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Short communication

Unveiling soil food web links: New PCR assays for detection of prey DNA in the gut of soil arthropod predators

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ABSTRACT

Molecular gut content analysis provides a highly specific and sensitive tool to examine the diet of soil invertebrates. Here, we present new polymerase chain reaction (PCR) assays for the detection of twelve prey taxa common in Central European forest soils. The assays target five species of collembolans as well as dipterans, gamasid and oribatid mites, lithobiid centipedes, spiders, staphylinid beetles and woodlice at the group level, amplifying 123–299 bp long DNA fragments. Cross-reactivity tests against 119 soil invertebrate taxa confirm their specificity. These new PCR assays were found to be highly sensitive, revealing the consumption of five different prey taxa in field-collected centipedes. Thus they provide a ready-to-use approach for unravelling trophic interactions among soil arthropods.

Molecular techniques have become increasingly popular to study predator–prey interactions under natural conditions (King et al., 2008; Pompanon et al., 2012) including those below-ground (e.g. Juen and Traugott, 2007; Heidemann et al., 2011). This is because they allow tracking feeding interactions which are inaccessible with conventional methodology (Symondson, 2002). Using polymerase chain reaction (PCR) assays it is possible to detect DNA of animal prey (including carrion; Juen and Traugott, 2005; Foltan et al., 2005) and of plant food sources (Staudacher et al., 2011) in a consumer's gut. This offers a new means to study the trophic linkages among soil-dwelling animals as well as between plants and root feeding animals, addressing an important compartment of the soil food web.

Most predators in soil are supposed to be generalists (Scheu and Setälä, 2002). Therefore, we intended to address trophic links on higher taxonomic levels (i.e., family and order rather than species level), to enable for a broad characterization of the predators' dietary spectrum.

The goal was to establish ready-to-use PCR assays which allow targeting a variety of prey groups which regularly might fall within the prey range of soil-dwelling generalist predators. Twelve new assays were designed and tested on field-collected specimens of *Lithobius* spp. Leach, 1814. Within these assays we target five species of collembolans using species-specific primers, whereas dipterans, gamasid and oribatid mites, lithobiid centipedes, staphylinid beetles, spiders, and woodlice are targeted by group-specific primers.

Invertebrates were collected in summers of 2008–2010 in beech forests of the national park Hainich (Thuringia, Germany) by sieving of litter and heat extraction of soil samples. To avoid amplification of ingested food DNA, all specimens were starved for 7–10 days before freeze-killing them. After identification to species level, total DNA was extracted using the blood & tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. In dipterans, gamasid mites, spiders and staphylinid beetles the nuclear 18S rDNA gene was sequenced; in collembolans part of the cytochrome C oxidase subunit I gene (COI) was sequenced (for DNA sequencing protocols see Supplementary material S1). All

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sequences were corrected manually and checked for similarity with sequences from GenBank using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). The new sequences were deposited in GenBank (JQ801570–JQ801608).

Based on these sequences and additional 18S rDNA sequences from GenBank (Table S1; Supplementary material), PCR primers, including ones with degenerated bases, were designed using PrimerPremier 5 (PREMIER Biosoft International, Palo Alto, CA, USA) following the guidelines of King et al. (2008).

The optimal annealing temperatures of each primer pair was determined by gradient PCR whereas the specificity was evaluated by cross-reactivity testing using ten individuals of each target taxon and up to 119 non-target taxa. The latter represent all major invertebrate groups at the study site (Table S2; Supplementary material). To test sensitivity of the PCR assays we employed a dilution series of DNA extracts: the DNA concentration of each target taxon (two individuals each) was measured using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA), adjusted to 200 pg μL^{-1}, two-fold serially diluted and then mixed with DNA of *Lithobius mutabilis* L. Koch, 1862 (200 pg μL^{-1}; for testing primers LIT S13/LIT A8, DNA of *Strigamia acuminata* (Leach, 1815) was used). This resulted in final prey DNA concentrations of 30,000, 15,000, 7500, 3750, 1875, 937.5, 468.75, 234.38, 117.19, 58.59, 29.29, 14.65, 7.32, 3.66, and 1.83 fg target taxa DNA per µL PCR and predator-to-prey DNA ratios of 1:1 to 20,000:1. Each 10 µL PCR consisted of 5 µL SuperHot Mastermix (2×), 1.25 mM $MgCl_2$ (both Geneaxxon, Ulm, Germany), 0.5 µL bovine serum albumin (3%; Roth, Karlsruhe, Germany), 0.5 µM of each primer and 3 µL of DNA extract. Thermocycling included 95 °C for 10 min, 35 cycles of 95 °C for 30 s, the primer-specific annealing temperature (see Table 1) for 30 s, 72 °C for 45 s, and a final step of 72 °C for 3 min. PCR products were separated using the capillary electrophoresis system QIAxcel (Qiagen, Hilden, Germany); fragments of the expected size and a relative fluorescent value of or above 0.1 RFU were scored as positive.

To test the new PCR assays on field-caught lithobiid predators, 50 *Lithobius* spp., collected in November 2008 at the beech forest sites mentioned above, were subjected to a CTAB-based DNA extraction protocol (Juen and Traugott, 2005). DNA extracts were purified using the Geneclean Turbo Kit (MP Biomedicals, Solon, OH, USA) yielding 150 µL of final DNA extract. One blank sample was included per 24 extracts to check for DNA carry-over contamination (none was found testing them with general COI primer (Folmer et al., 1994)).

We established specific COI primers for *Lithobius* spp. and the springtail species *Ceratophysella denticulata* (Bagnall, 1941), *Folsomia quadrioculata* (Tullberg, 1871), *Lepidocyrtus lanuginosus* (Gmelin, 1788), *Pogonognathellus longicornis* (Müller, 1776) and *Protaphorura armata* (Tullberg, 1869) as well as group-specific primers targeting the 18S rDNA gene of dipterans, gamasid and oribatid mites, spiders, staphylinid beetles and woodlice. The PCR assays amplified DNA fragments of the expected length in all targeted taxa. Only the woodlice primers ISO S6/ISO A3 showed a species-specific variation in amplicon size: *Trichoniscus pusillus* Brandt, 1833 was 123 bp, *Armadillidium vulgare* (Latreille, 1804) and *Ligidium hypnorum* (Cuvier, 1792) were 152 bp, *Oniscus asellus* Linnaeus, 1758 was 159 bp, *Philoscia muscorum* (Scopoli, 1763) was 160 bp, and *Porcellio scaber* Latreille, 1804 was 192 bp. The assays were highly specific as they exclusively amplified DNA of the target taxa. The only exception was the assay which targeted *P. armata,* which also amplified DNA of *Supraphorura furcifera* (Borner, 1901), another onychiurid springtail. Assay sensitivity was high across all twelve PCR systems: successful amplification ranged between 1875 and 1.83 fg target DNA per μL^{-1} PCR. Primers containing degenerated bases, however, were generally less sensitive (Table 1). Assay sensitivity was not adversely affected in the presence of excess predator DNA.

Fifty field-collected *Lithobius* spp. were tested for prey DNA using the newly established PCR assays. Five out of the eleven targeted prey taxa could be detected in 22 centipedes and 28 individuals had no amplifiable prey DNA in their guts. Most specimens (40%) had consumed *L. lanuginosus* followed by dipterans (16%), *F. quadrioculata* (6%), spiders (4%) and gamasid mites (2%). Simultaneous detection of two prey taxa in one predator was observed in 10 cases.

The present set of PCR assays allow testing for DNA of a wide range of possible prey of soil arthropod predators at a high level of

Table 1
Targeted taxa and genes, primer names and sequences, PCR product size, optimal annealing temperature (T_a), and PCR amplification threshold for the detection of common prey taxa within the gut content of soil-dwelling invertebrate predators. All primer pairs are used in singleplex PCR assays.

Taxon	Gene	Name	Sequence 5′–3′	Size (bp)	T_a (°C)	Detection threshold (fg μl^{-1} PCR)
Ceratophysella denticulata (Bagnall, 1941)	COI	CERDEN S5	ACTTCTTCCCCCCTCCTTAACCCTA	227	68	7.32
		CERDEN A3	CCCAGGATATTCCGGGGGC			
Folsomia quadrioculata (Tullberg, 1871)	COI	FOLQUA S4	CTGAACCGTTTATCCACCTCTC	169	62	29.29
		FOLQUA A1	AGTTCGGTCTCAAGTTATACCTACTGTG			
Lepidocyrtus lanuginosus (Gmelin, 1788)	COI	LEPLAN S3	CGATATAGCCTTTCCTCGTATAAAC	250	62	117.19
		LEPLAN A1	GGTTCGTATGTTAATGATAGTTGTG			
Pogonognathellus longicornis (Müller, 1776)	COI	POGLON S4	GATCAAATTTATAACGTTTTAGTAACC	202	62	7.32
		POGLON A4	CTAAACCTCCTGACAAGAGAAGC			
Protaphorura armata (Tullberg, 1869)[a]	COI	PROARM S3	GTAGAAAGAGGTGCAGGAACTGGC	268	68	3.66
		PROARM A3	TAATGGCTCCAGCAAGAACAGGTAAG			
Araneae group	18S	ARA S5	TAACRATACGGGACTCTTTYGAGA	255	68	468.75
		ARA A5	AGACAACCGGTGAAGATCATC			
Diptera group	18S	DIP S16	CACTTGCTTCTTAAATrGACAAATT	198	60	1.83
		DIP A17	TTyATGTGAACAGTTTCAGTyCA			
Gamasina group	18S	GAM S7	TTGGGGGCATTCGTATTGTT	230	63	29.29
		GAM A8	ATAACCCTACTTwGGTTTCCCGT			
Isopoda group	18S	ISO S6	GCwTTTrTTAGACCAAAAACCG	123–192	60	117.19
		ISO A3	CAGACACTyGrArGATACGG			
Lithobius Leach, 1814, group	COI	LIT S13	TGTTCwGCvGCwGTwGAAAG	293	54	1875.00
		LIT A8	GTdArkArTATdGTAATTGCTCC			
Oribatida group	18S	ORI S14	GCGCGCTACACTGAAGTG	299	68	29.29
		ORI A16	TCCTCTAAATGWTCAAGKTTGGG			
Staphylinidae group	18S	STA S6	TGCGGTTAAAAAGCTCGTAGTC	152	65	1.83
		STA A3	TCAATrAAGAGCACCGsGAT			

[a] PROARM S3/PROARM A3 are specific to onychiurid collembolans *P. armata* and *Supraphorura furcifera* (Borner, 1901).

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specificity and sensitivity. The current screening results on centipede predators suggest high consumption rates of decomposer prey, particularly collembolans while intra-guild prey may only be accepted occasionally. This fits to findings on the diet of other soil-dwelling generalist predators such as predatory beetle larvae (Eitzinger and Traugott, 2011). Still, a larger set of individuals would need to be tested to better characterize the lithobiids' feeding preferences. Note, however, that gut content analysis cannot discriminate between active predation, secondary predation and scavenging (King et al., 2008), necessitating additional feeding experiments to clarify centipede feeding strategies. Moreover, prey DNA digestion rates might differ between prey species (e.g., Greenstone et al., 2010) and depend on other factors such as meal size, physiological status of the predators or temperature (von Berg et al., 2008) which needs to be considered when interpreting the field-derived data. The new PCR assays complement already published assays targeting other important prey groups of soil-dwelling generalist predators (e.g. Harper et al., 2005; Kuusk and Agusti, 2007; King et al., 2011), which allows shedding light on complex animal–animal feeding interactions in soil food webs. The COI and 18S rDNA sequences generated in this study will also help extending DNA-libraries of soil organism to study the diversity of life in below-ground systems.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2012.09.001.

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