Friesea najtae n. sp. (Collembola, Neanuridae, Frieseinae) from southern Western Australia

Cyrille A. D'HAESE
MECADEV, UMR 7179 CNRS,
Muséum national d'Histoire naturelle, Sorbonne Universités,
case postale 50, 57 rue Cuvier, F-75231 Paris cedex 05 (France)

Mark I. STEVENS
South Australian Museum, North Terrace, GPO Box 234,
and, School of Pharmacy and Medical Sciences, University of South Australia,
SA 5000 Adelaide (Australia)
mark.stevens@samuseum.sa.gov.au

Wanda M. WEINER
Institute of Systematics and Evolution of Animals, Polish Academy of Sciences,
Sławkowska 17, 31-016 Kraków (Poland)
weiner@isez.pan.krakow.pl

Published on 31st March 2017

urn:lsid:zoobank.org:pub:2DE4E993-0837-4399-B075-73CC1AE867A8

D'Haese C. A., Stevens M. I. & Weiner W. M. 2017. — *Friesea najtae* n. sp. (Collembola, Neanuridae, Frieseinae) from southern Western Australia. *Zoosystema* 39 (1): 21-29. https://doi.org/10.5252/z2017n1a3

ABSTRACT
A new Frieseinae Massoud, 1967 semi-aquatic species, *Friesea najtae* n. sp., is described from southern Western Australia. The new species can be distinguished from all other *Friesea* Dalla Torre, 1895 species by the combination of 8 + 8 eyes, six anal spines without papillae on abdomen VI, absence of capitate chaetae on the abdomen and no capitate tenent hairs on tibiotarsi and fully developed furcula (stage 1-2). Barcode (mtDNA - COI) sequence is provided.

KEY WORDS
Poduromorpha,
southern hemisphere,
semi-aquatic,
DNA barcode,
SEM microphotographs
new species.

RÉSUMÉ
Friesea najtae *n. sp. (Collembola, Neanuridae, Frieseinae) une nouvelle espèce du sud de l'Australie-Occidentale.*
Une nouvelle espèce semi-aquatique de Frieseinae Massoud, 1967, *Friesea najtae* n. sp., est décrite du sud de l'Australie-Occidentale. Cette nouvelle espèce peut être distinguée parmi toutes les autres espèces de *Friesea* Dalla Torre, 1895 par la combinaison de 8 + 8 yeux, six épines anales sans embase sur l'abdomen VI, l'absence de soie capitées sur l'abdomen et des ergots capités sur les tibiotarses et la présence d'une furca pleinement développée (stade 1-2). La séquence barcode est fournie.

MOTS CLÉS
Poduromorpha,
hémisphère sud,
semi-aquatique,
barcode,
photographies MEB,
espèce nouvelle.

INTRODUCTION

Friesea Dalla Torre, 1895 is a speciose genus with nearly 190 species described to date. Most species are found in the northern hemisphere with 40 species strictly found in the southern hemisphere (e.g., Cassagnau & Rapoport 1962; Greenslade & Deharveng 1997; Weiner *et al.* 2009; Queiroz & Mendonça 2015) while six species only found in North/Central and South America with a few cosmopolitan ones. Interestingly of the 40 southern species, 13 are found in Antarctica or sub-Antarctic islands (Schaeffer 1891; Enderlein 1909; Denis 1947; Salmon 1949; Wise 1964, 1970; Weiner 1980; Deharveng 1981; Greenslade 1995). It remains unknown if this bias towards the northern hemisphere is due to a lack of studies of the southern hemisphere fauna or if this represents an accurate distribution for *Friesea.*

Greenslade & Deharveng (1997) listed seven species of *Friesea* recorded from Australia, even if they are not frequently encountered: *F. australica* Greenslade & Deharveng, 1997 (New South Wales, South Australia and Victoria), *F. bispinosa* Deharveng, 1981 (Crozet, Heard, Kerguelen and Macquarie Islands), *F.* cf. *claviseta* (Axelson, 1900) (Norfolk Island, cosmopolitan), *F. florifera* Greenslade & Deharveng, 1997 (Tasmania), *F.* cf. *mirabilis* (Tullberg) (South Australia, Western Australia, cosmopolitan), *F. neptunia* Greenslade & Deharveng, 1997 (Victoria), *F. tilbrooki* Wise, 1970 (Bishop, Bouvetoya, Heard, Macquarie and other sub-Antarctic Islands). An additional species has to be mentioned: *F. grisea* (Schäffer, 1891), known from various Antarctic and sub-Antarctic localities (Salmon 1962; Deharveng 1981; Dallai *et al.* 1988). It has also been recorded from the Russian station Molodyozhnaya (Wise 1967) situated in Australian territory.

In the present paper, we describe a new species from southern Western Australia, being only the second *Friesea* species now recorded from Western Australia and nineth from Australia.

MATERIAL AND METHODS

Specimens were collected with a mouth aspirator and preserved in 95% ethanol.

For morphological observations, they were cleared in successive lactic acid and KOH solutions and finally mounted on microscope slides using Marc André II mounting medium (close to Hoyer's medium). Specimens were examined using a compound microscope with differential interference contrast optics at magnifications ranging from 250 to 1000. Drawings were made with a drawing tube and vectorised with Inkscape. For SEM observations specimens were preserved in 100% ethanol before critical point drying (Emitech K850) and gold coating (Jeol JFC-1200).

DNA was extracted from a 95% ethanol-preserved specimen using a Qiagen DNeasy tissue extraction kit. The specimen was retrieved after 3 h digestion by proteinase k, and the extraction was carried out as advised by the manufacturer with final resuspension in 120 µl of elution buffer. The specimen was then further cleared in successive lactic acid and KOH solutions and mounted on microscope slides in Marc André II mounting medium. DNA amplification was carried out in a 25 µl volume reaction with Taq&LOAD Mastermix 5XC reagent (MP Bio- medicals). The thermocycler program consisted of an initial denaturing step at 94°C for 2 min, five amplification cycles with a 45°C annealing temperature (94°C for 40 s, 45°C for 40 s, 72°C for 1 min), 30 cycles with a 51°C annealing temperature, and a final step at 72°C for 5 min. PCR amplification and sequencing were carried out with primers we designed for Collembola (e.g., Greenslade *et al.* 2011; Schneider & D'Haese 2013).

Material is preserved in the Western Australian Museum (WAM), the South Australian Museum (SAMA), the Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences, Kracov (ISEA) and in the Muséum national d'Histoire naturelle, Paris (MNHN).

ABBREVIATIONS

A-H	ocelli A-H;
a, m, p	anterior, median and posterior rows of chaetae;
Abd.	abdomen segment;
Ant.	antennal segment;
av	apical vesicle;
hr	anal valve microchaetae;
i	chaeta i;
oc	ocular chaetae;
ms	microsensillum;
so	subapical organite;
S0-9	Ant. IV s-chaetae from 0 to 9 (= sensilla).

Nomenclature after D'Haese (2003).

Institutions

MNHN	Muséum national d'Histoire naturelle, Paris;
ISEA	Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences, Cracov;
SAMA	South Australian Museum, Adelaide;
WAM	Western Australian Museum, Perth.

SYSTEMATICS

Family NEANURIDAE Börner, 1901
Subfamily FRIESEINAE Massoud, 1967

Genus *Friesea* Dalla Torre, 1895

Friesea Dalla Torre, 1895: 14.

Polyacanthella Schäffer, 1897: 15.

TYPE SPECIES. — *Friesea mirabilis* (Tullberg, 1871).

Friesea najtae n. sp.
(Figs 1-6)

TYPE MATERIAL. — **Holotype**. South-Western Australia (WA), D'Entrecasteaux National Park, next to Windy Harbour road, 20 km South of Northcliffe and 2 km from the coast, 8 m a.s.l.; on the surface of swamps (Fig. 1A, B), sample au021a (Fig. 1B), 17.XI.2013, 34.82468°S, 116.06025°E, D'Haese leg, ♀, slide WAM – E 96053 (MNHN-EA013918).

FIG. 1. — Surface of ponds in D'Entrecasteaux National Park (southern Western Australia, WA), habitat where *Friesea najtae* n. sp. was collected walking on the surface of water: **A**, sampling site au47; **B**, sampling site au021a. Photograph: Cyrille A. D'Haese.

FIG. 2.— Live specimen of *Friesea najtae* n. sp photographed in its natural habitat. Photograph: Cyrille A. D'Haese.

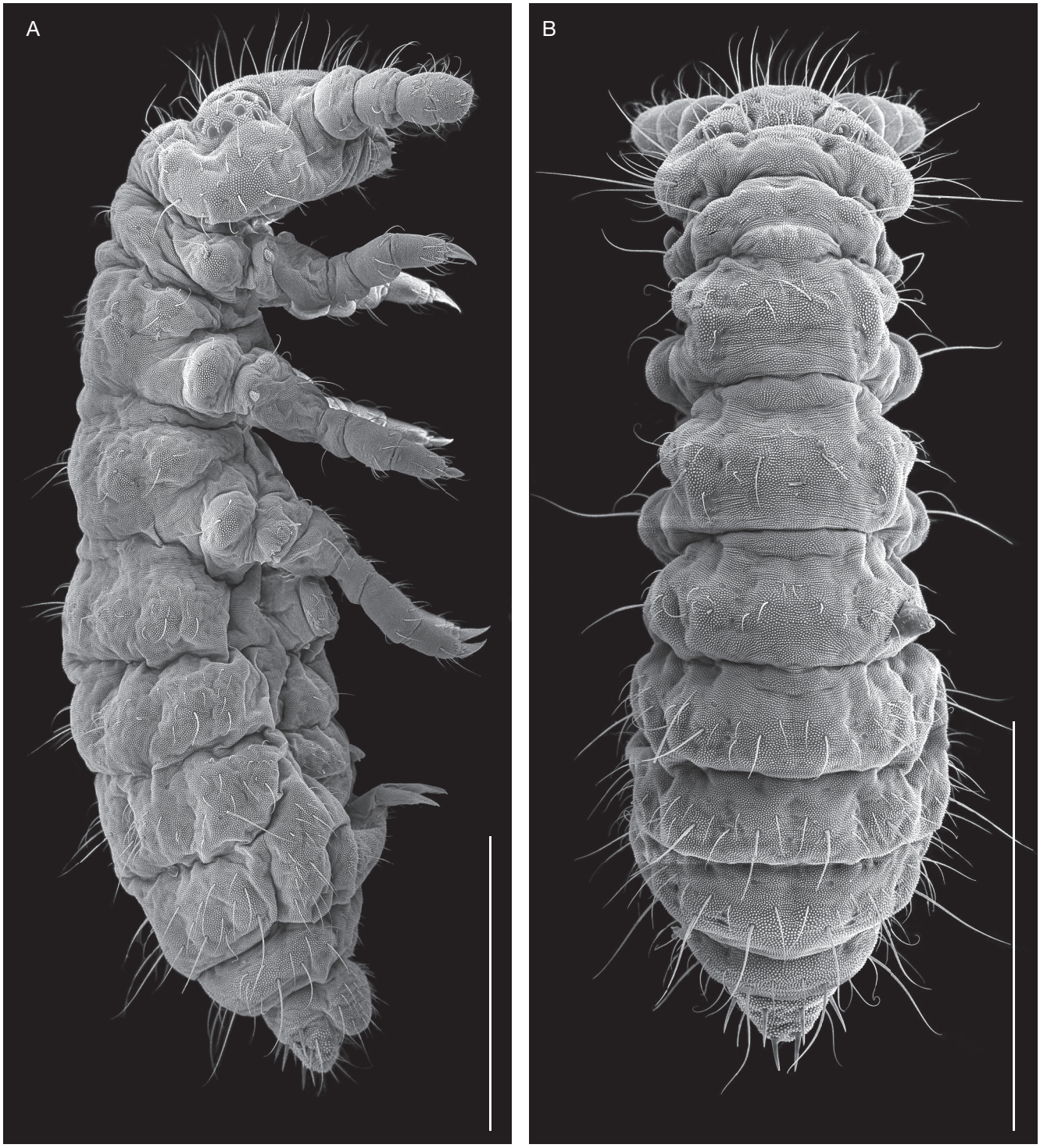

Fig. 3. — *Friesea najtae* n. sp. SEM microphotographs: **A**, habitus, lateral view; **B**, habitus, dorsal view. Scale bars: 500 µm.

Paratypes. Same data as the holotype, 3 ♂ and 1 ♀, slides WAM - E 96054-55 (MNHN-EA013921-22 and sample au047 (Fig. 1A), 25.XI. 2013, 34.82462°S, 116.06084°E, D'Haese leg, ♂ and ♀, slides WAM - E 96056-63, SAMA 01-001327-28, ISEA EA013923-26 and MNHN-EA013927-30.

Material examined. — Southern Western Australia (WA), D'Entrecasteaux National Park, next to Windy Harbour Road, 20 km South of Northcliffe and 2 km from the coast, 8 m a.s.l.; on the surface of swamps (Fig. 1A, B):

– sample au021a (Fig. 1B), 17.XI.2013, 34.82468°S, 116.06025°E, *c.* 30 specimens, D'Haese leg; slides EA013918-22 and MEB plate EA030026 with 4 specimens;
– sample au47 (Fig. 1A), 25.XI. 2013, 34.82462°S, 116.06084°E, *c.* 40 specimens, D'Haese leg; slides EA013923-27.

Diagnosis. — *Friesea najtae* n. sp. is characterized by a dark blue colour (Fig. 2), large size for the genus (up to 1.7 mm), 8 + 8 eyes (Fig. 4B), trilobated apical vesicle (Fig. 4A), 6 strong anal spines,

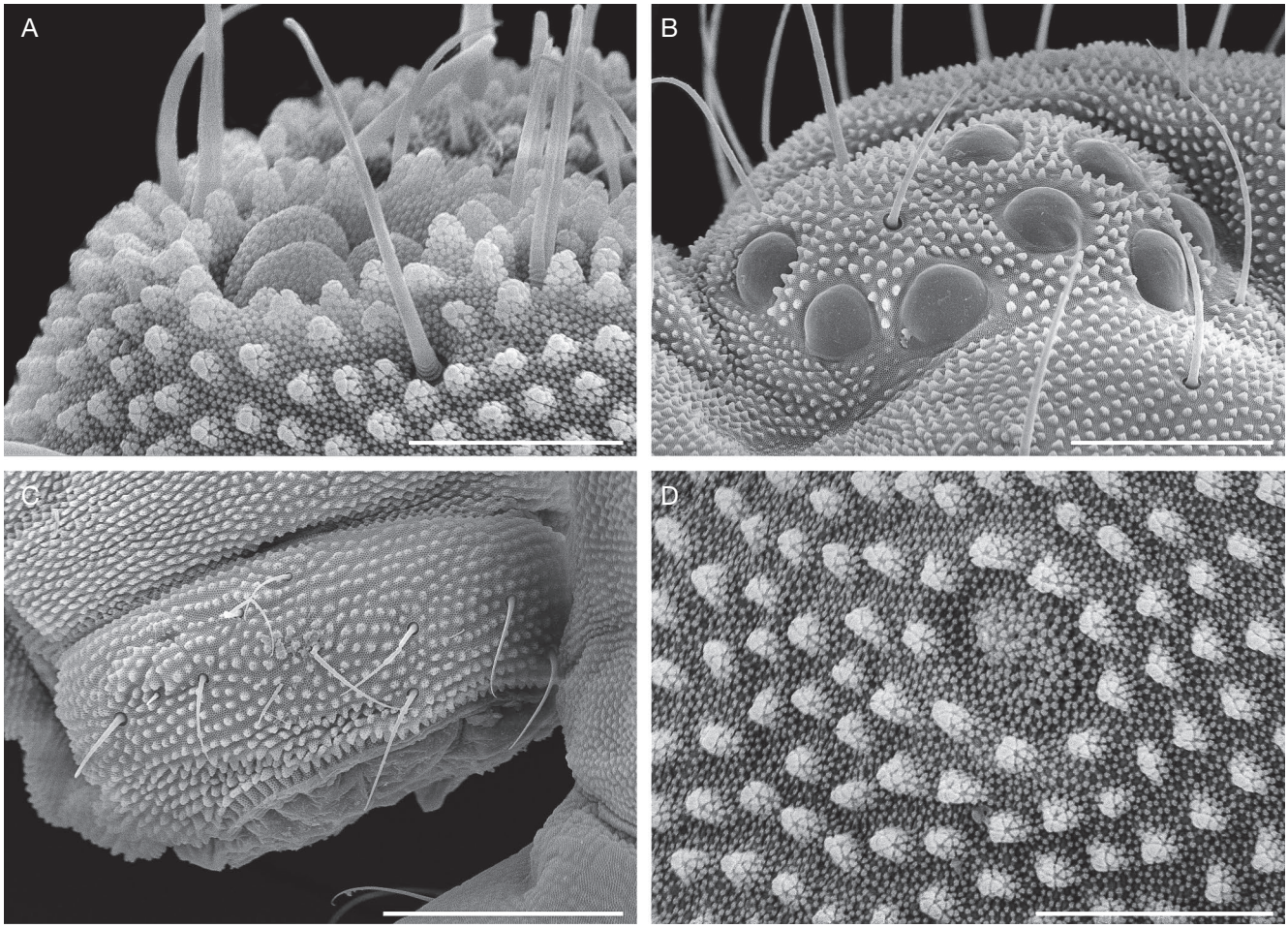

Fig. 4. — *Friesea najtae* n. sp. SEM microphotographs: **A**, tip of the antenna showing the trilobated apical vesicle; **B**, right side ocular patch showing eight ocelli and no PAO (front of the animal on the right side of the picture); **C**, ventral tube, lateral view; **D**, pseudopore on Abd. II. Scale bars: A, D, 10 µm; B, C, 50 µm.

symmetrical along their axis, without papillae on abdominal VI (Fig. 6A, B, D), no capitate tenent hairs on tibiotarsi or Abd. V-VI and a fully developed furcula with a large mucro slightly fused to the dens (stage 1-2 according to Cassagnau 1958: figs 3A, 6F).

Distribution. — To date, *Friesea najtae* n. sp. is only known from southern Western Australia from the two collecting sites (55 m apart from each other) au021a (Fig. 1B) and au047 (Fig. 1A).

Habitat. — These animals were found walking on the surface of the water in swamps (Fig. 1A, B). It is worth noticing that they were not found in Berlese-extracted samples of litter and soil collected on the side of the water. However, they could be from the other side of the pond and blown by the wind (surface water current). They are likely to inhabit the riparian zone, similar to *Chionobora amila* Greenslade & Potapov, 2015 for example (Stevens & D'Haese 2016).

Etymology. — Judith Najt was C. D'Haese's PhD advisor and friend, and a very long term colleague and dear friend of Wanda Weiner. The new species is named in her honour.

Description

General habitus

Large animal for the genus, habitus with strong head with diverging antennae shorter than head length and a large abdomen in midline (Abd. III-IV), ending by a pointed Abd. VI (Figs 2, 3). Body length: 1.0-1.7mm. Body colour dark blue, almost black (Fig. 2), slightly lighter on the ventral side with inner sides of ventral tube and furcula tip almost white. Dorsal clothing strong, with long and pointed ordinary chaetae as well as shorter chaetae, slightly crenulated (macro-, meso- and microchaetae; Fig. 3A, B).

Head

Antennae about ⅗ of head length. Ant. I with 7 chaetae, Ant. II with 13 chaetae. Ant. III and IV dorsally fused, ventrally weakly separated. Sensory organ of Ant. III consisting of 2 small subcylindrical internal microsensilla hidden by a fold, two subcylindrical guard sensilla, ventral microsensillum. Ant. IV truncated ventro-internally, sensilla difficult to distinguish from ordinary chaetae (Figs 3A; 5D); apical vesicle trilobated (Figs 4A; 5D).

Ocular area with 3 chaetae (oc1-3) and 8 + 8 ocelli, all subequal; postantennal organ absent (Figs 3A; 4B; 5B). Chaetotaxy of labrum: 2/5,3,4. Labium with papillated chaeta L.

Head of maxillae typical for the genus (Fig. 5E), mandibles as in Fig. 5F.

FIG. 5. — *Friesea najtae* n. sp.: **A**, dorsal chaetotaxy of th I and th II; **B**, dorsal chaetotaxy of the head; **C**, dorso-lateral chaetotaxy of Abdl to Abd. VI; **D**, chaetotaxy of Ant. III–IV (dorsal view); **E**, head of maxillae; **F**, head of mandible; **G**, third tibitarsus. Abbreviations: see Material and methods. Scale bars: A-C,100 µm; D-G, 25 µm.

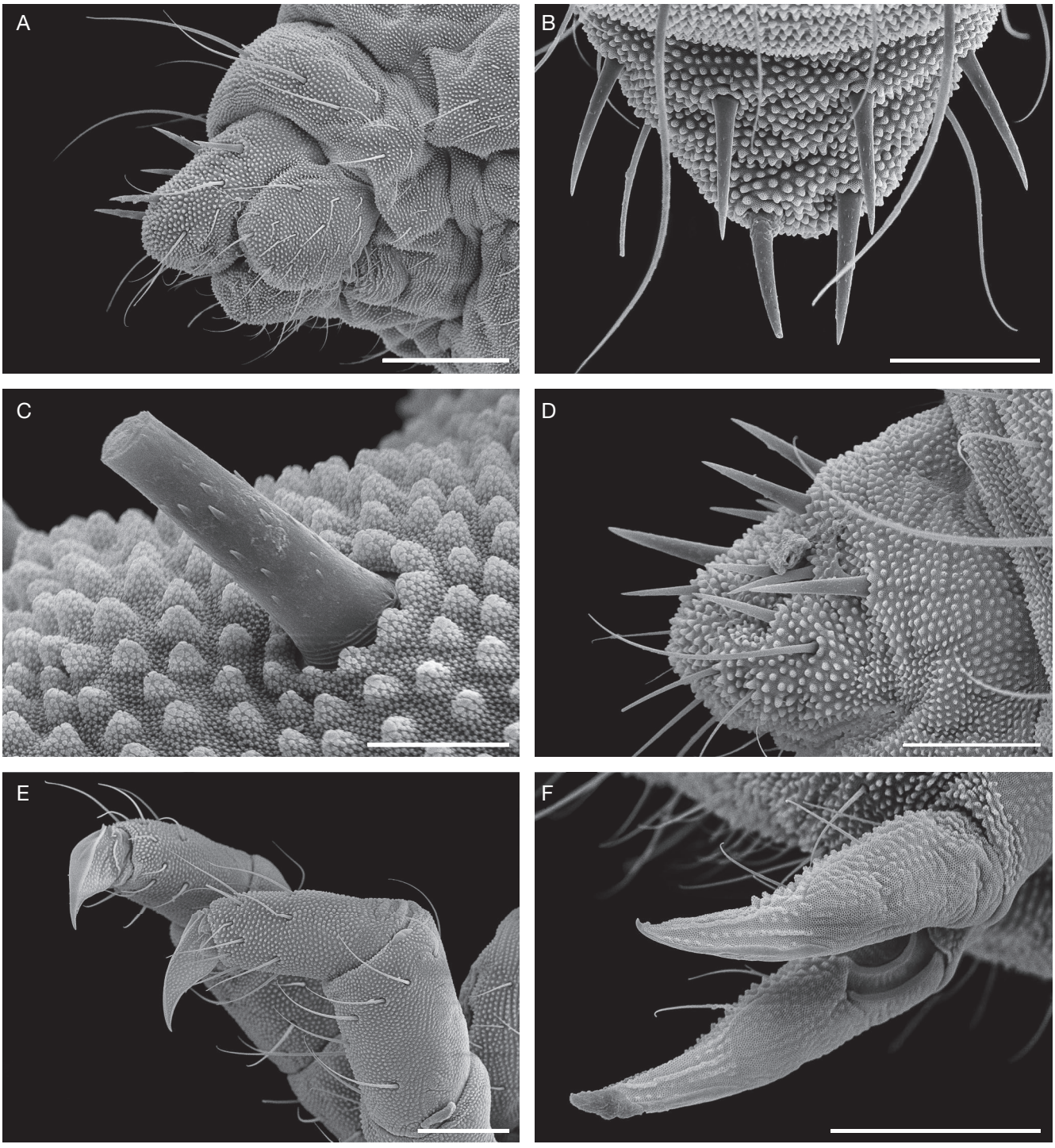

FIG. 6. — *Friesea najtae* n. sp. SEM microphotographs: **A**, Abd. V-VI ventro-lateral view showing female genital plate and anal valves; **B**, Abd. VI dorsal view with the six strong spine-like chaetae; **C**, detail of a spine-like chaetae on Abd. VI (tip of the chaetae broken); **D**, Abd. VI lateral view showing asymmetries and plurichaetosis of the spines; **E**, tibiotarsi III; **F**, furcula. Scale bars: A, 100 µm; B, D-F, 50 µm; C, 10 µm.

Chaetotaxy

Dorsal chaetotaxy as in Fig. 5A-C. Dorsal chaetae of various length, very slightly crenulated with *s*-chaetae meso-chaetae. *S*-chaetae formula per half tergum: 022/11111. Head with chaetae a0, d0 and three chaetae oc. Thoracic tergum I with 5 + 5. Dorsal chaetotaxy (Thoracic terga II to Abd. VI) with some asymmetries and slight plurichaetosis, see Figure 5A, B, making chaetae designation difficult. Thoracic sterna without chaetae, Abd. II and Abd. III sterna with 12 + 12 14 + 14 chaetae respectively. Ventral tube with 11 + 11 chaetae (Fig. 4C).

Body with several peudopores on various positions (Fig. 4D).

Six strong anal spines without papillae (Figs 5C; 6A-C), sometimes more due to plurichaetosis and asymmetries (Fig. 6D).

Thoracic sterna without chaetae.

Legs

Tibiotarsi I, II and III with 18, 18 and 16 chaetae respectively (Fig. 5G). No clavate tenent hairs. Claw with a very small median tooth (Figs 5G; 6E).

Furcula

Furcula fully developed, dens with 3 chaetae each, mucro large even if slightly fused with the dens (stage 1 - stage 2 according to Cassagnau 1958) (Fig. 6F). Tenaculum present with 2 + 2 teeth.

DNA barcode

A 658 base-pair (bp) fragment of the mitochondrial (mt) DNA cytochrome *c* oxidase I (COI) gene (DNA barcode) was amplified and sequenced for specimen EA013922 (see paratype information above) and deposited in BOLD database under accession number ADF3744. The base composition of the DNA barcode sequence is 26.4% A, 33.1% T, 22.2% C and 18.2% G (A + T = 59.6%):

5′ – ATCTATATATTTTCTTCTAGGTACATGAGCTGCTTT–
TACCGGGACATCTCTAAGTATCTTAATTCGATTAGAACTAG–
GTCAGGCAGGATCGTTTATCGGAAGAGATCAAACATATAATG–
TAATTGTAACGGCCCATGCTTTTATTATGATTTTCTTCATAGT–
TATACCTATAATAATTGGGGGGTTTGGAAACTGACTCGTTC–
CATTAATAATTGGAGCCCCTGATATAGCATTCCCCCGGA–
TAAATAATATAAGATTCTGGCTTCTTCCACCGTCTCTAACC–
CTTTTATTAGCAGGTAGAGTAGTAGAAAGAGGGGCAGGAACGG–
GATGAACAGTTTATCCCCCTCTTTCATCTAATCTAGCTCACGC–
CGGTAGCTCAGTGGACTTATCTATTTTTAGTCTACACCTCGC–
CGGGGCTTCTTCTATCCTAGGAGCAGTTAATTTTATTACCACTAT–
CATTAATATACGGTCAGAAGGGATGTCCTGAGACCAAATACCC–
CTTTTTGTTTGATCAGTTATATTAACGGCCATTCTTCTACTTC–
TATCTCTCCCAGTGCTGGCAGGTGCCATCACTATACTTCTAACT–
GATCGTAACCTAAATACCTCTTTTTTCGACCCAGCCGGAGGAGGG–
GACCCAATTTTATACCAACACTTATTT – 3′.

DISCUSSION

The habitus of *Friesea najtae* n. sp. is very similar to the habitus of *Friesea grisea* Schaffer, 1891 as drawn by Willem (1902, identified as *Achorutoïdes antarcticus* Willem, 1902, see his plate II, fig. 3). Willem's (1902) description differs from our specimens by the furcula (but the retinaculum seems to be identical), two anal spines instead of six and eyes seems to be bigger in our specimens. Also, *F. grisea* has clavate chaetae on tibiotarsi, not present in *F. najtae* n. sp.

The presence of more than four straight spine-like chaetae (anal spines) without papillae on Abd. VI defined the sub-genus *Polyacanthella* Schäffer, 1897 (e.g., Denis 1925, Womersley 1933, see Massoud 1967 for a discussion) to which *F. najtae* n. sp. would belong, if the subgenus was not deprecated. The distinction between true anal spines and spine-like chaetae should be mentioned here. One could consider that the anal spines in the present species are rather spine-like chaetae without papillae because of the symmetry of each "spine" along its axis, the words "anal spine" being used for asymmetrical "spines" (Massoud & Ellis 1977). Since the anal spines in the new species are very similar to the anal spines in *Friesea judithae* Palacios-Vargas, 1986, and since the author discussed the matter specifically in the context of the genus *Friesea* context (Palacios-Vargas 1986), we retain the use of anal spines.

Within the genus *Friesea*, only one species present a combination of 8 + 8 eyes, six anal spines and furcula well developed (stage 1 or 2): *F. acuminata* (Denis, 1925). *Friesea acuminata* is white with spots, has a simple apical bulb and is distributed in Europe. It is worth mentioning *F. tourratensis* Cassagnau, 1958 with five real anal spines (asymmetrical along the axis of the spine, see above) on papillae (and not six anal spines, symmetrical along the axis, without papillae as in *F. najtae* n. sp.), five or more capitate tenent hairs on each tibiotarsi and capitate chaetae on Abd. V-VI and is found in France and Spain. Other species also have 8 + 8 eyes and six anal spines: *F. carlota* Christiansen & Bellinger, 1988 (see Palacios-Vargas 2005 for a redescription), *F. lagrecai* Dallai, 1973, *F. mandibulata* Salmon, 1969, *F. palafoxaliciae* Palacios-Vargas, 2005, *F. parva* (Womersley, 1936), and *F. xitlensis* Palacios-Vargas & Acosta, 1994. However all these species are lacking a furcula. *Friesea handschini* Kseneman, 1938 is also close to *F. najtae* n. sp. by having six spine-like chaetae and 8 + 8 eyes, however even though the furcula is present, it is at stage four (mucro absent and dens reduced to a mamelon with two dorsal chaetae), apical vesicle is simple and capitate tenent hairs are present on tibiotarsi and capitate chaetae on Abd. V-VI. See Dányi *et al.* 2010 for a thorough redescription of *F. handschini*.

Acknowledgements

The laboratory work was done in D'Haese's BoEM laboratory. Microphotographs were taken at the Microscopies and Imagery Platform (MNHN). Field collection was supported by CaFoTrop (www.cafotrop.com) and Techmohygiène. Many thanks to Cristtina Fiera and José Palacios-Vargas for careful correction of the manuscript. All collecting undertaken with permits (SF009377) issued by the Department of Environment and Conservation of the Government of Western Australia.

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Submitted on 31st August 2016;
accepted on 13 February 2017;
published on 31st March 2017.