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A new species of *Bipolaris* from Iran

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Abstract— *Bipolaris salkadehensis* sp. nov., isolated from the leaves of *Sparganium erectum* (*Thyphaceae*) and *Cladium mariscus* (*Cyperaceae*) collected in Iran, is described and illustrated. The new species is characterized by straight to slightly curved subcylindrical to fusoid conidia with end cells demarcated by thick dark septa and with monopolar and bipolar germination. Morphological data and ITS+5.8SrDNA sequence analyses support *B. salkadehensis* as a distinct species of *Bipolaris*.

Key words — morphology, taxonomy, fungus, *Cochliobolus*, phylogeny

Introduction

Bipolaris species are known to infect many kinds of grasses causing spots or flecks on leaves or stems and sometimes cause diseases on herbaceous plants such as *Cactaceae* and *Musaceae* (Ellis 1971, Sivanesan 1987). Since Sivanesan (1987) monographed the graminicolous species of the genus, several new *Bipolaris* species have been described (Chiang et al. 1989, Peng & Lu 1989, Sisterna 1989, Alcorn 1990, Sivanesan 1992, Alcorn 1996, Chen et al. 2000, Zhu et al. 2000, Deng & Zhang 2002, Jiang & Zhang 2008, Zhang & Li 2009). Recent molecular phylogenetic analyses have shown that *Bipolaris* and *Curvularia* are closely related to each other and comprise a different clade from *Exserohilum* (Berbee et al. 1999, Olivier et al. 2000). The rDNA-ITS sequences of (particularly the internally transcribed spacers ITS1 and ITS2) are useful tools for resolving taxonomic relationships within *Bipolaris*.

In this study, we describe a new species of *Bipolaris,* which we compare both morphologically and phylogenetically with other species.

Materials & methods

Collection and isolation of the fungus

Plant material was obtained from the Salkadeh village, Khoy city, West Azerbaijan province, in northwestern Iran in the summer of 2010. Leaf samples of *Sparganium erectum* L. (*Thyphaceae*) and *Cladium mariscus* (L.) Pohl (*Cyperaceae*) with brown oval to elliptical lesions with approximately 2–3 mm in length were collected and stored dry in a refrigerator at 5°C until use. A single spore produced on the surface-sterilized leaf under NUV light on 12 h diurnal cycle at room temperatures 23 ± 2°C (Sivanesan 1987) was transferred to tap water agar with autoclaved wheat straw (TWA+wheat straw) plates. Five isolates were obtained: Bi-1–3 from *S. erectum*, and Bi-4–5 from *C. mariscus*. For morphological inspection each isolate was transferred to Petri dishes containing PDA using a thin glass needle and incubated at 23 ± 2°C under darkness; species descriptions are based on 10–14 day old cultures. Measurement and microphotographs were taken from slide mounts in lactophenol and lactophenol cotton blue using an Olympus light microscope (model BH2). The fungal isolates were compared with the descriptions of Ellis (1971, 1976), Sivanesan (1987) and reported species after 1987. Dried culture vouchers have been deposited in the herbarium in the Department of Plant Protection, Faculty of Agricultural Sciences & Engineering, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran (TUPP).

Molecular phylogenetic analyses

Whole genomic DNA was extracted from the mycelium of each isolate grown on V8 juice agar by homogenization in a standard sodium dodecyl sulfate (SDS) detergent lysis buffer followed by a phenol : chloroform extraction and precipitation in ethanol with sodium acetate (Sambrook et al. 1989). The ITS1+5.8S+ITS2 rDNA region was amplified using the polymerase chain reaction (PCR) conditions and the ITS1 and ITS4 primer pair (White et al. 1990). Purified PCR products were sequenced by ABI PRISM 3100 automated sequencers (Applied Biosystems, Foster City, CA, USA). For phylogenic comparison, the GenBank sequences of 26 *Bipolaris* species, 5 *Curvularia* species, 2 *Exserohilum* species, 2 *Drechslera* species, and *Alternaria alternata* (Fr.) Keissl. (as outgroup) were included (Berbee et al. 1999). The DNA sequences were aligned using Clustal X version 1.8 (Thompson et al. 1997). Further visual alignments were done in Sequence Alignment (Se-Al) Editor version 2.0 (Rambaut 2000). The data were analysed phylogenetically using distance methods. The distance matrix was calculated using Kimura's two parameter method (Kimura 1980) and analyzed with the neighbor-joining (NJ) method (Saitou & Nei 1987) using the program PAUP* 4.0 beta 10 (Swofford 2002). Bootstrap values were generated with 1000 replicate heuristic searches to estimate support for clade stability of the consensus tree using the same program. Sequences have been deposited in the DNA Data Bank of Japan (DDBJ).

Results

New species

Bipolaris salkadehensis Ahmadpour & Heidarian, **sp. nov.** FIG. 1

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Differs from *Bipolaris cynodontis* in conidia with both monopolar and bipolar germination and conidial end cells that are demarcated by thick dark septa.

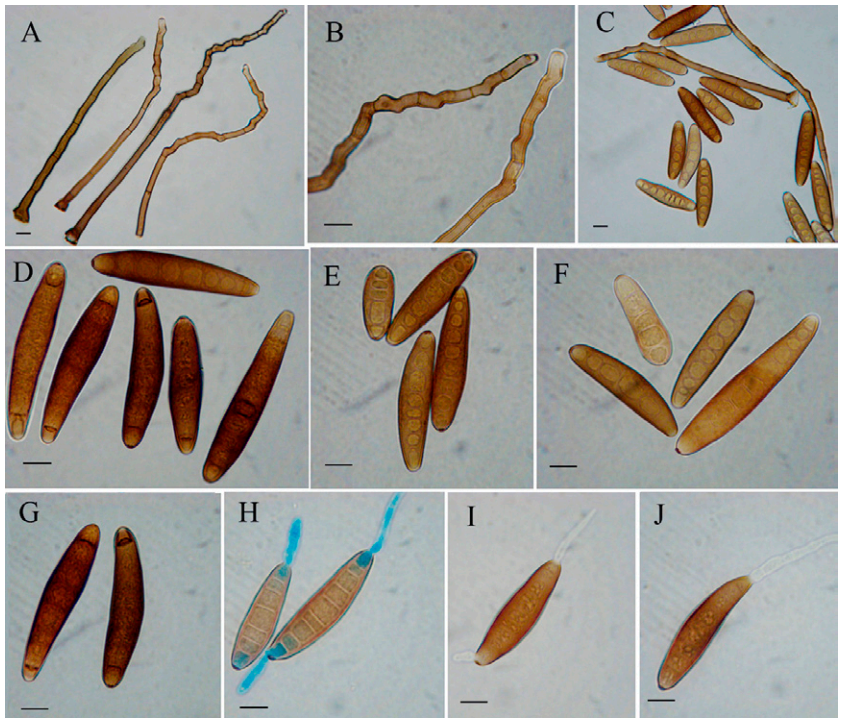

Fig. 1. *Bipolaris salkadehensis*. A, B. Conidiophores. C–G. Conidia. H–J. Germinating conidia. Scale bars = 10 µm.

Type: Iran. West Azerbaijan, Khoy City, Salkadeh village, on infected leaves of *Sparganium erectum*, 20 Sep 2010, coll. A. Ahmadpour Bi-1 (**Holotype**, TUPP1366 [dried culture]; DDBJ sequence, AB675490).

Etymology: referring to the type locality.

Colonies in 'TWA+wheat straw' agar brown, velvety, floccose. Hyphae brown, septate, branched, smooth, 5–7 µm wide. Stromata not formed. Conidiophores single or fasciculate in small groups, simple, pale brown to brown, cicatrized with scars often inflated and smooth, multiseptate, 225–590 µm long, swollen to 7.5–15 µm diam at base, then narrowing to 5–8.5 µm diam (middle) and 5–7.5 µm diam (apex). Conidia pale brown to dark brown, subcylindrical to fusoid, occasionally obclavate to clavate, straight to slightly curved, smooth, end cells rounded, very pale and demarcated by thick, dark septa, (4–)6–8 (–10) distoseptate, (32–)53–80(–93) × 12–15 µm, hilum ≤2 µm diam, sometimes slightly protruding. Germination of conidia is monopolar and bipolar. The primary septum in developing conidia is submedian, the second delimiting the basal cell, and the third distal.

Cultural characteristics: Colony velvety, floccose, olivaceous-brown on PDA.

Additional material examined: **IRAN**. **West Azerbaijan**, Khoy City, Salkadeh village, on infected leaves of *Cladium mariscus*, 20 Sep 2010, coll. A. Ahmadpour Bi-4 (TUPP1362; DDBJ sequence, AB675491) and Bi-5, on infected leaves of *Sparganium erectum*, Bi-2–3.

Comments: Its conidial morphology clearly establishes our specimen as belonging to *Bipolaris*. It is morphologically similar to *B. cynodontis*, *B. setariae* (Sivanesan 1987), *B. sesuvii* (Zhang & Li 2009), and *B. fusca* (Jiang & Zhang 2008). *Bipolaris cynodontis* is distinguished from the new species by its smaller conidia, absence of thick dark septa demarcating the end cells demarcated by thick dark septa, and with only bipolar germination (Sivanesan 1987). The conidia of *B. setariae* are somewhat similar — fusoid to navicular, pale to mid golden brown, 5–10 distoseptate, (45–)50–70(–100) × 10–15 µm (Sivanesan 1987) — but with end cells demarcated by unthickened hyaline septa and paler than those of *B. salkadehensis*. The conidia of *B. sesuvii* are olivaceous-brown to brown, basal cell concolorous or slightly pale, subcylindrical to broadly fusoid and rounded at ends, straight, smooth, 5–9 distoseptate, 52–77 × 13–16 µm (Zhang & Li 2009) and are narrower and have end cells demarcated by unthickened hyaline septa. The conidia of *B. fusca*, which are shorter and wider than those of *B. salkadehensis*, are straight or occasionally slightly curved, smooth, cylindrical or ellipsoidal, (3–)5–7(–11) distoseptate, 31–67 × 11–20 µm, yellowish brown to dark brown, with end cells often very pale and demarcated by thick dark septa (Jiang & Zhang 2008).

Phylogenetic analysis & discussion

PCR products of ITS+5.8S rDNA sequences were 506bp for isolate Bi-1 and 505bp for isolates Bi-2–5. Since the Bi-2–5 sequences matched completely, the Bi-1 sequence was registered as DDBJ AB675490 and the Bi-4 as DDBJ AB675491. According to the neighbor-joining tree derived from ITS+5.8S rDNA sequences made in this study, the *B. salkadehensis* isolates formed a monophyletic group with a 95% bootstrap value close to *B. cynodontis* (AF163093) (Fig. 2). However, the 98.2% (497/506 bp) sequence similarity between *B. salkadehensis* and *B. cynodontis* indicates that those two species clearly differ. Moreover, despite similar conidial morphologies, *B. salkadehensis* formed a different group from *B. setariae* and *B. sesuvii* (Fig. 2).

Berbee et al. (1999) used the ITS+5.8S rDNA sequences and a portion of the glyceraldehyde-3-phosphate dehydrogenase sequences to evaluate *Cochliobolus* (anamorphs *Bipolaris*) and proposed that *Bipolaris* be divided further, separating species with large, canoe-shaped, gently curving conidia (*Cochliobolus* Group 1) from those with short, either straight or curved conidia lacking a gentle curve along the whole spore length (*Cochliobolus* Group 2), which intermix

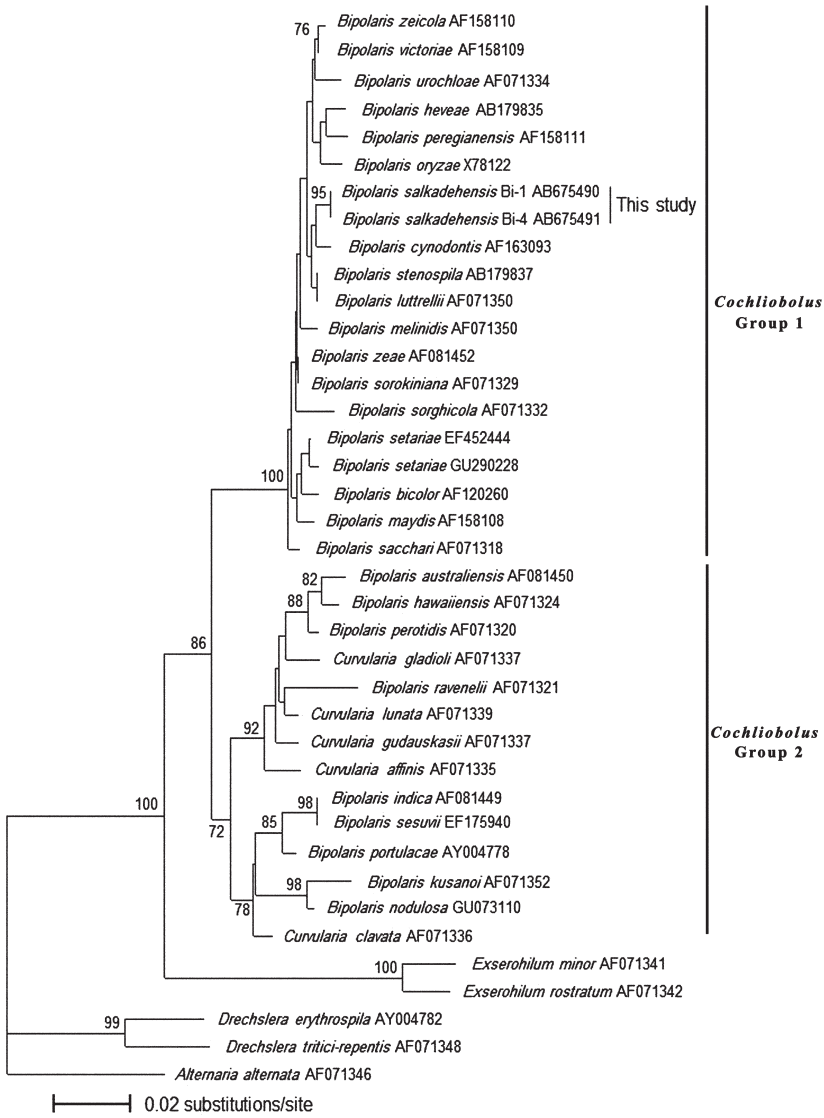

Fig. 2. A neighbor-joining tree inferred from the ITS+5.8S rDNA sequences from 35 taxa. Bootstrap values for 1000 replicates are shown on the branches, and rDNA-ITS DDBJ accession numbers stand after the species names. The branch length is proportional to the number of base changes as indicated by the scale bar. *Alternaria alternata* (AF071346) is an outgroup.

with *Curvularia* species. All *B. salkadehensis* isolates used in this study clustered in the *Cochliobolus* Group 1 subclade, which contains *Bipolaris* species large, canoe-shaped, gently curving conidia. Although the conidial morphology of *B. salkadehensis* is similar to *B. cynodontis*, *B. setariae* (*Cochliobolus* Group 1), and *B. sesuvii* (*Cochliobolus* Group 2), the phylogenetic analysis did not show a close relationship. The combined molecular-morphological analysis supports *B. salkadehensis* as an unreported new species.

Although the teleomorph was not observed in cultures or on the natural host, the *B. salkadehensis* teleomorph relationships can be compared by using differences or similarity in conserved DNA sequences (e.g. ITS, GPD, BRN1 and mating type genes) with other known *Bipolaris* species to provide a more reliable classification system at the generic and species levels (Berbee et al. 1999, Shimizu et al. 1998, Turgeon 1998). The MAT phylogenetic trees, along with the ITS and GPD trees, can be used as a database from which to look back into evolutionary history in order to understand how different reproductive lifestyles arose (Turgeon 1998).

Both morphological and phylogenetic evidence supports *B. salkadehensis* as a previously unreported species separate from other known *Bipolaris*. This is the first report of *Bipolaris* on the hosts *S. erectum* (*Thyphaceae*) and *C. mariscus* (*Cyperaceae*).

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