Reverse Taxonomy Reveals Pristionchus maupasi (Diplogasterida: Diplogastridae) Association with the Soil-Dwelling Bee Andrena optata (Hymenoptera: Andrenidae) in Turkey

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Reverse taxonomy reveals *Pristionchus maupasi* (Diplogasterida: Diplogastridae) association with the soil-dwelling bee *Andrena optata* (Hymenoptera: Andrenidae) in Turkey

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The diversity of nematode associates of soil-dwelling bees was recently surveyed in Turkey using molecular operational taxonomic unit (MOTU) profiling and culturing methods (Hazir et al. 2010). During that study, 4 MOTUs (= putative species) of diplogastrid nematodes (Diplogasterida: Diplogastridae) were recovered from the abdominal glands of adult soil-dwelling andrenid (Hymenoptera: Andrenidae) bees. These nematode MOTUs were tentatively assigned to genera based upon comparisons with GenBank as a potentially new species of *Allodiplogaster* (= *Koerneria*) (AY-165; D2/D3 GB# FJ661069) from *Andrena limata* Smith and 3 MOTUs from 4 *Andrena* species (1] AY-167 ex *A. parviceps* Kriechbaumer D2/D3 GB# FJ661070, mtCOI GB# FJ661032; 2] ADN-1 ex *A. limata* D2/D3 GB# FJ661065, mtCOI GB# FJ661025; and 3] AB-422, AB-429, and AB-449 ex *A. thoracica* (Fabricius) [D2/D3 GB#s FJ661062, FJ661063, FJ661064, and mtCOI GB# FJ661024] + KIR-1 ex *A. flavipes* Panzer D2/D3 GB# FJ661080, mtCOI GB# FJ661044 + KST-33 ex *A. limata* D2/D3 GB# FJ661081) belonging to a sister clade of nematodes designated as "*Mononchoides.*" These nematodes were all recovered as dauers from the abdominal glands of 0.6% (21/3,279) of female andrenid bees and none of the culturing attempts were successful for providing adult morphotypes or a type culture for further study (Hazir et al. 2010). Recent molecular phylogenetic work has broadened the database for more accurate MOTU sequence comparisons and phylogenetic matching for species identification and reverse taxonomy, and all of the putative "*Mononchoides*" species listed in Hazir et al. (2010) now appear to be species of *Pristionchus* (Atighi et al. 2013). In the study of Hazir et al. (2010), some of the dauers observed in the abdominal glands of andrenids were characteristically very long and thin, present in high numbers per gland (> 10), and thought to be fastidious members of the misidentified "*Mononchoides*" clade. Interestingly, both *Pristionchus* and *Allodiplogaster* (= *Koerneria*) are usually relatively easy to culture and have generally similar looking short and spindle-shaped dauers (Giblin-Davis et al. 1990; Kanzaki et al. 2013a). Given this confusion, we revisited this situation using reverse taxonomy (Kanzaki et al. 2012) to attempt to generate cultured isolates and morphotypes of the dauer nematodes from the abdominal glands of andrenid bees in Turkey.

Andrenid bees (n = 592; with 88% females and 12% males) were collected from flowers with a sweep net from 9 cities (Afyon, Aksaray, Ankara, Antalya, Aydin, Burdur, Isparta, Nevsehir, and Nigde), from 3 regions in Turkey (Aegean, Mediterranean, and Central Anatolia) from Mar to Jun, 2013. Adult bees were transported back to the laboratory alive in plastic vials and kept at 5 °C for a maximum of 3 days until dissected (Giblin-Davis et al. 1990). Each bee was examined and sexed before being dissected live in deionized water and observed for nematode associations on or in the body or in the abdominal glands (Giblin-Davis et al. 1990). In cases where nematodes were observed, the abdomen of the infested bee was transferred to 2.0% water agar, water agar seeded with the fungus *Botryotinia fuckeliana* (de Bary) Whetzel (= *Botrytis cinerea* Pers.), and/or nutrient broth agar and monitored weekly for at least one month to see if a culture established. When nematodes developed out of the dauer stage and began to propagate, they were identified morphologically using the methods of Kanzaki (2013), and handpicked to establish laboratory cultures on appropriate growth media for bacteria or fungus.

Only one of the 70 male bees was found to be associated with nematodes (a mermithid). Of the female bees, 9% (*n* = 522) were positive for nematodes. One of these females was abdominally infested with mermithid juveniles, but the rest were infested with diplogastrid dauers (range = 1–190 nematodes per bee) in one or more of the abdominal glands (total of 6 glands per female). Two general morphotypes of diplogastrid dauers were observed in the abdominal glands; 1) very long and thin dauers, often in numbers exceeding 10 per gland, or 2) relatively short and spindle-shaped dauers in low numbers per gland. The long and thin morphotype was the most common form recovered and the 2 morphotypes were not observed together in the same bee. Only one bee yielded a successful culture. This female bee was identified as *Andrena optata* Warncke and had 2 nematode-infested abdominal glands, each with a single (short and spindle-shaped) dauer nematode present. The bee (pinned voucher #09-35) was collected in the village of Bahcearasi, in the city of Aydin, Turkey (N 37°43.33' E 27°52.43') on 30 Mar 2013.

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The cultured nematode was tentatively identified as *Pristionchus* and transferred to NGM agar seeded with strain OP-50 *Escherichia coli* (Stiernagle 2006) for sub-culturing. Molecular samples were collected from cultured and morphotyped materials using the procedures of Tanaka et al. (2012). Molecular amplifications, sequencing, alignments and analyses were done using the methods of Kanzaki & Futai (2002) and Ye et al. (2007). The general morphology and morphometrics in temporary water mounts (see Table 1) fit the original description and previous observations for general morphometric values of the genus (Potts 1910; Herrmann et al. 2006; Kanzaki et al. 2013b). The newly determined molecular sequences for this isolate were deposited in GenBank with the accession numbers LC011448 (near-full-length small sub-unit ribosomal RNA gene [SSU]), LC011449 (D2/D3 expansion segments of the large sub-unit rRNA gene [LSU]), and LC011450 (fragment of the mitochondrial cytochrome oxidase I gene [mtCOI]). The partial SSU sequence (1658 bps excluding primer sequences) was identical to that of *P. maupasi* Potts (Holterman et al. 2006: FJ040443), and LSU and mtCOI were almost identical (98% identity) to those of one MOTU of "*Mononchoides* sp." (=*Pristionchus*) isolated from *A. limata* (FJ661081) and *A. flavipes* (FJ661044), respectively (Hazir et al. 2010).

We observed that this isolate of *P. maupasi* produces stenostomatous males and hermaphrodites as well as eurystomatous hermaphrodites (eurystomatous males also occur but are very rare), as has been found before for this species (Kanzaki, pers. obs.). In addition, typical diplogastrid dauers (short and spindle-shaped, not very long and thin) were abundantly produced on older plates.

Hazir et al. (2010) hypothesized that the few relatively short dauers observed in the abdominal glands probably matched a putative new species of *Allodiplogaster* (= *Koerneria*) that they sequenced (AY-165). Our present study using reverse taxonomy suggests that the story needs further work. Either the long thin dauers that were observed by Hazir et al. (2010) are an unusual and different species of *Allodiplogaster* (= *Koerneria*) or *Pristionchus*, or they are a completely different diplogastrid that failed to sequence and is unculturable. This situation challenges us to explore the nests of andrenid bees infested with the long thin dauers in Turkey to see if an adult morph can be recovered to link the adult and dauer morphotypes with a genotype.

Andrenids are typically univoltine (one generation per year) and overwinter as newly-eclosed adults inside their larval cells (Michener 2000). Nematode dauers may be transferred to an individual bee cell in the soil during the provisioning process via abdominal gland se-

Table 1. Morphometrics of *Pristionchus maupasi* from 2-week-old cultures and established from a population originally isolated from a single *Andrena optata* female from Turkey [mean + standard deviation with (range)].

	Stenostomatous males	Stenostomatous hermaphrodites	Eurystomatous male	Eurystomatous hermaphrodites
n	15	15	1	12
L	845 ± 120 (680–1120)	730 ± 69 (626–831)	734	806 ± 58 (709–803)
a	15.5 ± 2.1 (12.7–18.8)	16.0 ± 1.5 (13.6–18.8)	22.3	16.5 ± 1.0 (14.8–18.5)
b	5.2 ± 0.7 (4.4–7.2)	4.6 ± 0.3 (4.3–5.2)	4.6	4.9 ± 0.2 (4.5–5.2)
c	6.8 ± 1.2 (5.4–9.8)	5.2 ± 0.3 (4.5–5.7)	5.6	5.5 ± 0.2 (5.1–5.9)
c’	3.9 ± 0.7 (2.7–4.9)	6.7 ± 0.4 (5.9–7.3)	4.6	6.0 ± 0.5 (4.9–7.0)
V	— —	50.5 ± 1.1 (47.3-51.8)	—	49.8 ± 1.5 (46.8–52.3)
Stoma width	7.1 ± 0.6 (6.3–8.6)	6.5 ± 0.4 (5.7–6.9)	11.4	10.8 ± 1.1 (8.6–13.1)
Stoma depth	11.4 ± 1.0 (9.7–13.1)	11.7 ± 0.6 (10.9–12.6)	11.4	11.5 ± 1.1 (10.3–13.7)
Neck length	162 ± 8.3 (143–173)	160 ± 9.0 (146–174)	160	162 ± 7.6 (153–179)
Anterior pharynx	96 ± 8.1 (77–106)	93 ± 5.7 (84–103)	96	92 ± 4.1 (84–99)
Posterior pharynx	41 ± 4.3 (34–53)	57 ± 3.7 (51–63)	53	62 ± 4.1 (54–67)
Maximum body diam.	55 ± 11.9 (40–77)	46 ± 7.9 (36–59)	47	49 ± 4.7 (38–59)
Excretory pore from anterior end	130 ± 10.2 (106–149)	124 ± 7.5 (111–140)	119	125 ± 6.1 (116–136)
Nerve ring from anterior end	121 ± 8.1 (101–130)	117 ± 5.7 (107–129)	119	118 ± 5.1 (110–127)
Vulval body width	— —	43 ± 8.1 (33–56)	—	46 ± 5.0 (35–54)
Cloacal or anal width	33 ± 6.5 (26–49)	21 ± 2.0 (18–26)	29	25 ± 1.8 (23–27)
Tail length	125 ± 17.5 (96–161)	141 ± 14.4 (117–180)	131	148 ± 11.3 (127–163)
Spicule length (curve)	53 ± 4.2 (47–62)	— —	48	—
Gubernaculum length	19 ± 1.0 (17–22)	— —	17	—

cretions and then propagate on microbes associated with the larval food mass before it is consumed. The abdominal glands may provide a sticky material to hold pollen grains to the scopa (Altenkirch 1962). If so, nematodes could be phoretically transferred from one generation to the next when they are deposited during provisioning of the bee cells with pollen loads or some other housekeeping function for which the abdominal glands are used. Nematode propagation could occur rapidly and in competition with the growing bee larva and end with synchronized production of long-lived dauers that could wait on the overwintering prepupal stage or inside the abdominal glands of the newly-eclosed adult bee. Alternatively, the nematode might be necromenic, awaiting the death of the adult bee before entering into the propagative phase to consume the rotting carcass. This would require that the adult host dies in the nest or near the cells and that the nematodes invade the cells of the next generation of bees or await their emergence from the cells the following spring to associate again with the abdominal glands of the next generation of bees for reliable vertical transmission. *Pristionchus maupasi* could be associated with a completely different soil insect host, such as the known scarab beetle hosts of *Melolontha melolontha* (L.) (10% association frequency [AF]), *M. hippocastani* Fabricius (2.4% AF), or *Cetonia aurata* (L.) (0.4% AF) in Europe (Herrmann et al. 2006; Mayer et al. 2007) that might co-inhabit the soil around *Andrena* nests or aggregations. The apparently low association rate between *P. maupasi* and *Andrena* bees (less than 1%) suggests that andrenids are probably not the typical or "primary" hosts. This and the reverse taxonomy conclusion here suggest that *P. maupasi* is associated with at least 3 species of *Andrena.* These facts support the idea that it may have a more generalist invertebrate host association strategy similar to that observed for *P. pacificus*, which is sometimes associated with millipedes and termites (Kanzaki pers. obs.). Less stringent host carrier dynamics and reproduction via hermaphroditism might allow for greater geographical dispersion and establishment (Herrmann et al. 2010) enabling *P. maupasi* to be distributed more widely, i.e., *P. maupasi* is phylogenetically considered to be part of a North American *Pristionchus* clade, but found widely in Europe (Herrmann et al. 2006). The association of *P. maupasi* in the abdominal glands of female *Andrena* bees is intriguing in terms of the chemical ecology of dauer attraction (Hong & Sommer 2006; Hong et al. 2008). Perhaps there is an interesting "coincidence" in chemical ecology that leads to the cross attraction and association of *P. maupasi* dauers into the abdominal glands of andrenid bees from their "normal" cockchafer hosts in Turkey.

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Summary

Previous molecular operational taxonomic unit (MOTU) survey work with diplogastrid dauers from the abdominal glands of adult female soil-dwelling andrenid bees in Turkey had suggested commensal relationships between species of the genus *Andrena* and a new species of *Koerneria* and several species from an unidentified nematode clade near "*Mononchoides"* (based upon comparisons with GenBank at the time). We used reverse taxonomy on dauer nematodes from *Andrena optata* from Turkey to successfully culture, morphotype, and sequence adult nematodes that fully matched *Pristionchus maupasi* and one of the MOTUs previously isolated as dauers from the abdominal glands of *A. limata* and *A. flavipes*, and previously designated as belonging to the clade near "*Mononchoides."* This study demonstrates the value of reverse taxonomy for resolving MOTU identification issues as the depth of the reference sequence database increases and successful cultures or environmental samples of adults are made available for morphotypic and genotypic comparisons. In addition, it has helped expand our knowledge of the potential host range and biogeographical distribution of *P. maupasi* which was originally thought to be relatively host specific on scarab beetles, and has raised questions about the chemical ecology of dauers for this species in the wild.

Key Words*:* *Andrena optata*; bee; commensalism; dauer; host-specificity; necromeny; nematode-insect association; *Pristionchus maupasi*; Turkey.

Sumario

Investigaciones anteriores sobre la unidad taxonómica operativa molecular (UTOM) del dauer (un estadio larval resistente) de los diplogastridos en las glándulas abdominales de las hembras adultas de abejas andrenidas (Hymenoptera: Andrenidae) que viven en el suelo en Turquía habían sugerido que hay una relación comensal entre las especies del género *Andrena* y una nueva especie de *Koerneria* y varias especies de un clado nematodo no identificado cerca "*Mononchoides*" (en base a comparaciones con GenBank en el momento). Se utilizó la taxonomía inversa sobre las larvas nematodas en *Andrena optata* Warncke recolectos en Turquía para criarlos con éxito, determinar el morfotipo y secuenciar los nematodos adultos que coincidían con *Pristionchus maupasi* (F.A. Potts) y uno de las UTOM previamente aislado como un estadio de resistencia en las glándulas abdominales de *A. limata* y *A. flavipes*, y previamente designados como perteneciente al clado cerca "*Mononchoides*". Este estudio demuestra el valor de la taxonomía inversa para resolver problemas de identificación UTOM ya que incrementa la cantidad de secuencias de referencia en las bases de datos y la cria exitosa o muestras ambientales de los adultos se ponen a disposición para comparaciones morfotípicas y genotípicas. Además, ha ayudado a ampliar nuestro conocimiento de la variedad de hospederos posibles y distribución biogeográfica de *P. maupasi* que se creia originalmente que era relativamente específico a los hospederos Scarabaeidae y ha suscitado dudas sobre la ecología química de los individuos del estadio de resistencia para esta especie en el medio silvestre .

Palabras Clave: *Andrena optata*; abeja; comensalismo; dauer; host-especificidad; necromenia; asociación nematodo-insecto; *Pristionchus maupasi*; Turquía

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