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Original article

Genetic diversity of widespread moss-dwelling nematode species in German beech forests

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ABSTRACT

Molecular studies suggest that the number of microscopic animal species has been severely underestimated because of a high level of cryptic diversity. Using traditional methods of morphological species identification, a large number of species have remained undetected. In fact, many aquatic nematode morphospecies with an assumed widespread distribution instead may comprise cryptic species complexes. In terrestrial moss habitats, the diversity of nematode communities has yet to be evaluated in molecular surveys. Thus, the aim of this study was to assess the potential for cryptic diversity among the three dominant moss-dwelling nematode species (Plectus parietinus, Plectus cirratus, and Chiloplectus andrassyi) detected at five locations within four German beech forests. Analyses of the molecular variation in a mitochondrial (COI) gene and in two ribosomal (LSU and SSU) subunit genes were complemented by morphological identification of specimens. The morphological-based plectid species delineation was supported by the COI gene topology, but less by the analyses of nuclear marker genes. Furthermore, the results revealed a high level of high genetic diversity in terms of number of mitochondrial haplotypes (n = 24) detected overall for the three investigated morphospecies at the five locations, with no evidence of cryptic diversity. However, the large number of haplotypes only to be found at a single sampling location suggested a restricted level of gene flow even over short geographic distances (6 km).

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1. Introduction

Mosses are widespread in northern temperate zones and inhabit diverse locations, such as living or decaying trees, rocks, open mineral soil, and even water bodies. In addition to their ecological significance as water reservoirs mosses play an important role in the functioning of ecosystems [1]. However, mosses are also unstable environments, undergoing desiccation but also becoming inundated by rain.

The microscopic moss-dwelling faunal community, referred to as microfauna is strongly involved in ecological processes such as nutrient cycling and decomposition in addition to serving as a food source for higher trophic levels [2–5]. Generally, the moss-dwelling metazoan microfauna is dominated by mites, tardigrades, rotifers, and nematodes [6,7]. Amongst these, nematodes are the most abundant and diverse group, with densities as high as 1446

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http://dx.doi.org/10.1016/j.ejsobi.2016.03.002 1164-5563/© 2016 Elsevier Masson SAS. All rights reserved. individuals per 100 ml of moss [8]. As many as 52 species have been detected in a single habitat [9]. Interestingly members of the family Plectidae are generally very common and numerous in moss cushions in the northern temperate zone with shares up to 53% of the moss-dwelling nematode communities. By contrast, they are rather less abundant in freshwater ecosystems [6,10–14].

Similar to tardigrades and rotifers, a key feature of plectids is their ability to tolerate desiccation events, by means of anhydrobiosis. This metabolically inactive state is triggered by water scarcity and it allows plectids to thrive even in unstable moss habitats [15–17]. Additionally, during their active and dormant phase, nematodes might be passively transported over long distances by vectors such as wind or birds [18–21] which probably contributes to the stated widespread or even worldwide occurrence of some plectid species [22].

Molecular surveys of free-living nematodes have thus far been mostly restricted to aquatic species and they have often confirmed the existence of cryptic species [23,24]. By contrast similar studies on moss-dwelling nematodes are to our knowledge extremely rare. Cryptic species cannot be distinguished morphologically, whereas genetic analyses reveal the presence of distinct taxonomic entities







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[25]. Indeed, the results of recent molecular studies underline that the number of nearly 27.000 nematode species described thus far is multiple times higher, as it is expected from the morphological point of view as well [26,27]. Further genetic investigations of nematode morphospecies, including the so far neglected moss-dwelling representatives, will likely allow a substantial revision of this number [28].

In a first approach we analyzed the genetic structure of the three common moss-dwelling species, Plectus parietinus (Gerlach, 1963), Plectus cirratus (Bastian, 1865), and Chiloplectus andrassyi (Timm, 1971), extracted from moss cushions sampled at five different locations within four German beech forests located in Northern and Central Germany. P. parietinus is a terrestrial nematode frequently found in moss, leaf litter, and soil. Its geographic distribution extends from Europe over Africa, Canada, and the USA [10,11]. The broad geographic distribution of the nematode *P. cirratus* includes its widespread occurrence in Central Europe [10], although its species-level identification has been questioned because in some reports this species may have been confused with other nematode species [11]. C. andrassyi is a common moss-inhabiting nematode that has been detected across Europe as well as in Africa, and North and South America [10,11]. On average, the three studied species were the most abundant ones at the four different beech forest sites sampled in this study.

Genetic data were obtained and then analyzed for evidence of cryptic species diversity and to compare the dispersal patterns of the three investigated species. Thus, we focused on the genetic variance of two nuclear gene fragments (the large and small ribosomal subunits [LSU and SSU, respectively]) and one mitochondrial gene fragment (cytochrome *c* oxidase [COI]) among individuals of morphologically characterized *Plectus parietinus, Plectus cirratus* and *Chiloplectus andrassyi.* Specifically, we asked: 1) Does the morphological species identification match the genetic species delineation? 2) Do moss-dwelling nematodes share the same level of genetic diversity as aquatic nematodes, including signs of cryptic diversity? 3) Do moss-dwelling nematode species differ in their distribution and dispersal patterns?

2. Material and methods

2.1. Sample collection, extraction of organisms and DNA

Between May and July 2013, moss samples were taken at five forest locations in Germany (Table 1). The closest distance between two locations was 6 km (between Teutoburger Forest locations A and B); the greatest distance was 201 km (between Göhrde and Hainich). Moss samples distributed over an area of 20 m² were collected from the trunks of beech trees (*Fagus silvatica* L) at a height of 10–80 cm. At each location, at least five moss cushions were cut out using a plastic corer (internal diameter 3.7 cm); the samples were then pooled. At most forest locations, the moss communities were dominated by *Hypnum cupressiforme* (Hedw.), with the additional occurrence of *Brachythecium rutabulum*

(Hedw.), Mnium hornum (Hedw.) and Dicranum scoparium (Hedw.).

After field sampling, the moss samples were moistened with ultrapure water and stored for a maximum of 72 h in plastic boxes $(10 \times 10 \times 6 \text{ cm})$ at 4–8 °C. A previous experiment showed that this form of storage did not negatively impact the mortality of the microfaunal organisms but did allow anhydrobiotic individuals to become metabolically active.

For nematode extractions, each of the moss samples was washed with 0.5 l of ultrapure water for several minutes. The water was then filtered through a sieve (mesh size 3 mm) while the moss was held back and slightly squeezed in order to release as many individuals as possible. This procedure was repeated twice for each sample. The water, containing the moss-derived organisms, was divided into two equal fractions. One fraction was filtered onto a 10-µm sieve and the retained organisms were fixed with pure ethanol (final concentration 80%). The samples were stored at 4–6 °C until used in the molecular analysis, in which plectid species with the highest abundances were chosen. Among the 20 detected nematode species, P. parietinus, P. cirratus, and C. andrassyi were the most frequent (relative abundance from 10.1 to 18.4%; Supplementary Table 1). The other fraction was filtered onto a 10µm sieve, the retained organisms fixed with formalin (final concentration 4%), stained by the addition of Rose Bengal and finally processed for traditional light microscopy according to the method of Seinhorst [29]; the nematode composition in the moss was determined at a magnification of $>400 \times$ (Zeiss Axioplan 2).

Plectids were picked individually from ethanol-fixed samples and identified to the species level by using differential interference contrast microscopy (400–1000 × magnification). In addition, the characteristic morphological features (body length, body width, vulva position etc.) of adequate adult individuals were examined (Table 2). Each specimen was transferred into a 1.5-ml tube containing 20 μ l of lysis buffer (50 nM KCl, 10 mM Tris (pH 8.5), 2.5 mM MgCl₂, 0.5% Triton X-100, 0.5% Tween 20). After storage for 24 h at -80 °C, the samples were treated with 1.5 μ l of proteinase-K (20 mg/ml) for genomic DNA extraction. The incubation temperature was 65 °C for 70 min followed by 95 °C for 10 min. The DNA lysate was stored at -80 °C until further processing.

2.2. Morphological data

Morphological data of the measured speciment were concordant with the taxonomic description of the three species, *P. parietinus*, *P. cirratus*, and *C. andrassyi*, as reported by Zell [10] and Andrassy [11]. *P. parietinus* had the largest body size and was also the only species with gland cells. *C. andrassyi*, with its characteristic lip region, was the smallest species (Table 2).

2.3. PCR amplification and sequencing

To analyze the genetic structures of the three species, the fragments of two nuclear genes (LSU and SSU) and a mitochondrial gene (COI) were amplified in for ca. 50 plectid individuals. The

Table 1

Site	Location	Coordinates	P. parietinus				P. cirratus				C. andrassyi			
			n	Ν	h	π	n	Ν	h	π	n	Ν	h	π
Teutoburger Forest	Location A	52°01′54.1″N 8°29′32.6″E	3	3	1.0000	0.0176	9	4	0.8056	0.0526	10	6	0.7778	0.0281
Teutoburger Forest	Location B	52°02′56.0″N 8°24′52.6 ″E	2	2	1.0000	0.0344					2	1		
Arnsberger Forest	Möhnesee	51°28'10.1"N 8°09'07.1"E					6	2	0.3333	0.0009	4	3	0.8333	0.0594
Hainich National Park	Hainich	51°04′57.4″N 10°30′46.7″E	2	2	1.0000	0.013	4	3	0.0333	0.0433				
Göhrde State Forest	Göhrde	53°06′01.1″N 10°49′47.1″E	10	2	0.2000	0.001								

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