

Trimmatostroma salinum, a new species from hypersaline water

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Abstract: A new melanized meristematic fungus, *Trimmatostroma salinum*, is described. All strains known to date originated from saltern water along the Adriatic coast. On the basis of morphology, the species is classified in the anamorph genus *Trimmatostroma*. Its distinction from other meristematic fungi is discussed.

Key words: black yeasts, taxonomy, pleomorphism, ecology, hypersaline water, halotolerance, *Trimmatostroma*.

Introduction

During an investigation of fungal diversity in hypersaline water of Slovenian salterns along the Adriatic coast, we isolated numerous strains of fungi, the majority of which belonged to the black yeasts (Gunde-Cimerman *et al.*, 1999). They were found to be halotolerant or even halophilic (Zalar *et al.*, 1999a). Among these isolates, strains were encountered that were thought to represent a hitherto undescribed species. It is highly pleomorphic and hence it is difficult to classify in any hyphomycete genus. Because of its thick-walled, muriform cells which developed by conversion from undifferentiated hyphae, the genus *Trimmatostroma* was judged to be most suitable for its accommodation. It is described below as a new species.

Materials and methods

Water samples were taken from pools of different salinities in salterns in Seca at the Adriatic coast in Slovenia. Several isolation techniques were applied focussing on halophilic fungi (Gunde-Cimerman & Zalar, 1999). In the period of salt production (May – October), five strains of the new species were isolated from several crystallization pools: MZKI B-734 (sp. 659) = CBS 100461 and MZKI B-961 (sp. 1018), isolated by filtration on PDA + 4M NaCl; MZKI B-962 (1FI) and MZKI B-963 (7IVA), isolated by baiting; MZKI B-928, isolated by enrichment technique on MY50G agar medium.

Cultural characteristics were recorded on PDA, OA

and MEA at 25°C over a period of 2 months. For microscopy, slide cultures were made on the same media in humid chambers at 25°C. Slides were mounted in water and in lactic acid.

Growth, fermentative abilities and tolerance were established in duplicate according to Untereiner *et al.* (1999). Results were read after 3 weeks of growth. Assimilation of arbutin and ureum was tested in liquid and on solid media. A diagnostic ITS rDNA restriction map was prepared on the basis of data provided by de Hoog *et al.* (1999).

Trimmatostroma salinum Zalar, de Hoog & Gunde-Cimerman, *sp. nov.* – Fig. 1, Plate 1

Coloniae in agar maltoso flavido-virides vel brunneo-virides, post duos menses coeruleo-nigrescentes, siccae, cerebriformes, crustosae; margine irregulari, hyphis profunde in agarum penetrantibus; diameter coloniae maximus post duos menses 20 mm. Hyphae primum transverse septatae, 4–7 µm latae, maturae fusce olivaceo-virides, paulatim latiores et magis dense septatae, etiam in medio septis longitudinalibus divisae, sarcinas 12 × 10 µm formantes; acervi cellularum velut conidia meristematica separata, magnitudine variabilia, bicellularia 10 × 5 µm, quadricellularia 22 × 20 µm; cellulis modice asperulatis. Cellulae in omnibus stadiis crescentiae hypharum separari possunt, sarcinas 2- vel 4-(nonnumquam pluri-)cellulares, crassitunicatas formantes, saepe vestigiis parietis exterioris affixis conidiogenesim endogenam simulantibus. Blastoconidia etiam lateralia e denticulis hebitibus oriunda, obovoidea, 5–12 × 3–7 µm, olivaceo-viridia, catenis brevibus acropetalibus connexa.

Holotypus MZKI B-734 = CBS 100461, isolatus ex aqua

salina, a P. Zalar, Seca, Slovenia (herb. CBS; etiam cultura viva).

CULTURAL CHARACTERISTICS. – Colonies (MEA) yellowish green to brownish green, after 2 months becoming bluish black. Colonies at first shiny, becoming dry, cerebriform and crustose, raised to 5 mm at the centre, sometimes volcano-shaped with a central depression; margin irregular, with hyphae growing deeply into the agar; reverse black. Colonies on OA sometimes exuding a brown pigment into the agar. Daily growth rate (diam) on MEA, OA and PDA about 0.5 mm; maximum diam, reached after 2 months, on MEA 20 mm.

MICROSCOPY. – Cells germinating with hyphae which are 2–5 μm wide, pale greenish, profusely branched in all directions; branches septate about every 5 μm . Hyphae initially with transverse septation, 4–7 μm wide, consisting of nearly rectangular 7–10 μm long cells. With maturation hyphae becoming dark olive-green, gradually widening and developing more dense septation, cell duplets becoming recognizable due to an alternation of slightly thicker and thinner septa with hyphae being somewhat constricted at the thinner septum. Each cell duplet developing a central longitudinal septum with accentuated constrictions between duplets; cellular packets up to 10 \times 12 μm . Further septation progressing in all directions, hyphae reaching up to 20 μm in width; clumps of cells separating as meristematic conidia of variable size: 5 \times 10 μm when two-celled, 8 \times 10 μm when four-celled, 20 \times 22 μm when multicellular. Cells becoming slightly rough-walled and disarticulating into meristematic conidia. Cells separating in all stages of hyphal development, resulting mostly in two- to four- (occasionally many-) celled structures which are thick-walled, often with patches of outer cell-wall remains, reminiscent of endoconidial reproduction. Meristematic conidia sometimes producing secondary conidia which are spherical, up to 7 μm diam. Submerged hyphae slightly narrower, locally producing 1(–2) lateral blastoconidia on blunt denticles; conidia obovoidal, 5–12 \times 3–7 μm , olive-green, solitary or arising in short (up to 5), rarely once-branched acropetal chains (up to 5). Ramoconidium-like conidiogenous cells 7–12 \times 5–7 μm , producing spherical conidia up to 4 μm diam, rarely developing on denticles.

REMAINING CHARACTERS. – Physiological properties are listed in Table 1. rDNA characteristics are summarized in Fig. 2.

TYPE STRAIN. – MZKI B-734 = CBS 100461, isolated by P. Zalar from saltern, near Seca, Adriatic coast, Slovenia.

Discussion

The genus *Trimmatostroma* was introduced by Corda (1837) for the species *T. salicis* Corda. It was characterized by scattered, pulvinate, dark blackish brown to black sporodochia on wood of *Salix*. Sporodochia were formed on pseudoparenchymatous stromata. Descriptions of nearly all *Trimmatostroma* species in the literature (Hughes, 1953; Ellis, 1971, 1976) are based on specimens on the natural substratum because their differentiation in culture is more diffuse. Ellis (1971) distinguished *Trimmatostroma* from the superficially similar genus *Coniosporium* by acropetal vs. basipetal conidium production. This character is only visible in sporodochial specimens on wood. With substrata such as stony surfaces or water in salt ponds, or in culture, development is by gradual conversion of pre-existing hyphae into chains of multicellular conidia (Butin *et al.*, 1996; Yoshida *et al.*, 1996; Figueras *et al.*, 1996). Hence these fungi are very difficult to attribute to any genus of hyphomycetes. The present fungus was classified in *Trimmatostroma* mainly on the basis of a structural resemblance of the mature conidial apparatus to the *Trimmatostroma* species depicted by Ellis (1971, 1976).

Like most melanized meristematic fungi, *Trimmatostroma salinum* shows divergent morphological types. However, prevalent development is with hyphae which swell, develop transverse and longitudinal septa and eventually release 4-celled conidia by disarticulation. The classification in the anamorph genus *Trimmatostroma* was based on this type of morphology. Its halophilic nature and the structure of its conidia, which may have denticles when produced laterally, are unique features that warrant its description as a new species. The species was found to be phylogenetically related to the *Dothideales* and is well apart from other *Trimmatostroma* species sequenced thus far (de Hoog *et al.*, 1999). The genus *Trimmatostroma* currently contains about 15 species, mostly described from dead and living plant material, such as twigs, litter and lichens. Species nearly always have extended stromata from which the conidia arise. Stromata are immersed in the substratum and probably enable the fungi to penetrate specific hosts. Some superficial resemblance with the conidia of the following species is apparent. *Trimmatostroma betulinum* (Corda) S.J. Hughes (Ellis, 1971) is distinguished by larger cellular clumps which are more elongate in shape. *T. eriodyctionis* (Dearn. & Barth.) M.B. Ellis (Ellis, 1976) has much larger conidia and a longitudinal striation on the developing conidia. *T. indicum* Manoharachary *et al.* (1977), originating from soil, was described in culture as arising from a (sub)hyaline stroma. Conidia of *Trimmatostroma ma-*

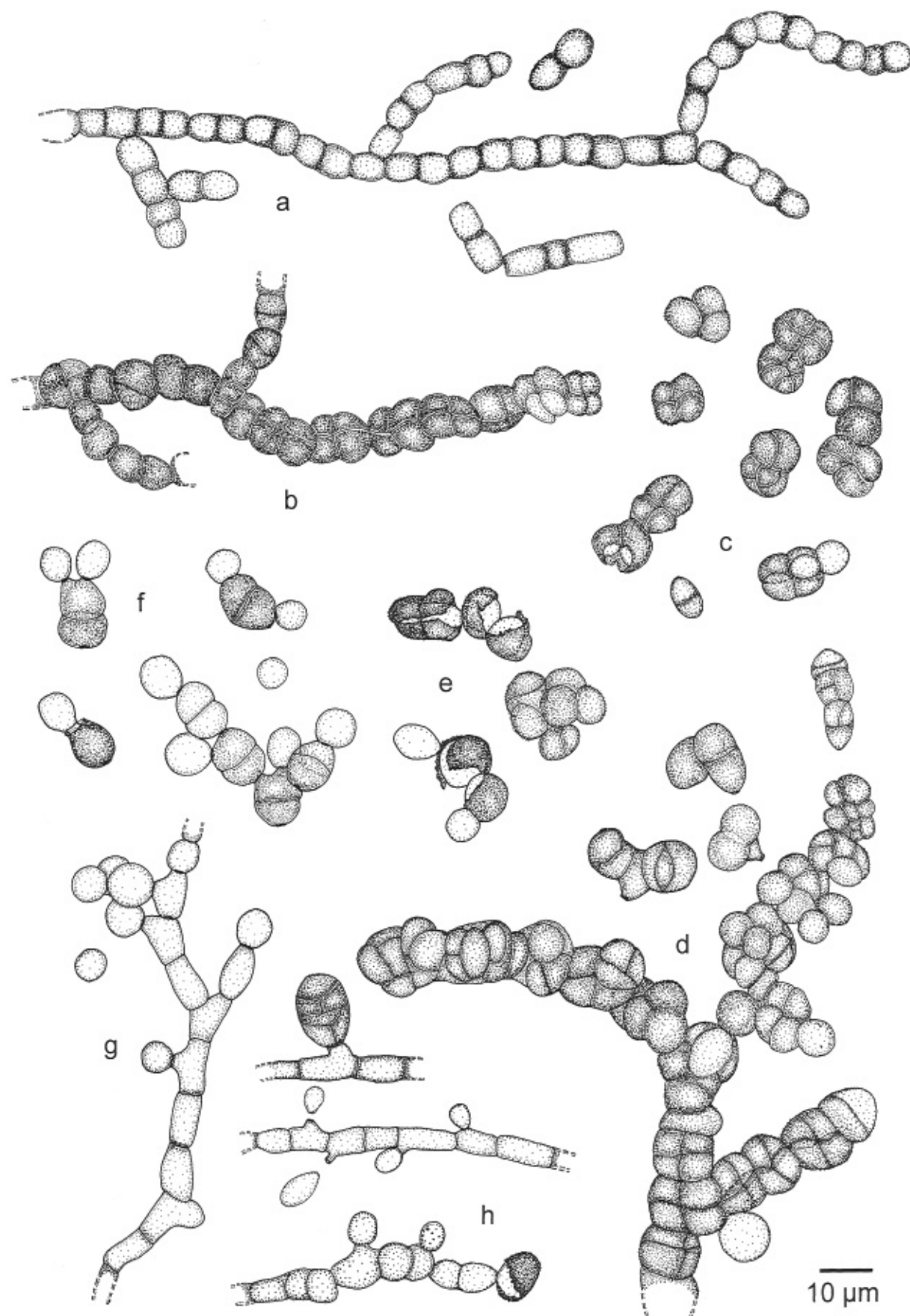


Fig. 1. *Trimmatostroma salinum*, CBS 100461 (MZKI B-734) on MEA. a. Hyphae in early stage of development; b. hyphae with longitudinal septation; c. disarticulated hyphae; d. hyphae during meristematic conversion; e. cells releasing endoconidia; f. single-celled catenulate synanamorph; g, h. lateral cells on hyphae.

cowanii (Sacc.) M.B. Ellis (1976) are heavily warted. *T. salicis* was described by Ellis (1971) as having curved conidia. Cultures identified with this species in the CBS collection showed much more expansion growth than *T. salinum*.

Coniosporium memorandum (Penz. & Sacc.) M.B.

Ellis (1976), found on dead leaves in the tropics, is characterized by heavily warted conidia. *C. diversum* (Cooke) Crane & Schoknecht (Schoknecht & Crane, 1977), originating from tropical plant leaves, also has ornamented conidia. *C. apollinis* Sterflinger (Sterflinger *et al.*, 1997), from calcareous rock in the Mediter-

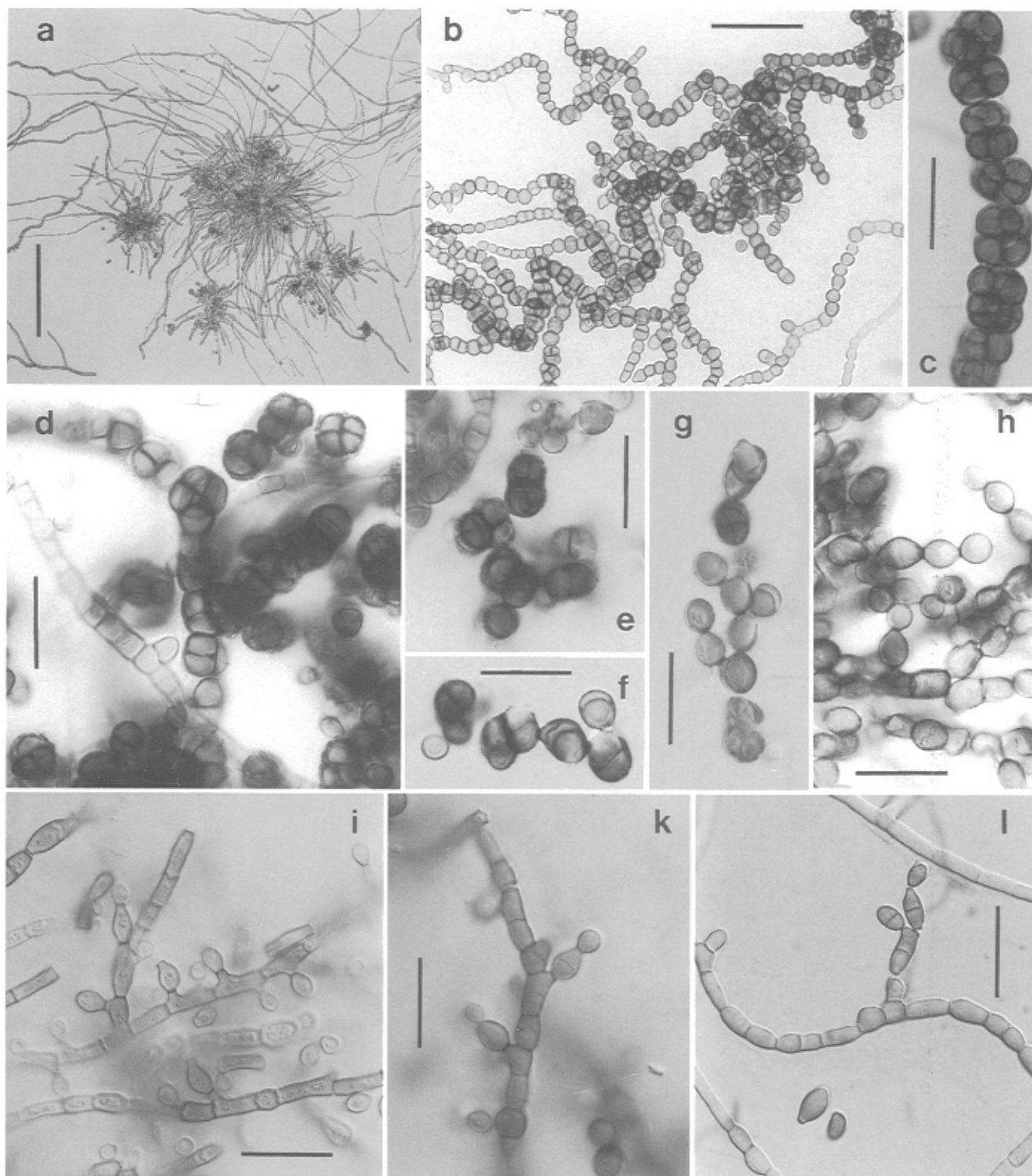


Plate 1. *Trimmatostroma salinum*, CBS 100461 (= MZKI B-734). a. Early development of colonies; b. young hyphae; c, d. meristematic conversion; e, f. disarticulated meristematic cells; g. single-celled catenulate synanamorph; h-l. lateral conidia on hyphae. Scale bar is 200 µm (a), 50 µm (b), 20 µm (d-l).

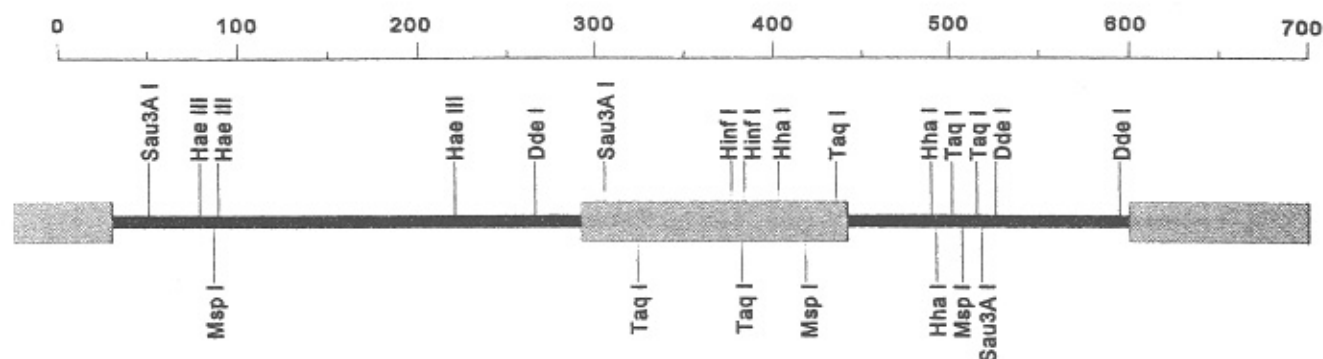


Fig. 2. Restriction map of ITS1-2 rDNA domain of *Trimmatostroma salinum*.

ranean, is structurally similar to *T. salinum* but has been proven to be an anamorph member of the *Chaetothyriales* rather than of the *Dothideales*.

Spilodochium species have non-catenate conidia which may be heavily warted. *Monodictys* can be superficially similar in culture but produces single, sessile conidia. *Taeniolella* and *Bispora* differ by having conidia with nearly exclusively transverse septa, few longitudinal septa occasionally being produced. The genus *Sarcinomyces* is heterogeneous (Sigler *et al.*, 1985; de Hoog & Rubio, 1982) and phylogenetically distant (Sterflinger *et al.*, 1999). *Sarcinomyces crustaceus* produces rectangular cell packets (Sigler *et al.*, 1985); it takes a phylogenetically isolated position (Sterflinger *et al.*, 1999). *S. phaeomuriformis* Matsumoto *et al.* (1986) is a member of *Chaetothyriales*, close to *Exophiala dermatitidis* (Kano) de Hoog (Uijthof *et al.*, 1998). *S. petricola* Wollenzien & de Hoog ultimately produces unicellular conidia (Wollenzien *et al.*, 1997).

Trimmatostroma salinum produces some synanamorphs in low abundance, that are unsuitable for its classification but might indicate relationships with other dothidealean fungi. Early stages of development, with melanized hyphae which are constricted at the septa (Fig. 2b, Plate 1b) are reminiscent of *Bispora*. A catenate anamorph (Fig. 2g, Plate 1k, l) resembles similar growth forms in *Hortaea werneckii* (Horta) Nishimura & Miyaji, *Stenella araguata* Syd. and *Cladosporium* species which are close relatives (de Hoog *et al.*, 1999). Strings of spherical cells may occur (Fig. 2f), that are reminiscent of *Sarcinomyces petricola* (Wollenzien *et al.*, 1997). Liberated cells may rupture and release endoconidium-like cells, as in *Phaeotheca* (Zalar *et al.*, 1999b).

Physiologically *Trimmatostroma salinum* somewhat resembles *T. abietis* Butin & Pehl (Butin *et al.*, 1996) in the absence of growth with D-glucosamine and poor assimilation of most carboxylated sugars, but *T. salinum* differs by weak growth with most sugar alcohols. Diagnostic differences are the absence of glycerol and xyli-

tol assimilation. The ITS1-2 sequence of *T. salinum* is clearly different from the remaining meristematic fungi studied thus far (de Hoog *et al.*, 1999); a restriction map for some widely used enzymes is given in Fig. 2.

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