A new species of *Pyricularia* (hyphomycetes) on *Cortaderia* (Poaceae) in New Zealand

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McKenzie EHC, Park D, Bellgard SE, Johnston PR. 2010 – A new species of *Pyricularia* (hyphomycetes) on *Cortaderia* (Poaceae) in New Zealand. Mycosphere 1(3), 223–228.

Pyricularia cortaderiae sp. nov., found on leaves of *Cortaderia selloana* in New Zealand, is illustrated and described and compared with related taxa. rDNA sequencing showed it to be distinct from other species. It was associated with a narrow, dark brown leaf streak.

Key words – anamorphic fungi – deuteromycetes – molecular phylogeny – taxonomy

Article Information
Received 22 September 2010
Accepted 28 September 2010
Published online 30 October 2010
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Introduction

Cortaderia species are tall, perennial, tussock grasses found mainly in South America. However, there are five endemic species (toetoe) in New Zealand and two species (pampas grass) naturalized from South America. The naturalized species commonly grow in waste places and scrub and invade *Pinus* plantations. While carrying out a survey of pampas grasses for possible biological control agents, a species of *Pyricularia* was found. It is described below as a new species.

Methods

Leaves and stems of a naturalized, widespread pampas grass, *Cortaderia selloana* (Schult. & Schult. f.) Asch. & Graebn. were collected from a scenic reserve. The *Cortaderia* was growing as a large clump within a patch of the endemic sedge, *Gahnia xanthocarpa*. The plant material was incubated under humid conditions and periodically examined for sporulating microfungi. Fungal fruiting structures were removed, mounted in lactophenol, and examined by light microscopy. Measurements were made on material mounted in lactophenol. Single conidia of the fungus were removed from the plant tissue and grown on potato dextrose agar (PDA). A living culture is preserved in the International Collection of Micro-organisms from Plants (ICMP). A dried herbarium specimen of the fungus was prepared and is deposited in the New Zealand Fungal Herbarium (Herb. PDD). The species is known only from the holotype.

Fungal DNA was extracted from mycelium of *P. cortaderiae* grown on a PDA plate using REDExtract-N-Amp Plant PCR Kits (Sigma, USA), following the manufacturer's instructions. The ITS region was amplified using the primers ITS1F and ITS4 (White et al. 1990). The newly obtained ITS sequence was aligned using Clustal W (Larkin et al. 2007) with sequences available from Bussaban et al. (2005) that represented the genetic breadth of the *Pyricularia* spp. sampled in that study, along with ITS sequences from other grass-inhabiting *Pyricularia* spp. available in GenBank. Phylogenetic analyses were performed using Bayesian maximum liklelihood in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) and a heuristic maximum likelihood analysis in PAUP* 4.01b (Swofford 2002) with the GTR+I+G model, selected using the AIC method in MrModelTest 2.3 (Posada & Crandall 1998, Posada & Buckley 2004). The Bayesian analysis was run with two chains for 10 million generations, trees sampled every 1000 generations with a burn-in of 25%. Bayesian posterior probabilities were obtained from 50% majority rule consensus trees. The PAUP ML analysis used addition sequence random and TBR branch swapping with 100 replicates to avoid local optima. A bootstrap analysis used the ML tree as a starting tree, each of the 100 bootstrap samples run with a single replicate.

Results

The genus *Pyricularia* Sacc. was described by Saccardo (1880) for a single species, *P. grisea* Sacc., found on grasses. A second species, *P. oryzae* Cav., was later described on rice (Cavara 1892). Various subspecific taxa have been described within both of these species, often based on differences in host pathogenicity. While these two species are morphologically similar, recent pathogenicity and mating studies (Kato et al. 2000), molecular studies (Couch & Kohn 2002) and descriptions of teleomorphs (*Magnaporthe grisea* (T.T. Hebert) M.E. Barrand and *M. oryzae* B.C. Couch), have led to the conclusion that these two species are distinct with *P. oryzae* parasitic on rice and many grasses, and *P. grisea* parasitic on *Digitaria* spp. (Couch & Kohn 2002). A molecular study by Hirata et al. (2007) revealed cryptic species within the *P. grisea* complex. Other species have been described on grasses: *P. didyma* M.B. Ellis, *P. dubiosa* (Speg.) Viégas, *P. leersiae* (Sawada) S. Ito, *P. panici-paludosi* (Sawada) S. Ito, *P. penniseti* Prasada & Goyal, *P. setariae* Y. Nisik, and *P. zizaniicola* Hashioka. In addition, several invalid species have been named for *Pyricularia* on grasses (Siwasin & Giatgong 1971). Additional species have been described, especially on monocotyledonous plants including Cannaceae, Commelinaceae, Cyperaceae, Musaceae, and Zingiberaceae. The species on monocotyledonous plants usually cause leaf spots, but some other species are saprobic on leaf litter of dicotyledonous plants.

The genus is characterised by the formation of solitary conidia on cylindrical denticles produced from usually slender, thin-walled conidiophores. The conidiogenous cells are terminal, sometimes becoming intercalary through sympodial extension of the conidiogenous cell and formation of new cross walls. The conidia are often 1- or 2-septate, and obpyriform, obturbinate or obclavate in shape (Ellis 1971a).

A specimen collected on *Cortaderia selloana* in New Zealand is distinct from all other known species, and is described as:

Pyricularia cortaderiae McKenzie, **sp. nov.** Fig. 1

MycoBank 512735

GenBank accession number: HQ283076

Etymology – named after the host substrate, *Cortaderia*.

Coloniae pilosae. Mycelium ex hyphis in substrato immersum, ramosis, septatis, laevibus, hyalinae vel luteus vel pallide brunneis, tenuitunicatis, 1.5–3 µm crassis compositum. Conidiophora macronematosa, mononematosa, solitaria, erecta, recta vel paulo flexuosa, nonramosa, laevia, 2–5-septata, pallide brunnea vel brunnea, apicem versus pallidiora, usque ad 250 µm longa, 5.5–7 µm lata. Cellulae conidiogenae holoblasticae, in conidiophora incorporatae, terminales, sympodiales, cylindricae, usque ad 155 µm longa, denticulis conico-truncatis vel cylindricis numerosis praedita. Conidia solitaria ex denticulis oriunda, sicca, acropleurogena, straminea vel pallide brunnea, laevia, obovoidea vel obpyriformea, basi truncate, apice late rotundato, 2-septatis, 25.5–32 × 11.5–15 µm.

Type: NEW ZEALAND, Auckland, Pakiri, Pakiri Scenic Reserve, 36° 12.44830'S, 174°39.03433'E, in foliis vivis poacearum *Cortaderia selloana* (Poaceae), 17 November 2008, R.E. Beever, S.E. Bellgard, N.W. Waipara (PDD 95061, **holotype**) (culture from type specimen, ICMP 17830).

Colonies in the form of scattered conidiophores. Mycelium immersed in the substratum. Hyphae branched, septate, smooth, hyaline to

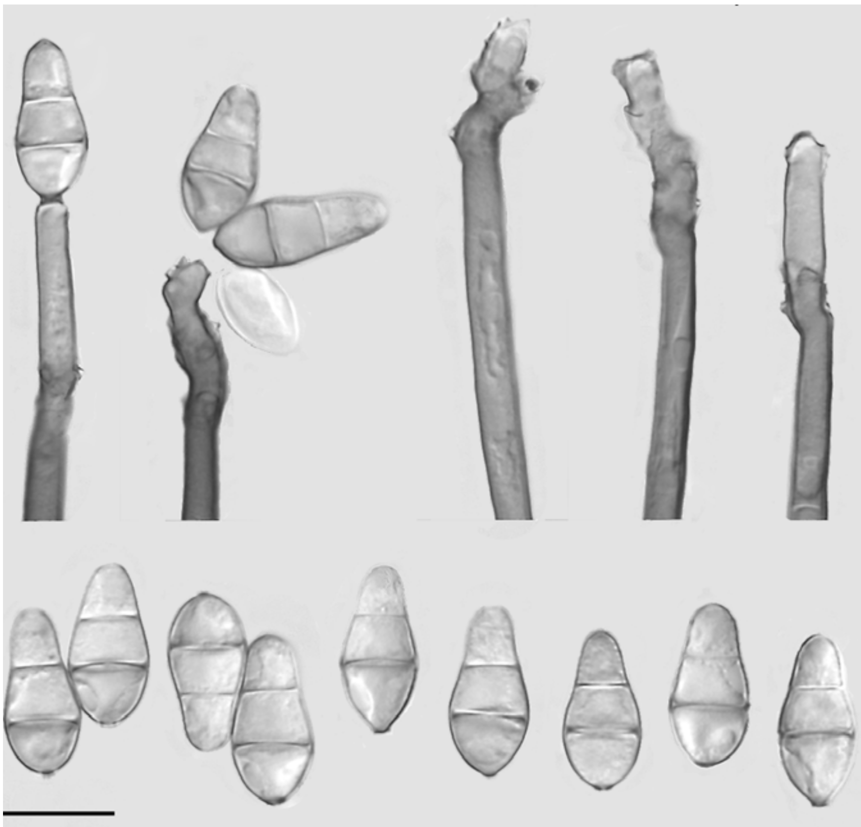

Fig. 1 – Conidia and conidiogenous cells of *Pyricularia cortaderiae* (from holotype). Specimen mounted in hydrous lactophenol. Scale bar = 20 µm.

yellowish, thin-walled, 1.5–3 µm diam. Conidiophores differentiated, mononematous, single, erect, straight or slightly flexuous, unbranched, smooth, 2–5-septate, pale brown to brown, sometimes paler towards apex, up to 250 µm long, 5.5–7 µm wide. Conidiogenous cells holoblastic, integrated, terminal, sympodially proliferating, cylindrical, up to 155 µm long, with conspicuous denticles; denticles conico-truncate, up to 3 µm high, 3 µm wide. Conidia solitary at ends of denticles, dry, acropleurogenous, straw-coloured or pale brown, smooth, obovoid to obpyriform, base truncate, apex broadly rounded, 2-septate, 25.5–32 × 11.5–15 µm (mean = 29.3 × 13.7 µm, n = 30).

Pyricularia cortaderiae was found sporulating exclusively on old, narrow, elongate, sometimes coalescing, dark brown leaf streaks (1–2 mm wide × 1–2 cm long) on *Cortaderia selloana*, and is presumably a pathogen.

Phylogenetic analyses

Both the ML and Bayseian analyses of ITS rDNA sequences produced a tree with the same topology. The Bayesian tree in shown in Fig. 2. The relationship of *P. cortaderiae* was unresolved within *Pyricularia*.

Discussion

The species of *Pyricularia* that cause leaf spots on grasses and cereals are morphologically similar to *P. cortaderiae*, possessing conidia that are pale coloured, 2-septate (except for *P. didyma*, which is 1-septate), and obovoid, obpyriform or obturbinate in shape (Table 1). However, the conidia of *P. cortaderiae* are larger than those of all of the other species, except for *P. zizaniicola.* The conidial size range and mean dimensions of *P. cortaderiae* and *P. zizaniicola* are virtually identical (Table 1). The main morphological difference between these two species appears to be in the conidiophores. The conidiophores of *P. zizaniicola* are predominantly 0–1-septate, rarely 2-septate, and measure 90–150 µm, rarely extending to 220 µm (Hashioka 1973). Those of *P. cortaderiae* are 2–5-septate and up to 250 µm in length.

CAB International (2002) gave conidial measurements of 19–31 × 10–15 µm for *P. setariae*. This is somewhat larger than the

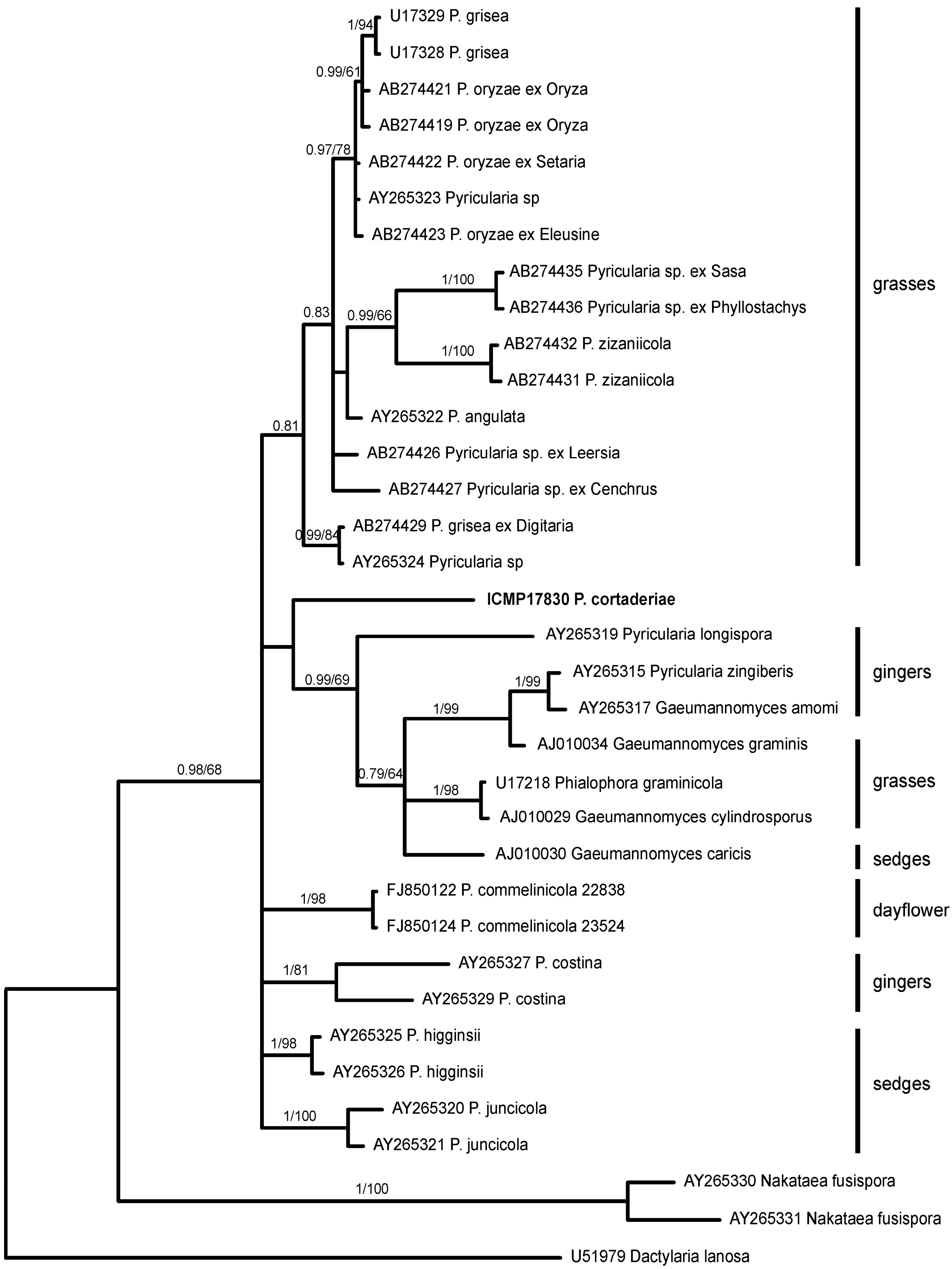

Fig. 2 – 50% majority-rule consensus phylogenetic tree based on Bayesian analysis of the ITS region. Bayesian posterior probabilities/ML bootstrap values are shown above the edges where they are greater than 50%. The sequence for *Pyricularia cortaderiae* newly generated in this study has been deposited in GenBank as HQ283076. *Nakatea fusispora* and *Dactylaria lanosa* were selected as outgroups to root the tree.

Table 1 Summary of features of *Pyricularia* species found on grasses.

Species	Conidia			Conidiophores
	Size (µm)	Mean (µm)	Septa	Size (µm)
P. cortaderiae (current material)	25.5–32 × 11.5–15	29.3 × 13.7	2	Up to 250 × 5.5–7
P. didyma (Ellis 1971b)	12–16 × 7.5–8.5	14 × 8	1	Up to 200 × 2.5–4.5
P. dubiosa (Viégas 1946)	15–22 × 8–10	–	2	80–100 × 3.5–4
P. grisea (Ellis 1971a)	17–28 × 6–9	20.9 × 7.7	2	Up to 150 × 2.5–4.5
P. leersiae (Tanaka 1920)	20–35 × 7–10	27 × 8.6	2	48–88 × 4–5
P. oryzae (Ellis 1971a)	17–23 × 8–11	21.2 × 9.6	2	Up to 130 × 3–4
P. panici-paludosi (Tanaka 1920)	17–26 × 8.5–12	22 × 10.2	2	80–160 × 4–5
P. penniseti (Prasada & Goyal 1970)	18.4–36.7 × 7.4–11.1	27.5 × 9.2	2	50–150 × 3.6–5.5
P. setariae (Nishikado 1917)	14–35 × 5–12	20 × 7.5	(1–)2(–3)	40–250 × 4-5
P. zizaniicola (Hashioka 1973)	24–33 × 10.5–15.5	27.7 × 13.5	2	90–150(–220) × 7–9

measurements given by Nishikado (1917) and places the size limits within the range of *P. cortaderiae*. Hirata et al. (2007) designated a cryptic, phylogenetic species as *Pyricularia* sp., within the *P. grisea* complex. It was isolated from both *Setaria geniculata* and *Leersia oryzoides*. Following DNA analysis, Yamagashira et al. (2008) designated *Pyricularia* isolates from both wild foxtails (*Setaria* spp.) and foxtail millet (*S. italica*) as the *Setaria* pathotype of *Magnaporthe oryzae*.

Sequencing of the ITS-region showed that *P. cortaderiae* is distinct from any of the other species recorded on grasses, for which sequences are available (Fig. 2). It is distinct from *P. zizaniicola* and isolates originating from *Setaria italica* and *S. geniculata* (Fig. 2). It is worth noting that the genus *Cortaderia* lies within subfamily Arundinoideae of the Poaceae while *Zizania* (host of *P. zizaniicola*) is in subfamily Bambusoideae, and *Setaria* (host of *P. setariae* and an unnamed *Pyricularia* sp. (Hirata et al. 2007)) is in subfamily Panicoideae (Clayton & Renvoize 1986). *Pyricularia cortaderiae* lies within a lineage that includes *P. juncicola* MacGarvie. However, it is morphologically distinct as conidia of the latter are 1-septate, obclavate, and measure 15–30 × 4–5 µm (Ellis 1976). *P. juncicola* was described from Ireland on dead leaves of *Juncus effusus*; in New Zealand it is common on dead leaves of Cyperaceae (*Uncinia* and *Carex* species).

The phylogenetic relationships within *Pyricularia* sens. lat. are poorly resolved, probably reflecting the limited gene sampling in studies on the genus to date (Bussaban et al. 2005, Hirata et al. 2007). The position of *P. cortaderiae* is unresolved, although appears to be far removed from other grass-inhabiting species, and somewhat related to lineages of *Gaeumannomyces* and to some of the *Pyricularia* spp. occurring on gingers.

Acknowledgements

Funds for this research were provided by the New Zealand Foundation for Research, Science and Technology through the Defining New Zealand's Land Biota OBI. Paul Cannon, CABI, UK is thanked for kindly providing comments on the manuscript.

References

Bussaban B, Lumyong S, Lumyong P, Seelanan T, Park DC, McKenzie EHC, Hyde KD. 2005 – Molecular and morphological characterization of *Pyricularia* and allied genera. Mycologia 97, 1002–1011.

CAB International. 2002 – Crop Protection Compendium, Global Module, 4th edn. CAB International, Wallingford, U.K.
Cavara F. 1892 – Fungi Longobardiae Exsiccati 1, no. 49.
Clayton WD, Renvoize SA. 1986 – Genera Graminum. Grasses of the World. Kew Bulletin Additional Series 13. 389 pp.
Couch BC, Kohn LM. 2002 – A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. Mycologia 94, 683–693.
Ellis MB. 1971a – Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, U.K. 608 pp.
Ellis MB. 1971b – Dematiaceous hyphomycetes. X. Mycological Papers 125, 1–30.
Ellis MB. 1976 – More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, U.K. 507 pp.
Hashioka Y. 1973 – Notes on *Pyricularia* II. Four species and one variety parasitic to Cyperaceae, Gramineae and Commelinaceae. Transactions of the Mycological Society of Japan 14, 256–265.
Hirata K, Kusaba M, Chuma I, Osue J, Nakayashiki H, Mayama S, Tosa Y. 2007 – Speciation in *Pyricularia* inferred from multilocus phylogenetic analysis. Mycological Research 111, 799–808.
Huelsenbeck JP, Ronquist F. 2001 – MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
Kato H, Yamamoto M, Yamaguchi-Ozaki T, Kadouchi H, Iwamoto Y, Nakayashiki H, Tosa Y, Mayama S, Mori N. 2000 – Pathogenicity, mating ability and DNA restriction fragment length polymorphisms of *Pyricularia* populations isolated from Gramineae, Bambusideae and Zingiberaceae plants. Journal of General Plant Pathology 66, 30–47.
Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007 – Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.
Nishikado Y. 1917 – Studies on the rice blast fungus, (I). Berichte des Ohara Instituts für Landwirtschaftliche Forschungen in Kurashiki, Provinz Okayama, Japan 1, 171–217.
Posada D, Buckley TR. 2004 – Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. Systematic Biology 53, 793–808.
Posada D, Crandall KA. 1998 – Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Prasada R, Goyal JP. 1970 – A new species of *Pyricularia* on bajra. Current Science 39, 287–288.
Saccardo PA. 1880 – Conspectus generum fungorum italiae inferiorum. Michelia 2, 1–135.
Siwasin C, Giatgong P. 1971 – Cytological study and cross inoculation of *Pyricularia* spp. International Rice Commission Newsletter 20, 13–19.
Swofford DL. 2002 – PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sunderland, Massachusetts, Sinauer Associates.
Tanaka T. 1920 – New Japanese fungi. Notes and translations—VIII. Mycologia 12, 25–32.
Viégas AP. 1946 – Alguns fungos do Brasil XIII. Hifomicetos. Bragantia 6, 353–442.
White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications (eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White). Academic Press, New York. 315–322.
Yamagashira A, Iwai C, Misaka M, Hirata K, Fujita Y, Tosa Y, Kusaba M. 2008 – Taxonomic characterization of *Pyricularia* isolates from green foxtail and giant foxtail, wild foxtails in Japan. Journal of General Plant Pathology 74, 230–241.