Marine Biodiversity (2025) 55:47
https://doi.org/10.1007/s12526-025-01531-2

SENCKENBERG

ORIGINAL PAPER

Application of DNA fingerprint to link megalopas and adults of *Johngarthia lagostoma* (H. Milne Edwards, 1837) (Decapoda, Gecarcinidae) and description and illustration of the megalopa stage

William Santana[1] · Isabella Dias-Silva[2] · Marcio C. A. João[2,3] · Marcos Tavares[4] · Marcelo A. A. Pinheiro[2,3]

Received: 22 January 2025 / Revised: 17 April 2025 / Accepted: 23 April 2025

Abstract
Johngarthia lagostoma is a terrestrial crab species endemic to a limited number of oceanic islands in the south Atlantic Ocean, separated by hundreds or thousands of open seas, namely Rocas Atoll, Fernando de Noronha, Trindade and Martin Vaz, and Ascension. Such a narrow distribution range adds to the vulnerability of this species. The remoteness of the islands, combined with a narrow breeding window, accounts for information gaps in its developmental biology so that, before this study, only the first zoeal stage of its larval development had been described. Here, a DNA fingerprint using partial sequences of the 16S and barcode regions of COI mitochondrial genes was successfully applied to link megalopas recovered in Trindade from inside burrows of *J. lagostoma* to adults of this species. Megalopas and adults were confirmed to be conspecific, with an average pairwise *p*-distance of 0.001% (range 0–0.004%). The current finding that the megalopas belong to *J. lagostoma* provides additional evidence for the megalopas being the stage of terrestrial recruitment in *J. lagostoma*, not the first crab stage as initially thought. The megalopa stage of *J. lagostoma* is herein described and illustrated, and a synoptic table is provided to facilitate the morphological comparison of the megalopa stage of *J. lagostoma* with those of its congeners and closely related species in the genus *Gecarcinus*.

Keywords Land crabs · Larval development · Recruitment · Trindade Island · Oceanic islands

Introduction

Brachyuran crabs of the family Gecarcinidae H. Milne Edwards, 1837, exhibit a high degree of terrestrial adaptation during the juvenile and adult life cycle, whereas the larval stage is spent in the ocean. The larval phase typically consists of five to six zoeal stages followed by a megalopa stage, which eventually further metamorphoses into a juvenile, completing the life cycle (Willems 1982; Hartnoll 1988; Anger 1995; Cuesta et al. 2002; Clark and Cuesta 2015). The larval stages serve as the dispersal phase, while the first crab stage has initially been considered the stage of terrestrial recruitment, such as in *Gecarcoidea natalis*

Communicated by B. Chan

✉ William Santana
willsantana@gmail.com

Isabella Dias-Silva
isabellayoukai@gmail.com

Marcio C. A. João
marcio.joao@unesp.br

Marcos Tavares
mdst@usp.br

Marcelo A. A. Pinheiro
marcelo.pinheiro@unesp.br

[1] Museu de Paleontologia Plácido Cidade Nuvens, Universidade Regional do Cariri – URCA, Crato, Ceará, Brazil

[2] Departamento de Ciências Biológicas e Ambientais (DCBA), Grupo de Pesquisa em Biologia de Crustáceos (CRUSTA), Instituto de Biociências (IB), UNESP - Universidade Estadual Paulista, Campus do Litoral Paulista (CLP), São Vicente, SP, Brazil

[3] Programa de Pós-Graduação em Ecologia, Evolução e Biodiversidade (PPG-EcoEvoBio), UNESP IB/Campus de Rio Claro, Rio Claro, SP, Brazil

[4] Museu de Zoologia, Universidade de São Paulo, São Paulo, SP, Brazil

Published online: 28 May 2025

(Pocock, 1889) (Hicks 1985; Hicks et al. 1990). More recently, however, it has been discovered that the megalopa is the stage of terrestrial recruitment in species of *Gecarcinus* Leach, 1814, and *Johngarthia* Türkay 1970, including *J. lagostoma* (H. Milne Edwards, 1837) (Hartnoll et al. 2014 and references therein).

Johngarthia lagostoma has a highly restricted geographic distribution, limited to four remote oceanic islands in the south Atlantic Ocean, separated by hundreds or thousands of kilometers of open sea: Rocas Atoll, Fernando de Noronha, Trindade and Martin Vaz, and Ascension Island. Despite this, many aspects of the species' biology remain poorly understood. However, knowledge concerning the breeding behavior has been broadened because of research conducted primarily on Ascension Island (Hartnoll et al. 2006, 2009, 2010) and Trindade (João et al. 2021, 2022; Tavares and Mendonça 2022; Pinheiro et al. 2024).

Logistic constraints associated with the island's remoteness complicate the rearing of larval stages under laboratory conditions. Consequently, only the first zoeal stage of *J. lagostoma* has been successfully obtained (Colavite et al. 2021; Lira et al. 2021), while the complete zoeal development and the megalopa morphology still need to be described. Although it has been reported that *J. lagostoma* emerges from the sea as megalopas, migrates inland, and subsequently metamorphoses into the first juvenile crab stage (Hartnoll et al. 2014; Pinheiro et al. 2024), a detailed morphological description is crucial to confirm the identity of the megalopa stage conclusively.

In recent years, DNA barcoding has become an essential tool for linking larval and adult stages of marine organisms, especially in cases where morphological identification is challenging or insufficient. This molecular approach allows researchers to match larvae collected from the wild with known adult reference sequences, thereby facilitating accurate species identification and enabling the characterization of otherwise poorly known developmental stages (e.g., Ueda et al. 2021). In crustaceans, such methods have been successfully applied to taxa including lobsters (Genis-Armero et al. 2023; Jiang et al. 2024), barnacles (Wong et al. 2014), and other decapods (Pantaleão et al. 2025), providing critical insights into larval morphology and life history. By integrating morphological and molecular analyses, we can now confidently describe larval forms, offering a more complete picture of species' developmental trajectories and aiding in taxonomic and ecological research.

This study employed morphological and molecular methods to link the megalopa stage to adult *J. lagostoma*, providing a comprehensive description and detailed illustrations of the megalopa based on specimens collected from burrows on Trindade Island. Additionally, a synoptic table is presented to facilitate morphological comparisons between the megalopa stage of *J. lagostoma* and those of its congeners, as well as species in the genus *Gecarcinus* with previously described megalopa stages.

Material and methods

Ten megalopas were hand-collected on 10 November 2022 from burrows excavated by adults of *Johngarthia lagostoma* at Andradas Beach (20°30′24″S–29°18′57.5″W), approximately 200 m inland from the coastline. Megalopae were manually collected from shallow burrows (10–20 cm deep) excavated in dry, loose sandy substrates along the supralittoral zone, always associated with sandhill vegetation. Each burrow contained up to ten recruits, typically one to three megalopae and the remainder juvenile crabs. Megalopae were found either co-inhabiting with adults or occupying burrows abandoned by the constructing adult.

Eight megalopas preserved in 70% ethanol were selected for morphological descriptions and illustrations. Specimens were stained with methylene blue or acid fuchsin when necessary and dissected in a 1:1 solution of glycerin and 70% ethanol. Drawings and measurements were conducted using a Leica® MZ12.5 stereomicroscope. Two complete specimens were mounted on excavated slides in different positions to check for the presence of setae on the carapace and to illustrate the entire megalopa. The sequence of appendages description follows the pattern established in previous studies (Colavite et al. 2016; Santana et al. 2016); setal descriptions were made under light microscopy (LM) and follow the system proposed by Pohle and Telford (1981) according to the general terminology. All megalopas were measured for carapace length (CL), from the basis of the rostrum to the posterior margin of the carapace and carapace width (CW). Abbreviations include P1 for the cheliped, and P2 to P5 for pereiopods two through five, respectively. Eight specimens were deposited at the Museu de Zoologia, Universidade de São Paulo (MZUSP 47507).

Molecular analysis

Collected larvae presumed to belong to *Johngarthia lagostoma* were preserved in 96% ethanol. Genomic DNA was extracted from larval muscle tissue using the Omega Biotek EZNA Tissue DNA Kit at the Laboratory of Molecular Biology, Museu de Zoologia, Universidade de São Paulo (MZUSP). Partial sequences of the 16S and barcode regions of COI mitochondrial genes were amplified with the following primers 16SF/16SR (Hultgren and Stachowicz 2008) and LCO1490/HCO2198 (Folmer et al. 1994), respectively.

PCR amplification was performed in 20-µL reactions with the following reagent volumes and concentrations: 0.4 µL of each primer (10 µM), 2 µL of Taq buffer (10X), 0.6 µL of MgCl2 (50 mM), 0.4 µL dNTPs (10 mM) and 0.2 µL Taq (PlatinumR Taq DNA Polymerase) (5 U/µL), 1 µL of genomic DNA, and 15 µL of ultrapure water. PCR cycles were as follows: 16S 94 °C for 30 s (denaturation), 48 °C for 30 s (annealing), and 72 °C for 60 s (extension); and COI 95 °C for 30 s (denaturation), 52 °C for 30 s (annealing), and 72 °C for 45–60 s (extension). Sequencing reactions were purified using ExoSAP-IT™ (Thermo Fisher Scientific Inc.), and sequencing was carried out at the Human Genome and Stem-Cell Research Center, University of São Paulo. Sequencing reactions consisted of 1 µL of purified PCR product in a 20-µL reaction containing 5 µL primer (0.5 pmol/µL), and sequences were obtained using an ABI 3500 automated DNA sequencer (Applied Biosystems) according to the manufacturer's instructions.

Sequences were assembled, trimmed of primers, and checked for quality using *Geneious* (versions 8.0.5 and 9.1.8, Biomatters Ltd, Auckland, New Zealand). The obtained fragments were aligned, and BLAST searches were conducted to identify significant matches with sequences from the megalopa morphologically assigned to *Johngarthia lagostoma*.

The new sequences were analyzed along with 13 previously published sequences from *GenBank*, including 16 s and COI barcode regions from adults of *J. lagostoma*, and its close relative *Gecarcinus lateralis* (Table 1). Alignments were performed using *MAFFT 7.310* (Katoh and Standley 2013). Both gene datasets were concatenated (COI: 1–516 bp; 16S: 517–946 bp) using *Geneious 8.0.5* (Kearse et al. 2012). As *Johngarthia lagostoma* is the only species of the genus present on the Atlantic islands and *Gecarcinus lateralis* does not occur in this region, the risk of misidentification is negligible; the latter was included in the phylogenetic analysis as a closely related outgroup to provide taxonomic context.

Table 1 Taxa included in the pairwise analysis with GenBank accession numbers

Species	GenBank accession nos	
	COI	16S
Johngarthia lagostoma	**PV650909**	**PV650910**
Johngarthia lagostoma	KM578831	KT159734
Johngarthia lagostoma	KM578832	KT159735
Johngarthia lagostoma	KM578833	KT159736
Johngarthia lagostoma	KM578834	KT159737
Johngarthia lagostoma	KM578835	KT159738
Johngarthia lagostoma	KM578836	KT159739
Johngarthia lagostoma	KM578837	KT159740
Johngarthia lagostoma	KM578838	KT159741
Johngarthia lagostoma	KM578839	KT159742
Johngarthia lagostoma	KM578840	KT159743
Johngarthia lagostoma	KM578841	KT159744
Johngarthia lagostoma	KM578842	-
Gecarcinus lateralis	-	AJ130804.2
Gecarcinus lateralis	-	OR448144.1

Newly sequenced specimens are highlighted in bold. –, sequences not available

Pairwise distances were calculated using MEGA version 11 (Tamura et al. 2021) by using the "Distances" option and "Nucleotide: p-distance" model option to calculate the distances. Positions with less than 95% site coverage were excluded (partial deletion option), allowing fewer than 5% alignment gaps, missing data, and ambiguous bases at any given position. Pairwise distances are summarized in Table 2.

The sequences of 16S from this study and from GenBank (Table 1, except NC 057475) were aligned (MAFFT alignment algorithm) and phylogenetic relations were estimated through a Bayesian inference method using a GTR substitution model with the MrBayes (Huelsenbeck and Ronquist 2001) plugin in Geneious 8.0.5, with 1,100,000 chain length, 100,000 burn-in length.

Results

Morphological description

Carapace length (CL): 2.62 ± 0.14 mm (2.41–2.76 mm); carapace width (CW): 2.43 ± 0.13 mm (2.22–2.56 mm).

Carapace (Fig. 1A). Longer than wide, subrectangular. Rostral spine short, acute, deflected ventrally. Frontal region with two small elevations near rostrum. Hepatic region distinct, gastric region swollen with distinct protogastric and metagastric regions. Protogastric region with two knob-like elevations. Branchial, cardiac, and intestinal regions well defined. Carapace surface covered mostly with simple setae. No yolk granules observed in the cephalothorax.

Antennule (Fig. 1B). Peduncle with three articles, proximal without seta; medial segment with two; and distal segment with one simple seta. Unsegmented endopod with one subterminal, long simple seta. Four-segmented exopod with proximal segment bearing 4 aesthetascs. Second segment with 7 aesthetascs, third segment with 4 aesthetascs, distal segment with aesthetasc-like distal seta.

Antenna (Fig. 1C). Articles proximally to distally with 2, 1, 2, 0, 0, 4, 0, 0, 4, 2 simple setae, respectively. Basal article without exopod bud.

Mandibles (Fig. 1D). Closely similar, scoop-shaped process with cutting edge with middle depression. Palp with two articles, 10 plumodenticulate setae on the distal segment.

Maxillule (Fig. 1E). Coxal endite with 7–8 setae (4 graded plumodenticulate, 3–4 plumodenticulate). Basial

Table 2 Pairwise evolutionary divergence between sequences of partial 16S rDNA and COI

	J. lagostoma	*J. lagostoma*	*J. lagostoma*	*J. lagostoma*	*J. lagostoma*	*J. lagostoma*	*J. lagostoma*	*J. lagostoma*	*J. lagostoma*	*J. lagostoma*	*J. lagostoma*	*J. lagostoma*	*J. lagostoma*
Johngarthia lagostoma	0.000												
Johngarthia lagostoma	0.000	0.000											
Johngarthia lagostoma	0.002	0.002	0.002										
Johngarthia lagostoma	0.002	0.002	0.002	0.004									
Johngarthia lagostoma	0.002	0.002	0.002	0.004	0.004								
Johngarthia lagostoma	0.002	0.002	0.002	0.004	0.004	0.004							
Johngarthia lagostoma	0.002	0.002	0.002	0.004	0.004	0.004	0.004						
Johngarthia lagostoma	0.002	0.002	0.002	0.004	0.004	0.004	0.004	0.004					
Johngarthia lagostoma	0.002	0.002	0.002	0.004	0.004	0.004	0.004	0.004	0.004				
Johngarthia lagostoma	0.002	0.002	0.002	0.004	0.004	0.004	0.004	0.004	0.004	0.004			
Johngarthia lagostoma	0.002	0.002	0.002	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004		
Johngarthia lagostoma	0.004	0.004	0.004	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	
Gecarcinus lateralis	0.103	0.103	0.103	0.103	0.101	0.105	0.105	0.105	0.101	0.105	0.103	0.101	0.103

The number of base differences per site from between sequences is shown. This analysis involved 14 nucleotide sequences. Species marked in bold represent the larval data

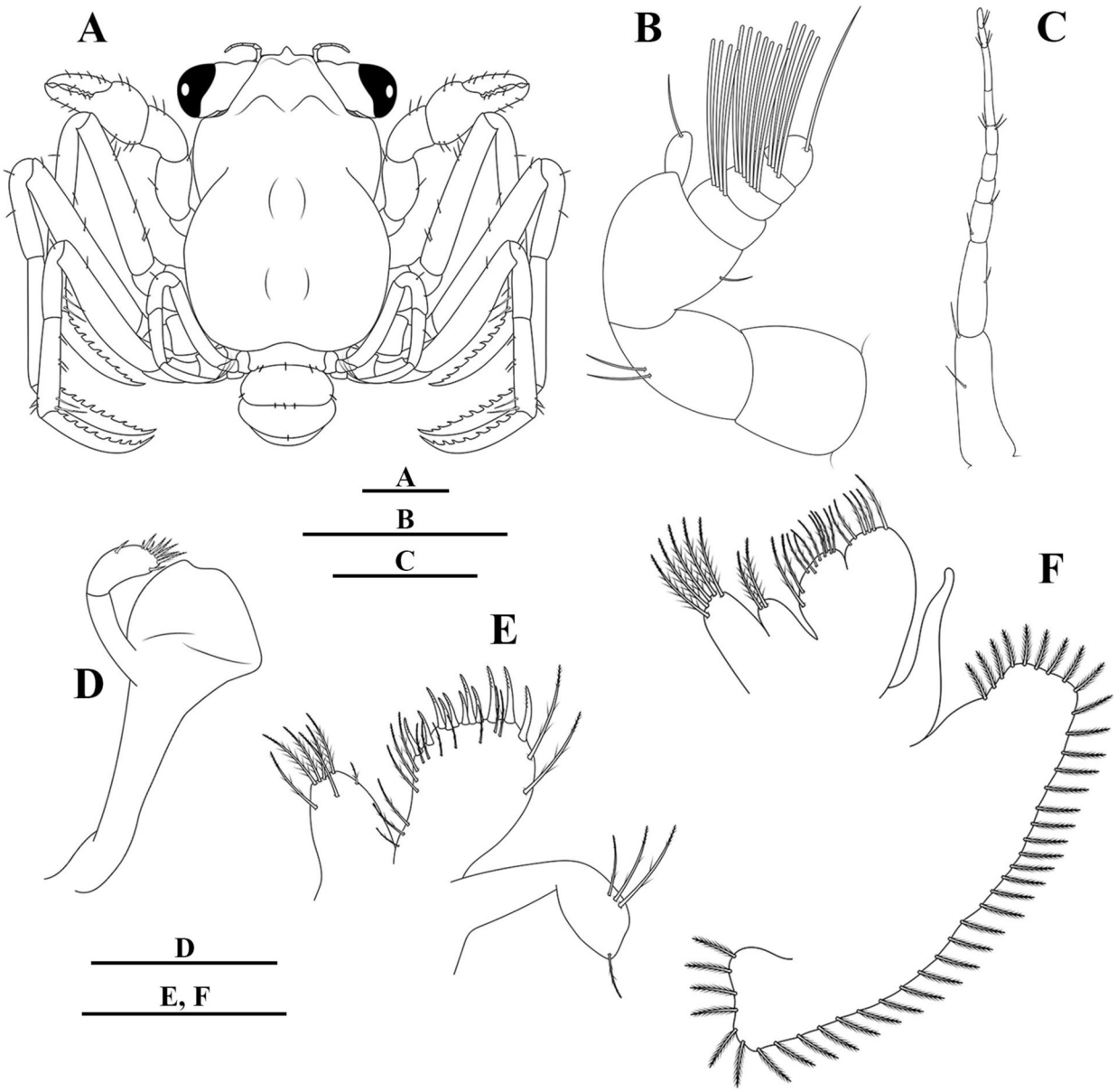

Fig. 1 Megalopa of *Johngarthia lagostoma* (H. Milne Edwards, 1837). (**A**) Habitus – dorsal view; (**B**) antennule; (**C**) antenna; (**D**) mandible; (**E**) maxillule; and (**F**) maxilla. Scale bars: A, 1 mm; B–F, 0.2 mm

endite with 25–27 setae (11 terminal plumodenticulate cuspidate, 11–13 subterminal plumodenticulate, 3 plumodenticulate setae on proximal margin). Endopod long, unsegmented, with 3 long subterminal and 1 long terminal plumodenticulate setae. Exopod without seta.

Maxilla (Fig. 1F). Coxal endite bilobed, proximal lobe with five long plumodenticulate setae, distal lobe with two plumodenticulate setae. Basial endite enlarged, bilobed, with 10 plumodenticulate setae on proximal and 6 plumodenticulate setae on distal lobe. Endopod long, narrowing distally, without setae. Scaphognathite with 38–42 marginal plumose setae, blade without seta.

First maxilliped (Fig. 2A). Coxal endite bearing 19–21 setae (1 long simple, 2 long, and 16–18 short plumodenticulate). Basial endite with 15 plumodenticulate setae (4 long and 11 short) arranged as illustrated. Endopod unsegmented 3 simple setae (1 long, 2 shorter) distally. Exopod not completely bisegmented, proximal segment without setae, distal with 3 long plumose setae. Epipod elongated with 4 long plumodenticulate setae medially.

Second maxilliped (Fig. 2B). Coxa and basis not differentiated with 1 plumodenticulate setae. Endopod with five articles, proximally to distally with 1, 1, 2, 7, 7 setae, respectively (segments 1 to 4 all with plumodenticulate setae, fifth

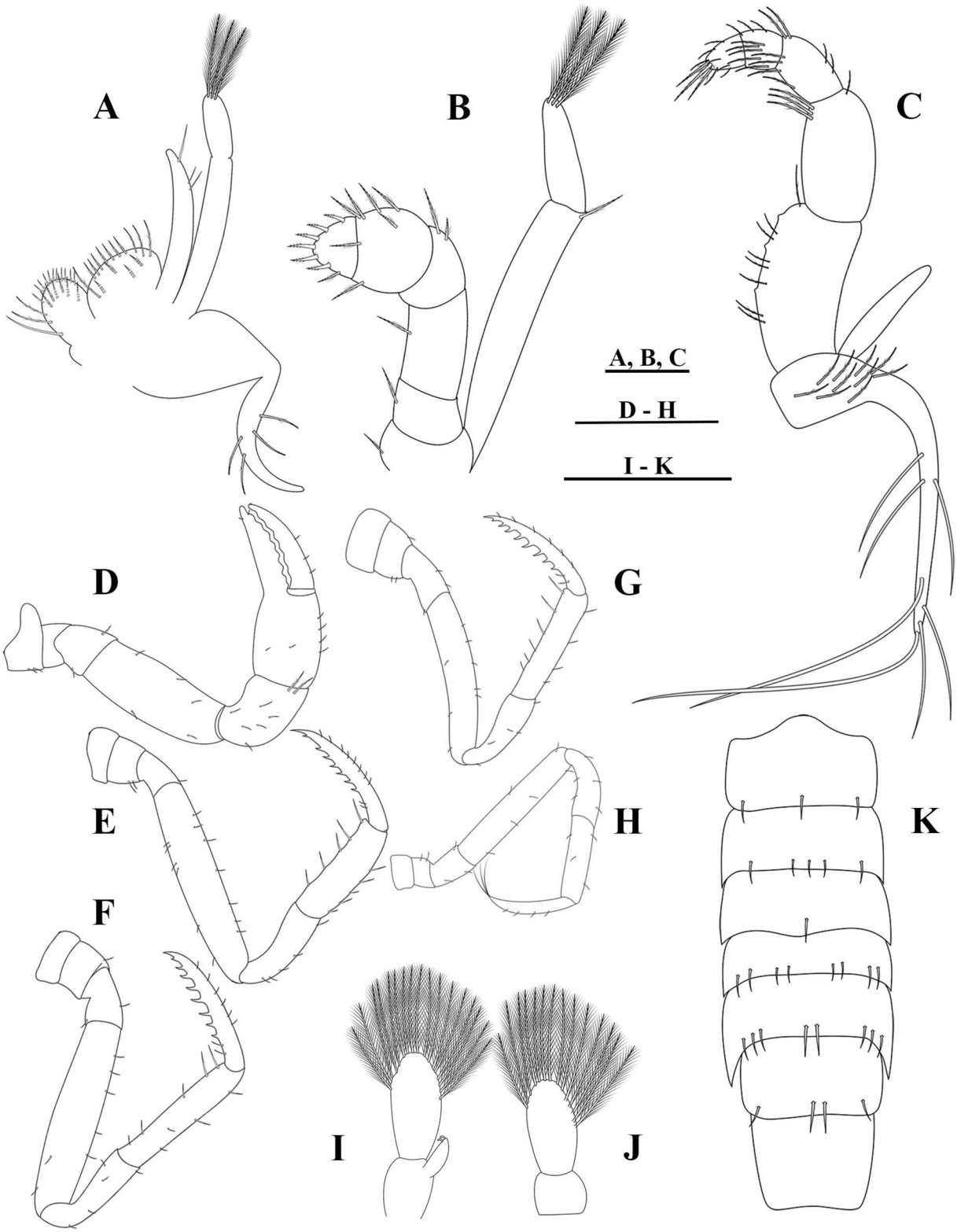

Fig. 2 Megalopa of *Johngarthia lagostoma* (H. Milne Edwards, 1837). (**A**) Maxilliped I; (**B**) maxilliped II; (**C**) maxilliped III; (**D**) cheliped; **E**–**H**, pereiopods P2 to P5; (**I**–**J**) first and fifth pleopods, respectively; (**K**) dorsal view of the pleon and telson. Scale bars: A–C, 0.5 mm; D–K, 1 mm

segment with 6 graded plumodenticulate and 1 plumodenticulate setae). Exopod with two articles, proximal with long plumodenticulate seta, distal with 3 long plumose setae. Epipod not present on examined specimens.

Third maxilliped (Fig. 2C). Coxa with 10 plumodenticulate setae. Basis fused to ischium with 3 protuberances on mesial margin, indicative of crista dentata. Endopod with five articles, proximally to distally with 8, 3 + 1, 4 + 4, 5 + 3, 9–11 plumodenticulate setae. Exopod reduced, unsegmented, without setae. Epipod unsegmented, with 3 long medial, 2 long, and 2 shorter distal setae.

Pereiopods (Fig. 2D–H). Cheliped and pereiopods with mostly simple setae as figured. Cheliped fixed and movable fingers dentated. Propodi of P2–4 with two strong, long plumodenticulate setae ventrodistally. Dactyls of P2–4 with a row of strong spinules ventrally. P5 propodus without long plumodenticulate setae distally, dactylus without spinules, 3 long simple setae distally.

Pleon (Fig. 2K). Pleonites I–VI with 3, 5, 1, 8, 8, 4 simple setae arranged as illustrated.

Pleopods (Fig. 2I, J). Pleonites II–V with pair of biramous pleopods. Exopod of pleopods II–V with 17, 17, 17, 14–15 plumose natatory setae, respectively. Endopod with two cincinnuli each. Pleonite VI with a pair of uropods, uniramous, two-segmented, with 14 natatory setae on distal segment.

Telson (Fig. 2K). Subrectangular, without setae.

Molecular analysis

A single megalopa of *Johngarthia lagostoma* from Trindade Island was sequenced, successfully amplifying the mitochondrial loci COI and 16S. BLAST searches confirmed that the sequences used were closely related to published sequences of adult *J. lagostoma*. In the evolutionary divergence analysis, 515 positions were included in the final dataset. The average pairwise *p*-distance between the megalopa identified as *J. lagostoma* and 12 adult specimens of the species was 0.001% (range 0–0.004%), in stark contrast to the divergence between the *J. lagostoma* megalopa and its close relative *Gecarcinus lateralis*, which was 0.103% (Table 2). These results unequivocally confirm that the megalopa analyzed in this study belongs to *J. lagostoma*.

The Bayesian inference analysis based on 16S rRNA gene sequences yielded a well-resolved phylogenetic tree (Fig. 3), supporting the monophyly of *Johngarthia lagostoma*. Sequences obtained from megalopae clustered with high posterior probability (PP = 1.0) alongside adult *J. lagostoma* sequences from GenBank, confirming their conspecificity. All *J. lagostoma* sequences formed a distinct and well-supported clade, clearly separated from *Gecarcinus lateralis*. This molecular evidence also corroborates the morphological identification and establishes a reliable link between larval and adult stages.

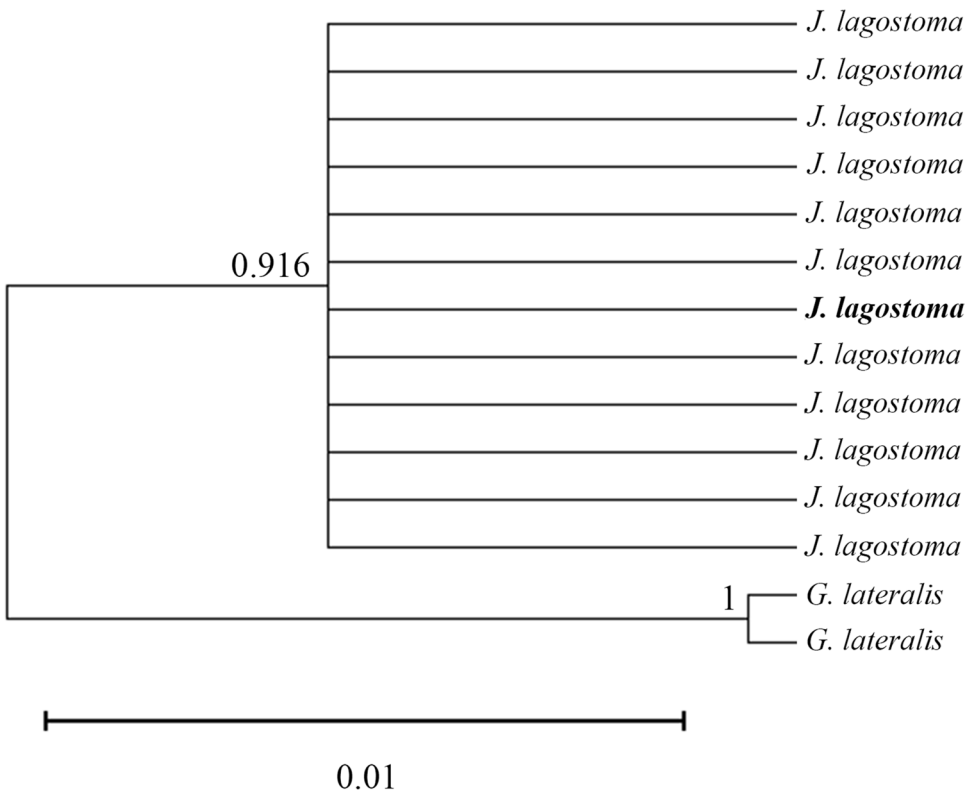

Fig. 3 Bayesian phylogenetic tree of *Johngarthia lagostoma* based on 16S rRNA sequences. Analysis used the GTR model in MrBayes (1,100,000 generations; 100,000 burn-in). Posterior probabilities are shown at nodes. The larval material sequenced from this study is shown in bold

Discussion

To the best of our knowledge, this is the first attempt to use DNA fingerprinting to link undocumented larval stages and adults in the Gecarcinidae family. Unambiguously, the molecular analysis performed here revealed that the megalopas recovered from adult burrows of *Johngarthia lagostoma* on Trindade Island are conspecifics, showing nearly identical sequences (average pairwise *p*-distance of 0.001%, ranging from 0 to 0.004%). These findings provide strong evidence that the megalopas found in this terrestrial habitat on Trindade indeed belong to *J. lagostoma*, reinforcing their role as the terrestrial recruitment stage for this species (Hartnoll et al. 2014; Pinheiro et al. 2024).

It has been shown that the gecarcinid genera *Gecarcinus*, *Gecarcoidea*, and *Johngarthia* form a clade, sharing several synapomorphic modifications in the adult maxillipeds. Among these, the exopods of the maxillipeds I–III substantially reduced with the complete loss of the flagellae (see Tavares 1989; 1991; 2002). In *Gecarcinus* and *Johngarthia*, the modification of the maxilliped exopods begins in the megalopa stage, characterized by the expressive reduction of the exopod and loss of the flagellum in the third maxilliped, whereas the maxillipeds I–II retain regular exopods and unsegmented flagellae (as illustrated by Willems 1982; Hartnoll and Clark 2006; present study, Fig. 2A–C).

The morphology of the megalopa of *Gecarcoidea lalandii* H. Milne Edwards, 1837, needs to be better documented (Webb 1922); however, judging from adult morphology, the megalopas of the two species in *Gecarcoidea* are expected to follow the same maxilliped observed in *Gecarcinus* and *Johngarthia*. Otherwise, megalopas are known for three out of six species of *Johngarthia* and two out of four species of *Gecarcinus* (Table 3). Hartnoll and Clark (2006: p. 161, tab. 1) referred to the megalopa of [sic] *Gegarcinus hydrodromas*, whose prezoea stage (not the megalopa) was poorly illustrated by Thompson (1836: p. 373, Fig. 3) as [sic] *Gegarcinus hydrodromus*. Hartnoll and Clark (2006) remarked that the validity of this species is in doubt, referring most probably to its belonging to the family Gecarcinidae. In fact, that species actually refers to the parathelphusid *Spiralothelphusa hydrodroma* (Herbst 1794), which was nicely illustrated in color by Herbst (1794: Tab. 41, Fig. 2).

The monophyly of *Johngarthia* has yet to be confidently established. Indeed, as discussed elsewhere, no synapomorphies have been identified in the adult and first zoeal morphology of *Johngarthia* to support its monophyly (Tavares 1989; 1991; Colavite et al. 2021) and, likewise, no candidate characters from the megalopas can be set forth so far as diagnostic to *Johngarthia*.

As with some other gecarcinids, the narrow distribution range of *J. lagostoma*, restricted to a few remote oceanic

Table 3 A comparison of *Gecarcinus* Leach, 1814, and *Johngarthia* Türkay 1970 megalopas

Species	Antennule		Antenna	Maxillule			Maxilla			Maxilliped I			Maxilliped II		Maxilliped III	
	Peduncle	Exopod		Coxa	Basis	Endopod	Coxa	Basis	Endopod	Coxa	Basis	Endopod	Endopod	Exopod	Endopod	Exopod
Gecarcinus lateralis1	3,7,1*	12,4,4aes*	4,3,2,0,2,4,1,4,2,3	21–24	30–32	2,5	20–24,16–20	14–18,16–20	8–10	24–29	24–29	4	2,1–2,9–11,12–14	0,1	31–33,23–25,15–17,12,8–9	4–5
Gecarcinus ruricola2	8,8,3	5,4,3aes	4,3,4,0,0,4,0,5,3,4	25	34	2,4	22,10	16,20	5	24	27	2–3	0,1,1,8,9*	5,5	30,26,15,11,8	1
Johngarthia lagostoma3	0,2,1	4,7,4aes	2,1,2,0,0,4,0,0,4,2	7–8	25–27	4	5,2	10,6	0	19–21	15	3	1,1,2,7,7	1,3	8,4,8,8,9–11	0
Johngarthia planata4	4,7,1	10,4,6aes	7,2,2,0,2,4,1,4,2,3	23	30	1,5	24,13	12,18	8*	27	26	3,5	2,2,9,15	5,6	28,23,15,9,12	7

Larval development data retrieved from the following: 1, Willems (1982); 2, Hartnoll and Clark (2006); 3, present study; 4, Cuesta et al. (2007). Numerals refer to the number of setae on each appendage; *, data from figure; *aes*, aesthetascs

islands (Rocas Atoll, Fernando de Noronha, Trindade and Martin Vaz, and Ascension), increases the vulnerability of this species (Pinheiro et al. 2016; Rodríguez-Rey et al. 2016). Despite significant information gaps regarding its breeding biology — an area of logical interest for enhancing conservation efforts — the remoteness of these islands, coupled with the lack of suitable laboratory facilities and the species' narrow oviposition time window from October to April on Trindade Island (Tavares and Mendonça 2022; João et al. 2023) all converge to limit opportunities for studying the complete larval development of *J. lagostoma* (see Pinheiro et al. 2024).

Future studies addressing recruitment parameters, such as those detailed here, are essential to delineate the reproductive dynamics of *Johngarthia lagostoma*. The insights into co-inhabitation behaviors and growth patterns provide foundational knowledge for conservation strategies. Furthermore, understanding the morphology of the megalopa stage is critical for field recognition, facilitating more effective monitoring and management of recruitment processes. These efforts will significantly contribute to conserving this endangered species on remote oceanic islands, ensuring its persistence amidst environmental and anthropogenic pressures.

Acknowledgements We thank the Brazilian Navy (First District), Inter-ministerial Secretariat for Marine Resources (SECIRM) and "Programa de Pesquisas Científicas da Ilha da Trindade" (PROTRINDADE), under the commander C. C. Vitória-Régia, who guaranteed our presence in Trindade Island and helped with the project logistic. We thank the members of the Crustacean Biology Research Group (CRUSTA) at UNESP IB/CLP for their assistance during field sampling, with special thanks to Nicholas Kriegler. We thank Jaqueline Batilana (MZUSP) for the DNA amplification and Jessica Colavite (MZUSP) for conducting the Bayesian analysis. We also thank the two anonymous reviewers for their constructive comments and suggestions, which greatly improved the quality of this manuscript.

Funding WS is supported by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) under grants PQ2 # 315185/2020–1 and PQ1D # 312823/2023–1, as well as by the *Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico* (FUNCAP) through fellowships (# 6647309/2017 and # PV1-0187–00033.01.00/21). MAAP and MT thank CNPq for the Research Productivity Fellowships (PQ2 # 305957/2019–8 and # 307482/2022–7; and PQ1 A # 443278/2019–9), respectively. Molecular analyses and sequencing were funded by CNPq grant 441794/2024–6 to MT. IDS acknowledges support from the *Programa Institucional de Bolsas de Iniciação Científica* (PIBIC) for the scientific initiation fellowship (# 143295/2020–9). MAAP coordinates and secures financial support for the "Projeto Caranguejos de Ilhas Oceânicas" from CNPq, funded through the Universal Project (CNPq # 404224–2016).

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval No animal testing was performed during this study.

Sampling and field study The sampling activities were authorized under permit #65446, issued by the Sistema de Autorização e Informação em Biodiversidade (SISBIO).

Data Availability All data generated or analyzed during this study are included in the manuscript.

Author contributions *William Santana:* methodology, formal analysis, writing — original draft, writing — review and editing. *Isabella Dias-Silva*: investigation, writing — review and editing. *Marcio Camargo Araujo João:* sampling, writing — original draft, writing — review and editing. *Marcos Tavares:* molecular analysis, writing —review and editing. *Marcelo Antonio Amaro Pinheiro:* conceptualization, term, writing — review and editing, project administration, funding acquisition.

References

Anger K (1995) The conquest of freshwater and land by marine crabs: adaptations in life-history patterns and larval bioenergetics. J Exp Mar Biol Ecol 193:119–145. https://doi.org/10.1016/0022-0981(95)00114-X

Clark PF, Cuesta JA (2015) Larval systematics of Brachyura. In: Castro P, Davie P, Guinot D, Schram F, von Vaupel Klein C (eds) *Treatise on zoology – anatomy, taxonomy, biology. The Crustacea*, vol 9 part C. Brill, pp 981–1048. https://doi.org/10.1163/9789004190832_020

Colavite J, López R, Hernández J (2016) First zoeal stage of *Macrocoeloma subparallelum* (Stimpson, 1860) (Decapoda: Brachyura: Majoidea) described from laboratory hatched material. Nauplius 24:e2016016. https://doi.org/10.1590/2358-2936e2016016

Colavite J, Tavares MDS, Mendonça JB, Santana W (2021) The first zoeal stage of *Johngarthia lagostoma* (H. Milne Edwards, 1837) (Crustacea: Brachyura: Gecarcinidae), with comments on the validity of the genus. Thalassas 37:767–773. https://doi.org/10.1007/s41208-021-00343-y

Cuesta J, Liu H, Schubart C (2002) First zoeal stages of *Epigrapsus politus* Heller, *E. notatus* (Heller) and *Gecarcoidea lalandii* H. Milne Edwards, with remarks on zoeal morphology of the Gecarcinidae Macleay (Crustacea: Brachyura). J Nat Hist 36:1671–1685.

Cuesta JA, García-Guerrero MU, Hendrickx ME (2007) The complete larval development of *Johngarthia planatus* (Brachyura: Grapsoidea: Gecarcinidae) described from laboratory reared material, with notes on the affinity of *Gecarcinus* and *Johngarthia*. J Crustac Biol 27:263–277. https://doi.org/10.1651/C-2642.1

Folmer O, Black M, Hoeh R, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3(5):294–299.

Genis-Armero R, Groeneveld J, Singh S, Clark PF, Corbari L, Palero F (2023) Identification of phyllosoma larvae of the lobsters *Acantharctus* and *Biarctus* (Crustacea: Scyllaridae) from the SW Indian Ocean. Zool Stud 62:33. https://doi.org/10.6620/ZS.2023.62-33

Hartnoll RG (1988) Evolution, systematics, and geographical distribution. In: Burggren WW, McMahon BR (eds) Biology of the land crabs. Cambridge University Press, New York, pp 6–53

Hartnoll RG, Clark PF (2006) A mass recruitment event in the land crab *Gecarcinus ruricola* (Linnaeus 1758) (Brachyura: Grapsoidea: Gecarcinidae) and a description of the megalop. Zool J Linnean Soc 146(2):149–164. https://doi.org/10.1111/j.1096-3642.2006.00195.x

Hartnoll RG, Mackintosh T, Pelembe TJ (2006) *Johngarthia lagostoma* (H. Milne Edwards, 1837) on Ascension Island: a very isolated land crab population. Crustaceana 79:197–215

Hartnoll RG, Broderick AC, Godley BJ, Saunders KE (2009) Population structure of the land crab *Johngarthia lagostoma* on Ascension Island. J Crustac Biol 29:57–61. https://doi.org/10.1651/08-2992.1

Hartnoll RG, Broderick AC, Godley BJ, Musick S, Pearson M, Stroud SA, Saunders KE (2010) Reproduction in the land crab *Johngarthia lagostoma* on Ascension Island. J Crustac Biol 30(1):83–92. https://doi.org/10.1651/09-3143.1

Hartnoll RG, Régnier-McKellar C, Weber N, Weber SB (2014) Return to the land; the stages of terrestrial recruitment in land crabs. Crustaceana 87:531–539. https://doi.org/10.1163/15685403-00003294

Herbst JFW (1794) Versuch einer Naturgeschichte der Krabben und Krebse nebst einer systematischen Beschreibung ihrer verschiedenen Arten. Zweyter Band. Mit XXV Kupfer-Tafeln und Register. Krebse. Gottlieb August Lange, Berlin und Stralsund, viii + 225 + [1] pp. 25 pls.

Hicks JW (1985) The breeding behaviour and migrations of the terrestrial crab *Gecarcoidea natalis* (Decapoda: Brachyura). Aust J Zool 33(2):127–142. https://doi.org/10.1071/ZO9850127

Hicks JW, Rumpff H, Yorkston H (1990) *Christmas crabs (2nd ed.)*. Christmas Island Natural History Association, Christmas Island, Australia, p:1–81.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Hultgren KM, Stachowicz JJ (2008) Molecular phylogeny of the brachyuran crab superfamily Majoidea indicates close congruence with trees based on larval morphology. Mol Phylogenet Evol 48(3):986–996. https://doi.org/10.1016/j.ympev.2008.05.004

Jiang GC, Yang CH, Wakabayashi K, Chan TY (2024) An integrated taxonomy approach identified the final stage of giant phyllosoma of *Parribacus antarcticus* (Lund, 1793) (Crustacea: Decapoda: Scyllaridae) from Taiwan waters. Zool Stud 63:56. https://doi.org/10.6620/ZS.2024.63-56

João MCA, Duarte RC, Bispo da Silva LS, Freire AS, Pinheiro MAA (2022) Sexual maturity of an endemic insular land crab: priority information toward the conservation of *Johngarthia lagostoma*. Biol Bull 243:14–27. https://doi.org/10.1086/720581

João MCA, Kriegler N, Freire AS, Pinheiro MAA (2021) Mating strategies of the endangered insular land crab *Johngarthia lagostoma* (H. Milne Edwards, 1837). Invert Reprod Dev 65(4):256–267. https://doi.org/10.1080/07924259.2021.1961885

João MCA, Duarte RC, Kriegler N, Freire AS, Pinheiro MAA (2023) Population biology of the endangered land crab *Johngarthia lagostoma* (H. Milne Edwards) in the Trindade Island, Brazil: identifying crucial areas for future conservation strategies. *Mar Ecol* 45:e12778. https://doi.org/10.1111/maec.12778

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12):1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Leach WE (1814) Crustaceology. In*:* Brewster D (ed) The Edinburgh encyclopaedia. Balfour, Edinburgh, 7(1):383–437. available online at https://www.biodiversitylibrary.org/page/37187640

Lira SMA, De Lima CDM, Teixeira IDA, Schwamborn R (2021) The first zoeal stage of the yellow land crab *Johngarthia lagostoma* (Milne Edwards, 1837) (Decapoda: Brachyura: Gecarcinidae). Zootaxa 4990:192–200. https://doi.org/10.11646/zootaxa.4990.1.13

Milne Edwards H (1837) *Histoire naturelle des crustacés, comprenant l´anatomie, la physiologie et la classification de ces animaux*. Encyclopédique Roret, Paris; vol I (1834): i–xxxv + 1–468, vol II (1837): 1–532, vol III (1840): 1–638, pls 1–42.

Pantaleão JA, Peres PA, Mantelatto FL (2025) Mapping the congruence between larval morphology and molecular phylogeny: convergent adaptations of freshwater shrimps (Decapoda, Caridea). Zoolog Scr 54(3):376–393. https://doi.org/10.1111/zsc.12709

Pinheiro MAA, Santana W, Rodrigues ES, Ivo CTC, Santos LCM, Torres RA, Boos H, Dias-Neto J (2016) Avaliação dos caranguejos Gecarcinídeos (Decapoda: Gecarcinidae). In: Pinheiro MAA, Boos H (eds) *Livro vermelho dos crustáceos do Brasil: avaliação 2010–2014*. Sociedade Brasileira de Carcinologia, Porto Alegre, pp 167–181

Pinheiro MAA, Dias-Silva I, Kriegler N, Santana W, João MCA (2024) Beneath the surface: co-habitation of recruits of the land crab *Johngarthia lagostoma* and its relevance for conservation on oceanic islands. J Mar Biol Assoc UK 104(e102):1–9. https://doi.org/10.1017/S0025315424000869

Pocock RI (1889) On the Arachnida, Myriopoda and land-Crustacea of Christmas Island. Proc Zool Soc Lond 1888(4):556–564

Pohle G, Telford M (1981) Morphology and classification of decapod crustacean larval setae: a scanning electron microscope study of *Dissodactylus crinitichelis* Moreira, 1901 (Brachyura: Pinnotheridae). Bull Mar Sci 31:736–752

Rodríguez-Rey GT, Hartnoll RG, Solé-Cava AM (2016) Genetic structure and diversity of the island-restricted endangered land crab, *Johngarthia lagostoma* (H. Milne Edwards, 1837). J Exp Mar Bio Ecol 474:204–209. https://doi.org/10.1016/j.jembe.2015.10.016

Santana W, Colavite J, Bolaños J, Hernández JE, Canepa M (2016) Morphology of the larval stages of *Pitho aculeata* (Gibbes, 1850) (Crustacea, Brachyura, Majoidea) and its implications on the taxonomic position of the genus. Mar Biol Res 12(8):854–863. https://doi.org/10.1080/17451000.2016.1209524

Tamura K, Stecher G, Kumar S (2021) MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol 38(7):3022–3027. https://doi.org/10.1093/molbev/msab120

Tavares M (1989) Sistemática filogenética dos Gecarcinidae (Crustacea, Decapoda, Brachyura). Museu Nacional, Universidade Federal do Rio de Janeiro, Dissertação de Mestrado

Tavares M (1991) Cladistic analysis and classification of the Gecarcinidae (Crustacea Brachyura). Mem Queensl Mus 31:213

Tavares M (2002) True crabs. In: Fischer W (ed) FAO species identification sheets for fishery purposes: Western Central Atlantic (Fishing Area 31). FAO, Rome, pp 327–352

Tavares M, Mendonça JBD Jr (2022) Brachyuran crabs (Crustacea, Decapoda) from the remote oceanic Archipelago Trindade and Martin Vaz, South Atlantic Ocean. Zootaxa 5146:1–129. https://doi.org/10.11646/zootaxa.5146.1.1

Teschima MM, Ströher PR, Firkowski CR, Pie MR, Freire AS (2016) Large-scale connectivity of *Grapsus grapsus* (Decapoda) in the Southwestern Atlantic oceanic islands: integrating genetic and morphometric data. Mar Ecol 37:1360–1372. https://doi.org/10.1111/maec.12347

Thompson J (1836) Art. XXXIII.-of the double metamorphosis in Macropodia Phalangium, or Spider-Crab, with proofs of the Larvae being zoëa in Gegarcinus hydrodomus, Thelphusa erythropus, Eriphia carribæa, and Grapsus pelagicus. Entomol Mon Mag 3:370–375

Türkay M (1970) Die Gecarcinidae Amerikas. Mit einem Anhang über *Ucides* Rathbun (Crustacea: Decapoda). Senckenb Biol 51(5–6):333–354.

Ueda K, Yanagimoto T, Chow S, Kuroki M, Yamakawa T (2021) Molecular identification of mid to final stage slipper lobster phyllosoma larvae of the genus *Chelarctus* (Crustacea: Decapoda: Scyllaridae) collected in the Pacific with descriptions of their larval morphology. Zool Stud 60:75. https://doi.org/10.6620/ZS.2021.60-75

Webb GE (1922) LIII.—note on some young stages of *Gecarcoidea lalandii*, Milne-Edwards. Ann Mag Nat Hist 10(59):530–534. https://doi.org/10.1080/00222932208632799

Willems KA (1982) Larval development of the land crab *Gecarcinus lateralis lateralis* (Fréminville, 1835) (Brachyura: Gecarcinidae) reared in the laboratory. J Crustac Biol 2:180–201. https://doi.org/10.2307/1547998

Wong JY, Chen H-N, Chan BKK, Tan IKP, Chong VC (2014) A combined morphological and molecular approach in identifying barnacle cyprids from the Matang Mangrove Forest Reserve in Malaysia: essentials for larval ecology studies. Raffles Bull Zool 62:317–329