuda being the northernmost extension of its range (Provenzano, 1959; de Wilde, 1973; Walker, 1994). *Coenobita clypeatus* inhabits supralittoral areas but can also penetrate long distances inland (de Wilde, 1973; Wolcott, 1988; Greenaway, 2003; Harzsch and Hansson, 2008). This species has been used as a model system to understand sensory, physiological, and behavioral adaptations, among others, to semi- and/or terrestrial life in crustaceans (de Wilde, 1973; Harzsch and Hansson, 2008; Krång et al., 2012).

The mole crab *Emerita talpoida* (Say, 1817) also inhabits along the east coast of North America, from Massachusetts to Florida, and is also present in the Gulf of Mexico (Efford, 1976; Tam et al., 1996). *Emerita talpoida* inhabits the wave wash zone of exposed sandy beaches, seasonally occurring in considerable numbers (Wenner, 1977). Reproduction in *E. talpoida* typically occurs from late winter through early fall with a maximum number of ovigerous females in late summer (Diaz, 1980). *E. talpoida* is most relevant to the food web dynamics of coupled aquatic-terrestrial ecosystems (Tewfik et al., 2016) and has been used as a model system to understand the mechanisms for burial in sand-dwelling crustaceans (Trueman, 1970), appendages (i.e., limb) regeneration (Weis, 1982), and the effect of parasites on the biological rhythms of their host individuals (Loh, 2017).

This study reports, for the first time, the complete mitochondrial genome of the terrestrial hermit crab *Coenobita clypeatus* and the mole crab *Emerita talpoida.* We have assembled, annotated, manually curated, and characterized in detail the complete mitochondrial genome of *Coenobita clypeatus* and *Emerita talpoida* following recommendations in Baeza (2022). Lastly, we examined the phylogenetic position of the two studied species in the infraorder Anomura based on mitochondrial PCGs.

2. Methods

2.1. Sampling, DNA extraction, and sequencing

The raw sequence data used to assemble the mitochondrial genome of the two studied species of anomuran crabs was generated by one of us (HBG) together with other colleagues as a part of a project focusing on developing new genomic resources for decapods (see Wolfe et al., 2019). Information on specimen sampling, DNA extraction, and sequencing methods are detailed in Table S3 in Wolfe et al. (2019).

2.2. Mitochondrial genome assembly

The totality of the raw Illumina reads we retrieved from GenBank (SRA accession number SRX5574073 [*C. clypeatus*] and SRX5559866 [*E. talpoida*]) were used to assemble the mitochondrial genome of the two studied species. The mitochondrial genomes were assembled *de novo* using the software NOVOPlasty v. 1.2.3 (Dierckxsens et al., 2017). In the case of *C. clypeatus*, NOVOPlasty was run using a fragment of the *16S* ribosomal RNA gene from the same species available in Genbank (accession number KF182531, voucher number ULLZ 9968, length 577 bp) as a seed and the complete mitochondrial genome of the congeneric *C. brevimanus* (NC_050386.1) as a reference. In the case of *E. talpoida*, NOVOPlasty was run using a fragment of the *cox1* protein coding gene from the same species available in Genbank (accession number KT959463, voucher number USNM:IZ:1286817, length 658 bp) as a seed. The two assembly runs used a kmer size of 39. Reads were not cleaned before the assembly following the developer's suggestions (Dierckxsens et al., 2017).

2.3. Mitochondrial genome annotation and manual characterization

The newly assembled mitochondrial genomes of *C. clypeatus* and *E. talpoida* were first annotated using the web servers MITOS (https://mitos.bioinf.uni-leipzig.de) and MITOS2 (https://mitos2.bioinf.uni-leipzig.de) (Bernt et al., 2013; Donath et al., 2019) using the invertebrate genetic code. Manual curation of the *in silico* annotated mitochondrial genomes, including start and stop codons corrections, were conducted using the tool ExPASy translate in the web server ExPASy (https://web.expasy.org - Artimo et al., 2012; Donath et al., 2019) and MEGA X (Kumar et al., 2018). We also compared the manually curated annotations of the new mitochondrial genomes with other anomuran mitochondrial genomes available in the NCBI web server. The two mitochondrial genomes were visualized with the web server GenomeVx (https://wolfe.ucd.ie/GenomeVx/ - Conant and Wolfe, 2008).

2.4. Mitochondrial genome characterization

Nucleotide composition and codon usage profiles of the PCGs in the

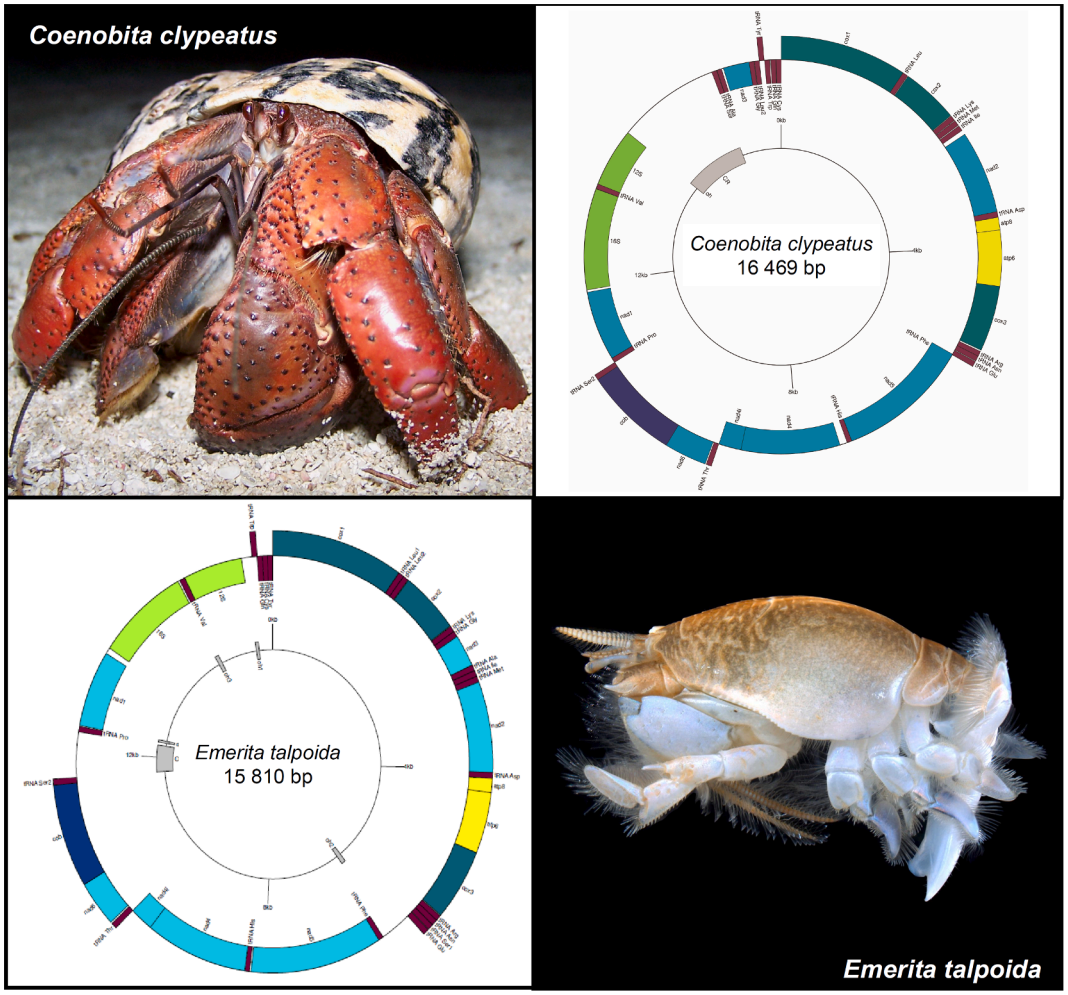

Fig. 1. Circular mitochondrial genome maps of the terrestrial hermit crab, *Coenobita clypeatus*, and the mole crab, *Emerita talpoida*. The annotated maps depict 13 protein-coding genes (PCGs), two ribosomal RNA genes (rrnS: *12S* ribosomal RNA and rrnL: *16S* ribosomal RNA), 22 transfer RNA (tRNA) genes, and the putative control region. Photo credit: *Coenobita clypeatus* photo by Antonio Baeza. "*Emerita talpoida* (YPM IZ 085053); photo by Eric A. Lazo-Wasem 2016-10-06″ - *Emerita talpoida* (Say, 1817) 2020 Collected in United States of America (licensed under https://creativecommons.org/publicdomain/zero/1.0/).

two mitochondrial genomes were analyzed. Nucleotide composition of each mitochondrial chromosome was estimated in the software MEGA X (Kumar et al., 2018). Codon usage was estimated using the invertebrate mitochondrial code in the web server Sequence Manipulation Suite (SMS) (https://www.bioinformatics.org/sms2/codon_usage.html - Stothard, 2018). Relative synonymous codon usage (RSCU) of all (concatenated) protein coding genes was estimated and visualized using the EZcodon tool in the web server EZmito (https://ezmito.unisi.it/ezcodon - Cucini et al., 2021).

In order to test for selective pressures in the different PCGs, values of K_A (the number of non-synonymous substitutions per non-synonymous site: $K_A = d_N = SA/L_A$), K_S (number of synonymous substitutions per synonymous site: $K_S = d_S = S_S/L_S$) and ω (the ratio K_A/K_S) were estimated with the software K_AK_S_Calculator2.0 (Wang et al., 2010). The K_A/K_S ratio is considered a measure of selective pressures acting on a gene; it indicates neutrality when $K_A/K_S = 1$, negative or purifying selection when $K_A/K_S < 1$, and positive or diversifying selection when $K_A/K_S > 1$ (Wang et al., 2009b). These values were calculated based on a pairwise comparison between *C. clypeatus* and the congeneric *C. brevimanus* (GenBank: MK310257) and between *E. talpoida* and the cofamilial mole crab *Stemonopa insignis* (GenBank: KY352240). The γ-MYN model (Wang et al., 2009a) was used during calculations to account for variable mutation rates across sequence sites. Significant differences ($P < 0.05$) in the ω were calculated by the software K_AK_S_Calculator2.0 to indicate either negative (purifying) selection or positive (diversifying) selection.

The transfer RNAs genes (tRNA) were identified using the software MiTFi (Jühling et al., 2012) as implemented in the MITOS web server. The secondary structure of each tRNA gene was visualized with the tool Forna in the web server ViennaRNA (https://rna.tbi.univie.ac.at/forna/ - Kerpedjiev et al., 2015).

The putative control region (CR) in the two studied mitochondrial genomes was also studied in more detail. MEGA X was used to analyze its nucleotide composition and the web server RNA Secondary Structure Prediction (https://tandem.bu.edu/trf/trf.html - Reuter and Mathews, 2010) was used to predict the secondary structure of this region and to identify hairpin structures along the sequence. The web servers Microsatellite Repeats Finder (https://insilico.ehu.es/mini_tools/microsatellites/ - Bikandi et al., 2004) and Tandem Repeats Finder (https://tandem.bu.edu/trf/trf.html - Benson, 1999) were also used to detect microsatellite and satellite sequences, respectively, in this region.

2.5. Phylogenetic position of the Anomura

We examined the phylogenetic position of the hermit crab *C. clypeatus* and the mole crab *E. talpoida* based on translated PCGs. We used amino acids instead of nucleotides in our phylogenetic analyses considering that the former characters have a higher phylogenetic

Table 1
Mitochondrial genome of *Coenobita clypeatus*. Arrangement and annotation.

Name	Type	Start	Stop	Strand	Length (bp)	Start	Stop	Anticodon	Continuity
cox1	PCG	1	1534	+	1534	ATG	T(AA)		0
tRNA Leu	tRNA	1535	1600	+	66			tag	7
cox2	PCG	1608	2297	+	690	ATG	TAA		7
tRNA Lys	tRNA	2305	2369	+	65			ttt	7
tRNA Met	tRNA	2377	2443	+	67			cat	10
tRNA Ile	tRNA	2454	2519	+	66			gat	55
nad2	PCG	2575	3573	+	1000	ATT	T(AA)		0
tRNA Asp	tRNA	3575	3643	+	69			gtc	0
atp8	PCG	3644	3802	+	159	ATT	TAG		−7
atp6	PCG	3796	4470	+	675	ATG	TAA		−1
cox3	PCG	4470	5261	+	792	ATG	TAG		16
tRNA Arg	tRNA	5278	5340	+	63			tcg	−1
tRNA Asn	tRNA	5340	5405	+	66			gtt	7
tRNA Glu	tRNA	5413	5478	+	66			ttc	2
tRNA Phe	tRNA	5481	5546	–	66			gaa	1
nad5	PCG	5548	7272	–	1725	ATG	T(AA)		0
tRNA His	tRNA	7273	7338	–	66			gtg	99
nad4	PCG	7438	8778	–	1341	ATG	TAA		−7
nad4l	PCG	8772	9074	–	303	ATG	TAA		2
tRNA Thr	tRNA	9077	9141	+	65			tgt	13
nad6	PCG	9155	9688	+	534	ATG	TAA		43
cob	PCG	9669	10,803	+	1071	ATG	T(AA)		1
tRNA Ser2	tRNA	10,804	10,869	+	66			tga	−1
tRNA Pro	tRNA	10,869	10,935	–	67			tgg	1
nad1	PCG	10,937	11,875	–	939	GTG	TAA		30
16S	rRNA	11,906	13,271	–	1366				2
tRNA Val	tRNA	13,274	13,341	–	68			tac	−2
12S	rRNA	13,340	14,133	–	794				0
CR		14,134	15,523	+	1390				0
[OH]		14,169	14,249	+	81				
tRNA Ser	tRNA	15,524	15,589	–	68			tct	3
tRNA Ala	tRNA	15,595	15,659	–	65			tgc	26
nad3	PCG	15,686	16,036	–	351	ATG	TAA		0
tRNA Gly	tRNA	16,037	16,102	–	66			tcc	5
tRNA Leu2	tRNA	16,108	16,174	–	67			taa	−1
tRNA Tyr	tRNA	16,174	16,240	+	67			gta	4
tRNA Trp	tRNA	16,245	16,313	–	69			tca	14
tRNA Gln	tRNA	16,328	16,395	–	68			ttg	4
tRNA Cys	tRNA	16,400	16,466	–	67			gca	3

information to noise ratio compared to nucleotides to reveal monophyletic clades deep in evolutionary time (Ahyong et al., 2009). Phylogenetic analyses were conducted using the two newly assembled and annotated mitochondrial genomes plus those of 42 species available in GenBank, including 37 anomurans belonging to 13 families (Pylochelidae, Lomisidae, Albuneidae, Chirostylidae, Aeglidae, Lithodidae, Coenobitidae, Paguridae, Diogenidae, Porcellanidae, Munidopsidae, Munididae, and Kiwaidae) and five species of brachyuran crabs (infraorder Brachyura) used as outgroups. We conducted a maximum likelihood (ML) analysis using the software MitoPhAST (Tan et al., 2015). MitoPhAST first extracted all PCG nucleotide sequences from species available on GenBank and others provided by the user (e.g., *C. clypeatus* and *E. talpoida*), translated each PCG nucleotide sequence to amino acids, conducted alignments for each PCG amino acid sequence using Clustal Omega (Sievers and Higgins, 2014), removed poorly aligned regions with trimAl (Capella-Gutiérrez et al., 2009), partitioned the dataset and selected best fitting models of sequence evolution for each PCG with ProtTest (Darriba et al., 2011), and used the concatenated and partitioned PCG amino acid alignments to perform a ML analysis in the software IQ-TREE version 1.6.10 (Nguyen et al., 2015). The robustness of the ML tree topology was assessed by 1,000 bootstrap iterations of the observed data.

3. Results and discussion

The software NOVOPlasty assembled and circularized the mitochondrial genome of the hermit crab *C. clypeatus* (ON203128) and the mole crab *E. talpoida* (ON164669) with a coverage of 226x and 242x, respectively. In *C. clypeatus* and *E. talpoida*, the length of the mitochondrial genome is 16,469 bp and 15,810 bp, respectively. The studied mitochondrial genomes were compact with few intergenic spaces and overlaps among gene junctions in *C. clypeatus* (Fig. 1a, Table 1); and with a few relatively long intergenic spaces (and gene overlaps) in *E. talpoida* (Fig. 1b, Table 2). In the two species, the mitochondrial genome comprised 13 protein-coding genes (PCGs), two ribosomal RNA genes (*rrnS* [*12S* ribosomal RNA] and *rrnL* [*16S* ribosomal RNA]), and 22 transfer RNA (tRNA) genes. A relatively long intergenic space with a length equal to 1,390 and 553 bp in *C. clypeatus* and *E. talpoida*, respectively, was assumed to be the D-loop/Control Region (Tables 1, 2).

In *C. clypeatus,* most (n = 8) of the PCGs and 11 tRNA genes were encoded on the heavy strand, while only five PCGs (in order from 5′ to 3′: *nad3*, *nad1*, *nad4l*, *nad4*, and *nad5*), the two rRNA genes, and the remaining 11 tRNA genes (*tRNA-Cys*, *tRNA-Gln*, *tRNA-Trp*, *tRNA-Leu2*, *tRNA-Gly*, *tRNA-Ala*, *tRNA-Ser1*, *tRNA-Val, tRNA-Pro, tRNA-His* and *tRNA-Phe*) were encoded in the light strand (Fig. 1a and Table 1). In *E. talpoida*, most of the PCGs and tRNA genes were encoded on the heavy strand while only four PCGs (in order from 5′ to 3′: *nad1*, *nad4l*, *nad4*, and *nad5*), the two rRNA genes, and seven tRNA genes (*tRNA-Tyr*, *tRNA-Cys*, *tRNA-Gln*, *tRNA- Val*, *tRNA-Pro*, *tRNA-His* and *tRNA-Phe*) were encoded in the light strand (Fig. 1b and Table 2).

Mitochondrial gene order observed in all hermit crabs *Coenobita* spp., including the studied species *C. clypeatus*, is the same, and mitochondrial synteny in these hermit crabs is also identical to that reported before in the closely related 'robber' or 'coconut' crab *Birgus latro* (Fig. S1) (Tan et al., 2018; Wang et al., 2019; Gong et al., 2020; Veldsman et al., 2020). In turn, gene order in *E. talpoida* is identical to that reported before in the

Table 2
Mitochondrial genome of *Emerita talpoida*. Arrangement and annotation.

Name	Type	Start	Stop	Strand	Length (bp)	Start	Stop	Anticodon	Continuity
cox1	PCG	1	1534	+	1534	ACG	T(AA)		0
tRNA Leu	tRNA	1535	1600	+	66			tag	0
tRNA Leu2	tRNA	1601	1665	+	65			taa	5
cox2	PCG	1671	2357	+	687	ATG	TAA		7
tRNA Lys	tRNA	2365	2430	+	66			ttt	0
tRNA Gly	tRNA	2431	2495	+	65			tcc	0
nad3	PCG	2496	2849	+	354	ATT	TAA		1
tRNA Ala	tRNA	2851	2914	+	64			tgc	2
tRNA Ile	tRNA	2917	2980	+	64			gat	1
tRNA Met	tRNA	2982	3047	+	66			cat	0
nad2	PCG	3048	4043	+	996	ATT	TAA		−2
tRNA Asp	tRNA	4042	4106	+	65			gtc	0
atp8	PCG	4107	4265	+	159	ATG	TAG		−7
atp6	PCG	4259	4933	+	675	ATG	TAA		−1
cox3	PCG	4933	5724	+	792	ATG	TAA		9
tRNA Arg	tRNA	5734	5796	+	63			tcg	−2
tRNA Asn	tRNA	5795	5861	+	67			gtt	1
tRNA Ser1	tRNA	5863	5928	+	66			tct	5
tRNA Glu	tRNA	5934	6002	+	69			ttc	210
OH		6213	6306	+	94				100
tRNA Phe	tRNA	6407	6473	–	67			gaa	−1
nd5	PCG	6473	8185	–	1713	ATA	TAA		18
tRNA His	trna	8204	8267	–	64			gtg	0
nad4	PCG	8268	9603	–	1336	ATG	T(AA)		−7
nad4l	PCG	9597	9899	–	303	ATG	TAA		2
tRNA Thr	tRNA	9902	9967	+	66			tgt	19
nad6	PCG	9987	10,496	+	510	ATT	TAA		−1
cob	PCG	10,496	11,647	+	1152	ATG	TAA		−17
tRNA Ser2	tRNA	11,631	11,697	+	67			tga	0
CR		11,698	12,250	+	553				0
tRNA Pro	tRNA	12,251	12,317	–	67			tgg	−4
OH		12,314	12,363	+	50				−34
nad1	PCG	12,330	13,268	–	939	ATG	TAA		75
16S	rrna	13,344	14,543	–	1200				−41
OH		14,503	14,584	+	82				−18
tRNA Val	tRNA	14,567	14,638	–	72			tac	1
12S	rrna	14,640	15,406	–	767				21
OH		15,428	15,496	+	69				47
tRNA Trp	tRNA	15,544	15,609	+	66			tca	−3
tRNA Gln	tRNA	15,607	15,674	–	68			ttg	3
tRNA Cys	tRNA	15,678	15,742	–	65			gca	0
tRNA Tyr	tRNA	15,743	15,808	–	66			gta	202

cofamilial mole crab *Stemonopa insignis* (Fig. S1) (Tan et al., 2018). Mitochondrial synteny varies mostly above the genus level in anomuran crustaceans (Yang and Yang, 2008; Kim et al., 2013; Sun et al., 2019; Tan et al., 2018; Gong et al., 2019, 2020).

The overall nucleotide composition of the mitochondrial genome in *C. clypeatus* was as follows: A = 28.1 %, T = 37.4 %, C = 14.4 %, and G = 20.2 %, with a 65.5 % AT-content and a 34.6 % GC-content. In *E. talpoida*, overall nucleotide composition of the mitochondrial genome was as follows: A = 36.2 %, T = 35.9 %, C = 16.0 %, and G = 11.8 %, with a 72.1 % AT-content and a 27.8 % GC-content. Overall AT-content observed in the mitochondrial genomes of the two studied species is within the range described for other anomuran crustaceans (Table S1).

In the mitochondrial genome of *C. clypeatus* and *E. talpoida*, PCGs comprise a total of 11,277 and 11,148 codons, respectively. In *C. clypeatus*, the start codon most frequently used was ATG (10 PCGs used it: *cox1, cox2, atp6, cox3, nad5, nad4, nad4l, nad6, cob, nad3*). Two PCGs used ATT (*nad2, atp8*) and a single PCG (*nad1*) used GTG as a start codon. In turn, the stop codon most frequently used was TAA (10 PCG: *cox1, cox2, nad2, atp6, nad5, nad4, nad4l, nad6, nad1, nad3*), two PCGs used TAG (*atp8, cox3*) and one PCG presented a truncated stop codon T (*cob*) (Table 1). The observed use of start and stop codons agree with that reported before in other congeneric species (e.g., *C. variabilis* [KY352236.2], *C. rugosus* [MN030161], *C. perlatus* [KY352234], and *C. brevimanus* [NC_050386, MK310257, KY352233]). The alternative start codon GTG in *nad1* is also commonly observed in the aforementioned congeneric species as well as in the cofamilial coconut crab *Birgus latro* (Veldsman et al., 2020). In turn, in the mole crab *E. talpoida*, ATG was also the most frequently used start codon (present in eight PCGs: *cox2, atp8, atp6, cox3, nad4, nad4l, cob, nad1*). Three PCGs used ATT (*nad3, nad2, nad6*), one PCG used ATA (*nad5*), and another PCG used AGC (*cox1*) as start codons. Two different stop codons were used by PCGs in *E. talpoida*. TAA was found in 10 PCGs (*cox2, nad3, nad2, atp6, cox3, nad5, nad4l, nad6, cob, nad1*) while TAG was used in a single PCG (*atp8*). Also, two truncated stop codons (T) were detected; in *cox1* and *nad4* (Table 2). Incomplete stop codons are likely the result of post transcriptional polyadenylation (Yang and Yang, 2008; Sun et al., 2019; Gong et al., 2019; Hickerson and Cunningham, 2000).

Relative synonymous codon usage (RSCU) and amino acid composition in the PCGs of *C. clypeatus* and *E. talpoida* are summarized in Fig. 2. The most frequently used codons (amino acids) in *C. clypeatus* were: TTT (Phe) used 236 times (69 %), ATT (Ile) used 225 times (73 %) and TTA (Leu) used 186 times (32 %). Codons (amino acids) that were the least commonly used (excluding stop codons) included TGC (Cys) used 11 times (28 %) and CCG (Pro) used 11 times (8 %). In *E. talpoida*, the most frequently used codons were TTA (Leu), used 335 times (59 %), ATT (Ile), used 301 times (87 %) ans TTT (Phe), used 272 times (84 %) whereas the least common were CGC (Arg) used one time (2 %), CGG (Arg) used one time (3 %) and TCG (Ser) used two times (1 %). RSCU and amino acid composition of PCGs have been reported before in a few other anomurans (e.g., *P. nigrofascia* - Gong et al., 2019; *P. longicarpus* - Hickerson and Cunningham, 2000; *M. laurensis* and *M. verrilli* - Sun et al., 2019; *B. latro* - Veldesman et al., 2020; *C. brevimanus* - Gong et al., 2020).

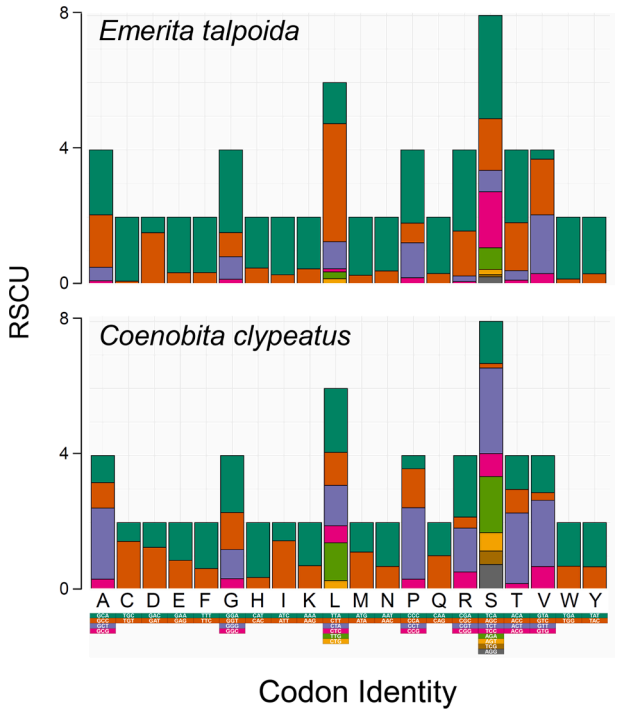

Fig. 2. Relative synonymous codon usage (RSCU) in *Coenobita clypeatus* and *Emerita talpoida*.

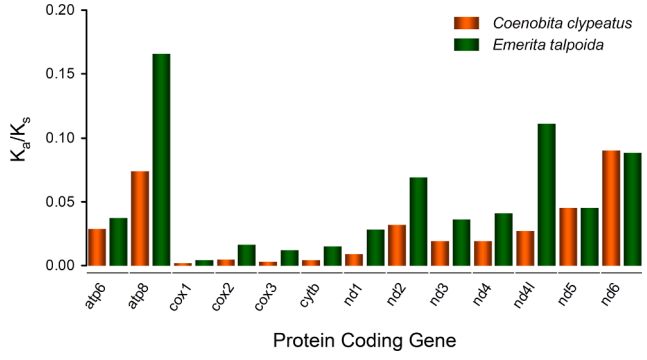

Fig. 3. Selective pressure analysis in the protein coding genes of *Coenobita clypeatus* and *Emerita talpoida*.

In all of these anomuran species, including *C. clypeatus* and *E. talpoida*, the most commonly used amino acids are Leu, Phe, and Ile. The abundance of A- and *T*-rich codons is congruent with the high AT-content observed in this study as well as in other anomuran crabs (Gong et al., 2020).

The Ka/Ks ratio calculated for each mitochondrial PCG in *C. clypeatus* and *E. talpoida* exhibits values < 0.17 (p value < 0.05 in all cases). In *C. clypeatus*, the *nad6* gene showed the highest Ka/Ks value (0.090), followed by *atp8* gene (0.074), while the *cox* family genes (*cox1* – 0.002, *cox2* – 0.005, *cox3* – 0.003) and *cob* (0.004) featured the lowest values (Fig. 3). Similarly, in *E. talpoida*, the *atp8* gene showed the highest value (0.166) and the *cox* family genes (*cox1* – 0.004, *cox2* – 0.016, *cox3* – 0.012) and *cob* (0.015) exhibited the lowest values (Fig. 3). In general, the observed Ka/Ks values indicate that, in the two studied anomuran crabs, all mitochondrial PCGs experience purifying selection and that this purifying selection is stronger in some (i.e., *cox* family genes and *cob*) compared to other PCGs (e.g., *atp8*). Selective pressure in PCGs have rarely been conducted in other anomuran crabs; the exceptions are the coconut crab *B. latro* and two species of hermit crabs belonging to the genus *Dardanus* (Veldesman et al., 2020; Zhang et al., 2021). In the latter three species, the same pattern observed in this study was reported: *atp8* exhibits the highest and the *cox* family genes exhibit the lowest Ka/Ks values (Veldesman et al., 2020; Zhang et al., 2021).

In the mitochondrial genome of *C. clypeatus*, 19 out of the 22 tRNA genes exhibited a 'cloverleaf' secondary structure (Fig. 4). Two tRNA genes, *tRNA-Phe* and *tRNA-Tyr*, exhibit a deletion of the dihydroxyuridine (DHU) loop, having only its arm while *tRNA-Ser1* lacks the thymine pseudouracil cytosine (TΨC) loop (Fig. 4). In *E. talpoida*, 19 out of the 22 tRNA genes exhibited a cloverleaf secondary structure (Fig. 5). *tRNA-Phe* and *tRNA-Arg* featured a deletion of the dihydroxyuridine (DHU) loop, having only its arm while *tRNA-Ser1* lack the thymine pseudouracil cytosine (TΨC) arm, having only its loop. tRNA secondary structure conservation varies considerably in the Anomura. For instance, at one extreme, in the squat lobster *Shinkaia crosnieri,* all tRNA genes exhibit the classical cloverleaf structure with the exception of tRNA-Ser that have a extremely short TΨC arm. This truncation of *tRNA-Ser* (either a short TΨC arm as we have observed in *E. talpoida* or the completely deletion of the TΨC arm, as we have observed in *C. clypeatus*) (Yang and Yang, 2008) is characteristics of eumetazoans (Bernt et al., 2013). On the other extreme, 14 out of the mitochondrial 22 tRNAs are missing at least one arm in the coconut crab *Birgus latro* (Veldesman et al., 2020). Whether or not truncated tRNA are functional remains to be addressed in the Anomura.

In *C. clypeatus* and *E. talpoida*, the two rRNA genes are located in the L-strand. In *C. clypeatus*, the *16S* rRNA, located between *nad1* and *tRNA-Val*, is 1,366 bp long while the *12S* rRNA, located between *tRNA-Val* and the CR, is 794 bp long. The AT-content estimated for the *16S* and *12S* rRNA is 71.1 % and 70.5 %, respectively. In turn, in *E. talpoida,* the *16S* rRNA, located between *nad1* and *tRNA-Val*, is 1,200 bp long while the *12S* rRNA, located between *tRNA-Val* and *tRNA-Trp*, is 767 bp long. The AT-content estimated for the *16S* and *12S* rRNA is 75.3 % and 75.2 %, respectively. Nucleotide usage of the rRNAs has rarely been reported in anomurans (Yang and Yang, 2008; Sun et al., 2019; Zhang et al., 2021). Still, the scarce available information indicates that the two studied species exhibit an AT-content within the range reported before for anomura crabs. In the infraorder Anomura, *Coenobita rugosus* (*16S* rRNA = 69.4 %, *12S* rRNA = 68.0 % - Tan et al., 2018) exhibits the lowest AT-content while the highest AT content has been reported for the yeti crab *Kiwa tyleri* (*16S* rRNA = 83.9 % and, *12S* rRNA = 83.1 % - Zhang et al., 2017).

In *C. clypeatus*, the 1,390 bp-long putative control region (CR) is located between the *12S* and *tRNA-Ser2* genes (Fig. 1), starting at position 14,134 and ending at position 15,523. The length of this non-coding region is similar to that previously reported for other species belonging to the family Coenobitidae (range: 1,368–1,381 bp - Tan et al., 2018). Also, the location of the CR in the mitochondrial genome of *C. clypeatus* is identical to that reported before for congeneric (i.e., *C. brevimanus*, *C. perlatus*, *C. rugosus*, and *C. variabilis* - Tan et al., 2018), cofamilial species (e.g., *Birgus latro*,- Veldesman et al., 2020), and other hermit crabs belonging to the family Diogenidae (*Dardanus arrosor* and *D. aspersus* - Zhang et al., 2021) within the same superfamily Paguroidea to which *C. clypeatus* belongs (Fig. S1).

In turn, in the mole crab *E. talpoida*, a 553 bp-long non-coding region is located between the *tRNA-Ser2* and *tRNA-Pro* genes (Fig. 1), starting at position 11,698 and ending at position 12,250 (Table 2). The location and length of this region is similar to the CR reported in *Stemonopa insignis* (605 bp - Tan et al., 2018), the only other species belonging to the family Albuneidae with a published mitochondrial genome (Fig. S1).

The ML phylogenetic tree (45 terminals, 3,641 amino acid characters, and 1,829 informative sites) confirmed the monophyly of the infraorder Anomura given that the two studied anomuran crabs together with all other anomuran species included in the analysis clustered into a single fully supported (bootstrap value [bv] = 100) clade (Fig. 6).

Within the Anomura, the mole crabs *Emerita talpoida* and *Stemonopa*

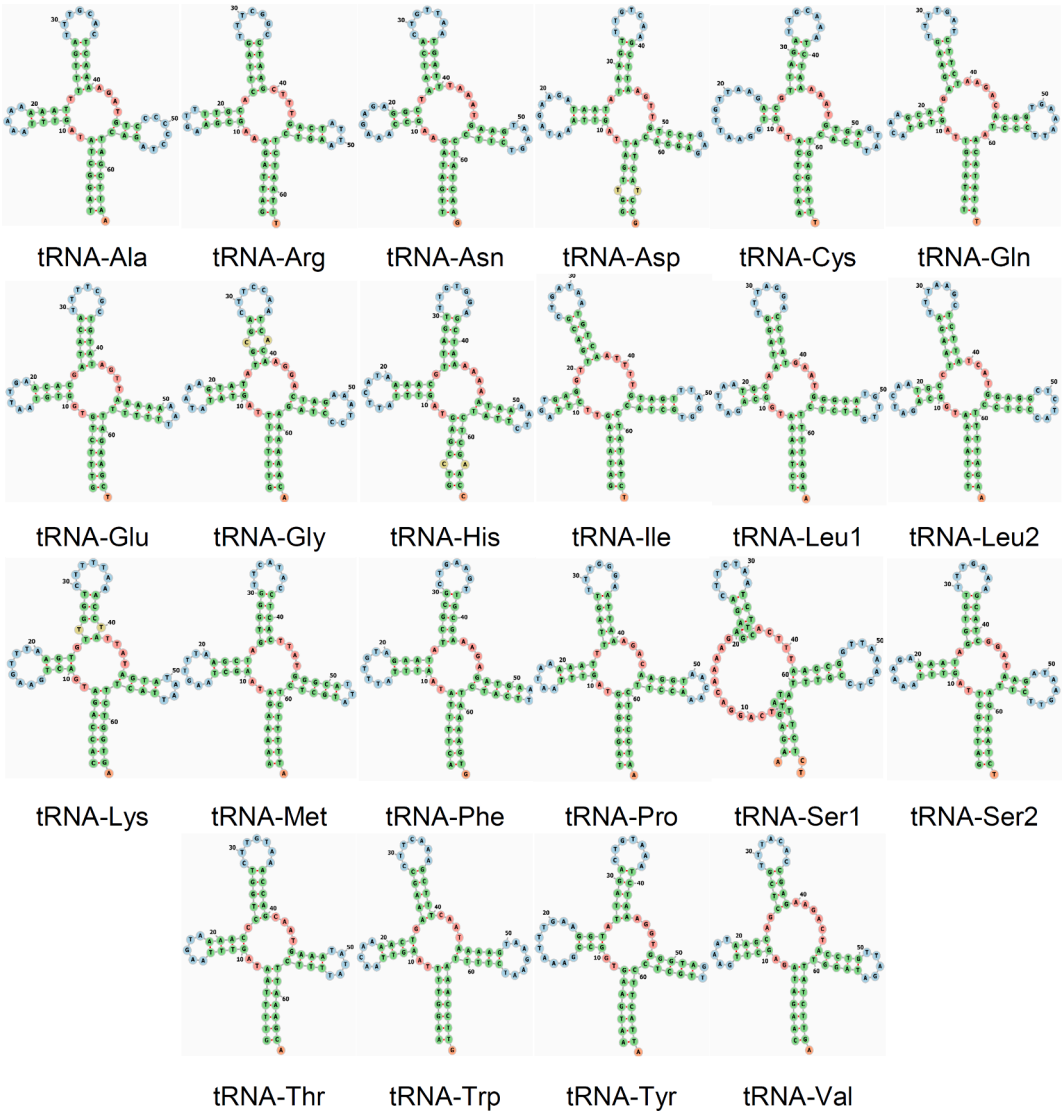

Fig. 4. Secondary structures of 22 transfer RNA genes in *Coenobita clypeatus*.

insignis clustered into a single fully supported clade (superfamily Hippoidea, family Albuneidae) that was sister to a second well supported clade (bv = 96) containing all other anomuran species included in our analysis (representing 14 families and 6 superfamilies). In the latter clade, representatives of the families Diogenidae (left-handed hermit crabs, represented by *Dardanus* spp. and *Clibanarius infraspinatus* in our analysis) and Coenobitidae (terrestrial hermit crabs, represented by *Coenobita* spp. and the coconut crab *Birgus latro*) clustered into a fully supported clade. Within this clade, *C. clypeatus* clustered together with all other congeneric species into a fully supported clade sister to *Birgus latro*, in line with previous studies (Bracken-Grissom et al., 2013; Tan et al., 2018), and additionally providing support for the monophyletic status (bv = 100) of the family Coenobitidae. In contrast to that observed for representatives of the Coenobitidae, *Dardanus* spp. and *Clibanarius infraspinatus*, all belonging to the family Diogenidae, did not clustered together into a monophyletic clade. The ML tree topology indicated that *Dardanus* spp. was sister to Coenobitidae (represented by *Coenobita* + *Birgus* in our analysis). However, the sister relationship between *Dardanus* and Coenobitidae was poorly supported by the bootstrap value (bv = 46). In turn, *Clibanarius infraspinatus*, was found to be sister to Coenobitidae + *Dardanus* spp. (bv = 100). The aforementioned relationships indicate that the family Diogenidae is not monophyletic, in line with recent findings by Zhang et al. (2021) and older studies (Bracken-Grissom et al., 2013).

In the ML tree, the monophyletic status of the family Lithodidae, represented by the genera *Lithodes*, *Paralithodes*, and *Cryptolithodes* in our analysis, was fully supported. This result agrees with Gong et al. (2019). In turn, the monophyletic status of the family Paguridae (represented by 10 species in our analysis) was not supported, considering that two species of *Pagurus*, *P. longicarpus* and a second unidentified species in Sultana et al. (2018), did not clustered together with the rest of its congeneric species, that in turn, comprised a fully supported clade sister to the family Lithodidae supported with bv = 72. The paraphyletic status of *Pagurus* and the family Paguridae has been called into question before (Hwang et al., 2019; (Zhang et al., 2021)).

The deep-water symmetrical hermit crab *Pylocheles mortensenii* (superfamily Paguroidea, family Pylochelidae) did not cluster together with any other representative of its own superfamily represented in our

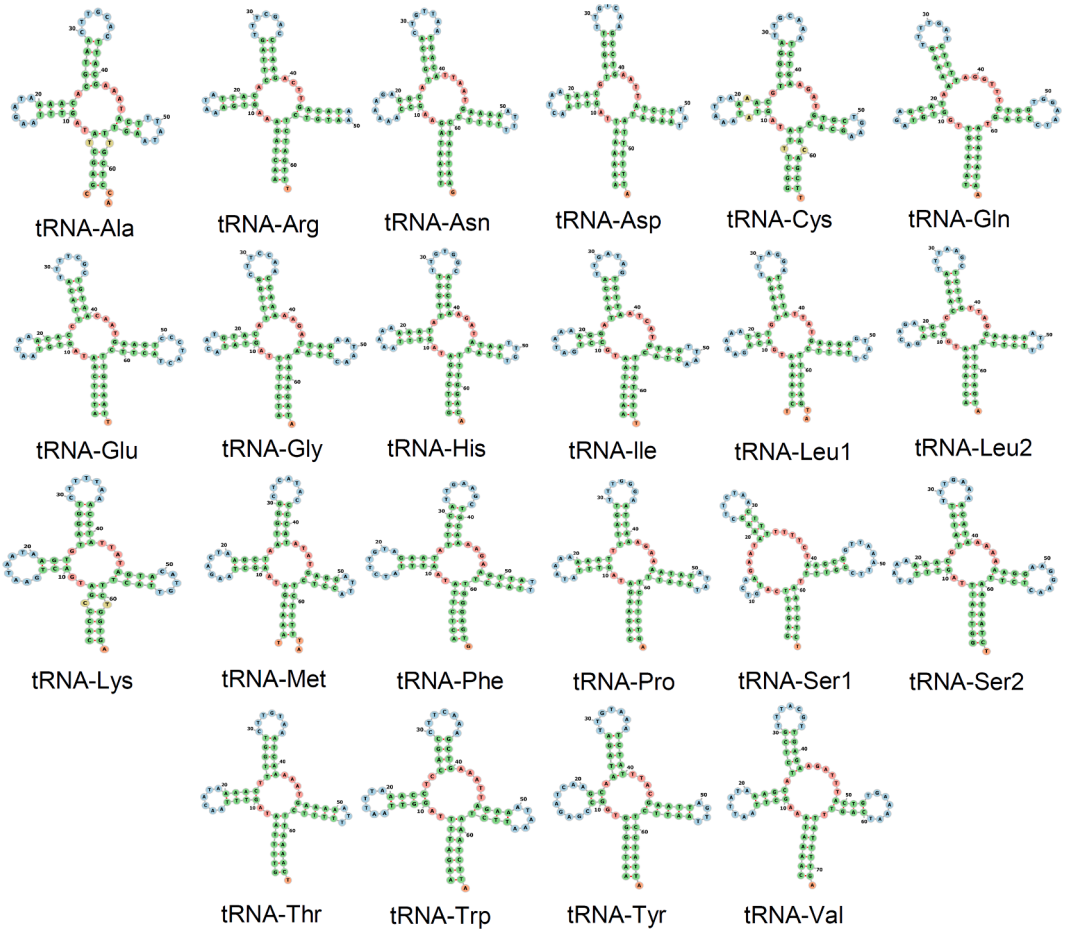

Fig. 5. Secondary structures of 22 transfer RNA genes in *Emerita talpoida*.

analysis, but instead, was found in a position sister to a fully supported clade comprised of all species belonging to the superfamily Galatheoidea, represented by 5 genera and 3 families, Munididae (squat lobsters), Munidopsidae (squat lobsters-like), and Porcellanidae (porcelain crabs), in our analysis. However, the sister relationship between *Pylocheles mortensenii* and Galatheoidea was poorly supported by the bootstrap value (bv = 45). Still, the phylogenetic placement of *Pylocheles* out of the Galatheoidea has been previously noticed by other researchers (Tsang et al., 2011). In the Galatheoidea, Munididae, represented by two species of *Munida* in our analysis, was sister to Munidopsidae, represented by 2 species of *Munidopsis* and *Shinkaia crosneiri* in our analysis. In turn, the family Porcellanidae was sister to Munididae + Munidopsidae.

Lastly, representatives of the families Kiwaidae (*Kiwa tyleri*), Chirostylidae (*Gastroptychus* spp.), Lomisidae (*Lomis hirta*), and *Aegla* aff. *longirostris* (Aeglidae) clustered into a single fully supported monophyletic clade. In this clade, Kiwaidae was sister to Chirostylidae (bv = 100). In turn, the tree topology indicated a sister relationship between Lomisidae and Aeglidae. However, the latter sister relationship was only moderately supported by the bootstrap value (bv = 67).

4. Conclusions

This study sequenced and characterized the mitochondrial genome of the hermit crab *Coenobita clypeatus* and the mole crab *Emerita talpoida*. Characterization of the complete mitochondrial genomes of *C. clypeatus* and *E. talpoida* enhances the genomic resources available for the infraorder Anomura and the families Paguroidea and Hippoidea, respectively. Selective pressure analysis in PCGs, rarely conducted in anomuran crabs, indicate that all these mitochondrial PCGs experience purifying selection and that this purifying selection is stronger in some (i.e., *cox* family genes and *cob*) compared to other PCGs (e.g., *atp8*). A phylogenomic analysis based on translated PCGs confirms the monophyly of the infraorder Anomura and retrieves most/all relationships at the superfamily and family level previously reported for anomurans. The analysis supports the monophyletic status of the families Albuneidae, Lithodidae, Coenobitidae, and Porcellanidae. In turn, the superfamily Paguroidea, and the families Paguridae and Diogenidae are polyphyletic.

CRediT authorship contribution statement

Angélica Colín: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Carolina Galván-Tirado:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Laura Carreón-Palau:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Heather D. Bracken-Grissom:** Methodology, Validation, Investigation, Resources, Writing – original draft. **J. Antonio Baeza:** Conceptualization, Methodology, Formal analysis,

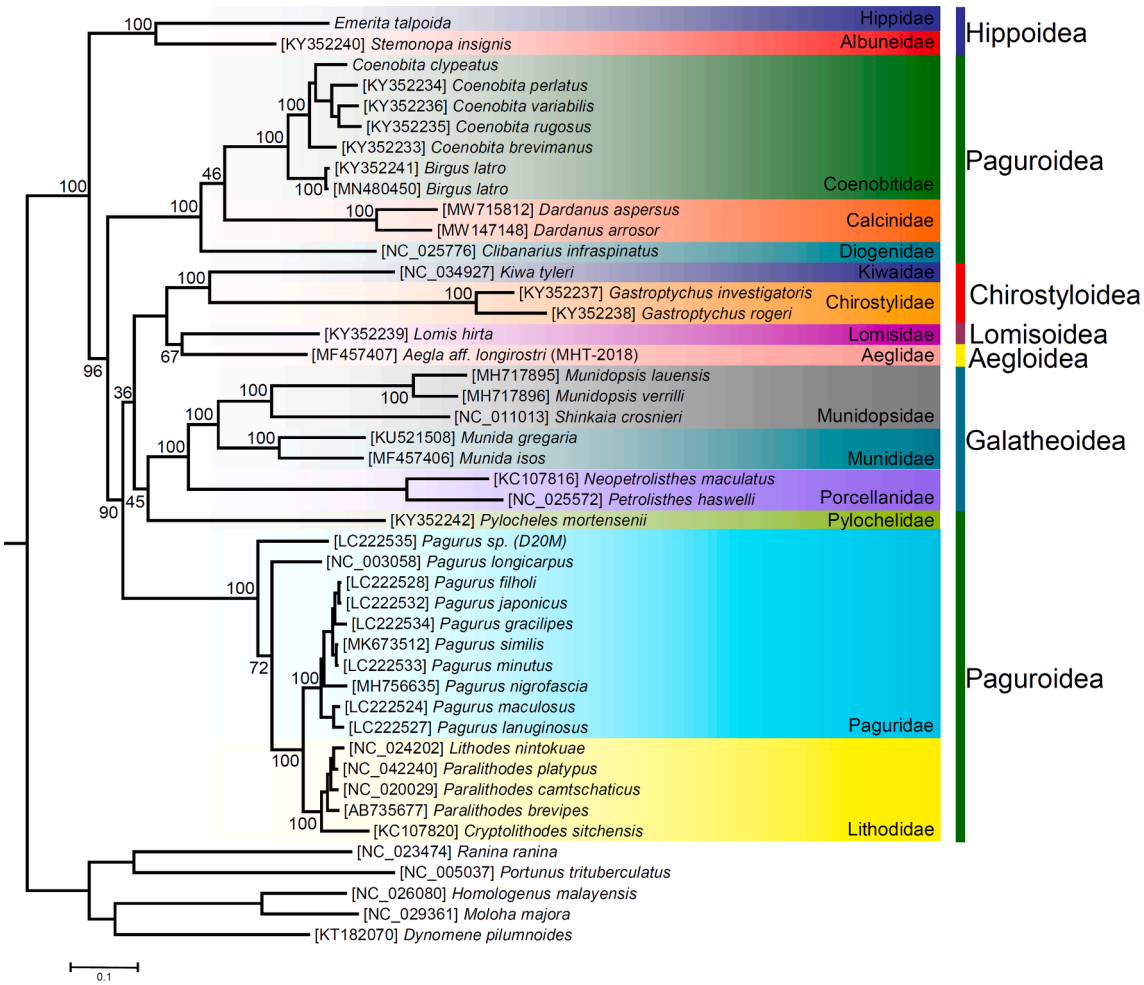

Fig. 6. Total evidence phylogenetic tree obtained from ML analysis based on a concatenated alignment of amino acids of the 13 protein-coding genes present in the mitochondrial genome of the terrestrial hermit crab *Coenobita clypeatus*, the mole crab *Emerita talpoida*, and other representatives of the infraorder Anomura. Outgroups included five species belonging to the infraorder Brachyura (true crabs). The robustness of the ML tree topology was ascertained by 1,000 bootstrap pseudoreplicates (numbers above or below the nodes) of the tree search.

Validation, Investigation, Data curation, Writing – original draft, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data has been deposited in GenBank and that information is clearly indicated in the text of the manuscript

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2022.146896.

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