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## NEMATODES THAT RIDE INSECTS: UNFORESEEN CONSEQUENCES OF ARRIVING SPECIES<sup>§</sup>

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### ABSTRACT

Insects that arrive in new regions can be hosts for a variety of unseen metazoans, including microscopic nematodes, which are carried phoretically as dauer juveniles or as internal/external parasites in various stages. This includes insects that arrived by natural means, were purposefully introduced for biological control before strict APHIS/PPQ provisions, were inadvertently introduced as hitchhikers or stowaways, or were brought in as part of the pet trade or for food. In some cases, the host associations are so specific that they may pose little threat, but in other cases where host specificity is relatively wide and/or host transfer opportunities exist, the nematode associates can expand, colonize and establish associations with native insects causing various downstream environmental effects. Because nematodes are mostly microscopic, the consequences of such introductions are usually not considered in the pet trade or as an added consequence as introduced or invasive insects arrive in the state and establish themselves. These arrival scenarios are discussed with real-world examples, including one with damage potential for Florida and the southeastern U.S., i.e. the red ring nematode that is associated with palm weevils.

Key Words: Biocontrol, entomophilic nematodes, environmental effects, establishment, invasion, trade

### RESUMEN

Los insectos que llegan en nuevas regiones pueden ser hospederos para una variedad de metazoarios invisibles, incluyendo nematodos microscópicos, que son llevadas foréticamente como juveniles del estado dauer o como parásitos internos/externos en varios estadios. Este incluye los insectos que llegan por forma natural, los que fueron introducidas a propósito para el control biológico antes de las estrictas disposiciones del APHIS / PPQ, los que fueron introducidos inadvertidamente conjuntamente con los medios de transporte, o los que fueron traídos como parte del comercio de mascotas o de alimentos. En algunos casos, la asociación con su hospedero es tan específico que puede presentar una amenaza muy leve, pero en otros casos cuando la especificidad del hospedero es relativamente amplia y/o existen oportunidades de transferencia del hospedero, los organismos asociados con los nematodos pueden ampliar, colonizar y establecer asociaciones con los insectos nativos causando diversos efectos ambientales posteriores. Debido de que los nematodos son en su mayoría microscópicos, generalmente no se consideran las consecuencias de su introducción en el comercio de mascotas o como una consecuencia adicional que introducen o insectos invasores llegan en el estado y se establecen. Se comenta sobre estos escenarios de llegadas con ejemplos del mundo real, incluyendo uno con el potencial de hacer daño en la Florida y el sureste de los EE.UU., como el nematodo del anillo rojo que esta asociada con el gorgojo de la palma.

Palabras Clave: control biológico, nematodos entomofílicos, efectos ambientales, establecimiento, invasión, comercio

As Jonathan Swift (1733) so eloquently put it, “So, naturalists observe, a flea has smaller fleas that on him prey; and these have smaller still to bite ‘em; and so proceed *ad infinitum*.” It is therefore not surprising that insects that arrive in new regions can potentially be hosts for a variety of unseen metazoans and microbes, including microscopic nematodes (typically ranging in size from about 160-2000 microns in length [fitting typical mesofaunal dimensions] and about 2-100 microns in width [fitting typical microfaunal dimensions]), which are carried phoretically as dauer juveniles or as internal/external parasites in various life stages. These stealth nematode riders have various potential ecological consequences ranging from negative to neutral depending upon downstream colonization effects. What might not be so obvious is just how prevalent these types of associations are.

Nematodes are mostly microscopic roundworms comprising the most abundant metazoans in the world. As E. O. Wilson points out, 4 out of every 5 metazoans on earth are nematodes (Rosenberg 2003). Most nematodes are free-living bacterivores, fungivores and predators considered to be major “churners” that help to facilitate the decomposition of dead organisms and organic matter by bacterial and fungal decomposers. They are aquatic organisms that live in hydrated terrestrial soils, freshwater and marine sediments and many species are adapted for various symbiotic associations with other animals, including commensalism, mutualism, and parasitism of larger (meso- to macrofaunal-sized) terrestrial arthropods (Giblin-Davis 2004). The diversity of such associations is still highly speculative, since the taxonomy of the phylum is in its infancy with only about 25,000 of the predicted one million or so species having been described (Hallan 2007). Also, most of these descriptions are based upon highly conserved and potentially homoplasious morphological characters without any molecular data to help digitize or corroborate species status. In addition, there appears to be unevenness in the association rates of nematodes with various insect groups making simple predictive models unreliable.

#### How Prevalent Are Associations between Nematodes and Insects?

Estimating the number of entomophilic nematodes is difficult given the predicted prevalence and diversity of insects, cryptic nature of the associations with nematodes, difficulty in identifying nematodes to species, and the issues with collecting and reporting negative data. Poinar (1975) summarized the literature on a variety of entomophilic nematodes, but this type of survey only focused on the positive results where someone took the time to identify and report the nema-

tode and insect pair. For example, Poinar (1975) reported that the beetle family Scarabaeidae had the largest listing of insect-associated nematodes. However, it is difficult to assess the relevance of this statement because of issues with the unevenness of sampling and the unevenness of what was ultimately observed but not published for this and other groups of insects. The basic information summarized by Poinar (1975) was mostly from previously published monographic and alpha-taxonomical reports. For these types of papers, negative data (insect species observed without a nematode association) are rarely included. Thus, there is a real need for rigorous transect sampling to help establish baseline nematode association rates for various natural groupings of insects.

Empirically, we have a general idea of the prevalence of unique entomophilic associations in some families. For example, in fig-wasps (Agaonidae) it appears that every species is probably intimately associated with between one to 5 host-specific nematode species (Giblin-Davis et al. 1995, 2006c; Kanzaki et al. 2009a; Davies et al. 2010a). In Scolytidae and Platypodidae, the species-specific association rate is probably closer to 3 or more unique nematode species per species of bark or ambrosia beetle (Rühm 1956; Massey 1974; Poinar 1975). In certain Cerambycidae, it is about 2 unique nematode species per long-horned beetle species with certain subfamilies having no known associates (e.g. Lepturina) and other subfamilies having 2 or more unique species per beetle species (e.g. Lamiinae) (Poinar 1975; Kanzaki unpubl. obs.). In the lucanids (stag beetles) all species examined were associated with one or more unique nematode species (Kanzaki et al. 2011). In the scarab beetles (Scarabaeidae), there appears to be significant variability for association rates, with some species having one or more unique nematode species and others with many shared nematode species and some without any known associates (Herrmann et al. 2006; Poinar 1975). In the carrion beetles (Silphidae), nematode association rates vary among genera and subfamilies, with the average number of unique associated species per insect species being about one with some overlapping species (Kanzaki et al. 2011, 2013). In termites (Isoptera), the association rate is probably closer to one unique nematode species per termite species (Kanzaki et al. 2012). Also, the one to one association rate is probably true for many fly families (Diptera) such as the Fergusoniidae (*Fergusonina*) (Davies et al. 2010b) as well as many of the flea species (Siphonaptera) (Poinar 1975). Because of the highly host-specific associations with thelostomatid nematodes (pinworms), the association rates in many of the cockroaches and crickets may also be close to one to one.

In other insect orders, especially those known from very dry environments, nematode associa-

tions are much rarer. For example, Lepidoptera (butterflies and moths), Orthoptera (grasshoppers) and Hemiptera (true bugs), are generally not associated with nematodes, and when associations occur, they mostly involve mermithid parasitoids that may not be particularly host specific, with rare instances of phoretic or parasitic associations. Soil-dwelling bees in Turkey appeared to have a species-specific nematode association rate of about 0.46 unique nematode species per infested bee species with less than a 6% association rate for all of the sampled bee species (Hazir et al. 2010). Some groups appear to be relatively devoid of nematode associates in the literature, such as the Formicidae (ants) (Poinar 1975), but this is probably due to under-sampling and/or under-reporting (Zhao et al. 2013).

For the purpose of this paper, we conservatively estimate that there are somewhere between 40,000-500,000 species of insect-associated nematodes globally using the equation;  $P_{in} * N_{ni} * N_i = T_{ni}$ ; where  $P_{in}$  = the proportion of all insect species in the world with nematodes [ $\approx 0.10$ - $0.20$ ],  $N_{ni}$  = number of unique nematode species per associated insect species in the world [ $\approx 0.4$ - $1.0$ ],  $N_i$  = total number of insect species in the world [ $\approx 1.0$  to  $2.5 * 10^6$ ], and  $T_{ni}$  = the number of insect-associated nematode species in the world. Unfortunately,  $T_{ni}$  is very difficult to accurately assess because of the lack of precision in all of the input variables. For example, inaccuracies in the measurement of the degree of host specificity among various phoretic and parasitic species of nematodes could easily confound estimates of  $N_{ni}$ , lack of clarity concerning the level of non-specific phoretics with free-living populations of cosmopolitan species could lead to over- or under-estimations of  $N_{ni}$ , rarity of associations and low sampling coverage could lead to underestimates of  $P_{in}$  and/or  $N_{ni}$ , and our general lack of knowledge of insect species diversity could lead to inaccuracies in the estimates of  $N_i$ . Regardless, the number of insect-associated nematode species ( $T_{ni}$ ) is much larger than most people currently imagine. "Ballparking" this estimate should help illustrate the potential for unseen introduction and movement of nematodes into new environments and microniches as their larger (meso- or macrofaunal) insect and other terrestrial arthropod hosts are moved or move globally.

#### Establishment Bottlenecks

Even when there is a unique nematode species associated with a particular species of insect, it does not necessarily mean that nematode species will be successfully introduced and become established in a new region with its original host. This is because association/parasitism rates for phoretic (commensal) and parasitic organisms usually range from about 1-50% (Massey 1974;

Poinar 1975; Herrmann et al. 2006). An insect by chance could easily be introduced without its normal nematode associates depending upon the size of the colonizing group and/or number of introduction events that have occurred. This is analogous to a bottleneck or a founder effect in population genetics where a small sampling of the original population can lead to reduced genetic variability and a non-random sampling of genes in the founder population. In terms of nematode symbiont establishment, there can be reduced nematode associate variability in the founding population that leads to local extinction events or non-random sampling of the associates that could lead to "missing the boat". A recent example appears to be *Parapristina verticillata* (Waterston), the pollinator wasp for *Ficus microcarpa* L. fil., which has become established in southern Florida and is pollinating *F. microcarpa* and helping to make it an invasive terrestrial weed by allowing it to reproduce (Nadel et al. 1992). However, one of its known nematode associates from its original range, *Schistonchus microcarpa* Zeng, Ye, Giblin-Davis, Li, Zhang & Du (Zeng et al. 2011), has apparently not become established (RGD unpublished). Davies et al. (2010a) have shown host-switching of some *Schistonchus* lineages in Australia where there is evidence of long term incursions of apparently Laurasian *Ficus* lineages and their associated fig wasps and nematodes supporting the possibility of nematode host-switching as fig wasp lineages are moved into new areas.

#### Host Specificity as a Determinant of Downstream Ecological Effects

Host specificity is a critical factor in the establishment and potential downstream ecological effects of the invasion or introduction of an organism into a new region. The highly host specific and exceedingly interesting *Fergusonina turneri* Taylor/*Fergusonobia quinquenerviae* Davies & Giblin-Davis (shoot and flower bud gall fly/mutualist nematode symbiont) complex was the first such pair to receive permission for release into Florida as a biological control agent against the invasive terrestrial weed *Melaleuca quinquenervia* (Cav.) S.T. Blake (Davies & Giblin-Davis 2004; Ye et al. 2007b; Blackwood et al. 2005). In this case, the pair was vetted extensively for host specificity on *M. quinquenervia* and then released twice without successful establishment (Blackwood et al. 2005; Pratt et al. 2013). If it had been successful, the mutualistic complex would have been another of a group of classical biological agents released and established against this weed for density dependent management where host specificity would have relegated it to a microniche in Florida that had been created by man through the introduction of *M. quinquenervia* over 100 years earlier.

An example of a highly host specific phoretic and necromenic association involves *Caenorhabditis japonica* Kiontke, Hironaka & Sudhaus, which has very stringent physiological requirements for survival in association with its fruit bug transport host, *Parastrachia japonensis* Scott in Japan. In such a case, the specific requirements for synchrony of life cycles to extend life in association as dauer juveniles with its bug host are sufficiently stringent to preclude survival on any other host (Tanaka et al. 2012). None of the many other bug species sharing the host bug's environment were found to be associated with the nematode (= all "negative" data) (Tanaka et al. unpubl. obs.). Establishment of *C. japonica* in a new environment would be linked inextricably to its fruit bug transport host and the potentially narrow microniche that the host can carve out in a newly colonized environment. Thus, a high level of host specificity due to physiological, developmental, or life history stringencies can limit the survivability options during transfer to a new environment regardless of whether the association involves a freeliving phoretic or parasitic nematode.

In the case of some thelastomatid and oxyurid pinworms known as obligate phoretics or parasites from the hindguts of terrestrial arthropods, especially those potentially involved in the pet trade such as the Madagascar hissing cockroach, *Gromphadorhina portentosa* (Schaum), and some *Blaberus* species close to *B. giganteus* (L.) from South America (Thomas 1995) and large millipedes such as the giant African millipede, *Archispirostreptus gigas* (Peters), similar stringencies probably apply. In these cases, the host is the niche regardless of the macro-environment and the egg is a transient infective stage. Thus, without their natural host, the nematodes will probably fail to survive or thrive in the new environment because of a lack of a suitable host unless a host switch is possible.

It is clear that there is significant variability in host specificity of different phoretic and parasitic nematodes within genera (Poinar 1975; Herrmann et al. 2006, 2010; D'Anna & Sommer 2011). Being able to assess host specificity not only challenges our current understanding of species boundaries in nematodes, especially as it pertains to cryptic species, but also our knowledge of the evolutionary processes that can lead to successful host switching (Page 2003). For example, recent work with the entomophilic diplogastrid genus *Acrostichus* suggests that cryptic species may be relatively common with biogeographical and host-associated endemism being the driver (McFrederick & Taylor 2012). However, this could change as the hosts are moved around and become established in new areas. For example, D'Anna & Sommer (2011) provided compelling evidence that the gonochoristic *Pris-*

*tionchus uniformis* Fedorko & Stanuszek is a European native scarab associate that arrived in North America and expanded its host range to scarabs there and possibly to the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) belonging to an entirely different beetle family (Chrysomelidae). The destructive CPB was introduced into Europe in the late 1800s where it either brought *P. uniformis* back with it or obtained it fresh from European scarabs. In either case, *P. uniformis* can now be recovered from scarabs and CPB in both regions where it has remained true to both families of hosts in a fascinating example of geographical and host range expansion. In addition, the hermaphroditic *P. pacificus* Sommer, Carta Kim & Sternberg has been moved around the world from Japan and now occurs in North America and many other locations where it appears to have switched hosts in some of the newly colonized areas (Herrmann et al. 2006, 2010). The ecological effect of such insect host switching of nematode bacterivores/omnivores over time and geographical space still remains largely unexplored.

Ralf Sommer's group at the Max Planck Institute is using the "mostly" scarab-associated genus *Pristionchus*, and in particular, the model nematode *P. pacificus* to further explore the evolutionary ecology and evolutionary developmental biology of these interesting insect associates. This work is elucidating the mechanisms of dauer juvenile formation and timing for synchronization with an insect host, and the importance of dauer nictation behavior and dauer chemotaxis to host semiochemicals for evolution, host-switching and maintenance of nematode/insect symbioses in varying environments (Hong et al. 2008a, b; Brown et al. 2011; D'Anna & Sommer 2011).

#### Case studies: A Tale of 2 *Bursaphelenchus* Species

The most notorious examples of entomophilic nematode introductions around the world in the past 100 years involve 2 lethal plant diseases, pine wilt disease (PWD), caused by the pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Buhrer), and red ring disease (RRD) caused by the red ring or coconut palm nematode (RRN or CPN), *B. cocophilus* (Cobb). We might not know about them except for the significant lethal pathogenic effects on their tree hosts, which led to years of exhaustive searching for causative relationships and determination of the countries of origin of these pathogenic nematodes (reviewed in Mota et al. 2008; Zhao et al. 2008; Giblin-Davis 1993; Griffith et al. 2005). These 2 examples demonstrate how introduction of unseen nematodes on a terrestrial arthropod host can have serious ecological repercussions.



## Pine Wood Nematode

Pine wilt disease was first reported from Nagasaki, Japan in 1905, but the causal agent and vector association were not understood until much later (Yano 1935). Originally, wood-boring insects were suspected to be the cause of massive tree deaths in the area leading to the common Japanese name for the disease of “matsu-kui-mushi” (pine-feed-bug) until a nematode was implicated as the causal agent (Tokushige & Kiyohara 1969; Kiyohara & Tokushige 1971). Mamiya & Kiyohara (1972) described the facultative plant/fungus parasitic nematode as *Bursaphelenchus “lignicolus”* and Morimoto & Iwasaki (1972) implicated the longhorn beetle, *Monochamus alternatus* Hope as the primary vector of the nematode in Japan. Pine wilt disease moved rapidly through Japan reaching the northern-most part of the island by ca 1980 after destroying large areas of native pine forests (e.g. *Pinus thunbergii* Parl. [Japanese black pine] and *P. densiflora* Sieb. & Zucc. [Japanese red pine]) (summarized by Futai 2008; Kishi 1995), then spreading to China in ca 1982 (Cheng et al. 1986; Zhao 2008), Taiwan in ca 1983 (Zhao 2008), and South Korea in ca 1988 (Yi et al. 1989). In 1999, pine wilt disease was reported from Portugal (Mota et al. 1999) and eventually in Spain in 2008 (Abelleira et al. 2011) where it has continued to destroy extremely large areas of native (and therefore naïve and susceptible) pine plantations (e.g. *Pinus pinaster* Aiton [Maritime pine]).

It turns out that *Bursaphelenchus “lignicolus”* was not an endemic nematode species to Japan, nor was *Monochamus alternatus* the natural vector for the pathogen in its region of origin (North America). The original range of *M. alternatus* is Japan, Taiwan and some parts of China and Korea. The eventual comparison of southeastern U.S. populations and Japanese populations of the nematode from pine wood led to the sinking of *B. “lignicolus”* as a junior synonym of *B. xylophilus*, which had been described many years earlier from Louisiana without reference to pine pathology (Steiner & Buhner 1934, Nickle et al. 1981). *Monochamus titillator* (Fabricius) and *M. carolinensis* (Olivier) were determined to be the natural vectors of *B. xylophilus* in North America where the relative importance of the disease was very low. This is because North America is the presumed center of diversity and point of origin for the pathogen where it had time to co-evolve with its pine hosts leading to resistance (summarized by Sutherland 2008). We now know that the destructiveness and general invasiveness of *B. xylophilus* derives from several key factors. First, *B. xylophilus* has a relatively wide fungal host range with the added versatility of being able to move through and feed on plant tissue. Second, the pre-dauer and dauer juvenile stages

of *B. xylophilus* can survive diverse biotic and abiotic challenges for relatively long periods of time (months to years) either on or off the insect host. Third, the dauer stage of *B. xylophilus* is versatile in its ability to switch to and be synchronized with the life history of congeners of *Monochamus* in newly invaded regions of the world where native pine hosts are available. Fourth, the nuptial feeding behavior of *Monochamus* species (which can expose *B. xylophilus* to healthy pine tissue) and their breeding behavior (which can expose *B. xylophilus* to fungus deep in a log and creates an opportunity for cerambycid host switching) allow for mass production of nematodes and distribution to naïve pine hosts. Fifth, *B. xylophilus* is microscopic and both it and its *Monochamus* vectors are deep wood inhabitants, which make them difficult to detect in imported/exported wood products. Lastly, there has been a strong and steady global demand for wood products over the past century, which has created many opportunities for introductions of both *B. xylophilus* and *Monochamus* increasing the chances for a successful invasion.

Thus, *B. xylophilus* represents a phoretic fungal-feeding nematode associated with *Monochamus titillator* and *M. carolinensis* in its native range (North America) where it does not cause pathology to the native pine species hosts. Both *B. xylophilus* and its cerambycid host(s) were introduced into Japan in wood where the nematode switched hosts to a better adapted local *Monochamus* host (*M. alternatus*) and began causing epiphytotic in the local naïve pine forests (*Pinus thunbergii* and *P. densiflora*). This same mode of invasion has been repeated several times in Asia and now in Europe involving different naïve pine hosts and locally-adapted *Monochamus* species.

Given that cerambycids such as *Monochamus* are usually hosts to several unique nematode species each (e.g. *Diplogasteroides*, *Rhabditolaimus*, and *Neodiplogaster* [Kanzaki & Futai 2004; Kanzaki et al. 2002; 2008b]), and that *M. titillator* and/or *M. carolinensis* were the “invading host(s)”, then there is also the possibility that other phoretic or parasitic nematodes might have been introduced into Japan (Asia) from North America at the same time as *B. xylophilus*. Ecological effects of this type of introduction are unknown because of the intense effort required to sort them out using traditional methods, but the effect could be significant in terms of increased local competition and possible displacement and local extinction of some species.

Contemporary examples of recently introduced wood-boring insects into North America with the same potential for unseen nematode phoretic or parasitic riders include the Asian long-horned beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Cerambycidae), which was first reported from maple in New York in 1996 and the Emerald

ash borer (EAB), *Agrilus planipennis* Fairmaire (Buprestidae), which was first reported attacking ash trees in Michigan in the early 1990s. *Rhabditolaimus* sp. and *Neodiplogaster* sp. have been recovered from *An. glabripennis* in Asia, but to our knowledge ALB has not been examined for nematode associates in North America (Kanzaki unpubl. data). In addition, we have received an apparently new species of insect-parasitic tylenchid nematode from EAB for identification that may have been brought in from its country of origin as a natural enemy when it was introduced or could be a host switch from a native buprestid following invasion (RGD unpubl. data). In Florida, the recently introduced redbay ambrosia beetle (RAB), *Xyleborus glabratus* Eichhoff (Scolytinae), which is the vector of the fungus *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva that causes laurel wilt in redbays and avocados is an excellent potential host for the mycophagous nematode, *Ruehmaphelenchus* sp. among others that occur naturally on this beetle in its region of origin (Kanzaki unpubl. data).

#### Red Ring Nematode

Originally, red ring disease (RRD) was reported from coconut from Trinidad in 1905 and was suspected to be caused by a fungus (Griffith et al. 2005). It was later shown to be a plant-parasitic nematode that was recovered from the diagnostic red ring of infested leaves, stems and red-colored roots of coconut (Rorer 1911; Nowell 1919). At first, the nematode was thought to be a root-attacking and soil-inhabiting species, but it was later shown to be tightly associated with the palm weevil *Rhynchophorus palmarum* (L.) by Cobb (1919) who described it as *Aphelenchus cocophilus* (eventually transferred to the current genus of *Bursaphelenchus* [see Griffith et al. 2005]). RRD has been reported from tropical America; from Mexico and the lower West Indies (Trinidad and Tobago, Grenada and St. Vincent) through Central America to northern South America (Venezuela, Guyana, Surinam, French Guyana, Colombia, Ecuador, Peru and Brazil) (Griffith et al. 2005). The environmental effect of RRD is small relative to PWD, but it does cause significant losses to cultivated coconut and African oil palm in tropical America of about 15% per year in many areas (Chinchilla 1988).

The insect host-vector range appears to be limited to palm and sugarcane weevils in the Dryophthorinae of Curculionidae, most importantly *Rhynchophorus palmarum*, but with evidence that *Dynamis borassi* (Fabricius) (Gerber et al. 1990), and possibly *Metamasius hemipterus* (L.) (Calvache 1994; Mora et al. 1994) are capable vector hosts in South America. The plant host range of this nematode is currently restricted to ~ 20 species of palms in the family Aracaceae,

including many palms native to South America (Griffith et al. 2005). The potential host range of *B. cocophilus* will likely include most of the Aracaceae because of the phylogenetic breadth of the current list of known hosts. *Bursaphelenchus cocophilus* does not appear to develop in non-Aracaceae hosts such as sugarcane or on the fungi that have been tested (Giblin-Davis et al. 1989) supporting its classification as an obligate internal palm parasite. Coconut has functioned as an excellent indicator host because of its widespread cultivation in the New World since the time of Columbus and its relatively high host susceptibility to both the nematode and palm weevils (Griffith et al. 2005). African oil palm has been a little less emblematic because it was introduced and cultivated throughout Central and South America only relatively recently and is apparently less susceptible to both the vector and nematode than coconut. The current RRD situation is analogous to what has been reported in parts of the native North American range of PWN, where susceptible imported pines such as *Pinus sylvestris* L. (Scots pine from Europe) or *P. thunbergii* (Japanese Black pine) have been cultivated for highway beautification and windbreak programs or planted in Christmas tree farms in the Midwestern U.S. only to be killed by PWD epiphytotics originating from PWN-infested beetles from resistant native forests (Gleason et al. 2000). In the case of RRD, coconut and African oil palm are the naïve and defenseless hosts that were introduced into an area (probably somewhere in northeastern Amazonia or Trinidad) where the native palm weevil and *B. cocophilus* partners were able to get together to produce ensuing RRD epiphytotics.

The center of origin of red ring disease appears to be in the vicinity of northeastern South America because of the history of the disease and the apparent spread of the RRN through the long standing and much more widely distributed *R. palmarum* populations (Giblin-Davis 1993; Gerber et al. 1990). In the first 50 years following the discovery of RRD, most studies and reports occurred within a radius of less than 1000 miles from Cedros, Trinidad. The chief vector *R. palmarum* was known to be broadly distributed from northwestern Mexico south to Bolivia long before that (Wattanponsiri 1966) and RRD appeared to move slowly outward and has still not arrived in the distal regions of *R. palmarum* distribution or in the southeastern United States where the allopatric congener, *R. cruentatus* (Fabricius) occurs. For example, RRD was only first reported in Ecuador in 1967 (Gerber et al. 1990), and Costa Rica and Honduras in the mid-1970s (Chinchilla 1988), whereas *R. palmarum* was known from these areas at least 40-50 years prior to these reports (Wattanponsiri 1966). Contrastingly, the phoretically-associated free-living bacterivorous nematodes, *Teratorhabditis palmarum* Gerber

& Giblin-Davis and *Acrostichus rhynchophori* Kanzaki, Giblin-Davis, Zeng, Ye & Center occur throughout the natural known distribution of both *R. palmarum* and *R. cruentatus* (Gerber & Giblin-Davis 1990a,b, Gerber et al. 1990; Kanzaki et al. 2009b). This suggests that RRN may be a recent acquisition by *R. palmarum*, perhaps from another insect (e.g. *D. borassi*) that used coconut as a new host or that it is the result of an entirely new type of relationship. Thus far, the putative sister species of RRN is the mycophagous *B. platzeri* Giblin-Davis, Kanzaki, Ye, Mundo-Ocampo, Baldwin & Thomas, which is vectored by nitidulid sap beetles in California to rotting fruit (Giblin-Davis et al. 2006b; Ye et al. 2007a). There is already clear evidence for *R. palmarum* being an occasional transport host for other species of nematodes besides RRN, *T. palmarum* and *A. rhynchophori* (Gerber & Giblin-Davis 1990a), i.e., the free-living mycophagous *Bursaphelenchus gerberae* Giblin-Davis, Kanzaki, Ye, Center & Thomas, which is probably normally associated with bark beetles or ambrosia beetles that occasionally co-occur in dying palms (Giblin-Davis et al. 2006a) and the free-living bacterivorous *Caenorhabditis angaria* Sudhaus, Kiontke & Giblin-Davis for which the chief phoretic carrier is the often co-occurring *Metamasius hemipterus* in dying palms (Sudhaus et al. 2011). Since *M. hemipterus* was first reported from Florida in the mid-1980s, we have recently dissected and cultured out the nematode associates from the now sympatrically occurring *M. hemipterus* and *R. cruentatus* in Davie, Florida, and thus far have not found any apparent host switching of *C. angaria* and/or *Diplogastrellus metamasius* Kanzaki, Giblin-Davis, Zeng, Ye & Center from *M. hemipterus* to *R. cruentatus* or any *T. palmarum*, *A. rhynchophori* or *Mononchoides* sp. from *R. cruentatus* to *M. hemipterus* (RGD preliminary unpubl. data). This suggests that the host transfer of these different bacterivorous and predatory species may take longer to occur, may be hampered by host compatibility differences, or may require deeper sampling to discover. Similarly, *M. hemipterus* was not observed to be a capable vector of RRN in Costa Rica even though it occurred sympatrically with *R. palmarum* in RRN-infested African oil palm plantations (Bulgarelli et al. 1998). The cross-transfer of other nematode associates was not examined in that study.

The potential invasiveness and destructiveness of *Rhynchophorus* species has been highlighted with the recent worldwide range expansion of the Asian red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Giblin-Davis et al. 2013). *Rhynchophorus palmarum* has recently been recovered in Texas and California (Giblin-Davis et al. 2013). This weevil by itself and in association with *B. cocophilus* poses a threat to other

tropical regions with palms including Florida. The risk partially derives from the possibility that all *Rhynchophorus* species may be capable vectors of RRN and that the weevil genus has a pan-tropical distribution (Wattanpongsiri 1966; Giblin-Davis et al. 2013). Thus, even if *R. palmarum* was not successful at establishment after introduction into a new area, as was the case for the North American *Monochamus carolinensis* in Japan for PWD where the native *Monochamus alternatus* became the vector of PWN leading to highly destructive epiphytotics (Kanzaki pers. obs.), other *Rhynchophorus* species could potentially pick up the RRN leading to a synergized new tree-killing population mutualism in areas with large numbers of naïve and potentially very susceptible palms (Giblin-Davis 2004). Alternatively, as species like *R. ferrugineus* invade areas such as the lower Antilles where both *R. palmarum* and *B. cocophilus* co-occur, a cross transfer is possible that might change the dynamics of the association and ultimately how RRD epiphytotics are manifested (Giblin-Davis et al. 2013).

#### Other Known Nematode Introductions

As described above, plant pathogenic nematode species can easily be recognized as invasive species. However, nematode species are being introduced to new localities as cryptogenic species associated with biological materials, such as wood and bark products, pet animals, and introduced pest insects. Additional examples are noted below.

Stag Beetles (Lucanidae) as Pets. International trade of insects has been increasing. For example, cockroaches are often used as animal feeding stock for vertebrate pets and stag beetles have become increasingly popular as pets in some Asian countries, such as Japan and Taiwan, where living insects of many species are being imported (Kameoka & Kiyono 2003). These introduced lucanids sometimes escape from their owners establishing their populations in natural field sites in Japan. Furthermore, their parasitic mites have been shown to parasitize native lucanids in Japan (Goka et al. 2004; Okabe & Goka 2008). As mentioned above, lucanid beetles are associated with many species of phoretic nematodes including diplogastrid bacterivores and *Bursaphelenchus* fungal feeders (Kanzaki et al. 2011). The second author (N. K.) considered the possibility of cryptogenic invasion of lucanid associated nematodes, and examined the nematode association of imported individuals. Although the research project is ongoing, almost all individual lucanids examined were found to be associated with one or more unique nematode species. Some of these introduced nematodes are morphologically similar to native species, i.e., close relative



or conspecific, and can be artificially associated with native beetle species (Kanzaki et al. unpubl. data). Currently, we do not have sufficient information concerning the ecological impact of these nematode species to the native nematode fauna. However, competition between introduced and native species, and genetic contamination of native fauna could easily occur, because these nematodes share the same micro-niches. Careful monitoring and evaluation of these cryptogenic species is necessary in the context of biodiversity conservation.

**Invasive Pest Insect Hosts.** Invasive pest insects sometimes carry and introduce phoretic nematodes. So far, 2 cases of nematode invasions accompanying invasive insect pests are suspected in Japan.

The first case involves *Teratorhabditis synpapillata* Sudhaus, associated with *R. ferrugineus*, a pest of garden and street palms in warm-temperate areas. During a field survey of its nematode associates in Kagoshima, Japan, *T. synpapillata* was isolated (Kanzaki et al. 2008a). Because the nematode had originally been reported from South and Southeast Asian countries as phoretic associates of *R. ferrugineus* (Muthukrishnan 1971) or associates of nutrition-rich soil, humus or sewage water (Sudhaus 1985; Tahseen et al. 2007), the population isolated from Japan was considered to be an introduced population accompanying its carrier weevil. The ecological ramifications of this introduction are unknown.

The second one is *Acrostichus* sp. (an apparently undescribed species) associated with a south-east Asian leaf beetle, *Sagra femorata* Drury (Kanzaki et al. 2012). The beetle was recently introduced to Mie, Japan from its native range and became established by feeding on leaves and shoots (veins) of kudzu, *Pueraria lobata* (Willd.) Ohwi (Fabaceae). Although the larvae form a large gall on the plant vein to create a pupal chamber, it has so far not caused ecological or economic damage. The nematode, *Acrostichus* sp., was isolated from the pupal chamber, body surface of the larvae and pupae and body of the adult insect. Because the nematode was not isolated from other insects (cerambycids and buprestids were also obtained in the survey) that share the same *P. lobata* vein, the nematode is considered to have high carrier specificity, and was most likely introduced with its carrier leaf beetle.

Both of these genera are bacterial-feeding nematodes. Further, the distribution of carrier insects in Japan is limited to the area growing the palm trees (*R. ferrugineus*-*T. synpapillata*) or a couple of prefectures (*S. femorata*-*Acrostichus* sp.). Therefore, the nematode is not likely to affect agricultural and forestry production and/or native ecosystems at the current status. Nevertheless, these kinds of cryptogenic invasions highlight the possibilities for future ecological effects.

## CONCLUSION

Nematodes are commonly associated with meso- and macrofaunal arthropods in various symbiotic relationships. We conservatively estimate that there are about 40,000-500,000 species of insect-associated nematodes globally. This provides opportunities for movement of these nematodes into new environments as their insect hosts expand their distributions, either on their own as climate changes or through the activities of humans. In the best case scenario, free-living phoretic or intimately-associated parasitic nematodes will go extinct after arriving in new regions as their host insect fails to establish. Alternatively, the introduced nematode symbionts become more widely distributed and only cause minor shifts in micro-habitat ecology with few downstream consequences (no extinctions of existing species but with further niche partitioning in the environment and on the insect host). However, in the worst case scenarios, such as the discussed pine wilt and red ring disease examples, plant-parasitic nematodes are associated with a vector in the country of origin where the pair has co-evolved with native plant hosts and causes only minor plant disease. Nematodes then transfer onto a native insect vector causing a new disease syndrome to develop on an introduced and naïve (unprepared) forestry/agricultural/horticultural host(s) causing major epiphytotic and major ecosystem service disruption and damage. These are natural consequences of increased globalization.

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