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Psammonobiotus dziwnowi and *Corythionella georgiana*, Two New Freshwater Sand-dwelling Testate Amoebae (Rhizopoda: Filosea)

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Summary. Two new species of the predominantly marine sand-dwelling testate rhizopod genera *Psammonobiotus* and *Corythionella* were discovered in sand beaches of the Canadian Laurentian Great Lakes. The most important differences between *P. dziwnowi* sp. n. its closest relative *P. communis* are (i) much smaller size of *P. dziwnowi*, (ii) greater length-to-width ratio in *P. dziwnowi*, (iii) greater angle of the plane of the oral aperture relative to the long axis of the test of *P. dziwnowi*. The only other known freshwater species of *Corythionella*, *C. golemanskyi* Nicholls, occurred together with *C. georgiana* sp. n. in samples from Wasaga Beach, Georgian Bay (Lake Huron) and enabled a direct comparison of test morphology of both populations occurring under the same environmental conditions. As well as a significantly smaller test size in *C. georgiana*, other differences with *C. golemanskyi* include smaller more elongate scales arranged in a more open non-overlapping pattern on the test surface of *C. georgiana*. Notwithstanding some uncertainties about the taxonomy of *C. acolla*, which is the only one of six marine species of *Corythionella* lacking a flared pseudostomal collar, the differences between it and *C. georgiana*, which has a minimally flared collar, include a generally shorter length, a much broader post-pseudostomal region and greater ratio of test width-to-pseudostome diameter in *C. georgiana*. These differences (ignoring, for the present, possible additional differences in the structure of the scale layer on the tests of *C. georgiana* and *C. acolla*), substantiate the designation of *C. georgiana* as an independent species.

Key words: *Corythionella georgiana* sp. n., Cyphoderiidae, freshwater testate amoebae, Psammonobiotidae, *Psammonobiotus dziwnowi* sp. n., Testaceafilosia.

INTRODUCTION

Although beach sand-dwelling testate rhizopod communities represented by many genera and species have long been recognized in the supra-littoral zones of the world's oceans (e.g. Chardez 1977, Golemansky 1998 and references therein), only recently has this community been investigated in freshwater lakes (Nicholls and MacIsaac 2004). Nicholls and MacIsaac (2004) encountered relatively abundant populations of a small unknown species of *Psammonobiotus* at several locations in the Great Lakes, which they referred to as "sp. # 2". Also, after publication of the of the Great Lakes survey, a previously undescribed species of *Corythionella* was discovered in beach sand samples from Wasaga Beach (Georgian Bay, Lake Huron), one of the original Great Lakes sampling locations included in Nicholls and MacIsaac (2004). This new species is similar in some respects to the only other known freshwater species, *C. golemanskyi* Nicholls (2003), but differs from it and its closest marine relative, *C. acolla* Gol. (Golemansky 1970a) in several ways relating to the shape and struc-

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ture of its test. Test morphology is the primary criterion in distinguishing among several species of both *Psammonobiotus* and *Corythionella* (Meisterfeld 2002). The purpose of this paper is to describe *Psammonobiotus dziwnowi* and *Corythionella georgiana* as two new members of the Testaceafilosia inhabiting freshwater beach sands.

MATERIALS AND METHODS

All field and laboratory methods were as previously described in Nicholls and MacIsaac (2004). Test dimensions and terminology were as portrayed in Figs 1 and 15. Statistical data describing test morphology (mean, standard deviation, median, minimum, maximum, coefficient of variation) and Mann-Whitney *U*-tests of significant difference between populations were done in *CoStat* (CoHort Software 1995) with reference to Neave (1981) for levels of significance.

In a Wasaga Beach (Georgian Bay of Lake Huron) sample containing both *Corythionella golemanskyi* and a smaller unidentified *Corythionella* (described herein as a new species), an objective measurement routine was set up to characterize each morphologic entity as follows: (i) a subsample was prepared from a few drops of the original sample mixed together with several drops of glycerine on a 22 × 50 mm No. 1 cover glass positioned on the stage of an inverted microscope; (ii) the subsample was allowed to evaporate over night to concentrate the specimens in 100% glycerine. This viscous medium enabled a more stable orientation of the specimens for height measurements and observation of optical cross-sections of tests after manipulation into position with a single hair brush; (iii) the subsample was searched systematically in a grid pattern using a 10× objective and dark ground illumination; (iv) all specimens of the two morphotypes were measured as they were encountered during the search regardless of which size group they were in; (v) after plotting test lengths *vs* widths of 37 measured specimens, it was apparent that there existed two distinct size categories, and the measurements were then grouped into the two groups, A (larger) and B (smaller); (vi) measurements were made on three additional randomly selected group B specimens to achieve a total of 20 measured specimens in each size group for more detailed statistical analysis.

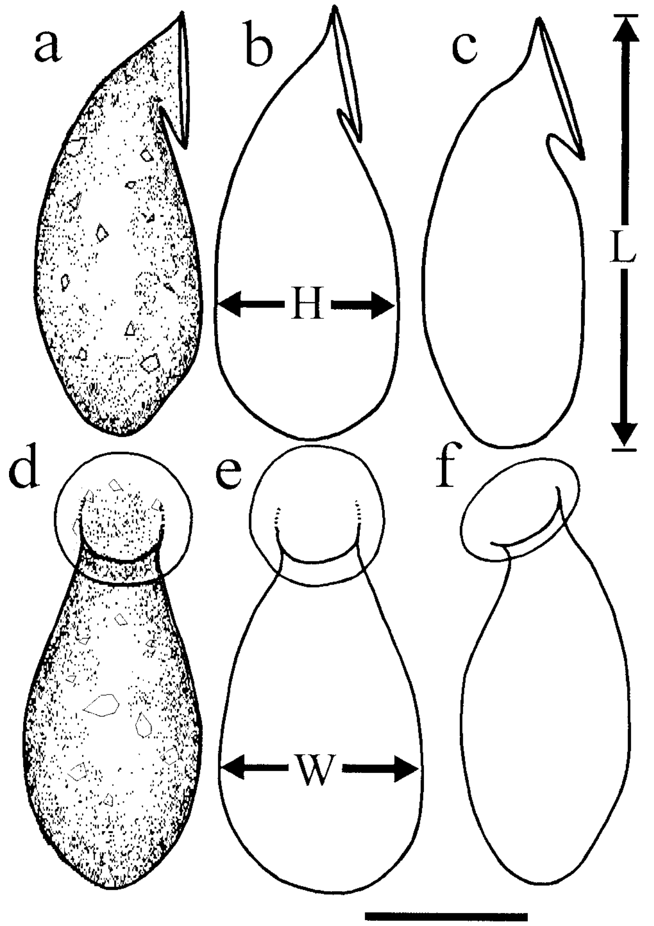


Fig. 1. Drawings of tests of *Psammonobiotus dziwnowi* sp. n. [from Nicholls and MacIsaac (2004; their "sp #2") with permission from *J. Great Lakes Res.*]. **a-c** - morphological variation of three tests in lateral views. **d-f** - morphological variation of three tests in ventral views. L, H, and W - test length, height, and width, respectively. Scale bar 10 µm.

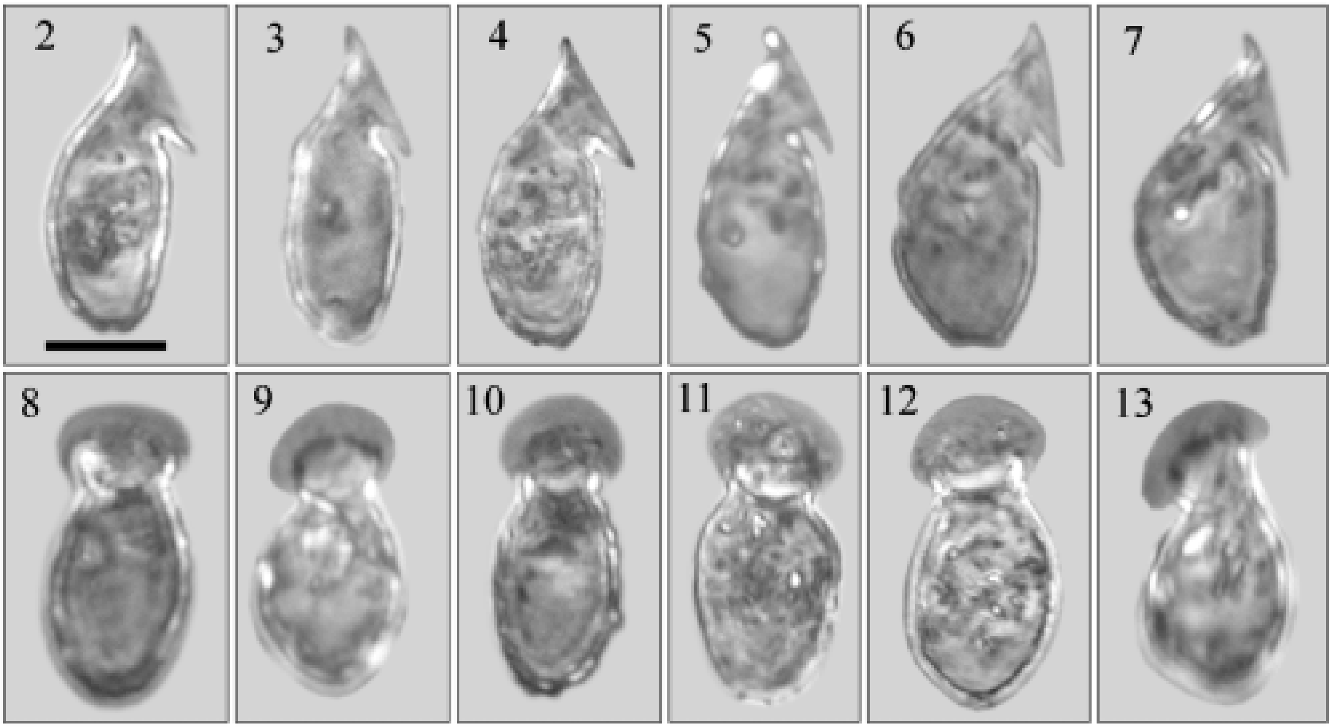
RESULTS

Psammonobiotus dziwnowi sp. n.; Figs 1-13

Phylum: Rhizopoda, Class: Filosea, Subclass: Testaceafilosia, Family: Psammonobiotidae.

Diagnosis: Protoplast was similar to that known for *Psammonobiotus communis* and emerged from the pseudostome as a single (less frequently, two, branched) long, filose pseudopod. Test shape was elongated-elliptical in outline in ventral and dorsal views and asymmetrically oblong-ovate in lateral view with a rounded dorsal edge and slightly flattened ventral edge. Median test length, width and height were 23, 11 and 10 µm, respectively (N=63). The oral aperture (pseudostome) was at the end of a bent neck-like anterior extension of the test and was surrounded by a flared collar. In ventral and dorsal views, the test body was always wider than the pseudostome collar. The test was covered with small, mainly angular, polymorphic and flat particles more easily seen in dried specimens impregnated with Canada Balsam or Styrax®. In lateral view, the angle of the plane of the pseudostome collar ranged from near zero degrees to about 45°.

Etymology: The specific epithet (*dziwnowi*) is derived from the name of the Polish town of Dziwnów on



Figs 2-13. Digital images of tests of *Psammonobiotus dziwnowi* sp. n. [from Nicholls and MacIsaac (2004; their "sp. #2") with permission from *J. Great Lakes Res.*]. **2-7** - lateral views showing range in form; **8-12** - ventral views showing range in form; **13** - a specimen in oblique-ventral view. Scale bar 10 µm applies to all figures.

the southern Baltic Sea, in the vicinity of which the first specimens of this species were apparently collected by Dr. V. Golemansky (see below). An approximate phonetic English pronunciation of the specific epithet is "dvee-nov-ee", with the accent on the second syllable.

Type specimen: The type specimen, mounted in Canada Balsam on a glass slide, was deposited with the Canadian Museum of Nature (Ottawa, Ontario, Canada) under catalogue No. CMNI-2005-0001.

Material from type locality: Retained by the author in sample No. V-1706, collected 4 September, 2002.

Type locality: Beach sand in the wave zone (supralittoral) at Providence Bay, south shore of Manitoulin Island, Lake Huron (45° 40' 0" N; 82° 16' 11" W).

Test measurements (Table 1) on 63 specimens [Nicholls and MacIsaac's (2004) original 47 + 16 additional specimens] did not change the median length, width and height of tests reported by Nicholls and MacIsaac (2004) for *P. dziwnowi* (their "sp. #2"). Using the size data summarized in Nicholls and MacIsaac's (2004) Table 2, the differences between *P. dziwnowi* and *P. communis* in their Rondeau Bay, Lake Erie sample, where both species were relatively abundant, were highly significant for all test dimensions (Mann-Whitney *U*-tests; $P<0.001$). Measurements of tests from the other Great Lakes locations were not made systematically; however, at none of the seven other locations where both *P. communis* and *P. dziwnowi* were found together was there any overlap in test size or shape.

There was some variation in the test shape of this species (Figs 1-13). Most notable was the angle of the plane of the pseudostomal aperture (compare, for example Figs 4 and 6). The main body of the test also showed some differences in shape, ranging from nearly cylindrical (Fig. 3) to those with very rounded dorsal surfaces (Figs 6, 7) and intermediate forms (Figs 2, 4, 5). The most typical ventral profiles found were like those in Figs 1d, 8, 10 and 11. Unusually wide tests (Fig. 9) or those with somewhat pointed aboral ends (Fig. 12) were less frequently encountered.

The most important differences between *P. dziwnowi* and *P. communis* can be summarized as (i) much smaller

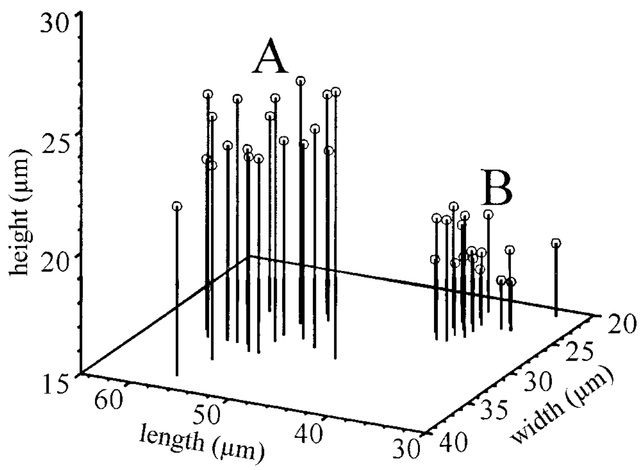


Fig. 14. Test dimensions in 20 specimens of *Corythionella golemanskyi* Nicholls (A) and *C. georgiana* sp. n. (B) co-existing in the same sample of Georgian Bay beach sand. Some specimens had measurements identical to those determined for other specimens, so they were not revealed in this plot (because of overlap).

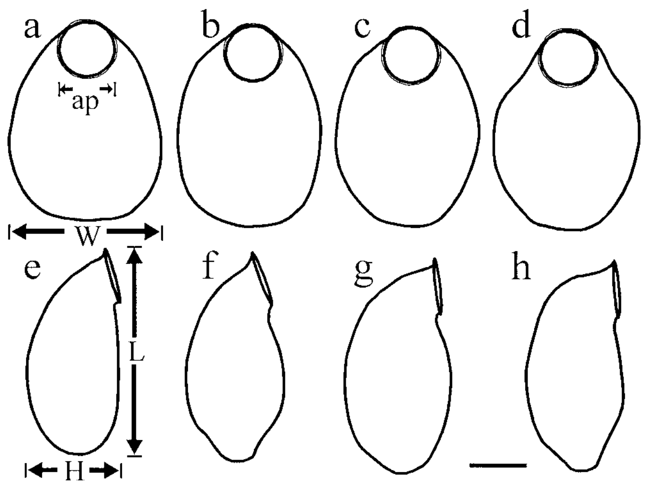


Fig. 15. Form variation in tests of *Corythionella georgiana* sp. n. **a-d** - ventral views of four tests; **e-h** - lateral views of four tests. L, W, H, and ap - test length, width, height and pseudostomal aperture diameter, respectively. Scale bar 10 µm.

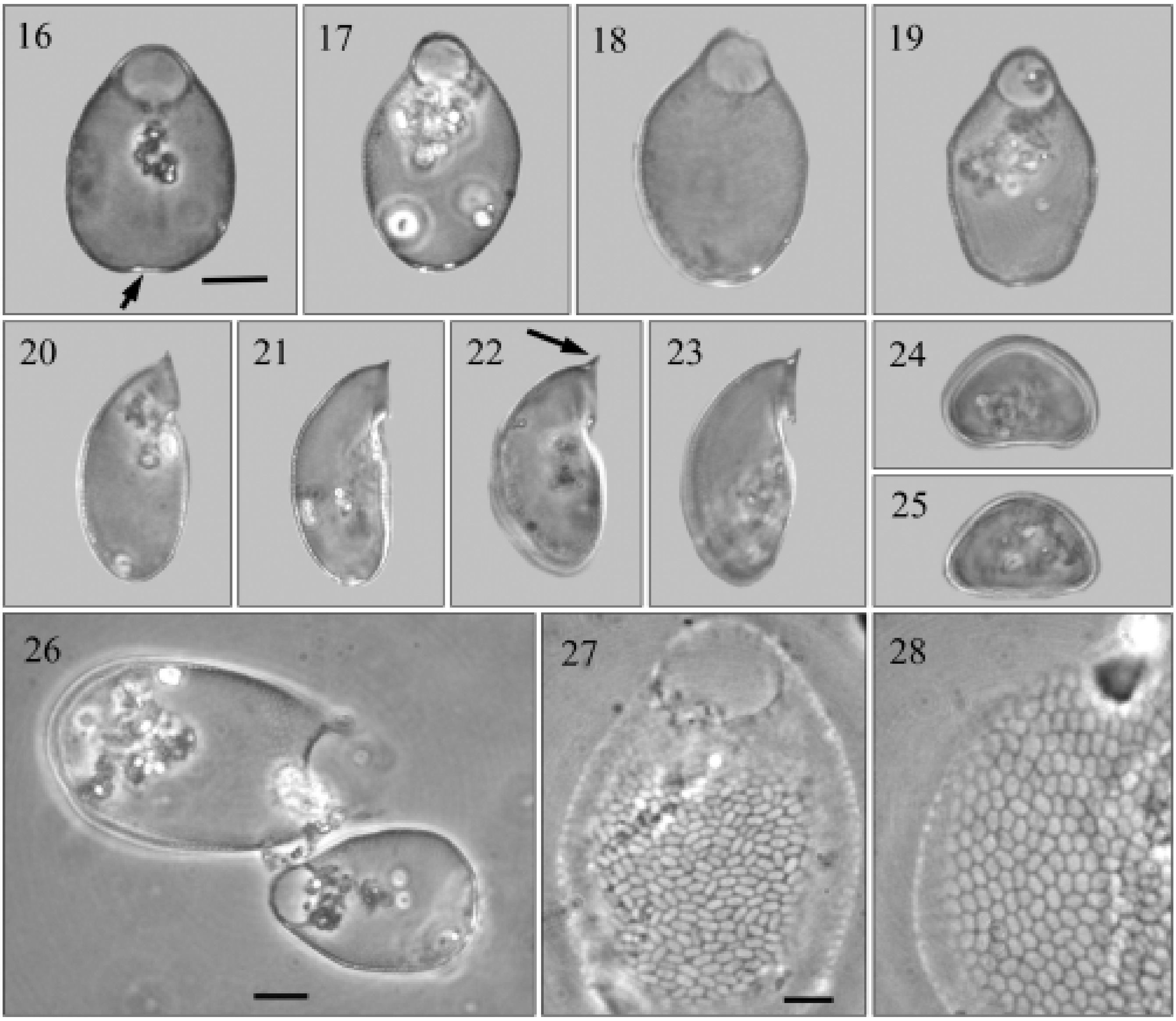
size of *P. dziwnowi*, (ii) greater length-to-width ratio in *P. dziwnowi*, (iii) *P. dziwnowi* shows much more variability in the angle of the plane of the oral collar relative to the long axis of the test, ranging from zero to about 45° (in contrast to zero to about 5° in *P. communis*). No *P. dziwnowi* tests with nearly circular outlines in ventral or dorsal views post-pseudostomal region were found; such shapes, although not common, were found occasionally in *P. communis* (see Nicholls and MacIsaac's Fig. 3e). Although it could not be measured reliably on all specimens for which the other morphologic contrast data

Table 1. Test size in *Psammonobiotus dziwnowi* sp. n. (N = 63), *Corythionella georgiana* sp. n. and *Corythionella golemanskyi* (N = 20 each). The diameter of the pseudostomal aperture in *P. dziwnowi* could not be clearly determined in many specimens, so this variable was not included in the descriptive statistics for this species. Definitions of L, W, H and ap are as depicted in Figs 1 and 15. Test measurements (in µm) for 47 of the 63 specimens of *P. dziwnowi* were included in Nicholls and MacIsaac (2004; their "sp. #2").

	length (L)	width (W)	height (H)	ap	L/W
P. dziwnowi					
median	23	11	10		2.2
minimum	21	9	9		1.7
maximum	27	14	12		2.6
mean	23.4	11	10.2		2.1
st.dev.	1.4	1	0.8		0.2
coef.var.(%)	5.9	8.8	8.1		7.5
C. georgiana					
median	39	24.5	18	10	1.56
minimum	33	21	17	9	5.7
maximum	41	27	20	11	1.8
mean	38.4	24.6	18.5	9.7	1.6
st.dev.	2	1.6	1.1	0.6	0.1
coef.var.(%)	5.2	6.7	5.8	6	5.7
C. golemanskyi					
median	54.5	32	23	12	1.7
minimum	46	26	22	11	1.4
maximum	60	38	26	13	2.1
mean	54.2	31.4	23.6	12	1.7
st.dev.	3.4	2.9	1.2	0.7	0.2
coef.var.(%)	6.3	9.4	5.1	6.1	9.9

Table 2. Mann-Whitney *U*-test values in tests of statistical difference between the median morphometric descriptors of Group A specimens (*Corythionella golemanskyi*) and Group B specimens (*Corythionella georgiana* sp. n.) in Fig. 14. The null hypothesis, that the medians of the two groups are not different (i.e. are drawn from the same "population"), was rejected for all *U* values < 127 (2-tailed $\alpha = 0.05$; Neave 1981).

Median	Group A	Group B	*U*-value
L	54.5	39	0
W	32	24.5	7.5
H	23	18	0
ap	12	10	2.5
L/W	1.7	1.6	67
L/H	2.3	2.1	199
W/H	1.3	1.4	165



Figs 16-28. Digital images of tests of *Corythionella georgiana* sp. n. **16-19** - images of four different specimens in ventral views (the arrow in Fig. 16 shows the aboral thickened depression in the test wall); **20-23** - images of four different specimens in lateral views (the arrow in Fig. 22 shows the small anterior flare of the pseudostomal collar; **24-25** - optical cross-sectional images of two different specimens; **26** - test of *Corythionella golemanskyi* (larger of the two) beside a test of *C. georgiana* illustrating size difference (scale bar 10 µm); **27** - scales on the test surface of *C. georgiana*; **28** - scales on the test surface of *C. golemanskyi*. Scale bars 10 µm (16 applies to 16-25, 26); 5 µm (27 applies to 28).

were obtained, the diameter of *P. dziwnowi*'s oral collar was usually in the range of about 8-10 µm, in contrast to that of *P. communis* which generally ranged between 15 and 20 µm.

***Corythionella georgiana* sp. n.**

Phylum: Rhizopoda, Class: Filosea, Subclass: Testaceafilosia, Family: Cyphoderiidae

The measurements of *Corythionella* tests in the Wasaga Beach sample clearly revealed the existence of two well defined groups (Fig. 14), one of which was *C. golemanskyi* since it conformed in size and shape to the original description of this species (Nicholls 2003). The other was clearly a distinct morphological entity that differed in other significant aspects of test form as well as length, width and height. It was for those reasons that a formal description of this taxon was needed.

Diagnosis: Tests were translucent, and colourless to pale yellow in colour, elongate-elliptical to nearly circular in outline (ventral and dorsal views). Test length, width and height were 33-41 µm, 21-27 µm and 17-20 µm, respectively. Tests had a rounded-triangular outline in optical cross-section. The pseudostomal aperture (9-11 µm) was separated from the main body of the test by a short bent neck having a slightly flared anterior rim (not readily observed in some specimens). Tests were covered with small (0.7-0.9 × 1-2.3 µm) elongated silica scales. Scales were randomly arranged with no overlapping of their margins. Protoplast with long, sometimes branching filose pseudopodia.

Etymology: The specific epithet is derived from the sample collection location (Georgian Bay of Lake Huron) where this species was relatively abundant.

Type specimen: The type specimen mounted in Canada Balsam on a glass slide, was deposited with the Invertebrate Zoology Division, Canadian Museum of Nature (Ottawa, Ontario, Canada), Catalogue No. CMNI-2005-0002.

Material from type locality: Retained by the author in sample No. V-1854, collected 2 September, 2002 as a composite of four samples of supra-littoral Wasaga Beach sand.

Type locality: Beach sand in the wave zone (supra-littoral) at Beach Number 2, Wasaga Beach, Georgian Bay, Lake Huron. Ontario, Canada (44° 29' 18" N; 80° 04' 17" W).

Variability in *C. georgiana* test dimensions was low; coefficients of variation for all size variables were all <7% (Table 1). Variability in test shape was also low with all specimens examined exhibiting a broadly rounded elliptical outline in ventral views (Figs 15a-d, 16, 17). Some tests also showed some minor constriction of the anterior region of the test immediately posterior to the pseudostome (Figs 15d, 18, 19). Others revealed a small depression and thickening in the test wall at the aboral end of the test (Fig. 16, arrow). In lateral views (Figs 15e-h, 20-23), there were also several different variations in form identified. The most commonly observed shape was a smooth rounded form (Figs 15e, 20), but variations included those with a flattening on both the dorsal and ventral surfaces near the posterior of the test. This resulted in a "pinched" appearance in lateral views (Figs 15f, g). Others showed a slightly more "hump-backed" appearance in the anterior-dorsal region (Fig. 15h) or in the mid-dorsal region of the test (Fig. 22). The angle of the plane of the pseudostomal aperture with the long axis of the test was also variable among specimens, ranging from a minimum of about -5° (Fig. 23) to a maximum of about +20° (Figs 15e, 20). Some specimens (Fig. 22, arrow; Fig. 23) revealed more flare in the pseudostomal collar, especially along the anterior margin, than others (e.g. Fig. 21). Little variability was seen in the optical cross-sectional views of the test of this species; all tests were wider than high and were in the form of rounded triangles (Figs 24, 25).

The size difference between *C. georgiana* and *C. golemanskyi* was highly significant for all test dimensions except the length-to-height and width-to-height ratios (Table 2). When specimens of each species were together in the same microscopic field of view (Fig. 26), such differences were immediately apparent. These species also differed significantly in the structure and arrangement of scales covering their tests. The scales of *C. georgiana* (0.7-0.9 × 1-2.3 µm; Fig. 27) were smaller and more elongate than those of *C. golemanskyi* (1-1.3 × 1.5-2.7 µm; Fig. 28). In addition to the size and shape differences, the scales of *C. georgiana* were also more loosely arranged and only occasionally overlapped at their margins, whereas those of *C. golemanskyi* were always densely arranged with scale edges overlapping those of adjacent scales.

DISCUSSION

Specimens fitting our description of *P. dziwnowi* were reported by Golemansky (1973) from samples of beach sand collected in 1969 near the SW Baltic Sea town of Dziwnów in Poland. Golemansky found only a few empty tests of this species, which, because of the absence of *P. communis* in the same sample, he believed might be a small atypical form of *P. communis*. There have been no other reports of this taxon in the intervening three decades (V. Golemansky, Zoological Institute, Sofia, Bulgaria, personal communication, December 14, 2002). With the discovery of *P. dziwnowi* in abundance at several Great Lakes beaches, including some where *P. communis* was also abundant (Nicholls and MacIsaac 2004), there can no longer be a question of its possible identity as *P. communis*. The major reasons for this include the significant differences between the dimensions and shapes of the tests of these two species coexisting under the same environmental conditions. Differences of the magnitude reported here in test shape and size observed in widely separated populations of these two species are therefore unlikely to be related to possible environmental effects on growth and development of the tests of these species.Among the test shape variables most discriminating between these two species was the very low angle of inclination of the plane of the pseudostomal aperature in *P. communis* and the contrasting greater angle *P. dziwnowi*.

Because *Corythionella acolla* Golemansky has a test length about equal to that of *C. georgiana*, and because of the nearly imperceptible flare on the rim of the pseudostomal aperture in *Corythionella georgiana*, its morphology must be discussed with reference to *C. acolla*, the only one of six marine species of this genus that lacks a flared rim. All but one of the published illustrations of *C. acolla* (Golemansky 1970b, 1973; Chardez 1971, 1977; Golemansky and Todorov 1999) are in mutual agreement with regard to general shape of the *C. acolla* test, although Golemansky (1973) illustrated a lateral view of a specimen with a slight flare along the posterior edge of the pseudostomal rim. Only Chardez (1977) included an illustration that is not in agreement with the others; his portrayal of this taxon shows a test with a curiously wide rim surrounding the pseudostome and relatively large scales on the surface of the test that were oriented more or less at right angles to the long axis of the test. Chardez (1977) also listed the test length of this *C. acolla* as 23-28 µm, well below the 33-68 µm given by Golemansky (1971). The other illustration provided by Chardez (Chardez 1971) illustrates densely arranged, very elongate scales oriented mainly along the long axis of the test. The other published depictions of this species illustrate smaller, more tightly arranged scales, but here too there are inconsistencies; compare, for example, the very small densely arranged scales in Golemansky (1970b) with the larger much more loosely arranged scales in Golemansky and Todorov (1999). None of the published illustrations of test scales in *C. acolla* is similar to that found for *C. georgiana*, although some of the previously published illustrations may be somewhat schematic and lacking in detail (no photographic images have been yet published for *C. acolla*).

With the exception of Chardez's (1977) illustration, the other published shapes of the test of *C. acolla* are in good agreement. In ventral view, all show a large pseudostomal aperture equal to about 1/2 the test width (in *C. georgiana* test width equals about 2.5 × aperture diameter) and a gradual widening of the test below the pseudostome towards the posterior one-half of the test. This shape is in marked contrast to that seen in *C. georgiana* which has a much wider test immediately posterior to the pseudostome.

In summary, there may be more than one taxon represented in the published literature under the *C. acolla* binomen. New reports of this species need to define more rigorously the range of form variation and provide photographic detail of the scale covering on the test. In particular, some further research on the taxonomy of *C. acolla* in view of its lack of a flared apertural collar is needed. Because all other species of the genus have a flared collar (but is reduced in the freshwater species *C. golemanskyi* and much more reduced in *C. georgiana*), all or part of what has been considered to be *C. acolla* in the past may have to be transferred to another genus defined, in part, by a non-flared apertural collar.

Notwithstanding the uncertainties surrounding the taxonomy of the marine *C. acolla*, the differences between it and *C. georgiana* in test size and shape (ignoring, for the present, possible additional differences in the structure of the scale layer on the test), are entirely adequate in my view to validate *C. georgiana* as an independent species. As well, the case for its independence from the only other known freshwater species, *C. golemanskyi*, has been clearly established on the basis of test morphology, and the microstructure of the test scale layer.

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