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Comparative analysis of diet in syntopic geophilomorph species (Chilopoda, Geophilomorpha) using a DNA-based approach

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ABSTRACT

Trophic niche partitioning between potentially competing species within the same coenosis has been little explored for most of the major groups of arthropod soil predators, among which are the geophilomorph centipedes. We performed a comparative study in nature on the diet of three species of Geophilomorpha living in the same site in Southern Europe. Through PCR-based molecular gut content analysis, we estimated trophic niche width and overlap with respect to three common prey groups: lumbricids, collembolans and dipteran larvae. Results show that apparently similar geophilomorph species differ significantly in prey spectrum, with quite different niche widths. Estimates of predator diet overlap gave moderate values, non-significantly different from null expectations. Within-species diet composition does not vary significantly with sex. This work, while providing the first evidence of trophic niche partitioning among coexisting geophilomorph species, contributes to recent progresses in the understanding of intra-guild interactions between predators in the soil food webs.

1. Introduction

A large number of arthropod generalist predators populate the soil of temperate forests (Polis, 1994), and guilds of similar species can live syntopically despite interspecific competition for prey (e.g., Toju and Baba, 2018; Peretti and Bonato, submitted). Although highly similar predator species may avoid competitive exclusion through nearly neutral dynamics (i.e., random demographic mechanisms and processes of spatial dispersal; Hubbell, 2001), several evidences support the idea that trophic differences are ultimately required for stable coexistence (e.g., Chesson, 2000; Levine and HilleRisLambers, 2009). To reduce competition for prey, syntopic predator species can differ in times of activity, in microhabitat and in the food they consume. This phenomenon is generally referred to as *niche partitioning* (Schoener, 1974).

In evaluating trophic niche partitioning, measures of *niche overlap* quantify the similarity of resources used by different species in terms of food consumption (Abrams, 1980). High values of niche overlap suggest high level of interspecific competition (see Krebs, 1999 and reference therein). In parallel, trophic *niche width* quantifies the range of food that a species uses (Levins, 1968). Extent and composition of trophic niche can vary with predator features like size and age, or with season (Bolnick et al., 2011; Haddad et al., 2016). In addition, sex differences in trophic niche can occur, possibly associated to sex-specific strategies to maximize fitness, through divergent habitat choice or nutritional requirements (Pekár et al., 2011).

Among arthropod soil predators, trophic niche partitioning has been largely investigated in a few groups only, especially spiders, where coexisting species have been found either to differ in prey use in relation to differences in foraging strategies, in microhabitat or in circadian activity (e.g., Michalko and Pekár, 2015), or to show substantial niche overlap, possibly explained by environmental constraints (Wirta et al., 2015). However, studies about the trophic interactions that regulate the coexistence of species of other predator major taxa, such as centipedes, are still lacking.

We performed a comparative study in nature on the diet of different species of geophilomorph centipedes. Geophilomorpha are one of the major groups of predators in the forest soil ecosystems worldwide (Albert, 1979; Petersen and Luxton, 1982) and up to ca. 20 species have been repeatedly found to coexist in the same soil community in temperate forests (Grgič and Kos, 2003; Peretti and Bonato, submitted). Some geophilomorphs live permanently inside the soil (strictly endogeic species) while others hunt regularly on the soil surface (regularly epigeic species). They are supposed to be generalist predators (e.g., Klarner et al., 2017), feeding on invertebrates of smaller or similar body size, including annelids, molluscs and arthropods (reviewed in: Lewis, 1981; Voigtländer, 2011). However, despite their important and widespread ecological role in forest soil ecosystems, very few data are available on their diet.

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In recent years, significant methodological advances have been made for studying feeding interactions in the soil webs, opening new prospective in trophic ecology. Fatty acids (FAs) can be traced over more than one trophic level, allowing to distinguish energy channels based on bacteria from those based on fungi in the soil (Pollierer et al., 2010). FAs analysis carried out on two geophilomorph species and two other centipede species from a forest soil community showed different FA composition between species, suggesting trophic niche differentiation (Ferlian et al., 2012). This approach can contribute to uncover the food web structure, but it is not sensitive enough to accurately define the prey spectrum of different predators. Instead, DNA-based gut content analysis allows the taxonomic identification of prey from small amounts of DNA detected in the predator gut, and is particularly useful for determining the prey spectrum in fluid-feeding predators (Pompanon et al., 2012), like geophilomorphs (see Koch and Edgecombe, 2012). DNA-based studies have been carried out on a few geophilomorphs and other centipedes through feeding laboratory experiments. These have shown that specific characteristics of the predator (such as the feeding mode) as well as of the prey (such as the tissue physicochemical qualities) may influence the post-feeding timing of DNA degradation, and thus the efficacy of the method in detecting prey DNA (Waldner et al., 2013; Eitzinger et al., 2014). However, it remains largely unexplored whether these insights may be generalized to other prey and predator species, and also to a natural environment context. Indeed, DNA-based gut content analyses have been carried out on some centipede species in the field (Günther et al., 2014; Eitzinger et al., 2018), but diet differences between coexisting geophilomorph species have not been investigated so far.

We used a PCR-based gut content analysis to comparatively investigate predation, through determining trophic niche width and overlap, in a sample of species of geophilomorphs living in the same site. Specifically, we analysed the frequency of detection of earthworms (Lumbricidae), springtails (Collembola) and fly larvae (Diptera), which are usually reported as the most frequently exploited prey groups (e.g., Eitzinger et al., 2018). At the intraspecific level, we tested for variation in prey consumption in relation to sex, while controlling for the effects of body size and sampling season. Finally, including a fourth predator species from a site nearby, we evaluated whether different predators feed on selected prey species, by identifying the prey DNA to the highest possible taxonomic resolution.

2. Materials and methods

2.1. Study site and sampling of the predators

Geophilomorphs were sampled within an area of about 2500 m^2 in a xerothermic wood located on Monte Cecilia near Baone (Euganean Hills, near Padova, Italy, 45.2503° N, 11.6919° E).

We selected the three species *Henia vesuviana*, *Himantarium gabrielis* and *Stimatogaster gracilis*, as these are among the species regularly coexisting in similar woody habitats throughout most of Southern Europe (Bonato et al., 2018). Moreover, they are locally abundant and are large enough to facilitate gut dissection and DNA extraction.

Sampling was performed over three years (2014–2016), but was restricted to 9 days within two short periods with similar climatic conditions, namely 6 days between mid-March and mid-May (spring) and 3 days between mid-October and the end of November (autumn), in order to minimize the effect of possible seasonal variation of diet. Additionally, spring and autumn offer the most suitable conditions for sampling geophilomorphs in the study site, because the soil is wet and animals spend more time in the upper layers of the ground. Vice versa, during the hot and dry summer most geophilomorphs retreat deep into the ground, as well as in winter, when the temperature of deeper soil levels is warmer than close to the surface. Centipedes were collected by standard methods for the geophilomorphs, i.e., by digging into the soil (to a depth of about 10 cm), sorting by hand and visually inspecting leaf litter and soil, and by turning stones, pieces of wood and barks found on the ground. The centipedes were fixed in 100% ethanol immediately upon collection.

An additional species, *Pleurogeophilus mediterraneus*, was sampled in a site with similar habitat and climatic conditions (Monte Castello, near Monselice, 45.2556° N, 11.7368° E, only 4 km apart from the other study area), during the same period (3 days in spring and 1 in autumn) and with the same methodology. Some specimens of *P. mediterraneus* were kept alive until they reached the laboratory, where their gut was dissected and fixed in RNAlater (Sigma Aldrich, St. Louis, USA) at −20 °C. The two storage methods (100% ethanol and RNAlater) gave DNA with comparable concentration and purity (considering A260/280 and A260/230 absorbance values).

Species were identified using ChiloKey (Bonato et al., 2014) and sex was determined based on gonopod morphology (Minelli, 2011). Head maximum width, used as a proxy for body size and age, was measured by means of a micrometre applied to a microscope to the nearest 10 µm.

In order to reduce the expected intraspecific variation of diet associated with body size and/or age (Eitzinger et al., 2018), we considered only adult and subadult specimens with head width in the following species-specific ranges (from the minimum size, up to twice the minimum size): 400–800 µm for *H. vesuviana* and *S. gracilis*, 1200–2400 µm for *H. gabrielis* and 800–1600 µm for *P. mediterraneus*.

2.2. DNA extraction

DNA was extracted from the dissected gut of large geophilomorph specimens, in order to reduce carry-over contamination from the animal body surface. Differently, for smaller specimens, DNA was extracted from the intermediate portion of the body trunk, as accurate gut dissection was problematic. Dissection was executed by a single person (FB) under sterile conditions, using flamed instruments.

DNA extraction was performed using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) and negative controls (i.e., no animal tissue) were included in each batch of samples to check for potential cross-sample contamination during extraction.

To ensure that DNA could be successfully detected in all specimens (i.e., to avoid false negative), samples were tested for the amplification of a selected gene (*COI*) of the predator species by employing species-specific primers (Table S1). These were designed on the *COI* sequences obtained from the amplification of DNA from legs (thus avoiding the incorporation of gut content) by general primers previously employed successfully in other geophilomorphs (Table S1). PCRs were performed in 20 µl reactions containing 4 µl of 5X Flexi Buffer, 0.4 µl of 10 mM dNTPs, 1 µl of 25 mM $MgCl_2$, 0.5 µl of 100% DMSO, 0.5 µl of 10 µM primers, 0.1 µl of 5U/µl GoTaq G2 Flexi DNA Polymerase (Promega, Madison, USA), 1–2 µl of template DNA and purified water. The reaction was carried out as follows: 1 step at 95 °C for 5 min, 28–32 cycles consisting of 1 min at 94 °C, 1 min at 41–47 °C, and 1 min 30 s at 72 °C, a final step at 72 °C for 7 min. The amplified fragments were purified using the MinElute PCR Purification Kit (Qiagen) and sequenced on both strands.

2.3. Screening of predators for prey DNA

Centipede specimens testing positive for species-specific predator primers were subsequently screened for DNA of Lumbricidae, Collembola and Diptera by the following markers and group-specific primers: Lumbricidae *12S* gene, 185F/14233R (Harper et al., 2005), Collembola *18S* gene, Col3F/Col5R (Kuusk and Agustì, 2008), Diptera *18S* gene, DIP S16/DIP A17 (Eitzinger et al., 2013). Each pair of primers had previously been tested positive on a selection of species representative of the taxonomic diversity of the target group (Harper et al., 2005; Kuusk and Agustì, 2008; Eitzinger et al., 2013), so that they were expected to be able to detect all the different species living in the study area. Moreover, their specificity for the target group had been

tested by amplification of DNA extracted from a broad range of other soil invertebrates (Harper et al., 2005; Kuusk and Agustì, 2008; Eitzinger et al., 2013).

The optimal PCR conditions were determined by testing lumbricid and dipteran primers on DNA extracted from the gut content of single specimens of geophilomorphs (*Stenotaenia* sp. and *H. vesuviana*) caught in the act of feeding on a Lumbricidae and a Tipulidae larva, respectively. For collembolans, where this option was not available, primers targeting *18S* of Collembola were tested with different conditions in PCR assays where serially diluted DNA of a collembolan specimen (Entomobryomorpha) was mixed with DNA extracted from a *H. vesuviana* specimen. Several mixing ratios were tested. The final concentration of collembolan DNA ranged from 3 to 0.0025 ng per µl PCR and the ratio between DNA of predator and collembolan ranged from 10/1 to 20000/1. DNA concentrations in the extracts was determined using NanoDrop (NanoDrop Technologies, Wilmington, DE, USA).

PCRs were performed in 10 µl reactions containing 2 µl of 5X Flexi Buffer, 0.2 µl of 10 mM dNTPs, 0.45–1 µl of 25 mM $MgCl_2$, 0.25 µl of 100% DMSO, 0.375–1 µl of each primer, 0.05 µl of 5U/µl GoTaq G2 Flexi DNA Polymerase, 1.25–3 µl of template DNA and purified water. For all screening PCRs the cycling conditions used were 95 °C for 10–15 min followed by 35–38 cycles of 95 °C for 30 s, 56–65 °C for 30–90 s, 72 °C for 20–45 s and a final cycle of 72 °C for 7 min. For protocol details see Table S2 in Supplementary data.

All PCRs included a positive control (DNA extracted from the gut content of the geophilomorph specimens feeding on Lumbricidae and Tipulidae in the field, or the DNA of a collembolan, see above) and a negative control (sterile water).

PCR products were visualized by electrophoresis on 1% agarose gel in 1X TAE stained with GelRed and fragments of expected length were scored as positive.

2.4. Diet quantitative analyses

To compare prey frequency distribution among the three syntopic predators, we used the Fisher exact test (Fisher, 1922). This essentially tests the association between two variables (in our case, predator species and prey species) in a contingency table against the null hypothesis that the percentages for one variable are the same for every category of the other variable (i.e., no difference in conditional distributions). A significant association between the two variables entails significant differences in prey frequency distribution among the predators. The same test was also used for testing within-species differences in prey detection rates of different prey groups between predator sexes and between collecting periods. Fisher exact test was performed using an on-line calculator (http://www.physics.csbsju.edu/stats/).

Trophic niche width of each centipede species was described by Levins metrics (Levins, 1968), as rescaled in the range 0–1 by Hurlbert (1978). Values of niche width close to 0 indicates extreme specialization in diet, while 1 denotes a complete generalist (Krebs, 1999). To evaluate the level of trophic niche overlap between the predator species, we adopted Pianka (1973) index, one of the most common measures of overlap in resource use. This was calculated for each species pair, and then averaged over all pairs of species. It takes values from 0 (no overlap) to 1 (complete overlap) (Krebs, 1999). For Pianka index calculation and testing we used the module 'Niche Overlap' of the R software package EcoSimR (ver. 1.0, Gotelli et al., 2015). This offers several options (named RA1 to RA4) for the generation of expected null distributions through randomization. Organizing data on resource utilization in a predator × prey matrix, RA1 replaces all matrix elements by a random uniform value in the interval (0,1), RA2 replaces the non-zero elements by a (0,1) random uniform value, RA3 reshuffles the row values, and RA4 reshuffles the non-zero row values. Both the last two algorithms retain the observed niche width of each species. Other two common indexes of niche overlap, namely Czekanowski (1909) and Morisita (1959), were also calculated for consistency check. Czekanowski index was implemented in the same package EcoSimR used for Pianka index, while for Morisita index we used the R software package SPAA (SPecies Association Analysis ver. 0.2.2, Zhang, 2016).

Within-species effect of body size on prey detection rate was inspected through Mann-Whitney *U* test. When not otherwise specified, statistical analyses and calculations were performed with the package STATGRAPHICS Centurion (ver. XVI.II) and Microsoft Excel 2010 spreadsheet.

2.5. DNA sequencing of Lumbricidae and Collembola prey

For Lumbricidae and Collembola we attempted to identify the prey to the most precise taxonomic levels through DNA sequencing. This was not carried out for Diptera because most PCR products were of insufficient quality to obtain good sequencing data.

All the amplified fragments of adequate quality of Lumbricidae *12S* and a subsample of Collembola *18S* fragments were purified using the MinElute PCR Purification Kit (Qiagen) and then directly sequenced on both strands with the same primer sets as used for amplification, by means of an ABI 3130 XL automatic capillary sequencer (Applied Biosystems, Branchburg, USA; service provided by BMR Genomics, Padova, Italy).

After merging the opposite strands, similar sequences were searched among all available sequences in GenBank dataset using MegaBlast. Because errors and imprecisions in annotations frequently occur in the major public DNA repositories (Nilsson et al., 2006), we revised and validated the taxonomic identification of the first 20 best scored matches in MegaBlast, by considering the most recent literature on taxonomy, phylogeny and geographical distribution of prey (Lumbricidae: Klarica et al., 2012, Dominguez et al., 2015; Collembola: Xiong et al., 2008 for Poduromorpha, Yu et al., 2015 for Tomoceroidea, Zhang et al., 2014, 2015, 2017 for Entomobryidae).

The taxonomic identification of our sequences was also checked against a list of the Lumbricidae and Collembola species known for, or expected to be found at the study sites, including all lumbricid species recorded from north-east Italy (Omodeo et al., 2004) and all collembolan species recorded from northern Italy (Dallai et al., 1995; Dalla Montà and Dall'Ara, 2000). The taxonomy of these sources was reviewed considering the recent literature (Lumbricidae: Pérez-Losada et al., 2012, Dominguez et al., 2015; Collembola: Xiong et al., 2008, Yu et al., 2015, Zhang et al., 2014, 2015, 2017) and the nomenclature was updated following DriloBASE for Lumbricidae (taxo.drilobase.org, last accessed April 2018) and Fauna Europaea for Collembola (www.faunaeur.org, last accessed April 2018).

3. Results

3.1. Prey detection in the gut of predators

DNA was successfully extracted from 111 geophilomorphs (28 *H. vesuviana*, 16 *H. gabrielis*, 38 *S. gracilis* and 29 *P. mediterraneus*). Of all these specimens, 75 were tested positive for at least one of the prey group considered (Lumbricidae, Collembola, Diptera), with a frequency of positive detection between 56% and 71% depending on the predator species (Fig. 1). DNA of two different prey groups was found together in the gut content of ten specimens of geophilomorphs and one specimen of *P. mediterraneus* contained DNA of the three different prey groups. Collembolans were found in all geophilomorph species, whereas lumbricids and dipterans were detected in three of the four species (Fig. 2).

3.2. Diet comparison between syntopic predators

Considering the three species sampled from the same site (M. Cecilia), only *H. gabrielis* was tested positive for all prey groups, while *H. vesuviana* was tested positive only for collembolans and dipterans, and *S. gracilis* only for lumbricids and collembolans (Fig. 3). Levins

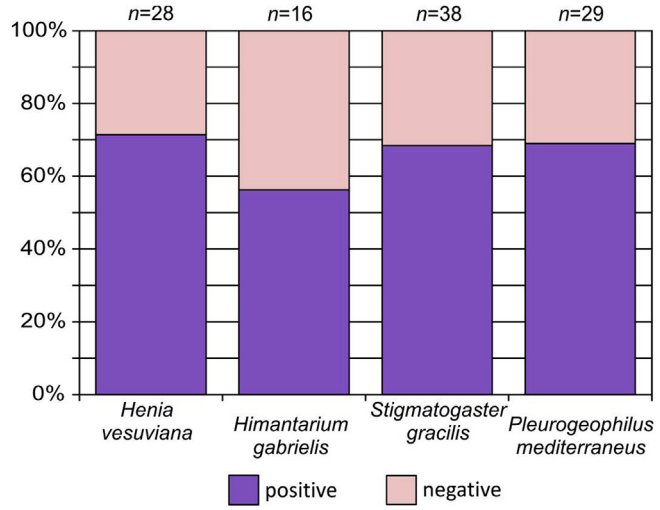

Fig. 1. Success in DNA detection of prey (Lumbricidae, Collembola and Diptera) in different species of geophilomorph centipedes, as percent of individuals testing positive for target prey DNA.

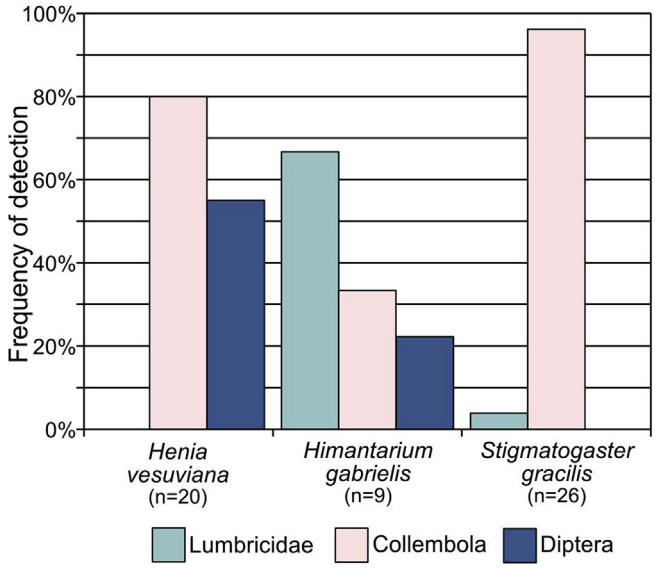

Fig. 3. Frequency of detection of Lumbricidae, Collembola and Diptera in three syntopic geophilomorph species with respect to the total number of predator specimens tested positively for prey DNA.

index of trophic niche width was 0.04 for *S. gracilis*, 0.47 for *H. vesuviana* and 0.73 for *H. gabrielis*. An even higher value (0.95) was found for *P. mediterraneus* in the other investigated site. Comparing the diet among the syntopic predators, differences in prey frequency distribution were highly significant among the three species (Fisher exact test 3×3, $p < 0.001$) and also between each pair of species (Fisher exact tests 2×3, $p < 0.001$; Fig. 3). Pianka index of trophic niche overlap resulted to be 0.60, with the three pairwise index values ranging from 0.46 to 0.82. This value is well within the 95% confidence interval of all null model distributions, but for RA1, where the value is only marginally higher than the lower 95% confidence limit. However, RA1 randomization, as well as RA2, is generally considered to be too liberal and not giving reliable results (Winemiller and Pianka, 1990). Estimates of niche overlap using different indexes gave values in the same range of a moderate overlap, 0.45 for Czekanowski index and 0.58 for Morisita index, and in any case non-significantly different from null expectations.

3.3. Intraspecific variation of diet

For each geophilomorph species, prey detection rates for different prey groups did not differ significantly between either predator sexes or sampling season (for both factors, Fisher exact test, two-tailed $p > 0.1$). This justifies the pooling of the data for sex and season in the interspecific analyses (see above).

Within the considered ranges of body size of each species (see Material and Methods), we found evidence of an effect of predator size on DNA prey detection only for collembolans, and only in *H. gabrielis* (Mann-Whitney *U* test on head width, $U = 0$, $p < 0.009$) and in the females of *H. vesuviana* ($U = 3.0$, $p < 0.004$). In both species, collembolans were found more frequently in the smaller specimens than in the bigger ones.

3.4. Diversity of prey species

We successfully sequenced 12 DNA fragments amplified with primers for Lumbricidae *12S* and 17 DNA fragments amplified with primers for Collembola *18S* (sequences available in Supplementary Data).

Considering the *12S* sequences of Lumbricidae (Table S3), in the gut content of 6 specimens of *H. gabrielis* we found 4 similar sequences confidently belonging to *Allolobophora rosea* and another sequence confidently belonging to a species of the complex "*Dendrobaena*" *cognettii/pygmaea* (see Domínguez et al., 2015). Instead, in the gut content of 6 specimens of *P. mediterraneus*, we found 5 different sequences, confidently belonging to the following five species: *Allolobophora rosea*, *Allolobophoridella eiseni*, "*Dendrobaena*"*cognettii/pygmaea*, *Dendrodrilus*

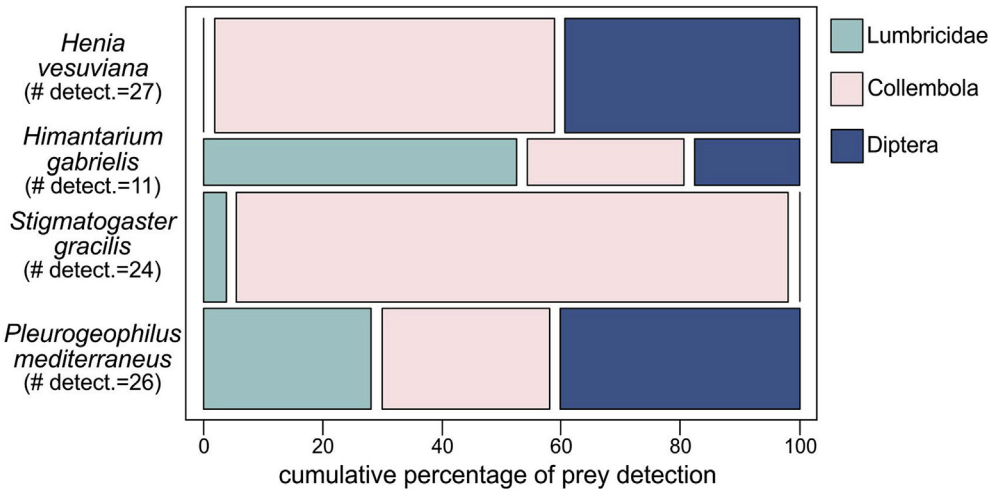

Fig. 2. Frequency distributions of prey groups in different species of geophilomorph centipedes. Box width is proportional to the relative frequency of a given prey with respect to total prey detections, box height is proportional to total prey detections in a given species of geophilomorph. Only geophilomorph specimens tested positively for prey DNA were considered. Differences in prey frequency distributions are all highly significant (see text).

rubidus and a species of *Octodrilus*. In the single *S. gracilis* specimen tested positive for lumbricids, a sequence of *Allolobophora rosea* was found.

Considering the *18S* sequences of Collembola (Table S4), in the gut content of 9 specimens of *H. vesuviana* we found 6 different sequences (alignment length 169 bp), all with pairwise p-distances < 2% and all closely matching up to 6 species of Entomobryomorpha. In particular, one sequence – found in several specimens – could be assigned confidently to the genus *Lepidocyrtus* and 4 sequences to other Entomobryidae (sensu Zhang and Deharverg, 2015). Similarly, in the gut content of 8 specimens of *S. gracilis* we found 7 different sequences (alignment length 205 bp), all with pairwise p-distances < 2%: 4 sequences could be confidently attributed to up to 4 species of Entomobryomorpha, either Isotomidae or Entomobryidae (of which one sequence identified as *Lepidocyrtus*), whereas the other 3 sequences remained unresolved as either Entomobryomorpha or Poduromorpha.

4. Discussion

Within-community dynamics depend prominently on how organisms utilize their environment, or, with different words, on the relation between their niche parameters. Since food is one of the most important dimensions of the niche, the analysis of animal diets contributes considerably to the organism's niche specifications (Krebs, 1999). Comparative studies on the diet of similar species within the same community are therefore the most direct way to investigate this subject, with the potential to disclose aspects of organism autecology and food-web relationships. As for arthropod predator guilds in soil ecosystems, trophic interactions have been extensively investigated, especially in agroecosystems (review in Furlong, 2015). However, most studies focused only on one taxonomic group of prey (e.g., Augustí et al., 2003) or one predator species (e.g., Krehenwinkel et al., 2017). Very few comparative studies were conducted on multiple predator species (e.g., Ferlian and Scheu, 2014; Šerić Jelaska et al., 2014), and even a lesser number on potentially competing predators within the same coenosis (e.g., Eitzinger and Traugott, 2011, carabid beetles; Toju and Baba, 2018, spiders; Günther et al., 2014, centipedes).

The present study is the first comparative study in nature on the diet of different syntopic species of geophilomorph centipedes. For this group, predation events in the field have been rarely reported, and only for very few species. Most observations were done in captivity, based on supplied food (reviewed in Lewis, 1981; see also Keay, 1986, Heaviside and Wallace, 1999), but prey consumption in natural conditions has remained almost unexplored. The main reason for this paucity of observational data is that most species prey inside the soil or leaf litter crevices, while the few regularly epigeic species creep on the forest floor only during the night. Additionally, as far as one can infer from the functional anatomy of the mouthparts, they mainly feed by sucking semi-fluid or externally-digested food upon killing (Manton, 1965; Koch and Edgecombe, 2012). All these features concur to make the traditional methods for diet investigation (direct observation of predation, morphological identification of prey remains in faeces or gut content) mostly unsuitable for these predators. To overcome these difficulties, we adopted a DNA-based approach to study geophilomorph gut content.

We found significant differences in the diet (described as the frequency distribution of prey groups) among all three investigated geophilomorph species from the same soil community during the same time period. The three species share a similar morphology of the poisonous claws when compared to other geophilomorphs, thus the observed differences in prey spectrum cannot be easily explained by any obvious specialization of their feeding apparatus. We also found that the three diets have a moderate degree of overlap, close to values reported in the few other investigations performed on soil predator arthropods (e.g., Wirta et al., 2015). The observed value, well within the 95% confidence interval of null model expectations, does not support neither the case of trophic niche separation due to interspecific competition for resources, nor that of foraging homogenization due to ecosystem limitation. However we cannot rule out that this result might derive from limitations of the present analysis: the statistical power of the tests may be flawed by the small size of our samples, only three major prey groups were assessed and the coarse-grain resolution of the taxonomic identification of prey can hide species-level diet differences. Last, we could not evaluate differences in prey size, for both the lack of species-level identification of prey and the relatively wide range of sizes the same prey species can traverse during its postembryonic development, especially for lumbricids.

Body-size is known to influence trophic resource partition within a predator guild (e.g. Günther et al., 2014) and can play an important role in reducing niche overlap among species (Woodward and Hildrew, 2002). Among the three investigated syntopic species, *H. vesuviana* and *S. gracilis* have a comparable body size, both in our sample and in general (up to 7 cm in length; Brolemann, 1930, Barber, 2009), whereas *H. gabrielis* is a much larger species (up to 20 cm; Brolemann, 1930). The latter, at variance with the other two species, fed on all target prey groups, in accordance with the hypothesis that large predators, to meet their energetic demand, tend to exploit prey communities more broadly (e.g., Woodward and Hildrew, 2002; Eitzinger et al., 2018).

We also found some evidence of a within-species variation in prey spectrum with body size in *H. gabrielis* and, to a lesser extent, in *H. vesuviana*, showing that also within-species size variation, mostly of ontogenetic origin (i.e., between developmental stages), may affect the composition of prey spectrum. Significant ontogenetic dietary shifts were reported for some Lithobiomorpha (Ferlian et al., 2012; Eitzinger et al., 2018), whereas little change of diet with growth was inferred by a fatty acids analysis in two Geophilomorpha (Ferlian et al., 2012).

Sex-differences in prey spectrum, when both males and females are active predators, have been poorly investigated in soil arthropods (e.g. Pekár et al., 2011; Šerić Jelaska et al., 2014), and not at all in centipedes. We did not find any evidence of difference in prey spectrum between the two sexes in our geophilomorph species. However, the small size of our sample might not provide enough statistical power to reveal subtle differences, so that we cannot exclude that males and females exploit trophic resources differently, as expected from sex-specific reproductive investment, including prolonged maternal care to brood (e.g., Lewis, 1981).

We found no evidence of diet differences between the two sampling seasons (spring and autumn). This fits our expectations, as sampling was limited to the two seasons that are locally the most similar for temperature and moisture conditions, in contrast with summer and winter. In general, some diet variation across the entire year is expected in seasonal climates, for different reasons including changes of prey availability and predator nutritional requirements. Indeed, Ferlian et al. (2012) found evidence of seasonal diet differences in two geophilomorph species (*Strigamia acuminata* and a species of *Geophilus* identified as *G. ribauti*) living in a beech forest (Hainich, central Germany). The fact that, contrary to Ferlian et al. (2012), we did not detect significant differences between our sampling periods may be explained by the different climatic regimes in the study areas (sub-mediterranean vs. sub-oceanic) and the different seasons that have been compared (only spring and autumn, rather than summer, autumn and winter). The different results could be also partially explained by the different methods used (DNA-based analysis vs. fatty acids analysis) and to the different species investigated (*Strigamia* and *Geophilus* are not strictly related to our species).

In the absence of knowledge about geophilomorph vertical distribution and movements inside the soil (see Voigtländer, 2011), we could not explore whether microhabitat structure, by affecting prey-predator interactions, might contribute to determine the difference in prey spectrum among the investigated geophilomorph species, as it has been showed for Lithobiomorpha (Günther et al., 2014). During sampling, *H. vesuviana* was regularly found in the upper litter layers or

under stone, as well as *H. gabrielis*, whereas *S. gracilis* was more frequently captured by digging into the soil. However, it is possible that all these species can move regularly through the soil column to prey.

About the specific diet of the investigated species the prey spectrum of *H. gabrielis* and *P. mediterraneus* included all the three taxa tested, whereas *H. vesuviana* and *S. gracilis* turned out to be less generalist, the latter with a sizably smaller value of niche width. The paucity of information about the trophic habits in these and other geophilomorph species hampers comparative evaluations. Some feeding observations in the field have been previously reported, but data were not collected methodically. Prey preferences of *H. vesuviana* were tested in a feeding trial (Keay, 1986), but the differences with respect to our results (earthworm consumption) question the reliably of laboratory experiments in inferring the natural prey spectrum of the species.

Collembolans resulted to be a major prey item in our sample, similarly to what was found for lithobiomorphs and carabid beetles in agroecosystems (Eitzinger and Traugott, 2011; Eitzinger et al., 2018). This may be related with their relative abundance in the soil and their high nutritive value (Bilde et al., 2000). However, DNA sequences of Collembola could be identified to the level of order or family only, owing to the poor resolution of the marker used, specifically devised for prey detection (Pompanon et al., 2012), and to the large number of collembolan species potentially living in the study area (many dozens; Dalla Montà and Dall'Ara, 2000).

Unlike collembolans, lumbricid DNA was frequently detected only in *H. gabrielis* and *P. mediterraneus*. The high taxonomic resolution of the DNA region amplified (Pompanon et al., 2012) and the availability of *12S* reference sequences for many of the species reported or expected in the study area (22 sequences available out of about 30 species; Omodeo et al., 2004) allowed a confident determination of most sequences at the species level. Our results show that these geophilomorphs caught different species of lumbricids, with no evidence for a selection between epigeic and endogeic species.

5. Concluding remarks

Our diet comparison between different species of geophilomorphs rests on DNA prey detection. This, however, can be biased by several biological factors, like differential prey digestion between predator species, different foraging frequency of predators on different prey taxa and variation of meal size (e.g., Greenstone et al., 2007; Hosseini et al., 2008). It is therefore advisable that further investigations, beyond involving a wider taxon sampling, proceed in parallel in both laboratory and natural conditions.

Nonetheless, these results show the higher value of species-specific gut content analysis performed on single specimens, rather than on a pool of specimens or species, when one aims at precisely characterizing the feeding strategies within a community.

The approach we used for sequence identification, which integrates genetic, geographic and taxonomic information, gave the best level of taxonomic resolution possible for the current, incomplete state of knowledge on the actual taxonomic diversity and geographic distribution of the prey groups. However, the limited number of reference sequences in repositories and the insufficient reliability of their taxonomic identification contributed to sizably reduce the resolution we achieved.

Soil communities are complex ecosystems, where the food-web relationships involving arthropod predators and their preys certainly represent a major component. Assessing the trophic niche of geophilomorphs and other centipedes is important not only to shed light on the biology of a group of predators so far little investigated, but also to clarify prey-predator interactions and intraguild predation in soil coenosis, to achieve an overall representation of soil food webs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2018.09.021.

References

Abrams, P., 1980. Are competition coefficients constant? Inductive versus deductive approaches. The American Naturalist 116, 730–735.
Agustí, N., Shayler, S.P., Harwood, J.D., Vaughan, I.P., Sunderland, K.D., Symondson, W.O., 2003. Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. Molecular Ecology 12, 3467–3475.
Albert, A.M., 1979. Chilopoda as part of the predatory macroarthropod fauna in forests: abundance, biomass, and metabolism. In: Camatini, M. (Ed.), Myriapod Biology. Academic Press, New York-London, pp. 215–231.
Barber, A.D., 2009. Centipedes. Synopses of the British Fauna, vol. 58 The Linnean Society of London.
Bilde, T., Axelsen, J.A., Toft, S., 2000. The value of Collembola from agricultural soils as food for a generalist predator. Journal of Applied Ecology 37, 672–683.
Bolnick, D.I., Amarasekare, P., Araujo, M.S., Burger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C., Vasseur, D.A., 2011. Why intraspecific trait variation matters in community ecology. Trends in Ecology & Evolution 26, 183–192.
Bonato, L., Lopresti, M., Minelli, A., Cerretti, P., 2014. ChiloKey, an interactive identification tool for the geophilomorph centipedes of Europe (Chilopoda, Geophilomorpha). ZooKeys 443, 1–9.
Bonato, L., Minelli, A., Zapparoli, M., 2018. Centipede communities (Chilopoda) of forest soils across Europe: abundance, species richness and species composition. Atti Accademia Nazionale Italiana di Entomologia 65, 113–120.
Brolemann, H.W., 1930. Éléments d'une faune des myriapodes de France. Chilopodes. Imprimérie Toulousaine, Toulouse.
Chesson, P., 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31, 343–366.
Czekanowski, J., 1909. Zur differentialdiagnose der Neandertalgruppe. Korrespondenzblatt der Deutschen Gesellschaft für Anthropologie 40, 44–47.
Dallai, R., Malatesta, E., Ramellini, P., 1995. Apterygota: Collembola, Protura, Microcoryphia e Zygentoma (= Thysanura s.l.), Diplura. In: In: Minelli, A., Ruffo, S., La Posta, S. (Eds.), Checklist delle specie della Fauna Italiana, vol. 33. Calderini, Bologna, pp. 25.
Dalla Montà, L., Dall'Ara, P., 2000. Contributo alla conoscenza della collembolofauna del Parco Regionale dei Colli Euganei. Bollettino del Museo Civico di Storia Naturale di Verona 24. pp. 107–122.
Domínguez, J., Aira, M., Breinholt, J.W., Stojanovic, M., James, S.W., Pérez-Losada, M., 2015. Underground evolution: new roots for the old tree of lumbricid earthworms. Molecular Phylogenetics and Evolution 83, 7–19.
Eitzinger, B., Micic, A., Körner, M., Traugott, M., Scheu, S., 2013. Unveiling soil food web links: new PCR assays for detection of prey DNA in the gut of soil arthropod predators. Soil Biology and Biochemistry 57, 943–945.
Eitzinger, B., Rall, B., Traugott, M., Scheu, S., 2018. Testing the validity of functional response models using molecular gut content analysis for prey choice in soil predators. Oikos. https://doi.org/10.1111/oik.04885.
Eitzinger, B., Traugott, M., 2011. Which prey sustains cold-adapted invertebrate generalist predators in arable land? Examining prey choices by molecular gut-content analysis. Journal of Applied Ecology 48, 591–599.
Eitzinger, B., Unger, E.M., Traugott, M., Scheu, S., 2014. Effects of prey quality and predator body size on prey DNA detection success in a centipede predator. Molecular Ecology 23, 3767–3776.
Ferlian, O., Scheu, S., 2014. Shifts in trophic interactions with forest type in soil generalist predators as indicated by complementary analyses of fatty acids and stable isotopes. Oikos 123, 1182–1191.
Ferlian, O., Scheu, S., Pollierer, M.M., 2012. Trophic interactions in centipedes (Chilopoda, Myriapoda) as indicated by fatty acid patterns: variations with life stage, forest age and season. Soil Biology and Biochemistry 52, 33–42.
Fisher, R.A., 1922. On the interpretation of $\chi 2$ from contingency tables, and the calculation of P. Journal of the Royal Statistical Society 85, 87–94.
Furlong, M.J., 2015. Knowing your enemies: integrating molecular and ecological methods to assess the impact of arthropod predators on crop pests. Insect Science 22, 6–19.
Gotelli, N.J., Hart, E.M., Ellison, A.M., 2015. EcoSimR: null model analysis for ecological data. R package version 0.1.0. http://github.com/gotellilab/EcoSimR.

Greenstone, M.H., Rowley, D.R., Weber, D.C., Hawthorne, D.J., 2007. Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two predators of the Colorado potato beetle. Bulletin of Entomological Research 97, 201–209.
Grgič, T., Kos, I., 2003. Centipede diversity in patches of different development phases in an unevenly-aged beech forest stand in Slovenia. African Invertebrates 44, 237–252.
Günther, B., Rall, B.C., Ferlian, O., Scheu, S., Eitzinger, B., 2014. Variations in prey consumption of centipede predators in forest soils as indicated by molecular gut content analysis. Oikos 123, 1192–1198.
Haddad, C.R., Brabec, M., Pekár, S., Fourie, R., 2016. Seasonal population dynamics of a specialized termite-eating spider (Araneae: Ammoxenidae) and its prey (Isoptera: Hodotermitidae). Pedobiologia 59, 105–110.
Harper, G.L., King, R.A., Dodd, C.S., 2005. Rapid screening of invertebrate predators for multiple prey DNA targets. Molecular Ecology 14, 819–827.
Heaviside, A., Wallace, A., 1999. Size-selective predation by *Geophilus insculptus* on *Blaniulus guttulatus*. Bulletin of the British Myriapod Group 15, 5–8.
Hosseini, R., Schmidt, O., Keller, M.A., 2008. Factors affecting detectability of prey DNA in the gut contents of invertebrate predators: a polymerase chain reaction-based method. Entomologia Experimentalis et Applicata 126, 59–66.
Hubbell, S.P., 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Monographs in Population Biology. Princeton University Press, Princeton.
Hurlbert, S.H., 1978. The measurement of niche overlap and some relatives. Ecology 59, 67–77.
Keay, A.N., 1986. Predation and prey in *Henia* (*Chaetechelyne) vesuviana* (Newport) (Chilopoda, Geophilomorpha). Bulletin of the British Myriapod Group 3, 21–25.
Klarica, J., Kloss-Brandstätter, A., Traugott, M., Juen, A., 2012. Comparing four mitochondrial genes in earthworms – implications for identification, phylogenetics, and discovery of cryptic species. Soil Biology and Biochemistry 45, 23–30.
Klarner, B., Winkelmann, H., Krashevska, V., Maraun, M., Widyastuti, R., Scheu, S., 2017. Trophic niches, diversity and community composition of invertebrate top predators (Chilopoda) as affected by conversion of tropical lowland rainforest in Sumatra (Indonesia). PLoS One 12 (8), e0180915.
Koch, M., Edgecombe, G.D., 2012. The preoral chamber in geophilomorph centipedes: comparative morphology, phylogeny, and the evolution of centipede feeding structures. Zoological Journal of the Linnean Society 165, 1–62.
Krebs, C.J., 1999. Niche overlap and diet analysis. In: Krebs, C.J. (Ed.), Ecological Methodology, second ed. Addison-Welsey Publishers, Menlo Park.
Krehenwinkel, H., Kennedy, S., Pekár, S., Gillespie, R.G., 2017. A cost-efficient and simple protocol to enrich prey DNA from extractions of predatory arthropods for large-scale gut content analysis by Illumina sequencing. Methods in Ecology and Evolution 8, 126–134.
Kuusk, A.K., Agustí, N., 2008. Group-specific primers for DNA based detection of springtails (Hexapoda: Collembola) within predator gut contents. Molecular Ecology Resources 8, 678–681.
Levine, J.M., HilleRisLambers, J., 2009. The importance of niches for the maintenance of species diversity. Nature 461, 254–257.
Levins, R., 1968. Evolution in Changing Environments: Some Theoretical Explorations. Princeton University Press, Princeton.
Lewis, J.G.E., 1981. The Biology of Centipedes. Cambridge University Press, New York.
Manton, S.M., 1965. The evolution of arthropodan locomotory mechanism. Part. 8. Functional requirements and body design in Chilopoda together with a comparative account of their skeleto-muscolar system and appendix on a comparison between burrowing forces of annelids and chilopods and its bearing upon the evolution of the arthropodan. Journal of the Linnean Society of London: Zoology 46, 251–484.
Michalko, R., Pekár, S., 2015. Niche partitioning and niche filtering jointly mediate the coexistence of three closely related spider species (Araneae, Philodromidae). Ecological Entomology 40, 22–33.
Minelli, A., 2011. Chilopoda – General morphology. In: Minelli, A. (Ed.), Treatise on Zoology – Anatomy, Taxonomy, Biology. The Myriapoda. vol. 1. Chilopoda Brill, Boston, pp. 43–66.
Morisita, M., 1959. Measuring of the Dispersion and Analysis of Distribution Patterns. Memoires of the Faculty of Science. vol. 2. Kyushu University, pp. 215–235 Series E: Biology.
Nilsson, R.H., Ryberg, M., Kristiansson, E., Abarenkov, K., Larsson, K.-H., Kõljalg, U., 2006. Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. PLoS One 1, e59.
Omodeo, P., Rota, E., Sambugar, B., 2004. Annelida Oligochaeta. In: Ruffo, S., Stoch, F. (Eds.), Ckmap 2004. Versione 5.1. Checklist e distribuzione della fauna italiana: 10.000 specie terrestri e delle acque interne. Ministero dell'Ambiente e della Tutela del Territorio In CD-ROM.
Pekár, S., Martišová, M., Bilde, T., 2011. Intersexual trophic niche partitioning in an ant-eating spider (Araneae: Zodariidae). PLoS One 6, e14603.
Peretti, E., Bonato, L., submitted for publication How many species of centipedes coexist in temperate forests? Estimating local species richness of Chilopoda in soil coenoses of the South-Eastern Prealps. European Journal of Soil Biology, under review.
Pérez-Losada, M., Bloch, R., Breinholt, J.W., Pfenninger, M., Domínguez, J., 2012. Taxonomic assessment of Lumbricidae (Oligochaeta) earthworm genera using DNA barcodes. European Journal of Soil Biology 48, 41–47.
Petersen, H., Luxton, M., 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos 39, 287–388.
Pianka, E.R., 1973. The structure of lizard communities. Annual Review of Ecology and Systematics 4, 53–74.
Polis, G.A., 1994. Food webs, trophic cascades and community structure. Australian Journal of Ecology 19, 12–136.
Pollierer, M.M., Scheu, S., Haubert, D., 2010. Taking it to the next level: trophic transfer of marker fatty acids from basal resource to predators. Soil Biology and Biochemistry 42, 919–925.
Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N., Taberlet, P., 2012. Who is eating what: diet assessment using next generation sequencing. Molecular Ecology 21, 1931–1950.
Schoener, T.W., 1974. Resource partitioning in ecological communities. Science 185, 27–39.
Šerić Jelaska, L., Franjević, D., Jelaska, S.D., Symondson, W.O.C., 2014. Prey detection in carabid beetles (Coleoptera: Carabidae) in woodland ecosystems by PCR analysis of gut contents. European Journal of Entomology 111, 631–638.
Toju, H., Baba, Y.G., 2018. DNA metabarcoding of spiders, insects, and springtails for exploring potential linkage between above- and below-ground food webs. Zoological Letters 4, 4.
Voigtländer, K., 2011. Chilopoda – Ecology. In: In: Minelli, A. (Ed.), Treatise on Zoology – Anatomy, Taxonomy, Biology. The Myriapoda, vol. 1. Chilopoda Brill, Boston, pp. 309–325.
Waldner, T., Sint, D., Juen, A., Traugott, M., 2013. The effect of predator identity on post-feeding prey DNA detection success in soil-dwelling macro-invertebrates. Soil Biology and Biochemistry 63, 116–123.
Winemiller, K.O., Pianka, E.R., 1990. Organization in natural assemblages of desert lizards and tropical fishes. Ecological Monographs 60, 27–55.
Wirta, H., Weingartner, E., Hambäck, P., Roslin, T., 2015. Extensive niche overlap among the dominant arthropod predators of the High Arctic. Basic and Applied Ecology 16, 86–92.
Woodward, G., Hildrew, A.G., 2002. Body-size determinants of niche overlap and intraguild predation within a complex food web. Journal of Animal Ecology 71, 1063–1074.
Xiong, Y., Gao, Y., Yin, W.-Y., Luan, Y.-X., 2008. Molecular phylogeny of Collembola inferred from ribosomal RNA genes. Molecular Phylogenetics and Evolution 49, 728–735.
Yu, D., Zhang, F., Stevens, M.I., Yan, Q., Liu, M., Hu, F., 2015. New insight into the systematics of Tomoceridae (Hexapoda, Collembola) by integrating molecular and morphological evidence. Zoologica Scripta 45, 286–299.
Zhang, J., 2016. SPAA: SPecies Association Analysis. R package version 0.2.2. https://cran.r-project.org/web/packages/spaa.
Zhang, F., Deharveng, L., 2015. Systematic revision of Entomobryidae (Collembola) by integrating molecular and new morphological evidence. Zoologica Scripta 44, 298–311.
Zhang, F., Chen, Z., Dong, R.-R., Deharveng, L., Stevens, M.I., Huang, Y.-H., Zhu, C.-D., 2014. Molecular phylogeny reveals independent origins of body scales in Entomobryidae (Hexapoda: Collembola). Molecular Phylogenetics and Evolution 70, 231–239.
Zhang, F., Ma, Y., Greenslade, P., 2017. New Australian Paronellidae (Collembola) reveal anomalies in existing tribal diagnoses. Invertebrate Systematics 31, 375–393.
Zhang, F., Sun, D.-D., Yu, D.-Y., Wang, B.-X., 2015. Molecular phylogeny supports S-chaetae as a key character better than jumping organs and body scales in classification of Entomobryoidea (Collembola). Scientific Reports 5, 12471.

Comparative analysis of diet in syntopic geophilomorph species (Chilopoda, Geophilomorpha) using a DNA-based approach

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Appendix A. Supplementary Data

Table S1. Primers used to amplify predator *COI* fragment.

Species	Primers employed for preliminary *COI* amplification			Specific primers developed for *COI*		
	For	**Rev**	**Source**	**For**	**Rev**	**Size of the amplified fragment**
Henia vesuviana	LCO1490	HCO2198	Folmer et al. 1994	Heniav_COI For: CGAATAAATAACCTAAGAT	Heniav_COI Rev: CACACAAATAAGGGAATG	272 bp
Himantarium gabrielis	COI Stigm For (5'-3'): GCAGCCTTATTGGAGACGAC	COI Stigm Rev (5'-3'): CGGTAAGGAGTATGGTGATTG	Original (amplified fragment 501 bp)	Himag_COI For: GCCGCAGTAGAAAATGGAG	Himag_COI Rev: TGGAAGGGAGAGGAGAAGA	255 bp
Stigmatogaster gracilis	LCO1490	HCO2198	Folmer et al. 1994	COI Stigm For: GCAGCCTTATTGGAGACGAC	COI Stigm Rev2: ACGGTTCATCCTGTTCCTG	250 bp
Pleurogeophilus mediterraneus	COI Strig For	COI Strig Rev	Bonato et al. 2017	Pleum_COI For: CCTCAATAGCCGTAGAAAG	Pleum_COI Rev: GACGGGTAGTGAGAGTAG	264 bp

Table S2. Molecular markers, primers and screening PCR conditions for targeted prey taxa.

Taxon	Gene	Primers	PCR cycles	Annealing and elongation conditions	$MgCl_2$ final concentration	Final concentration of each primer	DNA quantity	Notes
Lumbricidae	*12S*	185F 14233R	35-38	57 °C for 60 s, 72 °C for 20 s	1.125 mM	0.375 µM	1.25 µl of template, about 200-250 ng	protocol modified from Harper et al. (2005)
Collembola	*18S*	Col3F Col5R	35	65 °C for 90 s, 72 °C for 25 s	2.5 mM	2 µM	2.5-3 µl of template, about 700 ng	protocol modified from Günther et al. (2014)
Diptera	*18S*	DIP S16 DIP A17	38	56 °C for 30 s, 72 °C for 45 s	1.25 mM	0.5 µM	2 µl of template, about 150 ng	protocol modified from Eitzinger et al. (2013)

Table S3. *12S* DNA sequences of Lumbricidae detected in the Geophilomorpha gut content and tentative identification.

Sequence code	Frequency of detection in			Tentative identification	Sequence IDs of the best matches by MegaBlast	% identity with the best matches
	H. gabrielis (n=6)	*S. gracilis* (n=1)	*P. mediterraneus* (n=6)			
Lumbricidae 1	1	1	0	*Allolobophora rosea* (Savigny, 1826)	JN869657, AJ865005, KJ912392	90%
Lumbricidae 2	1	0	0	*Allolobophora rosea* (Savigny, 1826)	JN869657, AJ865005, KJ912392	90%
Lumbricidae 3	1	0	0	*Allolobophora rosea* (Savigny, 1826)	JN869657, AJ865005, KJ912392	90%
Lumbricidae 4	1	0	0	*Allolobophora rosea* (Savigny, 1826)	JN869657, AJ865005, KJ912392	90%
Lumbricidae 5	0	0	1	*Allolobophora rosea* (Savigny, 1826)	JN869657, AJ865005, KJ912392	90%
Lumbricidae 6	0	0	1	*Allolobophoridella eiseni* (Levinsen, 1884)	KJ912386	93%
Lumbricidae 7	2	0	2	*"Dendrobaena" cognettii* (Rosa, 1884) / *pygmaea* (Friend, 1923) [a]	KJ91240	95%
Lumbricidae 8	0	0	1	*Dendrodrilus rubidus* (Savigny, 1826)	JN869677	100%
Lumbricidae 9	0	0	1	*Octodrilus* sp.	KJ912453	86%

[a] *D. pygmaea* only recently distinguished as a different species from *D. cognettii* (Dominguez et al. 2015).

Table S4. *18S* DNA sequences of Collembola detected in the Geophilomorpha gut content and tentative identification.

Sequence code	detection frequency		Tentative identification	Sequence IDs of the best matches by MegaBlast	% identity with the best matches
	H. vesuviana (n=9)	*S. gracilis* (n=8)			
Collembola 1	1	0	Entomobryomorpha	KM978408, KM978401, KC236257, KC236252, KC236249, KC236232, U61301, KY052894, KY382773, KY382770, KY382765, KY382764, KY382762, KY382756, KX351376, KX351375, KX351374, KX351371, KX351369, KX351356	99%
Collembola 2	2	1	Entomobryidae	KM978408, KC236252, U61301, KY052894, KM978404, KM978401, KC236264, KC236249, KC236238, KC236233, KC236232, KC236229, KC236228, KC236226, KY382759, KM978405, KM978400, KM978399, KM978398, KC236266	99%
Collembola 3	1	0	Entomobryidae	KY382759, KM978408, KC236251, KC236242, KC236241, AJ605710 / KY052894, KY382766, KM978404, KM978401, JN981037, KC236264, KC236252,KC236250, KC236249, KC236238, KC236233, KC236232, KC236229, KC236226	99% / 98%
Collembola 4	1	0	Entomobryidae	KY382737, KC236254, KC236251, AY555514, U61301, KY382763, KC236250, EU368606 / KY382769, KM978408, KC236253, KC236252, KC236242,KC236241, KC236227, AJ605710, KY052894, KY382750, KM978404, KM978401	98% / 97%
Collembola 5	3	1	*Lepidocyrtus* sp. (Entomobryidae)	U61301 / KY382754, KM978408, KC236254, KC236253, KC236252, KY052894, KY382750, KM978404, KM978401, KC236264, KC236249, KC236248, KC236246, KC236245, KC236244, KC236238, KC236233, KC236232, KC236229	100% / 99%
Collembola 6	1	0	Entomobryidae	KY382759, KM978408, KC236252, KC236251, KC236242, KC236241, AJ605710, KY052894, KY382763, KM978404, KM978401, KC236264, KC236250, KC236249, KC236238, KC236233, KC236232, KC236229, KC236228, KC236226	99%
Collembola 7	0	1	Entomobryomorpha or Poduromorpha	DQ016556, JN981033, Z26765, AY555521, KX351371, KX351356, KT006882, JN981032, EU368601, DQ016561, DQ016557, DQ016555, DQ365774, AY037172, AY596361, AY555520, KY382756, JN981038 / KM978401, JN981039	99% / 98%
Collembola 8	0	2	Isotomidae or Entomobryidae (Entomobryomorpha)	KX351372, JN981036, KX351369, KX351356, KM978408, KM978401, JN981033, JN981032, KC236249, KC236232, DQ016557, DQ016556, EF023333, DQ365774, AY037172, AY555521, AY555520, KY382756, KX351376, KX351375	99%
Collembola 9	0	1	Entomobryomorpha or Poduromorpha	KC236254, DQ016557, DQ016556, AY037172, AY555521, AY555520, KY382769, KY382758, KX351356, KT003717, KM978408, KM978401, JN981035, JN981033, JN981032, JN981029,JN981027, KC236263, KC236249, KC236246	99%
Collembola 10	0	1	Entomobryomorpha or Poduromorpha	KX351356, JN981032, Q365774, AY037172, AY555520, JN981039, JN981037, JN981033, JN981023, JN981022, DQ016557, DQ365782, DQ365781, AY555521, JN981030, EU368602, KY382756, JN981038, JN981026, KC236239	99%
Collembola 11	0	1	Isotomidae or Entomobryidae (Entomobryomorpha)	KX351356, KM978408, JN981032, DQ365774, KY052894, KM978404, KM978401, JN981039, JN981037, JN981033, JN981023, JN981022, KC236264, KC236254, KC236252, KC236251, KC236249, KC236238, KC236233, KC236232	99%

Sequences

12S sequences of Lumbricidae detected in the Geophilomorpha gut content:

>Lumbricidae 1
CACCTCTAAAAGTATAAGTATGCAGCAATGATAATACTCATTTACGTCAGGTCAAAGTGC
AGCTTATGAGAAGGTGATGATGGGTTACAACCTAAATACAGATACGAAATATGTCACCAA
AAGTCATATAAAGGTGGACTTGGACGTAATAATTACATCAAGTTATAATGAAGACGAATC
TAAGACATGC

>Lumbricidae 2
CACCTCTAAAAGTATAAGTATGCAGCAATGATAATACTCATTTACGTCAGGTCAAAGTGC
AGCTTATGAGAAGGTGATGATGGGTTACAACCTAAATACAGATACGAAATATGTCACCAA
AAGTCATATAAAGGGGGACTTGGACGTAATAATTACATCAAGTTATAATGAAGACGAATC
TAAGACATGC

>Lumbricidae 3
CACCTCTAAAAGTATAAGTATGCAGCAATGATAATACTCATTTACGTCAGGTCAAAGTGC
AGCTTATGAGAAGGTGATGATGGGTTACAACCTAAATACAGGTACGAAATATGTCACCAA
AAGTCATATAAAGGTGGACTTGGACGTAATAATTACATCAAGTTATAATGAAGACGAATC
TAAGACATGC

>Lumbricidae 4
CACCTCTAAAAGTATAAGTATGCAGCAATGATAATACTCATTTACGTCAGGTCAAAGTGC
AGCTTATGAGAAGGCGATGATGGGTTACAACCTAAATACAGATACGAAATATGTCACCAA
AAGTCATATAAAGGTGGACTTGGACGTAATAATTACATCAAGTTATAATGAAGACGAATC
TAAGACATGC

>Lumbricidae 5
CACCTCTAAAAGTATAAGTATGCAACAATGGTAATACCCATTTACGTCAGGTCAAAGTGC
AGCTTATGAGAAGGTGACGATGGGTTACAACCTAAATATAGATACGAAATATGTCACTAA
AAGTCATATAAAGGTGGACTTGGACGTAATAATTACATCAAGTTATAATGAAGACGAATC
TAAGACATGC

>Lumbricidae 6
CACCTCTAAAAGAATTTAGTATGCAATAATGGCTTTCCCATTTACGTCAGGTCAAAGTGC
AGCTTATGAAAGGGAGACGATGGGTTACACCCTAAACAAAGATACCAATTGCAGCATTAA
AAGCTTCATGAAGTTGGACTTGGACGTAATTATAATTCAATATTACAATGAAAACGAATC
TAAGACATGC

>Lumbricidae 7
TACCTTTAAAAGAATCGTAGTATGCAAAAATGATAACCTCATACACGTCAGGTCAAAGTG
CAGCTAATGAAGGGGGGGGCGATGGGTTACACTATAAATAAAGATACGAATTATAATATA
AAAATTTATATGAAGGTGGACTTGGATGTAATCGCAACTCCAGTTTAAATGAAACTGAAC
CTAAGACATGC

>Lumbricidae 8
CACCTCTAAGAGTCTAAAGTGTGCAATAATGATTATTCTCATATATGTCAGGTCAAAGTG
CAGCTTATGAAAGGGAGATGATGGGTTACACCCTATATAAAGATACCAATTTTAGCATTA
AAAGCTAAATGAAGGTGGACTTGGACGTAATTATAATTCAATGTTATAGTGAAAACGAAT
CTAAGACATGT

>Lumbricidae 9
AACCTCTAAAAGTAGAAGTATGCAAGTATGATTAACTCATCTACGTCAGGTCAAAGTGCA
GCTAATGAAAAGGAGATGATGGGTTACACCCTAAATAAAGACACGAAATACAGCACTAAG
GTGCAGTATGAAGGCGGACTTGGATGTAATACTAATTAAATGTTATAATGAAGACGAATC
TAAGACATGT

18S sequences of Collembola detected in the Geophilomorpha gut content:

>Collembola 1
GTGCTCAACGCAGGCGCTACAGCCTGAACATTTGTGCATGGAATAATGGAATAGGATCTC
GGTTCTATTTCGTTGGCTTTCGGAGTCGAGGTAATGATTAATAGGGACAGACGGGGGCAT
TCGTACTGCGACGTTAGAGGTGAAATTCTTGGATCGTCGCAAGACGAAC

>Collembola 2
TCATGGTGCTCTTAACCGGGTGCCTTGAGTGGCCAGCACGTTTACTTTGAAAAAATTGGA
GTGCTCAACGCAGGCGCTACAGCCTGAACATTTGTGCATGGAATAATGGAATAGGATCTC
GGTTCTATTTCGTTGGCTTTCGGAGTCGAGGTAATGATTAATAGGGACAGACGGGGGCAT
TCGTACTGCGACGTTAGAGGTGAAATTCTTGGATCGTCGCAAGACGAAC

>Collembola 3
TCATGGTGCTCTTAACCGGGTGCCATGAGTGGCCAGCACGTTTACTTTGAAAAAATTGGA
GTGCTCAACGCAGGCGCTACAGCCTGAACATTTGTGCATGGAATAATGGAATAGGACCTC
GGTTCTATTTCGTTGGCTTTCGGAGTCGAGGTAATGATTAATAGGGACAGACGGGGGCAT
TCGTACTGCGACGTTAGAGGTGAAATTCTTGGATCGTCGCAAGACGAAC

>Collembola 4
TCATGGTGCTCTTAACCGGGTGCCTTGAGTGGCCAGCGCGTTTACTTTGAAAAAATTGGA
GTGCTCAACGCAGGCGCTACAGCCTGAACATTCGTGCATGGAATAATGAAATAGGACCTC
GGTTCTATTTCGATGGCTTTCGGAGTCGAGGTAATGATTAATAGGGACAGACGGGGGCAT
TCGTACTGCGACGTTAGAGGTGAAATTCTTGGATCGTCGCAAGACGAAC

>Collembola 5
TCATGGTGCTCTTAACCGGGTGCCTTGAGTGGCCAGCACGTTTACTTTGAAAAAATTGGA
GTGCTCAACGCAGGCGCTACAGCCTGAACATTTGTGCATGGAATAATGAAATAGGATCTC
GGTTCTATTTCGTTGGCTTTCGGAGTCGAGGTAATGATTAATAGGGACAGACGGGGGCAT
TCGTACTGCGACGTTAGAGGTGAAATTCTTGGATCGTCGCAAGACGAAC

>Collembola 6
TCATGGTGCTCTTAACCGGGTGCCTTGAGTGGCCAGCACGTTTACTTTGAAAAAATTGGA
GTGCTCAACGCAGGCGCTACAGCCTGAACATTTGTGCATGGAATAATGGAATAGGACCTC
GGTTCTATTTCGTTGGCTTTCGGAGTCGAGGTAATGATTAATAGGGACAGACGGGGGCAT
TCGTACTGCGACGTTAGAGGTGAAATTCTTGGATCGTCGCAAGACGAAC

>Collembola 7
TCTTGGTTCTCTTAATCGAGTGCCTTGAGTGGCCAGCACGTTTACTTTGAAAAAATTGGA
GTGCTCAAAGCAGGCGCTACAGCCTGAACATTAGTGCATGGAATAATGGAATAGGATCTC
GGTTCTATTTCGTTGGCTTTCGGAGTCGAGGTAATGATTAATAGGGACAGACGGGGGCAT
TCGTACTGCGACGTTAGAGGTGAAATTCTTGGATCGTCGCAAGACGAAC

>Collembola 8
TGAGTGGCCAGCACGTTTACTTTGAAAAAATTGGAGTGCTCAAAGCAGGCGCTACAGCCT
GAACATTAGTGCATGGAATAATGGAATAGGATCTCGATTCTATTTCGTTGGCTTTCGGAG
TCGAGGTAATGATTAATAGGGACAGACGGGGGCATTCGTACTGCGACGTTAGAGGTGAAA
TTCTTGGATCGTCGCAAGACGAAC

>Collembola 9
GCCTTGAGTGGCCAGCACGTTTACTTTGAAAAAATTGGAGTGCTCAAAGCAGGCGCTACA
GCCTGAACATTAGTGCATGGAATAATGAAATAGGATCTCGGTTCTATTTCGTTGGCTTTC
GGAGTCGAGGTAATGATTAATAGGGACAGACGGGGGCATTCGTACTGCGACGTTAGAGGT
GAAATTCTTGGATCGTCGCAAGACGAAC

>Collembola 10
TCATGGTTCTCTTTACCGAGTGCCATGAGTGGCCAGCACGTTTACTTTGAAAAAATTGGA
GTGCTCAAAGCAGGCGCTACAGCCTGAACATTAGTGCATGGAATAATGGAATAGGATCTC
GGTTCTATTTCGTTGGCTTTCGGAGTCGAGGTAATGATTAATAGGGACAGACGGGGGCAT
TCGTACTGCGACGTTAGAGGTGAAATTCTTGGATCGTCGCAAGACGAAC

>Collembola 11
TCATGGTGCTCTTAACCGGGTGCCATGAGTGGCCAGCACGTTTACTTTGAAAAAATTGGA
GTGCTCAAAGCAGGCGCTACAGCCTGAACATTAGTGCATGGAATAATGGAATAGGATCTC
GGTTCTATTTCGTTGGCTTTCGGAGTCGAGGTAATGATTAATAGGGACAGACGGGGGCAT
TCGTACTGCGACGTTAGAGGTGAAATTCTTGGATCGTCGCAAGACGAAC

Additional references

Bonato, L., Bortolin, F., Drago, L., Orlando, M., Dányi, L., 2017. Evolution of *Strigamia* centipedes (Chilopoda): a first molecular assessment of phylogeny and divergence times. Zoologica Scripta 46, 486–495.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3, 294–299.