

RESEARCH ARTICLE

# *Pristionchus* nematodes occur frequently in diverse rotting vegetal substrates and are not exclusively necromenic, while *Panagrellus redivivoides* is found specifically in rotting fruits

Marie-Anne Félix<sup>1\*</sup>, Michael Ailion<sup>2</sup>, Jung-Chen Hsu<sup>3</sup>, Aurélien Richaud<sup>1</sup>, John Wang<sup>3</sup>

**1** Institut de Biologie de l'Ecole Normale Supérieure, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Paris, France, **2** Department of Biochemistry, University of Washington, Seattle, Washington, United States of America, **3** Biodiversity Research Center, Academia Sinica, Taipei, Taiwan

\* [felix@biologie.ens.fr](mailto:felix@biologie.ens.fr)



**OPEN ACCESS**

**Citation:** Félix M-A, Ailion M, Hsu J-C, Richaud A, Wang J (2018) *Pristionchus* nematodes occur frequently in diverse rotting vegetal substrates and are not exclusively necromenic, while *Panagrellus redivivoides* is found specifically in rotting fruits. PLoS ONE 13(8): e0200851. <https://doi.org/10.1371/journal.pone.0200851>

**Editor:** Shawn Ahmed, University of North Carolina at Chapel Hill, UNITED STATES

**Received:** May 9, 2018

**Accepted:** June 5, 2018

**Published:** August 3, 2018

**Copyright:** © 2018 Félix et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files. The sequences are available at Genbank with accession numbers MH608216-608284 for 18S rDNA (all genera) and MH608291-608299 for 28S rDNA (*Panagrellus*).

**Funding:** MAF and AR are supported by the Ecole Normale Supérieure and the Centre National de la Recherche Scientifique. This work was supported by a National Science Foundation CAREER Award

## Abstract

The lifestyle and feeding habits of nematodes are highly diverse. Several species of *Pristionchus* (Nematoda: Diplogastridae), including *Pristionchus pacificus*, have been reported to be necromenic, i.e. to associate with beetles in their dauer diapause stage and wait until the death of their host to resume development and feed on microbes in the decomposing beetle corpse. We review the literature and suggest that the association of *Pristionchus* to beetles may be phoretic and not necessarily necromenic. The view that *Pristionchus* nematodes have a necromenic lifestyle is based on studies that have sought *Pristionchus* only by sampling live beetles. By surveying for nematode genera in different types of rotting vegetal matter, we found *Pristionchus* spp. at a similar high frequency as *Caenorhabditis*, often in large numbers and in feeding stages. Thus, these *Pristionchus* species may feed in decomposing vegetal matter. In addition, we report that one species of *Panagrellus* (Nematoda: Panagrolaimidae), *Panagrellus redivivoides*, is found in rotting fruits but not in rotting stems, with a likely association with *Drosophila* fruitflies. Based on our sampling and the observed distribution of feeding and dauer stages, we propose a life cycle for *Pristionchus* nematodes and *Panagrellus redivivoides* that is similar to that of *C. elegans*, whereby they feed on the microbial blooms on decomposing vegetal matter and are transported between food patches by coleopterans for *Pristionchus* spp., fruitflies for *Panagrellus redivivoides* and isopods and terrestrial molluscs for *C. elegans*.

## Introduction

The lifestyle and feeding habits of members of the nematode phylum are highly diverse. A single species may express diverse life cycles through production of free-living and host-

(MCB-1552101) to MA and the Ministry of Science and Technology, Taiwan and an Academia Sinica Career Development Grant to JW. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

associated stages. Many species of the nematode genus *Pristionchus* (Nematoda: Diplogastriidae), including the most studied *Pristionchus pacificus*, have been recently considered to be associated with beetles in a necromenic lifestyle, i.e. to associate with beetles in the nematode dauer diapause stage and wait until the death of the beetle to resume development and feed on microbes in the decomposing beetle corpse [1]. The necromenic lifestyle of *Pristionchus* nematodes now appears as a qualifier for the species, as in the "necromenic nematode *P. pacificus*" [2,3]. *Pristionchus* have been considered to share some attributes of parasitic nematodes and to have a very different lifestyle from the free-living nematode *C. elegans* [4].

Yet, to our knowledge, carcasses of naturally dead beetles have not been looked for and examined to assess whether *Pristionchus* feeds on them. Recent field studies have looked for *Pristionchus* in live beetles, and to a lesser extent in soil [5–8]. We detail below in three points what has been documented about the association of *Pristionchus* with beetles.

1. Many *Pristionchus* species including *P. pacificus* can be found on beetles [6,8–14]. (Note that a subclade of *Pristionchus* species has recently been found in fresh tropical figs in association with their pollinating wasps [15], in the same way that some *Caenorhabditis* species are found in other specific substrates [16]—we will not consider this fig subclade here).
2. *Pristionchus* can be found on live beetles in the dauer stage [12], based on collecting 114 live *Geotrupes stercorosus* beetles in a forest near Tübingen, Germany. The authors found a total of 17 *Pristionchus* individuals, 71 *Koerneria* (a genus close to *Pristionchus*), 466 other diplogastriids, 3927 *Pelodera* (Rhabditidae) and 508 parasitic Spirurida. *Pelodera* dauer larvae were present in 56 out of 114 *Geotrupes* beetles, with up to hundreds of *Pelodera* dauer larvae per beetle. *Pristionchus* was comparatively rarer: eight of 114 beetles (7%) yielded one *Pristionchus* individual, one carried two individuals and one had seven individuals, all in the dauer stage. *P. pacificus* is rare in Europe [9]. These beetle-associated *Pristionchus* were *P. sp. 6*, *entomophagus*, and *lheritieri*. In addition, a survey of 4242 beetles from Germany, France, Spain, and Switzerland again suggested that *Pristionchus* spp. are found in the dauer stage on beetles [9].
3. Finally, the data supporting the conclusion that *Pristionchus* non-dauer stages feed on carcasses of beetles come from experiments where beetles were killed on a Petri dish containing *E. coli* in the laboratory (e.g., [9], and for *P. pacificus*: [6]). When a beetle is killed on a Petri dish, some of the bacterial species that are inside the gut or on the surface of the animal will proliferate using the beetle or the Petri dish as food and *Pristionchus* can then feed on them or *E. coli* (cf. Fig 1 in [8]). In these experiments, *Pristionchus* appeared only after 7–10 days, suggesting that they took time to exit the dauer stage [9]. The delay may be explained by the fact that some beetles secrete chemicals that prevent their associated nematode species from exiting the dauer stage [17]. Because this delay is longer than the generation time of *Pristionchus* in this environment (3–4 days), it is not possible to assess either the number of individuals or their developmental stage on the beetle. Similarly, if the beetles are killed by the experimenter and then placed in a soil environment, *Pristionchus* will proliferate in the vicinity of the dead beetle only after 7 days [18]. These data suggest that *Pristionchus* may adopt a necromenic lifestyle by consuming the progeny of bacteria that were on the living host beetle, but they do not address whether this is an event occurring in the wild, and if so, how frequent it is for *Pristionchus*. The claim of a necromenic relationship does not take into account the possibility that the nematodes may disembark from their insect host well before it dies, should they encounter some food source. Necromeny may take place occasionally, but beetle corpses may be a very minor source of food for *Pristionchus*.

By contrast, older literature on *Pristionchus* species had associated them with a number of substrates. Sudhaus and Fürst van Lieven [19] reviewed the systematic work on species in this genus and summarized the habitat as "mostly decaying plants, associated with insects". Specifically, *Pristionchus* species were described from soil, humus, compost, moss, "diseased" stem, coffee berry, rotting bulbs of *Allium vineale*, damaged roots of coffee and garlic, around roots of several species, rotten potatoes, rotten wood, and decomposing fungi. Additionally, *Pristionchus* species were described to be associated with a wide range of insects: termites (two species), *Ostrinia* (Lepidoptera), "dead insects", and finally beetles (three species). The spectrum of habitats and associated insects thus appears much wider than the recent literature indicates.

It is interesting to compare the recent history of research on *Pristionchus* ecology with what is known of the ecology of the model organism *Caenorhabditis elegans* and other *Caenorhabditis* species. The history of collecting *Caenorhabditis* has been different, as are the conclusions. *Caenorhabditis* nematodes have been found to be rare in soil, but abundant in rotting vegetal matter, such as fruits and soft plant stems. In addition, *C. elegans*, *C. briggsae* and *C. remanei* have been found on terrestrial molluscs, isopods and millipedes [20–27]. In laboratory experiments, the nematodes were shown to be capable of climbing on or off these invertebrates [20,21,27–30]. Perhaps anecdotally, *C. nouraguensis* were found on cockroaches in a tropical forest and *C. tropicalis* once on a beetle [16]. Some species, such as *C. japonica*, have an apparently specific carrier insect [31–34]. These associations are considered to be mostly phoretic or may correspond to occasional "facultative necromeny" [35]. The possibility of phoresis or facultative necromeny has not been considered for *Pristionchus* spp.

We here provide data on nematode genera found in rotting vegetal matter, focusing on the genera *Pristionchus* and *Panagrellus*. By surveying different types of rotting vegetal and fungal substrates, we found diverse *Pristionchus* spp. at a similar frequency as *Caenorhabditis*, often in high numbers and in non-dauer feeding stages in rotting fruits and stems. In addition, we report that a single species of *Panagrellus* (Nematoda: Panagrolaimidae), which we identify as *Panagrellus redivivoides*, is found in rotting fruits but not in rotting stems and appears to be associated with *Drosophila* fruitflies.

## Materials and methods

### Sample handling and nematode genus identification

Collected samples were placed onto standard *C. elegans* Normal Growth Medium agar plates [36] previously seeded with *Escherichia coli* strain OP50 in the center of the plate. The samples were spread around the bacterial lawn. 1–2 ml water or M9 solution were added to humidify the samples [37].

For surveys in S1 Table, all plates were examined regularly under the dissecting microscope: in the most stringent surveys (Orsay, Santeuil), observations were made several times within the first hours, once or twice on the next two days, and at least on days 4 and 7. Nematodes that crawled out of the sample were identified to the genus or family level by morphological criteria, under the dissecting microscope with trans-illumination and sometimes further by Nomarski microscopy [37]. *Caenorhabditis*, *Pristionchus*, *Panagrellus*, *Oscheius*, *Mesorhabditis* species were identified as described in [26,37] (see pictures and drawings therein; and also [38,39] for identification keys). Our previous developmental evolution work provided us with experience on these various genera [40–48] and all strains then tested by rDNA sequencing confirmed correct identification to the genus level (S2 Table). In addition, *Caenorhabditis* were systematically identified to the species level as indicated in [26,37,49]. Given a generation time for these nematodes of about 3–5 days at 20°C, the number of individuals and developmental stages were noted for samples that were less than 48 hours from collection time.

These surveys were primarily aimed at studying *Caenorhabditis* populations, as reported in [22,25,26,49]. *Caenorhabditis* individuals tend to rapidly exit the sample to colonize the *E. coli* lawn, as do *Pristionchus*, *Panagrellus* and most other rhabditids. Some genera are more difficult to survey, either because they have a small body size, are present in small numbers, move slowly out of the sample or are less easy to recognize. The three genera *Caenorhabditis*, *Pristionchus* and *Panagrellus* that are highlighted in S1 Table, are those that may have been least missed in the samples. However, the number of samples that contained *Pristionchus* or *Panagrellus* spp. is likely an underestimate as they were less carefully looked for than *Caenorhabditis*.

Diplogastrids are easy to recognize by their short buccal cavity bearing strong teeth, the absence of a grinder in the basal bulb of the pharynx, the color pattern of the body stemming from the shape of female gonad arms generally bending back diagonally towards the vulva, and the pore appearance of the vulva. *Pristionchus* are easy to distinguish from other diplogastrids as adults, with their characteristic dumpyish body shape and cuticle with strong longitudinal ridges (Fig 1A). We also systematically checked that our morphological criteria corresponded to the *Pristionchus* genus by SSU rDNA sequencing of a subset of them (JU isolates, S2A Table).

*Panagrellus* species are recognized by their large body size, light brown gut color (like *Caenorhabditis*), posterior vulva and viviparous reproduction (Fig 2A). Young first-stage larvae exit through the female vulva and gravid females may contain several dozens of embryos in their uterus. We also sequenced SSU rDNA for some of them (S2B Table).

Genera of other rhabditids can be *Pelodera*, *Auanema*, *Rhabditella*, *Pellioiditis*, etc. In contrast to *Caenorhabditis* (or *Panagrellus*), their gut color is generally greyish/black, rather than brownish. Our notes are too scarce to distinguish them here and we placed them in a single category, with the exception of the ones for which we report 18S sequences.

*Oscheius* are very commonly found in rotting vegetal matter or soil and can be recognized by their greyish gut color, their lack of middle pharyngeal bulb, their thin female tail and their long and inflated rectum. The occurrence of *Oscheius* is likely underestimated as these animals are less striking, rarely reach large population sizes and develop more slowly than the above. They are here almost exclusively represented by small-size hermaphroditic *Oscheius* of the *Tipulae* and sometimes *Dolichura* subgroups (by contrast to large *Oscheius* of the *Insectivora* group; [45,50]).

*Panagrolaimus* are long and quite thin nematodes, with an only very slightly posterior vulva.

*Mesorhabditis* species are easier to find after one to two days on the plate and they may remain in the vicinity of the sample. They are recognized by their posterior vulva and dark body color. In our surveys, they were particularly looked for and isolated in 2015–17.

Animals of the *Protorhabditis/Prodontorhabditis/Diploscapter* clade [45,51] are small and tend to burrow in the agar and leave trails therein. Populations develop slowly and they can be missed.

*Rhabditophanes* has a black gut color and characteristically lay almost round eggs, instead of oval-shape ones. They were not systematically surveyed.

*Bunonema* individuals are easy to identify by their very small, spindle-shaped body, and a left-right asymmetric cuticle. They grow slowly and can easily be missed if their population is overwhelmed by other genera.

The "Other" category contains nematodes that are generally not bacterial eaters but fungi-eaters such as aphelenchs or parasitic nematodes that do not grow in our culture conditions.



**Fig 1. Rotting fruit and stem samples containing *Pristionchus*.** (A) *Pristionchus* adult female or hermaphrodite from rotting apple O824 from Orsay. The cuticle with longitudinal ridges and the pore-shaped vulva (arrowhead) are characteristic of *Pristionchus*. Bar: 100  $\mu$ m. (B) Apple O1194 in Orsay orchard, France with large (>1,000 individuals) populations including feeding individuals of *Pristionchus* sp. and *Caenorhabditis elegans*. (C) Pear CZ12 in Prague, Czech Republic with *Pristionchus* and *Panagrellus*. (D) Olives F8 near Firenze, Italy with *Pristionchus* sp. (E) *Arum* stem B09-6 in a wood near Le Blanc, France with *Pristionchus* and *Caenorhabditis*. (F) Stem S156 in a wood near Santeuil, France with a feeding population of several thousand *Pristionchus* individuals. (G) Banana pseudostem S9 in Palermo Botanical Garden, Italy, yielding *Pristionchus* and *C. elegans*.

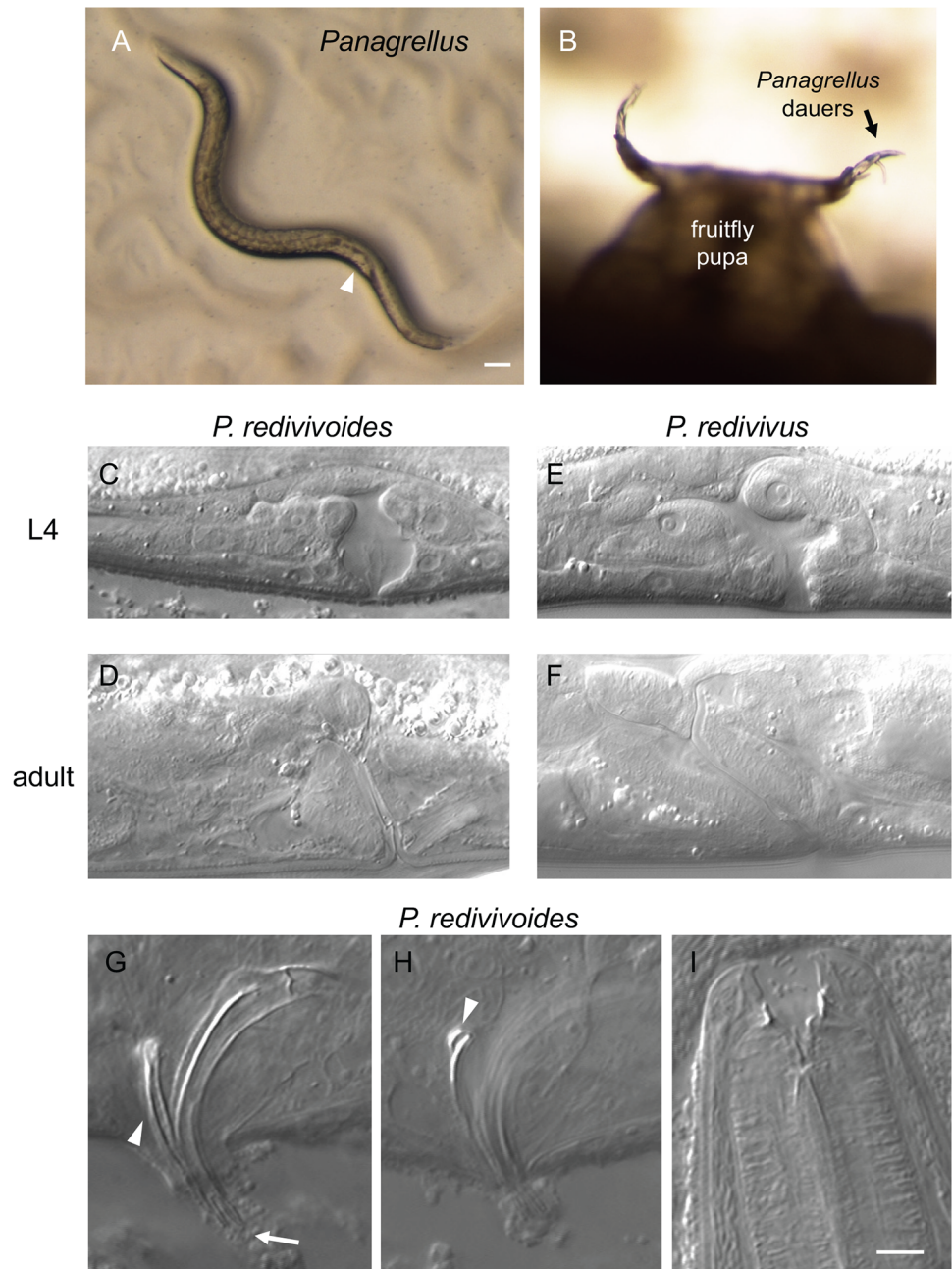
<https://doi.org/10.1371/journal.pone.0200851.g001>

## Culture and freezing

Strains were derived from single individuals in selfing species, or from a mated female or a male-female pair in male-female species, and cultured on standard *C. elegans* Normal Growth Medium agar plates. *Pristionchus* species were frozen with the *C. elegans* freezing protocol [36], sometimes adding 1 mM  $\text{CaCl}_2$  to the freezing solution. This protocol does not allow for a good retrieval of *Pristionchus* and *Panagrellus* nematodes. We recently adopted a DMSO--Dextran protocol that allows for better recovery (courtesy of Andre Pires da Silva), whereby a pellet of nematodes in M9 solution is resuspended in 7 ml of a mix of 1 g Dextran (Sigma D9260-500G), 1 ml DMSO and autoclaved  $\text{H}_2\text{O}$  to 10 ml. The nematodes are incubated for 10 min at room temperature before being placed in the  $-80^\circ\text{C}$  freezer in styrofoam boxes.

## Mating tests

For *Pristionchus*, we crossed 5 hermaphrodites from the *P. pacificus* JU1102 strain [11] with 5 males of our new isolates and monitored the proportion of males in the progeny as an evidence of crossing. For two of them, BRC20259 and BR20261, we further checked whether these F1 males were fertile by crossing them to hermaphrodites of either parental strain. All crosses were positive. Two replicates of a control with JU1102 animals only did not yield a high percentage of males.



**Fig 2. *Panagrellus* in rotting fruits.** (A) *Panagrellus* adult female from rotting apple O801 from Orsay. The quite posterior vulva (arrowhead) and late-stage embryos accumulating in front of it are characteristic of *Panagrellus*. Bar: 100 m. (B) *Panagrellus* dauers nictating on a *Drosophila* pupa in rotting pear B11-22 from Le Blanc. (C-F) Nomarski micrographs of L4 and adult vulvae of female *Panagrellus redivivoides* JU1476 (L4) and JU1798 (adult) and *Panagrellus redivivus* PS1163. Anterior is to the left, dorsal to the top for all panels, thus the uterus is on the left and the post-vulval sac is on the right in C-F. Most *Panagrellus* spp. display an anteriorly tilted vulva as shown for *P. redivivus*, while that of *P. redivivoides* is almost perpendicular to the ventral cuticle. (G) Spicule morphology of *P. redivivoides* (here strain JU1476). The forked ventral end of the spicules is indicated by an arrow, the gubernaculum by an arrowhead. (H) When in the proper focal plane, the dorsal side of the gubernaculum ends in the manner of a hook (arrowhead) (strain JU385). (I) Adult female mouth (here JU1055). Same scale for G-I. Bar: 5 μm.

<https://doi.org/10.1371/journal.pone.0200851.g002>

For *Panagrellus*, we crossed 3–5 L4 females with 3–5 males and monitored first and second generation progeny. A control with 5 L4 females of the tester strain JU385 without males did not yield any progeny (2 replicates).

## PCR and sequence analysis of rDNA

In some surveys indicated in [S2 Table](#), the nematodes were assigned to a genus using a molecular tag. The small subunit (SSU, 18S) of ribosomal DNA of *Pristionchus* isolates was amplified using primers SSU18A (5' -AAAGATTAAGCCATGCATG-3') and SSU26R (CATTCTTGGCAAATGCTTTCG), and sequenced using SSU18A or SSU9R (AGCTGGAATTACCGCGGCTG), as in [\[5–9,52\]](#); alternatively, as indicated in [S2 Table](#), primers RHAB1350F (5' -TACAATGGAAGGCAGCAGGC) and RHAB1868R (5' -CCTCTGACTTTCGTTCTTGATTAA) were used.

The large subunit (LSU, 28S) of ribosomal DNA of *Panagrellus* isolates was amplified using primers D2A (ACAAGTACCGTGGGAAAGTTG) and D3B (TCGGAAGGAACCAGCTACTA) as in [\[53\]](#).

The sequences were trimmed by visual inspection of the chromatograms, leaving the first four nucleotides of the downstream primer when present at the end of the sequence. A 'N' corresponds either to a low-quality sequence (including the possibility of a gap, especially when the same nucleotide occurred at consecutive positions) or a putative polymorphism of rDNA repeats. The sequences are available at Genbank with accession # (SUBMITTED SUB4276706 (18S) and SUB4277191 (28S)).

The sequences were run through NCBI Blast (April-May 2018) with default parameters. In case of the apparent polymorphism in our *Panagrellus redivivoides* 28S sequences (AGTTGATCGGGTGTGGCTTCGGY; cf. [S2B Table](#), column P), the highest peak was used in the blast analysis. Differences with the closest sequence in the database were manually checked.

## Data analysis

The abundance index is defined on a Log<sub>10</sub> scale as in [\[26\]](#). An index of 1 corresponds to one to 10 individuals, 2 for 11 to 100, 3 for 10<sup>2</sup> to 10<sup>3</sup>, 4 for 10<sup>3</sup> to 10<sup>4</sup>, and 5 for > 10<sup>4</sup>.

Statistical analysis was performed in R version 3.4.1 [\[54\]](#).

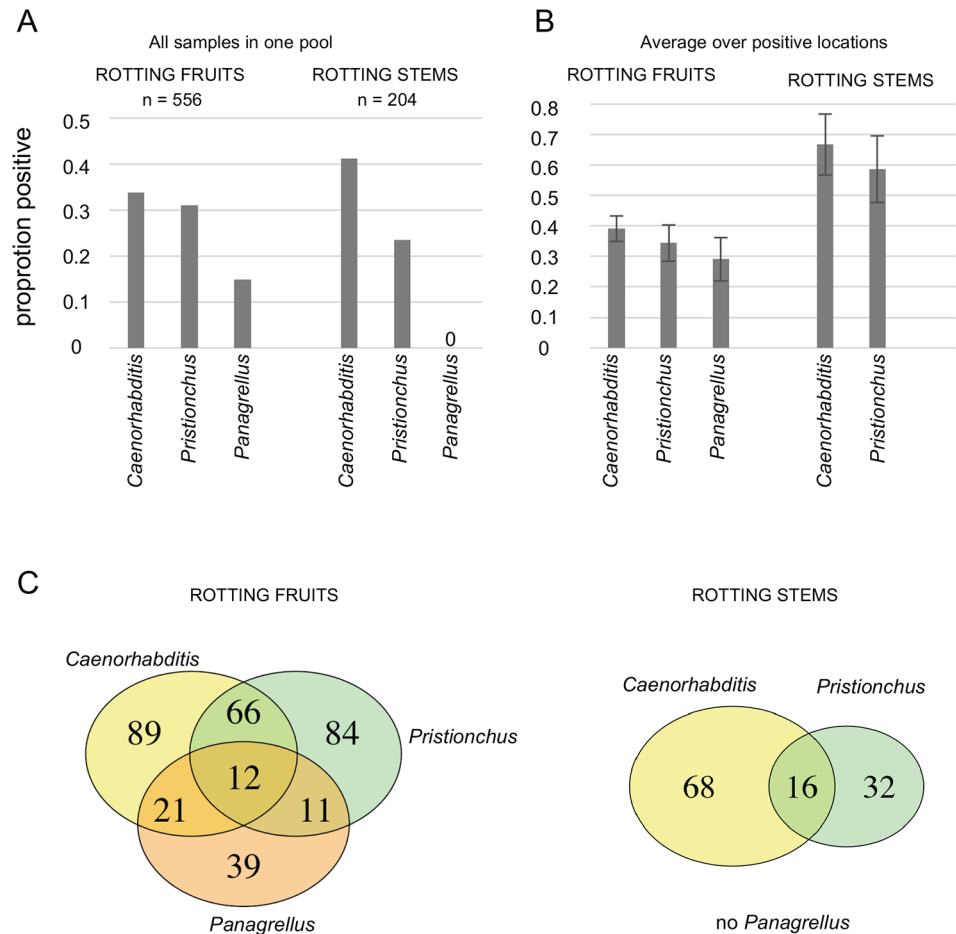
## Results

### Sampling for *Caenorhabditis*

We sampled rotting vegetal matter, mostly fruits and stems from herbaceous plants ([Fig 1](#)), and occasionally flowers, leaves, and wood as well as a few other sample types, such as fungi, soil, humus mixes and occasionally larger invertebrates ([S1](#) and [S2 Tables](#)). Our primary goal was to isolate *Caenorhabditis* spp. Around 20 years ago, we first had sampled soil and found some *Pristionchus* (see [S2A Table](#), first lines) but as we could not find *Caenorhabditis* we then focused on richer rotting vegetal matter [\[25\]](#). In 2015–2017, we also sought to isolate *Mesorhabditis* spp. and thus included samples of humus and rotting leaves where species of this genus are easy to find. We present here all samples for which we systematically noted the different nematode genera. Some locations (in France) were sampled on many occasions while others have been sampled once.

*Caenorhabditis* was found in 34% of rotting fruit samples (n = 556) and 41% of stem samples (n = 204) ([Fig 3](#), [Table 1](#)). As noted previously [\[26\]](#), we found them in these various samples either in a feeding stage or in the dauer diapause stage.

Among species of the *Elegans* supergroup [\[25\]](#), *C. elegans*, *C. briggsae*, *C. remanei* are commonly found in temperate areas. We also found *C. elegans* in samples from the Oku mountains



**Fig 3. Proportions of rotting fruit and stem samples containing *Caenorhabditis*, *Pristionchus* or *Panagrellus*.** (A) Proportions of samples positive for each genus, pooling all samples from rotting fruits or stems from 25 locations. (B) Proportions of samples positive for each genus, averaging over positive locations. Bars: standard error over locations. *Caenorhabditis*, *Pristionchus* are found in both types of sample, while *Panagrellus* are found in rotting fruits only. (C) Venn diagrams for the three genera in decomposing fruits (left) and stems (right).

<https://doi.org/10.1371/journal.pone.0200851.g003>

(Cameroon) and South Yunnan (China) and *C. elegans*, *C. briggsae* and *C. tropicalis* in samples from São Tomé. Among the *Caenorhabditis* species outside the *Elegans* supergroup, we found *C. virilis* in rotting apples (Orsay, also [25]) and in droppings from a small mammal having eaten fruits (Longueville), in both cases close to Paris, France. *C. portoensis* was found in several places in Western Europe in rotting fruits: in apples in Portugal [25] and France, and in oranges in Sicily. Finally, *C. monodelphis* [55] was found in one rotting wood sample including a tree fungus, from Oslo, Norway.

### ***Pristionchus* is commonly found in rotting vegetal matter**

*Pristionchus* was found in 31% of rotting fruit samples (n = 556) and 24% of stem samples (n = 204; Table 1), thus in a comparable proportion to *Caenorhabditis*, especially considering that the numbers for *Pristionchus* are underestimates (especially in the large set of Santeuil stem samples, where *Pristionchus* was not always distinguished from other diplogastrids; S1 Table). *Pristionchus* is thus commonly found in both rotting fruit and stem samples.



**Table 1. Occurrence of *Caenorhabditis*, *Pristionchus* and *Panagrellus* nematodes in rotting fruits and stems from various locations around the world.** Note that while *Caenorhabditis* was thoroughly searched for, the number of positive samples for *Pristionchus* and *Panagrellus* are underestimates, both because they may have been overlooked and because we may not have kept notes on batches of samples without *Caenorhabditis*. Detailed data for each sample are in S1 Table, with locations in France first in alphabetical order, then non-French locations.

Location	Total # rotting fruits	# fruits with <i>Caenorh.</i>	# fruits with <i>Prist.</i>	# fruits with <i>Panag.</i>	Total # rotting stems	# stems with <i>Caenorh.</i>	# stems with <i>Prist.</i>	# stems with <i>Panag.</i>
Crouy-sur-Ourcq, FR	4	0	0	0	14	10	3	0
Le Blanc & Indre	62	18	9	24	17	2	7	0
Longueville	9	1	3	0	2	1	2	0
Orsay	316	124	120	37	1	1	1	0
Plougasnou & Finistère	5	2	1	0	31	7	2	0
Santeuil & Vexin	39	8	2	5	89	55	17	0
Vaucluse & Simiane	28	7	11	7	0	0	0	0
Bangalore, India	7	4	1	0	1	1	0	0
Barcelona, SP	5	2	1	0	0	0	0	0
Oku, Cameroon	0	0	0	0	3	0	0	0
Cologne, DE	0	0	0	0	0	0	0	0
Cambridge, UK	0	0	0	0	1	0	0	0
Czech Republic	17	6	4	9	3	3	1	0
Heidelberg, DE	0	0	0	0	1	0	0	0
Kazakhstan	15	0	0	0	15	0	5	0
Los Angeles, USA	3	2	1	0	1	0	0	0
Norway	1	0	0	0	7	0	4	0
New Zealand	6	3	1	0	2	0	0	0
Potsdam, DE	0	0	0	0	0	0	0	0
Tuscany, IT	12	1	11	0	2	1	2	0
Vienna, Austria	3	2	0	0	0	0	0	0
São Tomé	3	1	1	1	3	2	0	0
Shanghai, China	7	3	4	0	9	0	3	0
Sicily, IT	11	3	1	0	1	1	1	0
U Warwick, UK	0	0	0	0	1	0	0	0
Yerevan, Armenia	3	1	2	0	0	0	0	0
<b>total</b>	556	188	173	83	204	84	48	0
<b>proportion</b>		0.34	0.31	0.15		0.41	0.24	0.00

<https://doi.org/10.1371/journal.pone.0200851.t001>

In addition to rotting fruits and stems, we noted the presence of *Pristionchus* in other types of decomposed vegetal matter (S1 and S2 Tables): soil/humus and leaf litter (over 20 samples), compost, flowers, iris and hyacinth bulbs, cacti, leaves, moss, and wood. We also found *Pristionchus* in fungi (on trees or on the ground), a *Geophilus* myriapod, a dead bee, a dead *Helix aspersa* snail (S1 and S2 Tables), and droppings from a small mammal. This list is quite similar to the samples where we found *Caenorhabditis*. The only exception may be that *C. elegans* and *C. briggsae* were found in live snails, slugs and isopods (here and [21,22,27,30]) and we never found *Pristionchus* in such samples.

*Pristionchus* was found in most geographic locations (23/26 locations; while *Caenorhabditis* was found in 21/26 locations). The three locations where *Pristionchus* was not found were those sampled for *Mesorhabditis* spp. (mostly humus/rotting leaves samples), where *Caenorhabditis* was also not found. The two locations where *Pristionchus* but not *Caenorhabditis* was

found were at Northern latitudes (Lofoten Islands) and on mountains with a continental climate and temperatures below freezing in winter (ca. 1500–2000 meters altitude near Almaty, Kazakhstan). Conversely, it would be interesting to establish whether *Pristionchus* may be less common than *Caenorhabditis* in equatorial regions (as could be suggested by the low frequency of *Pristionchus* in samples from São Tomé and Bangalore; [S1 Table](#)).

Within a single sample, the census size of *Pristionchus* appeared comparable to *Caenorhabditis*, with populations ranging from 1–few animals to over 1,000 (over 10,000 for *Caenorhabditis*) in one sample ([S1 Table](#); for example samples shown in [Fig 1B and 1F](#)). *Pristionchus* feeding stages were noted to be present alongside dauer larvae in both rotting fruits and stems (Orsay, Santeuil). *Pristionchus* was the predominant species in terms of numbers in some samples ([S1 Table](#)), while in others *Caenorhabditis*, *Oscheius*, other rhabditids, or *Panagrellus* were most abundant.

A variety of *Pristionchus* species were found, as indicated by our 18S sequencing ([S2 Table](#)), consistent with previous reports [7,8,11,56]. Among them, we found *P. pacificus*, as verified by crosses ([S2 Table](#)). Some of our 18S sequences do not match those of any *Pristionchus* species in the databases and may be new species. Conversely, some *Pristionchus* species may not be distinguished by this short fragment. Importantly, the sampled diversity covers all groups of *Pristionchus* species in [56], except the more basal 'Elegans' group. Indeed, we found 18S best hits in the *Pacificus* group to *P. pacificus*, *P. arcanus*, *P. japonicus* and *P. quartusdecimus*, in the *Maupasi* group to *P. atlanticus*, in the *Lheritieri* group to *P. uniformis* and *P. entomophagus*, and in the *Triformis* group to *P. triformis* and *P. hoplostomus*. In addition, in a rotting bulb we found a putative representative of the fresh fig clade [15] with a sequence resembling that of *Pristionchus* sp. 35.

### ***Panagrellus redivivoides* is found in rotting fruits but not rotting stems**

*Panagrellus* nematodes were found in 15% of rotting fruit samples ( $n = 556$ ; confidence interval using a binomial distribution 12–18%) and 0% of the rotting stem samples ( $n = 204$ ; confidence interval 0–2%) ([Table 1](#)). Thus, in our sampled substrate types, *Panagrellus* is specifically enriched in fruits versus stems (Fisher exact test rejecting homogeneity,  $p = 10^{-12}$ ). Rotting fruits contain bacteria and fungi and we observed that *Panagrellus* adults were often attracted to fungi (yeasts) on the culture plates.

Another invertebrate commonly found on these rotting fruits but not rotting stems is the fruit fly *Drosophila* (various species). We sampled live *Drosophila* in the field and on two occasions found *Panagrellus* ([S2 Table](#) and [26]). We also found *Panagrellus* in a *Drosophila willistoni* culture ([S2 Table](#)). We observed *Panagrellus* dauer larvae climbing on *Drosophila* pupae in our samples and waving, especially on the pupal appendages ([Fig 2B](#)). It is thus likely that fruit-flies constitute vectors for *Panagrellus* between rotting fruit food patches.

We performed pairwise mating tests between *Panagrellus* fruit isolates from various places in Europe, Canary Islands, Armenia and North America, and to our surprise, found that all tested combinations were compatible, thus representing a single biological species. Morphologically, the vulva slit appeared almost perpendicular to the ventral side of the females ([Fig 2C and 2D](#), [S2B Table](#)). This feature is characteristic of a single species of *Panagrellus*, called *P. redivivoides* [57,58]. In all other species such as *P. redivivus*, the vulva is strongly bent towards the anterior side of the animal ([Fig 2E and 2F](#)). Other morphological features of our isolates also matched the original and subsequent redescription of *P. redivivoides* [57,59,60], including the shape of the spicules and the gubernaculum dorsal end bearing a hook in lateral view ([Fig 2G and 2H](#)).

This *Panagrellus* species was collected from diverse fruit samples (apples, pears, peach, grapes, plums, cherries, tomatoes, walnuts, figs), but never from other substrates (except once in compost containing rotting fruits). Among the substrates we sampled, *P. redivivoides* thus appears specific to rotting fruits. It does not appear in every orchard or region where we have sampled, for example it was not found in Utah, while it was found in Oregon and Washington states (S2B Table). We recently found in China a new *Panagrellus* isolate (JU3343) in a compost heap of unclear composition but containing no fruits. As an exception that proves the rule, this isolate corresponds to a different *Panagrellus* species both through mating tests and morphology (S2B Table).

We amplified and sequenced 18S and 28S rDNA fragments for the *Panagrellus* isolates, which yielded the same sequence for all our rotting fruit *Panagrellus* isolates from eight locations spanning four continents, confirming our crossing tests. DNA sequence tags of *P. redivivoides* were not included in the Stock and Nadler article [59] that aimed to associate DNA sequence tags to *Panagrellus* morphological species. Two later articles with new *Panagrellus* sp. isolates [53,61] yielded closely related sequences but the authors did not attempt to identify the species morphologically or through mating tests. We found that the faster evolving 28S rDNA fragment was most similar but not identical to that of *Panagrellus* sp. MC2014 KM489128 (isolated from an aberrant specimen of the red palm weevil *Rhynchophorus ferrugineus* in [61]). The sequence of the 18S rDNA fragment is also similar but not identical to *Panagrellus* sp. MC2014 and to a newly described species *Panagrellus levitatus* [62] (see Discussion).

### Other nematodes

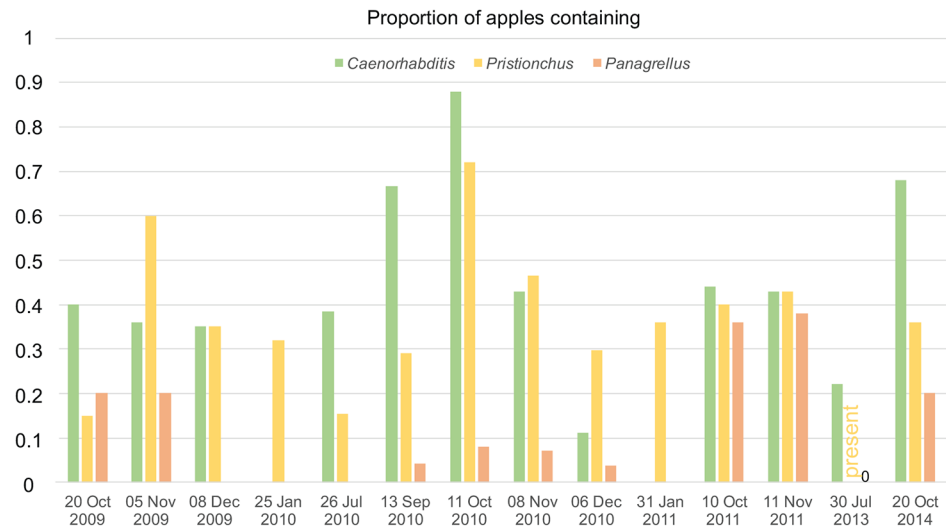
Other nematode genera were found in rotting fruits and stems. *Oscheius* (species of the *Dolichura* group including the common *Oscheius tipulae*) was present in all types of samples. Although it was found frequently in soil [63], we had never observed feeding stages until we started collecting rotting fruits and stems (S1 Table). Other rhabditids included *Rhabditella*, *Auanema*, *Pelodera*, etc. *Mesorhabditis* was often present in small numbers, and also found in humus and rotting leaves in forests. *Panagrolaimus* was also found in soil but often present in rotting fruits and stems, even if quite dry. *Rhabditophanes* was particularly observed in cold weathers and climates. Fruit samples also commonly contained fungi-eating nematodes such as aphelenchs.

### Co-occurrence

Many of the different nematodes co-occurred in the same sample (Table 1, S2C Table). Fig 3C summarizes the co-occurrence of the three genera *Caenorhabditis*, *Pristionchus* and *Panagrellus* in fruits and stems. A weak positive correlation was found in rotting fruits for the co-occurrence of *Pristionchus* and *Caenorhabditis* (correlation coefficient  $r = 0.16$  [0.08–0.24 confidence interval],  $p = 0.0001$ ). For example, in fruits, 41.0% of the samples with *Caenorhabditis* ( $n = 188$ , for a total of 556 fruits) also had *Pristionchus* and conversely, 44.5% of the samples with *Pristionchus* ( $n = 173$ ) contain *Caenorhabditis*. No other correlations were found significant (note however that sample sizes were smaller in stems and for *Panagrellus*).

### Seasonal pattern in the Orsay orchard

Fig 4 shows the abundance of the three genera over time in apples of the Orsay orchard. *Pristionchus* was present in rotting apples on every tested date of the year, spanning July to January. *Caenorhabditis* was not found on the two January dates (see further data in [26]) and *Panagrellus* was not found in July (twice in different years) nor on three dates in December and January.



**Fig 4. Seasonal pattern of presence of *Caenorhabditis*, *Pristionchus* or *Panagrellus* in apples of the Orsay orchard.** This figure shows the proportion of positive apples for *Caenorhabditis*, *Pristionchus* or *Panagrellus* at different dates of sampling. *Pristionchus* was noted present in a few apples on the 30 July 2013 timepoint but the number of positive apples was not scored. n = 20–28 apples per date.

<https://doi.org/10.1371/journal.pone.0200851.g004>

## Discussion

### Not only fond of beetles: *Pristionchus* also lives in rotting vegetal matter

Our results demonstrate that *Pristionchus* nematodes are found in rotting vegetal matter frequently and abundantly, in both dauer and feeding stages. This includes *P. pacificus* and other "beetle-associated" *Pristionchus* species, such as *P. uniformis*, *P. entomophagus*, *P. triformis*, *P. quartusdecimus* or *P. atlanticus* [8,9,12,56,64], or closely related species (S2 Table). From these data, we conclude that these *Pristionchus* species feed in rotting vegetal matter and are thus not exclusively necromenic, as previously reported by only sampling beetles. These decomposing invertebrates are likely not their main source of food compared to the microbial blooms and other prey nematodes that *Pristionchus* may encounter in rotting vegetal substrates.

From our literature review (see Introduction), *Pristionchus* have not been shown to be feeding in the wild on naturally decomposing beetle corpses. Thus, although it cannot be ruled out that *Pristionchus* may be necromenic on beetles in some instances, the relationship of *Pristionchus* species with beetles may be similar to the relationship of *Caenorhabditis* species with isopods or terrestrial molluscs. Indeed, when any of the carriers of *Caenorhabditis* are killed on a Petri dish as *Pristionchus*-bearing beetles have been, *Caenorhabditis* will exit the dauer stage and start reproducing. (Note that on live slugs and snails, other developmental stages of *C. elegans* are also found [26,27]). Evidence for a possible *C. elegans* necromenic behavior is anecdotal: non-dauer *C. elegans* were found once on a naturally dead *Helix* snail (Table 1 in [22]). Note that we found *Pristionchus* spp. on a live *Geophilus* myriapod, a dead snail and a dead honeybee (S2 Table; nematode developmental stage unknown), and some *Pristionchus* species also have been described on invertebrates other than beetles (e.g. *P. entomophagus* on a pamphilid wasp [65]). It is thus unclear for each *Pristionchus* species whether beetle species are the only specific associates. The present survey focused on rotting vegetal matter and a few other substrates and thus provides a narrow window on *Pristionchus* ecology, yet still considerably enlarging the sample diversity compared to only collecting beetles.

Each *Pristionchus* species (and possibly population) needs to be considered separately. Our survey found a variety of *Pristionchus* species covering several clades within the genus (S2A Table), with a biogeography consistent with that in previous reports in beetles [64]. Most of our sampling work was in Europe, where *P. pacificus* is rare compared to other *Pristionchus* spp. [8,9,11,66], but we found *P. pacificus* in decomposing vegetal matter in Asia (S2 Table).

### Consequences for the life cycle of *Pristionchus*

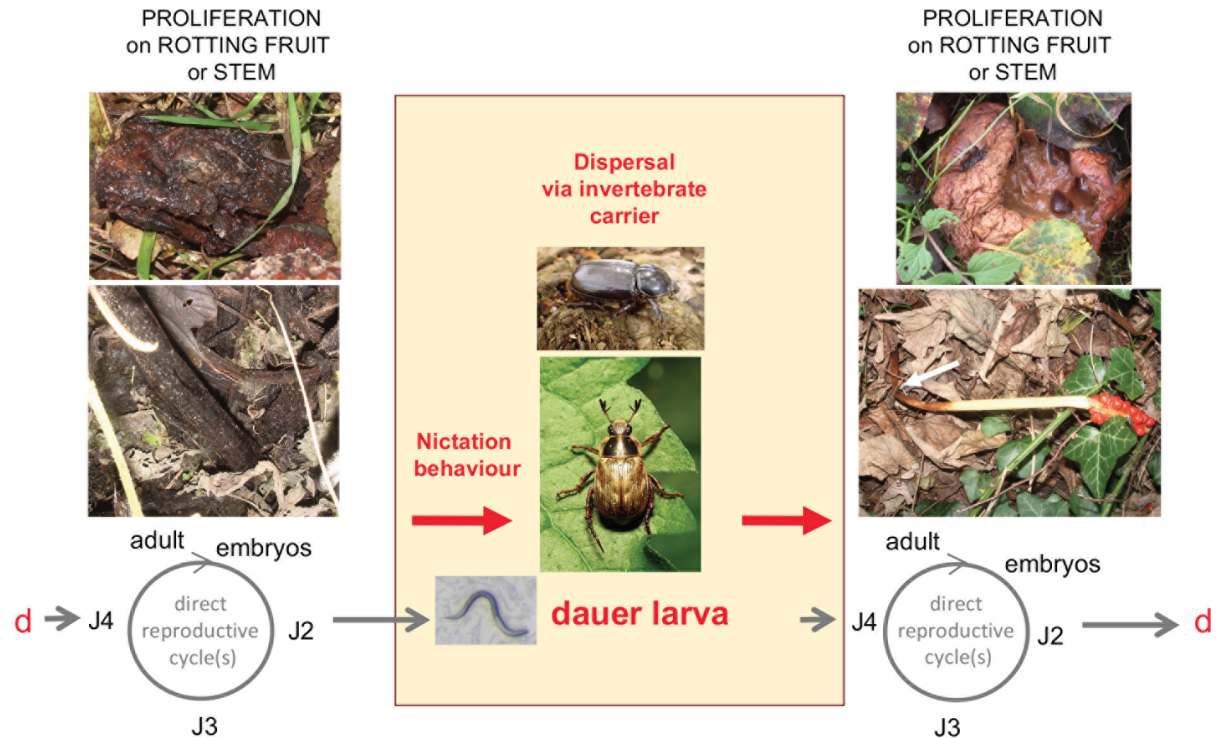
A representation of the *P. pacificus* life cycle has recently been proposed [17], based on the beetle association and laboratory findings. In this model, adult *P. pacificus* (and possibly dauers) are attracted to the oriental beetle *Exomala orientalis*. On the beetle, eggs and dauers are produced and developmentally arrested due to a beetle chemical studied in the article. Upon beetle death, *P. pacificus* exit the dauer stage and as adults may be attracted to another beetle. Alternatively, they adopt a "free" life cycle (likely meaning that they do not go through a dauer stage) and eventually may reenter the dauer stage or be attracted to a new beetle as adults. Presumably, the free life cycle is on the dead beetle.

Two features of this proposed life cycle are surprising: i. the stage that is attracted to the beetle is the adult nematode (which has not been observed so far on wild-caught beetles); ii. once on the beetle, the adult nematode is able to produce dauer progeny. This new view of the nematode life cycle was probably derived from the fact that most behavioral studies of chemotaxis towards beetles used *Pristionchus* adults [6,17,67,68], with the exception of [69,70], which studied dauer nictation.

We propose in Fig 5 an alternative life cycle for *P. pacificus* and other *Pristionchus* species that may be found associated with various beetles and other insects. This life cycle is similar to that proposed for *C. elegans* [30,71]. In our model, rotting vegetal matter is the feeding ground for *Pristionchus* spp., whose populations expand until the food is exhausted whereupon the young larvae enter the dauer stage. Beetles may transport dauer larvae between these patchy plant food sources. In this life cycle scenario, the association of dauer larvae with the larger invertebrate is phoretic. Necromeny may be occasional, but its occurrence remains to be demonstrated.

In addition to bacteria and fungi, *P. pacificus* and other *Pristionchus* spp. may feed on other nematodes as a food source in decomposing vegetal matter. The predatory behavior of many *Pristionchus* species towards other nematodes is rendered possible by the plasticity of development of the mouth form [15,72,73]. From our data, it is clear that many other nematodes co-occur with *Pristionchus* species in rotting vegetal matter, including but not restricted to *Caenorhabditis* and *Panagrellus* species (S1 Table). These other nematodes may compete for bacterial food or serve as prey for *Pristionchus*. We found a weak positive correlation for the co-occurrence of *Pristionchus* and *Caenorhabditis* in rotting fruits. Rather than a specific attraction of *Pristionchus* to *Caenorhabditis*-containing substrates (or conversely), we propose that this correlation may be simply explained by the degree of decomposition, humidity or microbial fauna in the fruits, which needs to be such as to sustain these nematode species.

*C. elegans* was shown to avoid sulfolipids secreted by *Pristionchus pacificus* [74]. Due to geographical sampling biases for each genus, we have so far not detected the co-occurrence of *C. elegans* and *P. pacificus* in the same sample. It would be interesting to sample further in areas where both *C. elegans* and *P. pacificus* were found, such as Southern California, Hawaii, South Africa or La Réunion [11,75]. *C. elegans* and *P. pacificus* were chosen as the two most studied species of each genus, and it is not known whether the sulfolipids and the reaction to them are specific for these two species. It is possible that the avoidance behavior of *C. elegans* has evolved as a response to several *Pristionchus* species, and that conversely, several *Caenorhabditis* species may show avoidance to *Pristionchus pacificus* or other *Pristionchus* species.



**Fig 5. Proposed life cycle of *Pristionchus* spp. in their natural habitat.** *P. pacificus* and other *Pristionchus* species proliferate in various types of rotting plant material, such as fruits. Dauer larvae are the stress-resistant, alternative third juvenile stage. (The first larval stage occurs within the embryo [97]). Dauer larvae may actively disperse to colonize new food sources. Alternatively, their nictation behaviour—standing on their tail and waving individually or in group—may allow them to attach and disperse via carriers, such as *Exomala orientalis* or *Oryctes borbonicus* for *P. pacificus*, until a new food source is encountered, where development resumes. J2–J4, juvenile stages; d, dauer larva. Modified after [71] drawn for *C. elegans*. Apples, stems and *P. pacificus* JU1102 dauer: Pictures by MAF. Bottom beetle: *Exomala orientalis*, by Katja Schulz via Wikimedia Commons CC BY-SA 2.0, [https://commons.wikimedia.org/wiki/File%3AOriental\\_Beetle\\_-\\_Flickr\\_treegrow\\_\(1\).jpg](https://commons.wikimedia.org/wiki/File%3AOriental_Beetle_-_Flickr_treegrow_(1).jpg). Top beetle: *Oryctes borbonicus*, by Jjargoud—Own work, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=5063614>.

<https://doi.org/10.1371/journal.pone.0200851.g005>

To explain the presence of *Pristionchus* spp. dauers phoretic on beetles, it must be assumed that the beetles visit the kinds of habitats where we found *Pristionchus* populations, i.e. rotting fruits and stems. Are the beetles that have been associated with *Pristionchus* spp. likely to visit fruits or a forest floor with rotting stems? Detailed studies would be needed to answer this important question, taking into account the seasonality of the beetle's life cycle itself. *Geotrupes stercorosus* was found to be the most reliable beetle source of *Pristionchus* nematodes in Europe by [9]. Indeed, *Geotrupes* adults may feed on forest litter or fungi [76] and lay eggs in nests they provide with forest litter [77]. The *P. pacificus*-carrying *Exomala orientalis* adults emerge from soil [78] and adult females may feed on flowers [79,80]. Indeed many of the scarab beetle species associated with *Pristionchus* were found to feed as adults on soft, high-quality diets and the micro-organisms therein [77] and many of the American tropical "dung beetle" species were found to feed on mature and rotting fruits [81]. Understanding the relationship between the rotting vegetal matter and the beetles is required to understand the biology of *Pristionchus* spp. and test our hypothesized life cycle (Fig 5). This includes answering the currently open question of whether *Pristionchus* is present on larval beetles.

### ***Panagrellus* in rotting fruits: A specific habitat for *Panagrellus redivivoides*?**

The *Panagrellus* genus was previously found on a variety of substrates. The 'sour paste nematode' *Panagrellus redivivus* is used in many studies because of its ease of culture in the

laboratory [82–89]. This species used to be commonly found in glues used to hang wallpaper and bind books [82,90]. Other *Panagrellus* species have been found in the slime flux or cankers of trees caused by bacterial or fungal diseases, in association with bark beetles or their frass, inside pitcher plants and, as a human associate, in beer mats and spoiled cider [59,60].

Taxonomic characterization of different *Panagrellus* species can be found in [38,59,60,91], with molecular data in [59]. The vagina of all but one of the *Panagrellus* species is tilted anteriorly towards the uterus, while a vagina that extends perpendicularly to the ventral cuticle of the female is specific to *Panagrellus redivivoides* [38,59,91]. *P. redivivoides* did not yet have a molecular tag to anchor the morphological description, which we provide here.

Our mating tests show that all the *Panagrellus* we isolated on rotting fruits or *Drosophila* belong to a single biological species, with a morphology matching *Panagrellus redivivoides* [57,58]. Its 28S rDNA sequence is identical to that of a *Panagrellus* sp. found in decomposing pomegranate in Italy [53] and both 28S and 18S sequences are similar but not identical to that of a *Panagrellus* found in a weevil in [61]. A new species of *Panagrellus* has been very recently described from a culture of *Drosophila melanogaster* and called *P. levitatus* [62]. This species presents similar but not identical 18S rDNA sequences to those we found (S2 Table) and has a weakly tilted vulva. The only morphological character of this isolate that is supposed to differ from *P. redivivoides* is the presence of circumcloacal papillae in *P. levitatus*, with a reference to [91] for their "absence" in *P. redivivoides* (to our knowledge only an absence of information). Further work would be required to make sure that there are two different species. Because of differences in rDNA sequence with *P. levitatus*, abundance on several continents and priority, we identify the biological species we found in rotting fruits as *Panagrellus redivivoides*.

Concerning phoretic associations, *P. redivivoides* may be associated with *Drosophila* fruit flies, in contrast to other *Panagrellus* sp. that appear associated with bark beetles. The fruit fly association relies on several reports. Early on, using *Drosophila* traps made of potato puree, Aubertot [92] found "*Panagrellus silusiae*", now synonymized with *P. redivivus* [90,93] (yet the species determination of Aubertot is unclear, especially since the species *P. redivivoides* was not described yet). *P. redivivoides* was found several times in laboratory cultures that were visited by fruitflies, including in the original description of the species by Goodey [57], which reports the arrival of the species in a banana maize-meal cider used to trap *Drosophila* flies. Lees [82] set up experiments to test the association of what he called "*P. silusiae*" (again, the species identification is unclear) and *Drosophila funebris* and showed that the latter could transport the former to new Petri dishes. "*Panagrellus zymosiphilus*", now synonymized with *P. redivivoides* [91] was also found twice in *Drosophila* cultures, the second time on *Drosophila obscuroides* recently isolated from nature [94,95]. "*P. zymosiphilus*" was also found in grapes, presumably brought by a *Drosophila* [96]. We added one data point to this association with *Drosophila* cultures with JU385, isolated in a *Drosophila willistoni* culture (S2B Table). In addition, *Drosophila* larvae are very often found in the rotting fruits we sampled and we could isolate *Panagrellus redivivoides* on *Drosophila* caught outside in two locations, Orsay and Le Blanc (reported in [26]), the latter yielding JU1055 (S2B Table).

We suggest the possibility that one species, *P. redivivoides*, is the most commonly associated with rotting fruits and *Drosophila* larvae, a distinguishing ecological feature compared to most *Panagrellus* species. Its life cycle may be similar to that depicted for *C. elegans* [71] or *Pristionchus* (Fig 5), but with *Drosophila* fruitflies carrying the dauer larvae from fruit to fruit.

In summary, based on our sampling and the observed distribution of feeding and dauer stages, we propose a life cycle for *Pristionchus* nematodes and *Panagrellus redivivoides* that is similar to that of many *Caenorhabditis* species, including *C. elegans*, whereby they feed on the microbial blooms on decomposing vegetal matter and are transported between these food

patches by larger invertebrates, which may be beetles for *Pristionchus* spp., fruitflies for *Panagrellus redivivoides* and isopods and terrestrial molluscs for *Caenorhabditis* spp.

## Supporting information

**S1 Table. Surveys of some nematode genera in various sample types from different worldwide locations.** Each sheet corresponds to a sampling location and is designated by a letter: A-G, locations in France, H-Z locations outside France, in alphabetical order. Presence of a nematode genus is indicated by 'x'. When the relative abundance of different genera has been scored, 1 indicates the most abundant, followed by 2, 3, etc. *Caenorhabditis* (green column), *Pristionchus* (yellow), *Panagrellus* (orange) are indicated first. The other groups are indicated roughly in order of ease of extraction from the sample and identification. On the right, in columns 'Caenorhabditis abundance log index' and 'Pristionchus abundance log index', is indicated the abundance log index (see [Methods](#)): here 5 is highest and 1 lowest. 'f' indicates feeding stages, 'd' dauer larvae, in order of abundance, with the caveat that dauer larvae are more difficult to identify than feeding stages. Note that the care and timing with which the different nematodes were identified and monitored for number and stages differ among the different sampling dates and locations. Some data for *Caenorhabditis* in sheets D-F are from [26,49]. *Pristionchus* are best distinguished from other diplogastrids in the adult stage; thus, a '\*' in the 'other diplogastrid' column indicates that these diplogastrids could have been *Pristionchus*. The *Caenorhabditis* species are abbreviated 'Cel' for *C. elegans*, 'Cbr' for *C. briggsae*, 'Cre' for *C. remanei*, 'Ctr' for *C. tropicalis*, 'Cvi' for *C. virilis*, 'Cpo' for *C. portoensis*, 'Cmo' for *C. monodelphis*. Latitude and longitude coordinates are indicated with the number of digits corresponding to the precision.

(XLSX)

**S2 Table. Wild isolates from various sample types.** Sheet A: *Pristionchus*. The table indicates the origin of the strains of *Pristionchus* (with JU or BRC standard strain names), and other samples that were noted to contain *Pristionchus*. The sample ID is a temporary ID for a given collection date. Other non-*Pristionchus* frozen strains from the same samples are indicated on the right. nd: not determined. Sheet B: *Panagrellus*. The table indicates the origin of *Panagrellus* strains. Sheet C: Other species of nematodes collected from rotten fruit and characterized by 18S rDNA sequencing.

(XLSX)

## Acknowledgments

We thank members of our labs and all other sample collectors listed in [S1](#) and [S2](#) Tables, especially Jim Thomas. We thank Irini Topalidou for assistance with PCR and sequencing. We thank C. Braendle, S. Chalasani, K. Kiontke, B. Schlager and H. Teotónio for comments on the manuscript.

## Author Contributions

**Conceptualization:** Marie-Anne Félix, Michael Ailion, John Wang.

**Funding acquisition:** Marie-Anne Félix, Michael Ailion, John Wang.

**Investigation:** Marie-Anne Félix, Michael Ailion, Jung-Chen Hsu, Aurélien Richaud, John Wang.

**Supervision:** Marie-Anne Félix, Michael Ailion, John Wang.



**Writing – original draft:** Marie-Anne Félix.

**Writing – review & editing:** Michael Ailion, John Wang.

## References

1. Sommer RJ, McGaughan A (2013) The nematode *Pristionchus pacificus* as a model system for integrative studies in evolutionary biology. *Mol Ecol* 22: 2380–2393. <https://doi.org/10.1111/mec.12286> PMID: 23530614
2. Cinkorpumin JK, Hong RL (2011) RNAi mediated gene knockdown and transgenesis by microinjection in the necromenic nematode *Pristionchus pacificus*. *J Vis Exp*: e3270. <https://doi.org/10.3791/3270> PMID: 22025167
3. Kroetz SM, Srinivasan J, Yaghoobian J, Sternberg PW, Hong RL (2012) The cGMP signaling pathway affects feeding behavior in the necromenic nematode *Pristionchus pacificus*. *PLoS One* 7: e34464. <https://doi.org/10.1371/journal.pone.0034464> PMID: 22563372
4. Dieterich C, Clifton SW, Schuster LN, Chinwalla A, Delehaunty K, Dinkelacker I, et al. (2008) The *Pristionchus pacificus* genome provides a unique perspective on nematode lifestyle and parasitism. *Nat Genet* 40: 1193–1198. <https://doi.org/10.1038/ng.227> PMID: 18806794
5. Floyd R, Abebe E, Papert A, Blaxter M (2002) Molecular barcodes for soil nematode identification. *Mol Ecol* 11: 839–850. PMID: 11972769
6. Herrmann M, Mayer WE, Hong RL, Kienle S, Minasaki R, Sommer RJ (2007) The nematode *Pristionchus pacificus* (Nematoda: Diplogastriidae) is associated with the oriental beetle *Exomala orientalis* (Coleoptera: Scarabaeidae) in Japan. *Zoolog Sci* 24: 883–889. <https://doi.org/10.2108/zsj.24.883> PMID: 17960992
7. Zauner H, Mayer WE, Herrmann M, Weller A, Erwig M, Sommer RJ (2007) Distinct patterns of genetic variation in *Pristionchus pacificus* and *Caenorhabditis elegans*, two partially selfing nematodes with cosmopolitan distribution. *Molecular Ecology* 16: 1267–1280. <https://doi.org/10.1111/j.1365-294X.2006.03222.x> PMID: 17391412
8. D'Anna I, Sommer RJ (2011) *Pristionchus uniformis*, should I stay or should I go? Recent host range expansion in a European nematode. *Ecol Evol* 1: 468–478. <https://doi.org/10.1002/ece3.28> PMID: 22393515
9. Herrmann M, Mayer WE, Sommer RJ (2006) Nematodes of the genus *Pristionchus* are closely associated with scarab beetles and the Colorado potato beetle in Western Europe. *Zoology* 109: 96–108. <https://doi.org/10.1016/j.zool.2006.03.001> PMID: 16616467
10. Herrmann M, Mayer WE, Sommer RJ (2006) Sex, bugs and Haldane's rule: the nematode genus *Pristionchus* in the United States. *Front Zool* 3: 14. <https://doi.org/10.1186/1742-9994-3-14> PMID: 16968539
11. Herrmann M, Kienle S, Rochat J, Mayer WE, Sommer RJ (2010) Haplotype diversity of the nematode *Pristionchus pacificus* on Réunion in the Indian Ocean suggests multiple independent invasions. *Biol J Linn Soc* 100: 170–179.
12. Weller AM, Mayer WE, Rae R, Sommer RJ (2010) Quantitative assessment of the nematode fauna present on *Geotrupes* dung beetles reveals species-rich communities with a heterogeneous distribution. *J Parasitol* 96: 525–531. <https://doi.org/10.1645/GE-2319.1> PMID: 20557197
13. Morgan K, McGaughan A, Villate L, Herrmann M, Witte H, Bartelmes G, et al. (2012) Multi locus analysis of *Pristionchus pacificus* on La Reunion Island reveals an evolutionary history shaped by multiple introductions, constrained dispersal events and rare out-crossing. *Mol Ecol* 21: 250–266. <https://doi.org/10.1111/j.1365-294X.2011.05382.x> PMID: 22126624
14. Darsouei R, Karimi J, Shokoohi E (2014) *Oscheius rugaoensis* and *Pristionchus maupasi*, two new records of entomophilic nematodes from Iran. *Russian Journal of Nematology* 22: 141–155.
15. Susoy V, Herrmann M, Kanzaki N, Kruger M, Nguyen CN, Rodelsperger C, et al. (2016) Large-scale diversification without genetic isolation in nematode symbionts of figs. *Sci Adv* 2: e1501031. <https://doi.org/10.1126/sciadv.1501031> PMID: 26824073
16. Ferrari C, Salle R, Callemeyn-Torre N, Jovelin R, Cutter AD, Braendle C (2017) Ephemeral-habitat colonization and neotropical species richness of *Caenorhabditis* nematodes. *BMC Ecol* 17: 43. <https://doi.org/10.1186/s12898-017-0150-z> PMID: 29258487
17. Cinkorpumin JK, Wisidagama DR, Rapoport V, Go JL, Dieterich C, Wang X, et al. (2014) A host beetle pheromone regulates development and behavior in the nematode *Pristionchus pacificus*. *Elife* 3.
18. Meyer JM, Baskaran P, Quast C, Susoy V, Rodelsperger C, Glockner FO, et al. (2017) Succession and dynamics of *Pristionchus* nematodes and their microbiome during decomposition of *Oryctes borbonicus*

- on La Reunion Island. *Environ Microbiol* 19: 1476–1489. <https://doi.org/10.1111/1462-2920.13697> PMID: 28198090
19. Sudhaus W, Fürst von Lieven A (2003) A phylogenetic classification and catalogue of the Diplogastriidae (Secernentea, Nematoda). *J Nem Morph Syst* 6: 43–90.
  20. Baird SE, Fitch DHA, Emmons SW (1994) *Caenorhabditis vulgaris* n.sp. (Nematoda: Rhabditidae): a necromenic associate of pill bugs and snails. *Nematologica* 40: 1–11.
  21. Baird SE (1999) Natural and experimental associations of *Caenorhabditis remanei* with *Trachelipus rathkii* and other terrestrial isopods. *Nematology* 1: 471–475.
  22. Barrière A, Félix M-A (2005) High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. *Curr Biol* 15: 1176–1184. <https://doi.org/10.1016/j.cub.2005.06.022> PMID: 16005289
  23. Caswell-Chen EP, Chen J, Lewis EE, Douhan GW, Nadler SA, Carey JR (2005) Revising the standard wisdom of *C. elegans* natural history: ecology of longevity. *Sci Aging Knowl Environ* 40: pe30.
  24. Barrière A, Félix M-A (2007) Temporal dynamics and linkage disequilibrium in natural *C. elegans* populations. *Genetics* 176: 999–1011. <https://doi.org/10.1534/genetics.106.067223> PMID: 17409084
  25. Kiontke K, Félix M-A, Ailion M, Rockman MV, Braendle C, Pénigault J-B, et al. (2011) A phylogeny and molecular barcodes for *Caenorhabditis*, with numerous new species from rotting fruits. *BMC Evol Biol* 11: 339. <https://doi.org/10.1186/1471-2148-11-339> PMID: 22103856
  26. Félix MA, Duveau F (2012) Population dynamics and habitat sharing of natural populations of *Caenorhabditis elegans* and *C. briggsae*. *BMC Biol* 10: 59. <https://doi.org/10.1186/1741-7007-10-59> PMID: 22731941
  27. Petersen C, Hermann RJ, Barg MC, Schalkowski R, Dirksen P, Barbosa C, et al. (2015) Travelling at a slug's pace: possible invertebrate vectors of *Caenorhabditis* nematodes. *BMC Ecol* 15: 19. <https://doi.org/10.1186/s12898-015-0050-z> PMID: 26170141
  28. Lee H, Choi M-k, Lee D, Kim H-s, Hwang H, Kim H, et al. (2011) Nictation, a dispersal behavior of the nematode *Caenorhabditis elegans*, is regulated by IL2 neurons. *Nature Neuroscience* 15: 107–112. <https://doi.org/10.1038/nn.2975> PMID: 22081161
  29. Lee D, Yang H, Kim J, Brady S, Zdraljevic S, Zamanian M, et al. (2017) The genetic basis of natural variation in a phoretic behavior. *Nat Commun* 8: 273. <https://doi.org/10.1038/s41467-017-00386-x> PMID: 28819099
  30. Schulenburg H, Félix M-A (2017) The natural biotic environment of *Caenorhabditis elegans*. *Genetics* 206: 55–86. <https://doi.org/10.1534/genetics.116.195511> PMID: 28476862
  31. Okumura E, Tanaka R, Yoshiga T (2013) Species-specific recognition of the carrier insect by dauer larvae of the nematode *Caenorhabditis japonica*. *J Exp Biol* 216: 568–572. <https://doi.org/10.1242/jeb.073593> PMID: 23077159
  32. Okumura E, Ishikawa Y, Tanaka R, Yoshiga T (2013) Propagation of *Caenorhabditis japonica* in the nest of its carrier insect, *Parastrachia japonensis*. *Zoolog Sci* 30: 174–177. <https://doi.org/10.2108/zsj.30.174> PMID: 23480376
  33. Okumura E, Yoshiga T (2014) Host orientation using volatiles in the phoretic nematode *Caenorhabditis japonica*. *J Exp Biol* 217: 3197–3199. <https://doi.org/10.1242/jeb.105353> PMID: 25063857
  34. Yoshiga T, Ishikawa Y, Tanaka R, Hironaka M, Okumura E (2013) Species-specific and female host-biased ectophoresy in the roundworm *Caenorhabditis japonica*. *Naturwissenschaften* 100: 205–208. <https://doi.org/10.1007/s00114-013-1011-z> PMID: 23325293
  35. Kiontke K, Sudhaus W (2006) Ecology of *Caenorhabditis* species. In: *WormBook* (ed. The *C. elegans* Research Community), [<http://www.wormbook.org/>] <https://doi.org/10.1895/wormbook.1.37.1> PMID: 18050464
  36. Stiernagle T (2006) Maintenance of *C. elegans*. In: *Wormbook* (ed. The *C. elegans* Research Community), [<http://www.wormbook.org/>] <https://doi.org/10.1895/wormbook.1.101.1> PMID: 18050451
  37. Barrière A, Félix M-A (2014) Isolation of *C. elegans* and related nematodes. In: *WormBook* (ed. The *C. elegans* Research Community), [<http://www.wormbook.org/>] <https://doi.org/10.1895/wormbook.1.115.2> PMID: 24803426
  38. Andrassy I (1984) *Klasse Nematoda*. Stuttgart: Gustav Fischer Verlag. 509 p.
  39. Sudhaus W (2011) A pictorial key to current genus groups of “Rhabditidae”. *J Nematode Morphol Syst* 14: 105–112.
  40. Félix M-A, Hill RJ, Schwarz H, Sternberg PW, Sudhaus W, Sommer RJ (1999) *Pristionchus pacificus*, a nematode with only three juvenile stages, displays major heterochronic changes relative to *C. elegans*. *Proc R Soc Lond B* 266: 1617–1621.

41. Félix M-A, De Ley P, Sommer RJ, Frisse L, Nadler SA, Thomas WK, et al. (2000) Evolution of vulva development in the Cephalobina (Nematoda). *Dev Biol* 221: 68–86. <https://doi.org/10.1006/dbio.2000.9665> PMID: 10772792
42. Félix M-A, Sternberg PW (1997) Two nested gonadal inductions of the vulva in nematodes. *Development* 124: 253–259. PMID: 9006085
43. Delattre M, Félix M-A (2001) Evolution and development of a variable left-right asymmetry in nematodes: the handedness of P11/P12 migration. *Dev Biol* 232: 362–371. <https://doi.org/10.1006/dbio.2001.0175> PMID: 11401398
44. Félix M-A (2004) Alternative morphs and plasticity of vulval development in a rhabditid nematode species. *Dev Genes Evol* 214: 55–63. <https://doi.org/10.1007/s00427-003-0376-y> PMID: 14730447
45. Kiontke K, Barrière A, Kolotuev I, Podbilewicz B, Sommer RJ, Fitch DHA, et al. (2007) Trends, stasis and drift in the evolution of nematode vulva development. *Curr Biol* 17: 1925–1937. <https://doi.org/10.1016/j.cub.2007.10.061> PMID: 18024125
46. Félix M-A, Vierstraete A, Vanfleteren J (2001) Three biological species related to *Rhabditis* (*Oscheius*) *pseudodolichura* Körner in Osche, 1952. *J Nematol* 33: 104–109. PMID: 19266004
47. Delattre M, Félix M-A (2001) Polymorphism and evolution of vulval precursor cell lineages within two nematode genera, *Caenorhabditis* and *Oscheius*. *Curr Biol* 11: 631–643. PMID: 11369226
48. Félix M-A, Sternberg PW (1996) Symmetry breakage in the development of one-armed gonads in nematodes. *Development* 122: 2129–2142. PMID: 8681794
49. Richaud A, Zhang G, Lee D, Lee J, Félix M-A (2018) The local co-existence pattern of selfing genotypes in *Caenorhabditis elegans* natural metapopulations. *Genetics* 208: 807–821. <https://doi.org/10.1534/genetics.117.300564> PMID: 29242287
50. Félix M-A (2006) “*Oscheius tipulae*.” In: WormBook (ed. The *C. elegans* Research Community), [<http://www.wormbook.org/>] <https://doi.org/10.1895/wormbook.1.119.1> PMID: 18050438
51. Fradin H, Kiontke K, Zegar C, Gutwein M, Lucas J, Kovtun M, et al. (2017) Genome architecture and evolution of a unichromosomal asexual nematode. *Curr Biol* 27: 2928–2939 e2926. <https://doi.org/10.1016/j.cub.2017.08.038> PMID: 28943090
52. Blaxter ML, De Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A, et al. (1998) A molecular evolutionary framework for the phylum Nematoda. *Nature* 392: 71–75. <https://doi.org/10.1038/32160> PMID: 9510248
53. Fanelli E, Troccoli A, Vovlas N, Scarcia G, Mincuzzi A, Sanzani SM, et al. (2017) Occurrence of *Sheraphelenchus sucus* (Nematoda: Aphelenchoidinae) and *Panagrellus* sp. (Rhabditida: Panagrolaimidae) associated with decaying pomegranate fruit in Italy. *J Nematol* 49: 418–426. PMID: 29353931
54. R Core Team (2015) R: A language and environment for statistical computing. In: Computing RFFS, editor. Vienna, Austria.
55. Slos D, Sudhaus W, Stevens L, Bert W, Blaxter M (2017) *Caenorhabditis monodelphis* n. sp.: defining the stem morphology and genomics of the genus *Caenorhabditis*. *BMC Zoology* 2: 4.
56. Ragsdale EJ, Kanzaki N, Röseler W, Herrmann M, Sommer RJ (2013) Three new species of *Pristionchus* (Nematoda: Diplogastridae) show morphological divergence through evolutionary intermediates of a novel feeding-structure polymorphism. *Zool J Linn Soc* 168: 671–698.
57. Goodey T (1943) On the systematic relationships of the vinegar eel-worm, *Turbatrix acetii*, and its congeners, with description of a new species. *J Helminth* 21: 1–9.
58. Goodey T (1945) A note on the subfamily Turbatricinae and the genus *Turbator* Goodey, 1943. *Journ Helminth* 21: 69–70.
59. Stock SP, Nadler SA (2006) Morphological and molecular characterization of *Panagrellus* spp. (Cephalobina: Panagrolaimidae): taxonomic status and phylogenetic relationships. *Nematology* 8: 921–938.
60. Abolafia J, Alizadeh M, Khakvar R (2016) Description of *Panagrellus ulmi* sp. n. (Rhabditida, Panagrolaimidae) from Iran, and comments on the species of the genus and its relatives. *Zootaxa* 4162: 245–267. <https://doi.org/10.11646/zootaxa.4162.2.3> PMID: 27615972
61. Camerota M, Mazza G, Carta LK, Paoli F, Torrini G, Benvenuti C, et al. (2016) Occurrence of *Panagrellus* (Rhabditida: Panagrolaimidae) nematodes in a morphologically aberrant adult specimen of *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae). *J Nematol* 48: 1–6. PMID: 27168645
62. Ivanova E, Perfilieva K, Spiridonov S (2018) *Panagrellus levitatus* sp. n. (Rhabditida: Panagrolaimidae), a nematode suppressing *Drosophila melanogaster* in laboratory cultures. *Nematology* 20: 285–297.
63. Baille D, Barrière A, Félix M-A (2008) *Oscheius tipulae*, a widespread hermaphroditic soil nematode, displays a higher genetic diversity and geographical structure than *Caenorhabditis elegans*. *Mol Ecol* 17: 1523–1534. <https://doi.org/10.1111/j.1365-294X.2008.03697.x> PMID: 18284567

64. Kanzaki N, Ragsdale EJ, Herrmann M, Roseler W, Sommer RJ (2013) Two new species of *Pristionchus* (Nematoda: Diplogasteridae) support the biogeographic importance of Japan for the evolution of the genus *Pristionchus* and the model system *P. pacificus*. *Zoolog Sci* 30: 680–692. <https://doi.org/10.2108/zsj.30.680> PMID: 23915163
65. Steiner G (1929) *Diplogaster entomophaga* n. sp., a new *Diplogaster* (Diplogasteridae, Nematoda) found on a *Pamphilius stellatus* (Christ) (Tenthredinidae, Hymenoptera). *Zool Anz* 80: 143–145.
66. Mayer WE, Herrmann M, Sommer RJ (2007) Phylogeny of the nematode genus *Pristionchus* and implications for biodiversity, biogeography and the evolution of hermaphroditism. *BMC Evol Biol* 7: 104. <https://doi.org/10.1186/1471-2148-7-104> PMID: 17605767
67. Hong RL, Sommer RJ (2006) Chemoattraction in *Pristionchus* nematodes and implications for insect recognition. *Curr Biol* 16: 2359–2365. <https://doi.org/10.1016/j.cub.2006.10.031> PMID: 17141618
68. Hong RL, Svatos A, Herrmann M, Sommer RJ (2008) Species-specific recognition of beetle cues by the nematode *Pristionchus maupasi*. *Evol Dev* 10: 273–279. <https://doi.org/10.1111/j.1525-142X.2008.00236.x> PMID: 18460089
69. Brown FD, D'Anna I, Sommer RJ (2011) Host-finding behaviour in the nematode *Pristionchus pacificus*. *Proc Biol Sci* 278: 3260–3269. <https://doi.org/10.1098/rspb.2011.0129> PMID: 21411455
70. Penkov S, Ogawa A, Schmidt U, Tate D, Zagorij V, Boland S, et al. (2014) A wax ester promotes collective host finding in the nematode *Pristionchus pacificus*. *Nat Chem Biol* 10: 281–285. <https://doi.org/10.1038/nchembio.1460> PMID: 24584102
71. Félix M-A, Braendle C (2010) The natural history of *Caenorhabditis elegans*. *Curr Biol* 20: R965–R969. <https://doi.org/10.1016/j.cub.2010.09.050> PMID: 21093785
72. Bento G, Ogawa A, Sommer RJ (2010) Co-option of the hormone-signalling module dafachronic acid-DAF-12 in nematode evolution. *Nature* 466: 494–497. <https://doi.org/10.1038/nature09164> PMID: 20592728
73. Serobyán V, Ragsdale EJ, Sommer RJ (2014) Adaptive value of a predatory mouth-form in a dimorphic nematode. *Proc Biol Sci* 281: 20141334. <https://doi.org/10.1098/rspb.2014.1334> PMID: 25080344
74. Liu Z, Kariya MJ, Chute CD, Pribadi AK, Leinwand SG, Tong A, et al. (2018) Predator-secreted sulfolipids induce defensive responses in *C. elegans*. *Nat Commun* 9: 1128. <https://doi.org/10.1038/s41467-018-03333-6> PMID: 29555902
75. Cook DE, Zdraljevic S, Tanny RE, Seo B, Riccardi DD, Noble LM, et al. (2016) The genetic basis of natural variation in *Caenorhabditis elegans* telomere length. *Genetics* 204: 371–383. <https://doi.org/10.1534/genetics.116.191148> PMID: 27449056
76. Hanski I (1991) North Temperate dung beetles. In: Hanski I, Cambefort Y, editors. *Dung beetle ecology*. Princeton: Princeton University Press. pp. 75–96.
77. Cambefort Y (1991) From saprophagy to coprophagy. In: Hanski I, Cambefort Y, editors. *Dung beetle ecology*. Princeton: Princeton University Press. pp. 22–35.
78. Facundo HY, Linn CEJ, Villano MG, Roelofs WL (1999) Emergence, mating and postmating behaviors of the Oriental Beetle (Coleoptera: Scarabaeidae). *J Insect Behavior* 12: 175–192.
79. Facundo HY, Villani MG, Linn CEJ, Roelofs WL (1999) Temporal and spatial distribution of the Oriental Beetle (Coleoptera: Scarabaeidae) in a golf course environment. *Environmental Entomology* 28: 14–21.
80. Choo HY, Lee DW, Park JW, Kaya HK, Smitley DR, Lee SM, et al. (2002) Life history and spatial distribution of Oriental Beetle (Coleoptera: Scarabaeidae) in golf courses in Korea. *Journal of Economic Entomology* 95: 72–80. <https://doi.org/10.1603/0022-0493-95.1.72> PMID: 11942767
81. Gill BD (1991) Dung beetles in Tropical American forests. In: Hanski I, Cambefort Y, editors. *Dung beetle ecology*. Princeton: Princeton University Press. pp. 211–229.
82. Lees E (1953) An investigation into the method of dispersal of *Panagrellus silusiae*, with particular reference to its desiccation resistance. *J Helminth* 27: 95–103.
83. Hieb WF, Dougherty EC (1966) Evidence for tricarboxylic acid cycle in *Panagrellus redivivus* and changes observed under varying conditions of culture. *Nematologica* 12: 93.
84. Hechler HC (1970) Reproduction, chromosome number, and postembryonic development of *Panagrellus redivivus* (Nematoda: Cephalobidae). *J Nematol* 2: 355–361. PMID: 19322325
85. Sternberg PW, Horvitz HR (1981) Gonadal cell lineages of the nematode *Panagrellus redivivus* and implications for evolution by the modification of cell lineage. *Dev Biol* 88: 147–166. PMID: 7286441
86. Sternberg PW, Horvitz HR (1982) Postembryonic nongonadal cell lineages of the nematode *Panagrellus redivivus*: Description and comparison with those of *Caenorhabditis elegans*. *Dev Biol* 93: 181–205. PMID: 7128930

87. Link CD, Graf-Whitsel J, Wood WB (1987) Isolation and characterization of a nematode transposable element from *Panagrellus redivivus*. *Proc Natl Acad Sci* 84: 5325–5329. PMID: [3037542](#)
88. Choe A, Chuman T, von Reuss SH, Dossey AT, Yim JJ, Ajredini R, et al. (2012) Sex-specific mating pheromones in the nematode *Panagrellus redivivus*. *Proc Natl Acad Sci U S A* 109: 20949–20954. <https://doi.org/10.1073/pnas.1218302109> PMID: [23213209](#)
89. Srinivasan J, Dillman AR, Macchietto MG, Heikkinen L, Lakso M, Fracchia KM, et al. (2013) The draft genome and transcriptome of *Panagrellus redivivus* are shaped by the harsh demands of a free-living lifestyle. *Genetics* 193: 1279–1295. <https://doi.org/10.1534/genetics.112.148809> PMID: [23410827](#)
90. Ferris H (2009) The beer mat nematode, *Panagrellus redivivus*: A study of the connectedness of scientific discovery. *J Nematode Morphol Syst* 12: 19–25.
91. Hechler HC (1971) Taxonomic notes on four species of *Panagrellus* Thorne (Nematoda: Cephalobidae). *J Nematol* 3: 227–237. PMID: [19322374](#)
92. Aubertot M (1925) Nématodes d'Alsace. Observations sur l'Anguillule de la bière (*Anguillula silusiae* de Man, 1914). *Bull Ass philom Als Lorr* 6: 333–342.
93. Rühm W (1956) Die Nematoden der Ipiden. *Parasitol Schriftenreihe* 6: 1–435.
94. Brunold E (1950) Über eine neue Nematodenart der Gattung *Anguillula* aus *Drosophila*-Nährböden. *Vierteljahrsschrift der Natur Gesellschaft in Zürich* 95: 148–150.
95. Brunold E (1954) Zur Morphologie, Biologie und Bakterienfreien Züchtung des Nematoden *Panagrellus zymosiphilus* Brunold 1950. *Z Morph u Ökol Tiere* 42: 373–420.
96. Smith MT, Shann C, Batenburg-van der Vegte WH, Schmitt R, Wehrli E, Roeijmans HJ, et al. (1992) *Botryozyma nematodophila* gen. nov., spec. nov. (Candidaceae). *Antonie Van Leeuwenhoek* 61: 277–284. PMID: [1497332](#)
97. Fürst von Lieven A (2005) The embryonic moult in diplogastrids (Nematoda)—homology of developmental stages and heterochrony as a prerequisite for morphological diversity. *Zool Anzeiger* 244: 79–81.