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## A new species, *Lophiostoma versicolor*, from Japan (Pleosporales, Dothideomycetes)

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### Abstract

*Lophiostoma versicolor* sp. nov. was found on *Acer* sp. in Japan. This species is characterized by ascomata with a laterally compressed apex; clavate, 2(–4)-spored asci with a long stipe; and verruculose, 3-septate, versicolored ascospores without a sheath or appendages. Phylogenetic analyses based on LSU nrDNA sequences supported the generic placement and species validity of *L. versicolor*.

**Key words** – ITS – Lophiostomataceae – *Lophiotrema* – LSU nrDNA – Pleosporomycetidae – Systematics – Taxonomy

### Introduction

During an investigation of bitunicate ascomycetes in Japan, an unidentified fungus was found on dead twigs of *Acer* sp. The morphological characteristics of the fungus, such as the presence of ascomata with a compressed beak and clavate asci, recall those of *Lophiostoma* (Hirayama & Tanaka 2011) belonging to the Lophiostomataceae. This fungus, however, is different from any of the existing species of the genus because it possesses 2(–4)-spored asci and verruculose, 3-septate, versicolored ascospores without a sheath or appendages.

As a generic placement of our obtained fungus, *Lophiotrema* was also considered. This genus is morphologically similar to *Lophiostoma*. For example, they share several characteristics including their carbonaceous ascomata with a laterally compressed apex (termed as a crest-like beak with a slit-like ostiole), fissitunicate asci, and hyaline to dark brown, uni- to multiseptate ascospores (Holm & Holm 1988). Some authors treated *Lophiotrema* as a synonym of the older genus *Lophiostoma* (Chesters & Bell 1970, Leuchtmann 1985), whereas most authors regarded them as distinct but closely related genera within Lophiostomataceae on the basis of morphological observations (Barr 1992, Yuan & Zhao 1994, Tanaka & Harada 2003a, b).

Hirayama & Tanaka (2011) revealed that *Lophiostoma* and *Lophiotrema* are distinct genera belonging to Lophiostomataceae and Lophiotremataceae, respectively, based on phylogenetic analyses of small subunit (SSU) and large subunit (LSU) nuclear ribosomal DNA (nrDNA) sequences. They concluded that the ascus shape including the length of the ascus stipe is the most important morphological criterion to delineate both genera. In *Lophiostoma*, the asci are clavate

with a relatively long stipe [mostly (10–)15–30 µm long], whereas in *Lophiotrema*, the asci are cylindrical with a short stipe (up to 15 µm long; Hirayama & Tanaka 2011). According to these generic circumscriptions, the collected fungus might appear to be a member of *Lophiostoma* because it has clavate asci with a relatively long stipe.

The objectives of this study were to evaluate the taxonomic significance of ascus shape and ascus stipe length which were used to distinguish both genera (Hirayama & Tanaka 2011) using the newly obtained fungus and to confirm its species validity based on phylogenetic analyses of LSU sequences.

## Materials & Methods

### Isolates and morphology

The methods for microscopy and single spore isolation followed those described by Hirayama et al. (2010). For the ascospore septum position, the decimal system (Shoemaker 1984) was used. The holotype specimen was deposited in the Herbarium of Hirosaki University (HHUF). The growth rate and colony characteristics were recorded from cultures grown on corn meal agar (CMA, Difco), malt extract agar (MEA, Difco), and potato dextrose agar (PDA, Difco) after 3 weeks at 20 °C in the dark. Colors were designated as described by Kornerup & Wanscher (1978). To validate isolations, sporulation was promoted by placing a small piece of mycelial culture on rice straw agar (RSA; Tanaka & Harada 2003a). The utilized fungal culture was deposited in the National Institute of Agrobiological Sciences (MAFF).

### Phylogenetic analyses

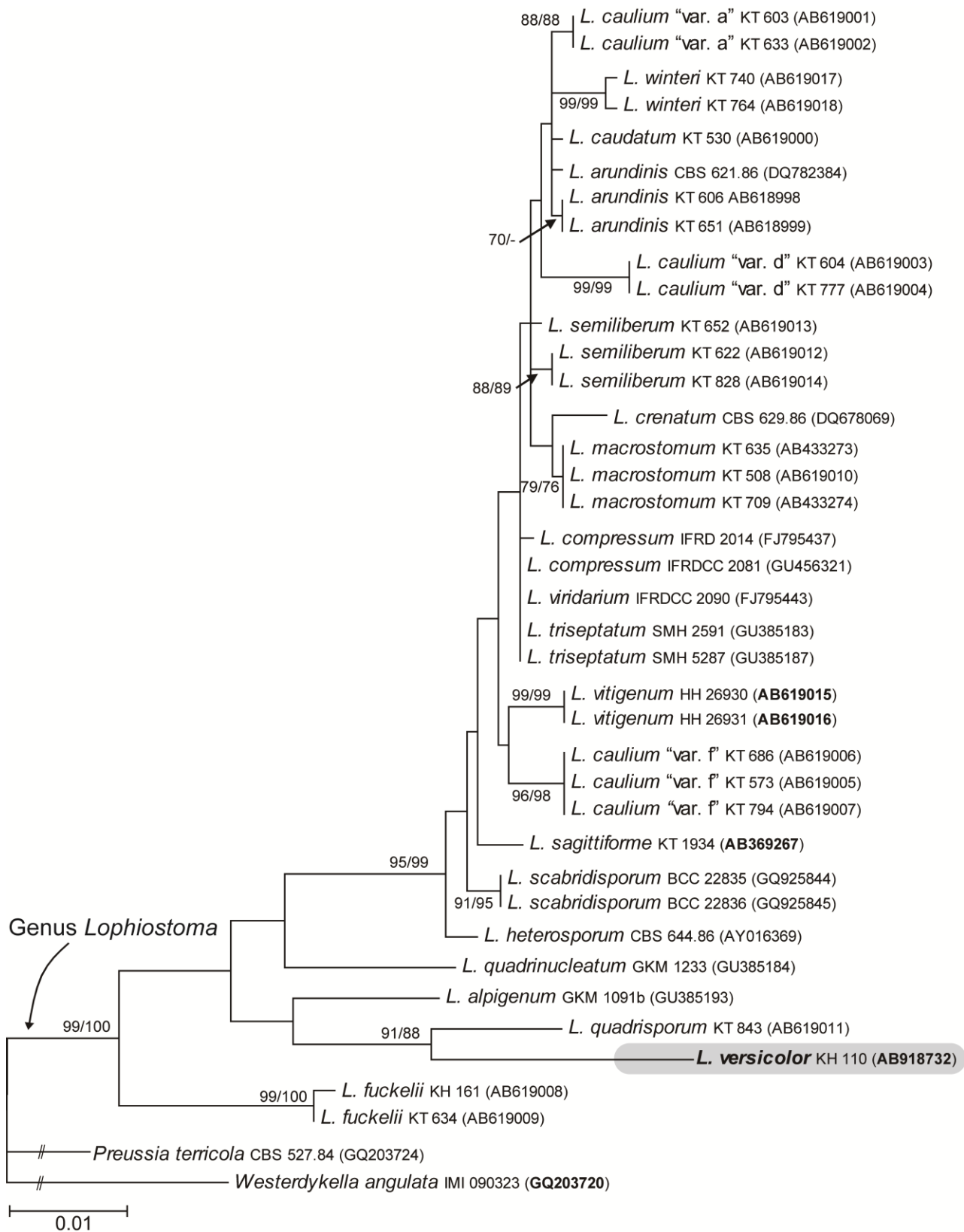
The detailed methods of DNA extraction and polymerase chain reaction (PCR) amplification were as those described by Tanaka et al. (2009). Partial LSU nrDNA and complete internally transcribed spacers (ITS) of nrDNA were amplified by PCR using the primer pairs LR0R–LR7 for LSU (Rehner & Samuels 1994) and ITS1–ITS4 for ITS (White et al. 1990). Newly obtained sequences were deposited in GenBank.

A BLAST search using the LSU sequence of the newly obtained taxon suggested that it is a member of *Lophiostoma* rather than *Lophiotrema*. Therefore, phylogenetic analyses of the collected fungus (*Lophiostoma versicolor*) with other *Lophiostoma* spp. were performed. The obtained LSU sequence of *L. versicolor* was manually aligned along with published sequences of *Lophiostoma* (e.g., Tanaka & Hosoya 2008, Mugambi & Huhndorf 2009, Suetrong et al. 2009, Zhang et al. 2009a, b, Hirayama & Tanaka 2011) in GenBank using the MUSCLE algorithm implemented in the program Molecular Evolutionary Genetic Analysis (MEGA) v5 (Tamura et al. 2011). Sequences of *Preussia terricola* and *Westerdykella angulata*, both members of Sporormiaceae (Kruys & Wedin 2009), were used as outgroup taxa. The aligned dataset was subjected to two phylogenetic analyses involving maximum likelihood (ML) and maximum parsimony (MP) using MEGA 5. Based on the Bayesian information criterion of MEGA 5, Kimura 2-parameter model with gamma distribution (K2 + G) was selected for the ML analysis. The ML tree was obtained using the default settings with “use all site” for gaps/missing data treatment. MP tree was generated using the close-neighbor-interchange heuristic search (level 1), the initial tree by random addition sequence (100 replicates), and the “use all site” option. The confidence in topologies was assessed using a bootstrap (BS) test involving 1000 replicates. The ITS sequence of *L. versicolor* was also obtained as a DNA barcode maker (Schoch et al. 2012), and it was not used for phylogenetic analysis.

## Results

### Phylogenetic analyses

A total of 37 LSU sequences of *Lophiostoma* spp. were aligned with outgroup taxa. Of 1228 characters, 143 (11.6%) were variable and 100 (8.1%) were parsimony-informative. The ML tree



**Fig. 1** – The ML tree of *Lophiostoma* spp. with the highest log likelihood (–3081.8178) based on the LSU dataset (1228 positions). ML and MP BS values greater than 70% are indicated at the nodes. A hyphen indicates BS values lower than 70%. The isolate number and GenBank accession number (in parentheses) are noted after the species name. Sequence derived from the type is shown in bold. The tree was rooted with *Preussia terricola* and *Westerdykella angulata* (Sporormiaceae).

with the highest log likelihood (-3081.8178) is shown in Fig. 1. The topology of the MP tree was almost identical to that of the ML tree. The newly obtained fungus (*L. versicolor*) nested within the strongly supported clade of *Lophiostoma*, which had high BS values (99% in ML, 100% in MP; Fig. 1). Phylogenetically, *L. versicolor* was most closely related to *L. quadrisporum*, and their relationship was moderately supported (91% in ML, 88% in MP; Fig. 1). These two species were sister taxa to *L. alpigenum*, but without support.

## Taxonomy

*Lophiostoma versicolor* K. Hiray. & Kaz. Tanaka, **sp. nov.**

Figs. 2–18

Mycobank: MB 808363.

Etymology – in reference to the versicolored ascospores.

Holotype: HHUF 30448.

Ascomata 330–350 µm high, 360–400 µm diam., scattered, immersed, erumpent at beak, subglobose, black. Beak 170 µm wide, crest-like, composed of globose, 2–4 µm diam., thick-walled cells, with a slit-like ostiole. Ascromatal wall 20–27.5 µm thick at sides, composed of 5–7 layers of rectangular, 7–10 × 2.5–6 µm, thin-walled cells, poorly developed at the base. Pseudoparaphyses numerous, 1–2 µm wide, septate, hyaline, branched and anastomosed, associated with gelatinous material. Asci 60–67.5(-77.5) × 10–12.5(-15) µm, fissitunicate, clavate, with a stalk of 12.5–22.5(-27.5) µm long, rounded at the apex, with an ocular chamber, with 2(-4) overlapping uni- or bi-seriate ascospores. Ascospores 18–23 × 6–9 µm (mean 20.2 × 7.7 µm, n = 30), L/W 2.3–2.9 (mean 2.6, n = 30), broadly fusiform, straight, with a primary septum median to submedian (0.50–0.55; mean 0.51, n = 30), 3-septate, constricted at primary septum, versicolored, brown to reddish brown except pale end cells, verruculose, guttulate, without sheath or appendage.

Colonies on CMA 13 mm after 3 weeks at 20 °C in the dark, Pastel Gray (1C1: Kornerup & Wanscher 1978), reverse Olive Gray (2F2). On MEA 11 mm, Olive Gray (2E2), reverse Olive Gray (1F2). On PDA 15 mm, Medium Gray (1D1) reverse Pastel Gray (1C1). No pigment was produced on these media. On RSA ascromatal state almost identical with those on the natural specimen was observed. Ascospores 19–22 × 6–7 µm (mean 20.8 × 6.5 µm, n = 50), L/W 2.9–3.7 (mean 3.2, n = 50).

Asexual state – unknown.

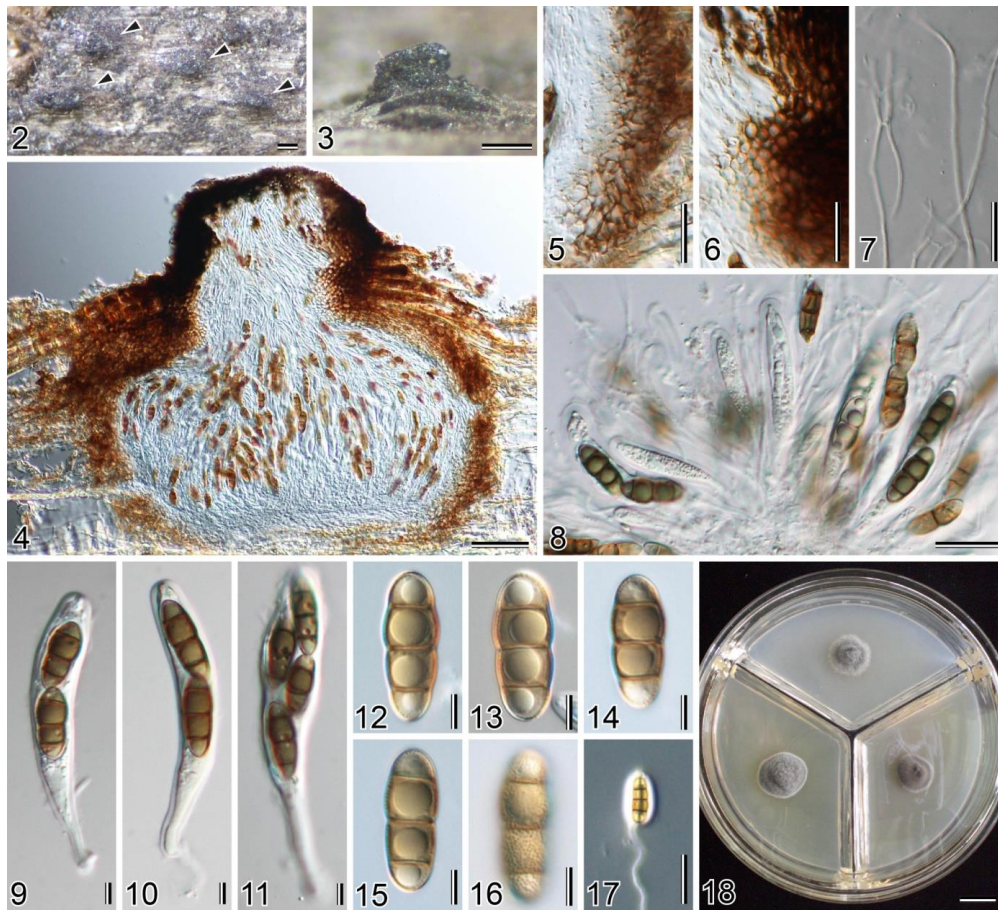
Known distribution – Japan.

Material examined – Japan, Mie, Tsu, Campus of Mie Univ., on twigs of *Acer* sp., 30 May 2008, coll. K. Tanaka and H. Yonezawa, KH 110 (HHUF 30448 holotype designated here) – ex-type culture MAFF 244508.

nrDNA sequences – AB918731 (ITS), AB918732 (LSU).

## Discussion

*Lophiostoma*, typified by *L. macrostomum*, belongs to Lophiostomataceae, Pleosporales, Dothideomycetes (Kirk et al. 2008, Zhang et al. 2012, Hyde et al. 2013). Species of this genus are found on various plants, e.g. woody plants, reeds, palms and bamboo, they and are inhabitants of terrestrial, freshwater and marine environments. This genus is characterized by ascomata with a laterally compressed apex, fissitunicate asci, and hyaline to dark brown, uni- to multiseptate ascospores (Holm & Holm 1988, Hirayama & Tanaka 2011). These ecological and morphological features are similar to those of *Lophiotrema*. Therefore, they have been considered as closely related genera belonging to the same family (Barr 1992) or congeneric (Chesters & Bell 1970). Recently, a molecular study of *Lophiostoma* and *Lophiotrema* revealed that they are phylogenetically distinct genera, and *Lophiotrema* is related to Testudinaceae rather than Lophiostomataceae (Zhang et al. 2009a, b). More recently, Hirayama & Tanaka (2011) concluded that these genera can be mainly distinguished by ascus shape including its stipe length, for example, clavate asci with a relatively long stipe [mostly (10–)15–30 µm] in *Lophiostoma* and cylindrical



**Figs 2–18** – *Lophiostoma versicolor* (2–17 from HHUF 30448. 18 from MAFF 244508). 2 Ascomata on host surface. 3 Compressed beak of ascoma, 4 Longitudinal section of ascoma. 5 Ascomatal wall at side. 6 Wall of beak. 7 Pseudoparaphyses. 8 Pseudoparaphyses and asci. 9–11 Asci. 12–16 Ascospores (16 Ascospore with verruculose ornamentation). 17 Germinating ascospore. 18 Colonies on CMA (upper), PDA (left) and MEA (right) after 3 weeks at 20 °C in the dark. – Bars 2, 3 = 200  $\mu$ m. 4 = 50  $\mu$ m. 5–8, 17 = 20  $\mu$ m. 9–16 = 5  $\mu$ m. 18 = 1 cm.

asci with a short stipe (up to 15  $\mu$ m) in *Lophiotrema*, and they established a new family Lophiotremataceae for *Lophiotrema*.

The collected fungus from *Acer* sp. has ascomata with a laterally compressed beak with a slit-like ostiole and clavate asci with a long stipe (Figs. 2, 3, 8). These morphological characteristics of this fungus suggest that it is a member of *Lophiostoma*, but it also has unusual features for the genus. For example, 2(–4)-spored asci and verruculose, 3-septate, versicolored ascospores without a sheath or appendages (Figs. 9–16) are atypical for this genus and there are no species with these characteristics in the monographs of *Lophiostoma* (e.g., Lehmann 1886, Berlese 1894, Chesters & Bell 1970, Holm & Holm 1988, Barr 1992, Yuan & Zhao 1994, Tanaka & Harada 2003a). *Lophiotrema*, morphologically closest genus to *Lophiostoma*, was also considered as a generic placement of the fungus. Therefore, molecular studies were conducted to confirm the generic classification of the collected fungus.

Preliminary analysis based on a BLAST search using LSU sequences, as well as those of ITS obtained for barcoding, suggested that the new species belongs to *Lophiostoma* rather than *Lophiotrema*. Subsequent phylogenetic analyses based on LSU nrDNA sequences for the obtained fungus (*L. versicolor*) and *Lophiostoma* spp. clearly indicated that *L. versicolor* is a member of *Lophiostoma* (Fig. 1). The characteristic versicolored ascospores observed in *L. versicolor* are known in *Byssothecium* (Boise 1983) and in *Passeriniella* (Berlese 1894), but these genera belong to the Massarinaceae (Zhang et al. 2012, Hyde et al. 2013) and to an unknown lineage comprising

marine fungi (Suetrong et al. 2009), respectively. *Lophiostoma* species including *L. versicolor* formed a monophyletic clade supported by strong BS values (99–100%; Fig. 1). *Lophiostoma versicolor* was most close to *L. quadrisporum* (similarity 978/1009 = 96.9% in LSU), and their relationship had moderate BS support (88–91%; Fig. 1). These results reconfirmed that the ascus shape including the length of ascus stipe, which was previously proposed for differentiating *Lophiostoma* and *Lophiotrema* (Hirayama & Tanaka 2011), is a reliable taxonomic indicator for distinguishing these genera.

*Lophiostoma versicolor* has several unique characteristics such as 2(–4)-spored asci and verruculose, versicolored ascospores (Figs. 8–16). Versicolored ascospores similar to those of *L. versicolor* are known to be present in *L. pileatum*, but this species differs from *L. versicolor* in having 8-spored asci and 6–10-septate, larger ascospores [(35–)40–60(–67) × 12–30 μm; Chesters & Bell 1970]. Most species of *Lophiostoma* have 8-spored asci, whereas species that have 2- or 4-spored asci are extremely rare. *Lophiostoma quadrisporum*, which is phylogenetically close to *L. versicolor* (Fig. 1), has 4-spored asci, but it has 1-septate, smooth, hyaline ascospores (Tanaka & Harada 2003b, misidentified as *L. nucula*, see also Hirayama & Tanaka 2011). The asci of *L. macrostomoides* and *L. mucosum* are (4–)8-spored, but the former has (4–)5–7(–8)-septate ascospores (Mathiassen 1993) and the latter has more large-sized ascospores, (27.5–)30.5–40(–46.5) × (5.5–)7–10.5(–12) μm (Tanaka & Harada 2003a), as compared to those of *L. versicolor*. Generally, the number of ascospores per ascus is considered as an important character for species discrimination (Voglmayr & Jaklitsch 2011). Therefore, we described here the fungus as a new species of *Lophiostoma*, *L. versicolor*.

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## References

- Barr ME. 1992 – Notes on the Lophiostomataceae (Pleosporales). *Mycotaxon* 45, 191–221.
- Berlese AN. 1894 – *Icones fungorum omnium hucusque cognitorum*. Vol. 1 (Reprint 1968, Bibliotheca Mycologia 16A, 1–243).
- Boise J. 1983 – On *Trematosphaeria circinans* and reinstatement of the genus *Byssothecium*. *Mycologia* 75, 666–669.
- Chesters CGC, Bell A. 1970 – Studies in the Lophiostomataceae. *Mycological Papers* 120, 1–129.
- Hirayama K, Tanaka K, Raja HA, Miller AN, Shearer CA. 2010 – A molecular phylogenetic assessment of *Massarina ingoldiana* sensu lato. *Mycologia* 102, 729–746.
- Hirayama K, Tanaka K. 2011 – Taxonomic revision of *Lophiostoma* and *Lophiotrema* based on reevaluation of morphological characters and molecular analyses. *Mycoscience* 52, 401–412.
- Holm L, Holm K. 1988 – Studies in the Lophiostomataceae with emphasis on the Swedish species. *Symbolae Botanicae Upsalienses* 28 (2), 1–50.
- Hyde KD, Jones EBG, Liu JK, Ariyawansa H, Boehm E, Boonmee S, Braun U, Chomnunti P, Crous PW, Dai DQ, Diederich P, Dissanayake A, Doilom M, Doveri F, Hongnan S, Jayawardena R, Lawrey JD, Li YM, Liu YX, Lücking R, Monkai J, Muggia L, Nelsen MP, Pang KL, Phookamsak R, Senanayake IC, Shearer CA, Suetrong S, Tanaka K, Thambugala KM, Wijayawardene NN, Wikee S, Wu HX, Zhang Y, Aguirre-Hudson B, Alias SA, Aptroot A, Bahkali AH, Bezerra JL, Bhat DJ, Camporesi E, Chukeatirote E, Gueidan C, Hawksworth DL, Hirayama K, Hoog SD, Kang JC, Knudsen K, Li WJ, Li XH, Liu ZY, Mapook A, McKenzie EHC, Miller AN, Mortimer PE, Phillips AJL, Raja HA, Scheuer C, Schumm F, Taylor JE, Tian Q, Tibpromma S, Wanasinghe DN, Wang Y, Xu JC, Yacharoen S, Yan JY, Zhang M. 2013 – Families of Dothideomycetes. *Fungal Diversity* 63, 1–313.
- Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008 – *Ainsworth and Bisby's Dictionary of the*

- Fungi, 10th edn. CAB International, Wallingford.
- Kornerup A, Wanscher JH. 1978 – Methuen handbook of colour, 3th edn. Methuen, London.
- Kruys Å, Wedin M. – 2009 Phylogenetic relationships and an assessment of traditionally used taxonomic characters in the Pleosporales (Pleosporales, Dothideomycetes, Ascomycota), utilising multi-gene phylogenies. *Systematics and Biodiversity* 7, 465–478.
- Lehmann F. 1886 – Systematische Bearbeitung der Pyrenomycetengattung *Lophiostoma* (Fr.) Ces. & Dntrs., mit Berücksichtigung der verwandten Gattungen *Glyphium*, (N.i.c.), *Lophium*, Fr., und *Mytilinidion*, Duby. *Nova Acta* 50, 47–152.
- Leuchtman A. 1985 – Kulturversuche mit einigen Arten der Gattung *Lophiostoma* Ces. & De Not. *Sydowia* 38, 158–170.
- Mathiassen G. 1993 – Corticolous and lignicolous Pleosporales s. lat. (Ascomycetes) on *Salix* along a mid-Scandinavian transect. *Sommerfelita* 20, 1–180.
- Mugambi GK, Huhndorf SM. 2009 – Molecular phylogenetics of Pleosporales: Melanommataceae and Lophiostomataceae re-circumscribed (Pleosporomycetidae, Dothideomycetes, Ascomycota). *Studies in Mycology* 64, 103–121.
- Rehner SA, Samuels GJ. 1994 – Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* 98, 625–634.
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spougea JL, Levesqueb CA, Chenb W, Fungal Barcoding Consortium. 2012 – Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America* 109, 6241–6246.
- Shoemaker RA. 1984 – Canadian and some extralimital *Nodulosphaeria* and *Entodesmium* species. *Canadian Journal of Botany* 62, 2730–2753.
- Suetrong S, Schoch CL, Spatafora JW, Kohlmeyer J, Volkmann-Kohlmeyer B, Sakayaroj J, Phongpaichit S, Tanaka K, Hirayama K, Jones EBG. 2009 – Molecular systematics of the marine Dothideomycetes. *Studies in Mycology* 64, 155–173.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011 – MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731–2739.
- Tanaka K, Harada Y. 2003a – Pleosporales in Japan (1): the genus *Lophiostoma*. *Mycoscience* 44, 85–96.
- Tanaka K, Harada Y. 2003b – Pleosporales in Japan (2): *Lophiotrema*. *Mycoscience* 44, 115–121.
- Tanaka K, Hirayama K, Yonezawa H, Hatakeyama S, Harada Y, Sano T, Shirouzu T, Hosoya T. 2009 – Molecular taxonomy of bambusicolous fungi: Tetraplospiraaceae, a new pleosporalean family with *Tetraploa*-like anamorphs. *Studies in Mycology* 64, 175–209.
- Tanaka K, Hosoya T. 2008 – *Lophiostoma sagittiforme* sp. nov., a new ascomycete (Pleosporales, Dothideomycetes) from Island Yakushima in Japan. *Sydowia* 60, 131–145.
- Voglmayr H, Jaklitsch WM. 2011 – Molecular data reveal high host specificity in the phylogenetically isolated genus *Massaria*. *Fungal Diversity* 46, 133–170.
- White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*, pp 315–322, Academic Press, San Diego.
- Yuan Z, Zhao Z. 1994 – Studies on lophiostomataceous fungi from Xinjianf, China. *Sydowia* 46, 162–184.
- Zhang Y, Schoch CL, Fournier J, Crous PW, De Gruyter J, Woudenberg JHC, Hirayama K, Tanaka K, Pointing SB, Spatafora JW, Hyde KD. 2009a – Multi-locus phylogeny of Pleosporales: a taxonomic, ecological and evolutionary reevaluation. *Studies in Mycology* 64, 85–102.
- Zhang Y, Wang HK, Fournier J, Crous PW, Jeewon R, Pointing SB, Hyde KD. 2009b – Towards a phylogenetic clarification of *Lophiostoma/Massarina* and morphologically similar genera in the Pleosporales. *Fungal Diversity* 38, 225–251.
- Zhang Y, Crous PW, Schoch CL, Hyde KD. 2012 – Pleosporales. *Fungal Diversity* 53, 1–221.