Studies on the cultural characterization of 16 species of *Hyphodontia* ERIKSSON and *Chaetoporellus* BOND. and SING ex. SING.

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Abstract. – The authors describe the principal cultural characters of 16 species of *Hyphodontia, (H. abieticola, H. efibulata, H. alutacea, H. alutaria, H. arguta, H. aspera, H. barba-jovis, H. crustosa, H. floccosa, H. juniperi, H. nespori, H. pallidula, H. quercina, H. spathulata, H. subalutacea* and *H. verruculosa)* and establish the homogenity of the genus. The above species are tetrapolar, secrete laccase enzyme and exhibit normal nuclear behaviour. Their mycelia present characteristic structures which are called malocysts and drepanocysts.

Chaetoporellus latitans exhibits the same characters and its transfer into the genus *Hyphodontia* is justified. Detailed descriptions of the cultural diagnosis are presented.

Introduction

Hyphodontia is a genus introduced by ERIKSSON (1958) encompassing many species from the genera *Odontia* and *Radulum* which have an odontoid and hydnoid hymenium in addition to those belonging to the genera *Corticium* and *Peniophora* which have a smooth hymenium. In their recent work, ERIKSSON & RYVARDEN (1976) metioned that *Hyphodontia* and *Chaetoporellus* are closely related although differences in spore morphology are evident.

The genus *Chaetoporellus* BOND. & SING. ex SING. created in 1944 referring to resupinate species with a poroid and cystidoid hymenium originally consists of two species: *Ch. latitans* and *Ch. greschikii.* Three more poroid species *(Ch. litschaueri, Ch. aureus* and *Ch. simanii)* were added to the genus by BONDARCEV (1953); subsequently the latter four species were re-named by several workers. LOWE (1966) reported *Ch. greschikii* as a synonym of *Amyloporia xantha* (FR. ex FR.) BOND. & SING. and *Ch. litschaueri* as a synonym of *Strangulidium sericeo-mollis* (ROMELL) POUZ. PASMASTO (1961) classified *Ch. simanii* in the genus *Tyromyces* and RYVARDEN (1973) created the genus *Auriporia* for *Ch. aureus.* Thus, the genus

Chaetoporellus was reported to have only one species (the type species), *Ch. latitans* (DOMANSKI, 1972). In 1976, ERIKSSON & HJORTSTAM introduced a species with a smooth hymenium, *Ch. curvisporus,* which originally was described by them in 1969 under the name of *Hyphodontia curvispora.*

JÜLICH & STALPERS (1980) transferred all the species of *Hyphodontia* which had been grouped by ERIKSSON (1958) into *Kneiffiela* KARST. 1889 (type species *K. barba-jovis);* they also included *Ch. curvisporus* but not *Ch. latitans.*

In 1982 these same species including *Ch. latitans* were transferred by JÜLICH into the genus *Grandinia.* The aim of this work is to show that by the study of its characteristics in culture, this last species *(Ch. latitans)* can be placed quite easily in a large assemblage of species which have been successively re-grouped in the genera *Hyphodontia* (ERIKSSON, 1958), *Kneiffiella* Karst (JÜLICH & STALPERS, 1980) and *Grandinia* Fr. (JÜLICH, 1982). Because of these incessant changes and while awaiting the unanimous confirmation of the systematists, the term *Hyphodontia* will be used as clearly defined by its original author, taking care not to propose the new combination of *H. latitans* which would prove necessary if the genus *Hyphodontia* was going to be conserved.

The present investigation describes the morphology, growth and other cultural characters of *H. latitans,* a rare species in the South of France. Comparisons between *Ch. latitans* and *Hyphodontia* species are also presented.

Materials and methods

Mycelial cultures and their descriptions were made according to BOIDIN (1958).

Spores of all the species concerned were collected on sterile glass slides, seeded onto a malt-agar medium the day of harvesting and left to germinate under favourable conditions. As soon as possible, confrontations of all species were carried out using monosporous mycelia representative of each pole. This coding system used is that of NOBLES (1965) and BOIDIN (1966).

I. Chaetoporellus latitans (Bourd. & Galz.) Bond. & Sing.

Description of fruitbody

Annual, resupinate and ochraceous white when dried. – Pores rounded or angular, 1–3 per mm with entire (on horizontal substrate) or dentate and irpicoid edges (on vertical substrate). Sterile margin subpruniose, narrow, often completely absent. – Subiculum very thin, 0,1 mm maximum thickness. Tubes – 1,5–2 mm long.

Monomitic system: regular hyphae, thinwalled or slightly thickened, 1,5–3 μm in diamter, with small, hemispherical clamps

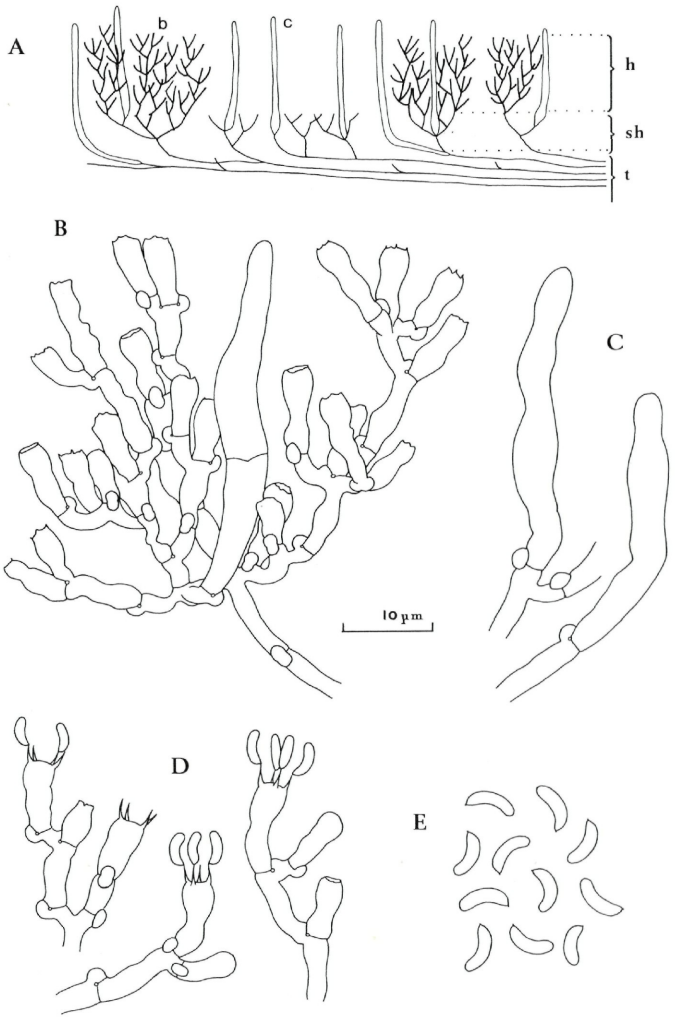


Fig. 1. *Chaetoporellus latitans:* A. Schematic view of hymenial organisation: b: basidia. – c: cystidia. – h: hymenium. – sh: subhymenium. – t: trama. – B. Fragment of the hymenium. – C. Cystidia. – D. Basidia. – E. Spores.

which are called hyphodontoid clamps. – Hymenium very specific. – Basidia produced successively with a corresponding increase in the thickness of the hymenium (Fig. 1, A–B) 7–10×3–4 μm, slightly constricted, with 4 sterigmata (Fig. 1, D). – Cystidia cylindrical, thinwalled, 40–60×5–6 μm, protruding from 2 to 10 μm, formed either from the hymenium or from the trama and bent in the latter case (Fig. 1, C). – Spores allantoid 3,5–4,5×0,75–1 μm, thinwalled, hyaline and non amyloid (Fig. 1, E).

Collections. – LY-AD 3028: collected from decayed stumps of *Pinus halepensis,* Gros Cerveau, Var, France, 5-12-1971; LY-AD 3074: collected from a stump of *Pinus halepensis* where mining by coleoptera was evident, Notre Dame des Anges, Var, France, 12-1-1972.

Habitat. – Samples were collected from the decayed stumps of *Pinus halepensis,* inside the holes made by the larvae of Cerambycidae (Coleoptera) and which were sometimes below ground level. This particular localization of *Ch. latitans* which is previously mentioned by Bourd. & Galz. (1928) is probably the reason for the rare appearance of this species.

Study of mycelia

(Observations are based upon the cultures LY-AD 3028 and LY-AD 3074)

Germination and monosporous cultures. – The germination time varied according to the experimental conditions. When using glass slides and 1,2% malt liquid under a collodion-film, the spores germinated within 3–4 days, whereas they germinated after 15 days when inoculated directly onto 1,2% malt-agar Petri dishes. In case inoculation was made seven days after the spores were harvested (1,2% malt-agar), the germination time was double. Both the germinating spores and monosporous cultures are unincleate.

Polysporous cultures

Growth: average growth (9 cm diameter Petri dish covered in 3 or 4 weeks).

Aspect: regular margin, aerial mycelium slightly developed, appressed, homogeneous, mat, milky, waxy; rare downy plages. Frequent fructifications with abundant sporulation on cultures older than six weeks. Odour slightly aromatic; reverse unchanged in colour.

Margin: Hyphae straight, slightly sinous, 3 μm in diameter, with very long articles (350–500 μm), slightly ramified and regularly clamped.

Aerial mycelium: similar to the marginal hyphae with small regular hemispheric clamps of hyphodontioid type (Fig. 2, A 1) hav-

ing two types of vesicles. Those born lateraly on the hyphae are sessile, spherical (7–8 μm diam.), carrying a narrow apical appendage (2–2,5 μm length), red colored with Giemsa and separated from the hyphae by a septum and a hook-like structure (Fig. 2, A 2). Although many slides were examined, true clamps were not observed. Because of its appearance (apple-like), we termed this type of vesicle a malocyst (from: *malus* =apple). The second type of vesicle is formed at the end of a short lateral ramification, is curved and forms a tapered hook-like structure ressembling a sickle (Fig. 2, A 3). Such vesicles we named drepanocysts (from: δρεπανον = sickle). They are separated from the lateral branches on which they are borne by a septum. Both the vesicle and its lateral branch have a tendency to entwine the neighbouring hyphae. These two types of vesicles are also present in the primary mycelium but the malocysts lack the hook-like structures.

Submerged mycelium: identical to aerial mycelium.

Cytology: the articles of the hyphae and the drepanocysts are binucleate.

Oxydases: Guaiacol 0,2%: +++ Tyrosine: –
2%: +++
Gallic acid: +++ P.-cresol: –

Polarity: This species is tetrapolar

A_1B_1	1–3	A_2B_2	4-5-7-8-10
A_1B_2	2–6	A_2B_1	9

Nuclear behaviour: Normal, the monosporous mycelium is of uninucleate articles, while the polysporous mycelium are of binucleate articles.

Key code: 2-3-7-15-26-32-36-38-43-(48)-55-60-61

II. Mycelial characters of the species grouped by Eriksson in the genus *Hyphodontia*

16 species of *Hyphodontia* were studied by Hassan (1981). These are *H. abieticola, H. alutacea, H. alutaria, H. arguta, H. aspera, H. barba-jovis, H. crustosa, H. efibulata, H. floccosa, H. juniperi, H. nespori, H. pallidula, H. quercina, H. spathulata, H. subalutacea* and *H. verruculosa.*

Spores and monosporous mycelium: Germination time of spores is variable, 1–2 weeks for *H. nespori, H. quercina, H. spathulata* and *H. verruculosa,* 2–3 weeks for *H. alutaria* and *H. juniperi* and 4 weeks for *H. crustosa.* Spores and articles of the monosporous mycelium are uninucleate.

Polarity: Five species were already known to be tetrapolar (*H. arguta, H. barba-jovis, H. crustosa, H. quercina* and *H. verruculosa*) and tetrapolarity was established by Hassan (1981) for seven

other species (*H. abieticola, H. alutacea, H. alutaria, H. floccosa, H. nespori, H. spathulata* and *H. subalutacea*). It was found that all clamped *Hyphodontia* species studied were tetrapolar.

Polysporous mycelium:

Growth: Slow or extremely slow growth for the majority of species.

Aspect: the aerial mycelium is slightly developed, leaving the medium transparent and giving a slight smell in *H. alutacea, H. barbajovis, H. quercina* and *H. verruculosa,* while *H. floccosa* and *H. subalutacea* give a strong but pleasant smell.

Microscopy: the mycelium consists of generative hyphae with clamps. The hyphae measure 2–6 μm in diameter, frequently containing numerous oil droplets, and surrounded by more or less thick walls except in *H. crustosa* and *H. pallidula* where the hyphal walls are thin. In the case of *H. aspera, H. crustosa, H. spathulata, H. verruculosa, H. sambuci* and *H. quercina,* the hyphae are covered with crystals.

The characteristic hyphodontioid clamps are seen in most species while *H. aspera, H. juniperi, H. quercina* and *H. verruculosa* show slightly less distincitve clamps. Almost all *Hyphodontia* species form different structures associated with the hyphae, these characteristic structures are:

Malocysts-present in 13 species: (*H. abieticola, H. alutacea, H. arguta, H. aspera, H. barba-jovis* (Fig. 2, B), *H. crustosa,*

Table 1. – Interspecific affinities in the genus *Hyphodontia* based on cultural characters as compared with the subgroups of ERIKSSON.

Group	Species	Lagénocysts	Malocysts	Drépanocysts	Allocysts
I	*H. pallidula*	+		+	+
	H. alutaria	+		+	+
	H. arguta	+	+		–
	H. aspera		+		+
	H. nespori		+		+
II	*H. spathulata*		+		+
	H. vesiculosa		+		+
	H. barba-jovis		+	+	
	H. abieticola		+	+	
III	*H. efibulata*		+	+	+
	H. alutacea		+	+	
	H. latitans		+	+	
	H. subalutacea		+		+
	H. floccosa		+	+	
	H. crustosa		+		+
IV	*H. juniperi*		+	+	+
	H. quercina				+

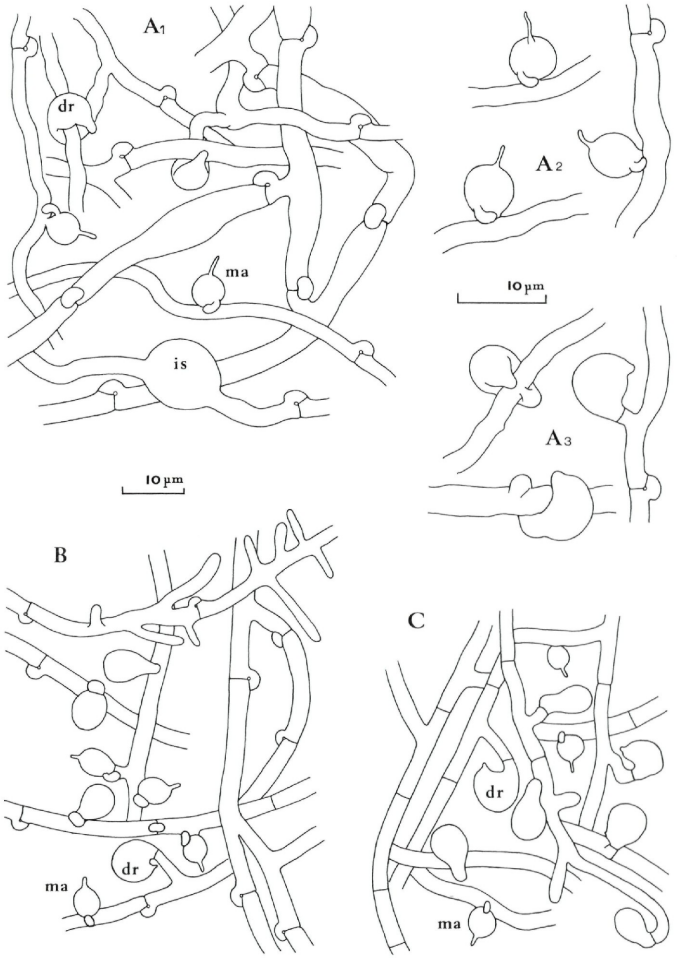


Fig. 2. A. Polysporous mycelium of *Chaetoporellus latitans;* A_1 general view, A_2 malocysts, A_3 drepanocysts. – B. Polysporous mycelium of *Hyphodontia barbajovis*. – C. Polysporous mycelium of *Hyphodontia efibulata:* dr: drepanocyst. – ma: malocyst. – is: intercallary swelling.

H. efibulata (Fig. 2, C), *H. floccosa, H. juniperi* (Fig. 3, A), *H. nespori, H. spathulata, H. subalutacea* and *H. verruculosa.*

Drepanocysts-present in 8 species: *H. abieticola, H. alutacea, H. lutaria* (Fig. 3, B), *H. barba-jovis, H. efibulata, H. floccosa, H. juniperi* and *H. pallidula.*

Allocysts-present in 11 species: *H. alutaria, H. aspera, H. crustosa, H. efibulata, H. juniperi, H. nespori, H. pallidula, H. quercina, H. spathulata, H. subalutacea* and *H. verruculosa.*

Lagenocystidia-present in *H. alutaria, H. arguta* and *H. pallidula* (Fig. 3, B).

Oxydases: Laccase is produced by all *Hyphodontia* species. In the case of *H. arguta, H. floccosa, H. quercina* and *H. verruculosa,* both laccase and tyrosinase are secreted.

Cytology: In all species (except for *H. efibulata* where the poly- and monosporous mycelium consist of uninucleate, septate hyphae similar to those forming the fruitbody; Fig. 3, C) the polysporous mycelium consists of binucleate hyphae. For this reason, the present investigation confirms the existence of parthenogenesis in this species.

III. Interspecific affinity in *Hyphodontia*

Hyphodontia species are grouped in table I according to their cultural characteristics. These groups correspond to those reported by ERIKSSON & RYVARDEN (1976) which were based on studies of the fruitbody. Four groups are distinguished:

Group I: this group includes species with lagenocystidia and allocysts and sometimes malocysts *(H. arguta)* or drepanocysts (*H. pallidula, H. alutaria*). It corresponds to group *H. pallidula,* subgroup *pallidula,* of ERIKSSON.

Group II: this group corresponds to ERIKSSON's second subgroup of *pallidula.* In culture, the mycelium produces malocysts and allocysts. Cystidioid capitate hyphae and torulose cystidia are often present in the hymenium.

Group III: this group includes species with mycelia bearing malocysts and drepanocysts (except for *H. subalutacea*).

According to the morphological features of the fruitbody, it can be divided into the following subgroups (see ERIKSSON, 1976):

- Subgroup A corresponds to the *H. barba-jovis* group of ERIKSSON, with a corticioid to odontioid fruitbody, long, tubular cystidia which are thickwalled except at the apex, and subglobose to ellipsoid spores.
- Subgroup B corresponds to ERIKSSON's *H. efibulata* group: same characters as the previous subgroup but without clamps: it exhibits parthenogenesis (HASSAN, 1981).

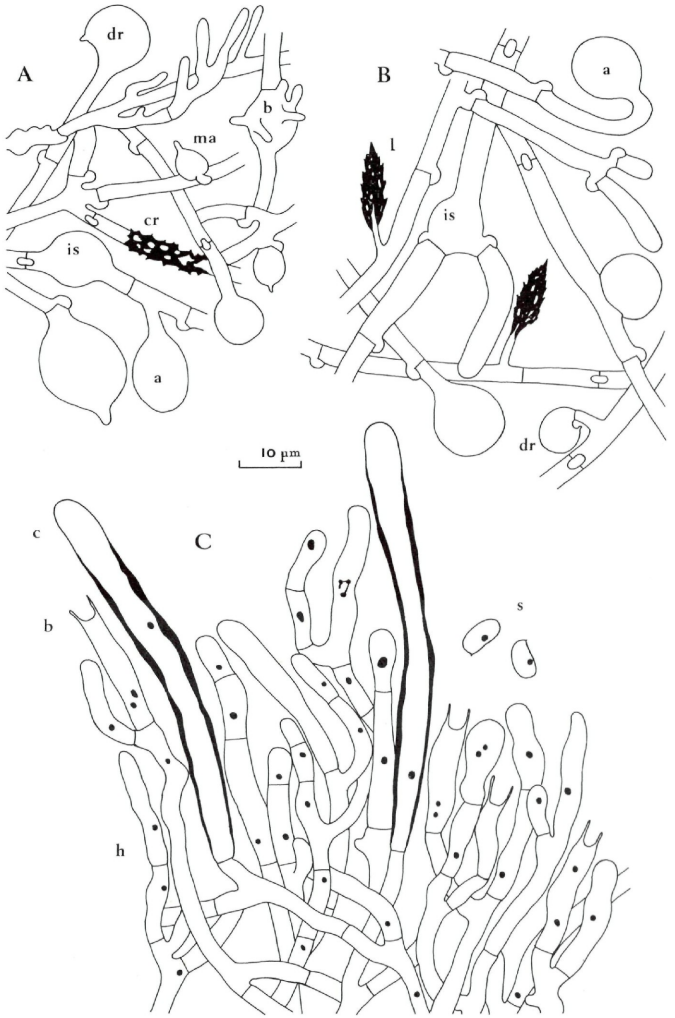


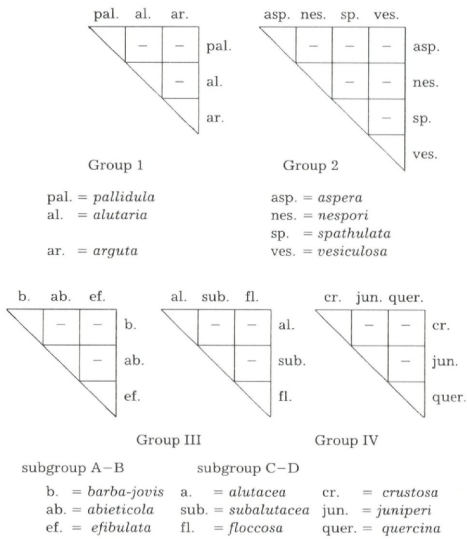
Fig. 3. A. Polysporous mycelium of *Hyphodontia juniperi*. – B. Polysporous mycelium of *Hyphodontia alutaria:* a: allocyst. – b: bulbil. – cr: hyphae covered with cristals. – dr: drepanocyst. – is: intercallary swelling. – l: lagenocyst. – C. *Hyphodontia efibulata:* fragments of hymenium colored by Giemsa's solution. The nuclei are represented by small dark spots. b: basidia with two sterigmata and two residual nuclei after spore discharge. – c: cystidia. – h: hyphae with simple septa and uninucleate articles. – s: uninucleate spores.

- Subgroup C includes the *H. alutacea* group of ERIKSSON and *Ch. latitans* with allantoid spores and thin-walled cystidia.
- Subgroup D corresponds to the *subalutacea* group of ERIKSSON with long, tubular cystidia and allantoid spores (with the exception of *H. microspora*). In this group, the three species *H. altaica, H. cineracea* and *H. microspora* were not studied for lack of material.

Group IV: based on the cultural characterization of the three species studied (*H. crustosa, H. juniperi* and *H. quercina*), this group is less well defined when compared with the other groups. The mycelia of *H. crustosa* and *H. juniperi* resemble those of group II in having malocysts and allocysts.

In all the above mentioned groups, the studied species are closely related and not easily distinguished. Such problems were also encountered by Eriksson (1976) and in an attempt to resolve this difficulty in species recognition, confrontations between the monokaryons of the species were carried out. It was found that all the species studied are intersterile (i. e. distinct species) (Table 2) and although several collections of *H. crustosa* showed differences in spore size, it was found that they were intercompatible.

Table 2. – Results of interfertility studies between species of similar *Hyphodontia*.



pal. = *pallidula*
al. = *alutaria*
ar. = *arguta*

asp. = *aspera*
nes. = *nespori*
sp. = *spathulata*
ves. = *vesiculosa*

subgroup A–B

b. = *barba-jovis*
ab. = *abieticola*
ef. = *efibulata*

subgroup C–D

a. = *alutacea*
sub. = *subalutacea*
fl. = *floccosa*

cr. = *crustosa*
jun. = *juniperi*
quer. = *quercina*

Conclusion and discussion

This study of mycelial characteristics establishes the affinities of *Hyphodontia* species. The results obtained confirm, conclusively, those of ERIKSSON & RYVARDEN established on morphological characters. They also confirm the great affinity between *Ch. latitans* and several species of *Hyphodontia,* viz. *H. abieticola, barba-jovis, efibulata* and *alutacea.* All examined species produce particular stuctures in culture, so-called malocysts (sessile spherical structures carrying a narrow apical appendage) and drepanocysts (sickle-like vesicles). *H. efibulata,* a species without clamp connections and with mononucleate articles, is parthenogenetic.

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