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Morphological and Molecular Identification of *Octolasmis* spp. Ectoparasites Infesting *Scylla serrata* in Southwest Papua, Bird's Head Island Peninsula

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Abstract

Despite existing reports, the cryptic diversity and species-specific ecological impacts of the parasitic barnacle Octolasmis on the economically important mangrove crab *S. serrata* remain poorly understood, particularly in understudied regions such as Papua. This study provides the first integrative taxonomic analysis (combining morphology and mitochondrial COI gene sequencing) of *Octolasmis* spp. infesting *S. serrata* from the Bird's Head Peninsula. Two species, *O. cor* and *O. angulata*, were identified. Phylogenetic analysis confirmed their strong genetic distinctness, which correlated with key morphological divergence, most notably in the development of the capitular disc (28.3% coverage in *O. cor* versus 10.5% in *O. angulata*). These fundamental morphological differences are consistent with the contrasting ecological strategies observed. *O. cor* demonstrated clear ecological dominance, exhibiting the highest prevalence (85%) and the greatest single-species infestation intensity (40 individuals per infested host), whereas *O. Angulata* showed lower prevalence (72%) and significantly lower intensity (15 individuals per infested host). Crucially, co-infestation was associated with the highest parasitic load, showing an intensity of 62 individuals per infested host individual that exceeded the sum of individual infestations, suggesting a synergistic effect. Our findings indicate that interactions between morphologically distinct ectoparasite species may contribute synergistically to the total parasitic burden on the host.

Introduction

Parasitic barnacles of the genus *Octolasmis* (Class Maxillopoda) are ectoparasites that infect crustaceans. *Octolasmis* spp. infestation of respiratory organs (gills) can induce severe physiological impacts. This infestation causes oxidative stress, a significant reduction in hyaline cells (a non-specific immune marker) by up to 14.75%, and triggers severe tissue inflammatory responses, which can ultimately lead to host mortality (Bahtiar et al., 2024).

Octolasmis spp. infestations have been reported in various regions of Indonesia, including Sulawesi, Java, Lombok, Maluku, and Sumatra (Nur et al., 2021; Pattipeiluhu et al., 2024; Sarjito et al., 2025; Yusni & Haq, 2020). Most previous studies have relied on morphological identification, which only resolved taxa to the genus level. A key limitation of this approach is its inability to reveal cryptic species complexes, groups of morphologically identical yet genetically distinct species that may differ in pathogenicity, host preference, and ecology. Consequently, data on the prevalence,

intensity, and impact of specific *Octolasmis* species have become biased and inaccurate, hindering the development of targeted control strategies for this ectoparasite. To address this gap, an integrative taxonomic approach combining classical morphological data with molecular markers is now recognized as the international standard for identifying cryptic species. Analysis of the Cytochrome C Oxidase subunit I (COI) gene sequence from mitochondrial DNA has proven effective for distinguishing parasitic crustacean species, including *Octolasmis* (Wijayanti et al., 2024). This study will not only apply this approach but also aim to generate the first COI DNA barcode sequences for *Octolasmis* spp. associated with *S. serrata* from the Southwest Papua region, specifically the Bird's Head Peninsula.

The research location in Teminabuan, South Sorong, Southwest Papua Province, was strategically selected. This region features extensive mangrove ecosystems and is a developing center for mangrove crab fisheries; however, it is beginning to face pressure from climate change and human activities. These conditions create an ideal 'natural laboratory' for studying host-parasite dynamics within an ecologically transitioning environment. A comprehensive understanding requires not only accurate species identification but also in-depth epidemiological analysis, including prevalence, intensity, and abundance in the gills. Data on these parameters are fundamental for assessing the threat level and understanding the parasite life cycle in nature.

Based on the above rationale, this study was designed to identify *Octolasmis* spp. on *S. serrata* from Southwest Papua's Bird's Head Peninsula using an integrative approach combining morphological and molecular (COI gene) methods. Furthermore, this research will determine the prevalence, intensity, and abundance of *Octolasmis* spp. infestation on the gills of mangrove crabs and interpret whether the morphology of the *Octolasmis* species influences its infestation pattern on the crabs. By achieving these objectives, this study is expected not only to fill a critical taxonomic knowledge gap but also to contribute directly to the development of sustainable aquaculture in mangrove ecosystems.

Material and Methods

Sample Collection

A total of 120 individuals of *S. serrata*, with a carapace length range of 12-15 cm and a weight range of 258-421 g, were collected from the waters of Teminabuan, Southwest Papua, Indonesia, from January to August 2025 (Figure 1). Subsequently, the gills of each crab were examined to isolate *Octolasmis* spp. Specimens were collected in microtubes containing different fixative solutions: 95% ethanol for molecular

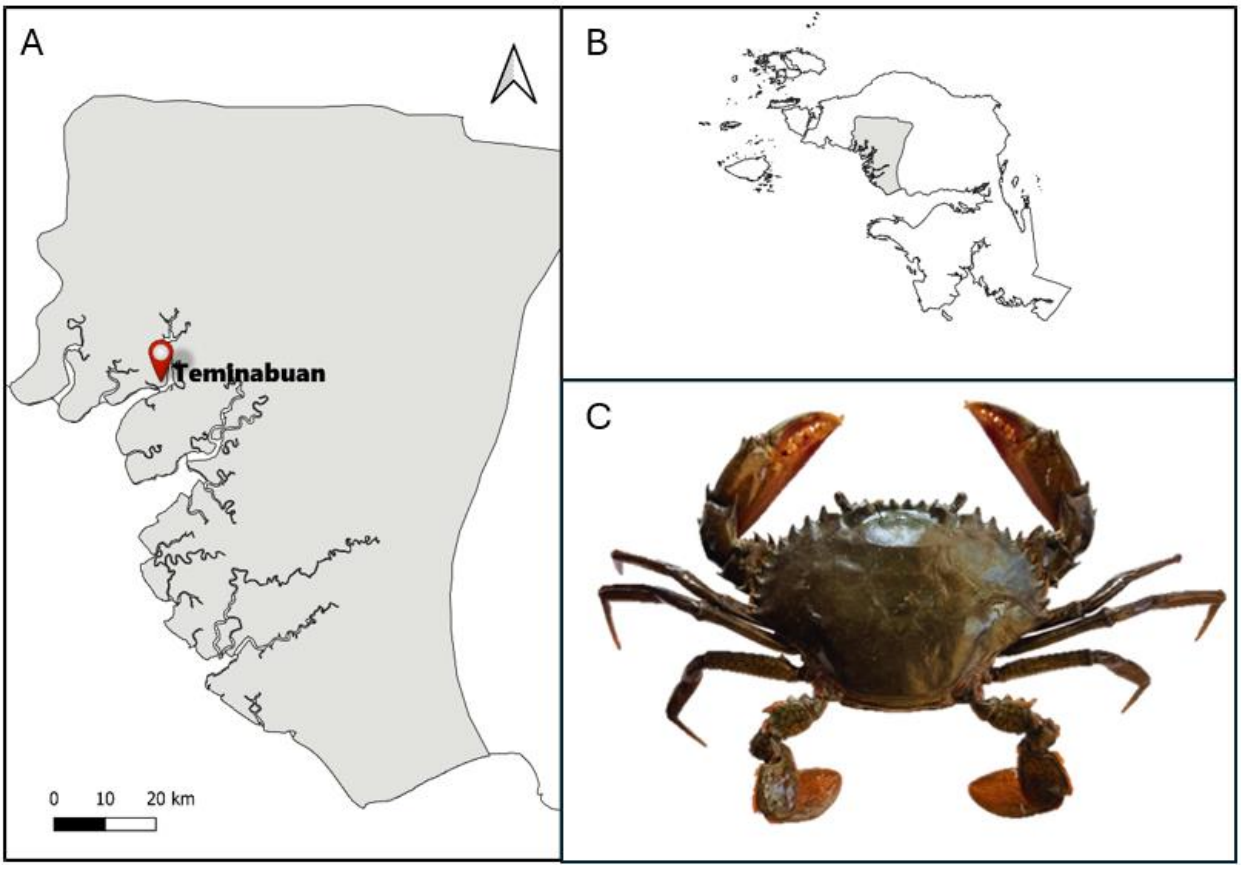

Figure 1. Sampling sites and *S. serrata* in the Teminabuan region, Southwest Papua. (A and B) Map showing sampling locations in the Teminabuan area, Southwest Papua, Bird's Head Peninsula (C) Dorsal views of collected mud crab individuals (*S. serrata*).

analysis and 10% formalin for morphological analysis (Dang et al., 2021; Yap et al., 2015).

The authorization for the utilization of *S. serrata* in this study has been regulated and complies with the criteria set forth by the Ministry of Marine Affairs and Fisheries of the Republic of Indonesia, with reference to Regulation of the Minister of Marine Affairs and Fisheries Number 7 of 2024, Article 5, Point 2, enacted on March 21, 2024. The research method is a non-invasive observation of the gills, employing ice-chilling for anaesthesia, and thus does not involve life challenge tests or inflict suffering on the crabs. Therefore, this study was exempt from requiring specific ethical approval.

Morphological Identification

Octolasmis spp. were identified based on the identification key developed by Jeffries et al. (2005). Specific morphological characteristics, including tergum, scutum, carina, cirri, peduncle, and capitulum, were observed using an Olympus Stereo Microscope SZX7. Additionally, the shape and size of the capitulum and the presence or absence of calcareous plates (and their variations) were recorded.

Molecular Identification

Molecular identification was performed on two Octolasmis specimens with distinct morphologies. DNA isolation and extraction were performed using the gSYNC DNA Extraction Kit (GeneAid Biotech Ltd., Taipei, Taiwan), following the manufacturer's protocol. Initially, *Octolasmis* sp. samples were placed in a sterile 1.5 mL tube and homogenized using a micropestle. DNA was extracted from the tissue using a Geneaid DNA Isolation Kit (Geneaid, New Taipei, Taiwan). The process began by mixing 200 µL of GST buffer, 20 µL of Proteinase K, and 10 mg of tissue sample. The mixture was incubated overnight at 60 °C after incubation, 200 µL of 100% ethanol and 200 µL of GSB buffer were added to the sample. The mixture was then transferred to a GS column mounted on a 2 mL collection tube. Next, 400 µL of W1 buffer was added to the column. The GS column was then treated with 600 µL of wash buffer and centrifuged. The filtrate was discarded, and an additional centrifugation step was performed to dry the column matrix. The spin column was transferred to a new microtube, and 100 µL of pre-heated elution buffer was added to the center of the column matrix. After standing for three minutes, a final centrifugation step was carried out. The purity and quality of the extracted DNA were determined using a Nanodrop spectrophotometer, and the DNA was stored at -20°C preservation and subsequent procedures.

The amplification step was performed using the Polymerase Chain Reaction (PCR) technique on a Peqlab PeqSTAR 96X (Standard) Thermal Cycler. PCR reactions were performed in a total volume of 50 µL containing the following components at specified concentrations: 2 µL of template DNA, 5 µL of 10X Ex Taq Buffer, 2 µL of each primer (10 µM), 0.25 µL of Go Taq DNA polymerase (Promega, Madison City, WI, USA), 2.5 µL of dNTP mix (10 mM), and 35.75 µL of distilled H_2O. The universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), which target the COI gene region, were used (Galan et al., 2018; Ivanova et al., 2007). The PCR mixture was run under the following conditions: initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, annealing at 42°C for 45 s, and extension at 72°C for 45 s. This was followed by a final extension at 72°C for 7 min before cooling the samples to 4 °C. All amplified PCR products were visualized on a 2% agarose gel stained with Gel Red® DNA dye (VWR International PBI, Milan, Italy) and documented using a Sony Xperia XZ3 Android smartphone.

Gene purification and DNA sequencing were conducted using the 1st Base in Malaysia via PT Genetika Science, Indonesia. DNA barcode nucleotide sequences were received as chromatogram trace files (. ab1). These chromatogram files were quality checked, and the forward and reverse nucleotide sequences for each specimen were cleaned, trimmed, and assembled using MEGA 11 software (Tamura et al., 2021) to produce a consensus DNA barcode sequence for each specimen. The NCBI GenBank BLASTn tool was used to obtain homologous sequences from related taxa. Phylogenetic trees were constructed in MEGA 11 using the Maximum Likelihood and Neighbor-Join methods (with 1000 bootstrap replicates). Representative trees were subsequently edited using the Interactive Tree of Life (iTOL) platform (Letunic & Bork, 2019).

***Octolasmis* spp. Infestation Parameters**

The gills of each crab were meticulously examined, with particular attention given to the hyperbranchial and hypobranchial regions to isolate and enumerate all attached individuals of *Octolasmis* spp. The left and right gills were dissected and placed in Petri dishes containing 70% ethanol for fixation and ease of handling, respectively. Each gill was subsequently observed under an Olympus SZX7 Stereo Microscope at a magnification range of 8× to 56× to ensure accurate identification and enumeration. All attached *Octolasmis* spp. individuals, including *O. cor* and *O. angulata*, found on either hyperbranchial or hypobranchial surfaces, were recorded using a handheld digital counter. The infestation pattern of *Octolasmis* spp. in mangrove crab gills was analysed by calculating the prevalence, intensity, and abundance, following the methodology recommended by Rózsa et al. (2000).

$$\text{Prevalence (\%)} = \left(\frac{\text{Number of infested crabs}}{\text{Total number of crabs examined}} \right) \times 100$$

$$\text{Intensity} = \frac{\text{Total number of } \textit{Octolasmis} \text{ spp. counted}}{\text{Number of infested crabs}}$$

$$\text{Abundance} = \frac{\text{Total number of } \textit{Octolasmis} \text{ spp. counted}}{\text{Total number of crabs examined}}$$

Results

Morphology

Morphological identification revealed the presence of two *Octolasmis* types: *O. cor* and *O. angulata*. Their morphological differences are shown in Figure 2. Morphometric analysis showed that *O. cor* had an average capitulum length of 2.62±0.23 mm, while *O. angulata* had a shorter capitulum length of 2.51±0.21 mm. These findings are consistent with the research of Voris et al. (2000), who reported an average capitulum length of 2.065 mm (range 0.572-4.719 mm) for *O. cor* and 1.88 mm (range 0.858-4.004 mm) for *O. angulata*. The morphological differences between the two species primarily lie in the development of their capitular structures. *O. cor* possesses a complete capitular disc with a plate coverage of 28.3±0.20% and a broad, robust scutum, whereas *O. angulata* has a reduced capitular disc with only 10.5±0.17% coverage and a tapered scutum tip. Furthermore, differences in the shape of the carina and length of the peduncle were observed between the two species. These morphometric measurements confirm the validity of identifying both species according to the classical descriptions of Voris & Jeffries (1997), where *O. cor* tends to be larger with a complete capitular structure, whereas *O. angulata* is smaller with incompletely developed capitular plates.

Phylogeny

Phylogenetic analysis revealed distinct clustering patterns, unequivocally identifying Sample A as *O. cor* through its monophyletic grouping with the reference sequences KC138499.1 and PV961088.1, while Sample B was confirmed as *O. angulata* based on its robust affiliation with the references MN336849.1 and MN336860.1 (Figure 3). The COI sequences generated in this study have been deposited in the NCBI GenBank database under accession numbers PX823314 (*O. cor*) with a length of 642 base pairs (bp) and PX876038 (*O.*

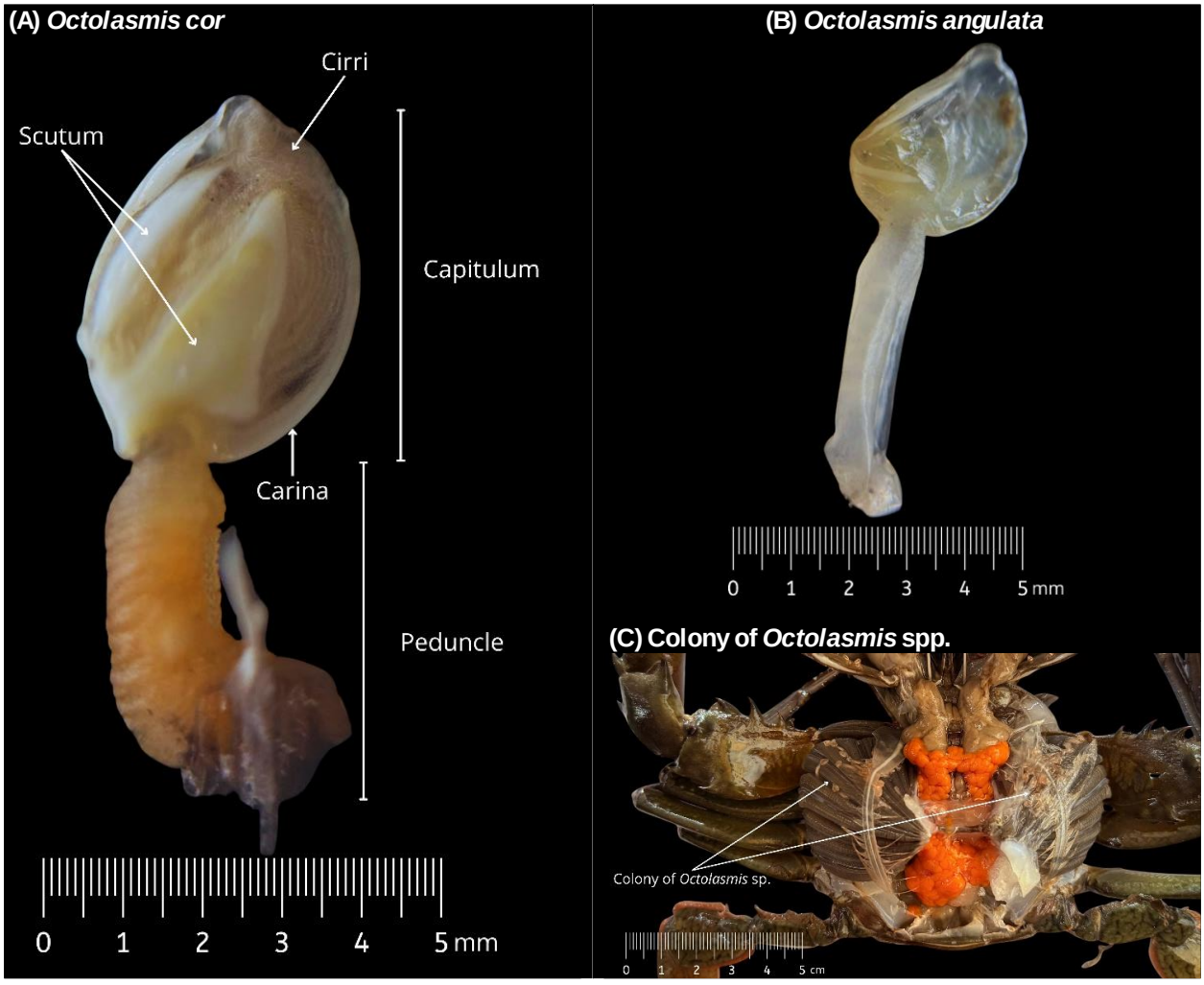

Figure 2. Morphology and colonies of *Octolasmis* sp. on crabs. (A) *O. cor*; (B) *O. angulata* with anatomical markers (Capitulum, Scutum, Carina, and Peduncle); (C) Colonies of *Octolasmis* spp. attached to the host.

angulata) with a length of 643 bp. The overall tree topology clearly delineated the evolutionary relationships among congeneric species and demonstrated significant genetic divergence (scale bar = 1.00 substitutions per site) within Octolasmis. These results are consistent with prior morphological identification and reinforce the taxonomic validity of the two Octolasmis species found on *S. serrata* in the waters of the Bird's Head Peninsula in Southwest Papua.

Octolasmis spp. Infestation

Prevalence data showed that *O. cor* had the highest infestation rate (85%), followed by *O. angulata* (72%), and co-infestation (72%) (Table 1). However, different patterns were observed for the intensity and abundance parameters. Co-infestation showed the highest values, with an intensity of 62 individuals per infested crab and an abundance of 45 individuals per crab. The infestation intensity of *O. cor* (40 individuals/infested crab) was significantly higher than that of *O. angulata* (15 individuals/infested crab), indicating that *O. cor* not only infects more hosts but also forms denser colonies on individual hosts compared to *O. angulata*. The highest abundance value in co-infections (45 individuals/crab) indicated that mixed infestations by both species resulted in the highest total parasitic burden in the crab population.

Statistical analysis revealed highly significant differences in the spatial distributions of the two *Octolasmis* species (Table 2). A paired t-test showed that both *O. cor* ($t = -15.53$, $P<0.001$) and *O. angulata* ($t = -16.03$, $P<0.001$) significantly preferred the inner gill surface (hyperbranchia) to the outer surface. A subsequent independent t-test confirmed that *O. cor* was consistently more dominant than *O. angulata* at

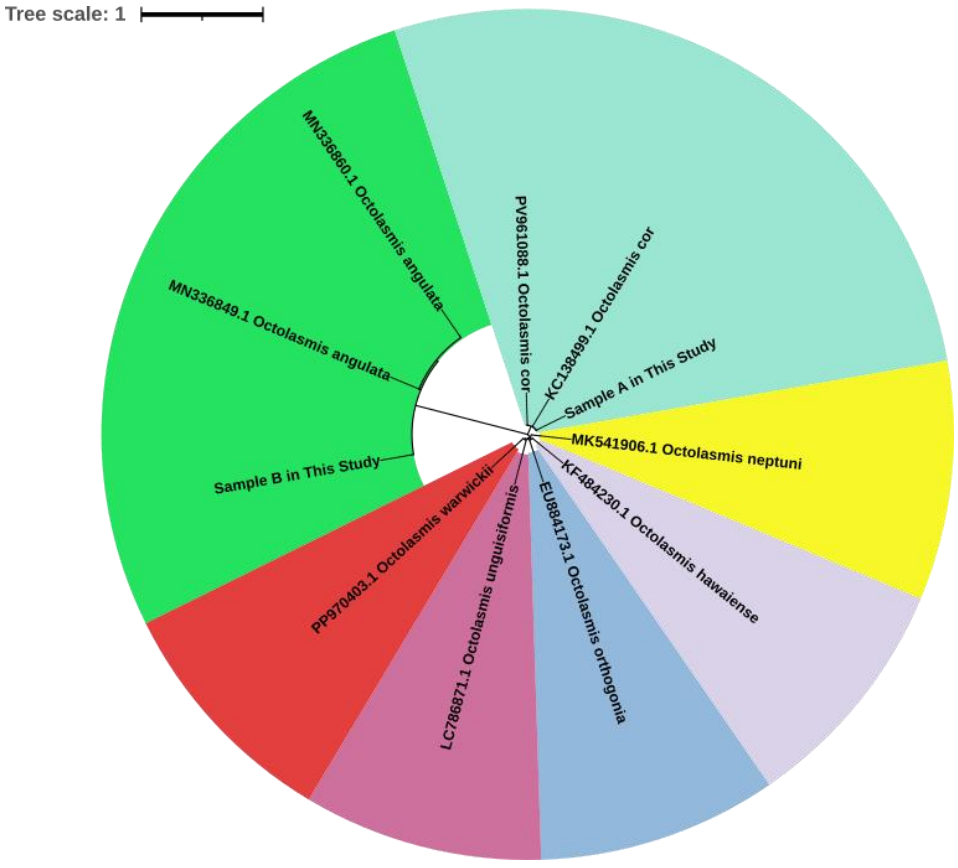

Figure 3. Phylogenetic tree of *Octolasmis* species based on genetic analysis. The tree shows the relationship between the collected samples and the reference sequences of *Octolasmis* species from GenBank. Sample A clustered with *O. cor*, while Sample B clustered with *O. angulata*. The scale bar represents 1.00 genetic distance.

Table 1. Ecological parameters of *Octolasmis* spp. infestation in mud crabs (*S. serrata*) (N=120). Co-infestation refers to a mixed infestation of *O. cor* and *O. angulata* on the same host. Prevalence represents the percentage of infested crabs, intensity indicates the mean number of *Octolasmis* per infested crab, and abundance indicates the mean number of *Octolasmis* per crab in the total sample

Infestation Type	Prevalence (%)	Intensity (Octolasmis / infested crab)	Abundance (Octolasmis / crab)
O. cor	85	40	34
O. angulata	72	15	11
Co-infestation	72	62	45

both surface locations (outer: t= 13.65, P< 0.001; inner: t= 12.26, P<0.001). The average infestation intensity of *O. cor* on the inner surface (26.5±14.6) was recorded to be three times higher than on the outer surface (7.5±4.7), further affirming a strong habitat preference for this location.

Discussion

This study successfully identified two Octolasmis species infesting *S. serrata* in the waters of the Bird's Head Peninsula in Southwest Papua using integrated morphological and molecular approaches. Morphological identification revealed clear differences, particularly in the capitular disc, where *O. cor* had a complete and robust structure (28.3% coverage), whereas *O. angulata* had a reduced structure (10.5% coverage). These morphometric findings, consistent with those reported by Voris et al. (2000), were further validated by COI gene analysis, which showed monophyletic grouping of *O. cor* and *O. angulata* with a significant genetic distance (0.10). This integration confirmed the taxonomic validity of *O. cor* and *O. angulata*, as emphasised by modern taxonomy (da Silva et al., 2011; Ferri et al., 2009). These findings provide a biological basis for interpreting the ecological patterns of *O. cor* and *O. angulata* infesting *S. serrata* in Southwest Papua.

The infestation parameters revealed a distinct ecological dominance by *O. cor*, a pattern that can be interpreted through the lens of its morphological superiority. *O. cor* exhibited the highest prevalence (85%) and, in single-species infestations, the highest intensity (40 individuals/infested crab). This dual advantage suggests a highly successful strategy. Its robust morphology, characterised by a complete capitular disc and broad scutum, likely provides a structural advantage for secure, high-density attachment, enabling it to colonise a majority of hosts and dominate the gill surface of individual crabs. This strategy resembles a "high-investment" model, where energy allocated to sturdy calcification pays off in competitive occupancy of prime attachment sites, a pattern of morphological adaptation to crowding observed in other barnacles (Hoch, 2010). In contrast, *O. angulata*, with its smaller size, reduced calcification, and narrow scutum (Ihwan et al., 2014). demonstrated a lower prevalence (72%) and a significantly lower infestation intensity (15 individuals/infested crab). Its minimalist and energetically efficient morphology may facilitate quicker initial attachment but appears to confer less competitive ability, resulting in a lower overall footprint within the host population.

The interaction between these species culminates in co-infestations, which was associated with the most severe parasitic burden. Co-infestations showed an intensity of 62 individuals per infested crab and the highest overall abundance (45 individuals per crab). This apparent additive effect may indicate a potential facilitative interaction or co-occurrence. We hypothesised that the initial settlement of *O. angulata* might modify the gill microenvironment (e.g. surface texture and water flow), potentially creating more favourable conditions for the subsequent dense settlement of *O. cor*, which requires stable foundations. A similar facilitative succession occurs in other ecological communities, where pioneer species modify habitats for subsequent colonists (Naves-Alegre et al., 2024). Alternatively, the dense clusters formed by *O. cor* may create microhabitats with altered flow dynamics that are opportunistically exploited by *O. angulata*, given that barnacle morphology and settlement are highly sensitive to local conditions (Hoch, 2010; Pereira et al., 2022). This observed co-infestation pattern, possibly reflecting facilitation, could contribute to an increased respiratory burden of *S. serrata*. Impairment due to gill fouling is well documented; for instance, infested blue crabs (*Callinectes sapidus*) show increased ventilatory effort to compensate for gill blockage, with mortality risks under heavy loads (Martin et al., 1992). The high infestation levels reported here and in other regions Ihwan et al. (2015) and Pattipeiluhu et al. (2024), underscore that the interaction between host ecology and parasite morphology may be an important factor influencing infestation severity and its physiological consequences.

Conclusions

This study presents the first integrative taxonomic analysis (morphology and COI sequencing) of *Octolasmis* spp. infesting *S. serrata* in Southwest Papua, confirming two distinct species *O. cor* and *O. angulata*. This reveals that their fundamental morphological divergence underpins contrasting ecological strategies *O. cor* dominates with higher prevalence (85%) and intensity (40 individuals/host), whereas *O. angulata* shows lower values (72%, 15 individuals/host). Critically, co-

Table 2. Distribution and infestation levels of *O. cor* and *O. angulata* in mud crabs (*S. serrata*) (N=120). Data show the total count, mean, and standard deviation of parasites found on the outer and inner surfaces of the host hyperbranchia. The mean values represent the number of parasites per crab, indicating hyperbranchial infestation intensity

Parameter	*O. cor*	*O. angulata*	t-statistic	p-value
Outside hyperbranchial	7.0 ± 4.7	2.0 ± 1.8	13.65	< 0.001
Inside hyperbranchial	26.5 ± 14.6	8.0 ± 5.2	12.26	< 0.001
Total Parasites (N=120)	4.070	1.294		
Paired T-Test				
t-value	-15.53	-16.03	-	-
p-value	< 0.001	< 0.001	-	-

infestation had a synergistic effect, producing the highest parasitic load (62 individuals/host). These findings demonstrate that species-specific identification and facilitative interactions are key to understanding parasitic burden, providing essential insights into the health management of this economically vital crab.

Ethical Statement

This study utilised crabs (Scylla spp.) in accordance with Article 5, Paragraph (2) of the Regulation of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia Number 7 of 2024. The research method is a non-invasive observation of the gills, employing ice-chilling for anaesthesia, and thus does not involve life challenge tests or inflict suffering on the crabs. Therefore, this study was exempt from requiring specific ethical approval.

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Author Contribution

Ahmad Albar: Conceptualization, Writing - Review & Editing.

Putri Meira Shyiang Sri: Data Curation, Formal Analysis, Investigation, Methodology, Visualization, Writing - Original Draft.

Uun Lestari: Funding Acquisition, Project Administration, Resources, Writing - Review & Editing.

Tawakkal: Supervision, Writing - Review & Editing.

Syahlan Anugra Taslim: Writing - Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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