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Observations on a *Geocentrophora* sp. (Lecithoepitheliata) flatworm from forest soils in Nova Scotia

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Abstract

The role of these free-living terrestrial flatworms in the soil food web is poorly known. We isolated individuals of a 1.5 mm long *Geocentrophora* sp. from forest soil samples near and about Halifax in Nova Scotia. The SSU rDNA sequence indicates 97% ML bootstrap support with *Geocentrophora* de Man, 1876, in the family Prorhynchidae Hallex, 1894, order Lecithoepitheliata Reisinger, 1924. We tried to determine the diet and role of the species in forest soil. The isolates were kept in the laboratory over several months, while fed a variety of diets. The only observed feeding strategy was on decomposing organic matter, but individuals could be maintained on agar plates with nematodes or amoebae. We did not observe any form of reproduction. The species somehow survives the frozen soil through winter months. These observations extend our scarce knowledge of the natural history of these flatworms in forest habitats.

Keywords: Flatworm, forest soil, *Geocentrophora*, *Lecithoepitheliata*, phylogeny, *Platyhelminthes*

Introduction

Terrestrial free-living flatworms are known to occur in Terricola (Tricladida), Prorhynchidae (Lecithoepitheliata), and Provorticidae (Rhabdocoela) (Yeates et al. 1997; Carbayo et al. 2002; Baguña and Riutort 2004). However, terrestrial free-living isolates are rarely sampled or described outside the Terricola species. They are relatively unknown from North American terrestrial soils. Those that are known are introduced species (Ogren and Kawakatsu 1998). We have encountered several times in our forest soil samples, one species of *Geocentrophora* de Man, 1876 which seems to be common in the forests around Halifax, Nova Scotia. Very little is known about the role of these small Lecithoepitheliata genera in the soil, and standard soil ecology texts tend to neglect them (Adl 2003; Coleman et al. 2004). We report observations on feeding preferences of this isolate, as well as the sequence of its SSU rDNA along with a phylogeny of existing Rhabditophora clades.

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Methods

Study site

The soil samples were collected from mixed coniferous forests around Halifax, Nova Scotia (44°53'N, 63°31'W, elevation 145 m), from A horizon soil. Samples were obtained between May and October 2003 and again in 2004. The sampling locations were at Point Pleasant Park, Purcell's Cove, and Regina Terrace Park. The mean annual temperature is 6.3°C and the mean annual precipitation is 1452 mm of which 231 mm is snow. The soil is a Gibraltar series, well-drained, coarse-textured sandy loam, derived from granite, classified as an Orthic Humo-Ferric Podzol.

Sampling and isolation

Soil was collected with a 5 cm diameter sampler to 10 cm depth, and intact cores were returned to the laboratory and extracted immediately. The flatworm specimens were isolated by sifting through soil placed in Petri dishes and flooded with water. About 2 g soil was placed in deionized water in a 10 cm Petri dish. The process was repeated with 10 subsamples from each core. Field abundances were estimated from the mean number of flatworms per soil core, using the nearest neighbour method and solving for density (Krebs 1998). On separate occasions, the flatworm was also found in Baermann funnel extractions for nematodes (Coleman et al. 1999). The specimens were hand-picked by micropipette and transferred on to agar plates (1.5% agar in distilled water, no nutrients). Alternatively, the specimens were kept in a Petri dish filled with loose forest soil and kept moist with deionized water.

Feeding study

Feeding preferences were assayed by placing one or more specimens in the presence of selected food items. Each feeding trial was independently replicated with new individuals at least three times. Organisms offered as food included bacteria, nematodes, fungal hyphae, enchytraeids, and amoebae. Bacterivorous nematodes were grown in Petri dishes with 1.5% agar and a thin lawn of bacteria. The mixed bacteria were previously cultured from the same soil samples on wheat grass medium (Adl et al. 2006). Fungivorous nematodes (*Aphelencus* spp.) were cultured on a lawn of *Hebeloma longicaudum* (Persoon, 1801) Kummer, 1871 and *Laccaria laccata* (Scop.: Fr.) Cooke fungal hyphae, on 1.5% agar. The fungi were grown separately on PDA medium (Fuller and Javorski 1987). Enchytraeids were hand-selected from soil samples and offered as prey in Petri dishes containing flooded soil or only water. Mixed species of amoebae were cultured on a thin lawn of bacteria. The amoebae for culture were extracted according to Adl et al. (2006) from the same soil samples. All observations were made with a dissecting microscope (Nikon SMZ with tilting mirror base, X1 Planapo with $\times 1$ –80 zoom magnification), and with a Zeiss inverted microscope (Axiovert 200M, phase contrast and DIC, Planapo $\times 5$, $\times 10$, $\times 20$, $\times 40$ objectives). Images were obtained with the Zeiss AxioCam and AxioVision software. Specimens were placed under a cover slide with or without ProtosloTM (Fisher Scientific) or 1% magnesium chloride.

SSU sequence and phylogeny

DNA for the SSU rDNA sequence was obtained from a single specimen, previously kept in deionized water for 2 days without food to clear the gut, and photographed. The specimen was hand-picked into a microcentrifuge tube with 100 µl 95% ethanol. Total DNA was isolated using the Qiagen DNA extraction kit. The SSU rRNA was amplified and sequenced as described in Carranza et al. (1996). The sequence is GenBank accession number DQ372928. Additional SSU rRNA nucleotide sequences of 80 Platyhelminthes (all belonging to the rhabditophoran clade) and nine other members of the Lophotrochozoa clade were retrieved from GenBank and included in the alignment. Alignment was done manually in Bioedit (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) according to a secondary structure model (Gutell et al. 1985). Positions that could not be unambiguously aligned were excluded from the analysis, leaving a total of 1491 homologous positions. Phylogenetic analysis for ML tree was calculated using the IQPNNI program (Vinh and von Haeseler 2004), with a GTR nucleotide model of evolution, taking into account across-sites rate variation (eight categories). A 100-replicates bootstrap support was calculated with the phym1 program (<http://atgc.lirmm.fr/phym1/>), using a GTR model with gamma distribution (four categories).

Results*SSU sequence analysis*

In search of a close out-group to infer the Platyhelminthes phylogeny, we used different representatives from other Lophotrochozoa phyla (such as Annelida, Mollusca, Phoronida, Echiura, Bryozoa, Pogonophora, Nemertini, and Brachiopoda) known to be closely related to Platyhelminthes (Ruiz-Trillo et al. 1999). Members from the Acoelomorpha (Acoela + Nemertodermatida) and the Catenulida clade were not included in the analysis to avoid potential phylogenetic noise. This is because Acoelomorpha have been shown not to be members of the Platyhelminthes (Ruiz-Trillo et al. 1999, 2002) and Catenulida have an unclear position relative to the Lophotrochozoa (Carranza et al. 1996). However, our analysis includes a wide and diverse representation of Rhabditophora Ehlers, 1985. The results (Figure 1) show the isolate to be a *Geocentrophora* species with a very high ML bootstrap support (97%), within the Lecithoepitheliata clade, and distinct from any other Rhabditophora clades.

Morphology and behaviour

The overall morphology is dorso-ventrally flattened, 0.9–1.5 mm long depending on individuals, with an anterior mouth (Figures 2, 3). It is very similar to descriptions of *G. sphyrocephala* de Man, 1876 (de Man 1875) but without the marginal row of pigments and a slightly lobed posterior end. The SSU rDNA sequence analysis shows it is distinct from *G. sphyrocephala*. The head region is slightly flared and spade-shaped, with ciliated pits located on either side of the head, with yellowish eye pigments on either side of the pharynx, and a curved copulatory stylet. The body is clear without pigmentation and internal organs are visible. The intestinal region is very dynamic when feeding and contracts. The mouth region is mobile and sucks food into the oesophagus. Occasionally food remains are eliminated from the mouth. The animal tends to crawl along surfaces, leaving behind a mucus trail, with bouts of swimming through the water. It avoids regions

Figure 1. Phylogenetic tree of the Platyhelminthes from SSU rRNA sequences as inferred by Iqnpni under a GTR+ γ model of evolution. Numbers above key nodes refer to the statistical support inferred from 100-replicates in phym, under a GTR+ γ model (and four gamma categories). The new isolate is indicated as *Geocentrophora sp. Nova Scotia*.

without soil in the Petri dish, in favour of soil into which it remains most of the time. The whole body is flexible, and the animal contracts into a tight ball if knocked or in the presence of strong light. In strong light, especially at the microscope, there was strong negative phototaxis. This made the individuals very difficult to photograph as they move away from the light, or contract into a tight ball, without relaxing in magnesium chloride or

Figure 2. Morphological features of *Geocentrophora* sp. isolated from Nova Scotia forest soil. (A) Ventral view showing curved copulatory stylet, eye pigments on either side of a bulbous pharynx, and one pair of anterior ciliated pits; (B) relaxed and (C) elongated form in locomotion.

Protoslo[™]. However, when exhausted and resting, individuals become completely immobile for several minutes.

Feeding study

There are reports of *Terricola* species feeding on earthworms (Yeates et al. 1997). The relative size of enchytraeids could have been suitable as potential prey for our isolates, if a

Figure 3. Photomicrographs of *Geocentrophora* sp. ($\times 100$ magnification). (A) Relaxed form of specimen used in Figure 2, transmitted light; (B) the same specimen under DIC settings; (C) the same specimen under phase contrast. More photographs can be viewed at <http://soilecology.biology.dal.ca/index.htm>.

similar feeding mechanism was involved. One flatworm was placed with 20 enchytraeids in a Petri dish with 3 ml of distilled water. When kept in water with enchytraeids, the flatworms ignored the enchytraeids and continued to search for food. Over 10 days, there was no reduction in the number of enchytraeids and no evidence of food in the flatworm guts. This was repeated four times with different individuals, and again with the same four specimens in soil in Petri dishes, with the same negative results. We discount this as a possible feeding strategy.

Feeding studies on mixed species of soil nematodes, amoebae, bacteria and fungi were attempted. Growth on each of the nematodes, amoebae, bacteria and fungi were compared to control culture plates without the flatworm.

When one or more flatworms were placed on agar with a mixture of bacterivorous nematodes and bacteria, or with fungivorous nematodes and fungal hyphae, the intestinal region contained food and there was evidence of feeding. The nematode abundances were one or two individuals per cm at the start of feeding studies. In both situations, the nematode numbers decreased and had to be replenished. However, we did not observe direct feeding on nematodes. The *Geocentrphora* individuals were kept on these agar plates for several months before being transferred back to soil, or discarded. They were not affected by being kept on agar for several months. Similarly, when placed on agar with a thin lawn of bacteria and amoebae, there was evidence of food in the gut and a decrease in the abundance of amoebae. Amoebae were seen to be removed after passage of a flatworm, as it sucked on the agar. However, these individuals did not appear well fed on amoebae plates after 1 week, and were returned to an organic soil diet. When individuals were kept on a thin lawn of bacteria or with the same hyphae alone, they were lost and assumed to have died.

When kept in moist soil, all specimens ingested organic matter readily and appeared healthy. At times, mineral soil particles of sand or silt would be in the gut. These would eventually be egested after a day or two. From our observations, we conclude that the preferred diet of this species is soil rich in decomposing organic matter, which includes soil organisms. The bacteria and fungal hyphae are inevitably ingested with whole soil, as are amoebae. However, neither bacteria alone nor hyphae alone would sustain this species, and we lost individuals on these diets. Nematodes and amoebae are probably adequate supplements to the organic soil diet. After 5–8 months in the same 5–7 g soil in a 10 cm Petri dish the flatworms were gradually lost. Some individuals formed cysts but we were unsuccessful in our attempts at excystment.

Discussion

Our phylogenetic analysis indicates this species to be closely related to *G. sphyrocephala* but distinct from a previous published sequence of that species.

In the forest soils where this species is found, the soil freezes in the winter for up to 5 months, to at least 50 cm depth, well below the organic horizon. We do not know the mechanism for surviving the winter. Individuals were found active in the summer months, but higher abundances were collected in October. We did not sample sufficiently to determine the abundance dynamics of this species at each site. However, the species was found in most soil cores (six cores per hectare) on every sampling trip in the fall. We believe this is a common species in this area, that is active at least during June to late October. Their abundance is usually below 1 per m², but increases to about 0.5 per g dry soil in late September to October.

We also encountered once a second species which was indistinguishable morphologically from *G. baltica* Kennel, 1883, known from northern European aquatic environments (<http://devbio.umesci.maine.edu/>), but also sampled in forest litter and moist habitats (U. Jondelius, personal communication). This specimen was photographed but not studied further.

We conclude that *Geocentrophora* species occur in forest soils around Halifax (Nova Scotia). The species studied ingests whole soil rich in organic matter, along with organisms that may be present in each mouthful. Although nematodes may be a component of the diet, protozoa, bacteria, and fungi on their own were insufficient food items to maintain activity.

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References

- Adl SM. 2003. The ecology of soil decomposition. Wallingford (UK): CABI.
- Adl SM, Acosta-Mercado D, Anderson TR, Lynn DH. Protozoa. 2006. In: Carter MR, Gregorich E, editors. Soil sampling and methods of analysis, chapter 36. 2nd ed. Boca Raton (FL): CRC Press, Forthcoming.
- Baguña J, Riutort M. 2004. Molecular phylogeny of the Platyhelminthes. *Canadian Journal of Zoology* 82:168–193.
- Carbayo F, Leal-Zanchet A-M, Vieira EM. 2002. Terrestrial flatworm (Platyhelminthes: Tricladida: Terricola) diversity versus man-induced disturbance in an ombrophilous forest in southern Brazil. *Biodiversity and Conservation* 11:1091–1104.
- Carranza S, Giribet G, Ribera C, Baguña J, Riutort M. 1996. Evidence that two types of 18S rDNA coexist in the genome of *Dugesia* (Schmidtea) *mediterranea* (Platyhelminthes, Turbellaria, Tricladida). *Molecular Biology and Evolution* 13:824.
- Coleman DC, Blair JM, Elliott ET, Wall DH. 1999. Soil invertebrates. In: Robertson GP, Coleman DC, Bledsoe CS, Sollins P, editors. Standard soil methods for long-term ecological research. Oxford: Oxford University Press. p 349–377.
- Coleman DC, Crossley DA, Hendrix PF. 2004. Fundamentals of soil ecology. 2nd ed. New York: Elsevier.
- de Man JG. 1876. *Geocentrophora sphyrocephala* n. gen. n. sp., eine landbewonende Rhabdocoele. *Tijdschrift voor Diergeneeskunde* 2:62–67.
- Fuller MS, Javorski A. 1987. Zoospore fungi in teaching and research. Athens (GA): Southeastern Publishing Corporation.
- Gutell RR, Weibser B, Woese CR, Noller HF. 1985. Comparative anatomy of 16S-like ribosomal RNA. *Progress in Nucleic Acid Research and Molecular Biology* 32:155–216.
- Krebs CJ. 1998. Ecological methodology. 2nd ed. San Francisco: Benjamin-Cummings.
- Ogren RE, Kawakatsu M. 1998. American Nearctic and Neotropical land planarian (Tricladida: Terricola) faunas. *Pedobiologia* 42:441–451.
- Ruiz-Trillo I, Paps J, Loukota M, Ribera C, Jondelius U, Baguña J, Riutort M. 2002. A phylogenetic analysis of myosin heavy chain type II sequences corroborates that Acoela and Nemertodermatida are basal bilaterians. *Proceedings of the National Academy of Science USA* 99:11246–11251.
- Ruiz-Trillo I, Riutort M, Littlewood DTJ, Herniou EA, Baguña J. 1999. Acoel flatworms: earliest extant bilaterian metazoans, not members of Platyhelminthes. *Science* 283:1919–1923.
- Vinh LS, von Haeseler A. 2004. IQPNNI: moving fast through tree space and stopping in time. *Molecular Biology and Evolution* 21:1565–1571.
- Yeates GW, Boag B, Johns PM. 1997. Observations on feeding and population structure of five New Zealand terrestrial planarians which prey on lumbricid earthworms. *Annals of Applied Biology* 131:351–358.