

A new species of *Notodiaptomus* Kiefer, 1936 (Copepoda, Diaptomidae) from the Amazon and Orinoco River Basins

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Abstract

A new species of *Notodiaptomus*, *Notodiaptomus simillimus* sp. nov., based on the female originally but erroneously assigned to *Rhacodiaptomus calatus*, and the corresponding newly discovered male, is described and illustrated in detail. The designation of the female as a new species of *Notodiaptomus*, and the recognition of the corresponding new male, is based on detailed morphological and biometrical studies, analysis of the known distribution and material from laboratory cultures. Morphological analysis demonstrated that the female shares the form of the external genital area and setal armature of the exopod 2 of leg 5 with other species of *Notodiaptomus* but they differ from those exhibited by species of *Rhacodiaptomus*. The distributions of the female and male assigned to *Notodiaptomus simillimus* sp. nov. overlap and include the Atabapo and Guaviare Rivers and Lago Calado. On the other hand, the distributions of the males and females originally assigned to *R. calatus* are disjunct and where they overlap, that is, in Lago Calado, each is accompanied by its respective mate. Laboratory cultures showed that, as expected, copulation did not occur between males and females described originally as *R. calatus*. This experiment demonstrated the existence of reproductive isolating mechanisms between these females and males, and confirmed the existence of two distinct genetic pools, i.e. two different species, the original males being of the valid *R. calatus* and the female a new *Notodiaptomus*. This female and the corresponding newly discovered male are N. *simillimus*. The new species is closely related to *N. coniferoides*.

Introduction

Brandorff (1973) described *Rhacodiaptomus calatus*, from Lago Calado, an Amazonian floodplain lake (3° 15' S, 60° 34' W) but without clear designation of the type specimens. Hardy et al. (1984) selected and designated from the syntypes, the male and female as lectotype and paralectotype, respectively.

Two independent studies undertaken by the authors in Venezuela and Brazil, indicated that the female and male originally described as *R. calatus* Brandorff, 1973 did not belong to the same species. Santos-Silva & Robertson (1993), in samples collected in Lago Calado (the type locality of *R. calatus*) from September, 1983 to December, 1984, found five species of Diaptomidae: *Argyrodiaptomus robertsonae* Dussart, 1985, *Notodiaptomus amazonicus* (Wright, 1935), *Notodiaptomus coniferoides* (Wright, 1927),

'Diaptomus' ohlei Brandorff, 1978, Rhacodiaptomus calatus Brandorff, 1973 and several unknown females and males diaptomids belonging to two different species, morphologically unrelated to each other. Based on morphological and biometrical analysis and on breeding experiments, Santos-Silva & Robertson (1993) concluded that the original female and male described as R. calatus Brandorff, 1973 were not conspecific. The true female of R. calatus was the unknown female referred to above, and consequently the correct male and female of R. calatus were matched. The paralectotype female of *R. calatus* designated by Hardy et al. (1984) did not belong to Rhacodiaptomus Kiefer, 1936, but is actually a species of Notodiaptomus Kiefer, 1936. This female was matched with the unidentified male cited above and recognized as a new species by Santos-Silva & Robertson (1993), but not formally described on that occasion. One of

us (G.C.) found females and males of the new *Notodiaptomus* species in plankton samples from Atabapo River, Venezuela and Guaviare River, Colombia, but the description of the new species was not formally published (results of the study were available only as an unpublished internal report).

We describe here this new species of *Notodiap-tomus*, *N. simillimus*. The new species results from of the removal of the female originally but erroneously assigned to *R. calatus*, and the corresponding new male. An undescribed new *Rhacodiaptomus* female assigned to the original *R. calatus* will not be treated in this paper.

Taxonomy

Family Diaptomidae Baird, 1850
Genus Notodiaptomus Kiefer, 1936
Notodiaptomus simillimus, sp. nov.
Rhacodiaptomus calatus, partim (Brandorff 1973: 345, pl. 1, Fig. 4d, pl. 4, Fig. 2d, e, pl. 5, Fig. 1a, b, e).

Type material: Holotype φ , fully dissected on a slide (Universidad Pedagógica Libertador, UPEL); paratypes: 1 \circ , fully dissected on a slide (UPEL), 10 φ and 14 \circ in ethanol (UPEL); all from plankton samples from Atabapo River, near San Fernando de Atabapo, Amazonas State, Venezuela (24 February 1974; leg. G. Colomine and L. Segovia).

Additional non-type material: $19 \ \circ \ \text{and} \ 22 \ \circ, \ \text{in}$ formalin (Instituto de Zoología Tropical, Universidad Central de Venezuela, IZT-UCV), from Guaviare River, Colombia, near San Fernando de Atabapo, Venezuela (24 February 1974, leg. E. Montiel, F. Michelangelly and L. Segovia). The following specimens, in alcohol, are from Lago Calado, Amazonas State, Brazil: 100 ♀ and 100 ♂. Instituto Nacional de Pesquisas da Amazônia (INPA-807), and separate lots of 20 \circ and 20 \circ deposited in the following museums: Museu de Zoologia da Universidade de São Paulo, São Paulo (MZUSP), Natural History Museum, London (NHM), Muséun National d'Histoire Naturelle, Paris (MNHM), National Museum of Natural History, Washington, DC (USNM), and Naturhistorisches Museum Wien, Austria (NHMW).

Material examined: 1 \circ dissected on slide (INPA), from Lago Calado, Amazonas State, Brazil (13 August 1941, leg. H. Sioli); 1 \circ and 1 \circ fully dissected on slide (UPEL); 10 \circ and 14 \circ , in ethanol (UPEL); all

from plankton samples from Atabapo River, near San Fernando de Atabapo, Amazonas State, Venezuela (24 February 1974, leg. G. Colomine and L. Segovia); 19 φ and 22 σ , in formalin (IZT-UCV), from Guaviare River, Colombia, near San Fernando de Atabapo, Venezuela (24 Febraury 1974, leg. E. Montiel, F. Michelangelly and L. Segovia); 229 φ and 50 σ ³ from Lago Calado, Amazonas State, Brazil, collected from September 1983 through December 1984.

Female: Length of holotype excluding caudal setae: 166 μ m of 10 paratypes, median = 1.69 μ m, mean = 1.69 μ m, range = 1.65–1.78 μ m. Length of 19 specimens from Guaviare River, median = 1.66 μ m; mean = 1.67 μ m, range = 1.58–1.75 μ m. In dorsal view, body (Fig. 1A) widest at first pedigerous somite. Rostrum (Fig. 4C) with paired rostral filaments, symmetrical, broader than in the male, with 1 pair of sensilla at incomplete proximal suture. Cephalosome with an incomplete suture dorsally. Suture between fourth and fifth pedigerous somite incomplete dorsally. Fifth pedigerous somite with large asymmetrical lateral wings, left wing larger than right, each with two sensilla, one small sensillum at dorsal surface and one larger sensillum at apex, directed laterally (Fig. 1A). Urosome 3-segmented (Fig. 1A). Genital double-somite (Fig. 4B) large, asymmetrical, longer than combined length of preanal and anal somite, swollen in the anterior region, forming two conical processes, each with a strong sensillum at tip, right expansion directed laterally and left directed posteriorly, left side bearing an additional semicircular inflated area (Fig. 4B). Pre-anal somite small, wider than long. Anal somite with weakly developed operculum. Caudal rami symmetrical, about 1.6 times longer than broad, with setules along inner margin. Genital area as shown in Figure 2A; genital operculum three times longer than anterior extensive area and with a short posteriorly-directed process.

Antennules (Fig. 3A, B) symmetrical, 25-segmented, extending to caudal rami. Number of setae (s), vestigial setae (vs), conical setae (cs), and aesthetascs (ae) of each segment as follows: 1 (1s,1ae), 2(3s, 1ae, 1vs), 3 (1s,1ae,1vs), 4 (1s), 5 (1s,1ae,1vs), 6 (1s), 7(1s,1ae), 8(1s,1cs), 9(2s,1ae), 10(1s), 11(1s), 12(1s,1ae,1cs), 13 (1s), 14(1s,1ae), 15(1s), 16(1s,1ae), 17(1s), 18(1s), 19(1s,1ae), 20(1s), 21(1s), 22(2s), 23(2s), 24(2s), 25(4s,1ae). Fusion in ancestral segments: II–IV; XVII–XVIIII. Tips of setae on segments 3, 7, 9 and 14 blunt. Vestigial setae on segments 2,



Figure 1. Notodiaptomus simillimus sp. nov., (A) habitus of female holotype, dorsal (B) habitus of adult male, dorsal. Scale bar = 0.1 mm.

3 and 5 consisting of one circle of thin cuticle and minute seta at center of circle.

Swimming legs and all other appendages as in *N. deitersi* (Poppe, 1981) (see Santos-Silva et al., 1999 for a detailed description), except leg 5. Fifth leg (Fig. 2B, C) symmetrical, coxa with conical posterior process in distal outer corner with robust sensillum at tip. Sub-triangular basis with outer postero-distal corner produced in rounded process, outer margin shorter

than inner margin, outer seta reaching middle of first exopod. Exopod 1 almost twice as long as broad, exopod 2 with short lateral spine near the base of third exopod, and with claw serrate along of both margin, exopod 3 with 2 terminal setae, outer seta smaller, surpassing middle of inner seta. Endopod (Fig. 2C) 2segmented, second segment longer than first, bearing 2 unequal setae on oblique tip plus row of spinules subterminally on anterior surface.



Figure 2. Notodiaptomus simillimus sp. nov., female holotype; (A) external genital area, (B) fifth leg, posterior, (C) fifth leg, detail of endopod. Adult male, (D) right fifth leg, postero-lateral, (E) fifth leg, posterior, (F) right fifth leg, detail of endopod, (G) left fifth leg, detail of endopod, (H) left fifth leg, detail of exopod and endopod, posterior. Scale bars = 0.1 mm.

Male: Length excluding caudal setae: 1270 μ m, median = 1410 μ m, mean = 1400 μ m, range = 1180–1520 μ m, n = 14. Length of 22 specimens from Guaviare River, median = 1410 μ m, mean = 1400

 μ m, range = 1340–1490 μ m. Body (Fig. 1B) smaller and more slender than female. Rostrum (Fig. 4D) with paired rostral filaments, asymmetrical with rounded process on right side of basal region, separated from



Figure 3. Notodiaptomus simillimus sp. nov., female holotype, (A) segments 1-15 of right antennule, (B) segments 16-25 of right antennule. Adult male, (C) segments 1-18 of right antennule, (D) detail of segment 19 of right antennule, (E) segments 19-22 of right antennule. Scale bars = 0.1 mm.

frontal margin of dorsal cephalic shield by complete suture, ornamented with 1 pair of sensilla. Cephalo-

some with incomplete suture dorsally. Fith pedigerous somite with small, slightly asymmetrical lateral wings,



Figure 4. Notodiaptomus simillimus sp. nov., scanning electron microscope pictures. (A) adult male right fifth leg, detail of endopod, anterior, (B) adult female fifth pedigerous somite and genital double-somite, dorsal, (C) adult female rostrum, ventral, (D) adult male rostrum, ventral, (E) adult male left fifth leg, detail of exopod and endopod, anterior. Scale bars: A, C–E = 10 μ m; B 100 μ m.

each with small sensillum at apex (Fig 1B). In dorsal view, body widest at first pedigerous somite. Urosome 5-segmented (Fig. 1B). Genital somite asymmetrical, right side expanded posteriorly over next urosomite. Genital aperture at ventro-lateral posterior corner of genital somite on left side. Caudal rami asymmetrical, 2.4 times longer than wide, with setules along inner margin.

Antennules asymmetrical, extending to middle of urosome. Right antennule (Fig. 3 C–E) 22-segmented. Number of setae (s), vestigial setae (vs), conical setae (cs), modified seta (ms), aesthetascs (ae), process (p), of each segment as follows: 1 (1s, 1ae), 2 (3s, 1ae, 1vs), 3 (1s, 1vs), 4 (1s), 5 (1s, 1ae, 1vs), 6 (1s), 7 (1s, 1ae), 8 (1s, 1cs), 9 (2s, 1ae), 10 (1s, 1ms), 11 (1s, 1ms), 12 (1s, 1ae, 1cs), 13 (1s, 1ae, 1ms), 14 (2s, 1ae), 15 (2s, 1ae, 1p), 16 (2s, 1ae, 1p), 17 (2s, 1ms), 18 (2s, 1ms), 19 (2s, 1ae, 2ms), 20 (4s), 21 (2s), 22 (4s, 1ae). Fusion in ancestral segments: II–IV; XXI–XXIII; XXIV–XXV; XXVII–XXVIIJ. Vestigial setae on segments 2, 3 and 5 as in female antennules; conical seta on segment 8 larger than conical seta on segment 12; modified seta on segments 10 and 11 similar to each other; modified seta on segments 17, 18

| | N. simillimus | N. coniferoides | | |
|--|---|---|--|--|
| Fourth pedigers | Without a median dorsal hump | Generally with a median dorsal hump | | |
| Genital somite: | | | | |
| (a) anterior half | Asymetrically expanded laterally in conical projection and the dorsal surface has a further round projection on the left side | Asymmetrically expanded laterally in rounded projection. Without projection on dorsal surface Expanded posteriorly over next urosomal segment | | |
| (b) dorsal right side | Not expanded posteriorly | | | |
| Genital area: | | | | |
| (a) anterior extensive area | One third as long as operculum | As long as operculum | | |
| (b) operculum | Distal margin (between lateral process) not convex | Distal margin convex | | |
| Leg 5: | | | | |
| (a) exopod 2, outer margin(b) exopod 3, terminalspines | With short lateral spinule Outer spine > 1/2 length of inner spine | Without lateral spinule Outer spine < 1/2 length of inner spine | | |

Table 1. Morphological differences between females of *Notodiaptomus simillimus* sp. n. (Fig. 2) and *N. coniferoides*

and 19 similar to each other; modified seta on segment 13 forming a strong process minutely bifid at apex, not surpassing next segment; spinous process on segment 15 larger than process on segment 16; antepenultimate segment with hyaline lamella. Tips of setae on segments 3, 7, 9 and 14 blunt. Left antennule 25-segment; setal formula armature and segmental fusion pattern as in the female.

Right fifth leg (Figs 2D–F and 4A): Coxa with posterodistal process not projecting over basis, process with a large and slender sensillum on tip; basis 2.4 times longer than broad, with outgrowth on posterior surface (Figs 2D and 4A), distal inner corner bearing endopod; exopod 1 with distal outer corner produced into triangular outgrowth; exopod 2 with anterior surface concave at midlength; lateral spine sigmoid, located in the distal quarter of segment, inserted on rounded protuberance; claw inserted distally, curved inward, tip curved outward, with spinules along only part of inner margin; endopod not separated from basis, with comb of spinules on inner anterior surface (Figs 2F and 4A). Left fifth leg (Fig. 2E, G, H) not reaching distal margin of right basis; coxa with small posterodistal process tipped with a large and slender sensillum; cylindrical basis, 1.8 longer than broader, inner and outer margin straight; exopod 1 sub-triangular, with curved outer margin, outgrowth on inner margin partly divided, bearing long setules. Exopod 2 with short spiniform process in outer margin, with field of denticles on posterior surface (Fig. 2H), with two small semicircular outgrowths and transverse row of denticles on anterior surface (Fig. 4E); semicircular outgrowth on inner margin bearing long setules, distal digitiform process heavily sclerotized and with 2 rows of denticles, spinulose seta (proximal process) inserted medially on inner surface (Fig. 2H). Endopod 2-segmented, conical, second segment with row of spinules on inner distal margin (Fig. 2G, H).

Etymology: The specific epithet is derived from Latin and refers to the similarity of the species with *Notodiaptomus coniferoides*.

| | N. simillimus | N. coniferoides | | | |
|---|--|--|--|--|--|
| Right leg 5: | | | | | |
| (a) exopod 1 | Longer than broad | Broader than long | | | |
| (b) exopod 2, margins | Internal and external margins convex | Internal margin straight, with a salient projection | | | |
| (c) exopod 2, posterior surface | Without knob | with knob, near inner margin | | | |
| (d) terminal claw | Curves inward with slightly recurved tip | Curves inward, tip not recurved | | | |
| Left leg 5: | | | | | |
| (a) exopod 2, outer margin | With spiniform projection | Without spiniform projection | | | |
| (b) exopod 2, inner setose pad | Prominently rounded | Not prominently rounded | | | |
| (c) exopod 2, distal digitform process | With two rows of denticles not bicuspidate. Anterior surface with transverse row of denticles | With two rows of denticles bicuspidate. Anterior surface with transverse row of denticles and curve row of 5 denticles | | | |
| (d) exopod 2, proximal process | With rows of small spines with helicoidal path and two subapical spinal projections | With linear rows of small denticles in proximal midlength and one subapical spinal projection | | | |

Table 2. Morphological differences between leg 5 of males of *Notodiaptomus simillimus* sp. n. and *N. coniferoides*

Discussion

Notodiaptomus simillimus is morphologically close to N. coniferoides. The differences which allowed us to separate these two species are presented in Tables 1 and 2. In short, the female of N. simillimus differs from that of N. coniferoides in that it never presents a dorsal conical blunt process on the fourth pediger somite, the left lateral projection on genital somite is conical and has a further round projection, and the setal armature of the exopod 2 of leg 5 includes a short lateral spine. Males of N. simillimus are distinguished from those of N. coniferoides by characters of the fifth leg: the first segment of the right exopod is longer than broad (not broader than long as in N. conferoides), and the small protusion on the inner margin, and the knob on the posterior surface of the second segment of the right exopod, both present in N. coniferoides, are lacking in N. simillimus.

Detailed biometrical analysis of the species of Diaptomidae from Lago Calado revealed that the species assigned to *R. calatus* presented an inexplicably high female:male ratio (Santos-Silva & Robertson, 1993) (see Table 3). This indicated that there might be a mismatch of males and females as had occurred in the original pair described as Dactylodiaptomus pearsei (Wright, 1927), because this species also presented a high ratio of females:males (Table 3). Brandorff et al. (1982) verified that Wright (1927) had matched the male with the wrong female, and he then described the correct female. Other authors (Dussart, 1984; Santos-Silva et al. 1989) also found and illustrated the correct pair. After this analysis and detailed morphological examination, we had a strong indication that the pair of R. calatus were mismatched, the female being a Notodiaptomus (not Rhacodiaptomus) species. The initially unknown males and females found in Lago Calado (Santos-Silva & Robertson, 1993) were allocated to Notodiaptomus and Rhacodiaptomus, respectively. Thereafter, we rearranged males and females of each species in their respective genera.

To test our hypothesis, we cultured ovigerous females of the original *R. calatus*, the former unknown female now recognized as a *Rhacodiaptomus*

| Species | Me len (m | ean gth m) o ^r | ♀:♂ ratio | nun exam | √ nber nined ♂ | References |
|-----------------------------|-----------------|------------------------------------|--------------|-------------|-------------------------|------------------------|
| Dactylodiaptomus pearsei | 2.26 | 1.53 | 1.45* | 01 | 01 | Wright, 1927 |
| Dactylodiaptomus pearsei | 1.57 | 1.42 | 1.11+ | 01 | 01 | Brandorff et al., 1982 |
| Dactylodiaptomus pearsei | 1.55 | 1.52 | 1.01+ | 13 | 11 | This paper |
| Rhacodiaptomus calatus | 1.52 | 0.98 | 1.55* | 8 | 7 | Brandorff, 1973 |
| Rhacodiaptomus calatus | 1.33 | 1.07 | 1.24 | 406 | 120 | This paper |
| Notodiaptomus simillimus | 1.53 | 1.48 | 1.03 | 229 | 50 | This paper |
| Notodiaptomus amazonicus | 1.68 | 1.48 | 1.13 | 1 | 1 | Wright, 1935 |
| Notodiaptomus amazonicus | 1.55 | 1.48 | 1.01 | 326 | 50 | This paper |
| Notodiaptomus coniferoides | 1.46 | 1.41 | 1.04 | 1 | 1 | Wright, 1927 |
| Notodiaptomus coniferoides | 1.50 | 1.44 | 1.04 | 445 | 50 | This paper |
| Argyrodiaptomus robertsonae | 2.30 | 2.00 | 1.15 | 01 | 01 | Dussart, 1985 |
| Argyrodiaptomus robertsonae | 2.07 | 2.02 | 1.02 | 97 | 50 | This paper |
| 'Diaptomus' ohlei | 1.07 | 0.97 | 1.10 | 104 | 74 | Brandorff, 1978 |
| 'Diaptomus' ohlei | 1.03 | 1.01 | 1.01 | 80 | 48 | This paper |

Table 3. Mean lengths and female:male ratio for Diaptomidae species occurring in Lago Calado plus lengths of *Dactylodiaptomus pearsei.* (*) ratio female:male of mismatched couple. (+) ratio female:male after correction

species, and Notodiaptomus conferoides. After obtaining adult males and females, we isolated several pairs of each species which reproduced as expected. We also mixed males and females of the three species. In some cases, as we expected, copulation did not occur, as between the original pair of R. calatus (now males of R. calatus and females of N. simillimus). In others, copulation was attempted but the spermatophore was not correctly placed; this occurred between males of N. conferoides and females of N. simillimus. Even when the spermatophore was correctly placed, the female produced an egg-sac without eggs. This experiment demonstrated the occurrence of reproductive isolating mechanisms and confirmed the existence of two distinct genetic pools, i.e. two different species, the original males being a valid R. calatus and the females N. simillimus.

The distribution records of the male and female assigned to *N. simillimus* overlap in the samples from Atabapo and Guaviare rivers where this species occurs alone. On the other hand, the distribution records of males and females originally assigned to *R. calatus* do not overlap, and where they co-occur, that is, in Lago Calado, each is accompanied by its respective mate. Dussart (1984) found females originally described as *R. calatus* in the Orinoco River around Barrancas and Ciudad Bolivar, in ponds near Tucupita

(Orinoco Delta), and in the Atabapo River. Dussart (1984) pointed out he that had found only females of *R. calatus* together with males of *N. coniferoides*. No males of *R. calatus* or females of *N. coniferoides* were found in those places where these species co-occurred. Now it is clear that the females and males identified by Dussart (1984) as *R. calatus* and *N. coniferoides*, respectively, are in reality *N. simillimus*. To conclude, we reaffirm to the best of our knowledge that there is no record of the true *N. coniferoides* from Venezuela and all known records of *R. calatus* are incorrect and should be referred to *N. simillimus*.

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