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First Report of Matricidal Hatching in *Bursaphelenchus xylophilus*

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Abstract: The reproductive strategy of the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is sexual amphimictic and oviparous. The incidence of intrauterine egg development and hatching in plant-parasitic nematodes is not a very common phenomenon. During the process of maintaining and breeding a *B. xylophilus* population isolated in Spain under laboratory conditions, evidence of matricidal hatching was observed. This is the first described case of this phenomenon in this species.
Key words: Bursaphelenchus xylophilus, detection, endotokia matricida, laboratory conditions, matricidal hatching, morphology, nematode reproduction, physiology, pinewood nematode.

Pine wilt disease, caused by the PWN *B. xylophilus* (Steiner et Buhrer) Nickle, is a major disease affecting conifer forests and resulting in significant economic losses (Mota et al., 2009). At the beginning of the 20th century, the disease was especially prevalent in East Asian countries; however, at the end of the century, the disease started to spread toward Europe and currently only affects the Iberian Peninsula, Portugal (Mota et al., 1999), and Spain (Abelleira et al., 2011; Robertson et al., 2011). In Galicia, one of the Spanish regions in which this nematode has been identified, a *B. xylophilus* population was isolated and reared under laboratory conditions with the aim of conducting further studies on its biology, pathogenicity, and control. Maintenance and multiplication of PWN populations are generally carried out in fungal cultures of *Botrytis cinerea* Pers., allowing for nematodes to multiply and complete their life cycle.

Nematodes have several reproductive strategies, although, in this particular case, the PWN reproduces sexually, through amphimixis, and is oviparous, both in natural and laboratory conditions (Hasegawa and Miwa, 2008). Intrauterine egg development and hatching are very common in many free-living and parasitic rhabditids, being more frequent in entomopathogenic nematodes and unlikely in plant-parasitic nematodes, in which case it is considered a rare phenomenon (Baliadi et al., 2001).

The purpose of this report is to describe, for the first time ever, a case of matricidal hatching in females of PWN reared under laboratory conditions.

Materials and Methods

Ever since the PWN was first identified in the autonomous community of Galicia (northwestern Spain) at the end of 2010, its population has been bred and multiplied to conduct future research on this pathogen. After testing different already-described methods allowing for the routine maintenance and multiplication of *B. xylophilus*, we selected the breeding process on potato dextrose agar (PDA) petri plates with *B. cinerea*.

Nematode multiplication

A culture of *B. cinerea* in PDA plates was used to obtain nematode growth media in petri plates measuring 55 and 90 mm in diameter and incubated at 25°C for 2 and 7 d, respectively. Once proper growth of the fungus had been verified, approximately 20 adult specimens of *B. xylophilus*, mostly females, were placed on petri plates (55 mm in diameter). To multiply this original population, the plates were incubated at 25°C for 1 wk. Then, once the first population had multiplied, two pieces of medium, with a density of more than 100 nematodes/cm^2, were cut and placed individually inside the PDA petri plates (90 mm in diameter), which had previously been incubated and covered entirely by the fungus.

Nematode storage

Once the population had multiplied in these plates, the medium was washed with distilled water to drag the nematodes toward the petri plates, which were then stored in a refrigerator at 7°C to maintain and preserve them for 3–4 mon. The plates were examined monthly to control the viability of the stored population. Whenever a high mortality rate was observed, the breeding and multiplication process was restarted.

Results and Discussion

During the control of the viability of the stored population, in which mobility and mortality were examined, a very unusual phenomenon of intrauterine egg development and hatching was recorded in *B. xylophilus*. First, a live gravid female containing three unhatched eggs at different embryonic stages was observed. One of these eggs was in an early embryonic stage and the other two were in a J2 juvenile stage (Fig. 1A). This phenomenon was later observed again in four females (only one being alive); one of the females contained an unhatched egg and the other three had hatched juveniles, which were actively moving inside the maternal body (Fig. 1C). The live female had disorganized inner organs and the dead females showed different levels of degradation (Fig. 1B–D), with the

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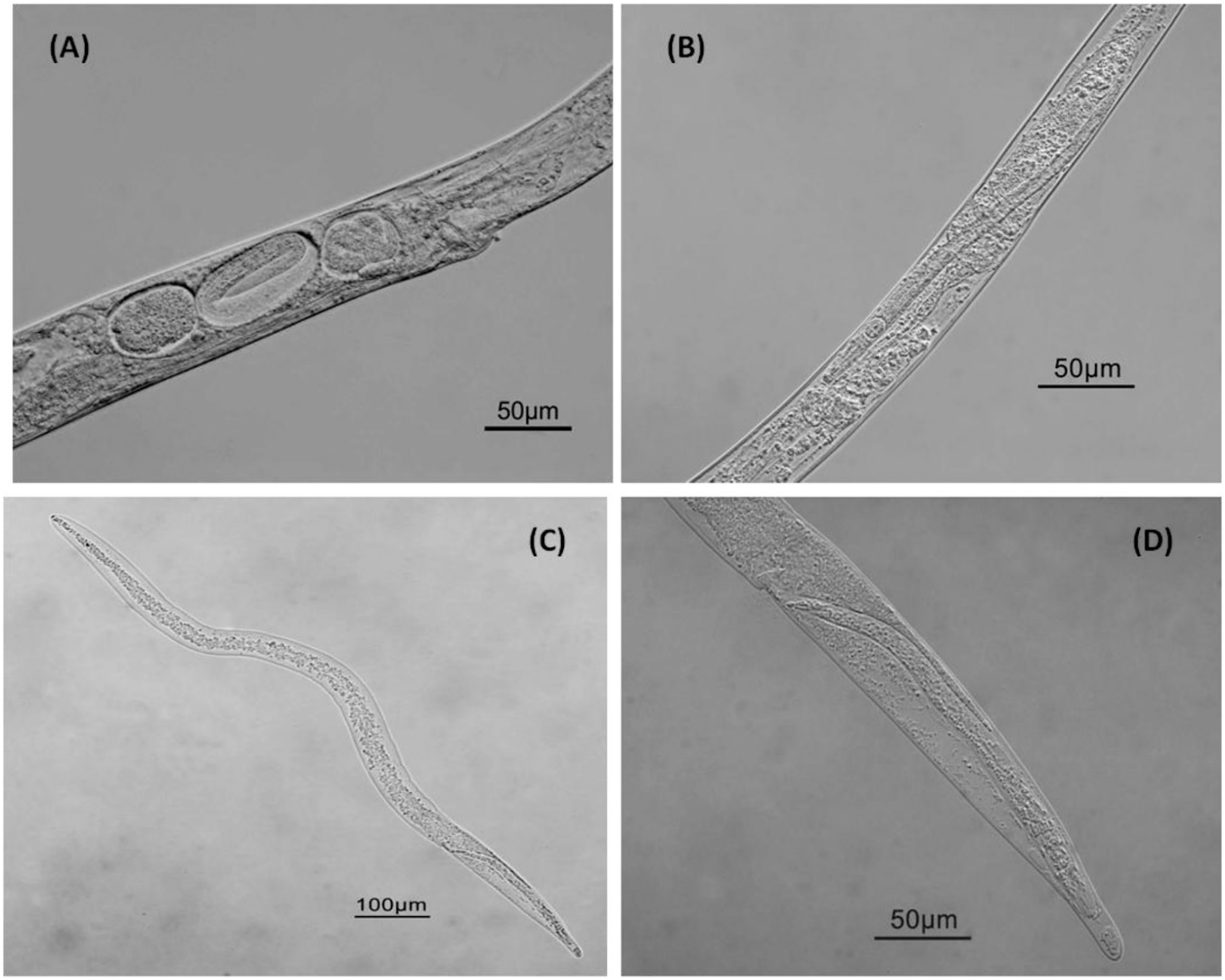

FIG. 1. Different *Bursaphelenchus xylophilus* females with matricidal hatching. A. Detail of the vulval region of a *B. xylophilus* female containing three eggs in different embryonic stages and without hatching (both female and juvenile were alive before photographed). B. Vulval region of a *B. xylophilus* female with a juvenile J2 (both were alive before photographed). C. *B. xylophilus* female with a juvenile J2 in the posterior region. D. Detail of the posterior part of a *B. xylophilus* female of the previous image.

stylet and the median bulb only remaining visible in one of them.

The phenomenon of intrauterine egg development and hatching, leading to the destruction of female nematodes by their juvenile stages (Maupas, 1899), was erroneously termed *endotokia matricida* (*endo* [Greek] = inside, -*tokia* [Greek] = birth, *matri* [Latin] = mother, -*cida* [Latin] = kill) by several authors, such as Southey (1969) or Laughlin et al. (1978). In an attempt to clarify the term, Luc et al. (1979) remarked the differences and suggested the use of *matricidal hatching* to refer to the intrauterine development and hatching of eggs, leaving the term *endotokia matricida*, defined by Seurat (1914), as a synonym of female encystment.

The first mention of matricidal hatching in plant-parasitic nematodes took place in the description of *Anguina tritici* Steinbuch by Scopoli in 1777 (cited as a female "vivipara & ovipara"). Over the last few decades, this phenomenon has been reported in a few species of other genera, as shown in Table 1. Specifically in the case of the *Bursaphelenchus* genus, and according to the available literature, this phenomenon was only recorded in *Bursaphelenchus eremus* (Rühm) Goodey (Carletti et al., 2007). In this case, an embrionated egg was observed within the postuterine branch of a female nematode that had been reared on cultures of *B. cinerea* and previously isolated from *Quercus robur* L.

Matricidal hatching in plant-parasitic nematodes is a very unusual phenomenon, mainly observed in routine analyses carried out under natural conditions, there being no available references on the culture conditions involved in the process. Previous studies of matricidal hatching in nonplant-parasitic nematodes, mainly in entomopathogenic and free-living nematodes, such as *Caenorhabditis elegans* Maupas, have cited several factors that could favor the incidence and development of matricidal hatching. Laughlin et al. (1978) and Singh and Khera (1976) gathered the opinions of

TABLE 1. Nematodes reported with intrauterine egg development and hatching.

Taxonomy classification			Plant parasitic nematodes	Other nematodes
Class Chromadorea				
Order Rhabditida	Suborder Rhabditina	Family Cephalobiidae		*Acrobeloides* sp. (Singh and Khera, 1976)
				Chiloplacus lentus (Roy, 1974)
				Metacrobeles amblyurus (Chiu et al., 2002)
		Family Heterorhabditidae		*Heterorhabditis bacteriophora* (Poinar, 1975)
				Heterorhabditis megidis (Ehlers et al., 1998)
				Heterorhabditis sp. (Johnigk and Ehlers, 1999)
		Family Mehdinematidae		*Mehdinema alii* (Luong et al., 1999)
		Family Neodiplogasteridae		*Pristionchus pacificus* (Gilarte et al., 2015)
		Family Rhabditidae		*Caenorhabditis elegans* (Mitchell et al., 1979)
				Oscheius chongmingensis (Liu et al., 2012)
				Rhabditis terricola (Pérez, 1866)
				Rhabditis tipulae (Lam and Webster, 1971)
				Rhabditis tripartita (Craveiro, 1985)
				Rhabditis sp. (Lordello, 1951)
	Suborder Tylenchina	Family Anguinidae	*Anguina* spp. (Ivanova, 1962)	
			Anguina sp. (Southey, 1969)	
			Anguina pacificae (McClure et al., 2008)	
			Anguina tritici (Scopoli, 1777; Marcinowski, 1909; Gupta and Swarup, 1968)	
			Ditylenchus destrutor (Duggan and Moore, 1962)	
			Ditylenchus dipsaci (Teploukhova, 1968)	
			Paranguina agropyri (Krall, 1967)	
		Family Aphelenchoididae	*Aphelenchoides fragariae* (Loof, 1959)	
			Aphelenchus avenae (Jairajpuri, 1964 [unpublised, Hechler])	
			Bursaphelenchus eremus (Carletti et al., 2007)	
			Bursaphelenchus xylophilus (this work)	
		Family Brevibuccidae		*Tarantobelus arachnicida** (Abolafia and Peña-Santiago, 2017)
		Family Hoplolaimidae	*Helicotylenchus* sp. (Yuen, 1965)	*Thecavermiculatus gracililancea* (Robbins, 1978)
			Helicotylenchus paxilli (Yuen, 1965)	
			Helicotylenchus vulgaris (Yuen, 1966)	
		Family Meloidogynidae	*Meloidogyne* sp. (Atkinson, 1889; Nagakura, 1930; Pinochet, 1978)	
			Meloidogyne incognita (Perlaza and Lopez, 1979)	
			Meloidogyne hapla (Monteiro et al., 2016)	
			Meloidogyne javanica (Lordello and Koguti, 1962)	
		Family Panagrolaimidae		*Panagrellus redivivus* (Samoiloff et al. 1980)
				Panagrolaimus tipulae (Lam and Webster, 1971)
		Family Pharyngodonidae		*Pharyngodon spinicauda* (Seurat, 1914)

(*Continued*)

TABLE 1. Continued.

Taxonomy classification			Plant parasitic nematodes	Other nematodes
		Family Pratylenchidae	*Pratylenchus agilis* (Martin and Riedel, 1982) *Pratylenchus crenatus* (Martin and Riedel, 1982) *Pratylenchus brachyurus* (Laughlin et al., 1978) *Pratylenchus coffeae* (Loof, 1959; Wehunt and Edwards, 1971) *Pratylenchus minyus* (Vovlas and Inserra, 1975) *Pratylenchus penetrans* (Martin and Riedel, 1982) *Pratylenchus scribneri* (Martin and Riedel, 1982) *Rabdopholus similis* (Loos, 1962)	
		Family Steinernematidae		*Neosteinernema longicurvicauda* (Nguyen and Smart, 1994) *Steinernema asiaticum* (Anis et al., 2002) *Steinernema carpocapsae* (Danilov, 1987) *Steinernema scapterisci* (Nguyen and Smart, 1990) *Steinernema cubanum* (Mráček et al., 1994) *Steinernema feltiae* (Wouts, 1980) *Steinernema glaseri* (Steiner, 1929) *Steinernema intermedium* (Poinar, 1985) *Steinernema litorale* (Yoshida, 2004) *Steinernema pakistanense* (Shahina et al. 2001) *Steinernema puertoricense* (Román and Figueroa, 1994) *Steinernema riobravis* (Cabanillas et al., 1994) *Steinernema ritteri* (De Doucet and Doucet, 1990) *Steinernema scapterisci* (Nguyen and Smart, 1990) *Steinernema sichuanense* (Mrácek et al., 2006) *Steinernema weiseri* (Mrácek et al., 2003) *Steinernema* sp. (Mráček and Růžička, 1990)
		Family Tylenchulidae	*Paratylenchus dianthus* (D'errico, 1980)	
Class Enoplea				
Order Dorylaimida	Suborder Nygolaimina	Family Longidoridae	*Xiphinema* sp. (Jatala, 1975) *Xiphinema insigne* (Jairajpuri & Bajaj, 1978)	

Taxonomic classification was done according De Ley et al. (2006) and for this species * Abolafia and Peña-Santiago (2017).

some authors on the possible factors or causes suspected to be involved in this phenomenon. The likely causes explained so far are limitation of food availability, high nematode population density, low temperatures in stored populations, advanced maternal age, and dysfunction of the reproductive organs of female nematodes due to their senescence or metabolic alterations or even to physiological factors, as pointed out by Pinochet (1978) and Perlaza and Lopez (1979) in root-knot nematodes as a result of the formation of a plug by the gelatinous matrix that renders the female nematode unable to discharge additional eggs.

Although the aim of this article is not to discuss the causes of matricidal hatching, most of the previously mentioned factors may explain the occurrence of matricidal hatching in our population, such as the limitation of food availability, the high nematode population densities, the low temperatures in stored populations, and the likely advanced age in the female nematodes. Nevertheless, further studies would be necessary to ascertain and understand the real causes of intrauterine egg development and hatching in *B. xylophilus*.

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