Sexual system in the tanaidacean *Falsapseudes bowmani* (Crustacea: Malacostraca: Peracarida)

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Abstract
With more than 40,000 species, Malacostraca is the most diverse crustacean class. Most malacostracans are gonochoristic, but simultaneous hermaphrodites are also known. Tanaidacea is one of two malacostracan orders that includes simultaneously hermaphroditic species; so far, simultaneous hermaphroditism has been confirmed externally and internally in only two tanaidacean species, both in the genus *Apseudes* (Apseudidae). Here we show, through external and internal morphological observations of fixed specimens, that the apseudid *Falsapseudes bowmani* is a simultaneous hermaphrodite, making *Falsapseudes* the second tanaidacean genus in which simultaneous hermaphroditism has been confirmed both externally and internally. In this species, the epistome (a projection on the clypeus) was thick and elongate in large specimens but was thin and spiniform in smaller specimens; the brooding of eggs or embryos was observed only in thin-epistome individuals, although a pair of ovaries was confirmed in both thick- and thin-epistome individuals. This suggests that individuals with a thick epistome may act as males while also retaining the female reproductive organs.

KEYWORDS
anatomy, cavernicolous, reproduction, sexual dimorphism, simultaneous hermaphroditism

1 INTRODUCTION

Malacostraca, the most diverse crustacean class, with more than 40,000 species (Ahyong et al., 2011), includes many familiar groups, such as mantis shrimps, crabs, prawns, krill, woodlice, and sandhoppers. Most malacostracans are gonochoristic, or dioecious (i.e., individuals are either male or female, and their sex does not change during their life), but

parthenogenetic (e.g., Scholtz et al., 2003) and hermaphroditic (e.g., Chiba, 2007) species are also known. Compared to sequential hermaphroditism (i.e., sex change), simultaneous hermaphroditism is less common in Malacostraca, and is so far known only in the orders Decapoda and Tanaidacea (Kakui & Hiruta, 2013). In Tanaidacea, simultaneous hermaphroditism has been inferred for around 20 species in which individuals have been observed bearing both oostegites (components of the marsupium, or brood pouch, a female trait) and a genital cone (a male trait) (Guţu, 2006; Rumbold, Obenat, Leonardi, & Spivak, 2015). However, internal reproductive organs of both sexes in single individuals have been confirmed in only two species in one genus, *Apseudes* (Kakui & Hiruta, 2013; Lang, 1953).

The monotypic genus *Falsapseudes* GUŢU 2006 contains only *Falsapseudes bowmani* (GUŢU & ILIFFE 1989), reported from a marine cave at Koror Island, Palau (Guţu, 2006). This species shows sexual dimorphism in the shape and size of the epistome (a projection on the clypeus); in males the epistome is “very big, lamellar, dagger-like and antero-ventrally directed,” whereas in females it comprises a “strong spine directed forward” (Guţu & Iliffe, 1989). However, we recently observed newly collected specimens of *F. bowmani* to have both oostegites and a male-type epistome, raising the possibility that this species is simultaneously hermaphroditic. In this study, we investigated the sexual mode of the species by examining the external and internal morphology.

2 METHODS

Ninety-nine tanaidaceans were collected on 23 January 2018 in Airai State, Babeldaob Island, Palau (7°20'N, 134°32'E), by scuba diving in a marine cave that opens at a depth of about 3 m. A mud sediment sample was taken by a 500-μm mesh net at a depth of about 14 m in the cave; the net was then put into a plastic bag. Tanaidaceans were sorted from the sample and fixed and preserved in 99% ethanol. Specimens were examined with a Nikon SMZ 1500 stereomicroscope to determine their developmental stage and epistome type; measurements were made with ImageJ (Rasband, 2018) from digital images taken by an Olympus OM-D E-M5 digital camera attached to the SMZ 1500 via Micronet NY-1S and NY1S-MOFA adapters. Body length (BL) was measured from the base of the antennules to the tip of the pleotelson, and body width at the widest portion of the carapace (CW, carapace width). As BL was highly correlated with CW (see Results section), the obtained regression was used to calculate the BL from CW measurements for eight specimens having a curved body.

Tanaidaceans lack a planktonic larval stage, and directly release juveniles called mancae from marsupia; the mancae resemble post-mancae, but their pereopod 6 and the pleopods are incompletely developed or lacking (Kakui, Hayakawa, & Katakura, 2017). Accordingly, we categorized specimens into one of two numbered developmental stages in this study: 1) “manca,” specimens without a fully developed pereopod 6; and 2) “post-manca,”

specimens with a fully developed pereopod 6. Each post-manca individual was further categorized into one of the following three conditions: 2A) “oostegite lacking,” without a marsupium, marsupium scars, or developing oostegites; 2B) “preparatory,” bearing developing oostegites; and 2C) “ovigerous,” having a fully developed marsupium. The shape of the epistome was classified into the “thick” and “thin” types (Figure 1), corresponding to the male and female types, respectively, according to Guţu and Iliffe (1989); the dorsal margin of the epistome is convex or almost straight in the former but strongly concave in the latter. We note that these authors described the male-type epistome as “lamellar,” but it is actually conical, rather than laterally flattened. Although we observed that the epistome varied relative to BL among thick-type specimens, we did not further subdivide the thick type. For analysis, 99 specimens observed were classified into six groups based on developmental stage and epistome type: mancae with a thin epistome (Group 1-tn); oostegite-lacking, preparatory, or ovigerous post-mancae with a thin epistome (Groups 2A-tn, 2B-tn, and 2C-tn, respectively); and oostegite-lacking or preparatory post-mancae with a thick epistome (Groups 2A-T and 2B-T, respectively). The CW data between post-mancae with a thin epistome (2A-tn, 2B-tn, and 2C-tn) and those with a thick epistome (2A-T and 2B-T) were analyzed with the Mann–Whitney *U*-test with R version 3.5.1 (R Core Team, 2018).

Specimens for scanning electron microscopy (SEM) were treated with hexamethyldisilazane, sputter-coated with gold, and observed at 15 kV accelerating voltage with a Hitachi S-3000 N SEM. For histology, ethanol-fixed specimens were soaked in Bouin’s fluid for half a day, embedded in paraffin, sectioned at 5 μm thickness, and stained with Mayer’s hematoxylin and eosin (HE). In total, eight post-mancae in five groups were sectioned and examined (CW in parentheses): two in Group 2A-tn (0.32, 0.36 mm); one in 2B-tn (0.40 mm); two in 2C-tn (0.41, 0.43 mm); one in 2A-T (0.42 mm); and two in 2B-T (0.49, 0.50 mm).

3 RESULTS

External observations showed a genital cone to occur on all post-mancae but not on mancae. Among 94 post-mancae, 56 specimens also had developing or fully developed oostegites (Figure 2); all ovigerous individuals bore a thin epistome. Groups 1-tn, 2A-tn, 2B-tn, 2C-tn, 2A-T, and 2B-T comprised 5, 35, 25, 16, 3, and 15 specimens, respectively.

A plot of CW against BL (Figure 3) shows a highly linear relationship across all groups (BL = 7.134CW + 0.026, R^2 = 0.974; n = 90). Using CW as a body-size index, the post-mancae with a thick epistome (2A-T and 2B-T) were significantly larger than post-mancae with a thin epistome (2A-tn, 2B-tn, and 2C-tn) (Mann-Whitney *U*-test, $p < 0.01$).

Internal morphology was examined for eight post-mancae in five groups. Although

histological resolution was not good because our specimens had been fixed initially in absolute ethanol, a pair of ovaries and a seminal vesicle filled with spermatozoa were evident in all specimens observed (Figure 4).

4 DISCUSSION

Our post-manca specimens all bore a genital cone. In eight specimens (ranging 0.32–0.50 mm in CW), ovaries and a seminal vesicle, which was filled with spermatozoa and lay inside the genital cone, were evident internally. We did not find testes; we speculate that they became deformed and simply were not evident in the sections, because fixation in absolute ethanol strongly deforms soft internal organs. These observations indicate that *Falsapseudes bowmani* is simultaneously hermaphroditic, making *Falsapseudes* the second genus in which simultaneous hermaphroditism has been confirmed by both external and internal observations. Although *F. bowmani* was originally reported as being gonochoristic (Guţu & Iliffe, 1989), the genital cone in this species is small and can be overlooked, and oostegite-lacking post-mancae with a thick epistome (Group 2A-T) can appear to be males. In other words, Guţu and Iliffe (1989) might have missed seeing a genital cone on their "females," and the two "males" they observed may have been post-mancae in Group 2A-T (oostegite lacking, thick epistome).

Our observations indicate that *F. bowmani* has a different sexual system from the simultaneously hermaphroditic apseudid *Apseudes* sp. *sensu* Kakui and Hiruta (2013) (hereafter, referred to as "*Apseudes* sp."). In our specimens of *F. bowmani*, the ovigerous state was found only in the thin-epistome-bearing post-mancae that were smaller than individuals with the thick epistome. In *Apseudes* sp., any individual greater than a certain body size (4.35 mm in BL; Kakui & Hiruta, 2013) can be ovigerous, as is the general case in females of other gonochoristic apseudoids (e.g., Messing, 1983; Pennafirme & Soares-Gomes, 2009; Schmidt, Siegel, & Brandt, 2002). In *F. bowmani*, individuals with the thick epistome might not enter into the ovigerous stage, but instead function as males while also retaining female reproductive organs. Future rearing experiments are needed to test this hypothesis.

Among the large specimens with a thick epistome, three lacked any traces of oostegites (Group 2A-T). Kakui and Hiruta (2013) did not observe this stage in *Apseudes* sp., ovigerous individuals of which lose their brood pouch without molting, leaving marsupium scars (Figure 5); they can then molt and enter into the preparatory stage, and finally become ovigerous again via another molt. Individuals in Group 2A-T were larger than those in Group 2C-tn. After the release of mancae, some ovigerous individuals might become the 2A-T condition via a molt.

This is the first report of a simultaneously hermaphroditic tanaidacean species from a marine cave. In the cave, many individuals of *F. bowmani* were observed swimming near the

surface of stalactites, the cave walls, and the silty bottom. The other two tanaidacean species in which simultaneous hermaphroditism has been confirmed by examination of internal organs are *Apseudes spectabilis* STUDER 1883, collected from 12–310 m depth around subantarctic South Georgia Island (Lang, 1953), and *Apseudes* sp., found in a tank housing giant clams (24–26 °C) in an aquarium (Kakui & Hiruta, 2013). To date, simultaneous hermaphrodites among tanaidaceans have been reported only from the family Apseudidae, although this may be due to a lack of relevant studies in other groups. Further descriptive studies of tanaidacean sexual systems will be necessary to eventually elucidate the evolutionary history and ecological significance of simultaneous hermaphroditism in Tanaidacea.

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FIGURE LEGENDS

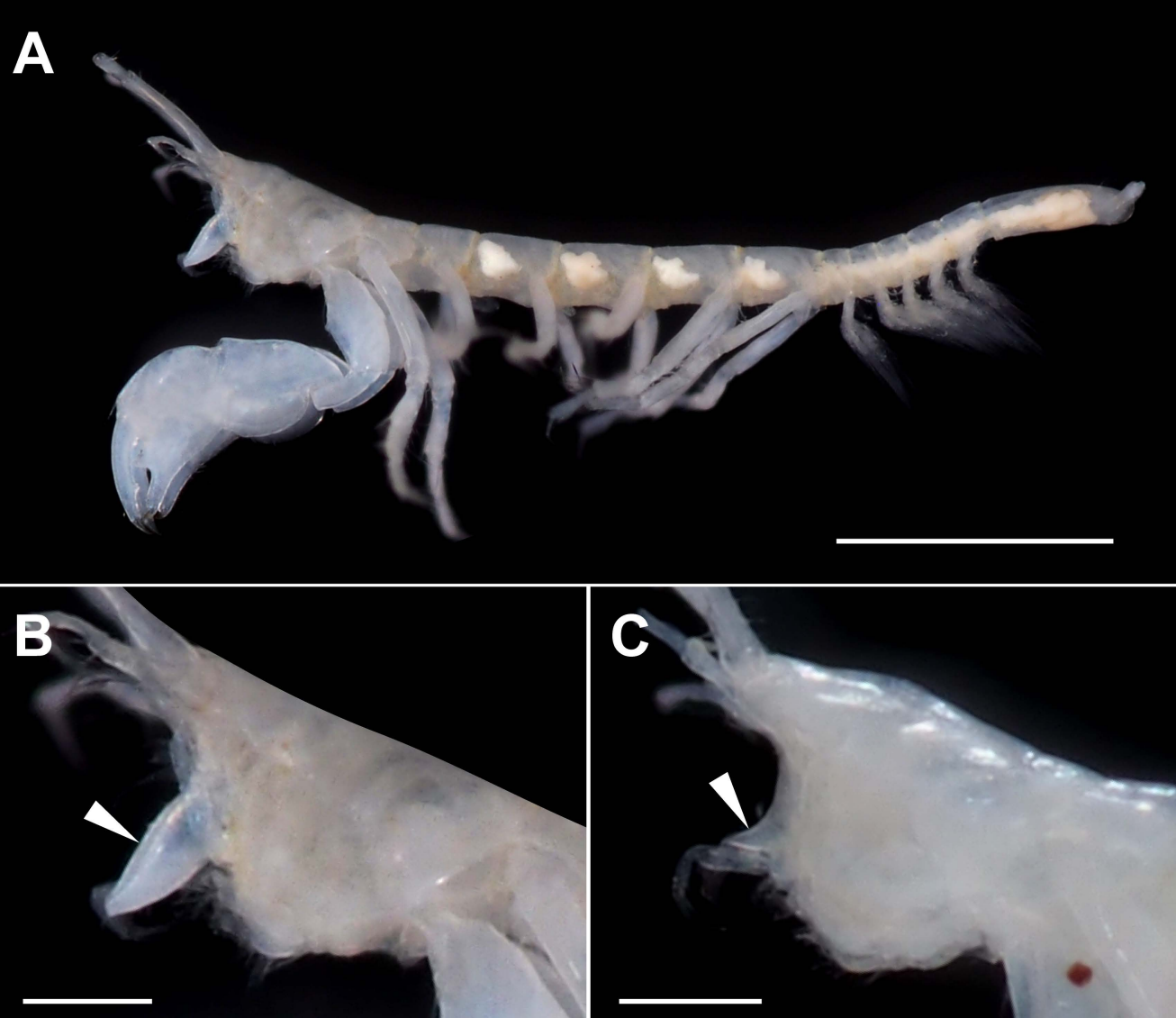
FIGURE 1 *Falsapseudes bowmani*, ethanol preserved specimens. **A.** Preparatory post-manca with a thick epistome, lateral view, distal tip of left antennule and both uropods broken (CW = 0.49 mm). **B.** Same, enlargement showing the thick epistome (arrowhead), lateral view. **C.** Ovigerous post-manca, enlargement showing a thin epistome (arrowhead), lateral view (CW = 0.43 mm). Scale bars: A = 1 mm; B,C = 0.2 mm

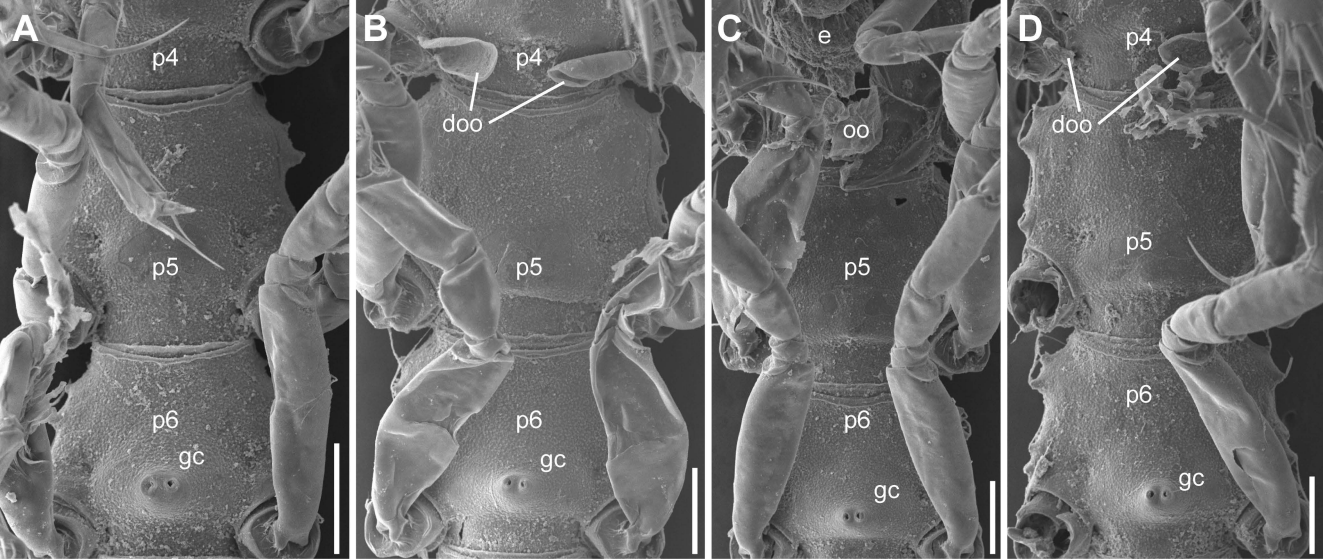
FIGURE 2 Female and male sexual traits in *Falsapseudes bowmani*, SEM images, ventral view. **A.** Oostegite-lacking post-manca with a thin epistome (Group 2A-tn; CW = 0.32 mm). **B.** Preparatory post-manca with a thin epistome (2B-tn; CW = 0.40 mm). **C.** Ovigerous post-manca with a thin epistome (2C-tn; CW = 0.39 mm); marsupium partly broken and egg exposed. **D.** Preparatory post-manca with a thick epistome (2B-T; CW = 0.43 mm); right oostegite on the pereopod 4 partly broken. Scale bars: 0.1 mm. doo, developing oostegites; e, egg; gc, genital cone with a pair of male gonopores; oo, fully developed oostegite; p4–6, pereonites 4–6, respectively.

FIGURE 3 Relationship among body size (body length and carapace width), developmental stage, and epistome type in *Falsapseudes bowmani*

FIGURE 4 Cross sections of a preparatory post-manca of *Falsapseudes bowmani*, with a thin epistome (Group 2B-tn; CW = 0.40 mm), showing internal reproductive organs; specimen fixed in ethanol, stained with HE. **A.** Section through pereonite 4. **B.** Section through pereonite 6; dashed rectangle indicates the area enlarged in C. **C.** Enlargement of the ventral portion of pereonite 6. Scale bars: 0.05 mm. d, dorsal, gc, genital cone; ov, ovary; sp, spermatozoon; sv, seminal vesicle; v, ventral

FIGURE 5 *Apseudes* sp. *sensu* Kakui and Hiruta (2013), with marsupium scars, ventral view. The arrow indicates the marsupium scar on the right pereopod-4 coxa. Scale bar: 1 mm


A
B
C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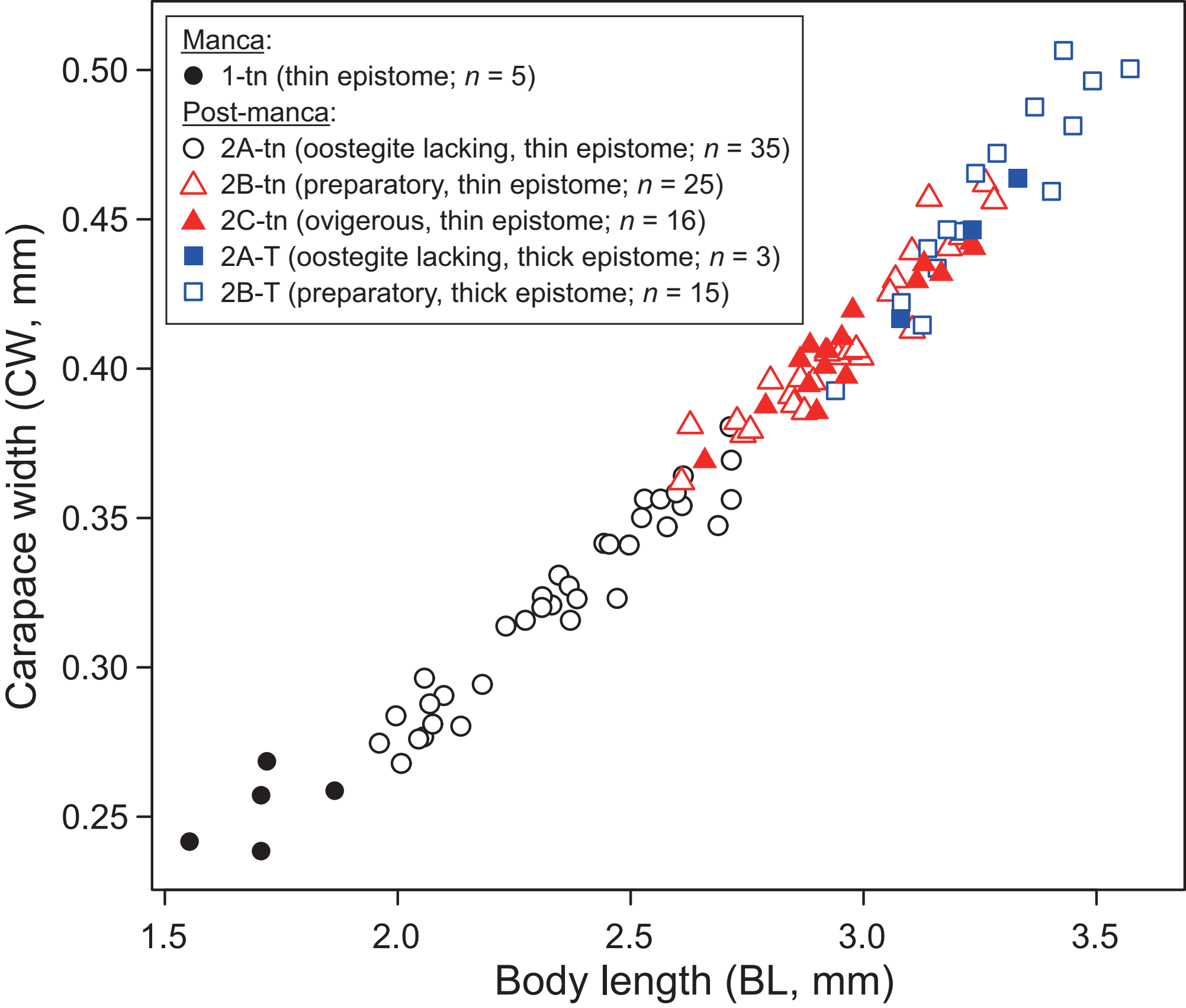
Manca:
1-tn (thin epistome; n = 5)
Post-manca:
2A-tn (oostegite lacking, thin epistome; n = 35)
2B-tn (preparatory, thin epistome; n = 25)
2C-tn (ovigerous, thin epistome; n = 16)
2A-T (oostegite lacking, thick epistome; n = 3)
2B-T (preparatory, thick epistome; n = 15)
Carapace width (CW, mm)
0.50
0.45
0.40
0.35
0.30
0.25
1.5
2.0
2.5
3.0
3.5
Body length (BL, mm)


A
ov
d
v
B
C
sv
sp
gc