

Cryptodiffugia leachi n. sp., a Minute New Testate Rhizopod Species (Rhizopoda: Phryganellina)

Kenneth H. NICHOLLS

S-15 Concession 1, RR # 1 Sunderland, Ontario, Canada

Summary. A previously undescribed, very small species of *Cryptodiffugia* has been discovered in a calcareous wetland on the Niagara Escarpment, a significant geologic feature in southern Ontario, Canada. *Cryptodiffugia leachi* n. sp. is characterized by its very small size, elliptical outline in optical cross-section (contrasting it with the similarly-sized *C. minuta* Playfair, which is strongly narrowed in the anterior pseudostomal region) and the presence of crystalline inclusions in the anterior region of the cell. The abundance of specimens in the collection afforded study of both living and encysted specimens.

Key words: *Cryptodiffugia*, *Diffugiella*, testate amoebae.

INTRODUCTION

The genus *Cryptodiffugia* [sensu Page (1966) and Hedley *et al.* (1977)] includes some 20 species of amoeboid protists housed in mostly small (< 30 µm long), smooth-walled tests. Until now, the smallest known species of *Cryptodiffugia* have included three species described by Playfair (1918) from Australia having test lengths in the range of 10-14 µm, a size that is similar to that found for *C. leachi*, the new species described here. The cylindrical, flask-shaped or angular shapes of the tests of those three Australian species

are, however, in marked contrast to the smooth elliptical-ovoid shape described here for *C. leachi*. Although test size and shape are the most important criteria for distinguishing species of this genus [see also Beyens and Chardez (1982) and Bobrov (2001) for accounts of more recently described species of *Cryptodiffugia*], other details of the live cell of *C. leachi*, notably pseudopod structure and behaviour, encystment structures and crystalline inclusion bodies have also been documented for this new species.

MATERIALS AND METHODS

Samples were collected from an extensive wetland complex at the base of limestone cliffs forming part of the Niagara Escarpment in an area known locally as "The Glen", located 10 km NNW of the City of

Address for correspondence: Kenneth H. Nicholls, S-15 Concession 1, RR # 1 Sunderland, Ontario, Canada L0C 1H0; E-mail: khnicholls@interhop.net

Owen Sound, Ontario, Canada (44°35'50"W; 80°55'50"N). Three samples of bottom sediments were collected by submerging wide-mouthed 500 ml polycarbonate bottles in the shallow water (<0.5 m depth) along the eastern edge of The Glen. These samples were combined into a single sample consisting of about 20-50 ml of loose organic sediment and about 400 ml of water. Samples were returned to the laboratory and examined periodically over the ensuing several days and weeks.

Subsamples of the collections, consisting of several drops of the sediment suspension placed on a 23 × 50 mm No. 1 cover glass, were examined with an inverted microscope. Measurements of tests were made with a ×100 oil immersion objective and a ×10 WF relay lens in the camera port, utilizing a pre-calibrated transparent ruler applied to the LCD monitor of the digital camera. Application of the 4 × digital zoom feature of the camera afforded a view of the image in the camera LCD with a magnification of about 3100×, from which the measurements were attained. For permanent slide preparations, including a specimen selected as the type of the new species described here as *Cryptodiffugia leachi* n. sp., isolations were made with a single hair brush and a micropipette drawn out in a flame to a working apex of about 100 µm ID. Specimens were air dried on No. 1 cover glasses and cemented to glass microscope slides with Canada Balsam.

RESULTS

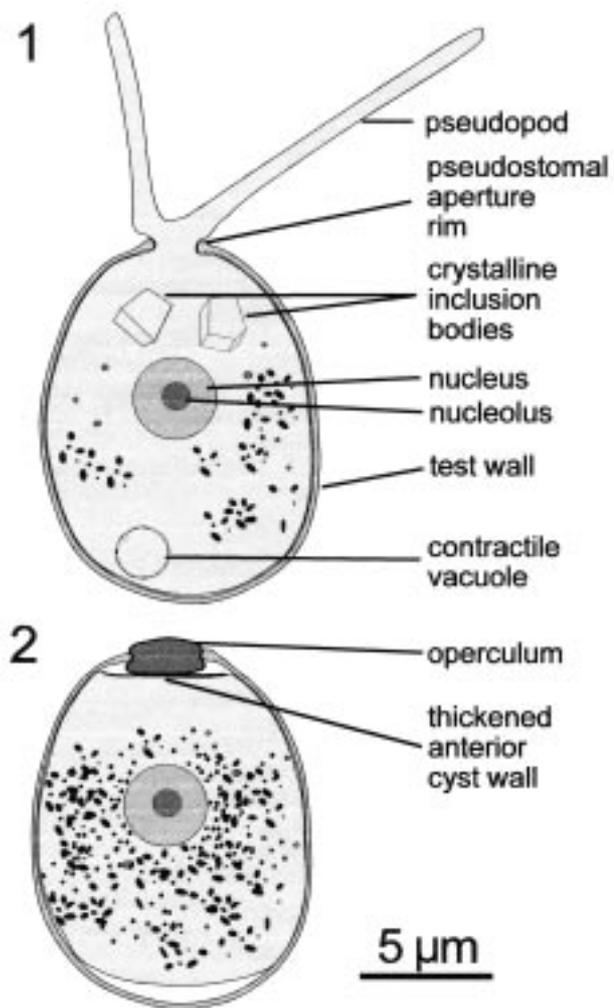
Cryptodiffugia leachi n. sp., Figs 1-14

Phylum: Rhizopoda, Class: Lobosea, Suborder: Phryganellina, Family: Cryptodiffugiidae.

Diagnosis: Cell with a centrally located nucleus about 4 µm in diameter and a single nucleolus 1.5 µm in diameter; a single contractile vacuole, 2-3 µm diameter, is located in the posterior region of the cell, surrounded by many small refractive granules and particles. Many cells also contain one or two large (up to 4 µm) crystalline inclusions in an antero-central position. Pseudopodia (1-3) long, straight and narrow, emanating from a thick-walled, transparent test. Tests with a smooth surface and an oval-elliptical shape in optical cross-section; 10-16 µm long by 8-12 µm wide, with a pseudostomal aperture 1.5-2.5 µm in diameter.

Etymology: The specific epithet (*leachi*) is in honour of Dr. J. H. Leach, who introduced me to the genus *Diffugia* in Lake Erie in 1970 - an exposure to testate rhizopods that only recently has developed into an important research interest.

Type specimen: The type specimen mounted in Canada Balsam on a # 1 cover glass and standard microscope slide, was deposited with the Invertebrate Zoology Division, Canadian Museum of Nature (Ottawa, Ontario, Canada), Catalogue No. CMNI-2006-0011.

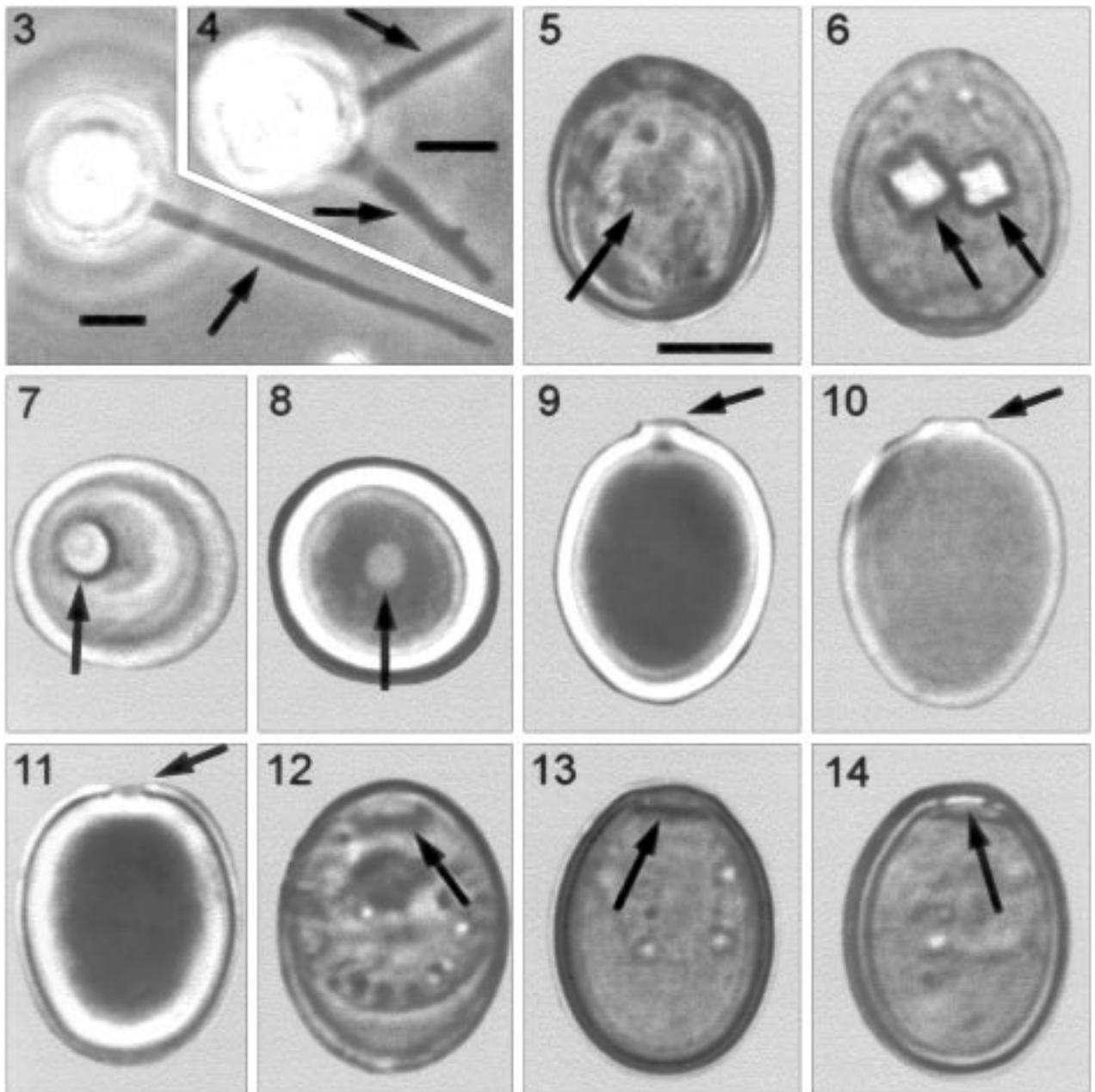


Figs 1, 2. Diagrammatic representations of *Cryptodiffugia leachi* n. sp. **1** - a free-living (non-encysted) cell housed in rigid thick-walled test with main cell components labelled. **2** - encysted cell showing the operculum and the thickened cyst wall.

Material from type locality: A preserved sample of aqueous sediment containing specimens of *C. leachi* collected May 25, 2002 was retained by the author as sample No. V-1919.

Type locality: An extensive wetland at the base of the Niagara Escarpment, an area known locally as “The Glen”, 10 km NNW of the City of Owen Sound, Ontario, Canada (44°35'50"W; 80°55'50"N).

With median test length, width and pseudostomal diameter of only 13, 10 and 2 µm, respectively (Table 1), *C. leachi* ranks among the smallest of the testate amoebae. Other distinctive features of living specimens include the narrow, cylindrical pseudopodia, which were



Figs 3-14. Digital images of *Cryptodiffugia leachi* n. sp. **3, 4** - living cells showing pseudopodia (arrows); **5** - cell within test showing anterior-central location of the nucleus (arrow). **6** - cell within test showing presence of two large crystalline inclusions (arrows). **7, 8** - polar views of tests showing the pseudostomal aperture (arrow). **9, 10** - two specimens with a slightly raised rim surrounding the oral aperture (arrow). **11** - lateral view of a test lacking apertural rim (arrow). **12** - early development of the anterior cyst wall (arrow). **13** - later development of anterior cyst wall (arrow) and operculum in pseudostomal aperture. **14** - operculum (arrow). Scale bars: 5 μ m.

never observed with major branching, but sometimes with small “buds”. Pseudopodia with rounded terminal apices extended up to 3-times the test length (Figs 1, 3, 4). Present also were one or two large refractive

crystal-like bodies in the anterior region of many cells (Figs 1, 6). The thick-walled test was generally elliptical in optical cross-section (Figs 6, 10, 13) with a small pseudostomal aperture, 1-2.5 μ m in diameter (Figs 7, 8,

Table 1. Test size in *Cryptodifflugia leachi* n. sp.

	median	minimum	maximum	mean	SD	CV (%)
Total test length	13	10	16	13.1	1.4	11.1
Test diameter at the widest part of the test	10	8	12	10	1.1	10.6
Internal diameter of the oral aperture	2	1	2.5	1.8	0.6	30.2

All measurements in μm , $n = 28$, SD - standard deviation, CV - coefficient variation.

11). In some specimens the aperture was bordered by a slightly raised rim (Figs 9, 10); in others this rim was lacking (Fig. 11). Encysted forms were easily identified by the simultaneous development of a thickened plug, or operculum, in the pseudostomal aperture and a thickening of the anterior cell membrane posterior to the operculum (Figs 2, 12-14).

DISCUSSION

The small size of *C. leachi* n. sp. sets this species apart from most other known species. A comparison with *C. oviformis* Penard in particular is useful because of its common and cosmopolitan occurrence. Hedley *et al.* (1977) reviewed the taxonomy and biology of the genus *Cryptodifflugia* and concluded that Page's (1966) *C. operculata* was a synonym of Penard's (1890, 1902) *C. oviformis* based on cultures of both taxa and the discovery that encysted forms of both may possess an operculum - an organic plug-like structure that occludes the oral aperture of the test. The presence of an operculum in *C. operculata* had been proposed by Page (1966) as the main feature distinguishing these two species. The presence of an operculum in *C. leachi* n. sp., suggests that this structure may be a unifying feature within the genus, but the life histories of other *Cryptodifflugia* species need to be studied before such a statement can be made with any degree of certainty.

The shape of the test of *C. leachi* n. sp. is elliptical in outline in contrast to the ovoid shape of *C. oviformis* where the posterior 1/3 is hemispherical and the anterior 1/3 is more conical in shape. Hedley *et al.*'s (1977) measurements of 100 specimens of *C. oviformis* revealed a range in test length of 14.5-22.2 μm , a width of 12.8-17.6 μm , and an apertural diameter of 3.2-6.4 μm . In an independent set of measurements of this species (but reported as *C. operculata*), Page (1966) found test lengths of 15.2-24.2 μm , widths of 13.8-20.7 and apertural

diameters of 3.4-6.9 μm . His averages for test length and width were 17.6 and 15.7 μm , respectively. Thus, the mean test length (13.1 μm) and mean test width (10.0 μm) of *C. leachi* n. sp. are smaller than the smallest *C. oviformis* tests. Similarly, the maximum apertural diameter found for *C. leachi* n. sp. was smaller than the largest aperture measured in previous studies of *C. oviformis* and further emphasizes the overall consistent and significant size difference in the tests of these species.

Hedley *et al.* (1977) determined by EM X-ray analysis that the test wall of *C. oviformis* was comprised of a thin outer organic layer and a much thicker inner, non-crystalline calcareous layer, possibly dominated by amorphous calcium phosphate (previous authors had presumed the wall to be "chitinous"). They found large mainly spherical calcareous inclusions within some mitochondria that may have some homologous relationship to the large crystal-like bodies found in the cytoplasm of many *C. leachi* n. sp. specimens. If calcareous, these crystals may serve in young thinner tests as a reservoir of calcium for reinforcement (thickening) of the test wall.

Among other small species of *Cryptodifflugia*, three species from Australia described by Playfair (1918) are comparable in size to *C. leachi* n. sp. One, *C. pusilla* Playfair, 1918, has tests 10-12 μm long but with nearly parallel sides; another, *C. minuta* Playfair, 1918 has test lengths of 10-13 μm , but is flask-shaped (markedly narrowing toward the anterior end); the third, *C. angulata* Playfair, 1918, is 13-14 μm long but has an angular shape in longitudinal view. All of these distinctions based on test shape were judged to be valid criteria for separation of *Cryptodifflugia* species as manifested in Page's (1966) review of the taxonomy and key to species.

Although there can be no confusion of living specimens of *Cryptodifflugia leachi* n. sp. with small species of related testate rhizopod genera such as *Microgromia* and *Difflugiella* with their distinctive

anastomosing filose pseudopodia, empty tests of these species might pose identification problems. The shape of the test of *Microgromia levipes* Penard, 1904, for example, resembles more that of *C. leachi* n. sp. than *C. oviformis*, but the average size given by Penard (1904), L = 18 µm; W = 15 µm, is within the range reported for *C. oviformis* (significantly larger than tests of *C. leachi* n. sp.). The test dimensions of *Diffugiella collum* Chardez, 1971 have been reported in two different size categories (i) 16-18 × 13-14 µm, with a pseudostome diameter of 5-6 µm (Chardez 1971) or (ii) 10-12 × 7-8 µm with a pseudostome diameter of 2.5-3 µm (Chardez and Thomas 1980). The latter specimens may represent a different species as judged by the much smaller size and the fact that they were collected in a seawater habitat, as opposed to a freshwater habitat for the original (Chardez 1971) specimens. In both cases, however, the tests of *D. collum* have a pseudostome situated at the apex of a short neck or collar - a structure lacking in *C. leachi* n. sp.

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