Description of *Bursaphelenchus rockyi* n. sp. (Nematoda: Aphelenchoididae) in peat moss from Russia

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Summary – *Bursaphelenchus rockyi* n. sp., isolated in Peking, China, from peat moss imported from Russia, is described. It is characterised by a lateral field with four lines, excretory pore located at the base of the nerve ring or slightly posterior, spicule 15.9 (15.1–17.3) μm long along the chord and with high condylus and a rounded tip, rostrum triangular or conical with bluntly pointed tip, lamina curvature becoming more pronounced at 60% of total length, six caudal papillae with P3 and P4 adjacent to each other, bursa small, starting posterior to P4, vulval lips hemispherical and protruding, and tail conical with finely rounded or mucronated terminus. The new species belongs to the *fungivorus*-group and is most similar to *B. arthuri*, *B. arthuroides*, *B. fungivorus* and *B. seani*, but can be distinguished from related species by morphological and sequencing results.

Keywords – *fungivorus*-group, molecular, morphology, morphometrics, new species, peat moss, phylogeny, SEM, taxonomy.

The economic significance of the pine wood nematode (*Bursaphelenchus xylophilus* (Steiner & Buhner, 1934) Nickle, 1970) and the red ring nematode (*B. cocophilus* (Cobb, 1919) Baujard, 1989), two serious pests of pines and palms, respectively, has recently generated great research interest in the genus. Due to globalisation and extensive international trade, the threat of introducing exotic species through trading material has increased. *Bursaphelenchus* currently consists of 125 mostly mycophagous species inhabiting newly dead wood or soil and utilising various groups of insects as their vectors (= transportation carrier without a direct nutritional relationship) (Kanzaki & Giblin-Davis, 2018). In recent years, many known and new species of *Bursaphelenchus* have been intercepted in China (Gu *et al.*, 2014; Wang *et al.*, 2018) and therefore imported materials are treated with great caution in order to detect the possible presence of pest species.

During nematological inspections by the plant quarantine service in Peking, China, an undescribed species of *Bursaphelenchus* Fuchs, 1937 was detected in peat moss imported from Russia. Preliminary investigations indi-

cated that this unknown species belong to the *fungivorus*-group of *Bursaphelenchus*. Literature searches revealed that several species had been reported from culture media (Braasch *et al.*, 2002; Čermák *et al.*, 2014) and so we performed detailed morphological and molecular analysis of the intercepted species. A comparative study of the *fungivorus*-group species indicated that this *Bursaphelenchus* was a new taxon which is described herein as *B. rockyi* n. sp.

Materials and methods

NEMATODE ISOLATION AND MORPHOLOGICAL OBSERVATIONS

Nematodes were extracted by a modified Baermann funnel technique for 24 h and multiplied successfully on *Botryotinia fuckeliana* (de Bary) Whetz. growing on potato dextrose medium for 2 weeks at 25°C. Measurements were made from the fungal-cultured specimens fixed in hot formalin and processed to glycerin follow-

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ing the method of Seinhorst (1959). Light micrographs were made using a Zeiss Imager M2 microscope equipped with a Zeiss Axiocam 506 mono CCD camera. For the SEM examination, the nematodes were fixed in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde, washed three times in 0.1M cacodylate buffer, post-fixed in 1% osmium tetroxide, dehydrated in a series of ethanol solutions and critical point-dried with CO₂. After mounting on stubs, the samples were coated with gold (Maria *et al.*, 2018). SEM micrographs were taken with a Hitachi SU8010 scanning electron microscope.

MOLECULAR ANALYSES

DNA samples were prepared according to Li *et al.* (2008). Three sets of primers (synthesised by Invitrogen, Shanghai, China) were used in the PCR analyses to amplify the partial 18S, ITS and 28S D2-D3 region of rDNA. Primers for amplification of the 18S region were forward primer K4f and reverse primer K1r (Penas *et al.*, 2006). Primers for amplification of the ITS region were forward primer F194 (Ferris *et al.*, 1993) and reverse primer 5368r (Vrain, 1993). Primers for amplification of the 28S D2-D3 region were forward primer D2A and reverse primer D3Br (De Ley *et al.*, 1999). PCR conditions were as described by Ye *et al.* (2007) and Li *et al.* (2008). PCR products were separated on 1% agarose gels and visualised by staining with ethidium bromide. PCR products of sufficiently high quality were purified for cloning and sequencing by Invitrogen, Shanghai, China.

The sequences of the near full length 18S, full length ITS and 28S D2-D3 region of *B. rockyi* n. sp. were compared with those of other *Bursaphelenchus* species available in GenBank using the BLAST homology search program. The selected sequences were aligned by MAFFT (Katoh & Standley, 2013) with default parameters. The alignments of sequences were manually edited and assembled in one dataset by using AliView (Larsson, 2014). The best-fitted model of DNA evolution was obtained using jModelTest2 (Darriba *et al.*, 2012) with the Akaike Information Criterion (AIC) and with GTR + I + G model for 18S region partition, ITS partition and 28S D2-D3 partition. BI analyses were done under a general time reversible of invariable sites and a gamma-shaped distribution (GTR + I + G).

The concatenated tree topology for rDNA genes was confirmed using MrBayes 3.2.3 (Ronquist & Huelsenbeck, 2003) with four chains (three heated and one cold). Model parameters were unlinked and the overall rate was allowed to vary across partitions. The number of

generations for the total analysis was set to 20×10^6 , with the chain sampled every 1000 generations, and the burn-in value was 25%. The Markov chain Monte Carlo (MCMC) method within a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic trees using 50% majority rule (Larget & Simon, 1999). The consensus trees were selected to represent the phylogenetic relationships with branch length and support level and visualised using TreeGraph 2 (Stöver & Müller, 2010).

Results

*Bursaphelenchus rockyi** n. sp. (Figs 1-3)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female

Body slightly arcuate ventrally when heat-relaxed. Cuticle marked by fine transverse striations, *ca* 1 μ m wide. Lateral field *ca* 2-3 μ m wide with four incisures, the inner two lines slightly narrower at mid-body, inner two lines sometimes fused, beginning between head and median pharyngeal bulb and extending nearly to tail. Lip region convex, offset. Stylet with small basal swellings, conus *ca* 40% of total length. Procorpus cylindrical. Median bulb well developed, rounded to oval-shaped, with conspicuous valve plates. Dorsal pharyngeal gland orifice opening into lumen of metacarpus mid-way between anterior end of metacarpal valve and anterior end of metacarpus. Pharyngeal gland lobe *ca* two body diam. long, overlapping intestine dorsally. Nerve ring located *ca* one metacarpal valve length posterior to metacarpus. Excretory pore located at base of nerve ring or slightly posterior. Hemizonid posterior to excretory pore, *ca* 60-93 μ m from head. Gonad single, outstretched, on right of intestine, comprising ovary, oviduct, spermatheca, crustaformeria, uterus, vagina and post-uterine sac. Developing oocytes arranged in multiple rows in anterior part of ovary, well developed oocytes arranged as single

* The specific epithet is a patronym named in honour of Rocky, the son of the first author, who was born in the same year that the species was discovered.

Fig. 1. *Bursaphelenchus rockyi* n. sp. A: Entire male; B: Entire female; C: Anterior body region; D: Vulval region; E, F: Female tail; G: Spicule; H: Male tail in lateral view; I: Male tail; J: Lateral lines. P + number = genital papillae (Scale bars = 10 μ m.)

Fig. 2. Light photomicrographs of *Bursaphelenchus rockyi* n. sp. A: Entire body of male; B: Entire body of female (V = vulva); C: Anterior region arrow showing position of excretory pore (exp); D: Vulval region; E, F: Female tail (a = anus); G: Lateral lines; H-L: Male tails (P + number = genital papillae). (Scale bars = 10 μ m.)

Fig. 3. Scanning electron micrographs of *Bursaphelenchus rockyi* n. sp. female. A: Head region; B: Lateral region and female tail tip; C: Male tail (with papillae P2-P4); D: Lateral field at mid-body. (Scale bars: A = 20; B, C = 10; D = 5 μ m.)

to double rows in posterior part of ovary. Oviduct tube-like, connecting ovary and crustaformeria. Spermatheca formed by distinctive thick tissue as an expansion of gonad, slightly ellipsoidal, sometimes filled with well-developed sperm. Crustaformeria inconspicuous, formed by relatively large cells, appearing somewhat glandular. Uterus short with a thick wall, sometimes containing a developing egg and several sperm. Vagina inclined anteriorly, vulva a transverse slit in ventral view, vulval lips hemispherical, slightly protruding, not forming vulval flap. A pair of three-celled structures in uterus nearest vagina (uterus/post-uterine sac junction) observed. Post-uterine sac well developed, extending for 23.2–50.3% of vulva to anus distance, sometimes filled with sperm. Anus appearing as a long transverse slit in ventral view. Tail conical, gradually tapering to distal end and forming a finely rounded terminus, sometimes mucron-like due to presence of ventral depression near tail.

Male

Body cylindrical, ventrally arcuate when heat-relaxed. Anterior body region and cuticle similar to female. Testis occupying ca 60% of body length, outstretched (sometimes anteriorly reflexed). Spermatocytes arranged in multiple rows. Tail ca two anal body diam. long, appearing claw-like at terminus in lateral view. Posterior cloacal lip slightly protruding. Spicules paired, not fused, wide in middle region with clear dorsal and ventral limbs. Condylus high with rounded tip, rostrum triangular or conical with bluntly pointed tip, capitulum with truncate depression near calomus/rostrum junction, lamina curvature becoming more pronounced at 60% of total length, distal end of spicules smooth, broadly rounded and lacking a cucullus. Six caudal papillae present comprising one ad-cloacal subventral papillae pair (P2), one postcloacal sub-ventral pair (P3) located in terminal region of tail, and an-

other subventral pair (P4) located slightly posterior to P3. Bursal flap small, starting posterior to P4, narrow oblong to irregular oval shaped in ventral view. Single precloacal papilla (P1) not observed.

TYPE HABITAT AND LOCALITY

The type material was isolated from a peat moss sample imported from Russia on September, 2017 and inspected at the Institute of Plant Quarantine, Chinese Academy of Inspection and Quarantine, P.R. China. Nematodes were cultured on a lawn of *Botryotinia fuckeliana* and it is this material that was designated as type.

TYPE MATERIAL

Holotype female, 16 male and 17 female paratypes (slide numbers WX-1 to WX-14) deposited in the nematode collection of Ningbo Entry-Exit Inspection and Quarantine Bureau, P.R. China. Three paratype females and three paratype males (T-7106p) deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, MD, USA.

DIAGNOSIS AND RELATIONSHIPS

Bursaphelenchus rockyi n. sp. is characterised by the lateral field with four incisures, short body length of 571 (518–629) μ m and 533 (480–565) μ m for females and males, respectively, excretory pore located at the base of the nerve ring or slightly posterior, spicule 15.9 (15.1–17.3) μ m long, condylus high with a rounded tip, rostrum triangular or conical with bluntly pointed tip, lamina curvature becoming more pronounced at 60% of total length, distal end of spicules rounded, cucullus absent, six caudal papillae present, P3 and P4 being

Table 1. Morphometrics of *Bursaphelenchus rockyi* n. sp. All measurements are in μm and in the form: mean $\pm$ s.d. (range).

Character	Female		Male
	Holotype	Paratypes	Paratypes
n	–	20	20
L	573	571 $\pm$ 31.3 (518-629)	533 $\pm$ 22.3 (480-565)
a	28.7	25.5 $\pm$ 1.5 (22.6-28.8)	27.9 $\pm$ 1.8 (24.6-31.9)
b	8.2	9.1 $\pm$ 1.1 (7.1-11.5)	7.8 $\pm$ 1.0 (6.2-10.5)
b'	6.1	6.4 $\pm$ 0.7 (5.2-8.3)	5.7 $\pm$ 0.8 (4.8-7.6)
c	17.9	17.1 $\pm$ 1.6 (14.3-19.8)	20.9 $\pm$ 1.7 (17.2-24.4)
c'	3.9	3.5 $\pm$ 0.5 (2.9-4.5)	2.2 $\pm$ 0.2 (1.9-2.6)
V or T	75.8	76.4 $\pm$ 1.6 (72.2-78.8)	61.5 $\pm$ 3.1 (55.4-66)
Max. body diam.	20.0	22.5 $\pm$ 2.0 (18-26)	19.2 $\pm$ 1.5 (15.8-22)
Lip diam.	6.9	7.1 $\pm$ 0.3 (6.6-7.7)	7.0 $\pm$ 0.3 (6.5-7.8)
Lip height	3.1	2.8 $\pm$ 0.4 (1.7-3.3)	2.9 $\pm$ 0.4 (2.3-3.6)
Stylet length	14.5	14.1 $\pm$ 0.8 (12.1-15.5)	13.5 $\pm$ 0.5 (12.5-14.9)
Median bulb length	16.8	15.7 $\pm$ 0.9 (14.1-17.5)	15.0 $\pm$ 0.6 (13.9-16.6)
Median bulb diam.	11.0	11.8 $\pm$ 1.0 (10.1-13.4)	10.8 $\pm$ 0.6 (9.2-11.5)
Excretory pore	66	67 $\pm$ 4.3 (61-74)	70 $\pm$ 5.1 (62-78)
Spicule (chord)	–	–	15.9 $\pm$ 0.6 (15.1-17.3)
Spicule (curved median line)	–	–	12.1 $\pm$ 0.5 (11.2-13.0)
Ovary (anterior end to vulva) or testis length	280	292 $\pm$ 51.4 (221-368)	328 $\pm$ 25.6 (269-362)
Post-uterine sac length	46	40 $\pm$ 8.4 (24-60)	–
Post-uterine sac length/vulva to anus (%)	43.4	37.2 $\pm$ 7.7 (23.2-50.3)	–
Tail length	32.0	33.6 $\pm$ 2.8 (27.9-38.9)	25.7 $\pm$ 1.5 (22.5-28.1)
Anal or cloacal body diam.	8.2	9.6 $\pm$ 1.3 (7.3-12.4)	11.7 $\pm$ 0.6 (10.8-12.6)

adjacent to each other, a small bursa starting posterior to P4, vulval lips hemispherical and protruding, and tail conical with finely rounded or mucronate terminus.

Based on the presence of four incisures in the lateral field, spicule morphology (broad spicules with highly rounded apex, conspicuous ventral and dorsal limb, rounded distal end without cucullus), and the number and arrangement of the male caudal papillae (Braasch *et al.*, 2009), the new species belongs to the *fungivorus*-group.

Currently, the *fungivorus*-group contains the following 16 species: *B. hunti* (Steiner, 1935) Giblin-Davis & Kaya, 1983, *B. sychnus* Rühm, 1956, *B. steineri* Rühm, 1956, *B. fungivorus* Franklin & Hooper, 1962, *B. gonzalezi* Loof, 1964, *B. seani* Giblin-Davis & Kaya, 1983, *B. thailandae* Braasch & Braasch-Bidasak, 2002, *B. arthuri* Burgermeister, Gu & Braasch, 2005, *B. willibaldi* Schönfeld, Braasch & Burgermeister, 2006, *B. braaschae* Gu & Wang, 2010, *B. kiyoharai* Kanzaki, Maehara, Aikawa, Masuya & Giblin-Davis, 2011, *B. arthuroides* Gu, Wang & Zeng, 2012, *B. parathailandae* Gu, Wang & Chen, 2012, *B. tadamiensis* Kanzaki, Taki, Masuya & Okabe, 2012, *B. penai* Kanzaki, Giblin-Davis, Carrillo, Duncan & Gonzalez, 2014, and *B. kesiya* Kanzaki, Aikawa, Maehara & Thu, 2016.

Kanzaki *et al.* (2016) divided the *fungivorus*-group into four subgroups based on the morphology of the spicule and male tail. Based on the relatively wide spicule with clear dorsal and ventral limbs, the new species is very close to species of subgroup 4, i.e., *B. hunti*, *B. gonzalezi*, *B. fungivorus*, *B. seani*, *B. arthuri* and *B. arthuroides*. *Bursaphelenchus rockyi* n. sp. can be distinguished from *B. kiyoharai*, *B. penai*, *B. sychnus*, *B. steineri*, *B. thailandae*, *B. willibaldi*, *B. braaschae*, *B. parathailandae*, *B. tadamiensis* and *B. kesiya* by the male spicule morphology (wide spicule *vs* relatively narrow spicule).

Bursaphelenchus rockyi n. sp. can be distinguished from *B. hunti*, *B. fungivorus*, *B. seani*, *B. arthuri* and *B. arthuroides* by the number of male caudal papillae (six *vs* seven) and from *B. gonzalezi* by the male caudal papillae (six *vs* eight). In addition, the new species can be distinguished from *B. hunti* by the shorter body length of 571 (518-629) and 533 (480-565) μm for females and males, respectively, *vs* 590-830 and 620-740 μm , shorter post-uterine sac length (less than half *vs* over two-thirds of vulva to anus distance), spicule shape (condylus high *vs* condylus low), and number of lateral lines (four *vs* three).

The new species can be distinguished from *B. gonzalezi* by shorter body length of 571 (518-629) and 533

(480-565) μm for females and males, respectively, *vs* 690-1100 and 580-970 μm , female tail terminus shape (finely rounded *vs* sharply pointed) and spicule shape (high rounded condylus and a pointed long rostrum in middle position *vs* low condylus and short rostrum); from *B. fungivorus* by shorter body length of 571 (518-629) and 533 (480-565) μm for females and males, respectively, *vs* 980 (770-1160) and 850 (570-1030) μm (Braasch *et al.*, 2002a), stouter body ($a = 25.5$ (22.6-28.8) *vs* 36 (28-43)), female tail shape (straight with a finely rounded terminus *vs* slightly ventrally bent with a finely rounded terminus) and length ($c' = 3.5$ (2.9-4.5) *vs* 6-7), and spicule shape (rostrum bluntly pointed *vs* sharply pointed); from *B. seani* by shorter body length of 571 (518-629) and 533 (480-565) μm for females and males, respectively, *vs* 1210 (770-1350) and 1030 (660-1290) μm , shorter tail length of 33.6 (27.9-38.9) and 25.7 (22.5-28.1) μm *vs* 71 (54-82) and 45 (22-57) μm for females and males, respectively, and female tail terminus shape (finely rounded *vs* sharply pointed); and from *B. arthuroides* by female tail shape (straight with a finely rounded terminus that is slightly ventrally bent *vs* distinctly ventrally bent with a finely pointed terminus) and length ($c = 17.1$ (14.3-19.8) *vs* 12.6 (1.0-15.9)), male papillae (six *vs* seven), shorter body length of 571 (518-629) and 533 (480-565) μm for females and males, respectively, *vs* 834 (726-1010) and 705 (653-779) μm , and stouter body ($a = 25.5$ (22.6-28.8) *vs* 31.5 (27.3-35.9)).

The new species is most similar to *B. arthuri*, from which it differs by male spicule length of 12.1 (11.2-13.0) *vs* 19.4 (16.0-20.9) μm , shorter body length of 571 (518-629) and 533 (480-565) μm for females and males, respectively, *vs* 962 (827-1069) and 923 (882-980) μm , stouter female body ($a = 25.5$ (22.6-28.8) *vs* 33.9 (31.2-35.7)), shorter post-uterine sac length (less than half *vs* about two-thirds of the vulva to anus distance), and shorter female tail ($c' = 3.5$ (2.9-4.5) *vs* 4.8 (4.2-5.4)).

MOLECULAR PROFILES AND PHYLOGENETIC STATUS

The partial 18S region, the full ITS and the 28S D2-D3 region sequences of *B. rockyi* n. sp. were deposited in GenBank with the accession numbers MH393459 (1669 bp), MH396468-MH396471 (1001-1007 bp) and MH396441 (726 bp), respectively. A total of 90 aphelenchid taxa (Supplementary Table S1) were selected to reconstruct the phylogenetic tree using *Aphelenchoides besseyi* as outgroup. The concatenated dataset was composed of 4643 total characters (1769 characters for partial 18S region, 1973 characters for full length of ITS and

Fig. 4. Phylogenetic relationships of *Bursaphelenchus rockyi* n. sp. and aphelenchid nematodes based on concatenated dataset (partial 18S rDNA + full length of ITS rDNA + partial 28S D2-D3 rDNA). The 10 001st Bayesian tree was inferred under GTR + I + G model; *Aphelenchoides besseyi* served as the outgroup species. Posterior probability values exceeding 50% are given on appropriate clades. The new species is in bold font.

901 characters for 28S D2-D3 region) after aligning and manually editing. In the phylogenetic concatenated tree (Fig. 4), *B. rockyi* n. sp. clusters with four closely related species: *B. arthuri*, *B. arthuroides*, *B. fungivorus*, and *B. seani*, which form a well-supported clade (100 posterior probability) with other species of the *fungivorus*-group. The 18S comparison of pairwise sequence identities between the new species and *B. arthuroides* (HQ599188), *B. fungivorus* (AY508016) and *B. seani* (AY508029) were 98.1, 97.3 and 96.8%, respectively. For the 28S D2-D3, the identities of *B. arthuri* (AM396564), *B. arthuroides* (HQ599190), *B. fungivorus* (AY508082, JN903921) and *B. seani* (AY508098, AY508097) were 90.3, 90.4, 88 and 84.7%, respectively. The ITS identities with *B. arthuri* (EU783918, AM157742), *B. arthuroides* (HQ599189), *B. fungivorus* (KF241745-47, HQ402559, AM179516) and *B. seani* (AM157745) were 90.3, 90.4, 88 and 84.7%, respectively. The molecular identities showed that the new species is distant from the related species and supports the status of *B. rockyi* n. sp. as a new species.

Discussion

The host range of the *fungivorus*-group nematodes is relatively complex. Members of this group have been detected in wood packaging materials (*B. arthuri*, *B. willibaldis*, *B. brasschae*, *B. thailandae*, *B. arthuroides*, *B. parathailandae* and *B. kesiyae*), plant tissues (*B. huntii* and *B. fungivorus*), rhizosphere soil (*B. gonzalezi*) and insects (*B. seani*, *B. tadamiensis*, *B. penai* and *B. kiyoharai*). This is the first species of this group to be originally described from peat moss samples. It is also possible that the host plant of the nematode grows in the peat moss. Peat moss is the dead fibrous material that forms when mosses and other living material decompose in peat bogs (Carrol, 2018). Previously, Braasch *et al.* (2002) reported *B. fungivorus* from a culturing medium composed of coniferous fibrous material and mentioned that the coniferous material was imported from Russia. The world's largest peat bogs resources are in the Russian Western Siberian Lowlands (Fraser & Keddy, 2005). In this context, it can be hypothesised that some of the Russian woodlands may be harbouring *Bursaphelenchus* species.

The insect associations for this group are unknown, only a few species have been reported associated with soil-inhabiting bees, wasps and beetles (Giblin-Davis & Kaya, 1983; Arias *et al.*, 2005; Kanzaki *et al.*, 2011, 2014a).

Since this species was reported from culturing medium the insect associates and relevant biology are unknown. As *Bursaphelenchus* is a mycetophagous genus, wood products with rapid colonisation of fungi could act as an ideal substrate for maintaining species of this genus. This indicates a potential way of introducing invasive species to new places, and we emphasise the need for additional work to improve the treatment procedures of all imported materials in order to avoid the dissemination of potentially damaging exotic species.

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Supplementary Table S1. Sequences of nematode species used for the phylogenetic analyses.

Species	18S	ITS	28S D2-D3
<i>Aphelenchoides besseyi</i>	AY508035	EF371500	AY508109
<i>Bursaphelenchus abietinus</i>	AY508011	EU707454	AY508074
<i>B. abruptus</i>	AY508010	AM400244	AY508073
<i>B. africanus africanus</i>	JF317266	AM397025	AM397024
<i>B. africanus rossicus</i>	–	HM623783	AM623784
<i>B. anamurius</i>	–	EU589048	FJ643489
<i>B. anatolius</i>	AY508025	–	AY508093
<i>B. andrassyi</i>	KF164829	KF164830	KF164833
<i>B. antoniae</i>	AM279709	AM279709	AM279710
<i>B. arthuri</i>	AM397010	AM157742	AM396564
<i>B. arthuroides</i>	HQ599188	HQ599189	HQ599190
<i>B. borealis</i>	AY508012	AM179511	AY508075
<i>B. braaschae</i>	GQ845409	GQ845407	GQ845408
<i>B. burgermeisteri</i>	JF317267	EU034530	EU159109
<i>B. chengi</i>	KT599480	EU384702	EU107359
<i>B. clavicaudatus</i>	AB299221	AB299221	AB299222
<i>B. cocophilus</i>	AY509153	KT156782	AY508076
<i>B. conicaudatus</i>	AB067757	AM179513	AM396565
<i>B. corneolus corneolus</i>	JQ765873	JQ765879	JQ765871
<i>B. corneolus taiwanensis</i>	HQ407406	JQ765877	HQ407405
<i>B. crenati</i>	KU683736	KU683738	KU683737
<i>B. debrae</i>	–	–	EF488813
<i>B. decraemerae</i>	MF800855	MF800857	MF807220
<i>B. doui</i>	AB299224	AM157743	AM396567
<i>B. eggersi</i>	AY508013	AM160663	AY508078
<i>B. eremus</i>	–	AM180515	AM396568
<i>B. fagi</i>	–	JX683685	JX683686
<i>B. firmae</i>	AB650015	AB663192	AB650014
<i>B. fraudulentus</i>	AY508015	AM179517	AY508081
<i>B. fungivorus</i>	AY508016	AM179516	AY508082
<i>B. geraerti</i>	MG264562	MG264563	MG264565
<i>B. gerberae</i>	AY508024	AM400238	AY508092
<i>B. gillanii</i>	KJ653442	KC347020	KJ653443
<i>B. hellenicus</i>	AY508017	AM400239	AY508083
<i>B. hildegardae</i>	AM397013	AM269736	AM396569
<i>B. hofmanni</i>	AY508018	AM180516	AY508084
<i>B. hylobianum</i>	AY508019	AM400240	KT806476
<i>B. kesiya</i>	LC087116	LC087116	LC087117
<i>B. kevinci</i>	AY753531	–	AY753532
<i>B. kiyoharai</i>	AB597255	–	AB597254
<i>B. koreanus</i>	JX154585	JX154583	JX154584
<i>B. luxuriosae</i>	AB097864	AM400245	AM396571
<i>B. macromucronatus</i>	–	EU256381	EU256382
<i>B. masseyi</i>	–	JQ287494	JQ287495
<i>B. mazandaranense</i>	JN153102	–	JN153103
<i>B. moensi</i>	MG205600	MF951103	MF952873
<i>B. mucronatus</i>	AY508023	AM400247	AM396573
<i>B. niphades</i>	AB849465	AB849470	AB849475
<i>B. obeche</i>	–	EU034529	EU159108
<i>B. okinawaensis</i>	AB358983	AB358983	AB358982
<i>B. osumiana</i> ¹⁾	AB918706	AB918706	AB918707

Supplementary Table S1. (Continued.)

Species	18S	ITS	28S D2-D3
<i>B. paraburgeri</i>	HQ727727	HQ727723	HQ727725
<i>B. paracorneolus</i>	AY508027	AM400241	AY508095
<i>B. paraluxoriosae</i>	JF966206	JF966205	JF966204
<i>B. parantoniae</i>	KT223041	KT223043	KT223042
<i>B. paraparvispicularis</i>	GQ421483	GQ421484	GQ429010
<i>B. parapinasteri</i>	KT878515	KT878517	KT878516
<i>B. parathailandae</i>	JN377724	JN377723	JN377722
<i>B. parvispicularis</i>	AB218829	AB218829	AB368537
<i>B. penai</i>	AB901293	–	AB901292
<i>B. piceae</i>	KT315781	KF772173	KF772174
<i>B. pinasteri</i>	AM397016	AM400242	AM396574
<i>B. pinophilus</i>	–	AM160664	–
<i>B. platzeri</i>	AY508026	AM400243	AY508094
<i>B. poligraphi</i>	AY508028	AM179512	AY508096
<i>B. populi</i>	HQ699855	FJ888483	FJ998281
<i>B. posteroovulus</i>	KF314804	KF314806	KF314807
<i>B. rainulfi</i>	AM397017	DQ257621	AM396575
<i>B. rockyi</i> n. sp.	MH393459	MH396468	MH396441
<i>B. rufipennis</i>	AB368529	–	AB368530
<i>B. sakishimanus</i>	LC027461	–	LC027462
<i>B. seani</i>	AY508029	AM157745	AY508098
<i>B. saudi</i>	KT806480	KT806481	KT806482
<i>B. sexdentati</i>	AY508031	AM269916	AY508103
<i>B. sinensis</i>	AB232162	EU400450	EU752257
<i>B. singaporensis</i>	AM397018	AM180514	AM396576
<i>B. sycophilus</i>	AB901291	–	AB901290
<i>B. taphrorychi</i>	–	–	MF422692
<i>B. tadamiensis</i>	AB635399	–	AB635398
<i>B. thailandae</i>	AM397019	DQ497183	AM396577
<i>B. tiliae</i>	–	KF736980	KF736955
<i>B. tokyoensis</i>	AB430445	AB430445	AB430446
<i>B. tryphloei</i>	–	FJ998282	FJ998283
<i>B. tusciae</i>	AY508033	AM269737	AY508104
<i>B. ulmophilus</i>	KR011752	KP331048	KP331049
<i>B. vallesianus</i>	AM397020	AM269922	AM396578
<i>B. willibaldi</i>	AM397021	AM180512	AM396579
<i>B. xylophilus</i>	AM397022	AM179515	AM396580
<i>B. yongensis</i>	AM397023	AM180513	AM396581

¹) *Bursaphelenchus osumiana* is a junior synonym of *B. wuae* (Huang & Ye, 2006; Kanzaki *et al.*, 2014b; Gu *et al.*, 2017).