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Short communication

Bursaphelenchus pinophilus Brzeski & Baujard, 1997 (Nematoda: Parasitaphelenchinae) associated with nematangia on *Pityogenes bidentatus* (Herbst, 1783) (Coleoptera: Scolytinae), from the Czech Republic

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The occurrence of *Bursaphelenchus* species in the Czech Republic is poorly known, the first report of the genus being made by Kubátová *et al*. (2000) who reported the association of *B. eremus* with the hyphomycetous microfungus, *Esteya vermicola*, and the bark beetle, *Scolytus intricatus*, collected from *Quercus robur*, in central Bohemia. To date, four other species have been reported from the country, namely *B. fungivorus* (Braasch *et al.*, 2002), *B. hofmanni* (see Braasch, 2001), *B. mucronatus* (see Braasch, 2001) and *B. vallesianus* (Gaar *et al.*, 2006). More recently, a survey for *Bursaphelenchus* species associated with bark- and wood-boring insects in the Czech Republic identified *B. pinophilus* Brzeski & Baujard, 1997 from the Moravia region. Although this represents a new country record, it was also associated with nematangia on the hind wings of a new insect vector.

A total of 404 bark- and wood-boring insects were collected from declining or symptomatic trees and screened for the presence of *Bursaphelenchus*. Bark and longhorn beetles were captured manually after debarking parts of the trunk displaying symptoms of insect attacks. Longhorn beetle larvae were also collected together with logs cut from the trunk. Logs were kept at room temperature in the laboratory until insect emergence. Each adult insect was individually dissected in water and examined for nematodes. All nematodes resembling dauer juveniles of *Bursaphelenchus* were collected and identified by molecular characterisation using a region of ribosomal DNA (rDNA) containing the internal transcribed spacer regions ITS1 and ITS2. ITS-RFLP analyses using five restriction enzymes (*Alu*I, *Hae*III, *Hin*fI, *Msp*I, *Rsa*I) were performed to generate the species-specific profile according to Burgermeister *et al.* (2009). Species identification was also confirmed by morphological data after culture of the dauers on *Botrytis cinerea* Pers. ex Ft., growing in 5% malt extract agar.

During this survey, only species belonging to the Curculionidae, subfamily Scolytinae, revealed the presence of nematodes belonging to *Bursaphelenchus.* Dauers of this genus were found aggregated under the elytra in nematangia formed at the root of the hind wings (Fig. 1). The dauers were identified from 12 individuals of *Pityogenes bidentatus* (Herbst, 1783) (Coleoptera: Scolytinae) collected under the bark of *Pinus sylvestris* trunks. Each insect carried *ca* 10-100 dauers. The ITS-RFLP patterns of the dauers so obtained confirmed the identification of *B. pinophilus* associated with this insect species.

Bursaphelenchus pinophilus has been found mainly in Europe and has been reported from various countries such as Poland (Brzeski & Baujard, 1997), Germany (Braasch, 2001), and Portugal (Penas *et al.*, 2007). The recent detection of this species associated with dead *P. koraiensis* in Korea (Han *et al.*, 2009) expands its geographical distribution and potential importance. It has been found associated only with *Pinus* species, but very little is known about the insect vector. The bark beetle, *Hylurgus ligniperda*, was initially suggested as the insect vector by Pe-

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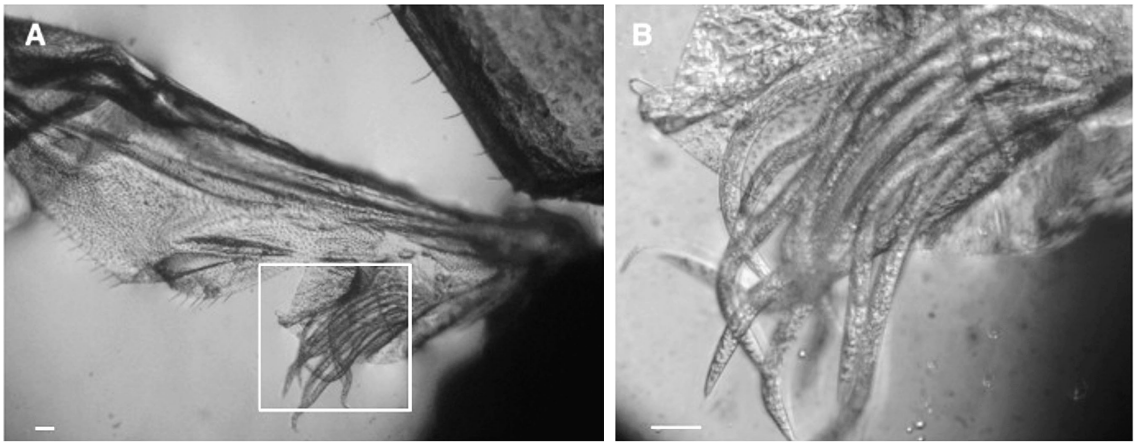

Fig. 1. Bursaphelenchus pinophilus *dauers associated with* Pityogenes bidentatus*. A: Light micrograph of dauers under insect vector hind wing; B: Enlarged view of delineated area in A, showing position of dauers. (Scale bars = 50 μm.)*

nas *et al.* (2006), although the nematode associated with this insect was later reclassified as *B. sexdentati* by morphological and molecular analysis (Penas *et al.*, 2007). According to the literature, *P. bidentatus* has been cited as a vector of *Ektaphelenchus* sp. (Kakuliya, 1966) in Georgia, and an unidentified nematode species in Spain (Roberston *et al.*, 2008). Interestingly, *B. pinophilus* was found in the nematangia formed at the root of the hind wings of *P. bidentatus.* Although this phenomenon is not so common in other *Bursaphelenchus* species, *B. rufipennis* has been found recently in such a structure on the hind wings of the insect *Dendroctonus rufipennis* (Kanzaki *et al*., 2008). Although other nematode species (*e.g*., *Ektaphelenchus* spp.) are frequently found associated within the same nematangia (see Kanzaki *et al*., 2008), in this particular case, only dauers of *B. pinophilus* were identified. The association between *B. pinophilus* and *P. bidentatus* represents the first report of this biological association and the association with the Scolytinae strengthens the tight and specific links between this group of *Bursaphelenchus* species and members of the Scolytinae (Ryss *et al.*, 2005).

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