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REDESCRIPTION OF A PHILIPPINE ENDEMIC FRESHWATER CALANOID
COPEPOD, *PSEUDODIAPTOMUS BREHMI* KIEFER, 1938 (COPEPODA,
CALANOIDA, PSEUDODIAPTOMIDAE)

BY

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ABSTRACT

The genus *Pseudodiaptomus* is represented by 19 species in Philippine coastal waters. Of all these species found in the Philippines, *P. brehmi* Kiefer, 1938 is the only known truly freshwater pseudodiaptomid endemic to this country, which was originally described from Lake Naujan, Mindoro Island. After numerous attempts to find it in its type locality, we concluded that the former is no longer extant in this lake and has now been displaced by the invasive Neotropical calanoid *Arctodiaptomus dorsalis* (Marsh, 1907). Sampling in Lake Taal, on the nearby island of Luzon, resulted in the surprise discovery of *P. brehmi* populations coexisting with *A. dorsalis*. This rediscovery of *P. brehmi* in Lake Taal and its absence from its type locality, necessitate this report as well as the species' morphological redescription.

RÉSUMÉ

Le genre *Pseudodiaptomus* est représenté par 19 espèces dans les eaux côtières des Philippines. De toutes ces espèces trouvées aux Philippines, *P. brehmi* Kiefer, 1938 est la seule espèce véritablement connue de Pseudodiaptomidae d'eau douce endémique de ce pays, décrite à l'origine du lac Naujan, dans l'île de Mindoro. Après de nombreuses tentatives pour trouver cette espèce dans sa localité-type, nous en avons conclu qu'elle n'était plus présente dans ce lac et qu'elle avait été remplacée par le calanoïde néotropical invasif *Arctodiaptomus dorsalis* (Marsh, 1907). L'échantillonnage dans le lac Taal, sur l'île proche de Luzon, a conduit à la découverte surprise de populations de *P. brehmi* coexistant avec *A. dorsalis*. Cette redécouverte de *P. brehmi* dans le lac Taal et son absence de la localité-type nécessitaient d'être rapportées de même que la redescription morphologique de cette espèce.

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INTRODUCTION

Kiefer (1938) described *Pseudodiaptomus brehmi* Kiefer, 1938 from Lake Naujan and later it was reported by Woltereck et al. (1941) during the Wallacea Expedition which surveyed numerous freshwater lakes in the Philippines including Lake Taal and Lake Naujan. Lake Naujan in the province of Mindoro Oriental in Mindoro Island, Philippines (13°10'N 121°21'E) is the fifth largest lake in the country, with a surface area of 81.25 km², a maximum depth of 45 m, 30 meters above sea level and is about one-third the size of Taal (NLNP, 2016). From 2012 to 2013 numerous attempts were made to re-confirm the presence of *P. brehmi* in Lake Naujan only to find out that it was no longer extant there. It was, however, found in Lake Taal (Papa et al., 2012), which is approximately 94.63 km north of Lake Naujan.

The genus *Pseudodiaptomus* Herrick, 1884 has been extensively studied throughout Southeast Asia and the Philippines (Walter, 1984, 1986, 1987; Walter et al., 2006). Currently there are 81 species recognized in the genus which is divided into 6 species-groups (Walter, 1986; Walter et al., 2006; Walter & Boxshall, 2015).

During the Wallacea Expedition of 1938, Kiefer collected an undescribed pseudodiaptomid in Lake Naujan, Mindoro; he then sent the specimens to Brehm, who confirmed that it was a new species. After the original description of *P. brehmi* by Kiefer (1938), this species was mentioned in the description of *P. terazakii* Walter, Ohtsuka & Castillo, 2006 where it was included in a key of Philippine *Pseudodiaptomus* species, as well as in the recent publications by Papa et al. (2011, 2012, 2017). Walter et al. (2006) provided a list of the 77 known species of the genus at the time with *P. brehmi* being one of the few and possibly the only freshwater pseudodiaptomid from the Philippines. According to Walter et al. (2006), *P. brehmi* is a member of the *lobus* species group under the *forbesi* subgroup, the latter is composed of 17 species, most originally from southeast Asia: *P. annandalei* Sewell, 1919 (India, Philippines), *P. binghami* Sewell, 1912 (India), *P. brehmi* Kiefer, 1938 (Philippines), *P. bulbosus* (Shen & Tai, 1964) (China), *P. forbesi* (Poppe & Richard, 1890) (China), *P. inflatus* (Shen & Tai, 1964) (China), *P. inopinus* Burckhardt, 1913 (China), *P. inopinus gordiodes* Brehm, 1952 (China), *P. inopinus saccupodus* (Shen & Tai, 1962) (China), *P. koreanus* Soh, Kwon, Lee & Yoon, 2012 (Korea), *P. lobipes* Gurney, 1907 (India), *P. malayalus* Wellershaus, 1969 (India), *P. mixtus* Walter, 1994 (Bangladesh), *P. nanseni* Sakaguchi & Ueda, 2010 (Japan), *P. poplesia* (Shen, 1955) (China), *P. spatulatus* (Shen & Tai, 1964) (China) and *P. terazakii* Walter et al., 2006 (Philippines). The species of this group are known for their preference for estuarine or freshwater habitats and are rarely found in saltwater environments, except

during rainy seasons and strong typhoons when flushing of the rivers and estuaries occurs.

Lake Naujan, Mindoro Island, Philippines has been known to be the type locality of *P. brehmi*. However, recent investigations by Papa et al. (2012) were not able to verify its presence in the lake. Instead, the species was found in Lake Taal, Luzon Island (Papa et al., 2012). During those studies, it was confirmed that both Lake Taal and Lake Naujan had established populations of a Neotropical invasive calanoid, *Arctodiaptomus dorsalis* (Marsh, 1907), and that in Lake Naujan, perhaps the presence of this species has led to the displacement of *P. brehmi* (cf. Papa et al., 2012). Given that *P. brehmi* populations from the type locality may have already been permanently displaced by *A. dorsalis* and that the Lake Taal population may be the only known remaining population of *P. brehmi*, it is considered appropriate to redescribe the species and to document it for a more comprehensive listing of copepod diversity in the Philippines. This paper provides a detailed redescription of *P. brehmi* since the original work of Kiefer and assigns a lectotype and paralectotypes based on Kiefer's collections deposited in the Staatliches Museum für Naturkunde Karlsruhe (Karlsruhe, Germany).

MATERIAL AND METHODS

Field sampling in both the limnetic and littoral zones were conducted bimonthly from January to September 2015 in Lake Taal, Batangas province, Luzon island (13.9750°N, 121.0220°E; fig. 1). Samples from the limnetic zone were collected using conical plankton nets with mesh sizes 100 μm and 45 μm at an average depth of 30 m. All samples were fixed in 95% ethyl alcohol, sieved using 70 μm and 30 μm sieves, and preserved in 70% ethyl alcohol with Rose Bengal dye. Calanoid copepods were sorted and tentatively identified to the lowest possible taxonomic classification. Dissection was done using fine tungsten needles with glycerine as a fixing medium and clear nail polish as a sealant. Body length measurements, further taxonomic observations, and the line drawings were made using an Olympus CX21 compound microscope with an attached drawing tube. A total of 20 male and 18 female *P. brehmi* were used to measure body lengths, particularly the total length, prosome length, and urosome length. An Optika B-500 Phase Contrast Microscope was also used to validate the presence of other morphological details.

Fortunately, Kiefer's collections and original drawings were located in the Staatliches Museum für Naturkunde Karlsruhe. We were able to borrow Kiefer's unsorted samples from Lake Naujan and found an adult male and two females from a small sample. There were also two slides, one male and one female labelled as

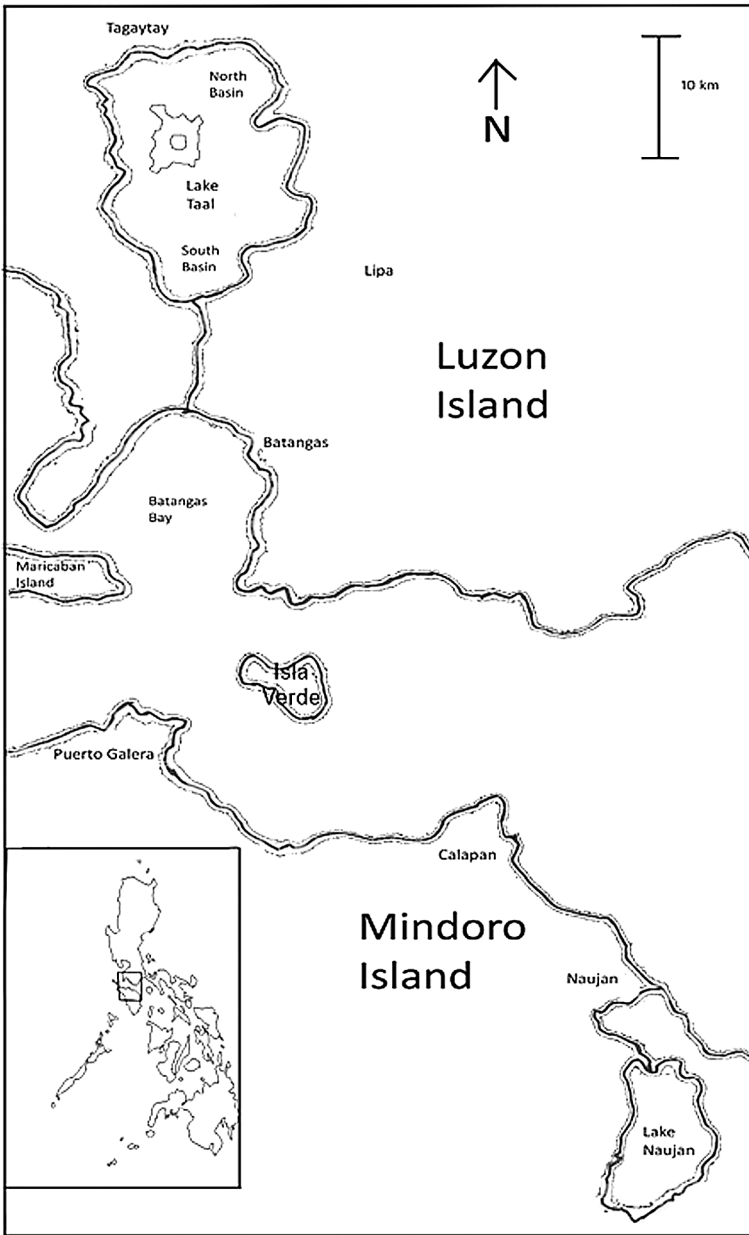


Fig. 1. Map showing Lake Taal where *Pseudodiptomus brehmi* Kiefer, 1938 was collected, and its proximity to Lake Naujan.

“Typus”, but these slides were not listed in the original publication as types. Using Kiefer’s slides and specimens, we are now able to designate a lectotype series.

Terminology of morphological characters is based on Huys & Boxshall (1991) and antennule armature formula: Segment = setae (spine) + aesthetasc. The armature formulae of swimming legs 1-5 follows Dussart & Defaye (2001), wherein each segment is separated by a semi-colon (;). Presence of seta/e is represented by Arabic numerals while presence of spine(s) is represented by Roman numerals. Writing the formula starts from left to right wherein the value on the left represents armature on the outer margin followed by a hyphen and then the next value represents the armature of the inner margin. Formula for the last segment is composed of three values representing the armature on the outer, medial, and inner margins each separated by a comma. Scanning electron microscopy was done using a JEOL JSM-5310 Scanning Microscope in order to see finer details. Dissected specimens were dehydrated by passing through increasing concentrations of ethyl alcohol. Specimens were then carefully transferred onto an SEM stub with an attached aluminium foil, air-dried for not more than 10 minutes and gold-coated at 30 Pa for 40 seconds using a JEOL JFC-1200 Fine Coater.

Abbreviations: SMNK, Staatliches Museum für Naturkunde, Karlsruhe; USNM, United States National Museum, Washington, D.C.; UST, University of Santo Tomas, Manila; ZRC, Zoological Reference Collection [of UST].

DESCRIPTION

Order CALANOIDA G.O. Sars, 1903

Family PSEUDODIAPTOMIDAE Sars, 1902

Genus *Pseudodiaptomus* Herrick, 1884

***Pseudodiaptomus brehmi* Kiefer, 1938**

Pseudodiaptomus brehmi Kiefer, 1938: 75-81, figs. 1-8. — Woltereck, 1941: 115. — Brehm, 1942: 273. — Papa & Zafaralla, 2011, table 2. — Papa, Tordesillas & Mamaril, 2012: 247-248, fig. 1G. — Papa, Li, Tordesillas, Han & Dumont, 2012: 2475, fig. 3, table 1. — Papa, Legaspi, Blaza, Lirio, Bangalan & Walter, 2017: 1-5.

Material examined.— Adults: 20 males and 18 females (UST-ZRC Slide Nos. 0079 to 0084 and 0215 to 0231; 0087-0093 and 0232 to 0237) collected during April-October, 2013 and January-July, 2015. UST-ZRC Slide Nos. 0215-0237. Thirty adult males and females (USNM 1405879). Lectotype — 1 male collected April 14, 1938 on slides (SMNK-Kiefer-M-3990, 3991 & 3992), Paralectotypes — 1 female on slides (SMNK-Kiefer-M-3923 & 3924) and 1 male and 2 females in alcohol (SMNK-Kiefer-G-1086). All UST and USNM specimens were collected by R. Papa in August 2009 and S. Guinto from January to July 2015 in Lake Taal, Batangas, Philippines (13.9750°N 121.0220°E).

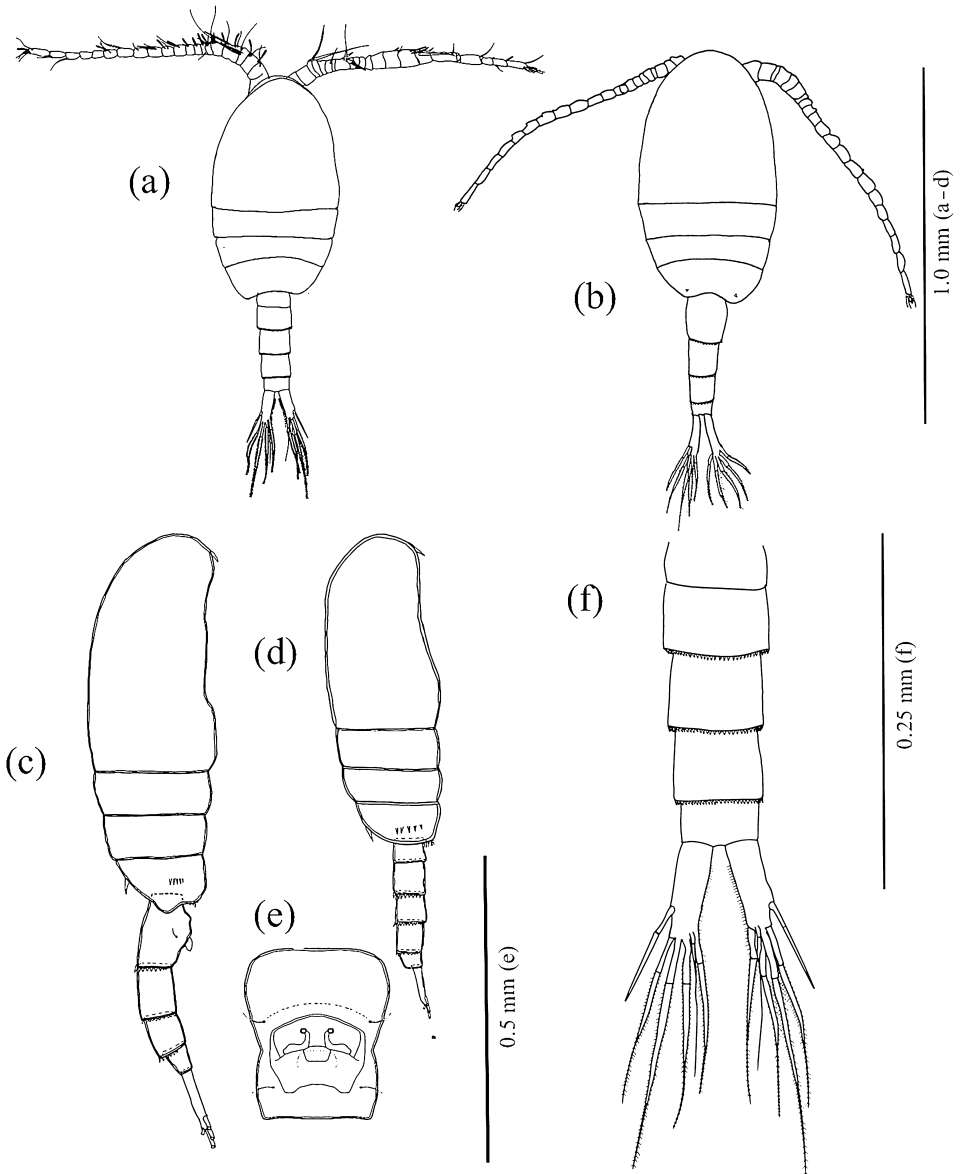


Fig. 2. *Pseudodiaptomus brehmi* Kiefer, 1938. a, male habitus (dorsal view); b, female habitus (dorsal view); c, female habitus (right lateral view); d, male habitus (right lateral view); e, female genital double somite (ventral view); f, male urosome (dorsal view).

Description of male.— Habitus (fig. 2a, d). Total body length (mean \pm SD) 0.77 ± 0.09 mm ($N = 20$) including caudal rami. Prosome to urosome ratio 1.7 : 1, prosome consisting of 4 somites, mean length 0.49 ± 0.04 mm, cephalosome about half as long as the entire prosome. Last thoracic somite (4-5 fused) rounded

with one small spine on dorsal sides (fig. 2a) and a small row of lateral fine spinules (fig. 2d). Urosome (fig. 2f) of five somites, mean length 0.28 ± 0.08 mm, urosomites 2-4 dorsoposterior margins lined with marginal spinules, urosomites and caudal rami with proportions $23 : 11 : 17 : 19 : 19 : 11 = 100$. Caudal rami with setules along inner margin, rami symmetrical, with a bare, dagger-shaped lateral seta on either ramus which is the shortest of the six setae, four plumose medial setae with the first and third about the same length, the second being the longest of the four, and the fourth being the shortest, and a weak and bare sixth seta located at the inner distal corner, about the same length as the fourth plumose seta.

Antennules (fig. 3a-b) asymmetrical not reaching beyond the prosome, segmentation and setation patterns follow that of the *lobus* group (Walter, 1986). Right antennule (fig. 3a) geniculate, indistinctly 20-segmented; segments 6-7 partly fused, 16-19 modified, geniculate at 18-19; armature (segment = setae + ae): 1 = 1 + ae; 2 = 4 + ae; 3 = 2 + ae; 4 = 1; 5 = 1 + ae; 6-7 = short spine + 2 + ae; 8-9 = spine + 1 + ae; 10 = 1 large hooked spine; 11 = spine + 1 + ae; 12 = spine + ae; 13-16 = spine + 1 + ae; 17 = broad modified dorsal spine + ae; 18 = setule comb row + ae; 19 = 2 spines + 2; 20 = 10 + ae. Left antennule (fig. 3b) 22-segmented, armature 1 = 1 + ae; 2 = spine + 3 + ae; 3 = 2 + ae; 4-5 = 2 + 2ae; 6-7 partial fusion, 1 spine + 2 + ae; 8-17 = 2 + ae; 18 = 1; 19 = 1 + ae; 20 = 1 + ventral plumose setae; 21 = 2 + ae; 22 = 6 + ae.

Antenna (fig. 4a) coxa with one seta, basis with two setae. Endopod with two segments, the first segment with two setae, second segment bearing 14 setae. Exopod 4-segmented setation pattern 1 : 3 : 2 : 5. Mandible (fig. 4b) basis with four medial setae; endopod 2-segmented, with first segment having four setae, second segment with eight setae and row of distal spinules; exopod 4-segmented with six setae. Coxal gnathobase with a triangular caudal tooth, a triangular subcaudal tooth, and a group of seven multi-cusped teeth, and one distal spinous seta located disto-laterally. Maxillule (fig. 4c) praecoxal arthrite with nine robust setae and six fine setae; coxa with four setae on endite and nine setae on epipodite; basis with four and five setae on proximal and distal endites respectively, exite with one seta; endopod 3-segmented with four setae on the first and second segments, and six on the third segment; exopod with ten setae. Maxilla (fig. 4d) first praecoxal endite with four setae, second praecoxal and two coxal endites each with three setae; basis with three setae, endopod bearing eight setae. Maxilliped (fig. 4e) praecoxa and coxa fused, endites with eight setae; basis and first endopod segment partly fused, with three setae on the earlier and two on the latter having medial setules; second to sixth endopodal segments with 2, 2, 2, 3, 4 setae; second segment with modified bifurcate setae.

Swimming legs 1-4 (fig. 5a-e) symmetrical, biramous, with 3-segmented endopods and exopods. Coxa and basis ornamentation same for legs 1-4, coxa with

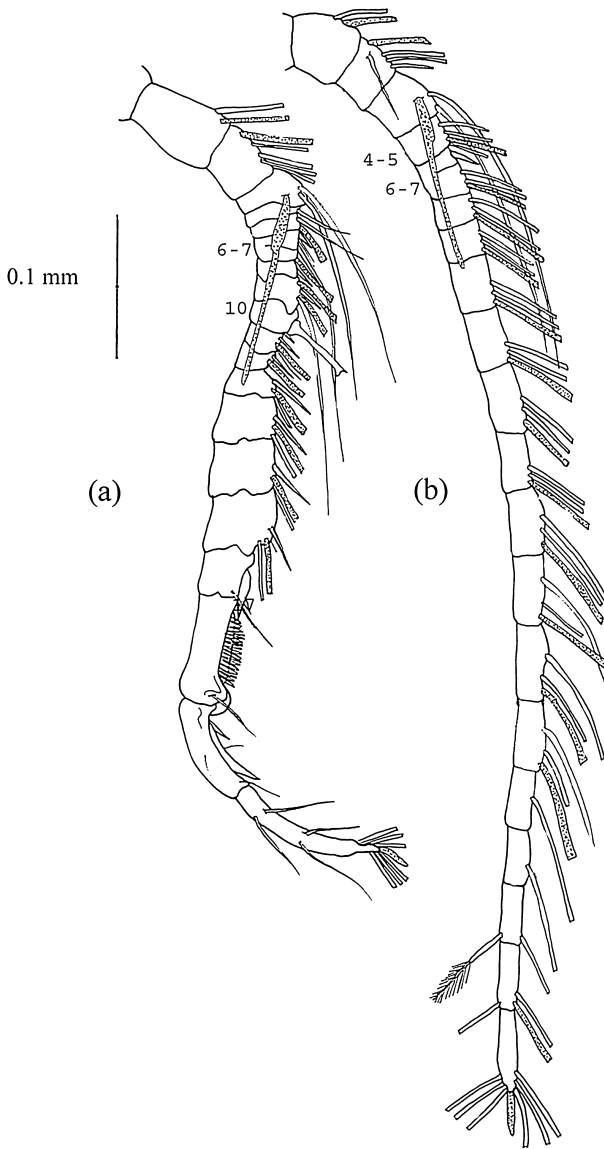


Fig. 3. Male antennule of *Pseudodiaptomus brehmi* Kiefer, 1938. a, right; b, left.

two medial setae and setule patch, lateral margin with setule patches. Basis medial margin with setules and distolateral seta. Armature patterns for legs 1-4 on table I.

Fifth legs (figs. 6a-c and 7a-d) with coxae fused, and with surface spinule row. Posterior view (fig. 6a). Coxa with spinule row. Right leg basis with pair of rounded projections with lateral spinule row extending to surface. Exopod segment 2 with medial setule row. Left leg, basis with small medial projection and lateral spinule

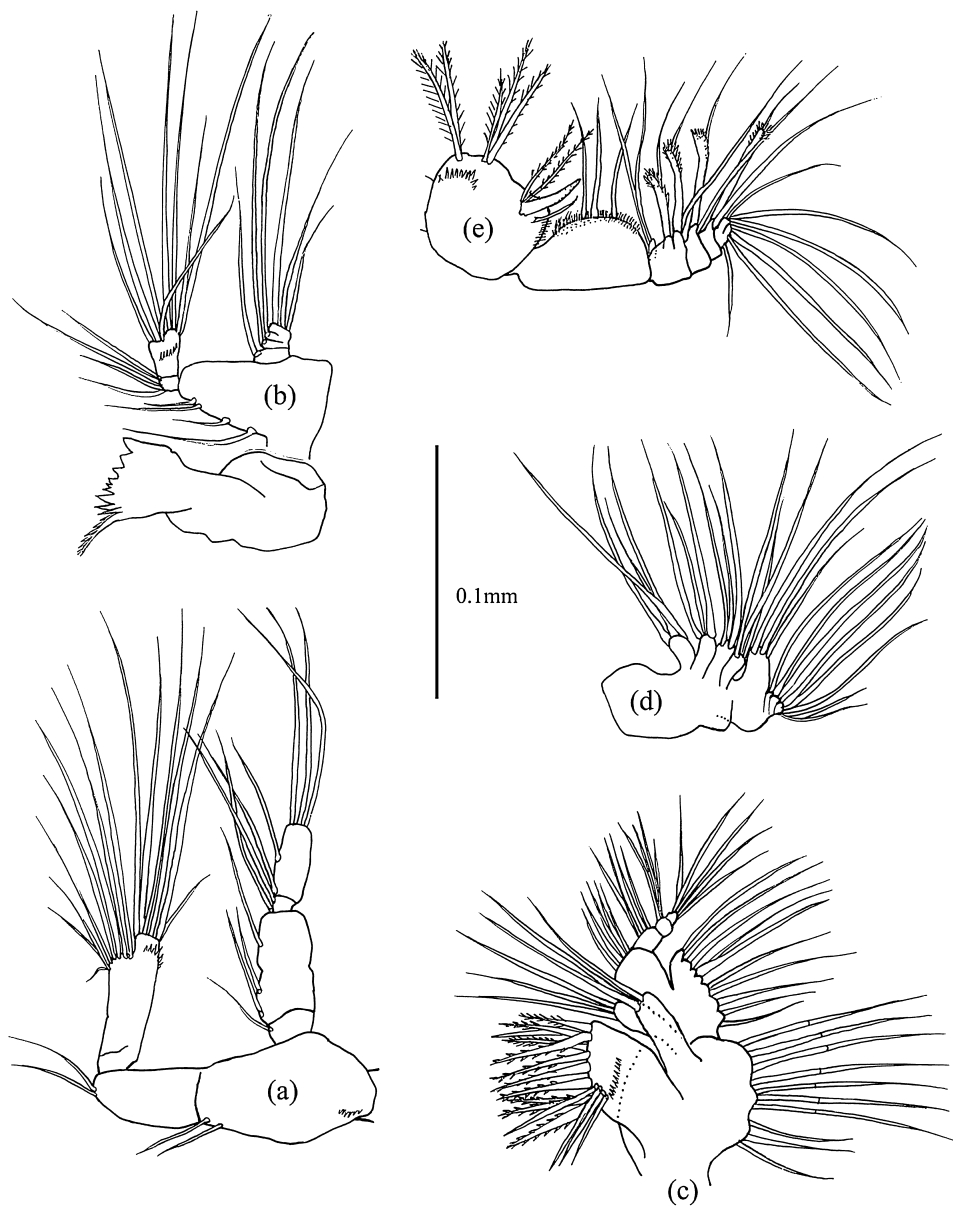


Fig. 4. Antenna and mouthparts of *Pseudodiaptomus brehmi* Kiefer, 1938. a, antenna; b, mandible; c, maxillule; d, maxilla; e, maxilliped.

row extending to surface. Exopod segment 2 with medial setule row. Anterior view (fig. 6b). Right leg basis with medial surface bristles. Exopod with 3 segments, first segment proximally ovate and distally produced into a spine-like process, with proximal seta. Second segment proximally rounded, distally ovate with 3

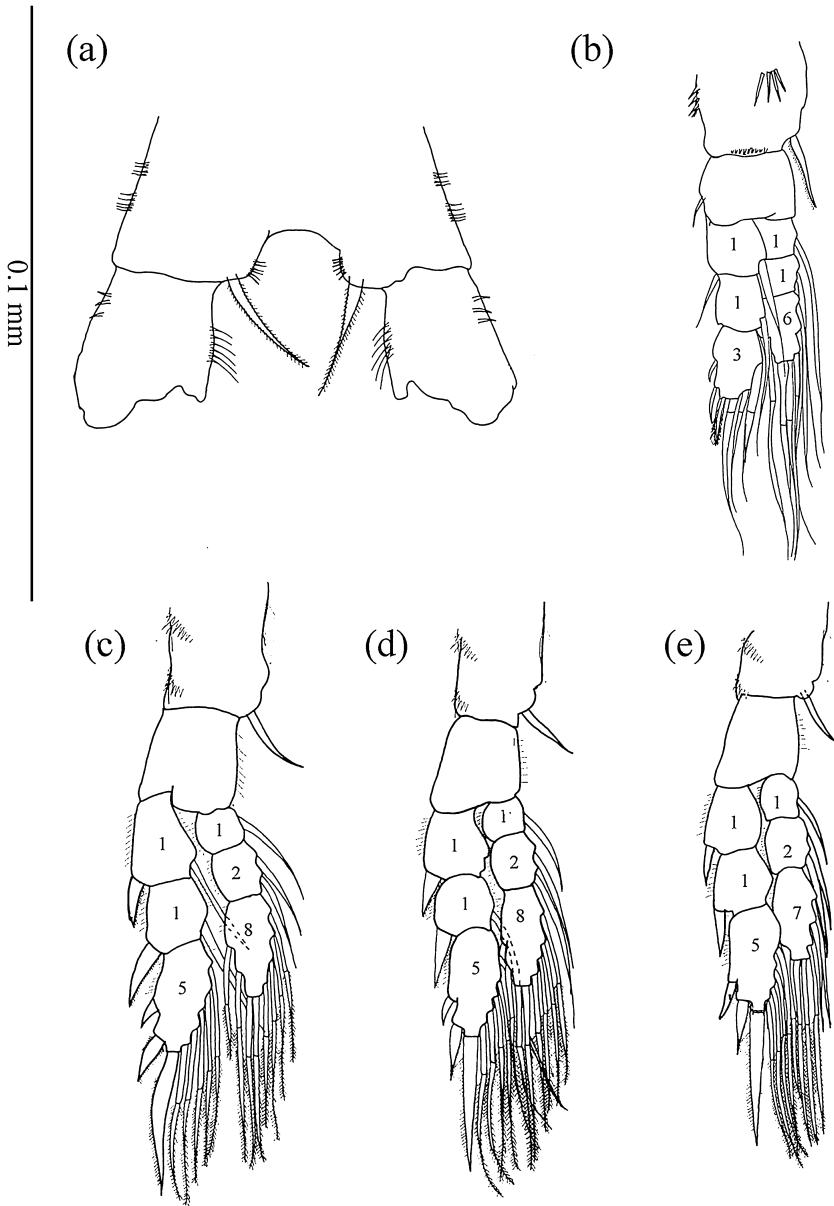


Fig. 5. Swimming legs of *Pseudodiaptomus brehmi* Kiefer, 1938 (the numbers on the endopod and exopod indicate the number of setae per segment). a, general coxa and basis for legs 1-4; b, leg 1; c, leg 2; d, leg 3; e, leg 4.

surface bristles and small distolateral spine. Third segment large, having a curved protrusion with setules located medially, produced distally into a curved spine-like process covered with setules. Left leg basis large with a medial seta and large laterally curved endopod with small medial process at midlength, distal tip slightly

TABLE I
Armature of swimming legs 1-4 of *Pseudodiaptomus brehmi* Kiefer, 1938

	Coxa	Basis	Exopod	Endopod
P1	0-1	0-0	I-1; 0-1; II, I-3	0-1; 0-1; 1, 3, 2
P2	0-1	0-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 4
P3	0-1	0-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 4
P4	0-1	0-0	I-1; I-1; II, 1, 4	0-I; 0-2; 1, 3, 3

pointed and 2 setae one at midlength, other at distal tip. Exopod 2-segmented, first segment ovate, with small proximomedial knob, 4 medial bristles and distolateral spine. Second segment glove-shaped, proximally rounded, medially notched and rounded distal end with 4 bristles and large lateral spine after midlength. Fig. 6c is reproduced from Kiefer's (1938) description.

Description of female.— Habitus (fig. 2b-c). Body length 1.02 ± 0.08 mm ($N = 18$) including caudal rami. Prosome to urosome ratio 1.6:1, prosome mean length 0.62 mm ± 0.05 mm, cephalosome and first pedigerous segment, and pedigers 4 and 5 fused, pediger 5 with rounded posterior corners with dorsal spine and lateral spinule row. Urosome of 4 somites, the genital double-somite swollen anteriorly, ventrally genital flaps are rounded and small with marginal setules (figs. 2e and 7e-f). Urosome (fig. 2a) symmetrical, 4-segmented (0.40 ± 0.03 mm), caudal rami with 5 typical terminal setae. Urosomites and caudal rami in a ratio of 32:9:14:19:26 = 100, with a row of triangular posterodorsal spinule row on margins of Ur1-3. Antennule 22-segmented and same as male left antennule, all mouthparts and swimming legs 1-4 as in male.

Fifth legs (fig. 6d-e) uniramous, symmetrical, exopod 3-segmented. Coxa and intercoxal sclerite fused, and with distinct row of posterior spinule row (fig. 6e). Basis with 1 surface seta and 1 bristle, and lateral spinule row (fig. 7d). First exopod segment with two medial bristles, a large distolateral spine, and one blunt distomedial spine. Second segment smallest with one medial bristle, one distolateral slender spine and a large serrate distomedial spine. Third segment largest medially curved and spiniform, with marginal bristles, and proximomedial spine.

DISCUSSION

Although Kiefer (1938) provided a good description of *Pseudodiaptomus brehmi*, he was not able to assign or mention the deposition of any type specimens in any institution. With the specimens from the type locality and additional specimens collected from Lake Taal, the redescription and designation of a

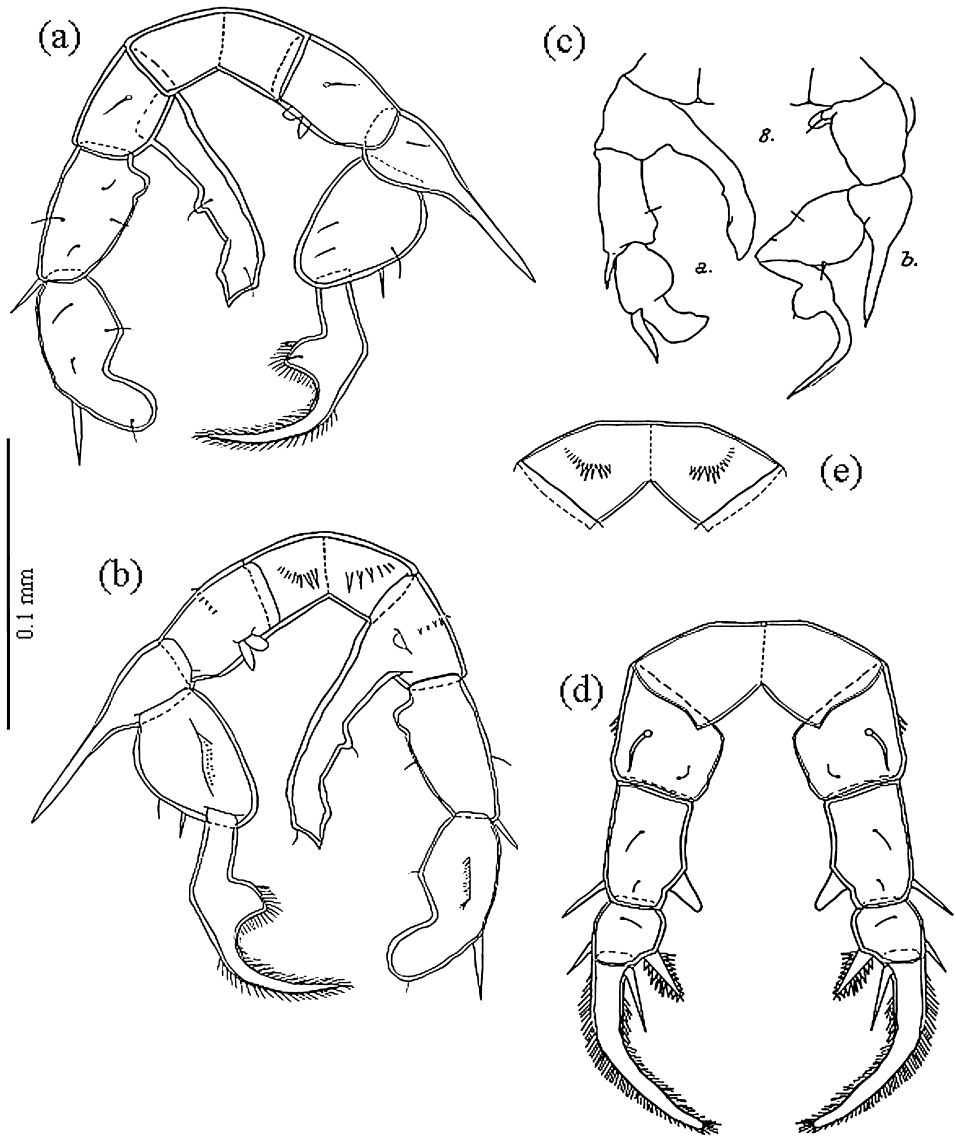


Fig. 6. Fifth legs of *Pseudodiaptomus brehmi* Kiefer, 1938. a, posterior view (male); b, anterior view (male); c, reproduced from Kiefer, 1938 (male); d, female (anterior view); e, female coxa (posterior view).

lectotype series of the species has been conducted. *Pseudodiaptomus brehmi*, the only freshwater pseudodiaptomid recorded for the genus, has already been displaced by the Neotropical invasive calanoid, *Arctodiaptomus dorsalis* in its type locality, Lake Naujan. Moreover, it now coexists with *A. dorsalis* in Lake Taal

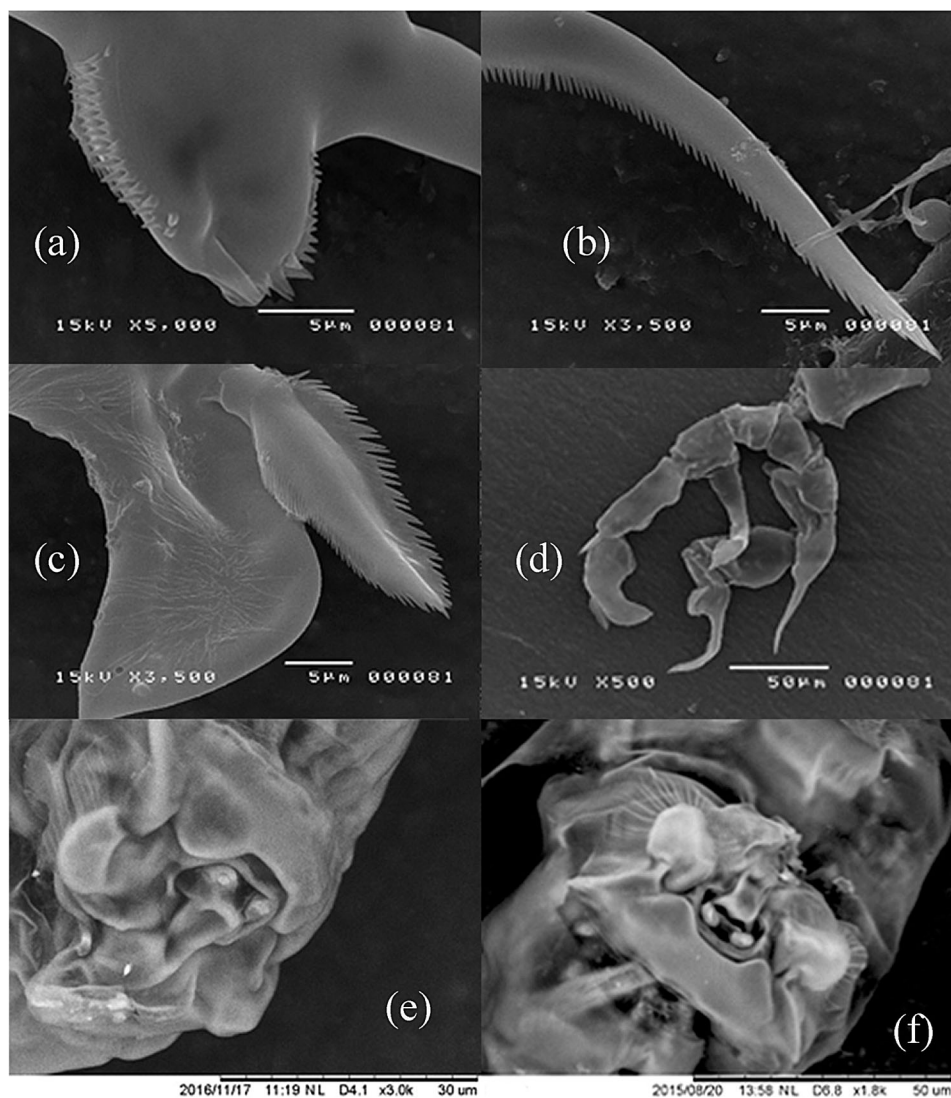


Fig. 7. SEM micrographs of *Pseudodiaptomus brehmi* Kiefer, 1938. Male: a-b, right leg third exopod, proximomedial process; c, left leg second exopod, distolateral spine; d, male leg 5 (posterior view); e-f, female ventral genital segment, seminal receptacle (lateral and ventral view, respectively).

which currently outnumbers it. This is made all the more important by the fact that *P. brehmi* is now only found in Lake Taal (Papa et al., 2017).

Kiefer (1938) described *P. brehmi* based on only a single male and female specimen, and our observations show slight differences from the original description and additional characters were described in this study. The fusion of the left endopod of the male P5 to its basis, resulting in a large medially curved process, and

the presence of a triangular process at the distomedial corner of the first exopod segment of the female P5 in the specimens observed confirms the inclusion of the species in the *forbesi* subgroup of the *lobus* species group, which is composed of 17 species originally found in Southeast Asia with its members preferring estuarine to somewhat freshwater habitats. Currently, there are three Philippine pseudodiaptomids under the *forbesi* subgroup: *P. annandalei*, *P. brehmi* and *P. terazakii*. The difference between *P. brehmi* and the other two species is that the first segment of the urosome of the female *P. brehmi* lacks lateral spines, while females of *P. annandalei* and *P. terazakii* possess them (Walter et al., 2006). Moreover, the first segment of the left P5 exopod lacks a medial pointed process while the other two species have a medially produced left P5 exopod first segment with 2 pointed processes (Walter et al., 2006). The successful documentation and redescription of this endemic Philippine pseudodiaptomid has been done with great urgency before it may have been gravely affected by the presence of the invasive calanoid *Arctodiaptomus dorsalis*, which has successfully displaced other native and endemic calanoid copepods in other inland aquatic ecosystems in other parts of the country.

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