



Identification criteria of the genus *Pseudopyricularia* on sedge plants

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Abstract: Pyriculariaceae family has newly been established several new genera and species. Indeed, some species originally described *Pyricularia* subsequently have been synonymized or transferred to other genera. In this study an overview of taxonomical and phylogenetic findings, symptomology of *Pseudopyricularia* species and an identification key to *Pseudopyricularia* species by morphological criteria are provided.

Key words: *Pseudopyricularia*, Phylogeny, New species, Identification key

Historical taxonomy

Pyriculariaceae has been established by Klaubauf et al. (2014) having several new genera including *Pyricularia s. str.*, *Pseudopyricularia*, *Macgarviomyces*, *Neopyricularia*, *Bambusicularia*, *Barretomyces*, *Deightoniella*, *Proxipyricularia*, and *Xenopyricularia*. Some species originally described in the genus *Pyricularia sensu lato*, have been synonymized or transferred to other genera. Previously, *P. higginsii* was transferred to the genus *Dactylaria*, and was designated as *D. higginsii* by Ellis (1976). Bussaban et al. (2005) suggested that *P. higginsii* should be maintained in *Pyricularia sensu lato* on the basis of ITS-nrDNA sequences. Newly, several isolates were identified as representative of *Py. higginsii* that belong to five new species and lie into *Pseudopyricularia* (Klaubauf et al., 2014). Species of

Pseudopyricularia are primarily distinguished from *Pyricularia s. str.* by having short, determinate, brown conidiophores with an apical rachis with flat-tipped denticles (Klaubauf et al., 2014).

Multigene-based phylogenetic analyses showed that *Pseudopyricularia* clades have species with different morphology so their description was revised (Pordel et al., 2017). Based on the new description, *Pseudopyricularia* conidia are obclavate, fusiform, cylindrical, and 1-2-septate (Pordel et al., 2017, Marin-Felix et al., 2017).

Isolation and morphological characterization of *Pseudopyricularia* isolates

Pseudopyricularia can readily be isolated from all the above-ground parts of weeds (Crous et al., 2015, 2018). Isolation of the pathogen from either seed lots or plant tissues with symptoms can be performed by cutting the marginal tissues of the visible lesions. Two min in 1% sodium hypochlorite, dry on filter paper, and then incubating on wet filter paper at 25 °C is favorable for isolation of *Pseudopyricularia* species (Pordel et al., 2017).

A standard method for purification is single hyphal tips emerging from germinating conidia and then moving to potato dextrose agar medium (PDA, Merck Co.) (Pordel et al. 2016). To describe the overall characters of colonies, grow on oatmeal agar (OA, Merck Co.) and PDA. Micromorphological characters are determined by growing colonies on synthetic nutrient-poor agar (SNA; Nirenberg 1976) and WA supplemented with pieces of rice leaves at 23–25 °C under a 12 h dark/12 h near-ultraviolet light regime after 1–3 wk (Pordel et al., 2017, 2019).

Symptoms of *Pseudopyricularia* species on sedges plants

The *Pseudopyricularia* species infect stems, leaves and heads of sedges, that the symptoms are leaf spots and blight and scorch of the stems (Bussaban et al., 2003, Pordel et al., 2017, 2019). Infected plants have light brown color, their heads are brown and will collapse. The favorite temperature and humidity for growing of the genus is 25 – 28 C and higher than 90% humidity.

Phylogenetic finding

Phylogenetic analyses established the new family Pyriculariaceae based on the genus *Pyricularia*

(Klaubauf *et al.* 2014). The Pyriculariaceae (Magnaporthales, Ascomycota) has several new genera and species. Main species is *Pyricularia oryzae* causing blast disease on rice and wheat plants, and many other species in this family are pathogen of Commelinaceae, Cyperaceae, Musaceae, and Zingiberaceae plants (Bussaban *et al.* 2003, Park & Shin 2009).

The new genera are described in Pyriculariaceae by phylogenetic and morphological criteria (Klaubauf *et al.* 2014), for example, *Pseudopyricularia* is a new

genus that infect the sedges plants (Cyperaceae and Juncaceae). Phylogenetic analyses revealed that *Pseudopyricularia* clade has species with different morphology (Pordel *et al.* 2017).

The *Pseudopyricularia* genus has eleven legitimated species that are identified by phylogenetics and morphological criteria (Table 1) (Klaubauf *et al.*, 2014, Crous *et al.*, 2015, Pordel *et al.*, 2017, Crous *et al.*, 2018, Marin-Felix *et al.*, 2017, Crous *et al.*, 2020, Ghorbani *et al.*, 2021, Crous *et al.*, 2021a).

Table 1. Legitimated species of the genus *Pseudopyricularia*.

Species	Holotype no.	Host	Country	References
<i>Pseudopyricularia bothriochloae</i> (Crous & Cheew.) Y. Marín & Crous,	CBS H-21436	<i>Bothriochloa bladhii</i>	Thailand	Marin-Felix <i>et al.</i> , 2017
<i>Pseudopyricularia cyperi</i> Klaubauf, M.-H. Lebrun & Crous	CBS H-21840	<i>Cyperus iria</i>	Japan	Klaubauf <i>et al.</i> , 2014
<i>Pseudopyricularia higginsii</i> (Luttr.) Klaubauf, M.-H. Lebrun & Crous	CBS 121934	<i>Typha orientalis</i> , dead leaves	New Zealand:	Klaubauf <i>et al.</i> , 2014
<i>Pseudopyricularia kyllingae</i> Klaubauf, M.-H. Lebrun & Crous	CBS H-21841	<i>Kyllinga brevifolia</i>	Japan	Klaubauf <i>et al.</i> , 2014
<i>Pseudopyricularia festucae</i> Crous	CBS H-24352	<i>Festuca californica</i>	USA	Crous <i>et al.</i> , 2021
<i>Pseudopyricularia hagahagae</i> Crous & M.J. Wingf.	CBS H-22400	Cyperaceae	South Africa	Crous <i>et al.</i> , 2015
<i>Pseudopyricularia hedjaroudii</i> M. Javan-Nikkhah, A. Pordel & G. Ghorbani	IRAN 18059F	<i>Cyperus</i> sp.	Iran	Ghorbani <i>et al.</i> , 2021
<i>Pseudopyricularia hyrcaniana</i> Pordel & Jav.-Nikkh.	UTFC PO10	<i>Cyperus alternifolius</i>	Iran	Pordel <i>et al.</i> , 2017
<i>Pseudopyricularia iraniana</i> Pordel & Jav.-Nikkh.	UTFC PO14	<i>Juncus</i> sp.	Iran	Pordel <i>et al.</i> , 2017
<i>Pseudopyricularia javanii</i> A. Pordel & G. Ghorbani,	IRAN 18060F	<i>Cyperus</i> sp.	Iran	Crous <i>et al.</i> , 2020
<i>Pseudopyricularia persiana</i> G. Ghorbani, Pordel & Jav.-Nikkh.	UTFC PO20	<i>Cyperus</i> sp.	Iran	Crous <i>et al.</i> , 2018

Macgarvieomyces is close genus to *Pseudopyricularia*, described three species occurring on *Juncus* in the genus *Diplorhinotrichum*. The *Macgarvieomyces* is morphologically different from *Pseudopyricularia* and *Pyricularia sensu stricto*. Non-

producing of chlamyospores, and having some branched conidiophores and conidia with different shapes, and septate separate *Pseudopyricularia* from *Macgarvieomyces* (Klaubauf *et al.*, 2014). Phylogenetic studies based on LSU, *RPBI* (genus

level), and *CAL*, ITS and *RPB1* or *ACT*, ITS and *RPB1* (species level) and morphological criteria describe different species having various conidia lies in *Pseudopyricularia* (Fig. 1) (Klaubauf et al., 2014, Pordel et al., 2017, Marin-Felix et al., 2017).

Identification Key

The below morphological key is presented for distinction of *Pseudopyricularia* from the *Pyricularia* and *Macgarvieomyces* genera:

Conidia pyriform to obclavate, narrowed toward tip, 2-septate, non-producing Chlamydo-spore *Pyricularia s. str.*

Conidia solitary, obclavate, fusiform, cylindrical, pale to medium brown, finely roughened, guttulate, 1–2 or 3 septate, chlamydo-spores present or absent.....2

2a. Conidia obclavate, hyaline, smooth, granular and guttulate, 1-septate, chlamydo-spores brown, ellipsoid, arranged in chains.....*Macgarvieomyces*.

2b. Conidia obclavate, obpyriform, fusiform, cylindrical, 1-2 or 3 septate, chlamydo-spores absent.....*Pseudopyricularia*.

Key for *Pseudopyricularia* species:

-Conidia 1- septate, obclavate or obpyriform1

-Conidia 2 -septate, obclavate.....2

-Conidia 2 or 3 -septate, obclavate.....3

1a. Conidia solitary, obclavate, pale brown.....*Pseudopyricularia bothriochloa* ((18–)19–22(–23) × (6–)7–8(–9) μm) and *Pseudopyricularia hyrcaniana* ((17–) 20–29(–31) × (5–) 6–8) μm) (Fig. 2, A and G).

1b. Conidia solitary, obpyriform, hyaline, (13–)15–25(–28) × (5–)6–9 μm.....*Pseudopyricularia hedjaroudii* (Fig. 2, B and H).

2a. Conidia solitary, obclavate, medium brown.....*Pseudopyricularia hagahagae* ((38–) 41–45(–49) × (7–) 8(–9) μm), and *Pseudopyricularia javanii* ((20–)25–35(–40) × 6–7 μm).

2b. Conidia solitary, obclavate, medium brown.....*Pseudopyricularia higginsii* (17.5–36.5 × 5.3–6.5 μm) (Fig. 2, C and I), *Pseudopyricularia cyperi* ((22–) 25–28(–35) × (4–)5(–6) μm), and *Pseudopyricularia kyllingae* ((23–)27–30(–35) × (5–)6(–7) μm).

3a. Conidia solitary, dry, obclavate, hyaline, (30–)36–52(–65) × 10–13 μm, 2(–3)-septate, conidia produce secondary conidiophore.....*Pseudopyricularia persiana* (Fig. 2, E and K)

-Conidia fusiform or cylindrical.....4

4a. Conidia solitary, fusiform or cylindrical, hyaline, (20–) 22–30 × 5–8 μm*Pseudopyricularia iraniana* (Fig. 2, F and M).

4b. Conidia solitary, fusiform, finely roughened, (25–)30–38(–40) × (6–)7 μm*Pseudopyricularia festucae*.

Conclusion

Pseudopyricularia species often infect the weed plants and could find on the northern border of Iran, including Golestan, Mazandaran, Guilan, Ardabil, and East Azerbaijan provinces (Pordel et al., 2015, 2017, 2019, and unpublished data). However, morphological species identification of *Pseudopyricularia* is challenging (Jayawardena et al., 2019). Therefore, identifying and describing all existing taxa by phylogenetic and morphological criteria should be considered. In this paper, we mentioned the isolation methods, typical symptoms, index genes, and important morphological criteria for identification.

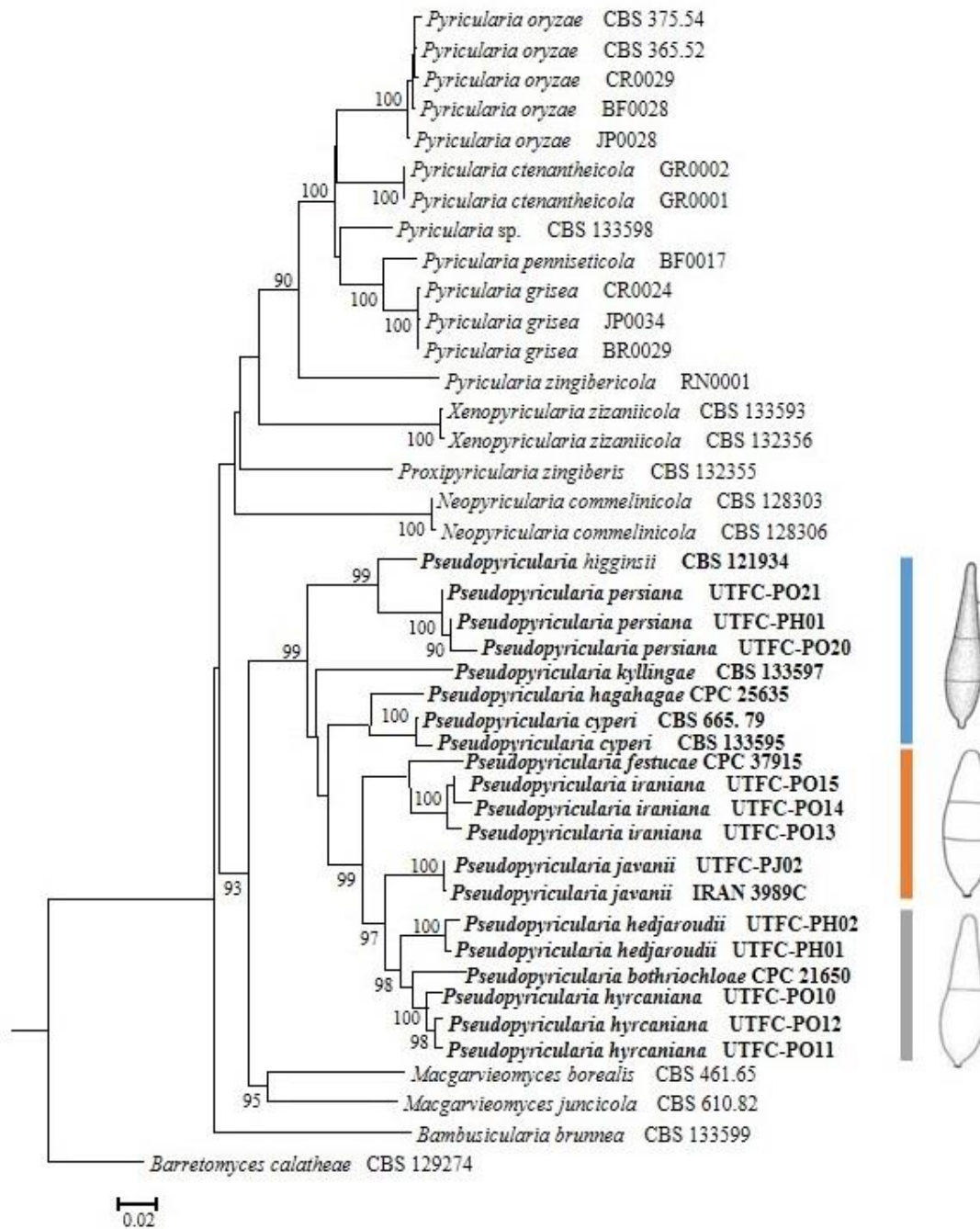


Fig. 1. Maximum likelihood (ML) tree inferred from combined *CAL*, *ITS*, and *RPB1* sequences. Bootstrap support values from ML analyses larger than $\geq 90\%$ are shown above internodes

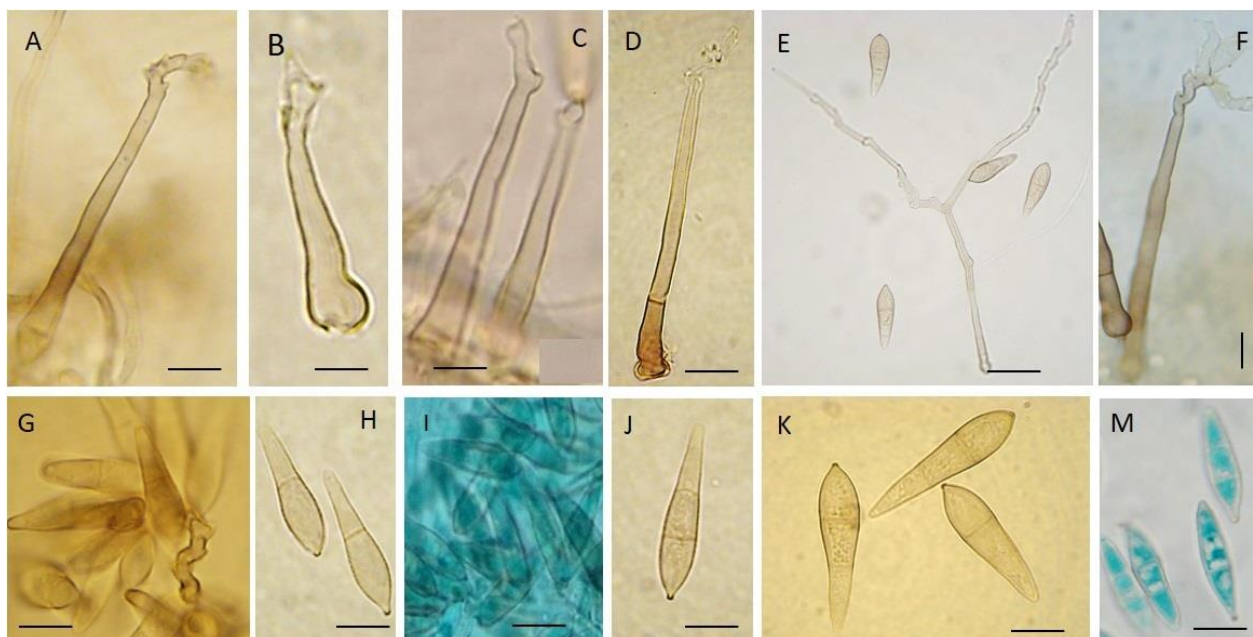


Fig. 2. *Pseudopyricularia*: Different types of conidiophores (A–F) and Conidia (F–J). Scale bars= 10 μ m

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معیارهای شناسایی جنس *Pseudopyricularia* روی گیاهان جگن

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چکیده: تیره Pyriculariaceae اخیراً با چندین جنس و گونه جدید ایجاد شده است. در واقع، برخی از گونه‌هایی که قبلاً در جنس *Pyricularia* معرفی شده بودند به گونه‌ها و جنس‌های جدید منتقل شدند. در این مطالعه مروری جامع بر یافته‌های تاکسونومی، فیلوژنی و علائم شناسی گونه‌های *Pseudopyricularia* انجام و کلید شناسایی گونه‌های *Pseudopyricularia* براساس معیارهای ریخت‌شناسی ارائه شده است.

کلمات کلیدی: *Pseudopyricularia*، فیلوژنی، گونه جدید، کلید شناسایی