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Integrative morphological and molecular insights into *Sacculina lata* (Rhizocephala: Sacculinidae) and its effects on the host crab *Podophthalmus vigil* in Nha Trang Bay

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Abstract

Rhizocephalans are highly specialized parasitic crustaceans commonly infesting decapod hosts, including swimming crabs, and often exert profound effects on host growth and development. Their extremely reduced morphology, however, makes taxonomic identification difficult. In this context, molecular genetic tools have become increasingly valuable for resolving taxonomic relationships within the group. Nevertheless, genetic information in this group remains limited, and only a few representative species have been investigated in detail regarding their biological effects on hosts. For many other rhizocephalans, the extent of host impact remains largely unexplored. The rhizocephalan *Sacculina lata* has been recorded parasitizing the portunid crabs *Charybdis miles* and *Podophthalmus vigil* in the western Pacific region, including Vietnam. Despite these records, the parasite's morphology has been documented only through schematic illustrations, while detailed photographic documentation, molecular data, and information on host impacts have not been reported. Accordingly, this study employed an integrative morphological and molecular approach to investigate *S. lata* infesting the swimming crab *Podophthalmus vigil* collected from Nha Trang Bay, Khanh Hoa, Vietnam. Direct observation of fresh specimens, scanning electron microscopy, and histological sections provided a more comprehensive characterization of the parasite's morphological features. Among 542 examined crabs, infestation prevalence was low (3.51%), showed a clear relationship with host size, and was not associated with host sex. Analyses of nuclear (18S rRNA) and mitochondrial (COI) markers clarified the phylogenetic position of *S. lata*, revealing a close relationship with *Sacculina angulata* and several congeners. Parasitism induced pronounced feminization of the abdomen and secondary sexual traits in male hosts, whereas infested females exhibited no significant abdominal modification. Overall, these findings provide new taxonomic, molecular, and ecological insights into rhizocephalan parasitism in an economically important swimming crab species.

Keywords

Excrescences; Feminization; Morphological Modifications; Prevalence; Retinacula.

Introduction

Rhizocephala represents a highly specialized group of parasitic barnacles that infest other crustaceans, including swimming crabs of the family Portunidae (Boschma, 1955; Høeg & Lützen, 1995). Rhizocephalans are characterized by having complex life cycles accompanied by extreme morphological and biological modifications associated with a parasitic mode of life. Infestation is initiated at the cypris larval stage, during which the parasite injects a vermigon into the host's body. Once inside the host, the parasite develops an internal root-like system (the interna) that spreads throughout host tissues to absorb nutrients, eventually giving rise to an external reproductive structure (the externa) that typically protrudes from the abdominal region of the crab (Glenner *et al.*, 2010; Høeg, 1995; Høeg *et al.*, 2020).

The taxonomy of Rhizocephala has traditionally relied largely on the morphology of the externa (Boschma, 1955; Glenner *et al.*, 2010). However, the extreme structural reduction associated with their highly specialized parasitic lifestyle has resulted in a paucity of reliable diagnostic characters, rendering species delimitation based solely on external features inherently problematic. In response to these limitations, integrative taxonomic approaches

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combining gross morphology, anatomical dissection, histological analysis, and scanning electron microscopy have been increasingly employed to reveal subtle yet informative characters (Golubinskaya *et al*., 2021; Lützen *et al*., 2016; Noever *et al*., 2016). In parallel, molecular genetic tools have become indispensable for accurate species identification and for resolving phylogenetic relationships within Rhizocephala (Glenner *et al*., 2003; Glenner & Hebsgaard, 2006; Glenner *et al*., 2021; Høeg *et al*., 2020). Nevertheless, despite their recognized value, genetic data remain strikingly limited, with publicly available sequences representing only a small fraction of the approximately 190 described rhizocephalan species (Høeg *et al*., 2020). Accordingly, expanding integrative morphological and molecular investigations across broader taxonomic and geographic scales is essential for refining species boundaries and achieving a more robust understanding of rhizocephalan diversity and evolution.

Beyond their taxonomic significance, rhizocephalan parasites exert profound effects on their crustacean hosts. Rhizocephalan parasitism induces a wide range of morphological, physiological, and behavioral alterations, frequently culminating in functional or complete parasitic castration (Goddard *et al*., 2005). Among these effects, modifications of the host reproductive system are particularly conspicuous (Kristensen *et al*., 2012; Waiho *et al*., 2017). Gonadal development is often suppressed, while the abdominal region, where the parasite's externa emerges and may mimic the host's egg mass, undergoes substantial restructuring. Such changes may involve alterations in both the segmentation pattern and relative size of the abdominal somites. In male hosts, these effects commonly include degenerative transformations accompanied by the acquisition of female-like morphological characteristics (Oanh *et al*., 2025; Yang *et al*., 2018).

Within this broader biological and taxonomic context, *Sacculina lata* author, date (Sacculinidae) has been reported parasitizing two portunid crab hosts, *Charybdis miles* (De Haan, 1835) and *Podophthalmus vigil* (Fabricius, 1798), across the western Indo-Pacific region, including Japan, China, Vietnam, and Singapore (Boschma, 1933, 1954; Yang *et al*., 2018). These host species are widely distributed and of considerable fisheries importance in the region. Although *S. lata* has been formally described and its diagnostic characters recognized, existing accounts are largely based on schematic line drawings, with a conspicuous absence of photographic documentation capturing the parasite's morphology in situ and in detail. Moreover, molecular genetic information for *S. lata* is currently unavailable, and both the infestation status and biological effects of this parasite on *P. vigil* have received limited attention to date.

To address these knowledge gaps, the present study provides a comprehensive redescription of *Sacculina lata* based on detailed morphological analyses supported by light microscopy and scanning electron microscopy, together with high-resolution photographic documentation. Molecular sequence data are further integrated to clarify its phylogenetic placement within Rhizocephala. In addition, the infestation status of *P. vigil* populations is systematically assessed, and parasite-induced morphological alterations in the host are examined. Collectively, these results contribute new insights into the taxonomy, phylogenetic relationships, and ecological implications of rhizocephalan parasitism in swimming crabs.

Materials and Methods

Crab sampling and Rhizocephalan collection

A total of 542 specimens of *Podophthalmus vigil* were collected from commercial fishing vessels operating in Nha Trang Bay, Khanh Hoa Province, Vietnam, between February and July 2025, with approximately 90 individuals sampled per month. In the laboratory, crabs were sorted by sex, and carapace width (CW) was recorded using digital calipers. Each individual was subsequently inspected for rhizocephalan infestation. When present, rhizocephalan parasites were gently removed from the host and preserved for detailed morphological examination and subsequent molecular analyses.

Female crabs possess seven clearly differentiated abdominal somites (segments 1–7), whereas males typically exhibit five externally visible abdominal somites (segments 1, 2, fused 3–5, 6, and 7). To quantitatively assess the effects of rhizocephalan infestation on host abdominal morphology, abdominal width was measured at two standardized positions. In individuals with seven abdominal segments, measurements were taken across the maximum transverse width of segments 6 and 4. In crabs with five abdominal units, measurements were recorded at the widest point of segment 6 and at the second lateral expansion of fused segments 3–5. All measurement landmarks are illustrated in Figure 6. Representative rhizocephalan specimens used for detailed morphological dissection and combined molecular analyses are listed in Table 1.

Morphological identification

Rhizocephalan parasites were described and identified through detailed examination of the externa, combining gross morphological observations, microanatomical analyses, and scanning electron microscopy (SEM). Gross morphology was assessed using fresh specimens or individuals preserved in 70% ethanol, examined under an Olympus SZ61 stereomicroscope and an Olympus CX41 compound microscope. Information on the specimens examined in detail is provided in Table 1.

Table 1: Measurements and sampling dates of rhizocephalan externae recorded from *Podophthalmus vigil* in Nha Trang Bay. An asterisk (*) indicates specimens used for molecular analysis.

Sample ID	Date collected	Externa dimensions: length × width × height (mm)	Host sex	Host CW (mm)	Intended examination
1	14 Mar 2025	25 × 16 × 9*	Male	48	Fix and stain, Microanatomy
2	22 Apr 2025	8 × 5 × 2	Female	53	
3	12 May 2025	14 × 7 × 3	Male	44	
4	16 May 2025	10 × 6 × 3	Male	57.5	
5	25 Jun 2025	20 × 12 × 6*	Female	64	
6	25 Jun 2025	13 × 8 × 3	Male	54	Fresh, Gross morphology
7	14 Jul 2025	26 × 14 × 8	Male	115	
8		21 × 4 × 8			
9	22 Jul 2025	16 × 9 × 4	Female	35	
10	22 Jul 2025	21 × 14 × 9	Female	58	

For microanatomical investigation, fresh externae were fixed in 10% neutral buffered formalin, dehydrated through a graded ethanol-xylene series, and embedded in paraplast. Transverse sections approximately 5 µm in thickness were prepared using a Slee 5062 microtome. Sections were stained with hematoxylin and eosin and examined with an Olympus CX41 compound microscope equipped with a Canon R digital camera for photomicrographic documentation.

For SEM analysis of the externa mantle cuticle, specimens were dehydrated through an ascending ethanol series, mounted on aluminum stubs, sputter-coated with gold using an EMS–550 coater, and examined with a FEI Quanta 250 scanning electron microscope. The final composite figures were prepared using PhotoFiltre.

For molecular analyses, a small ventral fragment (approximately 2 mm^3) of the Rhizocephalan visceral mass was dissected and preserved in 99.9% ethanol until DNA extraction.

Molecular extraction

Genomic DNA was isolated from preserved Rhizocephala tissue (specimens subjected to genetic analysis are indicated by an asterisk (*) in Table 1) using the DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's protocol. Partial fragments of the nuclear 18S rDNA and mitochondrial cytochrome c oxidase subunit I (COI) genes were amplified by polymerase chain reaction (PCR) using the primer pairs 329F/aR (Spears *et al*., 1994) and LCO1490/HCO2198 (Folmer *et al*., 1994), respectively. Each PCR was performed in a total reaction volume of 25 µL, consisting of 12.5 µL of GoTaq® Green Master Mix (Promega, USA), 1 µL of each primer (10 µM), 3 µL of genomic DNA template, and 7.5 µL of nuclease-free water.

PCR cycling conditions consisted of an initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 45 s, and extension at 72°C for 1 min for the 18S rDNA fragment. Amplification of the COI gene employed 35 cycles of 94°C for 30 s, 42°C for 45 s, and 72°C for 30 s. PCR products were electrophoresed on 1% agarose gels and visualized against an appropriate molecular size standard to verify amplification success and fragment length.

Successfully amplified products were purified using a commercial DNA purification kit (Promega) and sequenced bidirectionally with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), employing the same primers as used in PCR. Raw sequence reads were assembled and edited using Vector NTI Advance v11.5 (Invitrogen, Life Technologies), and multiple sequence alignments were performed in BioEdit v7.0.5.3 (Hall, 1999). All newly generated sequences were deposited in GenBank.

Sequence similarity was assessed through BLAST searches against the GenBank database. Phylogenetic reconstructions were conducted using maximum-likelihood (ML) and neighbor-joining (NJ) approaches. The best-fitting nucleotide substitution model for ML analysis was selected using ModelTest (Paradis & Schliep, 2019), and the ML tree was inferred in R (Schliep, 2011). NJ analyses were performed in MEGA v7.0 (Kumar *et al*., 2016). Both phylogenetic analyses incorporated newly obtained 18S rDNA and COI sequences from the present study together with reference sequences retrieved from GenBank (10 sequences for 18S rDNA and 6 for COI mtDNA), using *Parasacculina yatsui* (Polyascidae) as the outgroup (see Table 2).

Table 2. GenBank accession numbers of ingroup and outgroup taxa with corresponding host species included in the phylogenetic analyses.

№	Rhizocephalan species	Host species	Location	Genbank accession number of Rhizocephala	
				COI mtDNA	18S rDNA
1	*Heterosaccus lunatus* Phillips, 1978	*Charybdis (Charybdis) callianassa* (Herbst, 1789)	Australia	DQ059778	EU082414
2	*Heterosaccus papillosus* (Boschma, 1933)	*Charybdis (Charybdis) anisodon* (De Haan, 1850)	Vietnam	PV631193	OR644332
3	*Loxothylacus panopaei* Gissler, 1884	*Loxorhynchus grandis* Stimpson, 1857	USA	KU905819	AY265364
4	*Loxothylacus texanus* Boschma, 1933	*Callinectes sapidus* Rathbun, 1896	USA	–	L26517
5	*Sacculina angulata* Van Kampen & Boschma, 1925	*Charybdis (Charybdis) hellerii* (A. Milne–Edwards, 1867)	Vietnam	PQ776427	OR644327
6	*Sacculina carcini* Thompson, 1836	*Carcinus maenas* (Linnaeus, 1758)	Sweden	DQ059781	AY520656
7	*Sacculina lata*	*Podophthalmus vigil*	Vietnam	PZ261596	PZ263541
8	Boschma, 1933	(Fabricius, 1798)		PZ276408	PZ263542
9	*Sacculina pugettiae* Shiino, 1943	*Scyra ferox* (Ohtsuchi & Kawamura, 2019)	Russia	–	PP756299
10	*Parasacculina yatsui* (Boschma, 1936)	*Pachygrapsus crassipes* Randall, 1840	Japan	AB197809	MG604305

Prevalence analysis

Crabs were considered infested when a rhizocephalan externa was observed on the abdomen. For comparative analyses, *P. vigil* specimens were grouped by sex (male and female) and assigned to three carapace width categories (≤ 60 mm, 60.5–69.5 mm, and ≥ 70 mm), defined solely for statistical comparison and to maintain balanced sample sizes across groups. Infestation prevalence was calculated as the proportion of infested individuals relative to the total number of examined crabs. Differences in prevalence among host groups were assessed using Chi–square tests.

Effects of infestation analysis

The effects of rhizocephalan parasitism on host morphology were evaluated using a combination of morphological observations, morphometric measurements, and statistical analyses. Each specimen was examined both macroscopically and under a stereomicroscope (Olympus SZ61) to document abdominal segmentation patterns, including the number and relative dimensions of abdominal segments, as well as the developmental condition of the pleopods. Abdominal dimensions were quantified by measuring the width of the fourth and sixth abdominal segments (4AW and 6AW).

To explore size–related patterns in abdominal morphology, scatter diagrams were constructed to illustrate the relationship between carapace width (CW) and abdominal segment width. Analyses were conducted separately for four host categories: normal males (NM), infested males (IM), normal females (NF), and infested females (IF). For each category, CW was plotted against both 6AW and 4AW using Microsoft Excel 2016.

Before inferential analyses, data distributions were evaluated for normality using the Shapiro–Wilk test. The effects of infestation status on abdominal dimensions (6AW and 4AW) were assessed, with CW included as a covariate, using a one–way analysis of covariance (ANCOVA). Pairwise differences among groups were examined through post-hoc contrast analyses. All statistical procedures were conducted in SPSS version 20, and statistical significance was set at $p < 0.05$.

Results

Parasite Taxonomy

Subclass: Cirripedia Burmeister, 1834

Infraclass: Rhizocephala Müller, 1862

Family: Sacculinidae Lilljeborg, 1861

Genus: *Sacculina* Thompson, 1836

Species: *Sacculina lata* Boschma, 1933

Collection locality: Nha Trang Bay, Khanh Hoa Province, Vietnam

Host crabs: *Podophthalmus vigil* (Fabricius, 1798)

Examined samples: 10 individuals (Table 1)

Description

Externa ovoid to trapezoidal (Figure 1C-E). Mantle cuticle thin and densely ornamented with truncated, polygonal pyramid-shaped excrescences (height: 5-8 µm; width: 5-12 µm) (Figure 3A, B). Retinacula scattered, with 4-12 barbed spindles (length: 8-15 µm) arising from a common base (Figure 3C-F).

Visceral mass internally attached to stalk by thin mesentery (Figure 4B, C). Paired receptacles, situated near lower margin of visceral mass, are cylindrical and mostly straight, with slight anterior curvature at their dorsal ends; they are dorsally separate but ventrally confluent (Figure 4A-D). Vasa deferentia paired and straight, entering receptacles gradually (Figure 4A). Colleteric glands situated anteriorly, consisting of approximately 126 highly branched tubules (Figure 4E) lined with a dense layer of scaly chitin (Figure 4F).

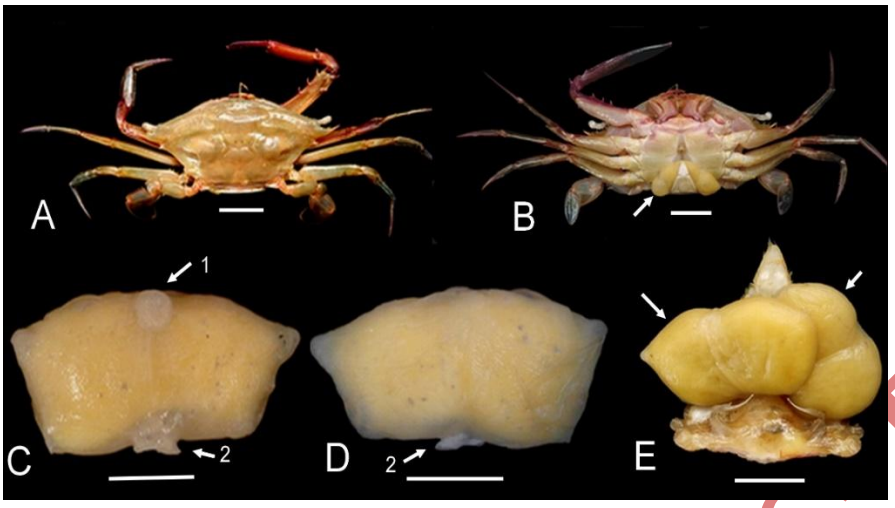

Figure 1. External morphology of *Podophthalmus vigil* infested by *Sacculina lata*: A. dorsal view of host crab; B. ventral view of host crab; externa of *S. lata* visible C. left lateral view of *S. lata*, D. right lateral view of *S. lata*, E. double infestation of *S. lata*. Arrows indicate *S. lata*; 1. mantle opening; 2. stalk. Scale bars: 5 mm.

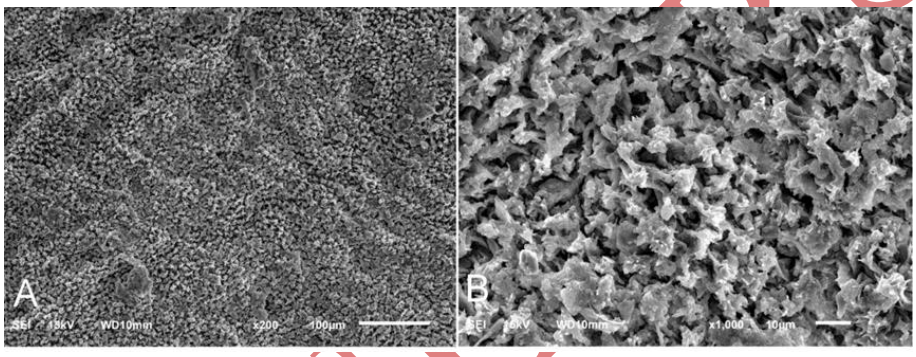

Figure 2. Scanning electron micrograph showing external cuticle of the mantle of *Sacculina lata* parasitizing on *Podophthalmus vigil*, densely ornamented with excrescences. A. magnification 200×; B. magnification 1,000×.

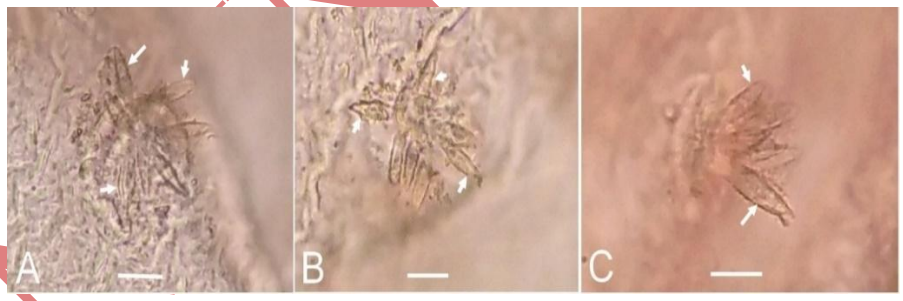

Figure 3. Micrographs of retinacula on the internal mantle cuticle of *Sacculina lata* parasitizing *Podophthalmus vigil*, showing 8 (A), 9 (B), and 6 (C) spindles (arrows), respectively. Scale bars: 10 µm.

Remarks

Morphological examination shows that the *Sacculina lata* specimens examined in this study conform closely to the original description and subsequent accounts from the same host species in the same geographic region. Within the genus *Sacculina*, *S. lata* exhibits the greatest morphological affinity with *Sacculina angulata*, a rhizocephalan parasite of *Thalamita sima* H. Milne Edwards, 1834, *Charybdis (Charybdis) orientalis* Dana, 1852 and *Charybdis (Charybdis) variegata* (Fabricius, 1798) (Boschma, 1955; Huang & Lützen, 1998). Nevertheless, the two species can be reliably distinguished by the structure of the externa excrescences and receptacles. In *S. lata*, the excrescences are papilla-shaped, with paired receptacles connected at both extremities and separated medially, whereas in *S. angulata* the excrescences are truncated and polygonal, with receptacles fused ventrally and separated dorsally.

Molecular validation based on BLASTn searches of both nuclear and mitochondrial markers did not retrieve any reference sequences in GenBank showing a match with the analyzed material. Sequence similarity to the closest available matches ranged from 95.5% to 99.7% for the nuclear 18S rRNA gene and from 83.7% to 94.2% for the mitochondrial COI gene. These results indicate a clear genetic separation between *Sacculina lata* and its congeners, at least those for which sequences are available. Consistent with patterns observed in comparative morphology, molecular analyses further suggest a close phylogenetic affinity between *S. lata* and *S. angulata*. Pairwise genetic distances between the two species reached 8.1% for the COI gene (Table 3) and 0.3% for the 18S rRNA gene (Table 4). In contrast, no intraspecific sequence divergence was detected among *S. lata* specimens for either genetic marker, providing strong support for the taxonomic coherence and genetic identity of the species.

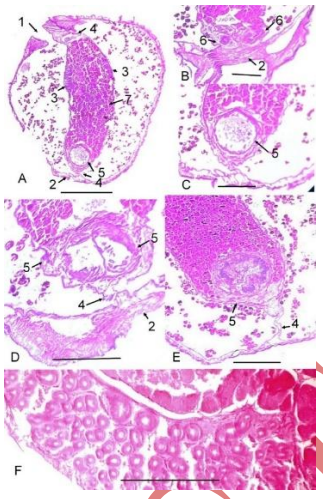

Figure 4. Longitudinal histological sections illustrating internal structures of the externa of *Sacculina lata*. A. Section passing through the central region of the externa; B. Section through the vas deferens; C. Section at the ventral region where the two receptacles are connected; D. Section at a level where the receptacles are separated; E. Section showing separated receptacles on the dorsal side; F. Colleteric gland. 1. mantle opening; 2. stalk; 3. colleteric glands; 4. mesentery; 5. receptacle; 6. vas deferens; 7. visceral mass. Scale bars: A = 1.0 mm; B-F = 0.5 mm

Phylogeny

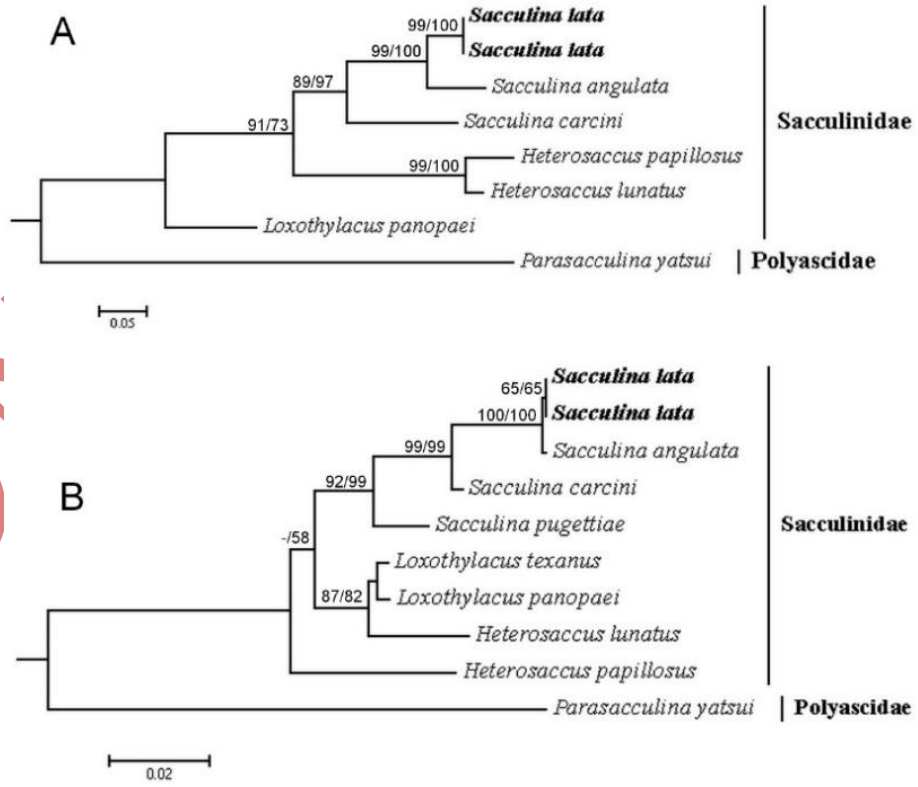

Figure 5. Inferred phylogenetic position of *Sacculina lata* (in bold) among representative Rhizocephala taxa from maximum-likelihood and neighbor–joining analyses. Trees were reconstructed from (A) COI mitochondrial sequences under the TVM+I+G substitution model and (B) 18S rRNA nuclear sequences under the GTR+G(4)+I model. Bootstrap support (ML/NJ; 1,000 pseudoreplicates) is indicated only for nodes receiving ≥ 50% support.

Phylogenetic relationships among selected Rhizocephala species were reconstructed using Maximum Likelihood (ML) and Neighbor–Joining (NJ) approaches based on mitochondrial and nuclear gene datasets. The COI–based phylogeny (Figure 5A) consistently recovered the genus *Sacculina* as being more closely related to *Heterosaccus* than to *Loxothylacus*. Within *Sacculina*, *S. lata* and *S. angulata* formed a strongly supported sister clade, clearly separated from other congeners with a high bootstrap value (100%), indicating robust mitochondrial divergence.

In contrast, the 18S rRNA tree (Figure 5B) revealed a clear genetic separation between *Sacculina* and the remaining genera within Sacculinidae. In this topology, *S. lata* clustered very closely with *S. angulata*, suggesting limited species–level resolution when inferred from the conserved nuclear marker alone. Nevertheless, the distinct separation observed in the COI phylogeny supports their recognition as separate species. Overall, the integration of mitochondrial and nuclear gene datasets yields a more complete and robust phylogenetic reconstruction of Rhizocephala than either marker analyzed independently.

Table 3. Genetic divergence based on COI p-distances (%) among analyzed rhizocephalan species

	Rhizocephala species	1	2	3	4	5	6	7	8
1	*Heterosaccus papillosus*	ID	21.6	23.0	22.6	22.6	6.1	24.6	30.8
2	*Sacculina carcini*		ID	17.5	16.1	6.1	21.3	22.1	31.9
3	*Sacculina angulata*			ID	8.1	8.1	21.9	22.6	30.7
4	*Sacculina lata*				ID	0.0	20.8	21.4	30.3
5	*Sacculina lata*					ID	20.8	21.4	30.3
6	*Heterosaccus lunatus*						ID	24.1	30.2
7	*Loxothylacus panopaei*							ID	29.2
8	*Parasacculina yatsui*								ID

Table 4. Genetic divergence based on 18S p–distances (%) among analyzed rhizocephalan species

	Rhizocephalan species	1	2	3	4	5	6	7	8	9	10
1	*Sacculina lata*	ID	0.0	0.3	2.3	5.0	7.3	6.7	5.9	5.8	13.6
2	*Sacculina lata*		ID	0.3	2.3	5.0	7.3	6.7	5.9	5.8	13.6
3	*Sacculina angulata*			ID	2.2	4.9	7.2	6.6	5.8	5.7	13.6
4	*Sacculina carcini*				ID	4.0	6.6	5.6	5.0	4.9	12.8
5	*Sacculina pugettiae*					ID	5.9	5.1	4.1	4.1	12.6
6	*Heterosaccus papillosus*						ID	5.7	5.0	4.8	12.5
7	*Heterosaccus lunatus*							ID	2.5	2.1	11.5
8	*Loxothylacus texanus*								ID	0.7	11.4
9	*Loxothylacus panopaei*									ID	11.4
10	*Parasacculina yatsui*										ID

Prevalence of infestation

Table 5. Overall, sex–specific, and size–class–specific prevalence of *Sacculina lata* infesting *Podophthalmus vigil*. Different lowercase letters (a, b) indicate statistically significant differences in infestation prevalence among crab size classes ($p < 0.05$).

Overall prevalence (%)	Prevalence by sex (%)		Prevalence by sizes categories (%)		
	Male	Female	≤ 60 mm	60.5–69.5 mm	≥ 70 mm
n = 542	n = 355	n = 187	n = 179	n = 184	n = 179
3.51	3.38	3.74	7.26[a]	2.72[b]	0.56[b]

A total of 542 specimens of *Podophthalmus vigil* were examined, comprising 355 males and 187 females. Among these, 19 individuals (12 males and 7 females) were found to be infested by *Sacculina lata*, resulting in an overall prevalence of 3.51% (Table 5). Single parasite infections predominated, with the exception of one male crab that harbored two rhizocephalans. Although the prevalence in females (3.74%) was slightly higher than that in males (3.38%), this difference was not statistically significant ($\chi^2 = 0.0477$, df = 1, $p > 0.05$). In contrast, infestation prevalence differed significantly among the three crab size classes ($\chi^2 = 12.40$, df = 2, $p < 0.01$). Specifically, the smallest size class exhibited the highest prevalence (7.26%), followed by the intermediate size class of 60.5–69.5 mm (2.72%), whereas only a single infested individual was recorded in the largest size class (≥ 70 mm), corresponding to a prevalence of 0.56% (Table 5). Overall, these results indicate that infestion by *S. lata* in *P. vigil* is more strongly associated with host size than with host sex.

Effects of infestation

In comparison of the abdominal morphology of uninfested *Podophthalmus vigil* (Figure 6A-D) with that of individuals infested by *Sacculina lata*, marked morphological alterations were documented. In all infested males (n = 12), pronounced feminization was observed, characterized by transformation of the abdomen into a distinctly seven–segmented form, with well–developed setae along the lateral margins of each segment, resembling the typical female condition. Nevertheless, the pleopods retained the diagnostic male morphology and did not exhibit branching or reduction (Figure 5E, F). In contrast, infested females (n = 7) showed no apparent morphological modifications, with both the number of abdominal segments and pleopod structure remaining comparable to those of uninfested females (Figure 5G, H). Overall, these observations indicate that infestation by *S. lata* induces substantial, sex-specific morphological modification of the abdomen in *P. vigil*, predominantly affecting male hosts.

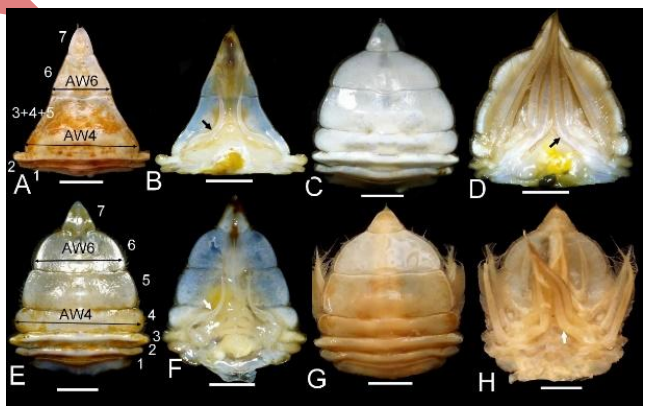

Figure 6. Morphological modifications of the abdomen of the crab *Podophthalmus vigil* induced by infestation by the rhizocephalan *Sacculina lata*. A–B. Dorsal and ventral views of the abdomen of a normal male. C–D. Dorsal and ventral views of the abdomen of a normal female. E–F. Dorsal and ventral views of the abdomen of an infested male. G–H. Dorsal and ventral views of the abdomen of a female infested with *S. lata*. Arrows indicate pleopods. Scale bars: 5 mm.

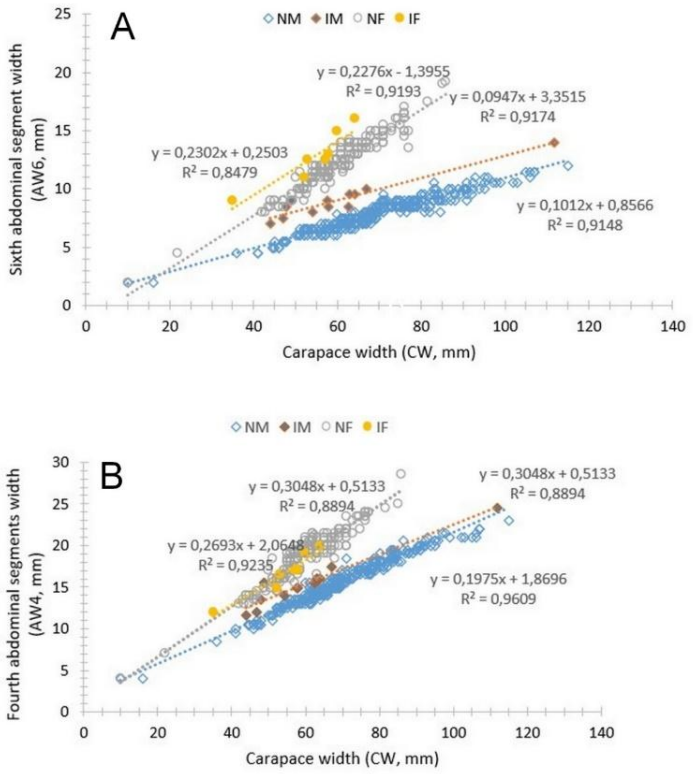

Figure 7. Relationship between carapace width (CW) and abdominal segment dimensions in *Podophthalmus vigil*. Scatterplots illustrate the association of CW with width of the sixth abdominal segment (6AW) (A) and width of the fourth abdominal segment (4AW) (B) in crabs infested and uninfested with *Sacculina lata*. IM. infested male; NM. normal male; IF. infested female; NF. normal female.

Scatterplot analyses revealed a strong positive relationship between carapace width and the widths of both the sixth and fourth abdominal segments across all examined groups, including uninfested males, uninfested females, infested males, and infested females ($r > 0$; $R^2 > 0.84$; Figure 7A, B). This consistent scaling relationship indicates that abdominal segment width generally increases with overall body size regardless of infestation status or sex. After accounting for the effect of carapace width, analysis of covariance (ANCOVA) detected significant differences in the adjusted mean width of the sixth abdominal segment between infested and uninfested individuals of both sexes, with marked effects observed in males ($F = 280.457$, $df = 1$, $p < 0.01$) as well as in females ($F = 48.157$, $df = 1$, $p < 0.01$). These results demonstrate that infestation by *Sacculina lata* is associated with an enlargement of the sixth abdominal segment in both male and female crabs.

In contrast, the response of the fourth abdominal segment exhibited a clear sex–specific pattern. A statistically significant difference in its width was detected only between infested and uninfested males ($F = 280.457$, $df = 1$, $p < 0.01$), whereas no significant difference was observed between infested and uninfested females ($F = 1.672$, $df = 1$, $p > 0.05$). Overall, these findings indicate that while *S. lata* infestation affects abdominal morphology in both sexes, its impact is more pronounced and consistently expressed in male than in female hosts.

Discussion

Rhizocephalans represent one of the most extreme examples of morphological and biological specialization among parasitic crustaceans. Their highly reduced body plan, coupled with the loss of most conventional diagnostic characters, has long posed challenges for species delimitation and comparative taxonomy. *Sacculina lata* has previously been reported from two portunid hosts, *Charybdis miles* and *Podophthalmus vigil*, distributed along the western Pacific coast (Boschma, 1933, 1954). However, detailed morphological

documentation of this species has remained limited. In the present study, we adopted an integrative morphological approach, which allowed a more comprehensive characterization of the parasite's structural organization and revealed several diagnostic features that have not been clearly illustrated before.

In particular, the excrescences on the external cuticle of the mantle were documented in detail and shown to possess distinct structural characteristics. These excrescences differ markedly from those described for *Sacculina angulata*, which has been regarded as the most morphologically similar congener (Boschma, 1955). The clear differentiation observed in these mantle structures provides new morphological evidence supporting the separation of the two species, complementing earlier taxonomic assessments that relied largely on line drawings and limited external descriptions. Consequently, the present study contributes novel, image-based morphological data that substantially refine the diagnosis of *S. lata*.

From a molecular perspective, phylogenetic analyses based on both nuclear 18S rRNA and mitochondrial COI genes revealed patterns that were largely congruent with morphological observations. Both markers consistently placed *S. lata* and *S. angulata* as closely related taxa, supporting their affinity within Sacculinidae. Notably, species-level separation was strongly supported by the COI phylogeny, in which the two taxa formed well-resolved, distinct clades with maximal bootstrap support (100%). In contrast, the 18S rRNA fragment (approximately 1,100 bp) exhibited very low genetic divergence between the two species (0.3%) (Table 4), reflecting the conservative nature of nuclear ribosomal genes. These results highlight the effectiveness of COI as a barcode marker for rhizocephalan taxonomy, while suggesting that nuclear markers may capture deeper evolutionary signals and require longer sequences or multiple primer sets to provide sufficient phylogenetic resolution. The combined use of mitochondrial and nuclear data therefore remains essential for robust species delimitation and evolutionary inference in this group.

Although molecular data for *Sacculina lata* are presented here for the first time, its infestation dynamics have not been entirely unexplored. A previous study provided a detailed and systematic assessment of rhizocephalan infestation in *Charybdis miles* from the Gulf of Tonkin, Vietnam (Yang *et al*., 2018). This work constitutes a rigorous baseline for comparison, enabling a more informed evaluation of infestation patterns observed in the present study. In comparison with that earlier investigation, the prevalence of *S. lata* infesting *Podophthalmus vigil* recorded here was relatively low (3.51%) and showed no significant difference between male and female hosts. In contrast, the previous study on *C. miles* reported a higher overall prevalence (7.4%), with female crabs exhibiting a significantly higher infestation rate than males. These discrepancies may reflect differences in geographic context and host–specific ecological traits, all of which may influence exposure risk and host-parasite interactions.

The study by Yang et al. (2018) (Yang *et al*., 2018) did not explicitly analyze infestation prevalence across host size classes; however, it demonstrated that infested crabs had a significantly smaller mean body size than uninfested individuals. This pattern is consistent with the well-established biological effects of rhizocephalans, which suppress molting and interfere with somatic growth, thereby preventing infested hosts from attaining larger sizes (Høeg, 1995). Although changes in abdominal segmentation were not addressed in the earlier study, male feminization was clearly documented, as evidenced by an enlargement of the abdomen relative to uninfested males. This modification has been interpreted as a parasite-induced alteration that enhances protection and functional accommodation of the externa.

When the host impacts of *S. lata* are compared with those induced by *Heterosaccus papillosus* infesting *Charybdis (C.) anisodon* in the same biogeographic region, notable differences emerge. While *H. papillosus* induces pronounced feminization in both male and female hosts, often resulting in the development of pleopods in males and obscuring sexual

dimorphism (Oanh *et al*., 2025), *S. lata* appears to exert disproportionately stronger effects on male hosts, with more limited morphological modification in females. Importantly, these rhizocephalans belong to two different genera, suggesting that the observed differences in host manipulation may reflect species-specific traits rather than a shared strategy at the generic or familial level. Consequently, further comparative studies encompassing multiple rhizocephalan species and host taxa are required to determine whether such host effects represent adaptive strategies at the species level or are indicative of broader evolutionary patterns at higher taxonomic ranks.

Conclusion

In conclusion, the present study provides the first integrative morphological and molecular characterization of *Sacculina lata* infesting *Podophthalmus vigil*. By integrating fresh observations, ultrastructural and histological analyses with mitochondrial and nuclear genetic data, diagnostic features that distinguish *S. lata* from closely related congeners are clarified and its phylogenetic position within Sacculinidae is confirmed. Moreover, the assessment of infestation prevalence and host impacts reveals host and sex specific patterns that differ from those reported in previous studies on other portunid crabs, highlighting variability in host-parasite interactions among rhizocephalans. Collectively, these findings not only refine the taxonomy of *S. lata* but also contribute new insights into the diversity of parasitic strategies and their ecological consequences in brachyuran hosts.

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