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ORIGINAL ARTICLE

Phylogenetic analysis of *Plenodomus lingam* and *Plenodomus biglobosus* isolates in Hungary

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Abstract

Blackleg (stem canker) of crucifers is a globally important disease caused by multiple genetic subclades of the fungi *Plenodomus lingam* (syn.: *Leptosphaeria maculans*) and *Plenodomus biglobosus* (syn.: *Leptosphaeria biglobosa*). In our study, we monitored the geographical distribution of these two pathogen species from rapeseed growing areas in Hungary. Multiplex PCR identified 48.7% of the isolates as *Plenodomus biglobosus*, which indicates the non-recent introduction of the pathogen into Hungary. In addition, multi-locus analysis revealed low genetic diversity within the species, as all isolates were clustered to the *Plenodomus lingam* 'brassicae' and *Plenodomus biglobosus* 'brassicae' subclades. The low genetic diversity of a population generally means reduced adaptation potential, which is essential information in breeding and in developing more effective management strategies.

Keywords *Plenodomus lingam* · *Plenodomus biglobosus* · *Brassica napus* · *tub2* · ITS rDNA · LSU · *rpb2* · Molecular phylogeny

Introduction

Oilseed rape (*Brassica napus* L.) is one of the world's most important oilseed crops. Stem canker also known as 'blackleg' of Brassica crops is the most damaging disease causing yield loss worldwide (Fitt et al. 2008). Stem canker is caused by two closely related fungal species sharing a similar life pattern: *Plenodomus lingam* (Tode) Desm. and *Plenodomus biglobosus* (Shoemaker & H. Brun) Gruyter, Aveskamp & Verkley (Dilmaghani et al. 2009). Their virulence differs significantly, with the greater yield loss being attributed to *P. lingam* (Williams and Fitt 1999). In the early 2000s, the species were distinguished by morphological differences of pseudothecia and classified in the *L.* genus (Shoemaker and Brun 2001), but later studies suggested that they rather belong to the *P.* genus (de Gruyter et al. 2012; Wijayawardene et al. 2014).

In Hungary, only *P. lingam* was previously reported as the causal agent of blackleg, but the presence of *P. biglobosus* was also confirmed a few years ago (Bagi et al. 2020). Despite some divergence, the pathogens cannot always be distinguished by morphological characteristics (Williams and Fitt 1999). DNA-based identification is required for reliable identification (Rouxel et al. 2004) and subclade differentiation. *Plenodomus lingam* can be divided into two subclades, whereas *P. biglobosus* includes seven distinct subclades (Mendes-Pereira et al. 2003; Vincenot et al. 2008; Zou et al. 2019). The subclade classification is based on geographical distribution, natural host range and phylogenetic analysis (Zou et al. 2019). The *Plenodomus* isolates previously reported from oilseed rape belong to *P. lingam* 'brassicae' (Mendes-Pereira et al. 2003), *P. biglobosus* 'brassicae' (Liu et al. 2014), 'canadensis' (Van de Wouw et al. 2008; Dilmaghani et al. 2009), 'australensis' (Plummer et al. 1994; Voigt et al. 2005) and 'occiaustralensis' (Vincenot et al. 2008; Dilmaghani et al. 2009) subclades. As pointed out by King and West (2022), the distribution patterns of *P. biglobosus* subclades need to be mapped because *P. biglobosus*

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is becoming an increasingly important pathogen of oilseed rape. The rDNA sequences, partial small subunit nrDNA (18S, SSU) and partial large subunit nrDNA (28S, LSU) are highly conserved and can discriminate at the levels of orders and kingdoms (Balesdent et al. 1998; de Gruyter et al. 2009). Molecular analysis of the ITS regions and the 5.8S rRNA gene has been used for many years to estimate diversity and classify Ascomycota fungi to species level (White et al. 1990; Capote et al. 2012). In the subclade related studies, ITS rDNA has been used for the phylogenetic analysis and reliable subclade identification of both species (Zou et al. 2019). Fragments of β-tubulin 2 gene sequences were also involved in the cluster analysis (Mendes-Pereira et al. 2003), but these sequences have not been published in international databases (NCBI, ENA). In order to distinguish closely related *Plenodomus* species, the RNA polymerase II second largest subunit (*rpb2*) region, which is more informative and variable than the ITS region, was also analyzed (Chen et al. 2015). This region can indicate the differences at species level (Drehmel et al. 2008).

We aimed to monitor and determine the distribution of the *P. lingam* and the newly reported *P. biglobosus* in Hungary, in order to estimate the arising importance of the fungi in Central Europe. To elucidate the genetic diversity within the *Plenodomus* population infecting oilseed rape in Hungary, our goal was to observe the phylogenetic relationship among the *Plenodomus* isolates by multi-locus sequence analysis of ITS1-5.8S-ITS2 region, partial sequences of the 28S nrDNA (LSU), the β-tubulin 2 gene (*tub2*) and the RNA polymerase second largest subunit (*rpb2* region) and provide available sequence data in the NCBI.

Materials and methods

Collection of *Plenodomus* isolates

Leaf and stem tissues of oilseed rape with blackleg symptoms were collected for the survey in major producing counties across Hungary in commercial fields in 2017–2021. The fungus was induced to sporulate by incubating unsterilized, diseased tissues in humidity chambers during 2–3 days to allow cirri exudation from pycnidia. After formation of pycnidiospores, the cirrus was transferred with a sterile glass needle (Goh 1999) and suspended in sterile distilled water. Suspensions of conidia were transferred to PDA (potato dextrose agar, BioLab Zrt., Hungary) plates and incubated at 24 ± 1 °C temperature for 3–4 days. The cultures were purified by single-spore colony isolation. Growing hyphal tips from germinating conidia were transferred onto fresh PDA plates with the aid of a sterile dissection needle. Identification was initially based on morphological criteria, and cultures identified as *Plenodomus* spp. (Mitrovic et al. 2016) were grown on PDA plates and maintained at 4 °C.

Identification and selection of isolates for analysis

In five years, 308 Hungarian *Plenodomus* isolates (data not shown) were obtained and identified to species level by multiplex PCR with specific primers: LmacR, LmacF and LbigF (Liu et al. 2006). The rapid and simultaneous detection of species is performed by the length of specific bands of the target sequence: 331 bp for *P. lingam* isolates, and 444 bp for *P. biglobosus* isolates.

For the detailed phylogenetic analysis and comparison, 26 *P. lingam* and 17 *P. biglobosus* isolates were selected from different locations (Table 1). We aimed to include at least one *P. lingam* and one *P. biglobosus* isolate from each studied county. For two counties, this was not possible because we could not identify *P. biglobosus* at all. From these counties, two *P. lingam* isolates were chosen for the analysis.

DNA isolation, amplification and sequencing

Hyphae were collected from a PDA plate of each isolate. Then, genomic DNA was extracted using the cetyl-trimethyl-ammonium-bromide (CTAB) method (Maniatis et al. 1983), followed by chloroform/isoamyl alcohol (24:1, v/v) extraction and isopropanol precipitation. The concentration and the purity of the DNA were evaluated by NanoDrop™ Spectrophotometer.

The PCR-based assay was conducted by amplifying the 18S-28S rRNA region (a partial sequence of the 18S ribosomal gene, the ITS1 region, the 5.8S ribosomal gene, the ITS2 region and a partial sequence of the 28S ribosomal gene), other section of 28S nrDNA (LSU), part of the *tub2* gene and partial *rpb2* region. Amplifications of fragments were carried out in a total reaction volume of 50 µL containing 15 ng of genomic DNA, 0.2–0.2 µM forward and reverse primers, Dream*Taq* Green PCR Master Mix (2X) (Thermo Scientific™).

To amplify the 18S-28S region, PN3 and PN10 primers and PCR conditions were performed according Mitrovic et al. 2016. The length of the target sequence without primers was 507 bp for *P. lingam* and 535 bp at *P. biglobosus*. The LSU region was amplified with the primers LR0R and LR7 (Rehner and Samuels 1994) according to the protocol of Chen et al. 2015. The target sequence length without the primers was 1328 bp for *P. lingam* and 1327 bp for *P. biglobosus*. The part of the *tub2* gene was amplified with the primer pair Btub2Fd and Btub4Rd (Woudenberg et al. 2009) by amplification conditions of Chen et al. 2015. The target sequence length without primers was

Table 1 *Plenodomus* isolates used in this study with the accession numbers of the gene sequences

Isolate ID	Species	Plant organ	Geographical origin	Year	GenBank No			
					ITS1-5.8S-ITS2	partial *tub2*	partial LSU	partial *rpb2*
L7	*P. lingam*	Stem	Nagylózs (47°55′ S/16°76′ W)	2017	OM098459	OM291849	OM102999	OM273759
L11	*P. lingam*	Stem	Nagylózs (47°55′ S/16°76′ W)	2017	OM098460	OM291850	OM103000	OM273760
L26	*P. lingam*	Leaf	Vadosfa (47°49′ S/17°12′ W)	2018	OM098461	OM291851	OM103001	OM273761
L30	*P. lingam*	Leaf	Osli (47°63′ S/17°07′ W)	2018	OM098462	OM291852	OM103002	OM273762
L34	*P. lingam*	Leaf	Bősárkány (47°69′ S/17°23′ W)	2018	OM098463	OM291853	OM103003	OM273763
L37	*P. biglobosus*	Stem	Szalánta (45°93′ S/18°23′ W)	2018	OM098464	OM273742	OM103004	OM291875
L40	*P. lingam*	Stem	Szalánta (45°93′ S/18°23′ W)	2018	OM098465	OM291854	OM103005	OM273764
L55	*P. biglobosus*	Leaf	Kuncsorba (47°11′ S/20°55′ W)	2019	OM098466	OM273743	OM103006	OM291876
L59	*P. biglobosus*	Leaf	Kuncsorba (47°11′ S/20°55′ W)	2019	OM098467	OM273744	OM103007	OM291877
L61	*P. lingam*	Leaf	Törökszentmiklós (47°20′ S/20°44′ W)	2019	OM098468	OM291855	OM103008	OM273765
L63	*P. lingam*	Leaf	Dombóvár (46°35′ S/18°13′ W)	2019	OM098469	OM291856	OM103009	OM273766
L71	*P. biglobosus*	Stem	Harkány (45°86′ S/18°23′ W)	2019	OM098470	OM273745	OM103010	OM291878
L74	*P. biglobosus*	Stem	Harkány (45°86′ S/18°23′ W)	2019	OM098471	OM273746	OM103011	OM291879
L105	*P. biglobosus*	Leaf	Martonvásár (47°34′ S/18°80′ W)	2019	OM098472	OM273747	OM103012	OM291880
L108	*P. lingam*	Leaf	Martonvásár (47°34′ S/18°80′ W)	2019	OM098473	OM291857	OM103013	OM273767
L110	*P. lingam*	Leaf	Baranyajenő (46°27′ S/18°06′ W)	2020	OM098474	OM291858	OM103014	OM273768
L112	*P. biglobosus*	Leaf	Baranyajenő (46°27′ S/18°06′ W)	2020	OM098475	OM273748	OM103015	OM291881
L128	*P. biglobosus*	Stem	Felsőnána (46°45′ S/18°53′ W)	2020	OM098476	OM273749	OM103016	OM291882
L131	*P. lingam*	Stem	Felsőnána (46°45′ S/18°53′ W)	2020	OM098477	OM291859	OM103017	OM273769
L144	*P. biglobosus*	Stem	Jászboldogháza (47°35′ S/19°98′ W)	2020	OM098478	OM273750	OM103018	OM291883
L150	*P. biglobosus*	Stem	Jászboldogháza (47°35′ S/19°98′ W)	2020	OM098479	OM273751	OM103019	OM291884
L155	*P. biglobosus*	Leaf	Kiskunlacháza (47°17′ S/19°06′ W)	2021	OM098480	OM273752	OM103020	OM291885
L166	*P. lingam*	Leaf	Kiskunlacháza (47°17′ S/19°06′ W)	2021	OM098481	OM291860	OM103021	OM273770
L174	*P. biglobosus*	Leaf	Meződ (46°28′ S/18°09′ W)	2021	OM098482	OM273753	OM103022	OM291886
L183	*P. lingam*	Leaf	Meződ (46°28′ S/18°09′ W)	2021	OM098483	OM291861	OM103023	OM273771
L197	*P. lingam*	Leaf	Palé (46°26′ S/18°07′ W)	2021	OM098484	OM291862	OM103024	OM273772
L198	*P. lingam*	Leaf	Palé (46°26′ S/18°07′ W)	2021	OM098485	OM291863	OM103025	OM273773
L201	*P. lingam*	Leaf	Gyirmót (47°62′ S/17°58′ W)	2021	OM098486	OM291864	OM103026	OM273774
L206	*P. biglobosus*	Leaf	Gyirmót (47°62′ S/17°58′ W)	2021	OM098487	OM273754	OM103027	OM291887
L214	*P. lingam*	Leaf	Esztergom (47°78′ S/18°76′ W)	2021	OM098488	OM291865	OM103028	OM273775
L217	*P. lingam*	Leaf	Esztergom (47°78′ S/18°76′ W)	2021	OM098489	OM291866	OM103029	OM273776
L228	*P. lingam*	Leaf	Sántos (46°34′ S/17°88′ W)	2021	OM098490	OM291867	OM103030	OM273777
L233	*P. lingam*	Leaf	Sántos (46°34′ S/17°88′ W)	2021	OM098491	OM291868	OM103031	OM273778
L238	*P. lingam*	Leaf	Gyomaendrőd (46°89′ S/20°79′ W)	2021	OM098492	OM291869	OM103032	OM273779
L245	*P. lingam*	Leaf	Gyomaendrőd (46°89′ S/20°79′ W)	2021	OM098493	OM291870	OM103033	OM273780
L257	*P. biglobosus*	Leaf	Püski (47°88′ S/17°40′ W)	2021	OM098494	OM273755	OM103034	OM291888
L258	*P. lingam*	Leaf	Püski (47°88′ S/17°40′ W)	2021	OM098495	OM291871	OM103035	OM273781
L265	*P. lingam*	Leaf	Igal (46°52′ S/17°93′ W)	2021	OM098496	OM291872	OM103036	OM273782
L277	*P. lingam*	Leaf	Tamási (46°64′ S/18°33′ W)	2021	OM098497	OM291873	OM103037	OM273783
L281	*P. biglobosus*	Leaf	Kiszombor (46°18′ S/20°45′ W)	2021	OM098498	OM273756	OM103038	OM291889
L283	*P. lingam*	Leaf	Kiszombor (46°18′ S/20°45′ W)	2021	OM098499	OM291874	OM103039	OM273784
L294	*P. biglobosus*	Stem	Gyékényes (46°23′ S/16°99′ W)	2021	OM098500	OM273757	OM103040	OM291890
L306	*P. biglobosus*	Stem	Gyékényes (46°23′ S/16°99′ W)	2021	OM098501	OM273758	OM103041	OM291891

345 bp for *P. lingam* and 337 bp for *P. biglobosus*. To amplify partial region of *rpb2*, RPB2F (5′-AGGCTTGTG GTTTGGTCAAGA-3′) and the RPB2R (5′-ATCATAGCR GTCTCTTCCTCCT-3′), we designed primers based on sequences from the *P. lingam rpb2* region (Accession Nos. DQ470894; KT389669; KY064047; XM_003841144) and *P. biglobosus rpb2* region (Accession Nos. KY064037; MT683512). For *rpb2* region, thermal cycling conditions

consisted of denaturation at 95 °C for 3 min, followed by 35 cycles of the following steps: denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 45 s, with a final extension step at 72 °C for 10 min. The target sequence length for both species was 492 bp without primers.

Amplification products were analyzed first by gel electrophoresis on 1% agarose gel (Sigma) stained with ECO Safe Nucleic Acid Staining Solution (Biocenter), then visualized under UV light. Amplicons were purified by High Pure PCR Product Purification Kit (Roche) according to the manufacturer's protocol. Fragments of the 18S-28S were sequenced by PN10 primer, fragment of the LSU, fragment of the *tub2* gene, fragment of the *rpb2* region were sequenced in both directions using the primers for PCR, in an ABI Prism automatic sequencer (BaseClear B.V.). Sequences were manually checked, edited and compared to reference sequences deposited in the NCBI BLAST database (Altschul et al. 1999).

Phylogenetic analyses of the *P. lingam* and *P. biglobosus* isolates

Further phylogenetic analyses were carried out with the MEGA11 program package (Tamura et al. 2021). The trees were obtained by applying neighbor-joining algorithms to matrixes of pairwise distances estimated using the maximum composite likelihood (MCL) approach (Tamura et al. 2004). The tree is drawn to scale, with branch length measured by the number of substitutions per site. The clade stability was assessed with 1000 replicates of bootstrap values, and *Leptosphaeria doliolum* (CBS 505.75) was designated as the outgroup in all rooted trees.

Sequences of the ITS regions including the 5.8S gene of rDNA used for subclade identification (Mendes-Pereira et al. 2003) were compared to *P. lingam* strain CBS 275.63, *P. biglobosus* 'brassicae' UBIP01000001 genome, ITS1-5.8S-ITS2 sequences of the *P. lingam* subclades 'brassicae' (AJ550885; AJ550887), 'lepidii' (AJ550890) and the *P. biglobosus* subclades 'thlaspi' (AJ550891), 'australensis' (AJ550869; AJ550870), 'erysimii' (AJ550872), 'canadensis' (AJ550868; FJ172238; AJ550867), 'occiaustralensis' (AM410082), 'americensis' (MG321243) and 'brassicae' (DQ133890). For the multi-locus analysis (ITS1-5.8S-ITS2, partial LSU, partial *tub2* gene, partial *rpb2* region), the whole-genome isolates (*P. lingam* 'brassicae' CBS 275.63 and *P. biglobosus* 'brassicae' UBIP01000001) were included, because only these two isolates had published sequences from all these regions.

Table 2 Number of locations with the isolate number from 2017 to 2021

Year	Location (*n*)	*P. lingam* (*n*)	*P. biglobosus* (*n*)
2017	1	13	0
2018	6	29	12
2019	7	27	28
2020	3	4	40
2021	12	85	70

Results

Across all sites and surveillance years, 308 Hungarian *Plenodomus* isolates were identified: 158 isolates were *P. lingam* (51.3%), while *P. biglobosus* was detected in case of 150 isolates (48.7%) (Table 2). The newly reported *Plenodomus biglobosus* was identified from six new counties, so it can be concluded that the pathogen is widespread and common in Hungary.

Phylogenetic analysis

For phylogenetic analyses, ITS1-5.8S-ITS2 sequences (468 bp for *P. lingam* and 496 bp for *P. biglobosus*), partial LSU sequences (877–881 bp for *P. lingam* and 874–881 bp for *P. biglobosus*), partial *tub2* gene sequences (343–345 bp for *P. lingam* and 336–337 bp for *P. biglobosus*) and partial *rpb2* region sequences (492–493 bp for *P. lingam* and 492–493 bp for *P. biglobosus*) of the examined 43 isolates were generated and deposited in GenBank.

Phylogenetic tree based on ITS1-5.8S-ITS2 region

The ITS1-5.8S-ITS2 sequences of Hungarian isolates were 100% identical in all 26 *P. lingam* isolates with the reference isolate from UK (JF740234 from CBS275.63 complete genome). Similarly, the ITS1-5.8S-ITS2 sequences of the 17 *P. biglobosus* isolates were also 100% identical with the reference sequence (UBIP01000001) (Fig. 1). Based on these results, it can be stated that all *Plenodomus* isolates investigated in the present study could be classified into *P. lingam* 'brassicae' and *P. biglobosus* 'brassicae' subclades. This region is highly conserved, as it has been determined by Mendes-Pereira et al. (2003).

Phylogenetic tree based on multi-locus phylogenetic analysis

Sequences of the *P. lingam* isolates: the partial *tub2* gene sequences were 99.42–100%, the partial LSU sequences 99.43–100% and the partial *rpb2* region sequences

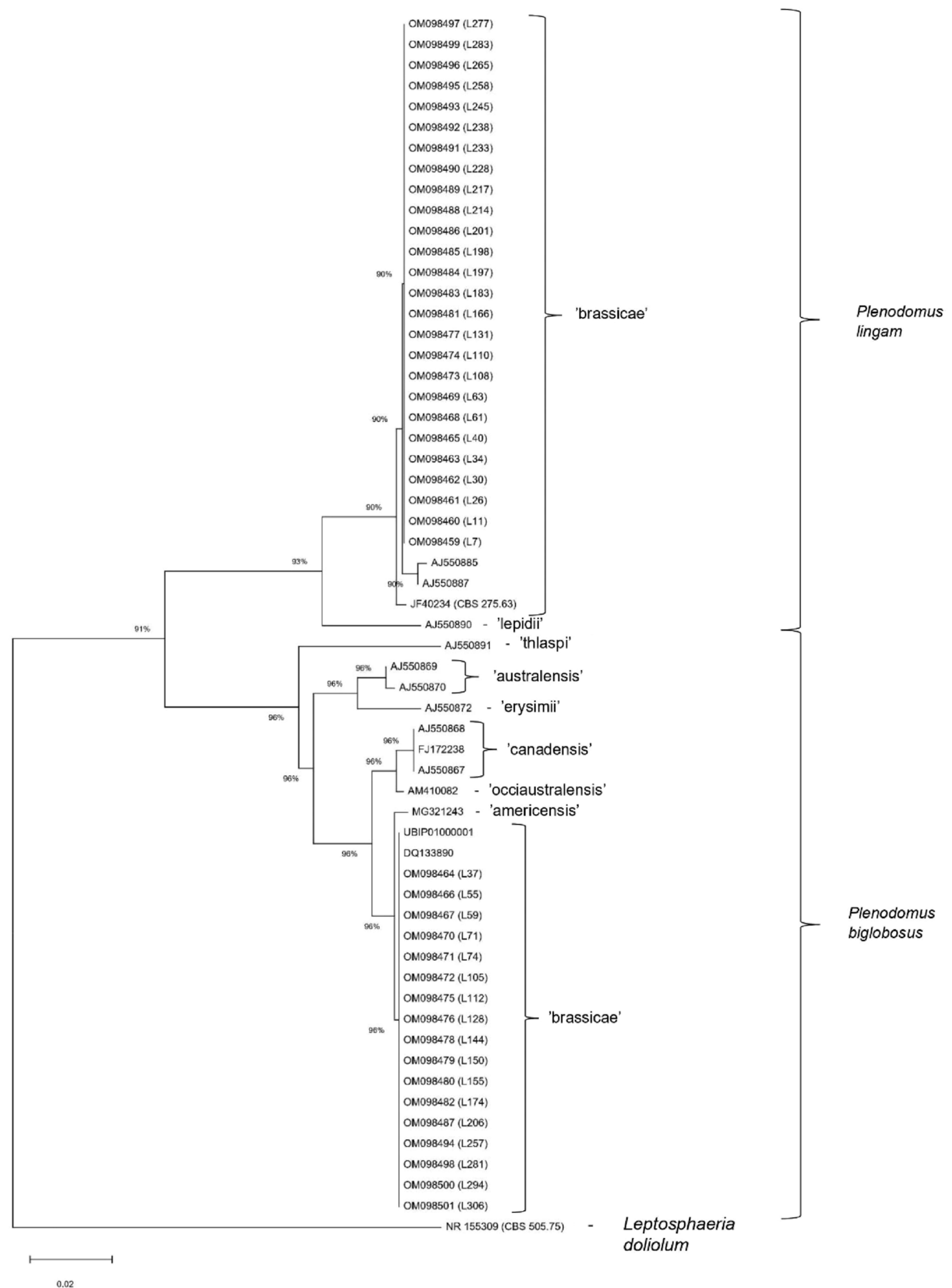

Fig. 1 Phylogenetic neighbor-joining tree based on ITS1-5.8S-ITS2 sequences of 26 isolates of *Plenodomus lingam* and 17 isolates of *Plenodomus biglobosus* from Hungary and isolates from NCBI database. The numbers are the percent bootstrap support for 1000 resampling and evolutionary analyses conducted in MEGA11. *Leptosphaeria doliolum* (CBS 505.75) was used as the outgroup

98.37–100% identical with the reference strain sequences (CBS 275.63). Sequences of the *P. biglobosus* isolates: the partial *tub2* gene sequences were 99.70–100%, the partial LSU sequences 99.09–100% and the partial *rpb2* region sequences 100% identical with the reference strain sequences (UBIP01000001) (Fig. 2). Noteworthy, these are the first sequence data in relation to the genes and regions of interest of the *P. lingam* and *P. biglobosus* in Hungary.

The combined four-locus data set consisted of 46 isolates with the whole-genome isolates of *P. lingam* 'brassicae' and *P. biglobosus* 'brassicae' and with *Leptosphaeria doliolum* as the outgroup taxon. In the tree (Fig. 2), the surveyed *P. lingam* and *P. biglobosus* isolates were clustered separately to their reference strains with 96% bootstrap support, respectively. Within species, we observed only parsimony-uninformative variability. The low genetic diversity among isolates in this investigation is not surprising, as the isolates belong to the same subclade (Fig. 1).

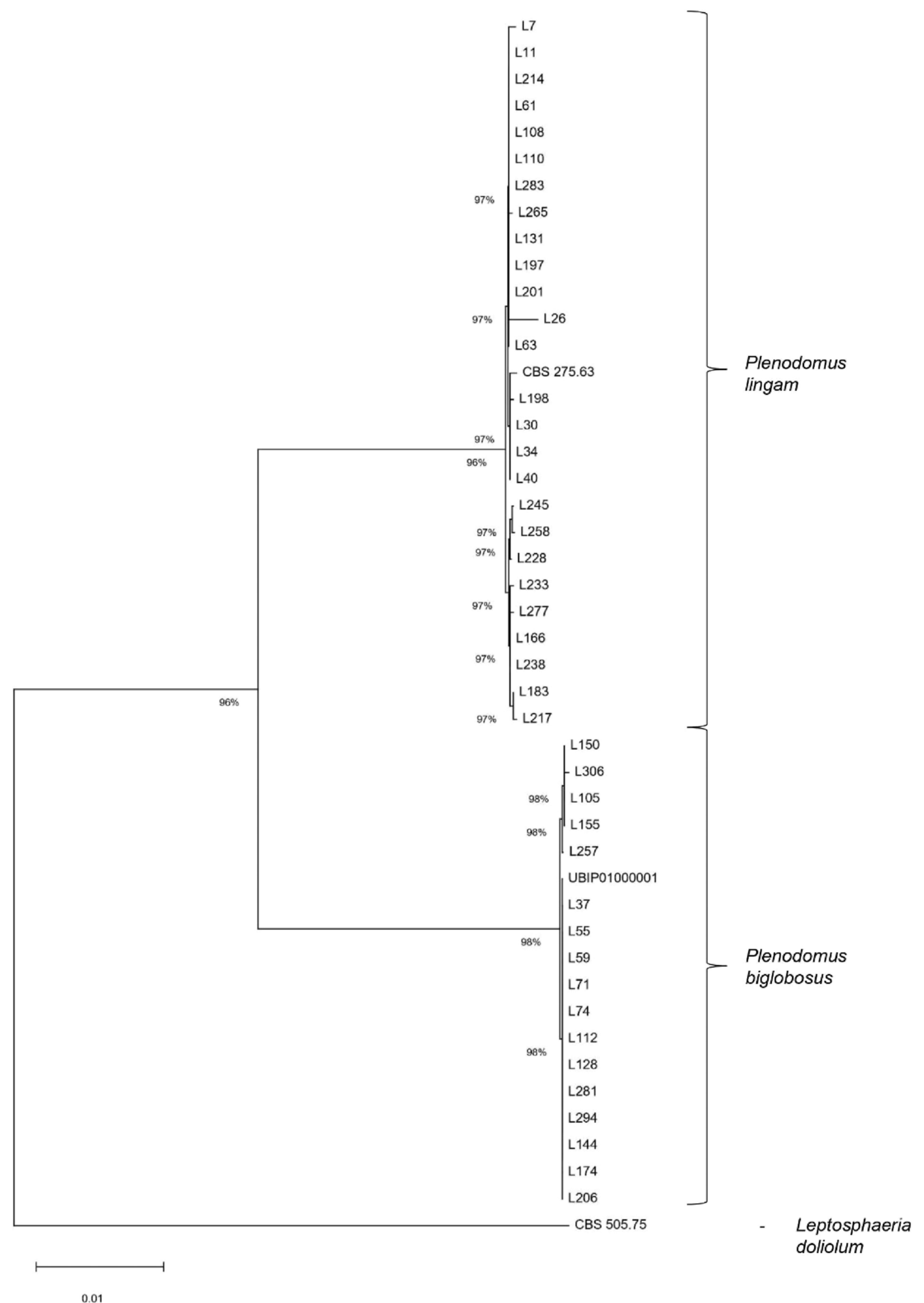

Fig. 2 Phylogenetic tree obtained from the combined partial *tub2* gene, ITS1-5.8S-ITS2, partial LSU, partial *rpb2* gene sequence alignment of 26 *Plenodomus lingam* and 17 *Plenodomus biglobosus* isolates under survey and reference strains. The tree was rooted using *Leptosphaeria doliolum* (CBS 505.75) as outgroup taxon. Bootstrap support values > 95% are indicated near the nodes

Discussion

Outbreak of a new pathogen or changes in genetic composition of a population could compromise the efficiency of established plant protection strategies. *Plenodomus biglobosus* was at first described in 2020 in Hungary (Bagi et al. 2020). The monitoring of new pathogens is of high importance as it can help to optimize breeding strategies and crop protection technologies (Huang et al. 2014). There is a lack of information about the distribution of *Plenodomus* species causing blackleg of brassicas in Central Europe; therefore, our goal was to identify and describe the local pathogens in oilseed rape cultivation. Based on the occurrence and frequency of *P. lingam* and *P. biglobosus*, it can be stated that *P. biglobosus* is more common and widespread in Hungary than previously thought.

Furthermore, we also tried to investigate the sources of variation in genetic diversity observed in the Hungarian *P. lingam* and *P. biglobosus* population. According to some views, in the UK additional genetic subclades may

be responsible for the growing importance of *P. biglobosus* (King and West 2022). Molecular identification and characterization clearly identified the Hungarian *P. lingam* isolates as members of the *P. lingam* 'brassicae' subclade. This subclade is distributed worldwide and can infect several *Brassica* species (Mendes-Pereira et al. 2003), while *P. lingam* 'lepidii' subclade has been only isolated from *Lepidium* sp. from Canada (Mendes-Pereira et al. 2003). Similarly, the analysis of Hungarian *P. biglobosus* isolates showed that all isolates belong to the *P. biglobosus* 'brassicae' subclade, admittedly. The subclade 'brassicae' that infects *Brassica* species (Mendes-Pereira et al. 2003) is the most widely distributed subclade of *P. biglobosus* (Liu et al. 2014). *Plenodomus biglobosus* 'canadensis' is the most closely related subclade to *P. biglobosus* 'brassicae' and has been isolated from oilseed rape and Chinese mustard (Van de Wouw et al. 2008; Dilmaghani et al. 2009). *Plenodomus biglobosus* 'australensis' (Voigt et al. 2005), 'occiaustralensis' (Vincenot et al. 2008) and 'americensis' (Zou et al. 2019) subclades can also infect *Brassica* species, incl. oilseed rape, while other subclades (Mendes-Pereira et al. 2003) 'thlaspi' (obtained from *Thlaspi arvense*) and 'erysimii' (isolated from *Erysimum* sp.) have not been reported from brassicas yet.

Molecular methods have been used in taxonomic studies of *Plenodomus* to reveal phylogenetic relationship among the species and subclades (Zou et al. 2019). Combined DNA phylogenetic analysis based on ITS, 28S nrDNA (LSU) and β-tubulin, sequences are often used to reconstruct these relationships.

Hungarian *P. lingam* and *P. biglobosus* isolates, based on four DNA regions, resulted consistent phylogenetic trees and the sequences were extremely similar to each other. The low molecular diversity among the *P. biglobosus* isolates also suggests that the pathogen was introduced to Hungary much earlier than it was first identified. Most likely its emergence has remained hidden due to very similar symptoms. These fungi coexist in hosts and cause leaf lesions, in addition *P. lingam* is associated with basal stem canker, while *P. biglobosus* rather causes upper stem lesions (Eckert et al. 2010; Sprague et al. 2017). Low genetic diversity in a population is more likely to mean reduced adaptation potential, which may also provide important information on the risk of fungicide resistance development.

Worldwide large-scale monitoring and surveys are required to prevent the extreme economic losses. Our results indicate that for both pathogens only the 'brassicae' subclades are present in the Hungarian populations at the moment. As a result of globalization, there is a risk that additional subclades will emerge in Hungary on oilseed rape, but in the meantime, it can be concluded that the importance of blackleg pathogens will not change in the near future.

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