Differential phoretic host use among sympatric *Caenorhabditis* nematodes and an association with invasive nitidulid beetles in southwestern Germany

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Abstract

Little is known about the natural history of most *Caenorhabditis* nematodes, despite their importance as a model system in biology. While these nematodes rely on invertebrate hosts to disperse to new habitats, host use for most species has not been characterized. We surveyed the invertebrate community of a habitat containing three sympatric *Caenorhabditis* in southwestern Germany, determining differential and specific host use for each of these co-occurring species. We documented a specific association between *C.* sp. 8 and two species of invasive nitidulid beetles, and a particularly strong association of the nematode with the beetle *Stelidota geminata*, where we recovered more nematodes per individual beetle and a higher proportion of beetles carrying nematodes for *S. geminata* than for the co-occurring *Epuraea ocularis*. Our results provide evidence for group dispersal using beetles in *C.* sp. 8, supporting previous observations of collective dispersal behavior in this species, and establish a starting point for further dissecting the evolutionary and mechanistic causes and consequences of interactions between *Caenorhabditis* nematodes and their host species in ecologically relevant conditions.

Keywords

Phoresy, dauer, invasive species, interspecific interactions, coexistence, Coleoptera

Introduction

Free-living nematodes are some of the most abundant and diverse animals on Earth, and have reached their ubiquitous status despite their small size and limited modes of locomotion (Schratzberger *et al.* 2019). Many free-living nematodes achieve relatively wide distributions for their body size as the result of phoresy, hitchhiking to new habitats by temporarily attaching to a larger and more mobile interspecific host vector (Bartlow & Agosta 2021). Phoresy in nematodes is often facilitated by an ability to enter diapaused or quiescent states, during which they can endure starvation and resist environmental extremes over extended time periods, allowing them to safely attach to vectors and disperse over greater distances than possible by crawling alone (Vlaar *et al.* 2021). The specificity of the relationship between phoretic nematodes and vector species varies widely across taxa, and is likely an important driver of nematode diversification and ecological success (Giblin-Davis *et al.* 2013; Bubrig & Fierst 2021; Vlaar *et al.* 2021).

Caenorhabditis nematodes are a key resource used to fuel a wide range of biological discovery over the last century, particularly the laboratory model organism *C. elegans* (Evans 2006). Despite their importance, the study of ecology and evolution in natural *Caenorhabditis* populations has only recently received significant attention thanks to the discovery of the natural habitat of these nematodes in the 2010s (Kiontke *et al.* 2011; Cutter 2015; Frézal & Félix 2015). We now know that *Caenorhabditis* are microbivorous nematodes with a boom-bust life cycle on ephemeral, microbe-rich resource patches, mostly decaying plant material (Kiontke *et al.* 2011; Cutter 2015). In the presence of stressors (*e.g.* starvation, high population density), *Caenorhabditis* nematodes develop a stress-resistant dauer larval form that facilitates dispersal and colonization of new patches after the rapid exhaustion of microbial resources (Barrière & Félix 2007; Félix & Duveau 2012; Schulenburg & Félix 2017; Vlaar *et al.* 2021). Experiments have suggested that *Caenorhabditis* appear to be reliant on phoretic associations with invertebrate carriers to colonize new resource patches (Li *et al.* 2014; Sloat *et al.* 2022). Although phoretic dispersal is a key component of the life cycle and ecology of *Caenorhabditis*, interactions with potential dispersal vectors remain unknown for the vast majority of *Caenorhabditis* species.

Even with limited study, a variety of invertebrate species have been documented hosting *Caenorhabditis* nematodes, with some species found in specialized associations with particular invertebrate taxa and others appearing to be opportunistic and generalist. The best characterized interaction between *Caenorhabditis* and vector is that of *C. japonica* with the shield bug *Parastrachia japonensis,* where the nematode is found exclusively in association with this host throughout its life-cycle, and has not been found on other invertebrates despite examination of numerous co-occurring taxa (Yoshiga *et al.* 2013). Though not as well characterized as the relationship between *C. japonica* and its vector, *C. niphades* and *C. inopinata* also have specific relationships with particular insects (*Niphades variegatus* weevils and *Ceratosolen spp.* fig pollinating wasps, respectively; (Woodruff & Phillips 2018; Sun *et al.* 2022). Other *Caenorhabditis* may have similarly specific relationships with vector species or taxonomic groups, having been originally isolated and described in association with particular insects (Figure 1A), such as *C. drosophilae* with the fruit fly *Drosophila nigrospiracula* (Kiontke 1997), *C. angaria* with the weevils *Metamasius hemipterus* and *Rhynchophorus palmarum* (Sudhaus *et al.* 2011), *C. plicata* with carrion beetles (Volk 1950)*, C. auriculariae* with fungus-feeding *Platydema* beetles (Dayi *et al.* 2021), and *C. monodelphis* with the ciid beetle *Cis castaneus* (Slos *et al.* 2017). However, for these species there does not appear to have been systematic examination of other co-occurring organisms that could also act as vectors.

In contrast to the specific interactions mentioned above, several *Caenorhabditis* species in the Elegans species group (Figure 1A) have regularly been found in association with various species of gastropods, isopods, and/or myriapods, including *C. remanei*, *C. latens, C brenneri, C. sinica*, *C. briggsae*, and *C. elegans* (Fitch *et al.* 1994; Baird 1999; Wang *et al.* 2010; Dey *et al.* 2012; Ross *et al.* 2012; Félix *et al.* 2013; Petersen *et al.* 2015; Diano *et al.* 2022). Both *C. briggsae* and *C. elegans* have also been occasionally isolated from insects (Kiontke & Sudhaus 2006; Félix & Duveau 2012), and *C. elegans* dauers will attach to and disperse on various invertebrate groups, as well as inanimate materials, in laboratory experiments (Lee *et al.* 2011, 2017; Chiba *et al.* 2023; Williams *et al.* 2023; Perez *et al.* 2025). Whether these species are truly generalists with regards to phoretic vectors or these less-frequent observations represent rare opportunistic or accidental attachment to non-vector species is unclear (Schulenburg & Félix 2017; Petersen *et al.* 2023). One study surveyed the broader invertebrate communities alongside *Caenorhabditis*-hosting gastropods, isopods, and myriapods but did not observe any additional invertebrate associations with *Caenorhabditis* (Petersen *et al.* 2015). Despite these described cases, vector use has not been characterized in any detail for the majority of the >80 known *Caenorhabditis* species. As a consequence, it is unclear how sympatric *Caenorhabditis* species vary or overlap in vector use, which has been hypothesized as an important aspect of niche variation among otherwise seemingly similar species, influencing metacommunity dynamics and coexistence by governing spatiotemporal aspects of dispersal, patch colonization, and competition (Cutter 2015; Schulenburg & Félix 2017).

In southwestern Germany, three species of *Caenorhabditis* (*C. briggsae*, *C. remanei*, *C.* sp. 8) can be found co-occurring at small spatial scales, even being found together on the same rotting fruits (Perez *et al.* 2025). Repeated sampling of the orchard meadows around the University of Konstanz campus has revealed that *C.* sp. 8, a little-studied species in the Angaria group of *Caenorhabditis* nematodes (Figure 1A), is the most abundant species in the area across multiple years, and can be readily observed forming dauer towers, a collective behavior hypothesized to increase dispersal success (Penkov *et al.* 2014; Perez *et al.* 2025). However, it remains unknown what vector *C.* sp. 8 uses for dispersal, and whether they are actually capable of collective dispersal in nature. To determine whether these three *Caenorhabditis* species rely on the same or different invertebrate vectors for dispersal, we conducted a broad survey of invertebrates found around rotting fruits. Based on the results from this general survey, we conducted a more detailed survey of two locally abundant species of nitidulid beetles that have recently invaded the European continent to test if either species serves as a more frequent host for *C.* sp. 8. We also assessed the life stage, number of individuals, and sex ratio of *C.* sp. 8 on these two beetle species across multiple sites and across a three month period at a single site to further characterize *C.* sp. 8 host specificity and determine whether this species disperses in groups.

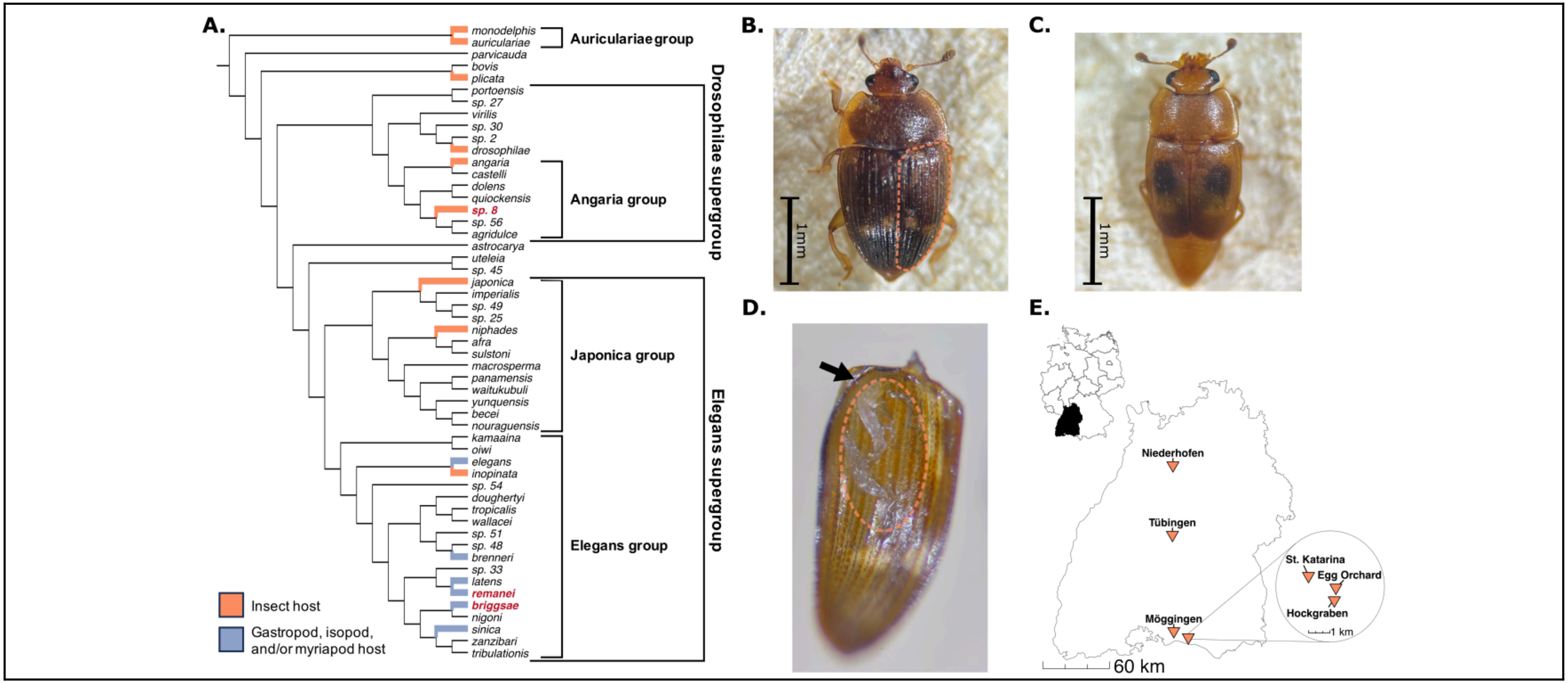

Figure 1. A. Phylogeny of *Caenorhabditis* nematodes, with the species groups and supergroups mentioned in this study indicated. Branches are colored by host taxonomic group for species with documented associations, and the three species included in this study are indicated by bold red text. Modified from (Fusca *et al.* 2025). **B.** Representative *Stelidota geminata*, with the right elytron outlined. **C.** Representative *Epuraea ocularis*. **D.** A cluster of >20 *C.* sp. 8 dauer larvae (circled area) attached to the inner surface of the right elytron dissected from *S. geminata*. The epipleuron is indicated by an arrow. **E.** Sampling locations in Baden-Württemberg, Germany where nitidulid beetles (*E. ocularis* and *S. geminata*) were collected. The inset map depicts sites around the University of Konstanz campus.

Materials and Methods

General invertebrate collection and nematode extraction

In our initial survey, we opportunistically collected invertebrates found on and around rotting fruits in orchard meadows near the University of Konstanz campus in Baden-Württemberg, Germany during the summer and autumn of 2025 (Table 1). Gastropods and large arthropods were individually collected into Whirl-pak bags or 2 mL microcentrifuge tubes, while smaller arthropods (*e.g.* dipterans, coleopterans, hymenopterans) were collected using an aspirator and deposited into 2 mL microcentrifuge tubes. Invertebrates were brought back to the lab within two hours for processing. Invertebrates were individually added to either 6 or 9 cm petri dishes containing nematode growth media (NGM) seeded with a 200 µL spot of *E. coli* OP50 and monitored for 48 hours to determine if any nematodes emerged onto the bacterial lawn. Emerged nematodes were identified to the genus or family level based on morphology (*e.g.* pharynx, reproductive structures, cuticle; (Barrière & Félix 2006), and *Caenorhabditis* were identified to the species level based on morphology, behavior, reproductive mode, and mating tests with isolates of known species when necessary (see below). The identity of *Caenorhabditis* that emerged over the 48 hour period was recorded for each sample.

Identification of Caenorhabditis *species*

Based on previous intense sampling and molecular barcoding of the nematode community from fruits in this area (Perez *et al.* 2025), we could identify *Caenorhabditis* species as either *C. remanei*, *C. briggsae*, or *C.* sp. 8, which can be relatively easily distinguished from each other based on established diagnostic criteria visible under a dissecting microscope, with additional morphological and behavioral distinctions we have observed during processing many samples from this region. In particular, *C.* sp. 8 are comparatively short and “dumpy” compared to the other two species, gonochoristic, and unique among local *Caenorhabditis* for their spiral mating behavior, as well as distinct male spicule and bursa morphology (Kiontke *et al.* 2011). Both *C. remanei* and *C. briggsae* have a slimmer body aspect ratio than *C.* sp. 8, but differ from each other in reproductive mode; *C. briggsae* is a self-fertilizing hermaphroditic species, producing males very rarely, while *C. remanei* is gonochoristic and produces many males (Ting *et al.* 2014). In addition, local *C. remanei* strains tend to form large aggregations on bacterial lawns and readily burrow into the NGM compared to the other two species, while *C. briggsae* forage individually and tend to remain on the agar surface in the presence of food. Any uncertain species identifications were confirmed with mating tests, where isolated males or offspring generated from isolated mothers were individually plated with the opposite sex of known species identity and assessed for reproduction, as each of these species is incapable of interbreeding with the other two (Greenway *unpublished data*, Ting *et al.* 2014). In the case of putative hermaphrodites, virgin worms were plated individually and assessed for reproduction over 72 hours.

Nitidulid beetle dissection and quantitative assessment of Caenorhabditis *occupancy*

Once we had identified beetles in the family Nitidulidae as the only invertebrates associated with *C.* sp. 8, we conducted more intense sampling of *Stelidota geminata* and *Epuraea ocularis*, two nitidulid species frequently observed and collected on rotting fruits in our prior sampling. A chance observation of an individual *S. geminata* with a particularly heavy nematode load provided evidence that *Caenorhabditis* can aggregate underneath the beetles' elytra. To determine the species, life stage, number of individuals, sex ratio, and locations of nematodes on both beetle species, we performed dissections of adult *E. ocularis* and *S. geminata* collected from 6 locations in Baden-Württemberg, Germany (Table 2). Beetles were collected from rotting fruits using an aspirator, individually placed in 2 mL microcentrifuge tubes, and stored on ice for a minimum of one hour prior to sacrifice by removing the head from the thorax. The beetles were dissected under a stereomicroscope on a 6 cm petri dish containing NGM seeded with 200 µL *E. coli* OP50. We first examined the external surface of the beetle for the presence of nematodes before removing both elytra and transferring them together to a new NGM petri dish, leaving the rest of the corpse on the original dish. We then examined the internal surface of the elytra and the exposed abdomen separately, washing 20 µL of M9 buffer over each sample to loosen attached nematodes, at which point the life stage (larvae, dauer larvae, or adult) of visible nematodes was recorded. After 24 hours, the species, total number, and, for a subset of samples, the proportion of males present in the detached nematode population was separately recorded for the elytra and corpse of each beetle. The proportion of males on the beetle was not able to be determined in all cases, for example when some individuals remained as dauers for several days while others exited dauer rapidly and began to reproduce within 24 hours, resulting in mixed generation larval populations. We further monitored the corpse of each beetle for an additional 48 hours (72 hours total) to qualitatively determine the presence of additional nematode species that emerged following beetle death. To assess seasonal patterns of nematode occupancy and abundance on the beetles, we also collected each beetle species from a single location near the University of Konstanz (Egg Orchard; Figure 1E) two to three times per month from September 2025 to November 2025. Beetles were no longer found on or around fruits after the first freeze in mid-November.

Statistics

We analyzed all data in R v4.3.2 (R Core Team 2023). To test whether the proportion of individuals carrying *C.* sp. 8 differs across beetle species and sites, we used a generalized linear model (GLM) as implemented in the stats package (R Core Team 2023), with presence/absence of *C.* sp. 8 as a binomial response variable and a logit link function, including beetle species and site as factors. To determine whether the number of extracted *C.* sp. 8 individuals differed between the sampled body parts of the two beetle species across sites, we used a generalized linear mixed model (GLMM) assuming a negative binomial response variable (nematode counts) and log link function with the glmmTMB package (Brooks et al. 2017), using beetle species, body part, and site as factors, and the interaction between species and body part. We also included a random effect of the individual ID nested within site to account for non-independence among body parts from the same individual. To test if the sex ratio of *C.* sp. 8 individuals on beetles differed between species or sites, we modeled the proportion of male nematodes to total nematodes per beetle using a binomial GLM with a logit link function in the *stats* package, including beetle species and site as factors. The interaction between species and site was non-significant for the GLMs investigating differences in the number of beetles carrying *C.* sp. 8 and sex ratio differences, so were excluded from the final models. For these among site analyses, beetles collected from the Egg Orchard site were subsampled to better match the collection dates and sample sizes from other sites, including only individuals collected from September 15 to November 1, 2025. Additionally, the Niederhofen site was excluded from all analyses due to the low number of beetles sampled from this location.

We also used GLMs to test whether the number of individual beetles hosting *C.* sp. 8 and the number of *C.* sp. 8 individuals extracted from the two beetle species differed over time at the Egg Orchard site. We used a GLM modeling *C.* sp. 8 presence/absence on each beetle as a binomial response variable and a logit link function with the *stats* package, including beetle species as a factor and sampling date as a number (binning samples collected within 5 days of each other). We used a GLM with *C.* sp. 8 counts per beetle modeled as a negative binomial response with a log link function in the glmmTMB package, including beetle species and sampling date as factors. The non-significant interaction between species and date was excluded from both models.

Results

General invertebrate survey for Caenorhabditis

We first surveyed a broad sample of invertebrates found on and around rotting fruits near the University of Konstanz, noting the presence or absence of the three *Caenorhabditis* species on each individual to determine which taxa serve as vectors for *Caenorhabditis* nematodes in southern Baden-Württemberg. In total, 478 individual invertebrates from various taxonomic groups were included in this general survey (Table 1). Confirming the results of previous studies, *C. briggsae* and *C. remanei* were both found on gastropods, and *C. remanei* on isopods. *C. briggsae* was isolated from two slugs (3.1% of gastropods), while *C. remanei* was isolated from four slugs (6.2% of gastropods) and 9 isopods (10.2% of isopods). The two species were never found occurring on the same vector individual in this survey. While we did not note the exact species of gastropods and isopods, most isopods belonged to the genera *Porcelio* or *Armadillidum*, and almost all gastropods were *Arion* slugs. In contrast, *C.* sp. 8 was never found on gastropods or isopods, but instead was only found in association with beetles in the family Nitidulidae, being isolated from 9 nitidulid beetles (4.6% of all nitidulids) in the general survey. This, however, represents an underestimation of the total occupancy rate of *C.* sp. 8 on nitidulids based on closer inspection of these beetles (see detailed survey below) and laboratory observations. We found *C.* sp. 8 occurring in several independent nitidulid breeding cultures started from beetles that were deemed “nematode free” after initial screening on agar plates. Several days after placing these beetles on sterilized apples, many of the cultures were covered in *C.* sp. 8 nematodes, indicating that *C.* sp. 8 may be reluctant to disembark hosts when presented only with *E. coli* OP50 as a food source. In light of this, we conducted closer inspection of isopods and gastropods to confirm that these taxa did not also host *C.* sp. 8 that were reluctant to depart onto the provided media. A subset of the isopods and gastropods from the general survey (*n* = 34 and 15, respectively) were killed with a scalpel and washed in water, plated on NGM petri dishes with the washing liquid, and observed for approximately one week. We did not find any additional *Caenorhabditis* emerging from these individuals, confirming the absence of *C.* sp. 8 on these taxa. Additionally, *C. briggsae* and *C. remanei* were never found on nitidulid beetles, and no *Caenorhabditis* species were found from any other invertebrate group in the survey (Table 1).

Table 1. Number and percentage of individuals from each invertebrate group from which *Caenorhabditis* species were isolated during an initial general survey of orchard meadows around the University of Konstanz.

Invertebrate Group	**No. Sampled Individuals**	***C. briggsae***	***C. remanei***	***C.* sp. 8**
Annelida	3	0	0	0
Arachnida	2	0	0	0
Blattodea	1	0	0	0
Coleoptera (Nitidulidae)	196	0	0	9 (4.6%)*
Coleoptera (Other)	14	0	0	0
Collembola	21	0	0	0
Diptera	80	0	0	0
Gastropoda	65	2 (3.1%)	4 (6.2%)	0
Hemiptera	1	0	0	0
Hymenoptera	3	0	0	0
Isopoda	88	0	9 (10.2%)	0
Myriapoda	4	0	0	0

*Actual occupancy rate of *C.* sp. 8 on nitidulids is higher, see main text and Table 2

Quantitative assessment of Caenorhabditis *sp. 8 from nitidulid beetles*

As an association between *Caenorhabditis* and nitidulids has not been previously described, we further studied this relationship by conducting additional sampling of *Epuraea ocularis* and *Stelidota geminata* (Figure 1B and C), the two nitidulid beetles most often encountered in our study area, for quantitative characterization of *C.* sp. 8 from these beetles. We dissected an additional 177 *E. ocularis* and 197 *S. geminata* collected between September 15 and November 1, 2025 across six sites in Baden-Württemberg (Figure 1E; Table 2) to determine the lifestage, prevalence, and sex ratio of *C.* sp. 8 on individual beetles, as well as where on the beetles the nematodes could be found. We found that the abundance of *C.* sp. 8 significantly differed among body parts, beetle species, and sites. Inspection of both the elytra and remainder of the beetle corpse revealed that only dauer larvae were found on the beetles (Figure 1D). The number of *C.* sp. 8 observed was higher on the elytra than on the rest of the beetle body ($\beta = 2.31 \pm 0.33$, $z = 7.10$, $p < 0.001$), with *S. geminata* having significantly higher abundances than *E. ocularis* on both the elytra and body (interaction $\beta = 0.97 \pm 0.40$, $z = 2.40$, $p = 0.016$; species $\beta = 1.14 \pm 0.37$, $z = 3.07$, $p = 0.002$; Table 2). Dauers were mostly found on the anterior portion of the elytra, stuck together into clusters in or near a cavity created by the epipleura (the portion of the elytron that folds back towards the abdomen (Goczał & Beutel 2023); Figure 1D, Figure 3), and were largely motionless until prodded with a wire pick or wetted with M9 solution, at which point they began to wriggle and detach from each other. Despite concentration on the elytra, low numbers of *C.* sp. 8 dauers were also found having emerged from the rest of the beetle body in some cases (Table 2). The number of *C.* sp. 8 observed on beetles was significantly lower at Möggingen ($\beta = -1.22 \pm 0.34$, $z = -3.55$, $p < 0.001$) and Tübingen ($\beta = -0.99 \pm 0.35$, $z = -2.84$, $p = 0.004$) compared to the Egg Orchard site (Table 2).

We also examined the sex ratio of *C.* sp. 8 individuals emerged from each beetle (combined across body parts), finding that the proportion of males on each beetle was significantly lower from an expected 1:1 ratio (Huang *et al.* 2023) for *E. ocularis* (Intercept $\beta = -0.68 \pm 0.23$, $z = -2.93$, $p = 0.003$) and was significantly different between species, closer to an even 1:1 for *S. geminata* ($\beta = 0.53 \pm 0.22$, $z = 2.46$, $p = 0.014$; Table 2), but did not differ among sites. Differences in the sex ratio of attached nematodes between the two species are likely driven by the low number of nematodes recovered per beetle for *E. ocularis* (Table 2).

In addition to carrying significantly more dauers per beetle, a higher proportion of *S. geminata* individuals were carrying *C.* sp. 8 dauers than were *E. ocularis* ($\beta = 1.57 \pm$

0.24, z = 6.59, $p < 0.001$), with *C.* sp. 8 found on 68.5% of *S. geminata* individuals compared to 35.6% of *E. ocularis* across all sites (Table 2). However, there were also differences in the proportion of all beetle individuals carrying *C.* sp. 8 dauers among sites, with lower overall rates of nematode recovery in both Möggingen (β = -1.03 ± 0.33, z = -3.09, p = 0.002) and Tübingen (β = -1.20 ± 0.35, z = -3.40, $p < 0.001$) compared to the Egg Orchard site (Table 2). As these sites were only sampled a single time later in the season, it is impossible to distinguish if the lower dauer abundance and proportion of beetle hosts is the result of site-specific or temporal variation in this interspecific interaction.

Temporal variation in nematode-beetle interactions

We examined temporal variation in the number of beetles hosting *C.* sp. 8 and the number of individual nematodes per beetle at a single site (Egg Orchard, Konstanz; 105 *E. ocularis*, 123 *S. geminata*) from September to November 2025 (Figure 2), spanning the season when *C.* sp. 8 is abundant and active in late summer until its disappearance in mid-autumn. Dauers of *C.* sp. 8 were found on a significantly higher number of *S. geminata* than *E. ocularis* over the entire period (β = 1.92 ± 0.31, z =6.25, $p < 0.001$), though the overall proportion of nitidulids hosting *C.* sp. 8 decreased over time (β = -0.43 ± 0.16, z =-2.73, p = 0.006). Likewise, *S. geminata* hosted significantly more *C.* sp. 8 dauers over the whole season than did *E. ocularis* (β = 2.13 ± 0.24, z =8.97, $p < 0.001$), and the number of dauers on both species decreased over time (β = -0.50 ± 0.14, z =-3.71, $p < 0.001$). Though this survey misses early season and over-winter dynamics, we recover a consistent pattern of higher *C.* sp. 8 dauer occupancy on *S. geminata* than on *E. ocularis* across time, and expect a qualitatively similar pattern exists across the year, particularly since nitidulid beetles overwinter as adults.

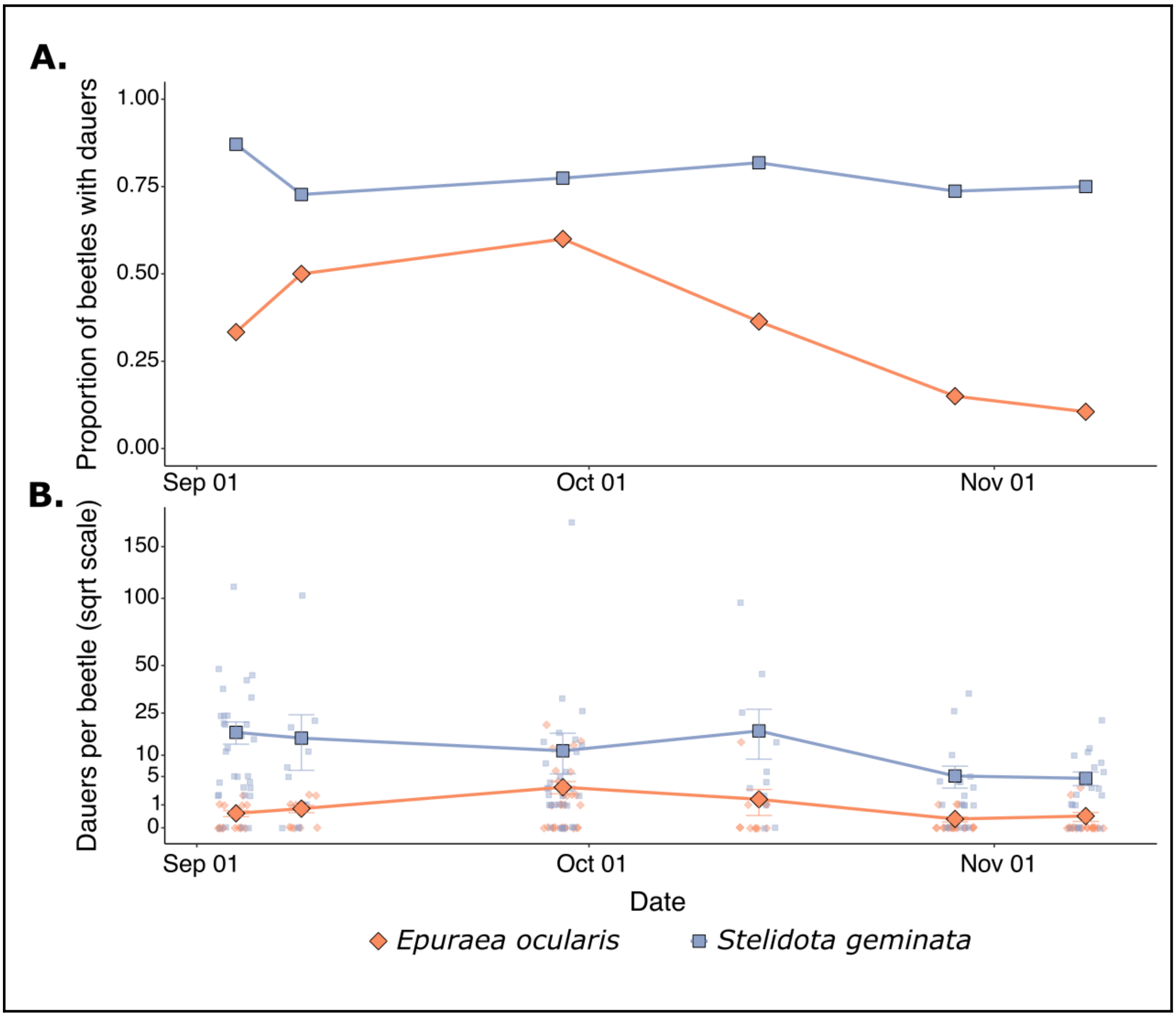

Figure 2. Temporal variation in the proportion of beetles hosting *C.* sp. 8 (**A**) and the number of *C.* sp. 8 dauer larvae extracted per beetle (square root scaled; **B**) for two nitidulid beetle species. In panel **B**, large points represent the mean number of nematodes per beetle for each species with error bars depicting the standard error, small points represent the number of nematodes extracted from individual beetles.

Other nematodes isolated from nitidulid beetles

While *C.* sp. 8 was the nematode species most often observed in our survey of these two nitidulid species, several additional nematode species were encountered on occasion. The next most frequently recovered was *Sheraphelenchus* (likely *S. sucus* based on frequent occurrence on fruits in the area [Perez *et al.* 2025]), a mycetophagous nematode in the family Aphelenchoididae (Kanzaki & Tanaka 2013). *Sheraphelenchus* were found on 62 *E. ocularis* (28.1% of sampled beetles) and 14 *S. geminata* (5.4% of sampled beetles), co-occurring with *C.* sp. 8 on 15 and 9 individual beetles, respectively. As seen with *C.* sp. 8, *Sheraphelenchus* were also frequently observed clustered into large groups on the underside of the elytra of *E. ocularis* (Figure 3). In addition to these commonly observed species, we also recovered a hermaphroditic *Pristionchus* species from 1 *E. ocularis* (0.45% of sampled beetles) and 2 *S. geminata* (0.77% of sampled beetles), and a hermaphroditic Panagrolaimid from 2 *E. ocularis* individuals (0.90% of sampled beetles), all co-occurring on beetles with *C.* sp. 8 with the exception of one of the Panagrolaimids.

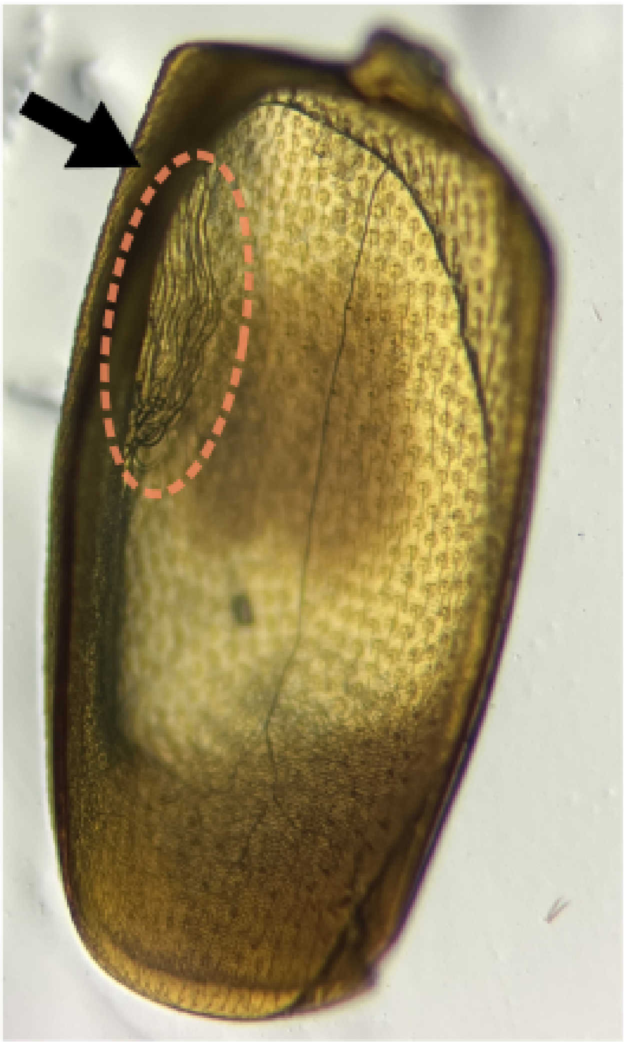

Figure 3. A cluster of >30 *Sheraphelenchus* larvae (circled area) attached to the inner surface of the right elytron dissected from an individual *E. ocularis*. The epipleuron is indicated by an arrow.

Table 2. Among site variation in the number of beetles hosting *C.* sp. 8 dauers (“No. positive”), the mean number of nematodes per beetle, the mean number of nematodes on the elytra and body, and the mean sex ratio of nematodes per beetle (calculated as the proportion of males in the sample). The same values are provided for the whole dataset across sites in the last two rows.

Site	Species	No. beetles	No. positive	Dauers/beetle Mean ± SE	Dauers on elytra Mean ± SE	Dauers on body Mean ± SE	No. beetles (Sex Ratio)	Sex Ratio Mean ± SE
Egg Orchard	*Epuraea ocularis*	61	25 (41.0%)	1.87 ± 0.53	1.84 ± 0.51	0.03 ± 0.02	13	0.34 ± 0.13
	Stelidota geminata	61	47 (77.0%)	10.52 ± 3.37	9.62 ± 2.85	0.90 ± 0.58	30	0.47 ± 0.06
Hockgraben	*Epuraea ocularis*	30	10 (33.3%)	1.37 ± 0.60	0.77 ± 0.23	0.60 ± 0.47	9	0.38 ± 0.14
	Stelidota geminata	30	24 (80.0%)	10.83 ± 2.68	9.23 ± 2.45	1.60 ± 0.72	22	0.52 ± 0.05
Möggingen	*Epuraea ocularis*	35	8 (22.9%)	0.51 ± 0.22	0.46 ± 0.21	0.06 ± 0.04	8	0.17 ± 0.09
	Stelidota geminata	35	18 (51.4%)	2.34 ± 0.65	2.29 ± 0.65	0.06 ± 0.06	17	0.54 ± 0.08
Niederhofen[‡]	*Epuraea ocularis*	1	1 (100.0%)	1.00				
	Stelidota geminata	3	3 (100.0%)	7.67 ± 5.24				
St. Katarina	*Epuraea ocularis*	30	16 (53.3%)	2.00 ± 0.59	1.70 ± 0.57	0.30 ± 0.09	12	0.49 ± 0.14
	Stelidota geminata	29	23 (79.3%)	8.24 ± 1.91	7.59 ± 1.76	0.66 ± 0.38	13	0.48 ± 0.12
Tübingen	*Epuraea ocularis*	20	3 (15.0%)	0.35 ± 0.22	0.35 ± 0.22	0	3	0.42 ± 0.30
	Stelidota geminata	39	20 (51.3%)	4.56 ± 1.10	4.56 ± 1.10	0	20	0.54 ± 0.05
All Sites[‡]	*Epuraea ocularis*	177	63 (35.6%)	1.36 ± 0.24	1.19 ± 0.22	0.18 ± 0.08	45	0.36 ± 0.06
	Stelidota geminata	197	135 (68.5%)	7.56 ± 1.20	6.92 ± 1.05	0.64 ± 0.22	102	0.51 ± 0.03

[‡] Beetles from Niederhofen were excluded from analysis due to low sample sizes
SE = standard error

Discussion

A better understanding of the relationships between *Caenorhabditis* nematodes and their phoretic vectors is necessary for characterizing the factors shaping the ecology and evolution of these model organisms. However, studies of variation in vector use among sympatric *Caenorhabditis* species, as well as basic characterization of vector use for most *Caenorhabditis* species, have remained limited. In this study, we quantified associations between three sympatric *Caenorhabditis* species and a variety of invertebrate taxonomic groups, finding variation in vector use among species, as well as a novel and specific vector use for *C.* sp. 8.

Variation in vector use among sympatric Caenorhabditis *species*

Despite all three *Caenorhabditis* species co-occurring at small spatial scales, even on the same rotting fruits (Perez *et al.* 2025), we found variation in vector use among species that may have implications for metacommunity dynamics among these likely competitors. In our general invertebrate survey of 478 invertebrates from the orchard meadows near the University of Konstanz, we found vector associations between *C. briggsae* and *C. remanei* with gastropods, as well as *C. remanei* with isopods, but none of the other surveyed taxa, consistent with vector associations previously reported for these species (Fitch *et al.* 1994; Baird 1999; Félix & Duveau 2012; Petersen *et al.* 2015; Ragsdale *et al.* 2022). *Caenorhabditis* sp. 8 was never recovered from gastropods or isopods, instead appearing to have specific associations with nitidulid beetles, and a particularly strong association with *S. geminata*. Interestingly, the recovery rates of the three nematode species from different hosts corresponds with the relative abundance of the nematode species in the sampled area (Perez *et al.* 2025). The rare *C. briggsae* was recovered from the lowest number of individual invertebrates (2 slugs, 3.1% of gastropods) and the more common *C. remanei* from relatively more individuals (4 slugs, 6.2% of gastropods; 9 isopods, 10.2% of all isopods), while the abundant *C.* sp. 8 was found on ~75% of the *S. geminata* and ~35% of the *E. ocularis* individuals in our detailed beetle survey of the same area (Egg Orchard). These vector use rates may simply reflect differences in the abundance of each species in the environment driven by other ecological factors. However, an intriguing hypothesis is that differences in local abundance and patch occupancy of *Caenorhabditis* species may be influenced by either the dispersal propensity of each species and/or the ability of the different vectors to shuttle nematodes to new resource patches (Cutter 2015), with slow moving terrestrial invertebrates limiting dispersal and highly mobile flying beetles rapidly covering larger distances. For *C. briggsae* and *C. remanei* the occurrence rates documented in our survey roughly correspond with a previous survey of vector-nematode associations from northern Germany (Petersen *et al.* 2015), perhaps indicating intrinsic differences in these species' propensity or ability for dispersal on invertebrates. Likewise, the high occupancy of *C.* sp. 8 on nitidulids that we recorded across multiple sites in Baden-Württemberg may indicate a higher propensity for dispersal in this species compared with the other two. Further work is necessary to determine the relationship between vector species use, patch colonization ability, and local abundance.

Our findings also provide evidence that *Caenorhabditis*-invertebrate interactions are governed by mechanisms facilitating host specificity across the genus. If Elegans group nematodes were truly vector generalists, we would expect to at least occasionally recover either species from the extremely common nitidulid beetles found on most fruits in the study area. However, neither *C. briggsae* nor *C. remanei* was found on any of the >450 nitidulid beetles sampled in this study. Likewise, *C.* sp. 8 was not recovered from any gastropods or isopods, despite frequent occurrence of these invertebrates on and around fruit containing *C.* sp. 8 dauers. It cannot be entirely ruled out that other beetle or insect species could act as hosts for *C*. sp. 8, particularly given the low sample size for some taxonomic groups; however, given occurrence rates of the sampled invertebrates on and around rotting fruit in our study area, we are confident that nitidulid beetles are the most often used hosts in this area. Laboratory experiments dissecting host preference for different invertebrate species among these *Caenorhabditis* could provide additional insight into the true degree of host specificity for each species and the mechanisms governing how they are able to differentiate among co-occurring vectors. Preference for host species has been experimentally demonstrated for the highly specialized *C. japonica*, mediated by chemotactic attraction to host cues (Okumura *et al.* 2013; Okumura & Yoshiga 2014), while experiments with *C. elegans* have failed to find any evidence of attraction to the chemical profiles of known host species (Archer *et al.* 2020; Petersen *et al.* 2023). Determining how chemosensory or other cues aid in vector detection and response will be of great value for better understanding the ecology, genetics, and evolution of *Caenorhabditis* nematodes. It has been hypothesized that Elegans group nematodes' host use, seemingly reliant on gastropods, isopods, and myriapods, may be governed by humidity, either controlling their ability to physically attach to hosts or due to physiological limits of desiccation during transit (Petersen *et al.* 2015). In line with this hypothesis, only Elegans group species are associated with moisture-dependent terrestrial invertebrates (Figure 1A). On the other hand, the known vectors of all characterized *Caenorhabditis* species from the basal Auriculariae group, Drosophilae supergroup, and Japonica group are insects (Figure 1A), seemingly with some amount of species-specificity. Even within the Elegans group, only the early diverging *C. inopinata* has been found in association with insects (Kanzaki *et al.* 2018; Woodruff & Phillips 2018). This pattern of host use across the *Caenorhabditis* phylogeny may point to a dependence on moist hosts, and potentially host generality, being a derived character in Elegans group species, in contrast to a hypothesized vector generalist ancestral state (Li *et al.* 2014; Cutter 2015). However, it remains difficult to assess support for this hypothesis due to a lack of characterized vector associations and information on dauer desiccation tolerance for most *Caenorhabditis* species (Figure 1A). Our finding that *C.* sp. 8 is specifically associated with nitidulid beetles adds another data point for insect association in the Drosophilae sugergroup, yet additional characterization of the natural history of Drosophilae supergroup and Japonica group species will be required to accurately reconstruct the evolutionary history of vector use in the genus.

C. *sp. 8 dispersal on nitidulid beetles*

Detailed analysis of the association between *C.* sp. 8 and the two nitidulid beetles in our study area highlighted that *C.* sp. 8 dauers frequently disperse in mixed sex groups on the underside of beetles' elytra, supporting our earlier finding that *C.* sp. 8 can form collective dauer towers for group dispersal (Perez *et al.* 2025). Several beetle-dispersed nematodes form similar clumps or clusters of individual worms on the inner surfaces of the elytra (Penas *et al.* 2006; Shimizu *et al.* 2013; Kanzaki *et al.* 2019; Polyanina *et al.* 2019; Goczał & Beutel 2023). The exact function of these elytral clusters remains unclear, as does whether they are the direct result of towering behaviors or can form once multiple nematodes have individually attached to the vector. We have previously observed *C.* sp. 8 dauer towers reacting to and briefly attaching to fruit flies in the laboratory (Perez *et al.* 2025); however, it remains to be tested how dauer towers directly interact with nitidulids, the apparent hosts of this species. Given the coordinated locomotion of dauers in the tower structure, it is plausible that the elytra clumps we observe here are analogous to the towers observed on rotting fruits, having collectively migrated underneath the elytra once attached to the beetle. Regardless of the origin, clustering together may increase individual nematodes' chances of surviving dispersal, as has been proposed for *Bursaphelenchus*, where only a few individuals located towards the sheltered inner part of the cluster survive and dehydrated nematodes on the surface form a protective sheath (Shimizu *et al.* 2013). Additionally, dispersing in groups may have evolved in sexually reproducing (gonochoristic) nematodes as means to increase the likelihood of successfully reproducing upon arrival to a new habitat patch (Cote *et al.* 2017). Our recovery of individual dauers and groups from each beetle species indicates *C.* sp. 8 is able to initiate dispersal on nitidulid beetles both as individuals and in groups, providing an excellent system for disentangling the benefits and costs of collective versus individual dispersal.

In addition to dispersing with members of the same species, we also observed co-occurrence of more than one nematode species on the same beetle. As the interactions between *C.* sp. 8 and co-occurring nematodes are yet unknown, it is unclear what the causes or consequences of multi-species phoresy may be. *Sheraphelenchus* and *Caenorhabditis* likely are not competing for resources, either feeding on fungal mycelia or free-living microbes, respectively. On the other hand, *Pristionchus* nematodes can be predatory, feeding on the larvae of other nematode species (Hiramatsu & Lightfoot 2023; Sommer 2025), and may take advantage of dispersing with other species in order to secure a meal upon arrival to a new habitat patch. Testing whether there are benefits to dispersing in mixed-species groups versus alone for any of these species would help shed light on whether co-occurrence is simply due to chance or if one species may seek out or avoid beetles hosting the others (Gupta & Borges 2021). Likewise, it remains to be seen if *C.* sp. 8 attachment and dispersal on nitidulid beetles may influence the fitness of the beetles. While often assumed to be harmless commensals, phoretic nematodes can have significant effects on their hosts, with evidence for both positive (Ledón-Rettig *et al.* 2018) and negative (Gupta & Borges 2019; Wang & Rozen 2019) effects on host fitness. Determining whether and how *C.* sp. 8 influences the ability of nitidulid beetles to disperse and reproduce may shed light on the evolution of the host-nematode interaction and of dispersal behavior in the nematodes.

Our results support an association between *C.* sp. 8 and nitidulid beetles, most strongly and consistently with *S. geminata* across sampling sites and time. Both nitidulid species we found hosting *C.* sp. 8 are also pest species that have recently invaded the European continent and spread rapidly since the 2000s (Konzelmann 2001; Callot 2007; Jelínek *et al.* 2016; Bibin 2017; Stan 2019; Grujić *et al.* 2020; Lasoń *et al.* 2023). It is an open question whether one of these species is the natural host of *C.* sp. 8, with which it has expanded its range to Europe by hitchhiking together, or instead both of these beetle species are novel hosts *C.* sp. 8 has recently begun to use. Several lines of evidence point towards the hypothesis that *C.* sp. 8 is also an invasive species. Despite heavy sampling for *Caenorhabditis* nematodes on the European continent relative to other areas of the globe (Cutter 2015), *C.* sp. 8 was first isolated and described from sites across North America in the 2000s (Kiontke *et al.* 2011), and has only been documented in Europe since the 2010s (World Wide Worms [justbio.com/tools/worldwideworms/]: accessed 15.11.2025). North America is also the native of range of *S. geminata* (Weber & Connell 1975), where it overlaps with the few initial collections of *C.* sp. 8 (Figure 4). *E. ocularis*, on the other hand, is native to Southeast Asia and the western Pacific (Cline & Audisio 2011; Rittner & Nir 2013). Furthermore, the closest relatives of *C.* sp. 8 and all known species in the Angaria group of *Caenorhabditis* nematodes occur only in the Americas (Kiontke *et al.* 2011; Sudhaus *et al.* 2011; Ferrari *et al.* 2017; Stevens *et al.* 2019; Sloat *et al.* 2022). The exception from this group is *C.* sp. 8, which has a wide and overlapping distribution with the invasive range of *S. geminata* in Europe (Figure 4). Taken together with the strong association we detected between *S. geminata* and *C.* sp. 8, this may indicate that *C.* sp. 8 is in fact a recent colonizer of Europe as a result of the expansion of its natural beetle host. Co-invasion on insect hosts has been documented for other phoretic nematodes (D'Anna & Sommer 2011), sometimes with severe ecological and economic consequences (Giblin-Davis *et al.* 2013). Currently, the poor sampling of *C.* sp. 8 in both North America and Europe, as well as a lack of ecological information for this species from North America, hinders further study of the history of this interaction.

Additional support for the invasion hypothesis will require documentation of a similarly specific phoretic interaction in the native range of *S. geminata*, as well as phylogeographic analysis of *C.* sp. 8 from populations originating from the putative native and introduced range. If *C.* sp. 8 is an invasive species in Europe, this system would provide an excellent opportunity for studying the ecological and evolutionary consequences of range expansion in *Caenorhabditis* nematodes, such as how native *Caenorhabditis* species respond to a novel competitor.

Conclusion

Invertebrate vector associations for *Caenorhabditis* nematodes in southwestern Germany correspond with current understanding of vector use in the genus. *C. briggsae* and *C. remanei* associate with moisture-dependent, terrestrial invertebrates, though the mechanisms governing this association remain unknown. The sympatric *C.* sp. 8, however, exclusively uses nitidulid beetles, exhibiting a strong association with *S. geminata* across both time and space. Whether dauers dispersing on these beetles gain any benefits from group dispersal, or have any consequences for their hosts are additionally unknown and represent fruitful avenues of future study. Further characterization of the association between *C.* sp. 8 and these invasive nitidulid species in Europe and the native ranges of the beetles will provide a better understanding of the ecology and evolution of this little-studied nematode species.

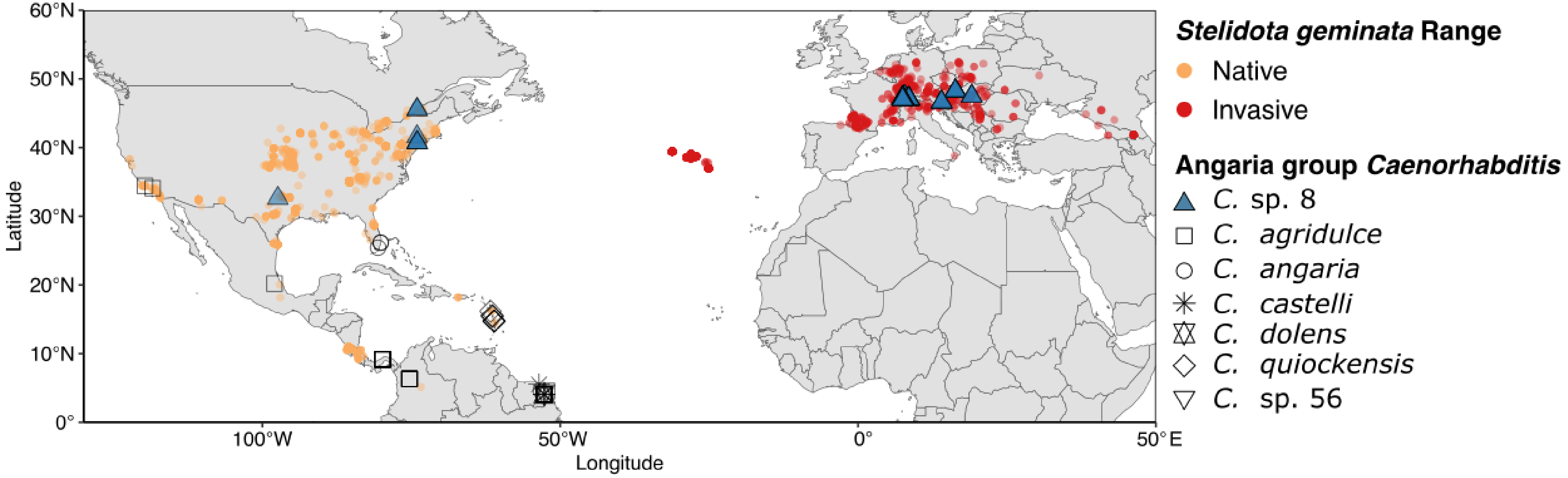

Figure 4. Global distribution of *S. geminata* and known occurrences of *Caenorhabditis* species in the Angaria group. *S. geminata* has spread from its native range in the Americas to Europe since the 2000s (orange versus red points, respectively; color intensity corresponds to the number of occurrences). All members of the Angaria group (black shapes) have only been found in the Americas, with the exception of *C.* sp. 8 (blue triangles). As most Angaria group species have been described and sampled from French Guiana, data points heavily overlap in this region. *S. geminata* distribution data was downloaded from GBIF (2025). *Caenorhabditis* occurrence data comes from World Wide Worms (justbio.com/tools/worldwideworms; accessed 15.11.2025).

Data Accessibility Statement

All data and code necessary to replicate the analysis and figures presented in this manuscript are available at https://github.com/SerenaDingLab/Greenway_et_al_Caen-Invert-Intxns.

Competing Interests Statement

The authors have no competing interests to declare.

Author Contributions

Conceptualization, data curation, formal analysis, visualization, and writing - original draft preparation: R.G. Investigation: R.G. and L.D. Writing - review and editing: R.G., L.D., and S.S.D. Supervision, project administration, resources, and funding acquisition: S.S.D.

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