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Research paper

Syntopy in subterranean fauna: Trophic specialisation in two new species of *Rhyacodrilus* Bretscher, 1901 (Annelida, Clitellata, Rhyacodrilinae)

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ABSTRACT

Trophic generalism in the subterranean fauna is assumed to be an adaptation to food scarcity or irregular availability, but studies on the feeding biology of subterranean species are almost nonexistent. Herein we (i) describe two new species, *Rhyacodrilus arenivorus* sp. nov. and *Rhyacodrilus moulis* sp. nov. (Annelida, Clitellata, Rhyacodrilinae), occurring in syntopy in the subterranean river of Labouiche, France; and (ii) report trophic and morphological specialisation in these two congeneric oligochaete species. The gut contents of *R. arenivorus* sp. nov. and *R. moulis* sp. nov. were similar, i.e., mineral particles and detritus. However, a detailed analysis revealed specialised foraging strategies in both species. Niche breadth value for ingested mineral particles was low (0.28) in *R. moulis* sp. nov. and intermediate (0.52) in *R. arenivorus* sp. nov. Moreover, slight interspecific morphological differences were found in two characters associated with foraging strategies in oligochaetes, the pharynx and the pharyngeal glands. A relatively high degree of niche overlap (≥0.58), however, was detected between *R. arenivorus* sp. nov. and *R. moulis* sp. nov. in relation to the size of ingested mineral particles.

1. Introduction

Subterranean ecosystems have long been considered to harbour only few, highly specialized species. However, over the last two decades, taxonomists have uncovered unexpectedly high biodiversity of subterranean invertebrates (Culver and Sket, 2000; Danielopol et al., 2002; Ferreira et al., 2007; Pipan and Culver, 2007; Deharveng et al., 2009; Achurra et al., 2015). Subterranean aquatic fauna is classified into three ecological categories (Sket, 2008): (i) stygobiont species are those strictly bound to a hypogean aquatic habitat; (ii) stygophile species show strong groundwater affinities; and (iii) stygoxene species occur sporadically in subterranean waters. The stygobiont fauna is generally dominated by crustaceans (Culver and Pipan, 2009; Stoch and Galassi, 2010), but oligochaetes (Annelida, Clitellata) are also abundant, widespread and taxonomically diverse (Martin et al., 2008; Des Châtelliers et al., 2009; Achurra et al., 2015). Groundwater oligochaete species are known in about half of the genera described up to date, with the families Naididae (sensu Erséus et al., 2008) and Lumbriculidae being the richest in stygobiont representatives (Martin et al., 2008). Several oligochaete genera are exclusively found underground, e.g., *Troglodrilus* (Juget et al., 2006), but the great majority occur in both epigean and subterranean waters (Brinkhurst and Jamieson, 1971). For example, the cosmopolitan genus *Rhyacodrilus* Bretscher, 1901 includes 14 stygobiont species and at least one stygophile species, but the great majority (about 36 species) are largely found in sandy substrates of epigean rivers and lakes. Regarding *Rhyacodrilus*, seven new species have been discovered over the last three years: 6 Neartic and 1 European species were newly described as *Rhyacodrilus* (Martinsson et al., 2013; Rodriguez and Fend, 2013), all from epigean habitats, and the stygobiont *Stochidrilus* (Martinez-Ansemil et al., 1997) has been proposed as a junior synomym of *Rhyacodrilus* (Rodriguez and Fend, 2013). The most common subterranean species in the genus, *Rhyacodrilus falciformis* Bretscher, 1901, is a stygophile oligochaete, occurring in freshwater bodies and wet soils, often associated with groundwater. *R. falciformis* is also the only species in the genus to be evaluated using molecular taxonomy (Martinsson et al., 2013).

Sympatry is the state of being in the same place (Poulton, 1903) and thus, two or more taxa are considered sympatric if their geographical ranges (entirely or partially) overlap, being largely used in reference to closely related taxa. If these taxa co-occur also in the same habitat, they are also said to be syntopic – a less common term. In other words, two or more species are syntopic if they

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are phylogenetically related, occupy the same macrohabitat, occur in the same locality and are observably in close proximity (Rivas, 1964). Syntopic species can be seen as a challenge to the competitive exclusion principle (Hardin, 1960). When two closely related species are limited by the same resources, competition will eventually lead to the exclusion of one species from the community unless they are able to use resources differently (resource partitioning or specialisation). Diet, microhabitat and time seem to be the most important niche axes, although most differentiation occurs along the first two (Schoener, 1974, 1986).

In the subterranean environment, most trophic resources are allochthonous and therefore, food can be rare and/or unevenly distributed (temporal and spatial variation, Gibert and Deharveng, 2002). Based on this limitation, trophic generalism is assumed underground; that is, subterranean organisms have adapted to exploit a wide range of resources and in that way, avoid food scarcity. However, knowledge on feeding biology of subterranean invertebrates is poor and studies on trophic niche differentiation are almost nonexistent (but see Bradford et al., 2014; Hutchins et al., 2014).

In this study, we describe two new stygobiont *Rhyacodrilus* species from a subterranean river in southern France, thereby contributing to the knowledge of the subterranean fauna. Also, we aim to evaluate if their syntopic occurrence could be explained via the evolution of foraging strategies and trophic niche partitioning; for this purpose, we (i) analysed the gut content of a number of specimens of both species and estimated the interspecific feeding overlap; and (ii) searched for interspecific morphological differences (if any) in characters associated with feeding.

2. Material and methods

2.1. Sampling and morphological analysis

Groundwater fauna was collected in the subterranean river of Labouiche (Rivière souterraine de Labouiche), Ariège, southern France, during several sampling surveys between 2013 and 2014. Coordinates for "Aygue perdent" entrance are 43°00′08.7″N, 1°34′38.7″E. Different groundwater habitats were sampled, including the river (7 sites over 1.5 km), small lentic pools associated with the river (2 sites) and epikarst (2 sites). Most sites were sampled once, but 4 sites were sampled 3–6 times. Samples were collected with hand-nets (sampled area approximately $30 \times 30\,cm^2$, maximum sampling depth 15 cm, net 100–200 μm mesh size), except for the epikarst, which was sampled indirectly by collecting the dripping water (Brancelj, 2004). Samples were sorted under a stereo-microscope in the laboratory; then, specimens were fixed in 70% ethanol or alternatively, in 4% formaldehyde and eventually transferred to 70% ethanol. Specimens were whole-mounted in glycerol for a preliminary identification and representatives of the subfamily Rhyacodrilinae were put aside for further examination. Anatomical characters were studied in rhyacodrilines stained with Ehrlich's hematoxylin, whole-mounted or dissected (see procedure in Timm and Martin, 2015) and mounted in Canada balsam after dehydratation in alcohol series and clearing in methyl salicylate. Several specimens were embedded in paraffin and sectioned at 7 μm. Histological sections were stained with Harris' hematoxylin and eosin and mounted in DPX.

For the species diagnoses, we focussed on characters related to the reproductive system (mostly internal characters) and chaetae (external characters). As a rule, only mature specimens (with eggs and/or sperm in the spermathecae) were used. Characters associated with the process of feeding were also described, although these are rarely considered to be diagnostic characters in Rhyacodrilinae. In aquatic oligochaetes, the gut extends through the entire body and it is divided into anterior ventral mouth, pharynx, oesophagus and intestine, although these sections may not be well differentiated. In the first segments, the dorsal epithelium of the pharynx forms the pharyngeal pad, which can be everted and retracted through the mouth by using pharyngeal muscles for food uptake. Pharyngeal glands are found from IV on, usually occupying 2–4 segments, and produce mucous secretion and digestive enzymes. The chloragogenous layer covers the intestine from V or VI on; its function is related to lipid storage and excretion of metabolites.

Type specimens are deposited in the Muséum national d'Histoire naturelle, Paris (MNHN). Other specimens are in the authors' collection at the University of the Basque Country (UPV/EHU).

2.2. Ingested items, niche breadth and feeding overlap

Gut content was analysed under the microscope in mounted specimens. Microscope mounts favour the observation of ingested items because the gut is flattened and the body wall is cleared. For each specimen, three postclitellar segments with filled gut were randomly selected. Ingested items were classified into two categories: mineral particles and detritus. Fungal spores were observed in a single specimen and thus, they were not considered; other potential food items, such as algae, were not observed. Mineral particles were counted, measured (length of the major axis, Juget, 1979) and classified into 11 categorical ranks according to their sizes (20–39 μm, 40–59 μm, 60–79 μm, etc., the maximum size being 220–239 μm). Grains with major axis <20 μm length were excluded from the analysis as they were difficult to count and bacteria do not colonize any grain smaller than about 10 μm (DeFlaun and Mayer, 1983). Detritus abundance was quantified by dividing the gut into 8 sections of equal area (in each segment) and expressed as the number of sections full of detritus (Robotmam, 1977). Detritus included undetermined organic matter, fragments of dead organisms and amorphous brownish substances (probably masses of microorganisms).

The level of specialisation in ingesting mineral particles of a given size range was calculated for each species using Levins' measure (B) (1968), which estimates niche breadth by measuring the uniformity of distribution of individuals among the resource states: $B = 1/\sum p_i^2$, where p_i is the proportion of individuals using resource state i. Niche breadth (B) was standardized (B') to express it on a scale from 0.0 to 1.0 using Hurlbert's formula (Hurlbert, 1978): $B' = B - 1/n - 1$, where n is the number of possible resource states. We used Pianka's index (Op) (Pianka, 1973) and Czekanowski's index (Oc) (Feinsinger et al., 1981) to estimate interspecific overlap in ingested mineral particles. Overlap values ranged from 0 (no resources used in common) to 1 (complete overlap). Values above 0.6 were considered high, between 0.4 and 0.6 intermediate, and less than 0.4 low (Grossman, 1986). We tested overlap indexes against the null model with RA3 and RA4 algorithms (Winemiller and Pianka, 1990). The null model proposes that niche overlap is statistically lower than expected. In both RA3 and RA4, resource states were used randomly in the null communities but the degree of specialization of each species was preserved; in RA3, resource states that were not used by a species in nature could be utilized in the null communities, while they could not be utilized in RA4. We interpreted that interspecific resource partitioning might be occurring when the observed mean overlap values were significantly lower than those expected by chance, whereas resource sharing would lead values higher than those expected by chance (Albrecht and Gotelli, 2001). For niche overlap calculations, we used the software EcoSim 7.0 (Gotelli and Entsminger, 2001).