Mycologia Iranica 2(1): 46 – 58, 2015

Original Article

A further contribution to the knowledge of sooty mould fungi from Iran

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Abstract: Sooty mold fungi are often associated with honeydew which insects secrete while feeding on the plant. A great variety of these fungi occur in the North of Iran, especially on *Citrus* spp which is one of the most widely cultivated fruit trees in this region. During last 10 years, several collections of these fungi examined and 15 species have been recorded. In this paper seven previously unreported species are treated and described for the first time from Iran. The treated species include *Catenuloxyphium heterosporum* (on *Salix aegiptiaca* and *Alnus glutinosa*); *Echinothecium* sp. (on *Eriobotrya japonica* and *Passiflora* sp.); *cf. Denisiella* sp. (On *Citrus sinensis* and *Malus pumila*); *Fumagospora capnodioides* (on *Alnus glutinosa*, *Populus deltoids*, *Salix aegyptiaca* and *Zelkova carpinifolia*); *Leptoxyphium fumago* (on *Alnus glutinosa*, *Rubus* sp. and *Phytolacca americana*); *Tripospermum juglandis* (on *Ligustrum* sp.) and *Scorias spongiosa* (on *Citrus sinensis*).

Key words: Capnodiales, Capnodiaceae, Chaetothyriaceae, Scorias, Fumagospora.

INTRODUCTION

Sooty mould fungi are a diverse group of ascomycetes that are common on the leaves, stems, twigs and fruits of many plants. These fungi are saprobic on the exudates produced by several insects living on the surface of plant structures (Hughes 1976; Chomnunti et al. 2011, 2014). Sooty mould fungi produce dark pigmented hyphae, usually with mucilaginous outer walls that form a thin network on the plant surface. Such thin network may be peeled readily from the surface with or without some treatment such as collodion solution (Hughes 1976). These fungi are common in tropical, humid subtropical or temperate climate. The first comprehensive monographic literature on these fungi extends back to over 50 years ago (Batista and Ciferri 1962; 1963a, b), however, taxonomy of the sooty mould fungi is much complicated and needs more studies. Identification of these fungi is largely based on the old literatures and new taxonomic treatments are rather scanty. However, some investigations and new approaches to solve taxonomic problems of these fungi have recently been done by some researcher (Cheewangkoon et al. 2009; Chomnunti et al. 2011, 2012, 2014; Bose et al. 2014; Yang et al. 2014). In north of Iran including Guilan, Gorgan and Mazandaran provinces, sooty moulds are common on living leaves of a great variety of plants (Khodaparast 1986, Byrami et al. 2013). According to previous studies about 15 species have been reported from Iran. This paper reports and describes more species of sooty mould found from this region.

MATERIALS AND METHODS

Infected plant materials were initially examined visually using stereomicroscope for mycelial appearance and development. Fungal structures were mounted in 50% lactic acid and examined using a BH2 Olympus light microscope equipped with a Sony digital Camera (DSC-HX1). Measurements were taken in lactic acid (50%) mounts, based on at least 25-30 conidiophores, conidia, etc. Morphological characters of fungal structures including hyphal type, conidia and conidiomata, ascoma, asci and ascospores, if present, were studied. Identifications of the taxa were largely based on the keys and descriptions available in several literatures including Batista, and Ciferri (1962;1963 a,b), Hughes (1976), Von Arx and Muller (1975), Kwee (1988), Reynolds (1999, 2000, 2010), Reynolds and Gilbert (2005, 2006), Chomnunti et al. (2011). Species descriptions, photographs of the conidiophores and conidia, ascoma, asci etc. are provided. Isolation of some sooty mould species was also attempted on water agar, potato dextrose agar (PDA) and 2 % malt

Submitted 3 April 2015, accepted for publication 5 June 2015
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extract agar (MEA). For isolation, a part of plant material containing pycnidia were incubated in a moist chamber for up to 14 h. Oozed conidial mass on top of ostiole of one pycnidium were removed with a sterile needle and streaked on agar containing media. Growing colonies were sub-cultured onto potato-dextrose PDA or MEA.

All collected specimens were deposited in the fungal collection of the Department of Plant Protection, Faculty of Agricultural sciences, University of Guilan (GUMC).

RESULTS AND DISCUSSION

Seven species of sooty mould fungi were identified, all of them are described as new records from Iran.

***Catenuloxyphium heterosporum* Bat., Nascim. & Cif., in Batista & Ciferri, Quad. Lab. crittogam., Pavia 31: 55 (1963)**

Saprobic on insect exudates and producing a black sooty-like covering on the surface of leaves. Mycelium consisting of a network of superficial brown septate hyphae. Hypahal cells cylindrical to subcylindrical, constricted at septa, measured 6-21 x 3.5-5 µm. *Conidiomata* pycnidial, superficial, scattered, brown, flask-shaped to cylindrical, without conspicuous stalk or stalk very short, wider at base or near the base, sometimes with oval fruiting zone at middle, tapering to the apex, producing ostiole at the apex, ostiole surrounded by hyaline hyphae, up to 35 µm in length, wall consisting of oblong cells, about 6-24 x 3-6 µm, total pycnidium length 200-350 (-420) µm, 36 -56 (-70) µm at wider part (usually near the base), Conidiogenous cells arising from the inner cell wall of the fruiting zone. Conidia ellipsoid, ovoid to cylindric, at first hyaline, aseptate or with 1-3 (usually 1) septa, 6.5-15 x 3-4 (-4.5) µm; mature conidia brown, with 1-3 (usually 1) septa, 10-17 x 5-7 µm (Fig. 1, 2).

Specimen examined: on *Salix aegiptiaca* L., Ramsar, Mazandaran Province, Iran, 27 Jul. 2007, S. A. Khodaparast (GUM 1309); on *Alnus glutinosa* (L.) Gaertn., Tonekabon, Mazandaran Province, Iran, 17 Jun. 2011, F. Byrami (GUM 1310).

In our specimen, pycnidia are longer and fimbriate versus shorter and not fimbriated ostiole which has been described in Batista and Ciferri (1963b) for *Catenuloxyphium heterosporum*.

***Cf. Dennisiella* sp.**

Mycelium consisting of superficial hyaline, septate hyphae. Hyphae are more or less cylindric, about 3-4 µm wide. Hyphae frequently become compact and form cushion of cells which are pigmented and develop two to several setae around cushion. Setae are nonseptate, thick-walled, subulate, robust and straight or sometimes curved and slightly flexible, usually pointed at apex, with a bulbous, lobed base, 50-250 µm long and 6-10 µm wide near the base.

Some setae are encircled by hyphae bearing conidia. The conidia are globose to subglobose, hyaline, about 3.5-6 µm (Figs. 3, 4). According to Hughes (1976) fungus with such conidia assigned to *Microxiphium* (anamorphic state of *Dennisiella*), however, It was difficult to discriminate if there was conidial state of this fungus or contamination from other fungi. No ascomatal state has been discovered during this study, however, it seems this specimen belong to *Dennisiella.*

Specimen examined: On *Citrus sinensis* Pers., Guilan, Sumaehsara 7 Feb. 2014, S.A. Khodaparast (GUM 1306); *Malus pumila* Mill., Guilan, Sumaehsara 16 Nov. 2011, S.A. Khodaparast (GUM 1307).

***Echinothecium* sp.**

Mycelium consisting of superficial pale brown to olivaceous brown septate hyphae. Hyphae are cylindric, constricted at septa. Ascoma globose, subglobose, setose, 65-180 µm, Peridium consisting of pale brown to olivaceous brown cells forming a textura angularis, setae dark brown, usually produced at upper part of ascoma, measured 13-95 x 2.5-9 µm, Asci elongate, fusiform, bitunictae, 50-90 x 8-20 µm, 8-spored, Ascospores fusiform, hyaline, 2-celled, 9-20 x 2.5-5.5 µm (Fig. 5).

Specimen examined: *Eriobotrya japonica* (Thunb.) Lindl., Mazandaran Province, Nashtaroud, Iran, 17 Jun. 2011, F. Byrami (GUM 1320); on *Passiflora* sp., Guilan Province, Rasht, Iran, 25 Oct. 2009, F. Byrami (GUM 1321).

According to Batista and Ciferri (1963a) this species is very close to *Capnobatistia serrulata* Cif. & Leal. This genus have been transferred to *Echinothecium* by von arx and Muller (1975).

***Fumagospora capnodioides* G. Arnaud, Annals d'École National d'Agric. de Montpellier, Série 2 10(4): 326 (1911)**

Saprobic on insect exudates and producing a black sooty-like covering on the surface of leaves. Mycelium consisting of a network of superficial brown septate hyphae. Pycnidia variable in shape, mostly cylindrical to subcylindrical, rarely flask shaped, with or without stalk, simple or branched,

usually 200-400 µm total length, fruiting zone 25-60 µm wide, stalk 25-95 x 25-46 µm, about 18-35 (-46) µm wide near the ostiole, ostiole with hyaline hyphae with 14-25 µm in length, conidia ellipsoid, at first hyaline, becoming pigmented at maturity, 2-3 (-4) transverse and 1-3 longitudinal septa, measured 15-25 x 8-11 µm (Fig. 6).

Specimen examined: on *Alnus glutinosa* (L.) Gaertn., Guilan, Poonel, 8 Aug. 2012, S. A. Khodaparst (GUM 1326); *Populus deltoides* W. Bartram ex Marshall, Sumaehsara, Guilan province, 20 Sept. 2007, S. A. Khodaparast (GUM 1318); *Salix aegyptiaca* L., Sumaehsara, Guilan Province, 28 Jun. 1997, S. A. Khodaparast (GUM 1316); L., *Zelkova carpinifolia* Dippel, Rasht, 7 Oct. 1997, S.A. Khodaparast (GUM 1317).

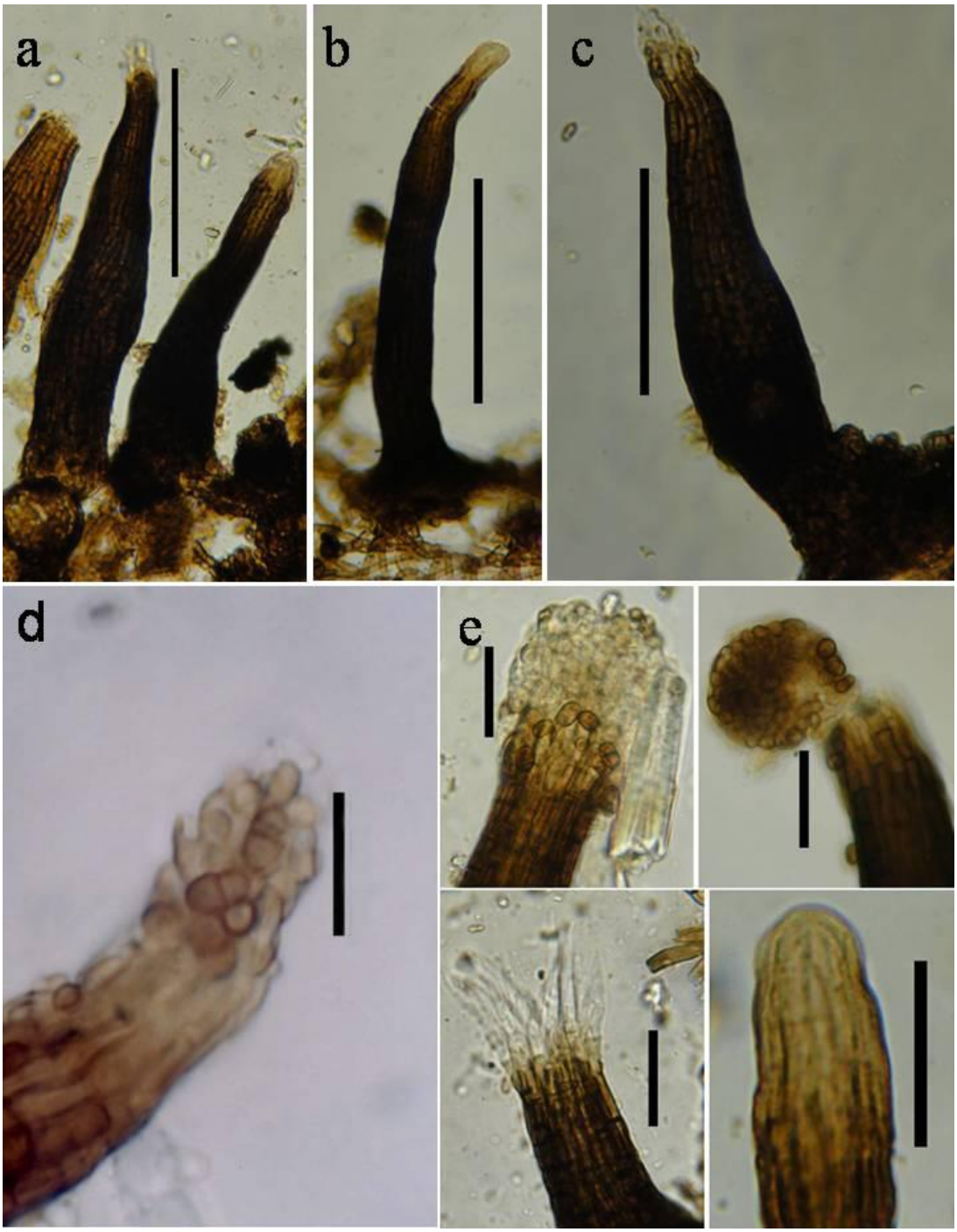

Fig. 1. *Catenuloxyphium heterosporum*: **a-c**. pycnidia, scale= 200 µm; **d-e.** Ostioles and mass of conidia, scale for D=20 µm, for E=30 µm.

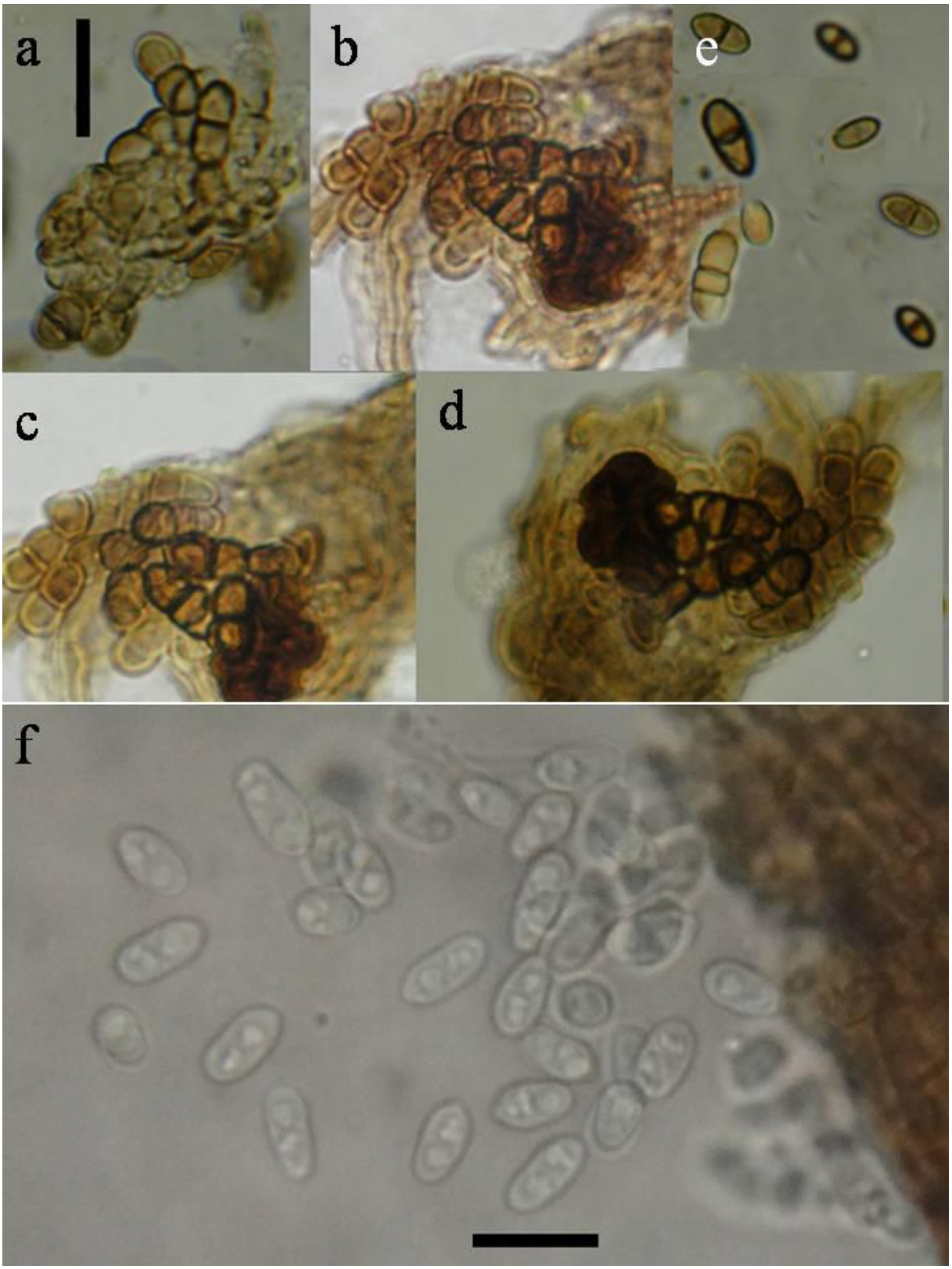

Fig. 2. *Catenuloxyphium heterosporum*: **a-f.** Different type of conidia, scale = 20 µm.

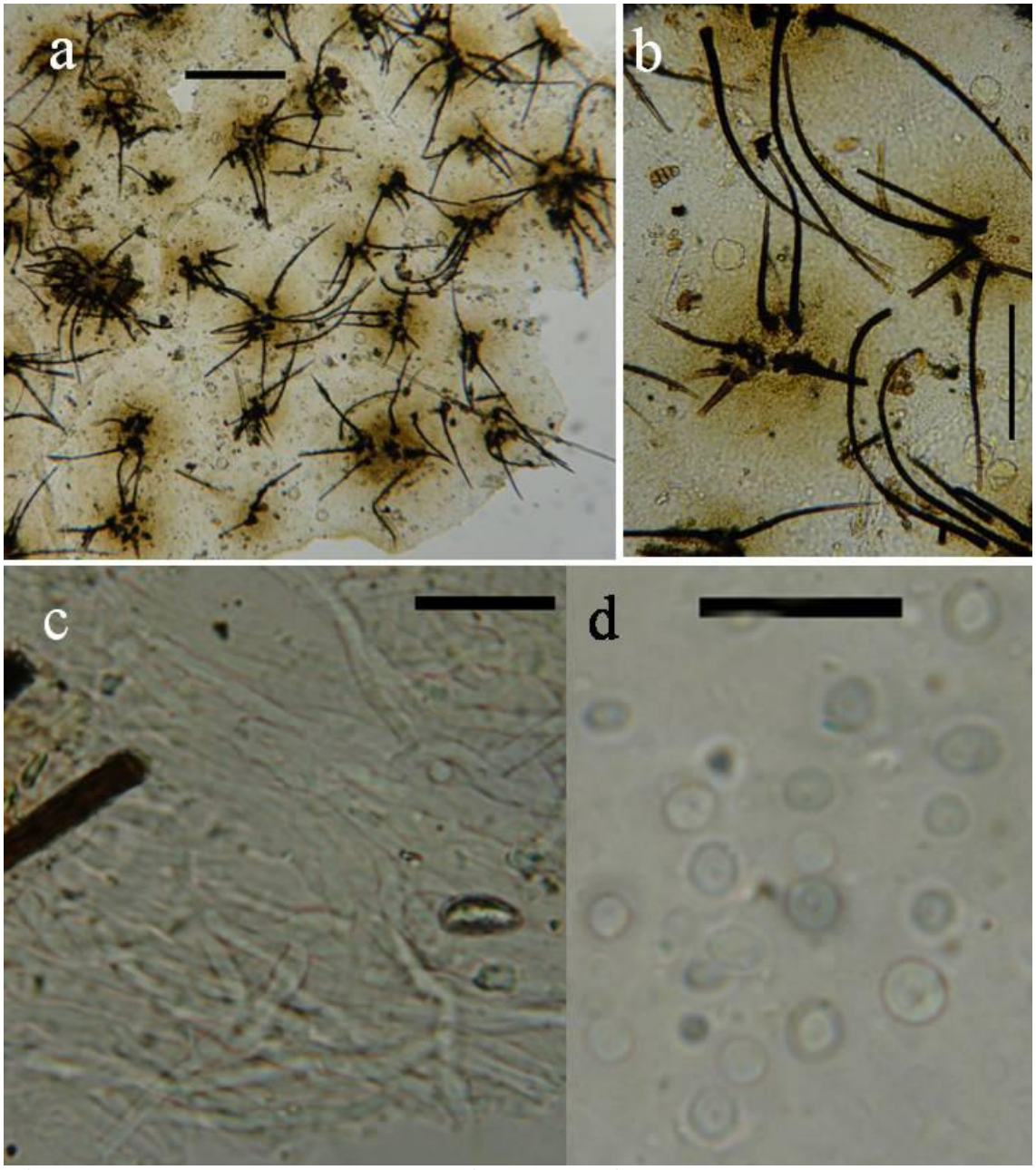

Fig 3. *Cf. Dennisiella* sp. **a-b** Setae on the mycelium, scale for A =200 µm, for B = 100 µm; **c.** mycelium, **d.** conidia, scale 20 µm.

***Leptoxyphium fumago* (Woron.) R.C. Srivast., Arch. Protistenk. 125 (1-4): 333 (1982)**

Saprobic on insect exudates and producing a black sooty-like covering on the surface of leaves. Mycelium consisting of a network of cylindrical and septate hyphae.

Conidioma a synnematal-like structure (pycnidia as stated by some authors such as Chomnunti et al. 2011), comprising 10-12 parallel, septate hyphae, cylindrical but usually slightly tapering toward apex, with long stalk, and a cup-like fruiting zone at the apex, total length up to 500 µm, usually proliferate to produce new conidiogenous zone, 13-55 µm at the base, cup-like fruiting zone measured 18-50 µm in wide. Conidiogenous cells arising from the inner cell wall of the fruiting zone. Conidia at first hyaline, ellipsoid, continuous, measuring 5-10 x 2-3 (-4) µm, finally they may enlarge, become 1-septate and pigmented on the host plant leaves (Fig. 7).

Specimen examined: On *Alnus glutinosa* (L.) Gaertn., Katalom-Tonekabon road, Mazandaran Province, Iran, 21 Nov., F. Byrami (GUM 1324); *Rubus* sp. Ramsar, Mazandaran Province, Iran, 21 Nov., F. Byrami (GUM 1308); *Phytolacca americana* L., Guilan Province, Sumaehsara, Iran, 27 Oct. 2007, S.A. Khodaparast (GUM 1325).

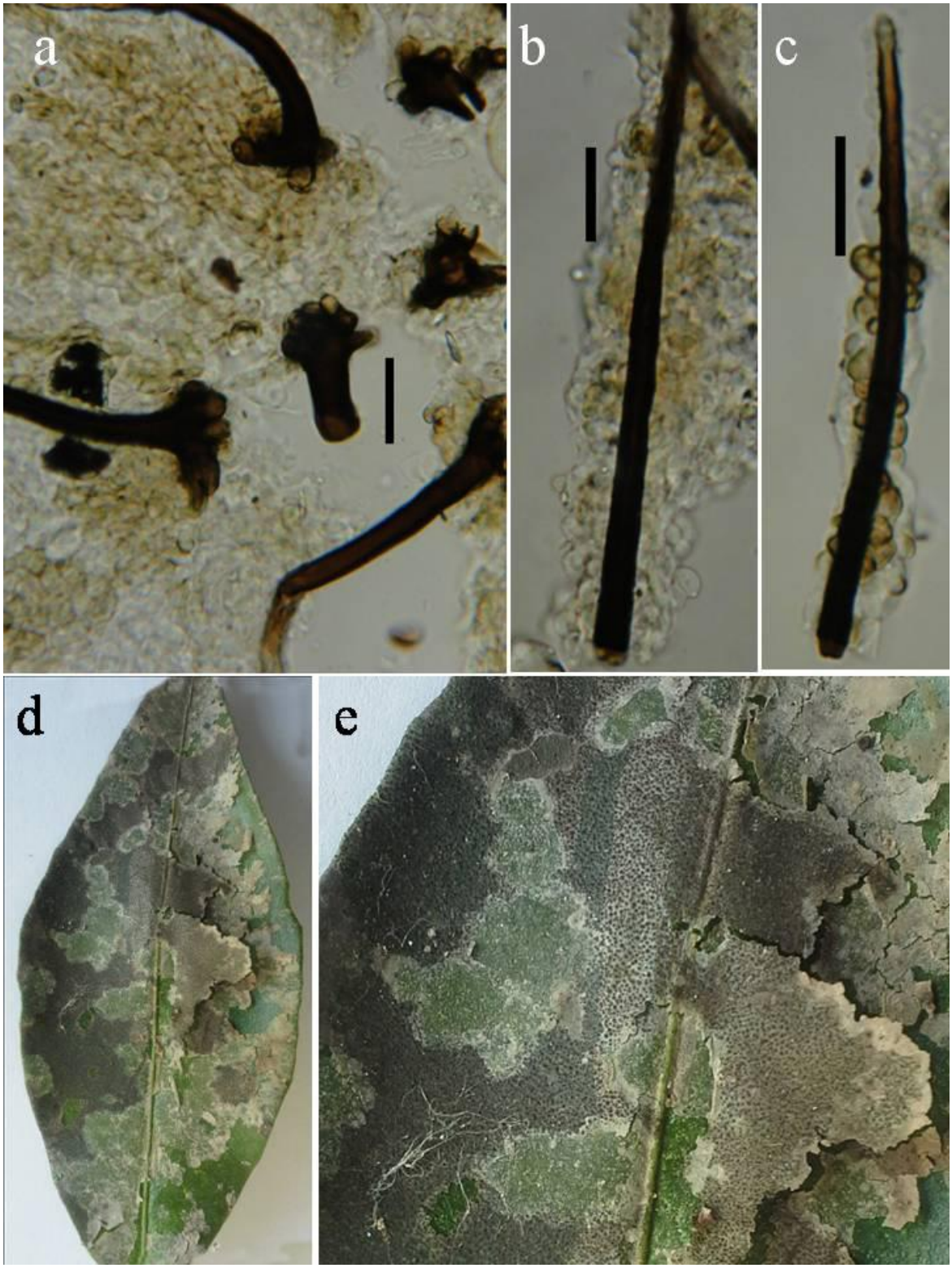

Fig. 4. *Cf. Dennisiella* sp. **a.** Lobed basal cell of setae on the mycelium, **b-c.** Conidia around setae assigned to *Microxiphium* (anamorphic state of *Dennisiella*), **d-e.** Colony on the leaf surface, scale 20 µm.

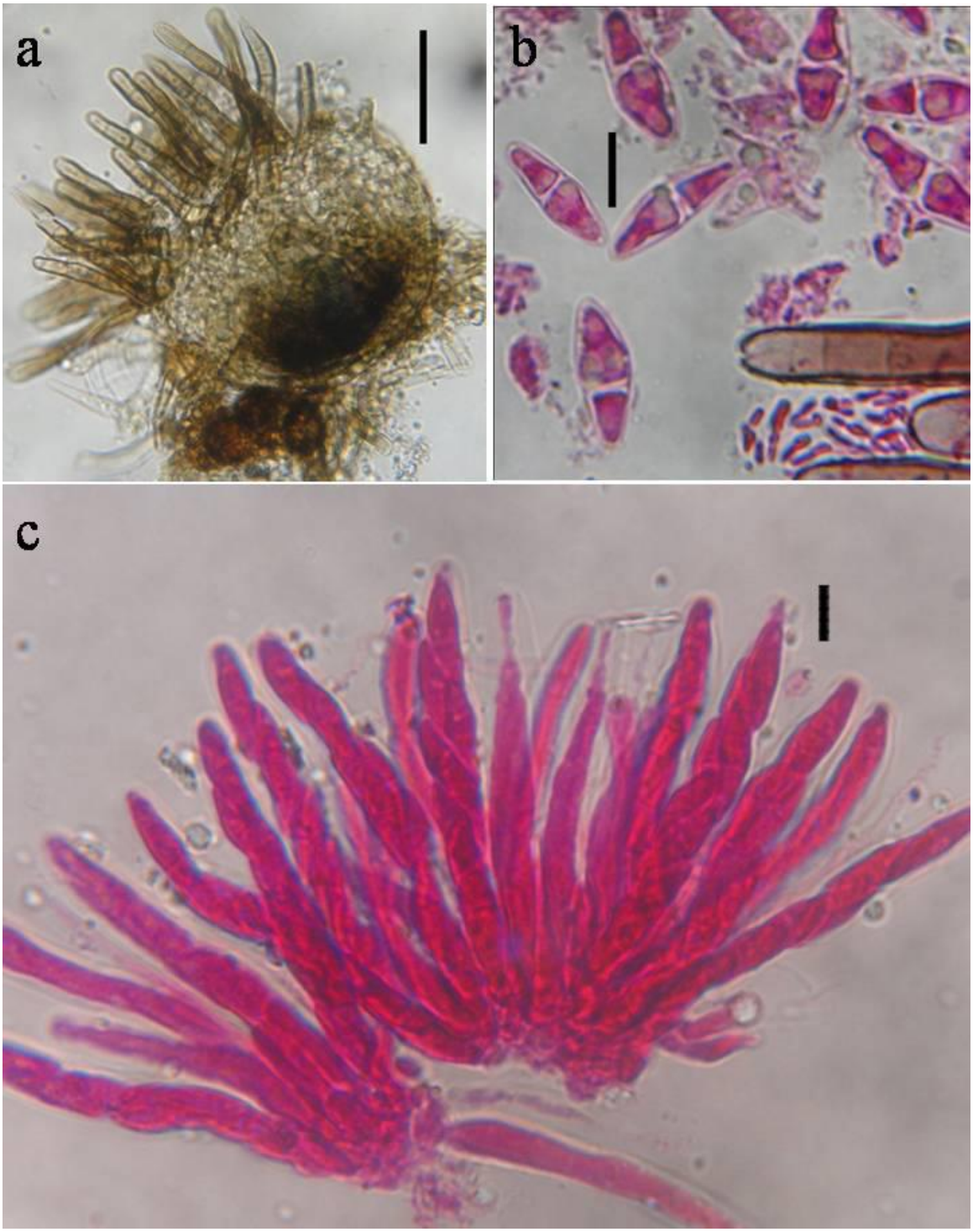

Fig. 5. *Echinothecium* sp.: **a.** Ascoma, **b.** ascospores, **c.** Asci, scale for A=50 µm, B=10 µm, C=20 µm.

***Tripospermum juglandis* (Thüm.) Speg., Physis, Rev. Soc. Arg. Cienc. Nat. 4 (no. 17): 294**

A dematiaceous Hyphomycetes that occurs solely or usually together with other sooty moulds as a superficial mycelium consisting of olivaceous septate hyphae on leaf surfaces that already infested with insect exudates. Hyphae are more or less cylindrical, constricted at septa, composed of cylindrical or barrel shape cells. Each conidium arises from a pyriform, stalked cell 5-15 µm long and 3-6 µm wide. Conidia are brown to olivaceous, star-shaped with four divergent arms which are wide at the base and attenuated toward apex, apex rounded, longest arm measures 23-55 µm and shortest arms 12-30 µm (Fig. 8).

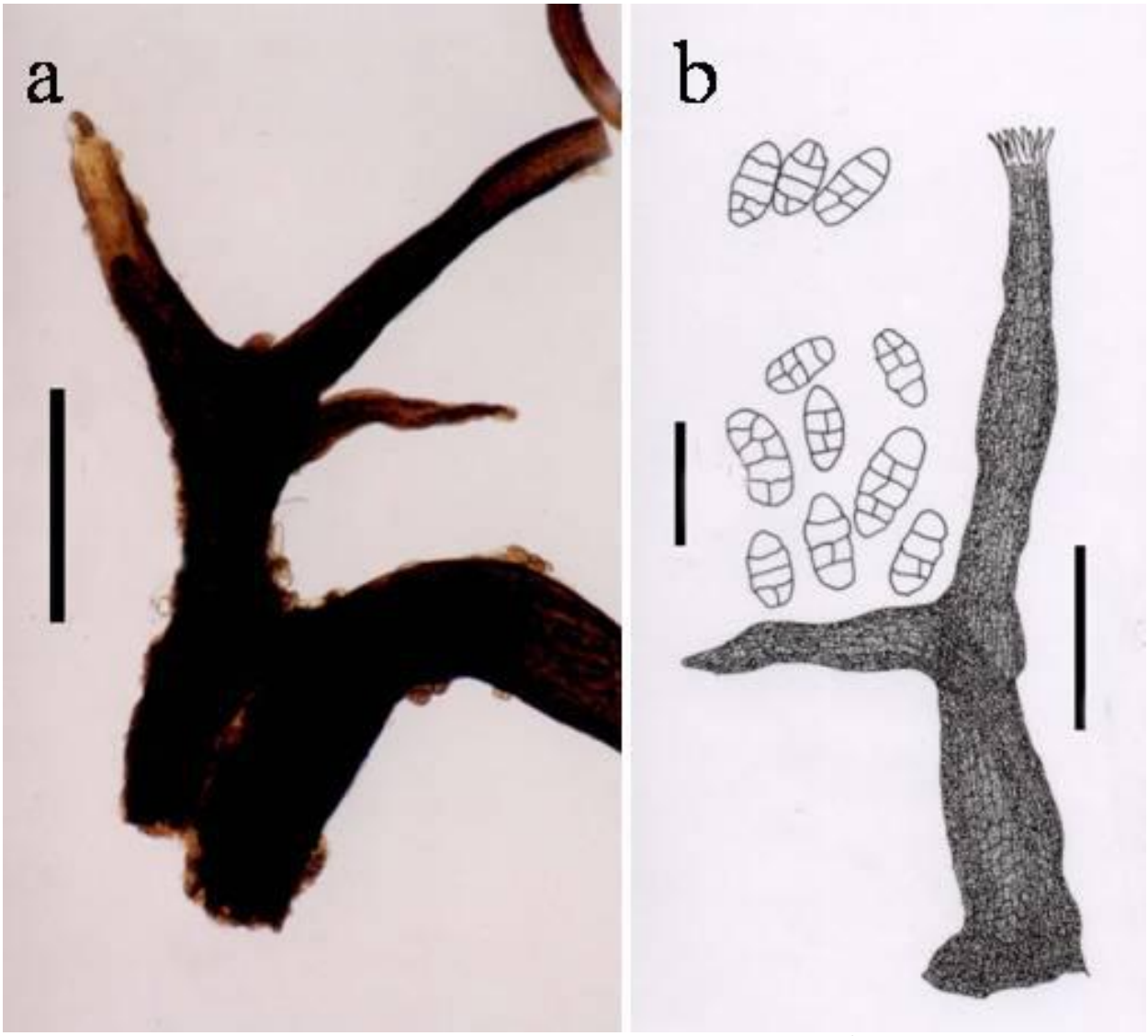

Fig. 6. *Fumagospora capnodioides*: **a.** Branched pycnidium, scale 50 µm, **b.** drawing of pycnidium, scale= 100 µm and conidia, scale =25 µm.

Specimen examined: *Ligustrum* sp., Katalom-Tonekabon road, Mazandaran Province, Iran, 22 Oct. 2010, F. Byrami (GUM 1323).

***Scorias spongiosa* (Schwein.) Fr., Syst. mycol. (Lundae) 3(2): 291 (1832)**

Saprobic on insect exudates and producing a black sooty-like covering on the surface of leaves. Mycelium consisting of a network of cylindrical and septate hyphae. *Conidiomata* pycnidial, scattered, brown to blackish brown, wall synnematous, comprising mostly of cylindrical cells, tapering to the apex, flask–shaped, total pycnidium length 250-550 µm, with a flattened stalk, stalks about 80-170 x 35-140 µm, continue to a conidiogenous zone, conidiogenous zone sub-ellipsoid, darker than other parts, (50-) 75-112 x 36-62 µm, extended into a neck, neck subcylindrical, 92-275 µm long, about 12-20 µm wide at base, and 10-13 µm apex; ostiole surrounded by hyaline hyphae about 10-25 µm. Conidia gathered in a slimy mass around ostiole, hyaline, ellipsoid, cylindric to subcylindric, rounded at both ends or pointed at the base, smooth, 1-celled, 2 guttules, 3.5-5.5 x 1.5-2 µm (Fig. 9).

Culture characteristics: Colony becoming up to 7 mm diam at 25 °C on PDA after 10 days, about 17.3 mm on MEA after 14 days in the darkness; superficial, flat, with entire edge, olivaceous to green, producing a characteristic spreading red –purple pigment in plate, showed a color change of the medium away from the original plate after subculturing; aerial hyphae at fiest with thin and inconspicuous septa, usually not

constricted, branched, smooth to slightly verrucose, pale brown to brown, cylindrical to sub-cylindrical, hyphae becoming thick-walled, pale broun to olivaceous, constricted at septa, about 3–5 µm wide.

Total pycnidium length 450-900 µm, stalks 120-600 µm long, and 50-125 µm wide, conidiogenous zone usually conspicuous, darked, sub-ellipsoid, ellipsoid, 150-230 x 55-150 µm, neck 137-375 µm long, 17- 30 near the base, tapering toward the apex, apex about 11-18 µm wide, hyaline hyphae around ostioles about 17-37 µm long. Conidia gathered in a slimy mass around ostiole, hyaline, ellipsoid, cylindric, subcylindric, 2 guttules, (-2.5) 3-4 x 1-2 (-2.5) µm. Pycnidia produced abundantly on the surface of the MEA plate (Fig. 10).

Specimen examined: On *Citrus sinensis* Pers., Sumaehsara, Guilan Province, Iran, 24 Jan. 2013, S. A. Khodaparast (GUM 1301).

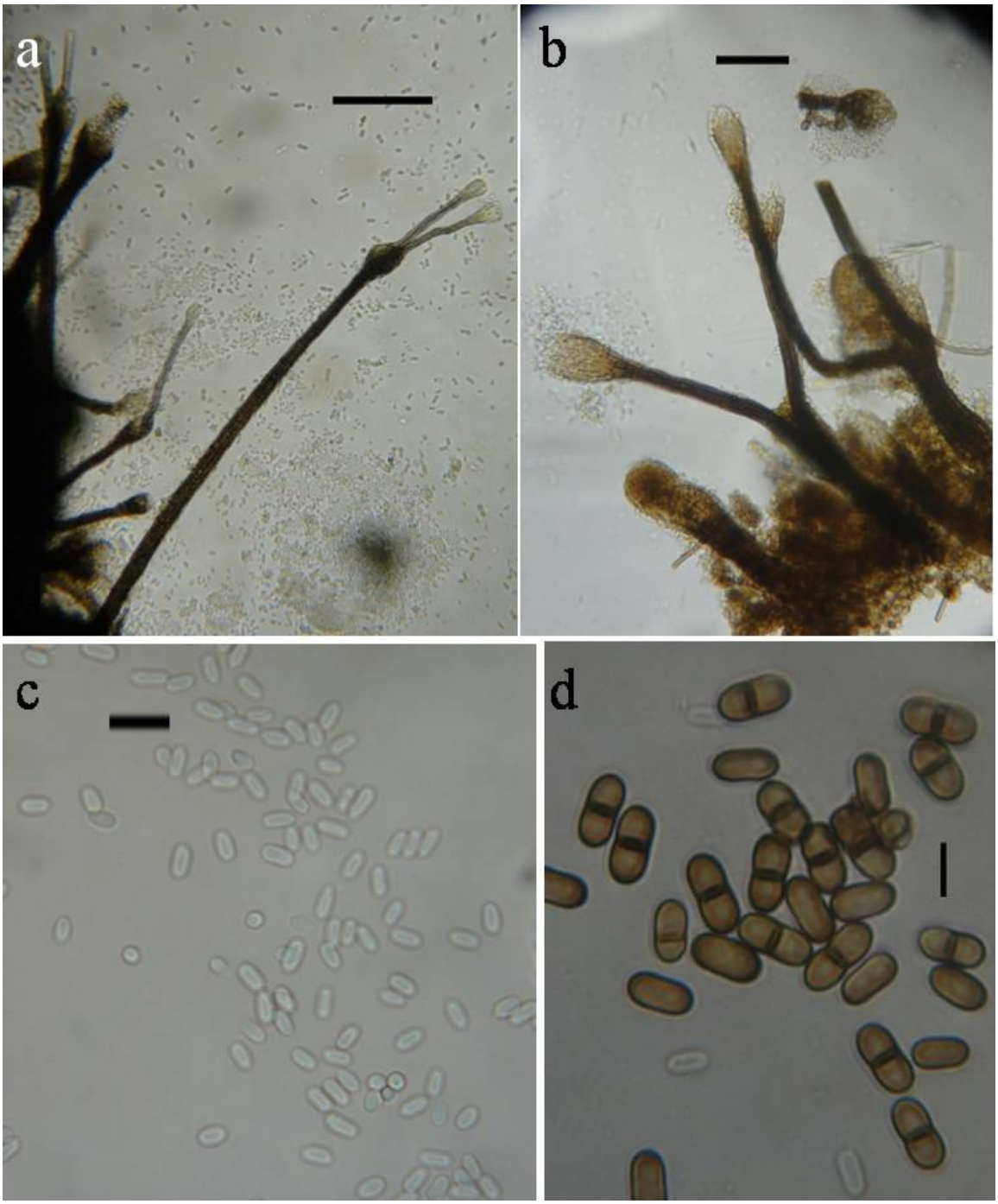

Fig. 7. *Leptoxyphium fumago*: **a-b.** Synnema, scale 50 µm, **c.** hyaline and immature conidia, scale 10 µm; **d.** mature pigmented conidia, scale 10 µm.

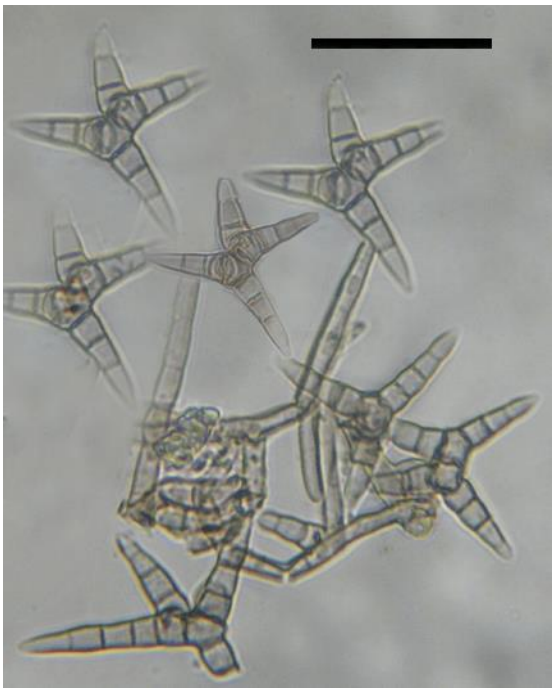

Fig. 8. *Tripospermum juglandis*: Conidia, scale =50 µm.

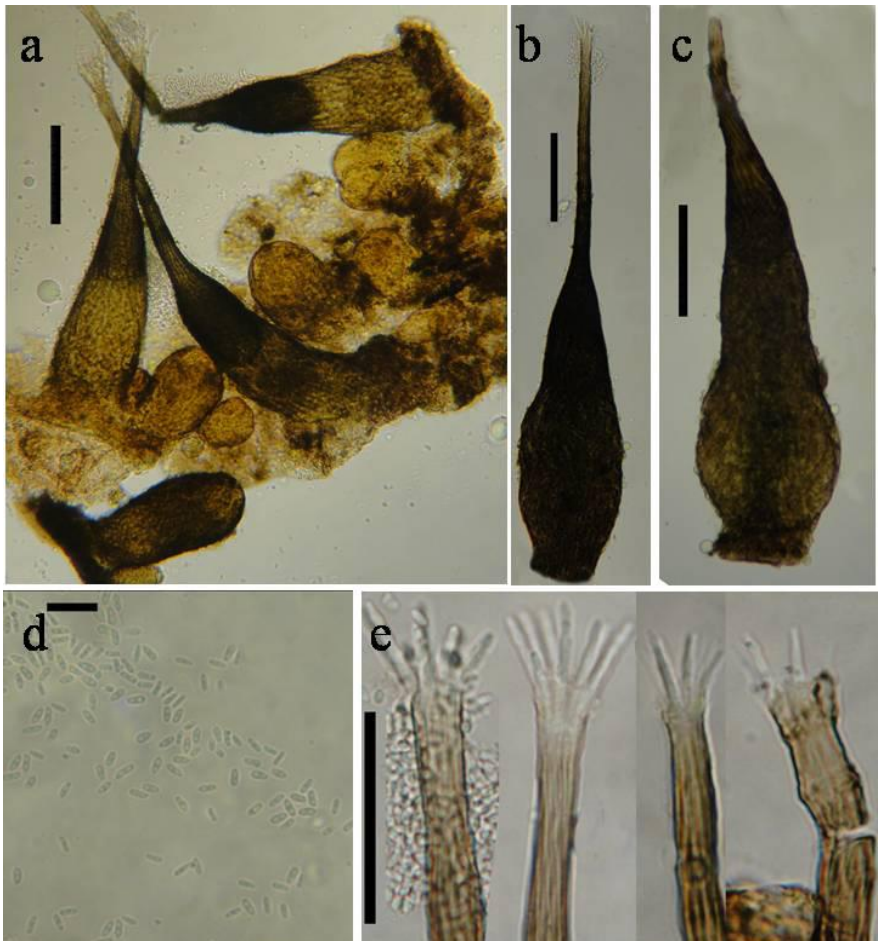

Fig. 9. *Scorias spongiosa*: **a.** pycnidial, some immature ascoma around pycnidial, scale= 100 µm; **b-c.** pycnidial, scale= 100 µm; **d.** Conidia scale =20 µm; **e.** pycnidium neck, scale =50 µm.

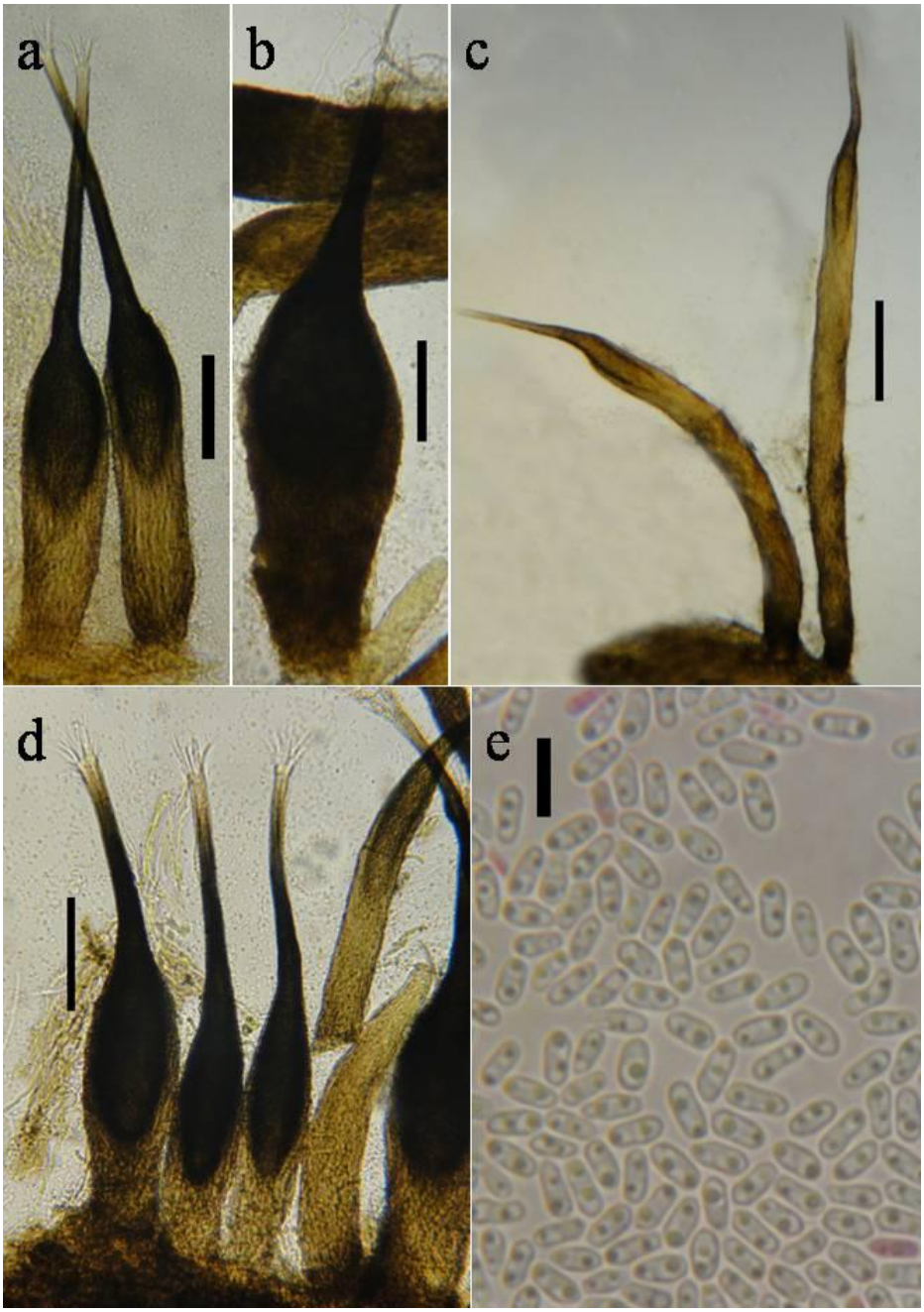

Fig. 10. *Scorias spongiosa*: **a-d.** Different type of pycnidia produced on Malt Extract Agar, scale for a, b and d = 100 µm, c = 200 µm; **e.** conidia, scale 5 µm.

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اطلاعات جدید در مورد قارچ های مولد کپک دوده ای در ایران

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چکیده: قارچ‌های مولد کپک دوده‌ای اغلب همراه با ترشحات عسلی حشراتی هستند که از گیاهان تغذیه می‌کنند. تعداد زیادی از این قارچ‌ها در شمال ایران به ویژه روی مرکبات گسترش دارند که از محصولات کشاورزی مهم این منطقه به شمار می‌آیند. طی ده سال گذشته نمونه‌های زیادی از این قارچ‌ها مطالعه شده‌اند و ۱۵ گونه از این منطقه گزارش شده است. در این مقاله نیز هفت گونه جدید از ایران گزارش می شود که عبارتند از :

Catenuloxyphium heterosporum (on *Salix aegiptiaca* and *Alnus glutinosa*); *Echinothecium* sp. (on *Eriobotrya japonica* and *Passiflora* sp.); *cf. Denisiella* sp. (On *Citrus sinensis* and *Malus pumila*); *Fumagospora capnodioides* (on *Alnus glutinosa*, *Populus deltoids*, *Salix aegyptiaca* and *Zelkova carpinifolia*); *Leptoxyphium fumago* (on *Alnus glutinosa*, *Rubus* sp. and *Phytolacca americana*); *Tripospermum juglandis* (on *Ligustrum* sp.) and *Scorias spongiosa* (on *Citrus sinensis*).

کلمات کلیدی: Capnodiales، Capnodiaceae، Chaetothyriaceae، Scorias، Fumagospora

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تاریخ دریافت: ۱۳۹۴/۰۱/۱۴ تاریخ پذیرش: ۱۳۹۴/۰۳/۱۵