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First record of Tardigrada from New Caledonia

short title Tardigrada from New Caledonia

Keywords Tardigrada, taxonomy, faunistics, seven species, Liefou Island, New Caledonia

Abstract Seven species of water-bears (Tardigrada) belonging to six genera (*Bryodelphax, Echiniscus, Pseudechiniscus, Mesobiotus, Minibiotus* and *Doryphoribius*) are reported for the first time from the New Caledonian archipelago, a region hitherto unexplored in aspect of this animal group.

Tardigrades occurred only in four samples from 21 occasionally sampled bryophytes and lichens, all coming exclusively from Lifou Island (20.9° S, 167.2° E). The island is located within the Layeuté Islands group of the archipelago. None of here described and illustrated species has been exactly identified due to very low abundance of extracted animals preventing evaluation of morphological variability and the absence of living specimens for analysis of molecular markers. Nevertheless, at least four of seven examined species from the genera *Echinicus, Pseudechiniscus, Minibiotus* and *Doryphoribius* represent supposedly still undescribed taxa.

Introduction

The tardigrade fauna of the Australasian Realm is very poorly known and from the New Caledonian archipelago no information about the presence of these animals has been published yet.

Twenty years ago Dr. Anne Bedos and Dr. Louis Deharveng collected in the archipelago some samples of bryophytes and lichens at the occasion of their studies of soil and cave fauna and kindly put these cryptogamic plants at my disposal for examination of tardigrades. In this paper I describe and illustrate several tardigrade taxa which occurred in the samples, as it seems opportune to provide such still unknown data.

Materials and Methods

Altogether 21 bryophyte and lichen samples have been collected. The material came mainly from the Layeuté Islands (Lifou, Tiga, Mara, Ouvea), two samples originated from the main island, La Grande-Terre. However, tardigrades were found only in four samples from Lifou Island (20.9° S, 167.2° E), all sampled on 23rd October 2000 and below 50 m a.s.l. The samples came from the following localities (the number of the animals found is presented in square brackets):

- A) Hnaeu, near Hunane Cave: moss on calciferous (coral) rock at the ground level, one sample [2 tardigrades];
- B) Mu, Cap des Pins (three samples): 1. Moss on calciferous ground near *Araucaria* forest [2];
 2. Mosses from *Araucaria* bark [20];
 3. Lichens from *Araucaria* trunk [32].

The collected plants were placed into small paper bags, dried and stored. Recently the animals have been extracted by the method described in Dastych (1980a). Sand, fine rocky residues, the remains of detritus and other parts of sediment which were washed out from the plants during the extraction were examined with 10 % hydrochloric acid (HCL) for the presence of calcium carbonate (compare e.g. Dastych 1988). Tardigrades were mounted on 14 microscope slides either in chloral gums (Faure's or Hoyer's medium) or in polyvinyl-lactophenol (PVL). One slide containing two tardigrades from the sample B1 was re-mounted due to poor preservation of the animals but one specimen has been lost during the procedure. Tardigrades were examined and microphotographs were taken with phase- and differential interference contrast microscope ZEISS "Photomikroskop III". The measurements were made under phase contrast using ocular micrometer. The slides (12) are deposited in the Zoological Museum, Centre of Natural History, University of Hamburg (the Accession No.: ZMH A1-12/2021).

Abbreviations and symbols used:

an – anus; cA – cirrus A (= seta A,=appendage A); cl – claw; dc – dentate collar; DIC – differential interference contrast; ey – eye-dot; go – gonoporus; HP – head plate; M1 – first median plate; M2 – second median plate; M3 – third median plate; n – sample size (=number of specimens); pc – papilla cephalica (=cephalic papilla,=secondary clava); PHC – phase contrast; PP – pseudosegmental plate; pt ss – stylet supports index (see Pilato 1981 for the index definition); SP – shoulder (=scapular) plate; ss – stylet support; st – stylet; TP – terminal plate; I – the first pair of dorsal paired plates; II – the second pair of dorsal paired plates; 2nd – second leg; 3rd – third leg.

Results

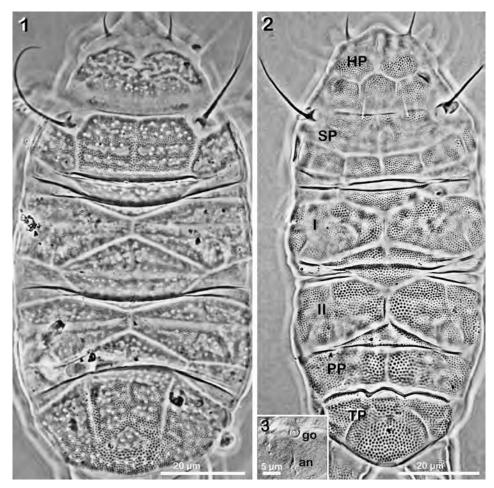
Altogether 56 tardigrades but no eggs have been found. The animals represent seven species within six genera, they are listed and described below.

Class: Heterotardigrada Marcus, 1927 Family: Echiniscidae Thulin, 1928 *Bryodelphax* cf. *arenosus* Gąsiorek, 2018 (Fig. 1)

Length $90-130 \mu m$ (mean = $90.9 \mu m$, n = 34); not mounted animals pale-greyish, transparent in preserving media. Dorsal plates well developed, characteristically sculptured (Fig. 1). Dorsum covered with internal cuticular pillars, noticeable in dorsal view as closely placed minute granules. They are the largest (ca. 0.5 μm in diameter) in the posterior part of terminal plate but indiscernible on venter. Small pore-like structures, strongly refracting the light and visible as bright dots/ points are distributed all over the dorsum, particularly on the shoulder and terminal plate (Fig. 1).

The cuticle in the central area of the shoulder plate thickened, thus darker and distinctly faceted. Its surface is covered with flat and mostly irregularly shaped thickenings which are more or less fused. The thickenings form 3–4 transversally oriented thicker and darker cuticular ridges (Fig. 1). The anterior ridge (transversal belt) is mostly the largest one and it covers almost a half of the central area of the shoulder plate. Additionally, a longitudinal but shorter and less discernible ridge occurs along the median line of the plate. Terminal plate with two elongated longitudinal sutures. The area between the sutures covered with several short, more or less distinct cuticular ridges. The ridges are mostly variably oriented (Fig. 1). Third median plate not divided. Ventral plates absent; venter smooth, i. e. without granulation or pore-like dots.

Cephalic papilla and primary clava short, head cirri slightly thickened at their bases (cirrophores?). The seta A about 25 % of the body length ($20.1-29.8 \mu m$, mean = $24.3 \mu m$, n = 23). No other body appendages present.

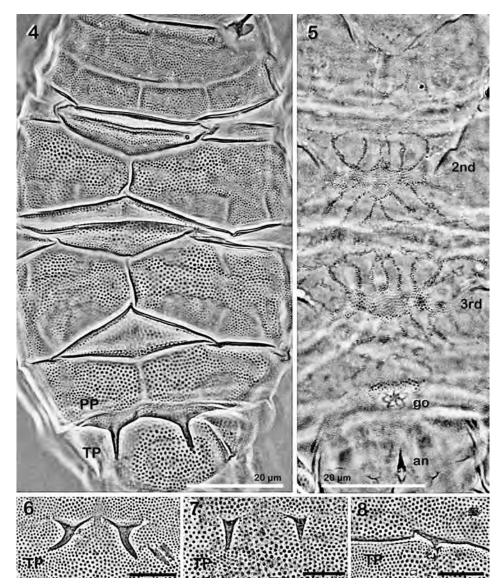


Figs 1-3 1. Bryodelphax cf. arenosus Gąsiorek et al., dorsal view. 2. Pseudechiniscus sp., male, dorsal view. 3. Same specimen as in Fig. 2, its genitoanal region. PHC-images. Abbreviations: see list in Materials and Methods.

No spine on leg I; leg IV with tiny dome-like papilla (ca. 1 µm in diameter), well visible only in several animals. Pulvini present but the leg plates I-III not discernible. The cuticle on legs IV more thickened and darker, but without dentate collar. External claws smooth, internal ones with tiny spines (spurs) directed downwards and located closely to the claw bases.

Measurements in a specimen $116 \mu m \log$ (Fig. 1, female): cephalic papilla and primary clava (i.e. the clava at seta A) $3.6 \mu m \log$, cirrus internus and externus 7.2 and $11.7 \mu m \log$, respectively. Shoulder plate $15.3 \mu m \log$, seta A $25.0 \mu m$, internal claw IV $5.9 \mu m$ in length.

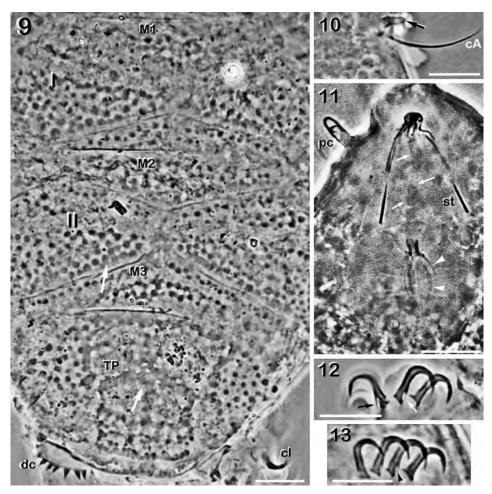
- Note The sculpturing of the cuticle (the ridges, sutures) is mostly well discernible in PHC, but in DIC microscopy sometimes hardly visible or not discernible. The internal cuticular pillars (i. e. "granulation") are clearly recognizable in these both optical techniques.
- Examined 37 animals (14 females, 23 specimens with unknown sex). Among them no males, no "larvae" (=2-clawed juveniles) were found.



Figs 4-8 Pseudechiniscus sp. 4. Female, dorsal view. 5. Same specimen as in Fig. 4, its ventral side with the net-like structure and genitoanal region. 6–8. Variability of spines on pseudosegmental plate. PHC-images. Scale bars in Figs 6–8=10 µm. Abbreviations: see list in Materials and Methods.

Localities B2, B3 (six and 31 specimens, respectively).

Remarks The sculpturing pattern on dorsal plates in examined specimens is very similar to that observed in two paratypes of *Bryodelphax arenosus* Gąsiorek, 2018. The micrographs in the original description of the species (see Gąsiorek 2018, particularly Fig. 3) are not optimal, thus they do not allow for exact comparison of these structures during identification. The pattern closely resembles also that one in *B. decoratus* Gąsiorek, Vončina, Degma & Michalczyk, 2020 (see Gąsiorek et al. 2020: Fig. 5). In the New Caledonian specimens no ventral plates have been found and they are also not reported in *B. arenosus*. However,



Figs 9-13 Echiniscus sp. 9. Posterior part of the body, dorsal view (arrows: cuticular sculpture). 10. Head region, latero-dorsal view (arrow: primary clava). 11. Bucco-pharyngeal apparatus (arrows: buccal tube; arrowheads: placoids), dorso-median view. 12–13. Claws on leg II (arrows and arrowhead: spurs on internal claws). PHC-images. Scale bars = 10 μm. Abbreviations: see list in Materials and Methods.

in *B. decoratus* they are described as "very dim and barely discernible" (see *l.c.*). The ventral plates (their presence or absence) belong to one of several key characters within the *Bryodelphax* (e.g. see Gąsiorek et al. 2020). The agreement of other characters observed in the examined material with those of *B. arenosus* suggests very close relationship or even identity of the New Caledonian animals with the latter species.

Echiniscus sp. (Figs 9–13)

Length 148 µm, the body pale but transparent in Faure's medium. Dorsal cuticle covered with roundish, dark (in PHC) and widely spaced small dome-like knobs ("granules") (Fig. 9). They are slightly larger on the paired plates, particularly in their posterior parts (Fig. 9, arrow), being rarely up to 2.0 µm in diameter, mostly slightly smaller. The granules are often interconnected with short, irregularly shaped cuticular striae/stripes (Fig. 9). When being more or less fused, then the granules form variously directed, more sclero-tized (darker) ridges on the cuticle surface. The edges of dorsal plates poorly marked. The second median plate transversally divided. The third median plate wide and its central part with two transversal and irregularly shaped rows of fused knobs (Fig. 9, M3). Terminal plate with two weak lateral incisions. The plate is characteristically faceted with short and variously directed ridges, originating from the fused cuticular knobs. These ridges form in the central part of the plate darker and thicker rectangular or square-like areas (Fig. 9, TP). Bucco-pharyngeal apparatus short (Fig. 11).Ventral plates could not be observed, stylet supports not visible (but see note below).

Head cirri short, cephalic papilla elongated (Fig.11). Primary clava short and elongated, seta A weakly developed and short (Fig. 10). No other lateral or dorsal body appendages (setae, spines) present.

Spine on leg I could not be discerned (see note), papilla on leg IV small. Dentate collar with short and sharp spines (Fig. 9 dc). Claws relatively small, the external smooth, the internal ones with a minute and thin spur strongly directed downwards (Figs 12, 13). The spur is located slightly above the claw base.

Measurements in a specimen 148 μ m: cephalic papilla 5.4 μ m, cirrus internus and externus 8.1 and 12.0 μ m long, respectively. Shoulder plate ca. 28.0, primary clava 4.5 and seta A 24.0 μ m long. Bucco-pharyngeal apparatus 32.0, internal claw IV 7.2 μ m in length.

- Note The sole specimen is unsuitably mounted on slide (far too much flattened), thus some of its structures are not discernible.
- Examined one animal, sex unknown.
- Locality B3.

Remarks The type of chaetotaxy of the specimen, i.e. the presence of only setae A on its trunk places the animal within the *"Echiniscus arctomys"*-group of species. This old working term encompasses numerous and variously related taxa. The species complex has been recently revised and most of its taxa are now separated formally into five new genera (Gąsiorek et al. 2019).

The markedly short setae A, the type of sculpture on dorsal plates and character of claws in the examined animal are similar to those described in *Echiniscus nigripustulus* Horning, Schuster & Grigarick, 1978, *E. vinculus* Horning, Schuster & Grigarick, 1978 and *Echiniscus kofordi* Schuster & Grigarick, 1966 (see Schuster & Grigarick 1966, Horning et al. 1978, Christenberry 1979, Pilato et al. 2005). (Now these species are known as *Claxtonia nigripustula, Claxtonia vincula and Kristensenius kofordi* – see Degma at al. 2021 and Gąsiorek et al. 2019, respectively). Among these three taxa the sculpturing of the dorsum in *C. nigripustula* resembles closely that one occurring in the New Caledonian specimen.

Pseudechiniscus (Meridioniscus) sp. (Figs 2–8)

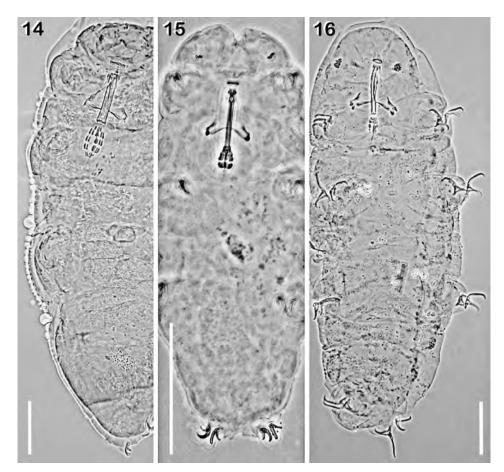
Length 84–164 µm (mean = 126.4, n = 13), mounted animals pale-yellowish. The eye-dots distinct, black. Dorsal plates well-formed. Dorsum with densely and regularly distributed granulation (Figs 2, 5). The granules (i.e. cuticular pillars) are the largest on paired plates II and on the pseudosegmental and terminal plate (Figs 2, 4). They are up to 1.2 µm in diameter, usually slightly below 1.0 µm. No cuticular connecting striae (stripes) occur between the particular granules. Head plate dorsally with distinctly marked wide "W"like pattern (Fig. 2, HP). The shoulder plate posteriorly faceted with transversal, elongated cuticular fold (ridge). The ridge forms a wide and separate transversal belt along and above the posterior edge of the plate (Fig. 2, 4). The belt is divided through five short longitudinal ridges into six areas (fields). The most lateral (marginal) fields are the smallest and visible as narrow platelets (e.g. being ca. 3 µm wide in a specimen 130 µm long). Four centrally located rectangular fields are well recognizable in all specimens (Figs 2, 4). In the median part of each paired dorsal plate occurs a wide transversal belt of slightly lowered cuticle (Fig. 4). Pseudosegmental and terminal plate not faceted, the latter one with two strong lateral incisions (Figs 2, 4). The posterior edge of pseudosegmental plate with a pair of variably sized spines (Figs 2, 4, 6-8).

Ventral plates absent. The granulation on venter is often hardly discernible. The ventral side of the body with a characteristic net-like pattern (Fig. 5) formed by variably oriented cuticular folds. The folds are covered with rows of minute, hardly discernible and densely placed granules. In the central area of the venter, between the second and third pair of legs, occurs additionally a small area (spot) composed of slightly larger granules (ca. 0.5 µm in diameter). Similarly sized granules are present around the female gonopores (Fig. 5, go). In the only available male (Figs 2, 3) hardly visible fragments of such a net-like pattern occurred only below the IInd and above the IIIrd pair of legs. Moreover, poorly discernible granulation was present in the subcephalic region, in centrally located, small area between legs of the IInd pair and around its gonopore. The venter surface of the male between the IIIrd pair of legs could not be examined due to unfavourable position of the extremities.

Cephalic papilla cone-like, slightly elongated and larger in the male. The papilla and primary clava medium-sized, head cirri with small cirrophores. Setae A short ($16.2-27.0 \mu m$, mean = $23.2 \mu m$, n = 13), about 18 % of the body length. No other lateral appendages present, the dorsal spines variable in size occurred only on the pseudosegmental plate.

Legs I without spine, legs IV with a small papilla. Leg plates covered with granulation smaller than that on dorsum, leg IV without dentate collar. Claws medium-sized, external smooth, internal ones with a small spur bent downwards. It is located at ca. 1/3 of the claw length, closer to the claw base.

Measurements in a female 120μ m long (Fig. 4): cephalic papilla and primary clava 4.5μ m, cirrus internus and externus 5.4 and 11.0μ m long, respectively. Seta A 23.4, shoulder plate 18.0μ m long. Papilla on leg IV 2.3, internal claws IV 7.2μ m in length.



Figs 14-16 14. Mesobiotus sp., habitus. 15. Minibiotus sp. 1, habitus. 16. Doryphoribius sp., habitus. PHC-images. All dorsomedian view. Scale bars = 50 µm.

Measurements in a male $115 \mu m$ long (Fig. 2): cephalic papilla 5.4, primary clava $3.6 \mu m$ long, cirrus internus and externus 6.0 and $13.5 \mu m$ in length, respectively. Seta A 24.3, shoulder plate $17.1 \mu m$ long, papilla on leg IV 1.8, internal claw 7.2 μm in length.

Examined 14 animals (5 females, 1 male: sex unknown in 8 specimens; no 2-claw juveniles).

- Locality B2.
- Remarks The distinct spines on pseudosegmental plates locate the examined animals within taxonomically perplexing group of taxa termed as *Pseudechiniscus novaezeelandiae*-complex (e.g. Tumanov 2020) or, traditionally known as *P. suillus-facettalis* group. Recently a new subgenus, *Meridioniscus* Gąsiorek, Vončina & Michalczyk, 2021, has been established for the members with elongated cephalic papilla (Gąsiorek et al. 2021), which includes also the New Caledonian specimens. The latter ones are similar to *Pseudechiniscus spinerectus* Pilato, Binda, Neapolitano & Moncada, 2001, but differ mainly by the absence of striae between cuticular granules, which occur in *P. spinerectus*. The New Caledonian animals

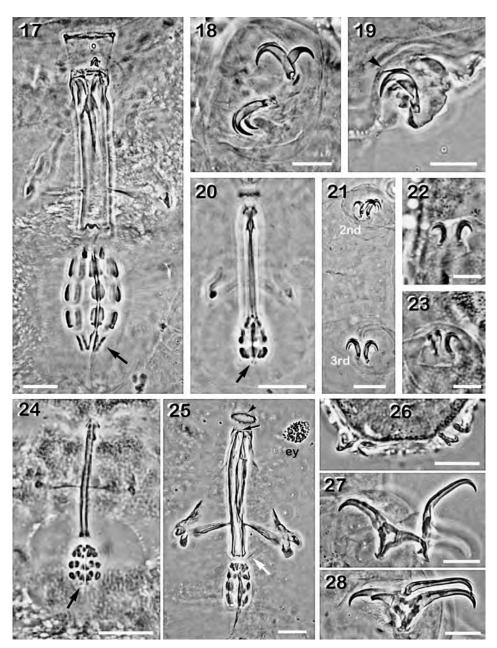
also bear resemblance to *P. brevimontanus* Kendall-Fite & Nelson, 1996, but the latter taxon has differently formed ventral net-like sculpturing, with more isometrically shaped meshes on the lateral sides of the trunk, compared to such elongated meshes in the examined specimens. In another species similar to the New Caledonian material i. e., *P. novae-zeelandiae* (Richters, 1908), the dorsal granules are connected by striae (comp. Pilato et al. 2005). Furthermore, the considered specimens are partly resembling *P. ramazzotti* Maucci, 1952 in having also dorsal granules without the striae. However, the venter of the latter species has no net-like pattern but is covered by more or less homogenous granulation.

One should note that the animals reported as *P. ramazzotti* by Dastych (1980b) from Caucasus, according to D. Tumanov (in litt.: 10 April 2021) who recently re-examined the material, represent yet undescribed species.

The specimens of *Pseudechiniscus* from New Caledonia belong supposedly to a new species, but an analysis of more suitably preserved material and molecular data are needed for secure identification. In recent times, the presence of putative cryptic species within *Pseudechinicus* has been discussed and such numerous taxa already listed, partly also as "unconfirmed candidate species" (= UCS) (see e.g. Cesari et al. 2020, Gobys et al. 2020, Gasiorek et al. 2021) or some have already been described (e.g. Roszkowska et al. 2020).

Class: Eutardigrada Richters, 1926 Family: Macrobiotidae Thulin, 1928 *Mesobiotus* sp. (Figs 14, 17–19)

Length 485 µm, body light-brownish in Faure's medium. The cuticle smooth. Eye spots located anteriorly and composed of small blackish-brown pigment granules. The buccopharyngeal apparatus relatively large, 108.0 µm long. The bottom of mouth cavity with well developed dorsal and ventral transversal ridges (Fig. 17) and a row of small and irregularly distributed granules (mucrones) above the latter structures. A ring of tiny granules occurs around the mouth opening. Buccal tube 46.8 µm long, the external width 8.1 µm, the internal one 7.2 µm (measured slightly above the bases of stylet supports). The supports are attached to the mouth tube at 34.7 µm of the tube length (thus pt ss index = 74.2 %). The tube with tiny posterior apophyses. Pharynx oval (53×44 µm), with three macroplacoids and large microplacoid (Figs 14, 17). Macroplacoids elongated, 5.4, 5.0 and 5.4 µm in length, respectively. The row of macroplacoids 21.6 µm long. The third macroplacoid with a distinct incisions in its caudal parts. The pharyngeal apophyses median-sized, microplacoids markedly long (5.0 µm) and thin (Fig. 17, arrow) and of the size of the second macroplacoid. Claws medium-sized, the external claw on leg II 11.7, the hind claw IV 13.1 µm long. Small, smooth lunules on legs I-III (Fig. 18). Those on legs IV slightly larger and their posterior edges with hardly visible corrugation (or barely discernible tiny, irregularly shaped teeth?). The main branches of claws with well



Figs 17-28 17–19: Mesobiotus sp. 17. Bucco-pharyngeal apparatus, ventral view (arrow: microplacoid). 18. Claws on the second leg. 19. External (?) claw on the fourth leg (arrowhead: accessory spines). 20–21: Minibiotus sp. 1. 20. Bucco-pharyngeal apparatus (arrow: microplacoid). 21. Claws of II and III leg, respectively. 22–24, 26: Minibiotus sp. 2. 22–23. Claws of II leg. 24. Buccopharyngeal apparatus (arrow: microplacoid). 26. Claws of IV leg. 25, 27–28: Doryphoribius sp. 25. Bucco-pharyngeal apparatus (arrow: pharyngeal rod). 27–28. Claws of II and IV leg, respectively. PHC-images. Scale bars = 10 µm. Abbreviations: see list in Materials and Methods.

developed, long, and thin accessory spines (Figs 18, 19). The distal parts (apices) of the spines on legs IV are distinctly standing away from the main claw's branch (Fig. 19, arrowhead).

Examined one animal; sex unknown.

Locality A.

Remarks The specimen is characterized by markedly long and thin microplacoids and distinctly divergent accessory spines from main claw branches on the IVth pair of legs (Figs 17, 19). The absence of eggs does not allow for more exact identification of the species. The recently established genus *Mesobiotus* includes a large group of species separated then from the polyphyletic genus *Macrobiotus* (see Vecchi et al. 2016). The former genus includes now 70 species (Degma et al. 2020), for which an identification key has been recently presented (Kaczmarek et al. 2020).

Minibiotus sp. 1 (Figs 15, 20–21)

Length 155 µm, body pale-whitish. Small eye-dots composed of several pigment granules and located anteriorly. Dorsum covered with small, roundish and slightly flat-topped cuticular knobs (protuberances), being up to 4.0 µm in diameter, though mostly smaller, and ca. 1.0 µm high. Their tops have centrally located roundish or elongated pit (porelike structure), the latter up to 1.4 µm in diameter. The knobs are arranged in transversal bands and their number and size increases towards the body rear. These protuberances are relatively widely spaced on dorsum at the level of the IInd and IIIrd pair of legs and most frequent and more closely placed in the caudal part of the body. At least five transversal bands with such knobs are discernible on dorsum, these being most distinct at the body rear and the level of the IInd and IIIrd pair of legs. In the anterior part of dorsum only poorly discernible two transverse bands of irregularly distributed and widely spaced pits (pore-like structures) occur but no knobs are recognizable. The knobs occurred also on venter, being there much smaller and very sparsely distributed. Widely spaced and smaller knobs or pits were present on legs as well. The cuticle between knobs is smooth.

Bucco-pharyngeal apparatus small, moderately sclerotized and $38.7 \,\mu$ m long. Mouth opening with a ring of tiny roundish structures (peribuccal papulae?), mouth cavity small and funnel-like (Fig. 20). The bottom of the cavity with hardly visible transverse ridge(s?). Buccal tube 21.6 μ m long and narrow: its external diameter 1.8 μ m, the internal one 1.2 μ m. Buccal crest (=lamina, strengthening bar) moderately long, of about a half of the tube length. The posterior apophyses of the tube minute. Stylet supports attached to the tube at the distance of 14.5 μ m from the tube outset (pt ss index=67.1 %). Pharynx spherical (ca. 20.0 μ m in diameter) with small pharyngeal apophyses, two short rounded macroplacoids and a minute microplacoid. The first macroplacoid closely located at the pharyngeal apophyse and distinctly constricted in its middle. The second one corn-like, with tiny caudal incision. The macroplacoids row 6.8 μ m; the first macroplacoid 3.6, the second one 2.3 μ m in length.

Claws well sclerotized, relatively large (Figs 15, 21). The external claws 5.4 μ m and 6.3 μ m long on legs I and III, respectively. Accessory spines on main claw branches small, also small

and smooth lunules on legs I-III. Claws on legs IV unfavourably positioned, thus the posterior edges of the lunules are not discernible, consequently no claws measurements are provided.

Examined one animal, sex unknown; possibly a juvenile.

Locality A.

Remarks The specimen resembles *Minibiotus fallax* Pilato, Claxton & Binda, 1989 in having two macroplacoids and dorsal protuberances (knobs) arranged in transversal bands. The stout claws, relatively long buccal crest and some other characters are also similar in both taxa (Pilato et al. 1989, Claxton 1998). The absence of eggs and a sole, not optimally preserved animal prevent however more exact identification. The species might represents *M. fallax* (recorded only from Australia: Claxton 1998) or it belongs to still undescribed taxon.

Minibiotus is considered as a possibly polyphyletic genus (e.g. Guidetti et al. 2007, Bertolani et al. 2014). It encompasses also the above mentioned both taxa, which represent a separate, not much speciose clade within the genus. The status of this clade in *Minibiotus* is long under debate and its exclusion from the genus is still under discussion (e.g. Pilato et al. 1989, Guidetti at al. 2007, Bertolani et al. 2014, Stec et al. 2015).

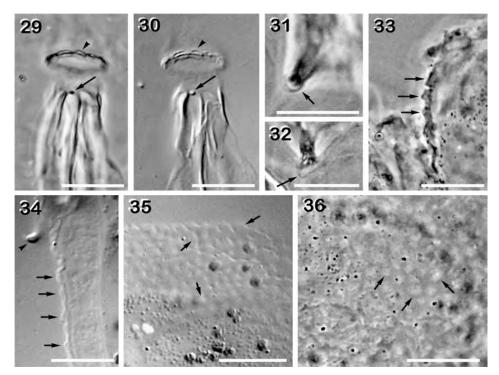
Minibiotus sp. 2 (Figs 22–24, 26)

Body 214 µm long, cuticle smooth, no pores. Eye-dots anterior, medium-sized. Densely distributed tiny (ca. 0.5–0.8 µm sized) epidermal pigment granules (brown-blackish in PHC, Fig. 23; bright and gold-brownish in DIC) occur under the dorsal cuticle. The granules are arranged on dorsum in 8–9 transverse pseudosegmental belts; 4–5 such belts/stripes, distinctly thinner, are present on the ventral side of the body.

Bucco-pharyngeal apparatus $37.8 \,\mu$ m long, mouth opening small, ca. $3.0 \,\mu$ m wide. Buccal tube $20.7 \,\mu$ m long, its stylet supports attached at the distance of $12.6 \,\mu$ m (pt ss index = $60.9 \,\%$). The tube narrow, its external diameter $1.4 \,\mu$ m, the internal one ca. $1.0 \,\mu$ m. The posterior apophyses of the tube distinct (Fig. 24). Buccal crest very short. Pharynx spherical (ca. $22.0 \,\mu$ m in diameter), with distinct pharyngeal apophyses close to the first macroplacoid, three corn-like macroplacoids and a tiny microplacoid (Fig. 24). Macroplacoid row $5.5 \,\mu$ m, pharyngeal apophyses 1.4, the first macroplacoid 1.4, the second 1.0 and the third $1.4 \,\mu$ m in length. Microplacoid located very closely to the third macroplacoid and $0.5 \,\mu$ m long.

Claws small, not much differentiated in size on leg I and IV, their main branches with minute accessory spines. The claws lunules small and on legs I-III smooth; on legs IV the lunules are strongly rolled up, thus their posterior edges are not discernible. External claws on leg I ca. 5.0 µm long, those on legs IV (= the hind ones) 5.5 µm in length.

Examined one animal, sex unknown. Locality B3.



Figs 29-36 Doryphoribius sp. 29–30. Mouth cavity region (arrowhead: mouth ring with oval structures, arrow: dorso-median ridge). 31. Lunula (arrow) of internal claw on leg III. 32. Lunula (arrow) of the claw on leg IV. 33–36. Cuticular sculpture (arrows: cuticular thickenings,="granules") on leg II (33), lateral view; below leg III (34), lateral view (arrowhead: claw of leg III); between II and III leg (35), latero-dorsal view; between III and IV leg (36), dorsal view. Figs 29, 31–33, 36 – PHC-images, Figs 30, 34, 35 – DIC-images. Scale bars = 10 µm.

Remarks The buccal apparatus of the *"Minibiotus intermedius"*-type which prevails among the members of the genus (i. e. the sets of three macroplacoids) is also present in the considered specimen. The absence of eggs makes more exact identification not possible.

Family: Doryphoribiidae Gąsiorek, Stec, Morek & Michalczyk, 2019

Doryphoribius sp. (Figs 16, 25, 27–36)

Length 395 µm, body light-brownish in Faure's mountant. Sparsely distributed and irregularly shaped small chunks of pigment granules occur within the body (Figs 33, 35, 36). The chunks are chiefly located in the caudal region (Fig. 16). Eye-dots relatively large, more or less oval (ca. 10 µm long). They are composed of variably sized blackish-brown tiny pigment granules (Fig. 25, ey).

The cuticle on dorsum with poorly discernible, small (up to $2.0 \,\mu$ m in diameter), flattened and more or less roundish thickenings (Figs 33-36). The thickenings (="granulation") being more distinct in the caudal part of the dorsum, occur also on legs but they could not be discerned on venter. Bucco-pharyngeal apparatus $81.0 \mu m \log R$. Mouth opening surrounded by a ring of oval, distinctly elongated small structures (Figs 29–30). Mouth cavity medium-sized, its bottom with dorso-median ridge shaped as a distinctive granule (tooth) of ca. 0.7 μm in diameter (Figs 25, 29, 30); dorso-lateral ridges strongly reduced in size. Ventro-median ridge formed as a thin and small bar (ca. 2.0 μm long), being fused (?) with the reduced ventro-lateral ridges. No mucrones in mouth cavity.

Buccal tube 45.9 µm long, 4.5 µm wide (its internal diameter 2.3 µm) and with small posterior apophyses. Stylets' supports attached in the posterior part of the tube at 32.4 µm of the tube length (pt ss index = 70.6 %). Buccal crest well-formed and long (Fig. 25). Pharynx sub-spherical (ca. 36 µm wide) with medium-sized pharyngeal apophyses and three macroplacoids, the latter increasing in length posteriorly. No microplacoids. Between the tube posterior apophysis and the phryngeal apophysis occurs thin and long cuticular bar (= pharyngeal rod, Fig. 25: arrow). Macroplacoid row 12.6 µm. The first macroplacoid 2.7, the second 3.6, the third one 4.1 µm long. In one of three sets of macroplacoid rows, the second and the third macroplacoid are aberrantly developed, i. e. strongly reduced in size, being abnormally shaped and almost absent (Fig. 25).

Claws relatively large, with slender main branches and with characteristic sculpture inside (Figs 16, 27, 28). The claw bases of legs I-III with small roundish lunules (Fig. 31); on external claws IV (i.e. the hind ones) the lunules are larger and distinctly wider (Fig. 32). Main branches with thin and medium-sized accessory spines. The claws on leg I and IV not much differentiated in size, the main branch of the external claw I and that of claw IV 17.9 and 19.8 µm long, respectively.

Examined only one of two animals found, as the second specimen has been lost (see "Material and Methods"). Sex unknown.

 ${\scriptstyle {\rm Locality}} \quad B{\scriptstyle 1}.$

Remarks The New Caledonian specimen represents distinct species with well defined morphological characters. They are: sculpturing of dorsal cuticle in a form of small and closely located thickenings, a marked tooth in the mouth cavity (dorso-median ridge), three macroplacoids increasing in size posteriorly, legs I-III without cuticular bars at the bases of internal claws and the presence of (small) lunules on claws. The animal resembles *D. taiwanus* Li & Li, 2008, however, no lunules have been found in the latter (Li & Li 2008). Moreover, in its original description no information about the armature of buccal cavity is provided (l. c.). This re-mounted animal might represent an undescribed species. The sole specimen, with some aberrant macroplacoids, does not allow for such a description.

Discussion

Members of six genera (*Bryodelphax, Echiniscus, Pseudechiniscus, Mesobiotus, Minibiotus* and *Doryphoribius*) have been discovered in this survey and each genus is represented only by a

sole species (but two in *Minibiotus*) and mostly characterized also by very low number of the specimens found. The tardigrades occurred only in ca. 20 % of all examined samples and were encountered only in one of five sampled islands of the New Caledonian archipelago, namely Lifou Island. These genera have a world-wide distribution (e.g. Ramazzotti & Maucci, 1983).

All examined tardigrades were extracted from bryophytes or lichens with a strong or weak calcium carbonate contents (the samples from rock habitats vs. those from the bark of trees, respectively). A simple analysis confirming such a content implemented by usage of hydrochloric acid indicates that all examined tardigrades belong supposedly to more or less calciphilous animals (see e.g. Dastych 1980, 1988).

Of all seven species found and described here, none could be reliably identified to a species level. It resulted mainly from the limited number of extracted specimens, the absence of eggs (in *Mesobiotus* and *Minibiotus*, where the morphology of eggs is also a one of the crucial identification criteria), the lack of distinct autapomorphic character state(s) for particular taxon and also the unavailability of their molecular markers. Nevertheless, presumably at least four of all listed here taxa, i. e., *Bryodelphax* cf. *arenosuss, Pseudechiniscus* sp., *Minibiotus* sp. 1 and *Doryphoribius* sp. might represent still undescribed species.

In the recent years, with series of papers which introduced molecular analyse to the taxonomic studies, particularly those at the species level (e.g. Guidetti et al. 2005, 2009, Jørgensen et al. 2007, Cesari et al. 2009), the knowledge of taxonomy, systematics and phylogeny of Tardigrada has been enormously expanded and these areas are now still radically changing. It is particularly well observable in a cascade of numerous important revisions and immensely increasing number of the descriptions of new taxa, especially new species (for review see e.g. Degma et al. 2020). These changes are continuation of previous taxonomic breakthrough based then chiefly on morphological studies. The immense information upswing resulting from those studies was initiated with the papers by Pilato (1969a, 1969b) over 50 years ago.

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