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The Complete Mitogenome of *Xeruca formosensis* (Rathbun, 1921) (Crustacea: Brachyura: Ocypodidae), a Fiddler Crab Endemic to Taiwan, with its Phylogenetic Position in the Family

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Xeruca formosensis is a species and genus of fiddler crab endemic to Taiwan, with limited distribution in western Taiwan and the offshore Penghu Islands. This study reports the complete mitochondrial genome (mitogenome) of this species using next-generation sequencing. The mitogenome contains 15,684 bp, comprising 13 protein-coding genes, 22 tRNAs, 2 ribosomal RNAs and a 750-bp intergenic space (control region). The nucleotide composition is biased toward A+T (69.4%). A phylogenetic analysis based on the concatenated protein-coding genes showed that the genera *Xeruca* Shih, 2015 and *Tubuca* Bott, 1973 are sister to each other. In addition, the phylogeny of the 16 available mitogenomes in the family Ocypodidae also supports the current systematics of this family based on one nuclear and two mitochondrial markers. As this species inhabits high intertidal mudflats with high temperature and high salinity, mitogenome analyses may help us understand the mechanisms of adaptation to extreme environments, as well as the connectivity of metapopulations based on mitogenomes from different populations.

Key words: Mitochondrial genome, Phylogenetic analysis, Endemic species and genus, Taiwan, Next generation sequencing.

BACKGROUND

Xeruca formosensis (Rathbun, 1921) is a species and genus endemic to Taiwan, with a narrow distribution in western Taiwan and the Penghu Islands in the Taiwan Strait (Rathbun 1921; Crane 1975; Shih et al. 1999 2015 2016a; Shih 2015). It has previously been placed in the genus *Tubuca* Bott, 1973 and the subgenus *Uca* (*Thalassuca*) Crane, 1975 (Bott 1973; Crane 1975; Shih 1999), and its current genus is *Xeruca* Shih, 2015, based on morphological and molecular evidence (Shih 2015; Shih et al. 2016b). Other studies of its natural history have included larval morphology, behavior, ecology and conservation (Shih et al. 1999 2005; Shih 2015; TY Chen et al. 2017 2019; YC Zhang and Shih 2022).

Previous studies of the molecular phylogeny of *Xeruca*, other genera of fiddler crabs, and other members of the family Ocypodidae were based on the combined mitochondrial 16S rDNA and cytochrome oxidase subunit I (~1250 base pairs [bp]), as well as the nuclear 28S rDNA (~690 bp) (Shih 2015; Shih et al. 2016b). Recently, there have been more studies

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on the mitochondrial genomes (mitogenomes) of brachyurans (*e.g.*, Q Wang et al. 2021; Liu et al. 2022), including fiddler crabs and ghost crabs within the family Ocypodidae (Karagozlu et al. 2016; Sung et al. 2016; Tan et al. 2016; JQ Chen et al. 2018; Guo et al. 2019; TT Yang et al. 2019; Ting et al. 2020; ZQ Wang et al. 2020; Conrad et al. 2021). In Conrad et al. (2021), the phylogeny of mitochondrial genomes supports the systematics established in Shih et al. (2016b), *i.e.*, two subfamilies as well as two clades within Gelasiminae.

To understand whether the phylogeny of the family Ocypodidae based on mitogenomes is consistent to that based on the combined 16S, cox1 and 28S (Shih et al. 2016b), mitogenomes from more species are necessary. In this study, the complete mitogenome of *Xeruca formosensis* is described, and the phylogenetic position of *X. formosensis* within the Ocypodidae is analyzed.

MATERIALS AND METHODS

Sample collection, library preparation and sequencing

A specimen of *Xeruca formosensis* (carapace width 27.7 mm, carapace length 17.9 mm, Fig. S1) was collected from the Yangang River estuary in Hsinchu City, northwestern Taiwan on 9 June 2020. The specimen was preserved in 95% ethanol and deposited into the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (NCHUZOOL 15080). Total genomic DNA (gDNA) was extracted from the tissue of one walking leg using the Tissue and Cell Genomic DNA Purification Kit (BioKit, Taiwan) in the laboratory of the Taiwan Ocean Research Institute. DNA quality and concentration were measured with a NanoDrop 2000c (Thermo Scientific) and Qubit 2.0 Fluorometer (Thermo Scientific), respectively.

The gDNA was extracted for whole genome sequencing (WGS) by next-generation sequencing (NGS). An NGS library was generated using a Truseq nano DNA Library Prep Kit (Illumina, USA) following the manufacturer's recommendations. Index codes were added and the library was sequenced on an Illumina Miseq platform, generating 150 bp paired-end reads (Genomics BioSci and Tech, Taiwan).

Mitochondrial genome assembly, annotation and analysis

The WGS data were trimmed, paired and de novo assembled using CLC Genomics Workbench (vers. 12.0, QIAGEN). The assembled mitochondrial genomic sequence was annotated in MITOS2 web (Donath et al. 2019, http://mitos2.bioinf.uni-leipzig.de/index.py) with the invertebrate genetic code (code 5). The start and stop codons were checked manually and compared with those of other crab mitogenomes by the software program MEGA 11 (Tamura et al. 2021). The tRNA genes were searched by tRNAscan-SE (vers. 2.0, Lowe and Chan 2016, http://lowelab.ucsc.edu/tRNAscan-SE/index.html). A circular genome map was constructed with GenomeVx (Conant and Wolfe 2008, http://wolfe.ucd.ie/GenomeVx/).

To understand the phylogenetic relationships between *Xeruca formosensis* and other related species of the family, the concatenated nucleotide sequences of 13 protein-coding genes (PCGs) of *X. formosensis* and those of species available in GenBank (Table 1) were aligned (Table S1) by the MUSCLE function of MEGA 11 (Tamura et al. 2021), constructed by Bayesian inference (BI) and maximum likelihood (ML) analyses. As all the available mitogenomes of fiddler crabs belong to the Gelasiminae, their phylogenetic relationship could be revealed by treating the *Ocypode* species in the Ocypodinae as outgroups (see Shih et al. 2016b). The best model was GTR+I+G, determined by PartitionFinder (vers. 2.1.1, Lanfear et al. 2017) and selected by the Bayesian information criterion (BIC). This model was subsequently used for BI analysis, which was performed with MrBayes (vers. 3.2.6,

Table 1. Species of fiddler crabs and ghost crabs in the family Ocypodidae, with the NCBI accession numbers of the complete mitogenomes used in this study

Species	Accession numbers
Subfamily Gelasiminae	
Austruca lactea	KY865330
Austruca lactea	MH796169
Gelasimus borealis	MH183126
Gelasimus borealis	MH796170
Cranuca inversa	MF457405
Minuca minax	MT012731
Tubuca arcuata	KX911977
Tubuca capricornis	MF457401
Tubuca rosea	MN072632
Tubuca polita	MF457400
Tubuca paradussumieri	MN072633
Xeruca formosensis	OL693688
Subfamily Ocypodinae	
Ocypode ceratophthalmus	LN611669
Ocypode ceratophthalmus	MW255974
Ocypode stimpsoni	MN917464
Ocypode cordimana	KT896743

Ronquist et al. 2012). The search was run with four chains for 10 million generations and four independent runs, with trees sampled every 1000 generations. The convergence of chains was determined by the average standard deviation of split frequency values below the recommended threshold of 0.01 (Ronquist et al. 2019), and the first 4,000 trees were discarded as the burnin. The ML analysis was conducted in RAxML (vers. 7.2.6, Stamatakis 2006). The model GTR+G (*i.e.*, GTRGAMMA) was used for all subsets with 100 runs to find the best ML tree by comparing likelihood scores. The robustness of the ML tree was evaluated by 1,000 bootstrap pseudoreplicates using the model GTRGAMMA.

RESULTS

The WGS of *Xeruca formosensis* gDNA produced a raw data of 4,318,278 reads (including 4,128,240 unique reads and 190,038 duplicate reads, the raw data is available by request). The average read length was 301 bases. The raw data were trimmed and paired, and produced 4,180,046 reads. After de novo assembly, the complete mitogenome of *Xeruca formosensis* (GenBank accession number: OL693688) was 15,684 bp in length, including 13 protein coding genes (PCGs), 2 ribosomal RNA genes (12S ribosomal RNA, rrnS and 16S ribosomal RNA, rrnL), 22 transfer RNA (tRNA) genes and a 750-bp intergenic space (putative control region, D-loop). Nine of the 13 PCGs and 14 tRNA genes were on the plus strand, while four PCGs (nad5, nad4, nad4l and nad1), eight tRNA and two ribosomal RNA genes were encoded on the minus strand (Fig. 1). Five types of conventional invertebrate mitochondrial start codon (ATG, ATA, ATT, ATC and GTG) were found, but only nad6 and nad4 used start codons ATC and GTG, respectively. Twelve PCGs used TAA as the stop codon, and the nad2 gene terminated with TAG as the stop codon. The cox1 and cob genes also terminated with the TAA stop codon, which was completed by the addition of 3' A residues to the mRNA. Two ribosomal genes, rrnS and rrnL, contained 836 and 1340 bp, respectively, and were located close to each other with trnV between them. The length of 22 transfer RNA (tRNA) genes in the mitogenome of *Xeruca formosensis* ranged from 64 to 73 bp, similar to other mitogenome of confamiliar species in the phylogenetic analysis. There were 12 intergenic spaces (1–33 bp) and 14 overlapping gene junctions (1–25 bp) (Fig. 1, Table 2). A long intergenic space (750 bp), located between the rrnS and trnI genes, was assumed to be the D-loop/control region for the replication function of the mitogenome. The PCGs base composition was A = 24.4–28.8%, T = 33.7–48.5%, C = 6.6–23.4%, G = 7.5–20.6%. The overall base composition of the PCGs is as follows: A = 34.7%, T = 34.7%, C = 19.3%, and G = 11.3%, with an A + T bias (69.4%) (Table 3).

Based on the concatenated PCG sequences, the topologies of phylogenetic trees reconstructed by BI and ML were consistent and only the BI tree was shown (Fig. 2). It showed that there were two main clades in the Ocypodidae, which correspond to the two subfamilies Gelasiminae and Ocypodinae. Within the Gelasiminae, *Minuca minax* was identified to be sister to the clade composed of the Indo-West Pacific taxa, with a sister relationship identified between the genera *Gelasimus* and *Cranuca* as well as between *Xeruca* and *Tubuca*. In addition, *Tubuca paradussumieri* and *T. capricornis* as well as *Ocypode ceratophthalmus* and *O. stimpsoni* were identified as sister-species pairs.

DISCUSSION

In this study, the gene order of *Xeruca formosensis* (Fig. 1) is as the same as those of most confamiliar species, including the genera *Austruca*, *Cranuca*, *Gelasimus*, *Tubuca* and *Ocypode* (see Table 1 for the genera and species). An exception is *Minuca minax*, in which the positions of the trnQ and trnI genes are switched (Conrad et al. 2021: fig. 4). In the mitogenome of *X. formosensis*, the 13 PCGs use five types of start codons: ATG (cox1, cox2, cox3, nad4l, nad5, cob, atp8), ATA (atp6), ATT (nad1, nad2, nad3), ATC (nad6) and GTG (nad4). Among these, ATG, ATA and ATT are commonly used in brachyuran mitochondrial genomes (*e.g.*, JQ Chen et al. 2018; Tan et al. 2018). ATC is used in *Austruca lactea*, but only in its nad3 gene (TT Yang et al. 2019; ZQ Wang et al. 2020), and GTG is also found in *A. lactea* in the same nad4 gene (TT Yang et al. 2019). Twelve PCGs use TAA as a stop codon, which is the same pattern observed in *Austruca lactea* (ZQ Wang et al. 2020). Cox1 and cob terminate with TAA stop codons that are completed by the addition of 3' A residues to the mRNA; this has also been observed in other decapods (*e.g.*, anomuran galatheid; JS Yang and WJ Yang 2008). In the American *Minuca minax*, the stop codon of cox1 is TAA, but cob terminates with TAA completed by the addition of 3' A residue to the mRNA (Conrad et al. 2021). The overall base composition in *X. formosensis* exhibits an A+T bias (69.4%), which is within the range (64.5–77.5%) reported for confamiliar species (Karagozlu et al. 2016; Sung et al. 2016; Tan et al. 2016; JQ Chen et al. 2018; Guo et al. 2019; TT Yang et al. 2019; Ting et al. 2020; ZQ Wang et al. 2020; Conrad et al. 2021).

The phylogenetic tree based on the PCGs

sequenced in our study (Fig. 2), as well as the phylomitogenomic relationship in Conrad et al. (2021), support the systematics of Ocypodidae based on the mitochondrial 16S rDNA and cox1 as well as the nuclear 28S rDNA (Shih et al. 2016b). This includes the monophyly of the subfamilies Gelasiminae and Ocypodinae, *Minuca* being sister to the Indo-West Pacific clade in Gelasiminae, and the further sister relationships between genera (*Tubuca* and *Xeruca*; *Gelasimus* and *Cranuca*) as well as species (*Tubuca paradussumieri* and *T. capricornis*; *Ocypode ceratophthalmus* and *O. stimpsoni*). Additional mitogenomes, including species in the genera *Paraleptuca*, *Leptuca*, *Petruca*, *Afruca*, *Uca* and

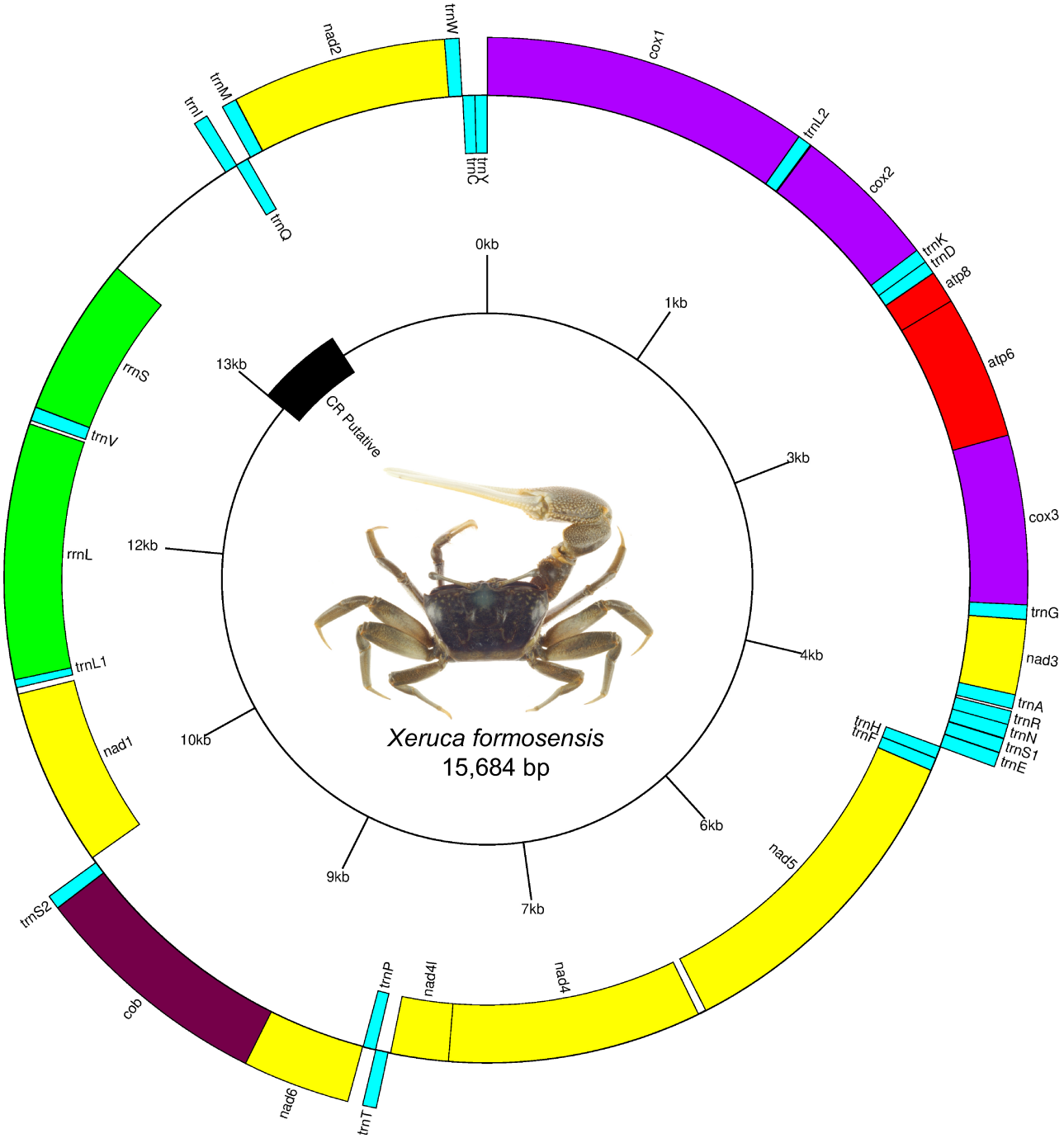

Fig. 1. Circular map of the mitogenome of *Xeruca formosensis*. Genome map contains 13 protein-coding genes (PCGs), 2 ribosomal RNA genes (12S ribosomal RNA and 16S ribosomal RNA), 22 transfer RNA (tRNA) genes, and a putative control region (D-loop).

Ucides, are necessary for a complete phylogeny of the Ocypodidae, which could provide important evidence for the systematics of this family.

Xeruca formosensis typically inhabits high intertidal mudflats with clay sediment, and the temperature and salinity of these habitats become high during the neap tide period (Shih et al. 1999 2005; Shih 2015). Further analyses of the mitogenome may help us understand the mechanisms involved in the ecological adaptation to extreme environments or requirements for extreme metabolic demands (Guo et al. 2018; Sun et al. 2019; B Zhang et al. 2019; Lau et al. 2021). Mitogenomes from different populations across the known range may reveal the connectivity of metapopulations of this species (Morin et al. 2010; Fieldsa et al. 2018).

CONCLUSIONS

In this study, the complete mitogenome of *Xeruca formosensis* was sequenced, which showed a close relationship between *Xeruca* and *Tubuca* within the subfamily Gelasiminae. The phylogenetic relationships

Table 2. Characteristics of the mitogenome in *Xeruca formosensis*

Gene	Start	Stop	Strand[1]	Length	Intergenic base[2]	Star/Stop codon	Anticodon
cox1	1	1534	+	1534	0	ATG/Taa[3]	
trnL2	1535	1600	+	66	4		TAA
cox2	1605	2294	+	690	-2	ATG/TAA	
trnK	2293	2359	+	67	-1		TTT
trnD	2359	2422	+	64	0		GTC
atp8	2423	2581	+	159	-4	ATG/TAA	
atp6	2578	3249	+	672	-1	ATA/TAA	
cox3	3249	4040	+	792	-1	ATG/TAA	
trnG	4040	4108	+	69	0		TCC
nad3	4109	4459	+	351	-1	ATT/TAA	
trnA	4459	4525	+	67	11		TGC
trnR	4537	4600	+	64	-1		TCG
trnN	4600	4666	+	67	2		GTT
trnS1	4669	4735	+	67	1		TCT
trnE	4737	4805	+	69	-1		TTC
trnH	4805	4868	-	64	0		GTG
trnF	4869	4935	-	67	0		GAA
nad5	4936	6669	-	1734	12	ATG/TAA	
nad4	6712	8049	-	1338	-7	GTG/TAA	
nad4l	8043	8345	-	303	12	ATG/TAA	
trnT	8358	8423	+	66	0		TGT
trnP	8424	8490	-	67	22		TGG
nad6	8493	8999	+	507	-1	ATC/TAA	
cob	8999	10133	+	1135	0	ATG/Taa[3]	
trnS2	10134	10200	+	67	30		TGA
nad1	10231	11163	-	933	33	ATT/TAA	
trnL1	11197	11263	-	67	-25		TAG
rrnL	11239	12578	-	1340	18		
trnV	12597	12669	-	73	0		TAC
rrnS	12670	13505	-	836	0		
CR	13506	14255	+/-	750	0		
trnI	14256	14323	+	68	-3		GAT
trnQ	14321	14389	-	69	16		TTG
trnM	14406	14476	+	71	0		CAT
nad2	14477	15487	+	1011	-2	ATT/TAG	
trnW	15486	15554	+	69	2		TCA
trnC	15557	15620	-	64	-1		GCA
trnY	15620	15684	-	65	0		GTA

[1] Plus strand (+)/ minus strand (-). [2] Negative values represent number of overlapping base pairs. [3] TAA stop codon is completed by the addition of 3' A residues to the mRNA.

reconstructed based on the mitogenomes of the family Ocypodidae also support the current systematics of this family based on one nuclear and two mitochondrial markers. We suggest further analyses that focus on the mechanisms of adaptation in habitats with high temperature and high salinity, as well as the connectivity of metapopulations.

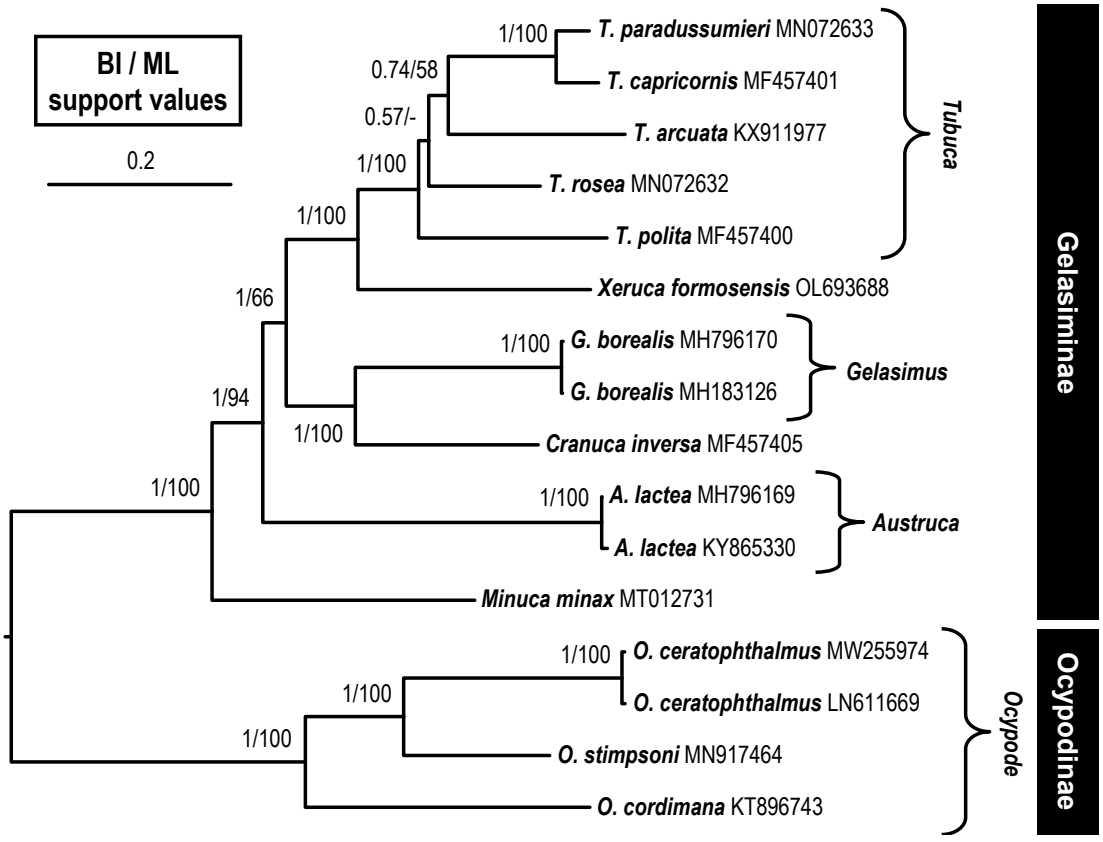

Fig. 2. A Bayesian inference (BI) tree for the species in the family Ocypodidae based on the PCG sequences. Posterior probability and bootstrap values for the BI (left) and maximum likelihood (ML) (right) are shown at the nodes. "-" means the support values < 50% in ML.

Table 3. Nucleotide frequencies of protein-coding genes in *Xeruca formosensis*

	Nucleotide frequency (%)					
Gene (strand)	A	T	C	G	A+T	C+G
cox1(+)	28.0	35.2	20.1	16.7	63.2	36.8
cox2(+)	29.9	34.3	21.6	14.2	64.2	35.8
atp8(+)	33.3	37.1	22.0	7.5	70.4	29.5
atp6(+)	28.1	36.2	22.8	12.9	64.3	35.7
cox3(+)	27.5	33.7	23.4	15.4	61.2	38.8
nad 3(+)	27.4	37.6	22.5	12.5	65.0	35.0
nad 5(-)	28.8	42.7	9.5	19.0	71.5	28.5
nad 4(-)	28.0	42.2	10.1	19.7	70.2	29.8
nad 4L(-)	26.7	48.5	6.6	18.2	75.2	24.8
nad6(+)	25.2	46.2	20.5	8.1	71.4	28.6
cob(+)	26.5	37.4	21.9	14.2	63.9	36.1
nad1(-)	24.4	42.9	12.1	20.6	67.3	32.7
nad2(+)	27.0	42.6	20.9	9.5	69.6	30.4
Overall	34.7	34.7	19.3	11.3	69.4	30.6

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Competing interests: The authors declare that they have no conflict of interest.

Availability of data and materials: The genome sequence data that support the findings of this study are openly available in GenBank of NCBI (National Center for Biotechnology Information) at https://www.ncbi.nlm.nih.gov under the accession no. OL693688. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA861006, PRJNA861006, and SAMN29880904, respectively.

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Supplementary materials

Fig. S1. The specimen of *Xeruca formosensis* (NCHUZOOL 15080, Yangang River estuary, Hsinchu City) used in this study. (download)

Table S1. The aligned sequences (FASTA format) of 13 protein-coding genes of *Xeruca formosensis* and those of species available in GenBank used for reconstruction of phylogeny. (download)