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RESEARCH

Ontogenetic allometry of the first gonopod in the land crab *Johngarthia lagostoma* (H. Milne Edwards, 1837) (Crustacea: Brachyura: Gecarcinidae): delayed development of the corneous unguis suggests morphofunctional specialization

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Abstract

In Brachyura, reproduction involves a range of behavioral, morphological, and physiological mechanisms that contribute to reproductive success. The land crab *Johngarthia lagostoma*, endemic to four oceanic islands in the South Atlantic, shows no clear pre- or post-copulatory guarding behavior, and the mechanisms involved in sexual selection remain uncertain. This study investigated the ontogenetic development of the first gonopod using relative growth analyses and high-resolution imaging techniques, with special emphasis on the corneous unguis, a distal non-calcified structure of unknown function. Morphometric analyses were performed using carapace width as the independent variable and measurements from different gonopod regions, including the corneous unguis, as dependent variables. Relative growth was evaluated using power function, and growth shifts were estimated from the intersection between juvenile and adult growth phases. Nano-computed tomography and X-ray analyses were used to describe the overall morphology of the gonopod and provided detailed visualization of its distal region, including the horny claw and associated setae field. Most biometric relationships showed growth shifts at similar sizes (50–55 mm *CW*). In contrast, the corneous unguis showed a delayed ontogenetic transition (64.9 mm *CW*), indicating a developmental trajectory distinct from that of the other gonopod regions. The coordinated maturation of most gonopod regions, together with the delayed development of the corneous unguis, indicates differential developmental timing among components of the copulatory apparatus. Although the functional role of the corneous unguis remains uncertain, its distinct ontogenetic pattern supports the hypothesis that it may be involved in post-copulatory reproductive processes associated with sperm transfer and/or ejaculate processing.

Keywords Accessory structures · Relative growth · Reproductive morphology · Sperm competition

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Introduction

Reproductive strategies comprise a set of behavioral, morphological, and ecological mechanisms shaped over evolutionary time that directly contribute to species reproductive success (Hartnoll 1969; Andersson 1994). In brachyuran crabs, sexual selection acts differently on males and females, shaping distinct morphological, physiological, and behavioral adaptations involved in reproduction (McLay and López-Greco 2011; Sal-Moyano et al. 2014; João et al. 2021). Females commonly exhibit anatomical adaptations, such as seminal receptacles, which allow sperm storage and enable polyandry, thereby increasing offspring genetic diversity (McLay and López-Greco 2011). Males, in turn, generally display strategies related to pre- and post-copulatory competition, aimed at maximizing the fertilization of female gametes and ensuring paternity, including agonistic behavior, female guarding, sperm transfer, and ejaculate positioning (McLay and Becker 2015; González-Pisani and López-Greco 2020; Becker and Bauer 2020).

Male reproductive success is often associated with phenotypic differences among competitors (Brockerhoff and McLay 2005; Reading and Backwell 2007). Body size and cheliped size are commonly considered dominance traits (McLay and Becker 2015; Fazhan et al. 2017), with larger males bearing more developed chelipeds often achieving higher mating rates than smaller individuals (Sainte-Marie et al. 2008; Waiho et al. 2015). However, when these external traits do not provide clear evidence of reproductive strategies, gonopod morphology may provide important clues about sperm transfer and other post-copulatory reproductive processes (Ewers et al. 2015; Lianos et al. 2021; Mollemberg et al. 2021). Gonopods, especially the G1, are essential diagnostic characters in brachyuran crab systematics (Guinot 1977), and their distal region often bears accessory structures such as setae, sensilla, spines, protuberances, and corneous projections (Beninger et al. 1991; Rorandelli et al. 2008; Kienbaum et al. 2017; Lianos et al. 2021). Such structures may assist in gonopod positioning, sperm transfer, and interaction with the female reproductive tract (Diesel 1991; McLay and López-Greco 2011), although their precise function is often difficult to demonstrate directly Becker and Bauer (2020). In brachyuran crabs, abrupt changes in the development of reproductive structures are commonly associated with the onset of morphological sexual maturity and shifts in reproductive investment during ontogeny (Hartnoll 1974, 1978, 1982; Pinheiro and Fransozo 1998). Therefore, analyses of allometric growth have been widely employed to investigate the development and possible functional specialization of reproductive structures, including gonopods (Oliveira-Rocha et al. 2019; Mollemberg et al. 2021).

For land crabs, the literature on mating strategies is still limited, and some species lack detailed accounts of copulatory behavior (João et al. 2021 and references therein). The superfamily Gecarcinoidea MacLeay, 1838, includes some of the most terrestrial species within infraorder Brachyura (Marin and Tiunov 2023) and was recently divided into the families Cardisomatidae Watabe, 2007 and Gecarcinidae MacLeay, 1838 (Guinot and Rodríguez-Moreno 2026). Within this group, *Johngarthia lagostoma* (H. Milne Edwards, 1837) is a land crab restricted to four oceanic islands in the South Atlantic: Ascension, Fernando de Noronha, Rocas Atoll, and Trindade (Rodríguez-Rey et al. 2016). This species is currently considered Endangered (EN) due to its reduced area of occupancy, high endemism, habitat degradation, and threats from exotic species (Cumberlidge and Sharp 2025). Recent studies have addressed population density and structure, mating behavior, sexual maturity, color variation, recruitment, megalopa description and reproductive potential in this species, especially on Ascension (Hartnoll et al. 2006, 2009, 2010, 2017) and Trindade island (João et al. 2021, 2022, 2023, 2024; Pinheiro et al. 2024; Santana et al., 2025; Mosna et al. 2025a; Kriegler et al. 2026).

Concerning copulation behavior, mating in *J. lagostoma* occurs near beaches, close to sand dune vegetation where sexual partners show similar sizes and share the same environment with other adult individuals (João et al. 2021, 2024). According João et al. (2021), only a few cases of male-male combat were recorded during copulation, raising the hypothesis of weak competition for receptive females, and no post-copulatory behavior (e.g., female guarding by the male) was observed, with partners separating at the end of copulation. These observations raise questions about whether sexual selection in this species may involve less conspicuous mechanisms, including sperm transfer, ejaculate placement, or other post-copulatory processes. The morphology of the first gonopod pair of *J. lagostoma* was previously described by Türkay (1970), and this structure acts to transfer sperm to females during copulation, with only its apical region being inserted into the female gonopores. More recently, Tavares and Mendonça (2022) described a distal non-calcified structure in the first gonopod, the "corneous unguis", whose function remains uncertain.

Despite recent advances in the reproductive biology of *J. lagostoma* (Mosna et al. 2025b), the functional significance of gonopod morphology and its ontogenetic development remain poorly understood. A few studies on land crabs show that the sperm content stored by a single female may comprise a genetic pool from several males (Jennings et al. 2000). Given the apparent absence of intense pre- and post-copulatory competition in *J. lagostoma* (see João et al. 2021), morphological traits associated with sperm

transfer may represent important targets of sexual selection, although direct functional evidence is still lacking. We hypothesize that gonopod structures exhibit differential allometric growth during ontogeny, reflecting the progressive acquisition of reproductive functionality and morphological sexual maturity. Accordingly, patterns of relative growth may reveal the timing and functional significance of gonopod development. The present study evaluates the ontogenetic growth patterns of the first gonopod through biometric relationships in order to investigate their morpho-functional development.

Materials and methods

Study area and sampling procedures

Male crabs were manually collected from Andradas Beach, one of the main breeding areas of *J. lagostoma* on Trindade Island (20º51'9.44″ S, 29º31'22.34″ W), following João et al. (2024). Sampling was conducted at night using red-light headlamps. Carapace width (*CW*, maximum width; Fig. 1A) was measured with a mechanical caliper (0.05 mm precision), to obtain a homogeneous distribution of individuals across the ontogenetic range of the species. For descriptive purposes, the crabs were grouped into size classes based on 10 mm carapace width (*CW*), covering the observed size range. Each specimen was individually stored in a plastic bag until return to the laboratory, where individuals were subjected to cryoanesthesia and subsequently dissected. The first pair of gonopods was removed using forceps and a stylus, individually placed in properly labeled vials, and preserved in 70% ethanol for further analyses.

Furthermore, considering that the first pair of gonopods of *J. lagostoma* bears, in its subdistal region a non-calcified structure called the corneous unguis, whose function remains uncertain (Tavares and Mendonça 2022), we sought to evaluate its ontogenetic development. To this end, the presence or absence of this structure was recorded for each individual. This procedure supported subsequent analyses of relative growth by restricting the sample to individuals bearing a fully developed and undamaged corneous unguis.

The morphological analysis of the gonopod was performed using a GE Phoenix v|tome|x s 240 industrial high-resolution computed tomography and X-ray system at the Center for Biodiversity Documentation – CDB, FFCLRP/USP. Sample preparation and image processing for nanotomography followed the protocol established by Mantelatto et al. (2022) and subsequent adaptations made by Oliveira-Rogeri et al. (2023). This system is equipped with a DXR250RT detector panel of 1,000 × 1,000 pixels, with a minimum voxel size of 2 µm and a maximum energy of 180 kV (nano-CT). A diamond target was used with the following parameters: source operating at 60 kV, 200 µA, 1 × 1 binning, 333 ms exposure time, 1,200 projections, 1 frame skipped, 4 frames averaged, default offset and gain corrections. Image processing and 3D volume reconstruction were performed using GE phoenix datos|x 2, and subsequent volume analysis was developed in Dragonfly Software (Comet Technologies Canada Inc 2026).

Relative growth

Relative growth is a morphometric analysis widely used for crustaceans, whose growth is discontinuous and dependent on the molting process (Clayton 1990; Syama 2009). This procedure is a relevant tool for evaluating and interpreting sexual characteristics, allometric growth patterns, and maturity sizes (Hartnoll 1974, 1978, 1982).

Gonopod measurements were obtained using a computer-assisted image analysis system (SAIC; KS-300® Zeiss®), coupled to a stereomicroscope (Zeiss® Stemi® SV-6) equipped with a digital camera (Zeiss® AxioCam® MRc5®), with a precision of 0.01 mm. Five dependent variables

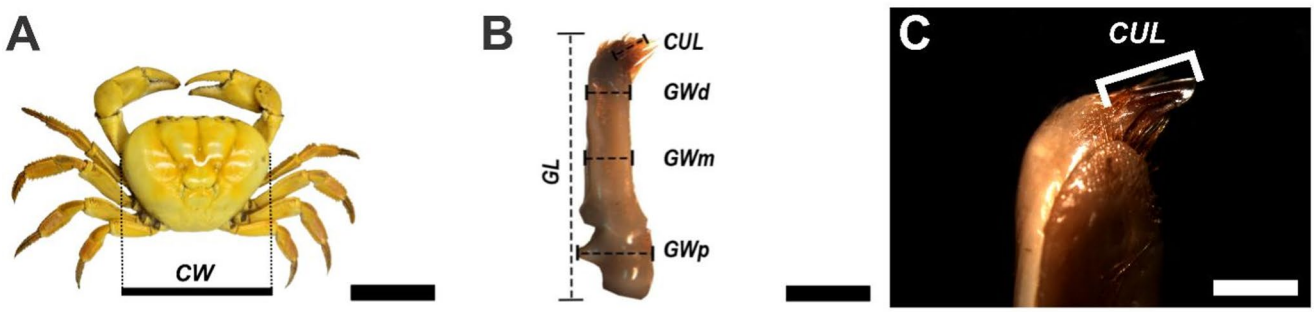


Fig. 1 Structures of *Johngarthia lagostoma* (H. Milne Edwards, 1837) used in morphometric analyses: **A** Carapace width (*CW*, mm) used as an independent variable in biometric relationships (scale = 60 mm); **B** dependent variables measured on the first pair of gonopods: *GL* gonopod length; *CUL* corneous unguis length; *GWd* width of the distal region of the gonopod; *GWm* width of the mesial region of the gonopod; *GWp* width of the proximal region of the gonopod (scale = 10 mm); **C** Detail of the distal region of the first pair of gonopods under a stereomicroscope, focusing on the apical structure called the corneous unguis (Tavares and Mendonça 2022) (scale = 2 mm)

(Y-axis; Fig. 1B, C) were measured for each specimen with previously recorded size data (*CW*, X-axis; Fig. 1A), namely: *GL*, gonopod length, measured from the coxo-basal fusion of the protopodite to its distal portion, excluding the apical corneous unguis; *CUL*, length of the corneous unguis (Fig. 1C); *GWd*, width of the distal region of the gonopod, immediately below the tuft of setae located anterodistally on the appendage; *GWm*, width of the mesial region of the gonopod, corresponding to the midpoint of the total appendage length; and *GWp*, greatest width of the proximal region of the gonopod protopodite. When a specific region of the gonopodium was worn or damaged and could not be measured, only the corresponding measurement was excluded, while valid measurements from other regions were retained, with the sample size varying between biometric ratios.

The empirical points of the five biometric relationships (*GL* × *CW*, *CUL* × *CW*, *GWd* × *CW*, *GWm* × *CW* and *GWp* × *CW*) were initially subjected to residual analysis to discard spurious points that could compromise the fit of the mathematical equations (Sokal and Rohlf 2012). We used the power function ($Y = aX^b$) to assess the relative growth of gonopod measurements in relation to male carapace width in *J. lagostoma* (Hartnoll 1974, 1978, 1982). Linear equations corresponding to the ontogenetic phases (juvenile and adult) were identified using the segmented package in R (Muggeo 2008; R Core Team 2026), after logarithmic transformation. Growth patterns were analyzed based on the relative growth constant *b* (model slope), which may indicate isometric growth ($b = 1$), positive allometry ($b > 1$), or negative allometry ($b < 1$), together with the respective coefficients of determination (R^2). Departures from isometry were tested for all relationships using Student's t-test. Subsequently, morphological maturity values were determined for each biometric relationship (see Somerton 1980) using the Mature routine in R (adapted from Ihaka and Gentleman 1996). When an abrupt breakpoint was observed between growth phases, the size at maturity corresponded to the point of intersection. However, when overlap between ontogenetic phases was observed, a sigmoid curve was fitted to the percentage of adult individuals in each size class (10 mm *CW*), and the size at which half of the individuals were morphologically mature ($CW_{50\%}$) was estimated.

To confirm whether one or two regression lines were required for the empirical points of each biometric analysis, the data were subjected to analysis of covariance (ANCOVA) in order to test the significance of contrasts between slopes (*b*, the allometric growth constant of the equations), as well as between intercepts (a, the Y-axis intercept constant of the equations) (Sokal and Rohlf 2012). Thus, the effect of the size covariate (*CW*), the categorical variable (ontogenetic phase: juvenile and adult), and the interaction between these two factors were tested on the dependent variables (*GL*, *CUL*, *GWd*, *GWm*, and *GWp*). When the interaction between factors (*CW* and ontogenetic phases) was statistically significant, the slopes of the mathematical equations were assumed to differ significantly and, therefore, were represented separately.

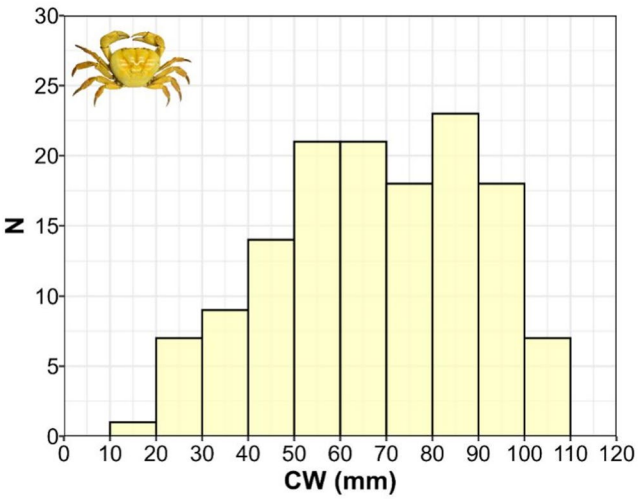


Fig. 2 Frequency histogram of male size (*CW*, carapace width) for specimens used in relative growth analyses of the first gonopod of *Johngarthia lagostoma* (H. Milne Edwards, 1837)

Results

A total of 139 male specimens of *J. lagostoma* were analyzed. Crab size ranged from 17.6 to 104.6 mm *CW* (mean ± standard deviation: 67.0 ± 21.6 mm *CW*) and was distributed across ten size classes from 10 to 110 mm *CW* (Fig. 2). The corneous unguis was recorded in all analyzed specimens, indicating its presence throughout ontogeny. Complementary analyses using nano-CT and X-ray imaging allowed the general morphology of the gonopod to be documented, as well as detailed visualization of the apical region where the corneous unguis projects. The gonopod showed an elongated and slightly curved shape (Fig. 3A, B, E, and F), with an apical tuft of setae and the corneous unguis projecting distally, allowing the relationship between the corneous unguis and the surrounding setal field to be clearly visualized (Fig. 3C, D, and G). Furthermore, the corneous unguis was structurally distinct from the distal region of the gonopod, appearing more radiolucent, consistent with its non-calcified nature, closely associated with the surrounding setal tuft (Fig. 3G).

Relative growth

All biometric relationships showed a biphasic growth pattern, with significant differences between juvenile and

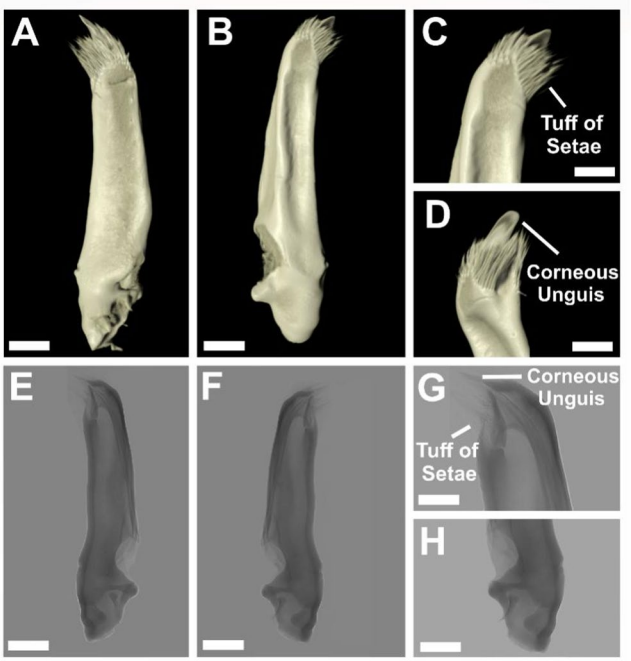


Fig. 3 Morphology of the first gonopod of *Johngarthia lagostoma* (H. Milne Edwards, 1837). **A–D** Images obtained by nanotomography; **A** mesoventral view; **B** mesodorsal view; **C** detail of the distal region, highlighting the setal tuft and the apical portion; **D** detail of the subdistal region showing the corneous unguis and associated setae; **E–H** images obtained by X-ray; **E** mesoventral view; **F** mesodorsal view; **G** detail of the subdistal region, highlighting setal tuft and the corneous unguis in the apical portion; **H** detail of the proximal region. Scale bars = 1 mm

adult ontogenetic phases (Table 1). Breakpoints occurred between 50 and 55 mm *CW* for the gonopod biometric relationships (Fig. 4A, C, D, and E), except for the corneous unguis, for which the breakpoint was estimated at 64.9 mm *CW* (Fig. 4B). The slopes of the regression lines differed between ontogenetic phases (Table 1), with juveniles showing positive allometry ($b > 1.10$; $p < 0.05$) and adults showing marked reductions in allometric growth, with negative allometry ($b < 0.90$; $p < 0.05$), mainly in the corneous unguis, whose allometric coefficient decreased from 1.12 to 0.43 (61.6%). The width of the mesial region was the only measurement that showed isometric growth in the adult phase ($b = 0.99$; $p \geq 0.05$). ANCOVA revealed a significant effect of the interaction between ontogenetic phase and carapace width for each dependent gonopod variable analyzed (Table 2).

Discussion

Ontogenetic changes in the relative growth of the first gonopod of *J. lagostoma* revealed a similar timing of growth shifts across most gonopod regions, with breakpoints occurring within a narrow range of body sizes (50–55 mm *CW*), making this one of the few studies to address gonopod allometry in a land crab. The convergence of maturity estimates for *GL*, *GWd*, *GWm*, and *GWp* within a narrow size interval indicates that most regions of the copulatory apparatus undergo coordinated morphological development during the transition from juvenile to adult stages. In contrast, the corneous unguis exhibited a later breakpoint, indicating a distinct developmental trajectory. This temporal decoupling suggests that the structure continues to develop after the remaining gonopod regions have already reached their adult growth pattern, possibly reflecting the later establishment of a specialized reproductive morphology. Therefore, our findings support the hypothesis that different gonopod regions follow distinct ontogenetic growth trajectories during reproductive maturation. However, direct functional evidence linking the corneous unguis to sexual selection or post-copulatory processes remains unavailable.

Table 1 Relative growth of the first gonopod of *Johngarthia lagostoma* (H. Milne Edwards, 1837)

Biometric relationships	OP	*n*	Power function ($Y = aX^b$)	Linearized function ($\ln Y = \ln a + b \ln X$)	R^2	t	A
GL × *CW*	J	32	$GL = 0.14CW^{1.11}$	$\ln GL = -1.96 + 1.11 \ln CW$	0.91	1.81*	+
	A	106	$GL = 0.51CW^{0.79}$	$\ln GL = -0.68 + 0.79 \ln CW$	0.94	–11.03*	–
CUL × *CW*	J	54	$CUL = 0.01CW^{1.12}$	$\ln CUL = -4.18 + 1.12 \ln CW$	0.88	2.24*	+
	A	63	$CUL = 0.27CW^{0.43}$	$\ln CUL = -1.31 + 0.43 \ln CW$	0.57	–7.37*	–
GWd × *CW*	J	31	$GWd = 0.004CW^{1.51}$	$\ln GWd = -5.33 + 1.51 \ln CW$	0.91	5.89*	+
	A	106	$GWd = 0.06CW^{0.84}$	$\ln GWd = -2.72 + 0.84 \ln CW$	0.85	–4.53*	–
GWm × *CW*	J	38	$GWm = 0.013CW^{1.27}$	$\ln GWm = -4.37 + 1.27 \ln CW$	0.87	3.43*	+
	A	88	$GWm = 0.03CW^{0.99}$	$\ln GWm = -3.28 + 0.99 \ln CW$	0.60	-0.06^{ns}	0
GWp × *CW*	J	38	$GWp = 0.03CW^{1.13}$	$\ln GWp = -3.43 + 1.13 \ln CW$	0.87	1.88*	+
	A	100	$GWp = 0.08CW^{0.89}$	$\ln GWp = -2.50 + 0.89 \ln CW$	0.89	–3.14*	–

Equations were obtained by regression analysis using the power function and its logarithmic transformation, with gonopod length (*GL*), corneous unguis length (*CUL*), distal gonopod width (*GWd*), mesial gonopod width (*GWm*), and proximal gonopod width (*GWp*) as dependent variables, and carapace width (*CW*) as independent variable. Abbreviations: A, allometric pattern (+, positive allometry; –, negative allometry; 0, isometric); n, sample size; OP, ontogenetic phase (J, juvenile; A, adult); R^2, coefficient of determination; t, calculated Student's t-value used to test departure from isometry ($b = 1$). The asterisk indicates statistical difference

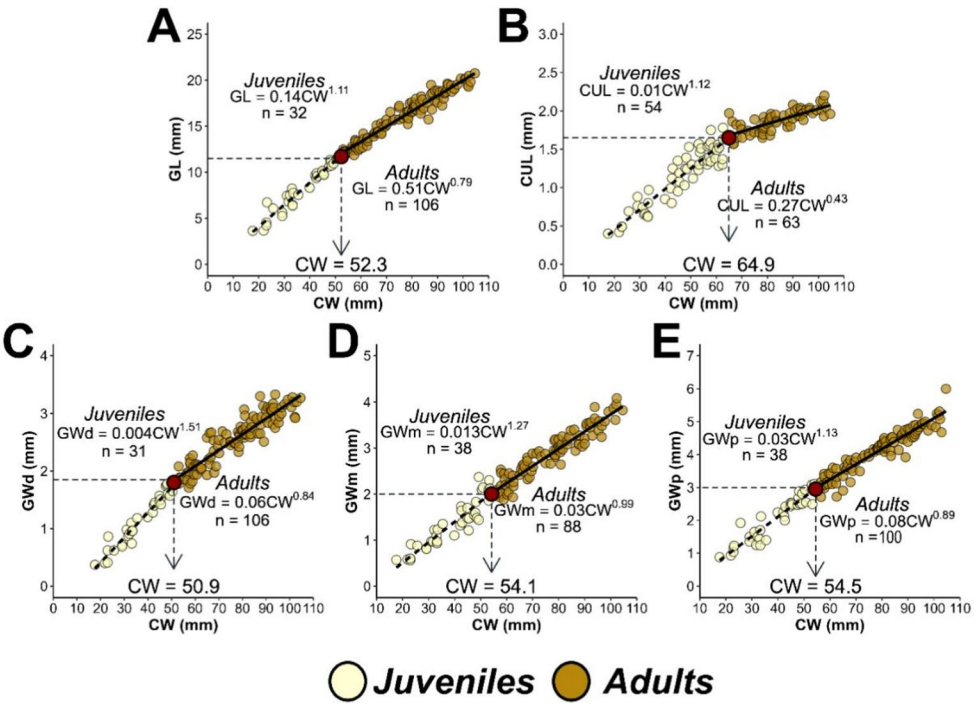


Fig. 4 Relative growth of the first pair of gonopods of *Johngarthia lagostoma* (H. Milne Edwards, 1837). Biometric relationships between gonopod length (*GL*: **A**), corneous unguis length (*CUL*: **B**), width of the distal gonopod region (*GWd*: **C**), width of the mesial gonopod region (*GWm*: **D**), and width of the proximal gonopod region (*GWp*: **E**) in relation to carapace width (*CW*). The variation in dot color indicates the different ontogenetic phases. The dashed arrow, and the red dot, indicates the change in growth between ontogenetic phases, as well as the size at sexual maturity based on the relative growth of the dependent variables for each relationship. Abbreviations: n, number of specimens; R^2, coefficient of determination

Table 2 Summary of the analysis of covariance (ANCOVA) testing the effects of carapace width (*CW*), ontogenetic phase (juvenile and adult), and their interaction on measurements of the first gonopod of *Johngarthia lagostoma* (H. Milne Edwards, 1837)

Dependent variable	Source of variation	df	SS	F
GL	*CW*	1	18.281	**5468.260***
	Phase	1	0.005	1.603
	CW × phase	1	0.157	**47.063***
	Residuals	134	0.448	–
CUL	*CW*	1	14.951	**1661.06***
	Phase	1	0.095	**10.55***
	CW × phase	1	0.431	**47.83***
	Residuals	113	1.017	–
GWd	*CW*	1	26.506	**3424.727***
	Phase	1	0.018	2.267
	CW × phase	1	0.675	**87.269***
	Residuals	133	1.029	–
GWm	*CW*	1	25.100	**2345.201***
	Phase	1	0.049	**4.595 ***
	CW × phase	1	0.153	**14.340***
	Residuals	122	1.413	–
GWp	*CW*	1	21.132	**3028.980***
	Phase	1	0.000	0.015
	CW × phase	1	0.088	**12.680***
	Residuals	134	0.935	–

Dependent variables: *GL* gonopod length; *CUL* corneous unguis length; *GWd* distal gonopod width; *GWm* mesial gonopod width; and *GWp* proximal gonopod width. *df* degrees of freedom, *SS* sum of squares; and *F* Fisher's F-value

**F*-values shown in bold and followed by an asterisk indicate statistical significance at 5% ($p < 0.05$)

In Brachyura, sperm transfer is performed by a pair of intromittent gonopods (*G1* and *G2*) acting together with the penis to inseminate a paired female receptacle, a synapomorphy supporting the monophyly of the infraorder (Guinot et al. 2013; McLay and Becker 2015). In several brachyuran groups, the first gonopod is the main copulatory structure, whereas the second gonopod and the penis are inserted through its proximal suture and act, respectively, in the release and guidance of the sperm mass (McLay and Becker 2015). The distal region usually concentrates projections, setae, sensilla, spines or other accessory structures, which may perform a mechanoreceptive function (Rorandelli et al. 2008), assisting in gonopod positioning, coupling during copulation, and sperm release into the female seminal receptacles (Beninger et al. 1991; Becker and Bauer 2020; Lianos et al. 2021). The mesial and proximal portions, in turn, contribute to the functional stability of the gonopod during copulation, by maintaining the sperm channel and accommodating the penis and the second gonopod (McLay and Becker 2015). In this context, the function of the corneous unguis and its associated setal tuft in the subdistal portion of the gonopod remains one of the most relevant unresolved aspects of the copulatory morphology of *J. lagostoma*.

The relative growth of the gonopod variables in *J. lagostoma* is consistent with patterns reported for other brachyurans crabs (Hartnoll 1974, 1982; McLay and Becker 2015; Oliveira-Rocha et al. 2019). In general, gonopods show positive allometry before maturity and reduced growth after the reaching adulthood (Fig. 5A), reflecting their reproductive function and lower proportional investment once functional size is reached (Pinheiro and Fransozo 1998; Freire

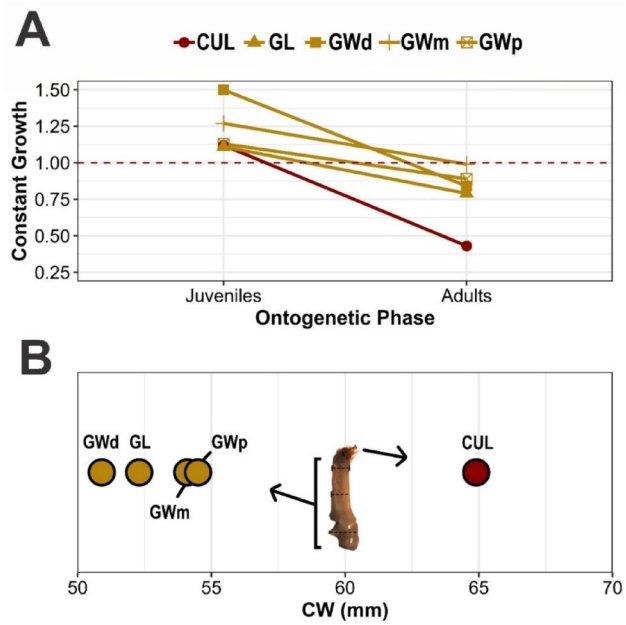


Fig. 5 Compilation of relative growth results for the variables of the first pair of gonopods of *Johngarthia lagostoma* (H. Milne Edwards, 1837). **A** Variation of the allometric constants (*b*) in each ontogenetic phase (juvenile and adult). The dashed red line indicates the isometry value ($b=1$). **B** Sequence of estimated maturity sizes for the variables of the first gonopod; *GL* gonopod length; *CUL* corneous unguis length; *CW* carapace width; *GWd* distal gonopod width; *GWm* mesial gonopod width; *GWp* proximal gonopod width

et al. 2011; González-Pisani and López-Greco 2020). This decrease may indicate that, after the copulatory apparatus reaches a functional configuration, energetic investment may be redirected to other reproductive traits, such as chelipeds (see João et al. 2022). Among gecarcinids, relative growth studies integrating biometric analyses of gonopods remain scarce, with most studies focusing on male cheliped variables (João et al. 2022). The convergence of multiple gonopod measurements within the same maturity size interval (50–55 mm *CW*; Fig. 5B) suggests coordinated development of the copulatory apparatus. This pattern is relevant because different gonopod regions may perform complementary mechanical roles during sperm transfer (Rowe and Arnqvist 2011), thereby contributing to the complete functionality of the copulatory apparatus.

The delayed shift in the growth pattern of the corneous unguis, observed after the maturity size estimated for the remaining gonopod regions (Fig. 5B) and after the functional maturity previously estimated for this species (56.4 mm *CW*; João et al. 2022), may indicate a distinct role after sexual maturity. This ontogenetic shift was particularly evident because the corneous unguis exhibited the greatest reduction in relative growth rate (b-values) between ontogenetic phases, from 1.12 in juveniles to 0.43 in adults. Such a pronounced decrease suggests that most structural investment in this appendage occurs before or around the onset of maturity, followed by a stabilization phase once its functional morphology has been established. Nano-CT and X-ray analyses further revealed that the corneous unguis forms a structurally distinct projection at the distal region of the gonopod, appearing poorly sclerotized and directly associated with the apical setal field. These features support the interpretation that the corneous unguis is not merely a continuation of the gonopod cuticle, but a distinct component of the distal copulatory region.

In brachyuran crabs, modifications in the distal region of these gonopods (e.g., curvatures, spines, denticles, hooks, and setae), have often been interpreted as structures adapted and associated with copulation and post-copulatory processes. However, their functional roles should be interpreted cautiously, because direct evidence for sperm removal or ejaculate manipulation by gonopods remains limited (Becker and Bauer 2020). Such structures may assist in positioning, sperm transfer, spermatophore rupture, or interaction with the female reproductive tract (Beninger et al. 1991; Rorandelli et al. 2008; Sal-Moyano et al. 2014; Antunes et al. 2018; Mollemberg et al. 2021). For *J. lagostoma*, the absence of plugs and sperm packages in the female reproductive system, together with the presence of wall-less spermatophores and free spermatozoa in the lumen of the seminal receptacle, has already been reported (Mosna et al. 2025b). In this context, the possibility that the corneous unguis participates in ejaculate processing or in the mechanical disruption of sperm packages is plausible, but remains hypothetical. Alternatively, the absence of sperm packages may result from dissolution by female secretions (Anilkumar et al. 1999), from male-derived secretions (see Hou et al. 2010; Mosna et al. 2025b), or from the combined action of different mechanisms.

The functional role of the corneous unguis remains uncertain. Nevertheless, its delayed ontogenetic development, distinct growth trajectory, strategic apical position, and marked reduction in allometric growth after maturity collectively support the interpretation that it represents a specialized reproductive structure. A comparable functional interpretation has been proposed for *Stenorhynchus seticornis* (Herbst, 1788), in which denticles occurring in opposite positions in the apical region of the gonopods may contribute to spermatophore rupture during sperm transfer (Kienbaum et al. 2017; Antunes et al. 2018). In this sense, whether the corneous unguis is associated with sperm competition, ejaculate positioning, spermatophore disruption, or other post-copulatory mechanisms remains to be tested experimentally. Future studies employing scanning electron microscopy, high-resolution nanotomography, and three-dimensional reconstructions should provide a more detailed characterization of the corneous unguis and its relationship

with adjacent setal fields and cuticular structures. In addition, genetic analyses for paternity assessment would be valuable to clarify whether post-copulatory sexual selection occurs in *J. lagostoma* and whether the corneous unguis plays any role in this process.

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Author contributions EEDM: conceptualization, original draft preparation, morphometric measurements, statistical analyses, and writing. MCAJ: sampling of specimens, dissection, biometrics, conceptualization of the idea, assistance with morphometric measurements and statistical analyses. DFRA: contribution to relative growth analyses . FLM: provision of Nano-CT and X-ray equipment and assistance in preparing morphological descriptions. FJZ: assistance in morphological description . DG: contribution to the morphological description of the structures throughout the manuscript. MAAP: funding acquisition, research coordination, supervision, and guidance of the first author. All authors reviewed and approved the final version of the manuscript.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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