Rhizocephalan-induced host feminization

Morphological feminization in hermit crabs (family Paguridae) induced by parasitic peltogastrid barnacles (Crustacea: Cirripedia: Rhizocephala)

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Abstract

Rhizocephalan barnacles are highly specialized parasitic crustaceans that profoundly alter the morphology, physiology, and reproduction of their decapod hosts. In hermit crabs (Paguridae), parasitism by peltogastrid rhizocephalans has been reported to induce feminization of male secondary sexual traits, such as the development of female-specific pleopods and the reduction of cheliped size; however, quantitative and comparative analyses across host–parasite combinations remain limited. In this study, we examined morphological feminization in four hermit crab species (*Pagurus lanuginosus*, *Pa. filholi*, *Pa. nigrivittatus*, and *Pa. japonicus*) parasitized by three peltogastrid rhizocephalans (*Peltogaster* sp., *Pe. postica*, and *Pe.* aff. *ovalis*). The occurrence frequency of a second pleopod, a female-specific structure for egg brooding, was significantly higher in parasitized males of *Pa. lanuginosus* and *Pa. nigrivittatus*, whereas no significant difference was detected in *Pa. filholi*. Allometric analyses revealed pronounced reductions in cheliped length in parasitized males of *Pa. lanuginosus* and *Pa. japonicus*, with regression slopes approaching those of normal females. Effect size analyses further demonstrated that cheliped lengths of parasitized *Pa. lanuginosus* and *Pa. japonicus* were similar to those of normal females, whereas those in parasitized *Pa. filholi* and *Pa. nigrivittatus* were not, indicating the magnitude of feminization differed markedly among host–parasite combinations. These results indicate that peltogastrid-induced morphological feminization in hermit crabs is highly species-specific and depends on both host and parasite identity. Our findings highlight the importance of integrating molecular identification of parasites with quantitative morphological analyses to elucidate the diversity of host manipulation strategies in rhizocephalan barnacles.

Key words: Parasitic barnacle, Peltogastridae, Cheliped allometry, Egg-carrying pleopod, Parasitic effect size

Introduction

Parasites play essential roles in marine ecosystems and can strongly influence host reproduction, behavior, and population dynamics (Kuris, 1974). Among marine parasites, rhizocephalans (Thecostraca: Cirripedia) infect a wide variety of crustacean hosts, including hermit crabs (Yoshida et al., 2014; Jung et al., 2019; Kajimoto et al., 2022, 2025a), true crabs (Lützen et al., 2018; Toyota et al., 2023; Kajimoto et al., 2025b; Oahn et al., 2025), and shrimps (Lützen et al., 2016). Rhizocephalan parasitism typically results in parasitic castration, rendering hosts infertile and redirecting reproductive energy toward parasite growth and reproduction (Hines et al., 1997). To achieve this, rhizocephalans exhibit remarkable morphological and physiological adaptations for parasitism (Høeg, 1995a). Adult females consist of one or more external reproductive bodies (externae) and an internal root-like system (interna) that absorbs nutrients from the host tissues. Rhizocephalans show pronounced sexual dimorphism, which dwarf males residing within the female externa (Høeg, 1995a, b; Høeg et al., 2019).

In crab hosts, rhizocephalan infection often induces morphological feminization of male secondary sexual characteristics (Alvarez et al., 1996; Kristensen et al., 2012; Toyota et al., 2023; Oahn et al., 2025). For example, parasitized male crabs may develop a broadened, female-like abdomen instead of the typical narrow male form. Such modifications enable parasitized males to carry one or more externae, potentially increasing parasite reproductive output (Rees and Glenner, 2014; Nagler et al., 2017; Mouritsen et al., 2018). In addition to morphological changes, parasitized males may exhibit female-typical behaviors, such as those associated with larval release (Takahashi et al., 1997). Other reported effects include cheliped's size reduction (Hartnoll, 1962), modification or degeneration of copulatory structures, and changes in pleopod number or morphology (Kristensen et al., 2012; Toyota et al., 2023; Oahn et al., 2025).

Comparable effects of rhizocephalans on host morphology have also been documented in hermit crabs (Shiino, 1931; Oguro, 1955; Nielsen, 1970; Kajimoto et al., 2025a). In the family Paguridae, the second pleopod is typically a female-specific structure functioning in egg attachment and brooding, whereas it is vestigial or absent in males. Nevertheless, the development of a second pleopod has been reported in males parasitized by peltogastrid or peltogasterellid rhizocephalans, including *Pagurus samuelis*, *Pa. lanuginosus*, *Pa. filholi*, *Pa. ochotensis* (Shiino, 1931; Oguro, 1955; Nielsen, 1970; Kajimoto et al., 2025a). Despite these observations, the mechanisms underlying second pleopod development remain unclear, and it is not yet established whether this trait is directly induced by rhizocephalan parasitism. However, rhizocephalan infection reduces hormone levels for crab reproduction and disrupts the gene expressions related to molting, growth, and gonadal development (Xie et al., 2025). These effects suggest that the rhizocephalan-induced morphological feminization may be mediated by endocrine disruption. In addition, parasitized males often show reduced right cheliped length relative to body size, whereas parasitized females exhibit little or no comparable change (Oguro, 1955; Kajimoto et al., 2025a).

Quantitative assessments on peltogastrid-induced feminization in hermit crabs have been limited, largely because parasitism is difficult to detect: infection prevalence is often low, and confirmation generally requires shell removal to identify the presence of externae. Furthermore, externa morphology alone has been frequently insufficient for reliable species identification. Recent advances in molecular techniques, however, have enabled accurate identification of rhizocephalan species using genetic markers

(Tsuchida et al., 2006; Yoshida et al., 2014; Jung et al., 2014; Høeg et al., 2019; Jung et al., 2021; Toyota et al., 2023). In the present study, we examined multiple hermit crab species parasitized by different peltogastrid rhizocephalans and compared the occurrence of the second pleopod and cheliped allometry between parasitized and unparasitized males. By integrating molecular parasite identification with quantitative morphological analyses, we aimed to clarify how peltogastrid rhizocephalans influence host sexual traits and to identify species-specific patterns of morphological feminization.

Materials and Methods

Ethical statement

All experimental procedures and sampling protocols compiled with the guidelines of the Institutional Animal Care and Use Committee of Kanagawa University. All animal experiments were conducted in accordance with the ARRIVE guidelines (Percie du Sert et al., 2020)

Sample collection

In the intertidal zone at Asari, Otaru City, Hokkaido, Japan (43.176°N, 141.068°E; Fig. 1), specimens of *Pa. lanuginosus* (Fig. 2A) and *Pa. filholi* (Fig. 2B) parasitized by peltogastrid rhizocephalans were collected in September and October 2025, along with normal (externa-free) individuals of *Pa. filholi* collected in September and November 2025. In the coastal area of Chikura, Minamiboso City, Chiba, Japan (34.924°N, 139.942°E; Fig. 1), parasitized individuals of *Pa. filholi* were obtained in June 2024 and monthly from March to August 2025, along with normal individuals collected in June 2024 and in September and October

2025. In coastal areas around the Noto Peninsula, Ishikawa Prefecture (Fig. 1), both normal and parasitized individuals of *Pa. nigrivittatus* (Fig. 2C) were collected between February–April, as well as in June and October 2025. In the coast near Sado Island, Niigata Prefecture, Japan (Fig. 1), parasitized individuals of *Pa. japonicus* (Fig. 2D) were collected in October 2024 and from the Noto Peninsula in April and October 2025, along with normal individuals of *Pa. japonicus* collected in February and October 2025. All parasitized specimens were preserved in absolute ethanol for subsequent molecular identification. Normal specimens of *Pa. lanuginosus*, *Pa. filholi*, *Pa. nigrivittatus*, and *Pa. japonicus* were fixed either in absolute ethanol or in 80% Esmile solution (Esmile WKII-75, Shinwa Alcohol Co., Ltd., Japan).

Species identification based on COI sequencing

A portion of each Peltogasterid externa was excised for DNA extraction. Genomic DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's protocol. For molecular identification of rhizocephalan species, a region of the mitochondrial COI was amplified by polymerase chain reaction (PCR) using the primer pair crust-cox1f (ACTAATCACAARGAYATTGG) (Podsiadlowski and Bartolomaeus, 2005) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al., 1994). PCR amplification was carried out under the following thermal cycling conditions: initial denaturation at 94 °C for 7 min; followed by 35 cycles of 94 °C for 30 s, 45 °C for 30 s, and 72 °C for 2 min; and a final extension step at 72 °C for 7 min. Reactions were performed in 50 μL volumes using either TaKaRa Ex Taq or TaKaRa Ex Taq Hot Start Version (Takara Bio, Shiga, Japan). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The resulting amplicons were sequenced by Eurofins Genomics (Tokyo, Japan). All Peltogasterid COI sequences obtained in this study were deposited in the DNA Data Bank of Japan (DDBJ) under accession numbers LC862897–LC862898, LC910526–LC910703.

Measurements of morphological traits

The sex of each hermit crab individual was determined by examining the presence or absence of female gonopores under a stereoscopic dissecting microscope. To assess morphological effects associated with rhizocephalan parasitism, the presence or absence of a second pleopod was examined in *Pa. lanuginosus*, *Pa. filholi*, and *Pa. nigrivittatus*, as this appendage is typically female-specific in these species. In *Pa. japonicus*, the second pleopod occurs in both sexes and was therefore excluded from this analysis. Shield length and the length of the right major cheliped were measured for all individuals using a digital caliper to the nearest 0.01 mm.

Statistical analysis

Data on the presence of the second pleopod, right cheliped length, and shield length in normal male and female *Pa. lanuginosus* from Asari were obtained from Kajimoto et al. (2025a). To evaluate the effect of rhizocephalan parasitism on morphological feminization in male hermit crabs, the frequency of individuals possessing a second pleopod was compared between normal and parasitized males of *Pa. lanuginosus*, *Pa. filholi*, and *Pa. nigrivittatus* using Fisher's exact test.

Cheliped length was used as an indicator of secondary sexual trait modification. To assess the effect of parasitism on relative right cheliped length across species, equality of regression slopes was tested using shield length as a covariate. Statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). For each species, analysis included normal males without a second pleopod, normal females, parasitized males (without a second pleopod in *Pa. lanuginosus*, *Pa. filholi*, and *Pa. nigrivittatus*), and parasitized females. When no significant interaction between factors was detected, analysis of covariance (ANCOVA) was performed with shield length as a covariate.

To quantify parasite-induced morphological feminization, standardized effect sizes (Hedges' g; Hedges, 1981) were calculated based on differences in the cheliped-to-shield length ratio between parasitized males (without a second pleopod in *Pa. lanuginosus*, *Pa. filholi*, and *Pa. nigrivittatus*) and normal females, which served as the reference group. Ninety-five percent confidence intervals (CIs) were estimated using nonparametric bootstrapping with 2,000 resampling iterations (Kirby et al., 2013). Effect sizes whose CIs did not include zero were considered statistically significant. Statistical analyses were performed in R (version 4.2.2; R Core Team, 2022), and data processing and visualization were performed using the BootES package (version 1.3.1).

Results

Occurrence of hermit crabs parasitized by peltogastrids

At Asari, 16 male and 28 female parasitized individuals of *Pa. lanuginosus* were collected. For *Pa. filholi* at the same site, 45 males and 41 females were normal, whereas 5 males and 2 females were parasitized. At Chikura, 40 males and 41 females of *Pa. filholi* were normal, while 59 males and 41 females were parasitized. Around the Noto Peninsula, 70 males and 37 females of *Pa. nigrivittatus* were normal, and 13 males and 6 females were parasitized. For *Pa. japonicus*, two parasitized individuals were collected from Sado Island. In addition, around the Noto Peninsula, 36 males and 78 females were normal, whereas 7 males and 2 females were parasitized.

Identification of rhizocephalan species

Mitochondrial COI sequencing identified all peltogastrids parasitizing *Pa. lanuginosus* as *Peltogaster* sp., based on sequence similarity to reference sequences (accession numbers: OR481989, LC865657). All peltogastrids parasitizing *Pa. filholi* from both Asari and Chikura, as well as those parasitizing *Pa. nigrivittatus*, were identified as *Peltogaster postica* based on sequence similarity (accession numbers: PP204808, LC865664). All peltogastrids collected from *Pa. japonicus* were identified as *Peltogaster* aff. *ovalis*, based on similarity to the reference sequence AB778063.

Presence of the second pleopod in parasitized males

In *Pa. lanuginosus*, *Pa. filholi*, and *Pa. nigrivittatus*, the elongated second pleopod is a female-specific morphological characteristic and is typically absent or vestigial in normal males, although *Pa. filholi* from Chikura, the presence of the second pleopod in 19 normal males were observed (Table 1). In this study, the second pleopod was observed in males of all three species (Table 1). The frequency of second pleopod occurrence was significantly higher in males of *Pa. lanuginosus* parasitized by *Peltogaster* sp. and in males of *Pa. nigrivittatus* parasitized by *Pe. postica* than in normal males (Fisher's exact test, $p < 0.05$; Table 1). In contrast, no significant difference in the frequency of second pleopod occurrence was detected between parasitized and normal males of *Pa. filholi* from either Asari or Chikura.

Allometric variation in cheliped length

Some normal males were observed to possess a second pleopod. For example, in *Pa. filholi* from Chikura, normal males with a second pleopod had significantly shorter cheliped length than those without a second pleopod (ANCOVA, F = 12.42, $p < 0.001$; Fig. 3). These individuals may represent previously parasitized hosts; however, confirmation was not possible because removal of the interna and molecular identification of potential parasites could not be performed. Consequently, these individuals were excluded from the ANCOVA and the standardized effect size. Similarly, parasitized males with a second pleopod were excluded because the mechanisms underlying second pleopod development remain unclear, and it could not be determined whether this trait was directly induced by peltogastrid parasitism.

In females of all examined hermit crab species, no marked differences were detected in the allometric relationship between right cheliped length and shield length when comparing normal and parasitized individuals (Supplementary Table 1; Supplementary Figure S1). In contrast, clear differences were obserbed in males. In *Pa. lanuginosus* parasitized by *Peltogaster* sp., males exhibited a pronounced reduction in right cheliped length relative to shield length compared with normal males, and the regression line overlapped with that of normal females (Table 3a; Fig. 4A). In *Pa. filholi* from Asari, significant differences in cheliped allometry were detected between normal males and females; however, no significant difference was detected between parasitized males and normal males (Table 3b; Fig. 4B). In contrast, in *Pa. filholi* from Chikura, parasitized males exhibited a significant shift in cheliped allometry relative to normal males, with regression slopes approaching those of normal females (Table 3c; Fig. 4C). In *Pa. nigrivittatus*, significant differences in cheliped allometry were detected between normal males and females; however, no significant difference was detected between parasitized males and normal males (Table 3d; Fig. 4D). In *Pa. japonicus*, parasitized males exhibited a marked reduction in cheliped length relative to shield length compared with normal males, and the regression line overlapped with that of normal females (Table 3e; Fig. 4E).

Comparison of parasitic effect among host-parasite combinations

In females, standardized effect sizes (Hedges' g) for cheliped-to-shield length ratios were close to zero or negative, and their 95% confidence intervals overlapped zero, indicating little morphological change associated with parasitism (Supplementary Figure S2). In contrast, in males, effect size estimates differed among host–parasite combinations. Pronounced negative effect sizes were detected in *Pa. lanuginosus* parasitized by *Peltogaster* sp. and *Pa. japonicus* parasitized by *Pe.* aff. *ovalis*, with confidence intervals approaching or overlapping the reference values of normal females (Fig. 5).

Discussion

Presence of a second pleopod in parasitized males

The second pleopod, which is absent or vestigial in normal males of most pagurid hermit crab, was observed in males parasitized by peltogastrid rhizocephalans in all host species examined in this study. In *Pa. lanuginosus* and *Pa. nigrivittatus*, the frequency of second pleopod occurrence was significantly higher in parasitized males than in normal males, consistent with previous observations of rhizocephalan-induced feminization in hermit crabs (Shiino, 1931; Oguro, 1955; Nielsen, 1970; Kajimoto et al., 2025a). However, the mechanisms underlying second pleopod formation remain unknown. If the formation of the second pleopod is induced by peltogastrid rhizocephalan, these results indicate that peltogastrid parasitism is frequently associated with the expression of female-specific pleopod traits in male hosts. In contrast, no significant difference in second pleopod frequency was detected between normal and parasitized *Pa. filholi* males, despite infection by the same rhizocephalan species (*Peltogaster postica*) at both Asari and Chikura. This suggests that the expression of second pleopods in parasitized males is not a uniform response to peltogastrid infection but may depend on host species–specific sensitivity or developmental constraints. Even when infected by the same rhizocephalan species, host responses may therefore vary substantially among hermit crab species. It should also be noted that second pleopods were occasionally observed in males without detectable externae. In hermit crab genera in which rhizocephalan parasitism has not been reported, such as Paguristes and Pseudopaguristes, males consistently lack second pleopods (McLaughlin, 2002; Rahayu, 2022). Accordingly, the presence of a second pleopod in externa-free males of *Pagurus* may reflect previous or incipient parasitism, such as loss of the externa or early-stage infection prior to externa emergence. Accurate estimation of infection prevalence therefore requires direct examination of the interna, ideally combined with molecular identification.

Host grooming behavior is essential for the maintenance of rhizocephalan externae, as poorly groomed externae can become fouled and necrotic (Ritchie and Høeg, 1981). Accordingly, the development of a second pleopod in parasitized males may enhance grooming efficiency and thereby increase externa survival (Kajimoto et al., 2025a). Notably, in *Pa. lanuginosus* and *Pa. filholi*, males parasitized by *Peltogasterella gracilis*, which produces multiple externae, exhibit a higher frequency of second pleopods than not only unparasitized males but also males parasitized by peltogastrid rhizocephalans (Kajimoto et al., 2025a). Changes in pleopod composition suggest that peltogstrid-parasitized males develop female-like pleopod that function to protect and support rhizocephalan externae.

Reduction of cheliped length in parasitized males

Cheliped length is a prominent male secondary sexual trait in hermit crabs and plays an important role in mating competition and agonistic interactions. In the present study, marked reductions in relative cheliped length were observed in males of *Pa. lanuginosus*, *Pa. filholi* from Chikura, and *Pa. japonicus* parasitized by peltogastrid rhizocephalans, whereas parasitized females showed little or no corresponding change. These patterns are consistent with previous reports of rhizocephalan-induced feminization in hermit crabs and brachyuran crabs (Oguro, 1955; Kristensen et al., 2012; Kajimoto et al., 2025a; Toyota et al., 2023). However, the magnitude of cheliped reduction varied among host–parasite combinations. In *Pa. filholi* from Asari and in *Pa. nigrivittatus*, parasitized males did not differ significantly from normal males in cheliped allometry, despite infection by the same rhizocephalan species (*Pe. postica*). Nevertheless, effect size analyses revealed that cheliped morphology in only parasitized *Pa. lanuginosus* and *Pa. japonicus* males tended to shift toward female-like values, indicating subtle morphological modification that was not always detected by regression-based comparisons alone. These results highlight the utility of standardized effect sizes for evaluating parasite-induced morphological changes that may be masked by inter-individual variation or limited sample size.

Differences in cheliped reduction among host–parasite combinations suggest that the extent of morphological feminization is determined not only by parasite presence but also by species-specific interactions between host and parasite. Nielsen (1970) and Kajimoto et al. (2025a) previously reported that even closely related rhizocephalans can induce distinct patterns of morphological modification in different hermit crab hosts, and the present study provides quantitative support for this view using molecularly identified parasite species.

Implications for host–parasite interactions in peltogastrid rhizocephalans

Rhizocephalans achieve reproductive success primarily through parasitic castration, redirecting host energy from reproduction toward parasite growth and reproduction (Lafferty and Kuris, 2009). The degree of morphological feminization observed in parasitized males may therefore reflect differences in the amount of energy extracted by the parasite (Nagler et al., 2017). In peltogastrid rhizocephalans, reproductive traits such as egg number, egg size, breeding frequency, and the number of reproductive externae remain poorly documented, and interspecific variation in these traits may contribute to differences in parasitic impact on host morphology. In the present study, species-level identification of all peltogastrid parasites revealed patterns that differed from those reported previously for *Pa. lanuginosus* in the same locality (Kajimoto et al., 2025a). This discrepancy may reflect the grouping of multiple *Peltogaster* species in earlier studies, emphasizing the importance of precise parasite identification when evaluating host responses. Integrating molecular taxonomy with quantitative morphological analyses is therefore essential for accurately characterizing the diversity of rhizocephalan-induced effects.

Despite accumulating evidence of rhizocephalan-induced morphological feminization, the proximate mechanisms underlying these changes in hermit crabs remain largely unknown. Recent transcriptomic analyses suggest that rhizocephalan parasitism may alter neuroendocrine signaling pathways in host eyestalks and thoracic ganglia, potentially leading to feminized morphology and behavior (Feng et al., 2023). Future studies combining comparative transcriptomics, neurobiological approaches, and controlled infection experiments will be necessary to elucidate how peltogastrid rhizocephalans manipulate host development and sexual traits.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

AK, TO, and KT designed the research. AK and AT conducted the sampling and measured the morphological parameters. AK and AT conducted the sequencing. AK analyzed all data and wrote the first draft, and all authors approved the final version of the manuscript.

References

Alvarez F, Calderon J (1996) Distribution of *Loxothylacus texanus* (Cirripedia: Rhizocephala) parasitizing crabs of the genus Callinectes in the Southwestern Gulf of Mexico. Gulf Caribb Res 9: 205–210

Feng C, Zhang J, Bao J, Luan D, Jiang N, Chen Q (2023) Transcriptome analysis of germ cell changes in male Chinese mitten crabs (*Eriocheir sinensis*) induced by rhizocephalan parasite, *Polyascus gregaria*. Front Mar Sci 10: 1144448

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

Hartnoll RG (1962) Parasitic castration of *Macropodia longirostris* (Fabricius) by a sacculinid. Crustaceana; 295–300

Hedges LV (1981) Distribution theory for Glass's estimator of effect size and related estimators. J Educ Stat 6: 107–128

Hines AH, Alvarez F, Reed SA (1997) Introduced and native populations of a marine parasitic castrator: variation in prevalence of the rhizocephalan *Loxothylacus panopaei* in xanthid crabs. Bull Mar Sci 61: 197–214

Høeg JT (1995a) The biology and life cycle of the Cirripedia rhizocephala. J Mar Biol Assoc U K 75: 517–550

Høeg JT (1995b) Sex and the single cirripede: a phylogenetic perspective. In: Schram FR, Høeg JT, editors. New frontiers in barnacle evolution. Crustacean Issues. Vol. 10. Rotterdam: A. A. Balkema: 195–208

Høeg JT, Rees DJ, Jensen PC, Glenner H (2019) Unravelling the evolution of the Rhizocephala: a case study for molecular-based phylogeny in the parasitic Crustacea. In: Smit NJ, Bruce NKL, Hadfield KA, editors. Parasitic Crustacea: State of knowledge and future trends. Zool Monogr. Vol. 3. Feldhar H, Schmidt-Rhaesa A, series editors. Cham: Springer: 387–419

Jung J, Yoshida R, Kim W (2019) Diversity of parasitic peltogastrid barnacles (Crustacea: cirripedia: Rhizocephala) on hermit crabs in Korea. Zool Stud 58: 33

Jung J, Yoshida R, Lee D, Park JK (2021) Morphological and molecular analyses of parasitic barnacles (Crustacea: Cirripedia: Rhizocephala) in Korea: preliminary data for the taxonomy and host ranges of Korean species. PeerJ *9*: e12281

Kajimoto A, Høeg JT, Kato K, Yusa Y (2022) Variations in life cycle and seasonal sex ratio in the rhizocephalan *Peltogasterella gracilis* (Boschma, 1927) (Cirripedia: Rhizocephala: Peltogasterellidae). J Crustac Biol 42: ruac057

Kajimoto A, Iwasaki A, Ohira T, Toyota K (2025a) Morphological feminization in hermit crabs (family Paguridae) induced by rhizocephalan barnacles. Zool Lett 11: 6

Kajimoto A, Toyota K, Yusa Y (2025b) Effects of morphological changes induced by the rhizocephalan parasite *Polyascus polygenea* on predation risk of the Asian shore crab *Hemigrapsus sanguineus*. Zool Stud 64: 59

Kirby KN, Gerlanc D, Boot ES (2013) An R package for bootstrap confidence intervals on effect sizes. Behav Res Methods 45: 905–927

Kristensen T, Nielsen AI, Jørgensen AI, Mouritsen KN, Glenner H, Christensen JT, Lützen J, Høeg JT (2012) The selective advantage of host feminization: a case study of the green crab *Carcinus maenas* and the parasitic barnacle *Sacculina carcini*. Mar Biol 159: 2015–2023

Kuris AM (1974) Trophic interactions: similarity of parasitic castrators to parasitoids. Q Rev Biol 49: 129–148

Lafferty KD, Kuris AM (2009) Parasitic castration: the evolution and ecology of body snatchers. Trends Parasitol 25: 564–572

Lützen J, Itani G, Jespersen Å, Hong JS, Rees D, Glenner H (2016) On a new species of parasitic barnacle (Crustacea: Rhizocephala), *Sacculina shiinoi* sp. nov., parasitizing Japanese mud shrimps *Upogebia* spp. (Decapoda: Thalassinidea: Upogebiidae), including a description of a novel morphological structure in the Rhizocephala. Zool Sci 33: 204–212

Lützen J, Jensen KH, Glenner H (2018) Life history of Sacculina carcini Thompson, 1836 (Cirripedia: rhizocephala: Sacculinidae) and the intermoult cycle of its host, the shore crab *Carcinus maenas* (Linnaeus, 1758)(Decapoda: Brachyura: Carcinidae). J Crustac Biol 38: 413–419

McLaughlin PA (2002) A review of the hermit crab (Decapoda: anomura: Paguridea) fauna of Southern Thailand, with particular emphasis on the Andaman Sea, and descriptions of three new species. PMBC Spec Publ 23: 385–460

Mouritsen KN, Geyti SN, Lützen J, Høeg JT, Glenner H (2018) Population dynamics and development of the rhizocephalan *Sacculina carcini*, parasitic on the shore crab *Carcinus maenas*. Dis Aquat Org 131: 199–211

Nagler C, Hörnig MK, Haug JT, Noever C, Høeg JT, Glenner H (2017) The bigger, the better? Volume measurements of parasites and hosts: parasitic barnacles (Cirripedia, Rhizocephala) and their decapod hosts. PLoS ONE 12: e0179958

Nielsen SO (1970) The effects of the rhizocephalan parasites *Peltogaster paguri* Rathke and *Gemmosaccus sulcatus* (Lilljeborg) on five species of Paguridan hosts (Crustacea Decapoda). Sarsia 42:17–32

Oanh, LE Thi Kieu, Anh Thi Ngoc Hoang, Thanh Quyet Vu, Vo Thi Ha, Sang Quang Tran, Oanh Thi Truong, Henrik Glenner, Binh Thuy Dang (2025) Morphological and molecular characterization of *Heterosaccus papillosus* Boschma, 1933 (Rhizocephala: Sacculinidae) and its effects on the swimming crab *Charybdis anisodon* De Haan, 1850 (Decapoda: Portunidae) in Nha Trang Bay, Vietnam. *Zootaxa* 5673: 213-226

Oguro C (1955) On the sacculinization of the hermit-crab, *Eupagurus ochotensis* (Brandt). Annot Zool Jpn 28:100–105

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M (2020) The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. J Cereb Blood Flow Metab 40:1769–1777

Podsiadlowski L, Bartolomaeus T (2005) Organization of the mitochondrial genome of mantis shrimp *Pseudosquilla ciliata* (Crustacea: Stomatopoda). Mar Biotechnol 7: 618–624

R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. URL: https://www.R-project.org/ Accessed 17 December 2025

Rahayu DL (2022) Hermit crabs of Singapore (Crustacea: decapoda: anomura: diogenidae, Paguridae), with description of two new species. Raffles Bull Zool 1: 70

Rees D, Glenner H (2014) Control region sequences indicate that multiple externae represent multiple infections by *Sacculina carcini* (Cirripedia: Rhizocephala). Ecol Evol 4: 3290–3297

Ritchie LE, Høeg JT (1981) The life history of *Lernaeodiscus porcellanae* (Cirripedia: Rhizocephala) and co-evolution with its porcellanid host. J Crustac Biol 1: 334–347

Shiino SM (1931) Studies on the modification of sexual characters in *Eupagurus samuelis* caused by a rhizocephalan parasite *Peltogaster* sp. Mem Coll Sci Kyoto Imp Univ Ser B. 7:63–101

Takahashi T, Iwashige A, Matsuura S (1997) Behavioral manipulation of the shore crab, *Hemigrapsus sanguineus* by the rhizocephalan barnacle, *Sacculina polygenea*. Crustacean Res 26: 153–161

Toyota K, Ito T, Morishima K, Hanazaki R, Ohira T (2023) *Sacculina*-Induced morphological feminization in the Grapsid crab *Pachygrapsus crassipes*. Zool Sci 40: 367–374

Tsuchida K, Lützen J, Nishida M (2006) Sympatric three-species infection by *Sacculina* parasites (Cirripedia: Rhizocephala: Sacculinidae) of an intertidal grapsoid crab. J Crustacean Biol 26: 474– 479

Xie J, Ying C, Tang Z, Yang Y, Liu K (2025) Eyestalk transcriptome analysis reveals sexually dimorphic host-parasite interactions: divergent molecular strategies of

Polyascus sp. for reproductive and growth regulation in *Eriocheir sinensis*. PeerJ 13: e20089

Yoshida R, Hirose M, Hirose E (2014) Hermit crab host prevalence by species of Peltogastridae (Cirripedia: Rhizocephala): hosts vary with locations on the Pacific coast in mainland Japan. J Crustac Biol 34: 467–480

Tables and Figures

Table 1. Number of normal or parasitized male and female Pagurus species, with or without the second pleopod.

Parasite	Sampling site	Hermit crab		No. of individuals	
		Species	Type	without second pleopod	with second pleopod
Peltogaster sp.	Asari	*Pagurus lanuginosus*	Normal male	61	4
			Parasitized male	12	4
			Normal female	0	88
Peltogaster postica		*Pagurus filholi*	Normal male	45	0
			Parasitized male	4	1
			Normal female	0	41
	Chikura		Normal male	40	19
			Parasitized male	47	12
			Normal female	0	41
	Noto	*Pagurus nigrivittatus*	Normal male	70	1
			Parasitized male	10	3
			Normal female	0	37

Table 2. ANCOVA of cheliped length in normal male and female Pagurus hermit crabs versus male individuals parasitized by *Peltogaster* species.

Sampling site	Parasite	Host	Comparison of the regression lines	Homogeneity of the slopes		Allometric changes	
				F value	p-value	F value	p-value
a. Asari	*Peltogaster* sp.	*Pagurus lanuginosus*	**Normal male vs.**				
			Normal female	0.29	0.589	19.18	<.0001
			Parasitized male vs.				
			Normal male	0.65	0.423	11.07	<.01
			Normal female	1.33	0.251	1.24	0.267
b. Asari	*Peltogaster postica*	*Pagurus filholi*	**Normal male vs.**				
			Normal female	2.99	0.087	31.03	<.0001
			Parasitized male vs.				
			Normal male	0.32	0.573	0.04	0.841
			Normal female	5.29	<.05	-	-
c. Chikura	*Peltogaster postica*	*Pagurus filholi*	**Normal male vs.**				
			Normal female	18.74	<.0001	-	-
			Parasitized male vs.				
			Normal male	4.04	<.05	-	-
			Normal female	2.30	0.132	77.10	<.0001
d. Noto	*Peltogaster postica*	*Pagurus nigrivittatus*	**Normal male vs.**				
			Normal female	7.420	<.01	-	-
			Parasitized male vs.				
			Normal male	0.270	0.602	3.170	0.079
			Normal female	0.490	0.486	37.210	<.0001
e. Noto Sado	*Peltogaster* aff. *ovalis*	*Pagurus japonicus*	**Normal male vs.**				
			Normal female	10.44	<.01	-	-
			Parasitized male vs.				
			Normal male	4.29	<.05	-	-
			Normal female	2.13	0.149	0.51	0.476

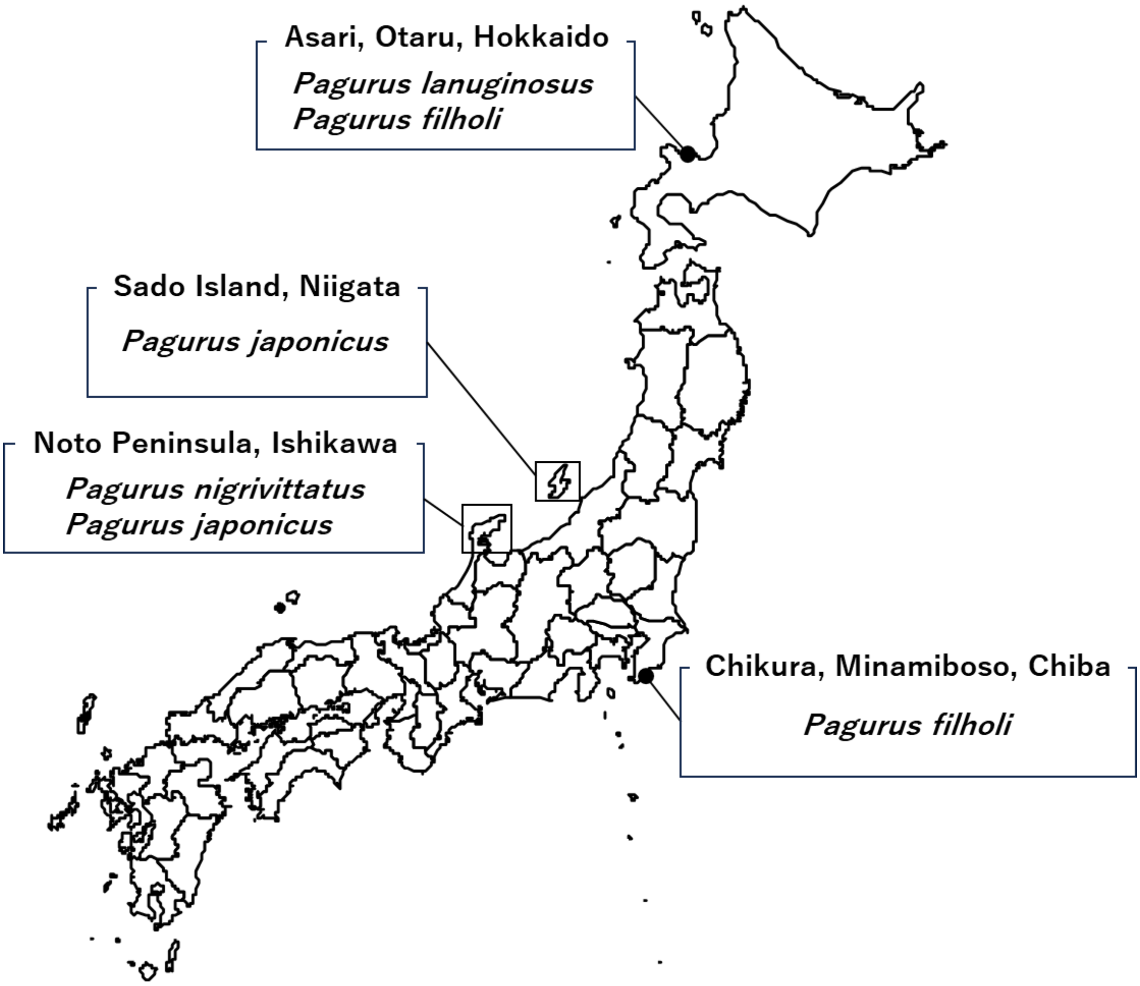


Fig. 1. Sampling sites of hermit crabs with/without peltogastrids.

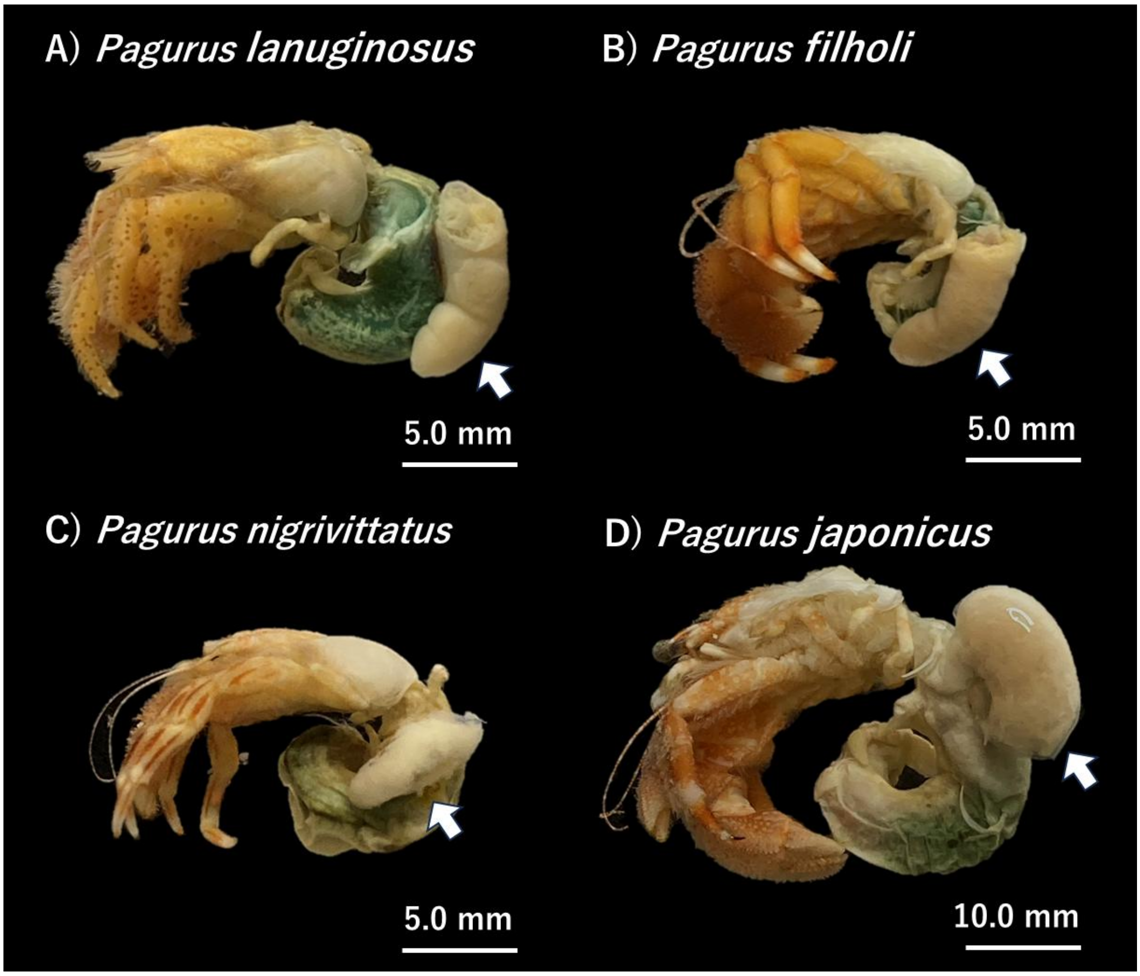


Fig. 2. Specimens of host hermit crabs and peltogastrid rhizocephalan parasites (indicated by white arrows) examined in this study: *Pagurus lanuginosus* (**A**); *Pa. filholi* (**B**); *Pa. nigrivittatus* (**C**); *Pa. japonicus* (**E**).

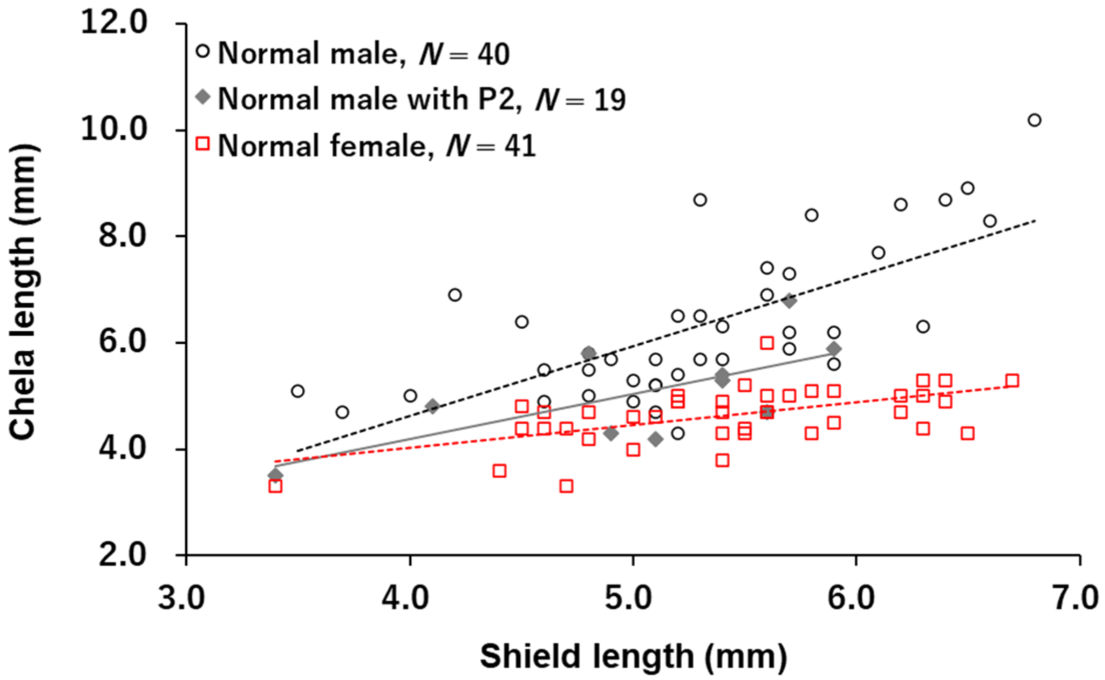


Fig. 3. Allometric variation in cheliped length relative to shield length in normal *Pagurus filholi* in Chikura.

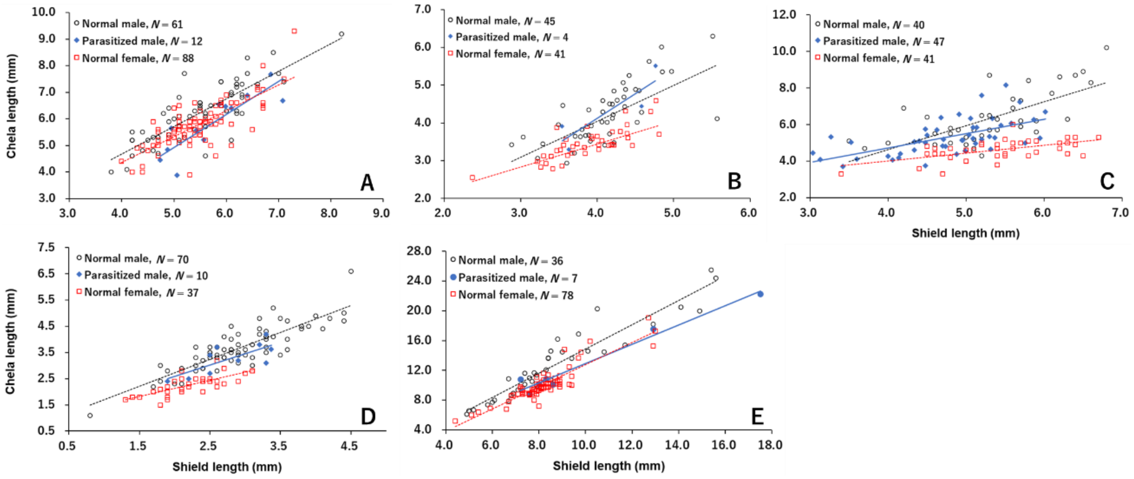


Fig. 4. Allometric variation in cheliped length relative to shield length in male hermit crabs, comparing normal and parasitized individuals: *Pagurus lanuginosus* parasitized by *Peltogaster* sp. **(A)**; *Pa. filholi* in Asari **(B)**, *Pa. filholi* in Chikura **(C)**, and *Pa. nigrivittatus* **(D)** parasitized by *Pe. postica*; *Pa. japonicus* parasitized by *Peltogaster* aff. *ovalis* **(E)**.

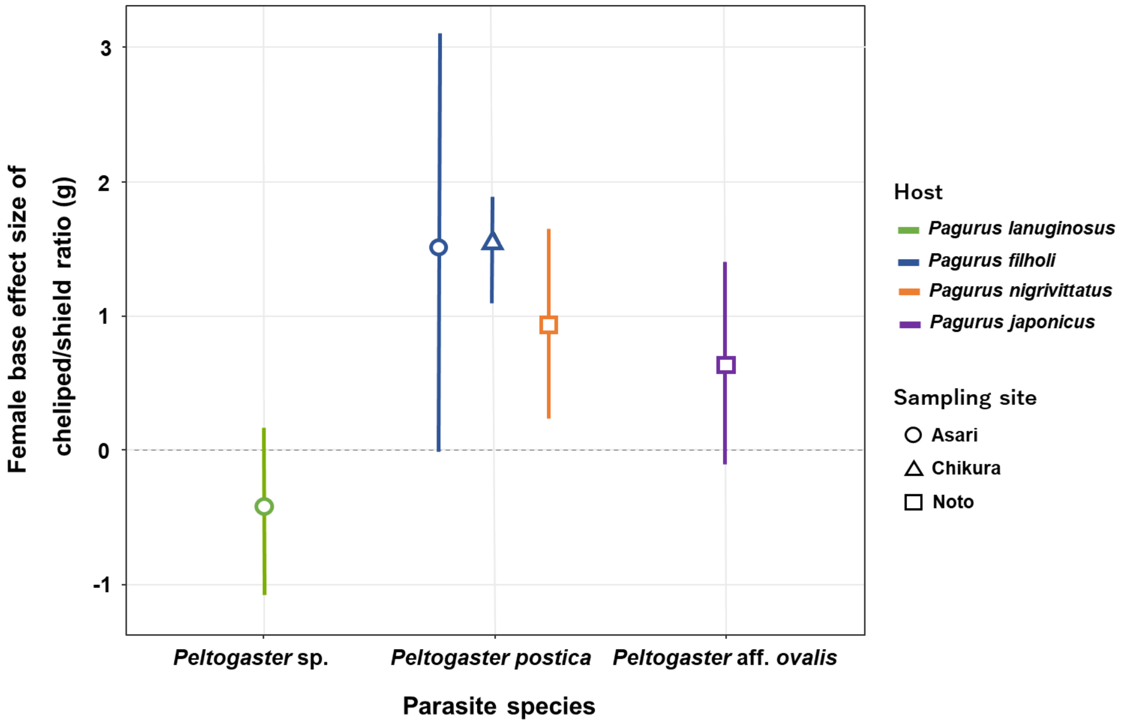
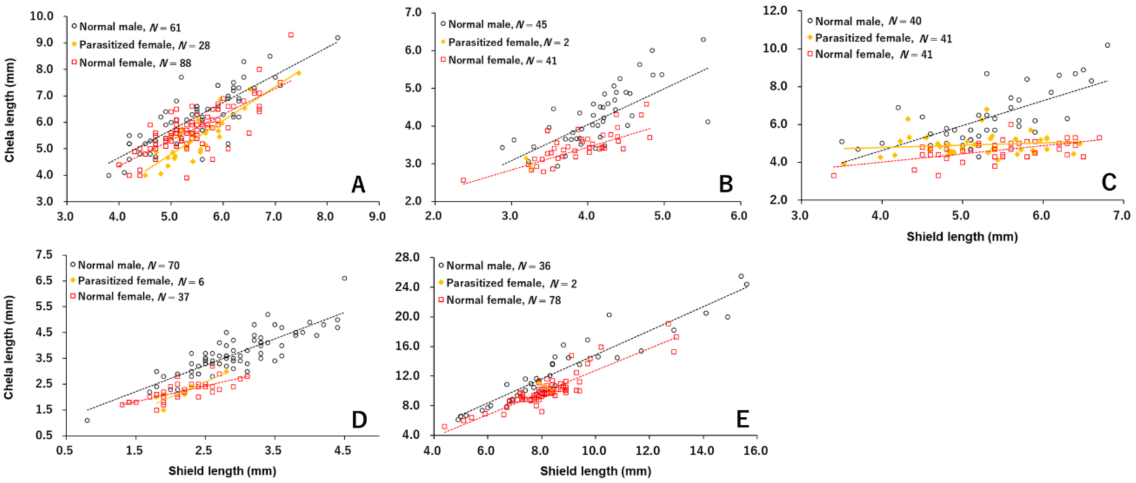


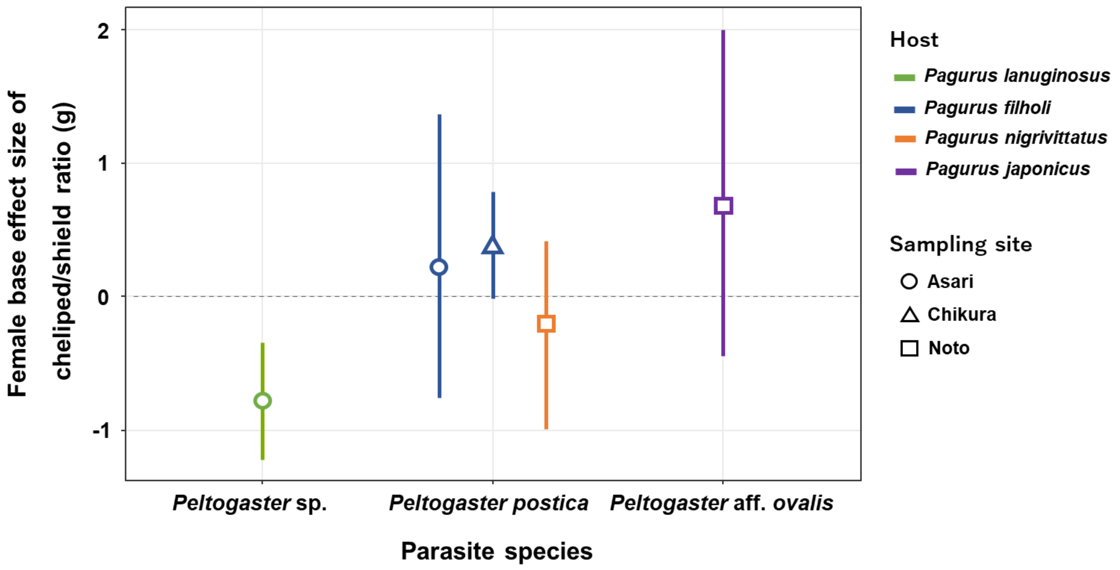
Fig. 5. Effect sizes of cheliped-to-shield length ratios in parasitized male hermit crabs relative to normal females.

Supplementary Table 1. ANCOVA of cheliped length in normal female Pagurus hermit crabs versus those parasitized by Peltogaster species.

Parasite	**Host**	**Homogeneity of the slopes**		**Allometric changes**	
		F value	**p-value**	**F value**	**p-value**
Peltogaster sp.	*Pagurus lanuginosus*	2.67	0.105	11.33	<.01
Peltogaster postica	*Pagurus filholi*	0.84	0.366	0.01	0.907
Peltogaster postica	*Pagurus filholi*	12.06	<.001	-	-
Peltogaster postica	*Pagurus nigrivittatus*	3.200	0.082	0.120	0.727
Peltogaster aff. *ovalis*	*Pagurus japonicus*	1.36	0.246	0.61	0.438



Supplementary Figure S1. Allometric variation in cheliped length relative to shield length in female hermit crabs, comparing normal and parasitized individuals: *Pagurus lanuginosus* parasitized by *Peltogaster* sp. **(A)**; *Pa. filholi* in Asari **(B)**, *Pa. filholi* in Chikura **(C)**, and *Pa. nigrivittatus* **(D)** parasitized by *Pe. postica*; *Pa. japonicus* parasitized by *Peltogaster* aff. *ovalis* **(E)**.



Supplementary Figure S2. Effect sizes of cheliped-to-shield length ratios in parasitized female hermit crabs relative to normal females.