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Taxonomy of *Daedalea flavida* Lev., *Lenzites adusta* Massee and *Lenzites japonica* Berk. and Curt.

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Morphological, anatomical and cultural characters of *Daedalea flavida* Lev. *Lenzites adusta* Massee and *Lenzites japonica* Berk. and Curt. have been studied in detail. Besides, conspecificity test has been carried out among these three species and total soluble proteins of them have been analysed through gel electrophoresis. All these studies together indicate that each of these three fungi is an individual species, one being different from the other.

Key words : *Daedalea flavida* Lev., *Lenzites adusta* Massee, *Lenzites japonirca* Berk. and Curt., taxonomy

INTRODUCTION

According to Ryvarden and Johansen(1980) *Daedalea flavida* Lev and *Lenzites adusta* Massee are the same as *Lenzites acuta* Berk. Ryvarden *et al.* (l986) reported that *L .acuta* is close to *Lenzites japonica* Berk and Curt. These reports, thus, clearly indicate that in the opinion of Ryvarden *D. flavida, L. adusta* and *L. japonica* are synonymous. All of these three species cause white rot on wood. But, Bakshi (1971) and Sen (1973) have considered *D. flavida* and *L. adusta* as distinct species. So, it is evident that there is great controversy regarding taxonomy of *D. flavida, L. adusta* and *L. japonica.* Therefore, morphological, anatomical, cultural studies as well as conspecificity test and gel electrophoretic analysis of total soluble proteins of these fungi have been carried out to resolve the taxonomic problem related to these fungi.

MATERIALS AND METHODS

Macroscopic and microscopic characters of the fungi were studied. For microscopic characters of basidiocarps observations were made on freehand sections and their teased portions mounted in distilled water, Melzer's reagent (Boidin 1958) and cotton blue in lactic acid (Boidin 1958). For cultural studies procedure of Nobles (1948, 1965) was followed. Sexuality of each species was determined through mating in all possible combinations of monosporous cultures made from a basidiocarp of any spec'es. Conspecificity test was carried out using mycelia from four monosporous cultures of each of the isolates compared which were grown together in pairs in all possible combinations with those of the other isolates and the resulting mycelia were examined for the presence of clamp connections. 9 isolates in 3 species (*D. flavida, L. adusta* and *L. japonica)* have been tested in this way. The results of the crosses are summarized in Table-1, in which a (+) sign is the symbol for results denoting fertility between the two isolates, while a (-) sign stands for sterility. Fertility is accepted as proof that the basidiocarps belong to the same species while sterility indicates that the basidiocarps belong to different species.

Investigations on electrophoretic study was made starting with young culture and for this purpose fungi were grown in liquid media. Liquid medium was inoculated with three mycelial discs (7 mm in diameter) from the advancing zone of culture plate solidified with 2% agar grown for 6-7 days. The liquid cultures were maintained at room temperature (31-36°C). Protein was extracted in phosphate buffer

(pH= 7.5) and precipitated by the addition of Ammonium sulphate to 80% saturation, followed by dialysis (MCO 3,500) of the precipitate overnight at 4°C. Proteins were estimated by the method of Lowrey *et al* (1951). Electrophoresis in SDS-Polyacrylamide was performed according to the method of Laemmli (1970) with slight modification. The separating gel was prepared by mixing acrylamide solution (7.5%), Tris-HCl (0.375M) pH 8.8, SDS solution (0.1%), Ammonium persulfate (0.1% W/V), TEMED (0.07% V/V) and water (doubled distilled).

After polymerization of the separating gel was complete, the stacking gel mixture (Acrylamide solution 4%, Tris-HCl 0.125M, pH 6.8, SDS 0.1%, TEMED 0.05%V/V, Ammonium persulfate 0.05%W/V) was pipetted into the mold. "Comb" providing a template for 13 wells was inserted into the liquid stacking gel solution before polymerization begins. After the polymerization of the upper gel was completed protein separation was carried out on a 7.5% gel. All protein samples were mixed to a ratio 1:1 with sample buffer (0.125M Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 0.1% Bromophenol blue, 10% 2-Mercaptoethanol) and boiled for 5 minutes. Samples (75-100) µg were subjected to SDS-PAGE with known molecular weight marker. Upper and lower buffer chambers were already filled with the required volume of Tris glycine electrophoresis buffer (0.02 M Tris Base, 0.192 M glycine, pH 8.3, SDS 0.1%). Electrophoresis was carried out on slab gel (12.5 X 15.5) cm, 1.5 mm thick at 200V for 4 hours. After electrophoresis, gel was removed from two pieces of glass plates. The gel was stained with staining solution (0.125%) Coomassie Blue R-250, 50% methanol, 10% Acetic acid) for 1 hour. Then the gel was destained with destaining solution (7% Acetic acid, 5% Methanol) with frequent changes of solution.

After complete visualization of the protein bands, relative mobilities were calculated for each of the unknown proteins of interest.

$$\text{Relative electrophoretic mobility (REM)} = \frac{\text{Distance migrated by protein}}{\text{Distance migrated by tracking dye}}$$

Photographs were taken to show the banding patterns of each species.

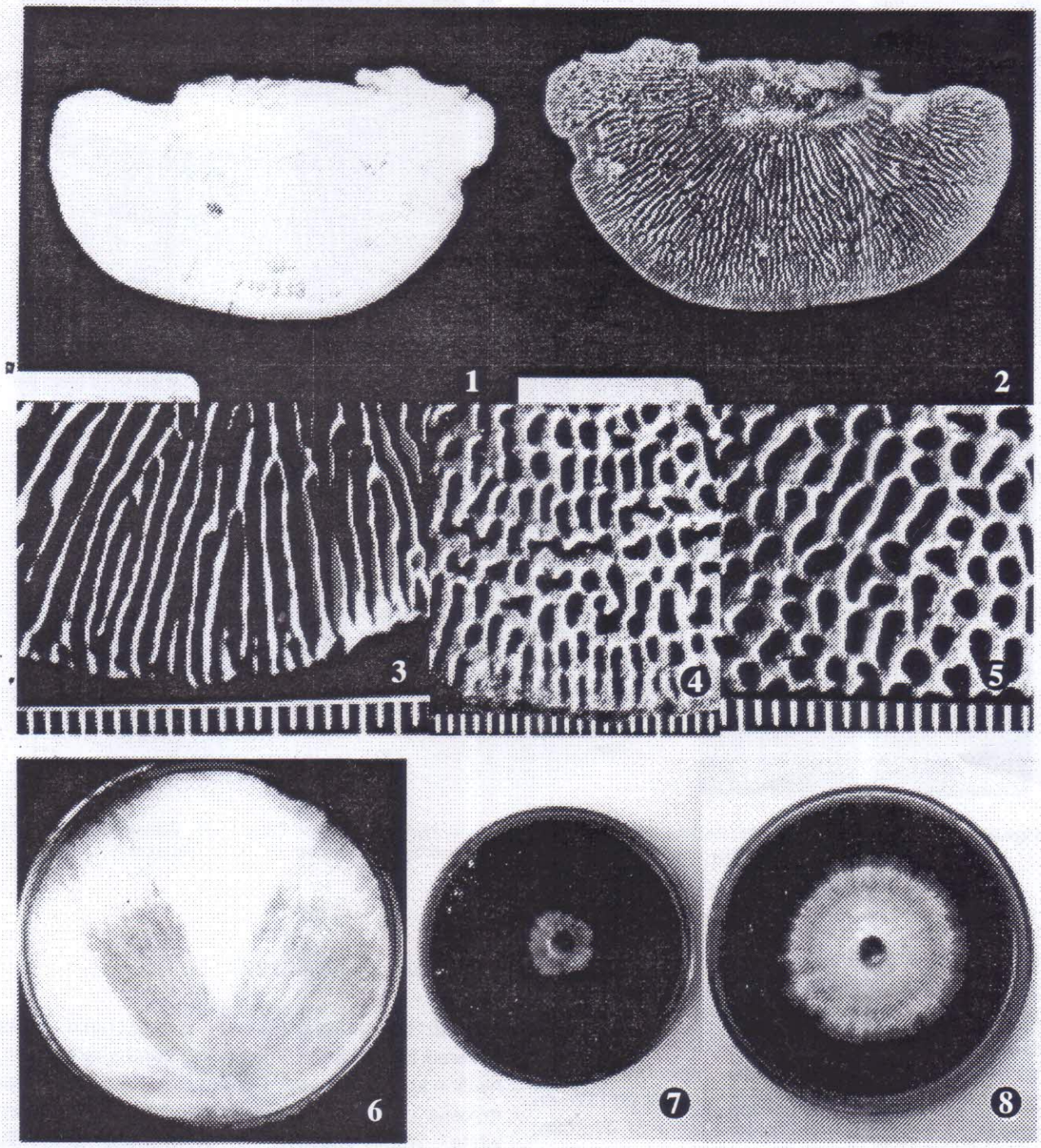
OBSERVATIONS

***Daedalea flavida Lev.Aim.Sci.Nat; Ser 3,* 2: 198 1844**

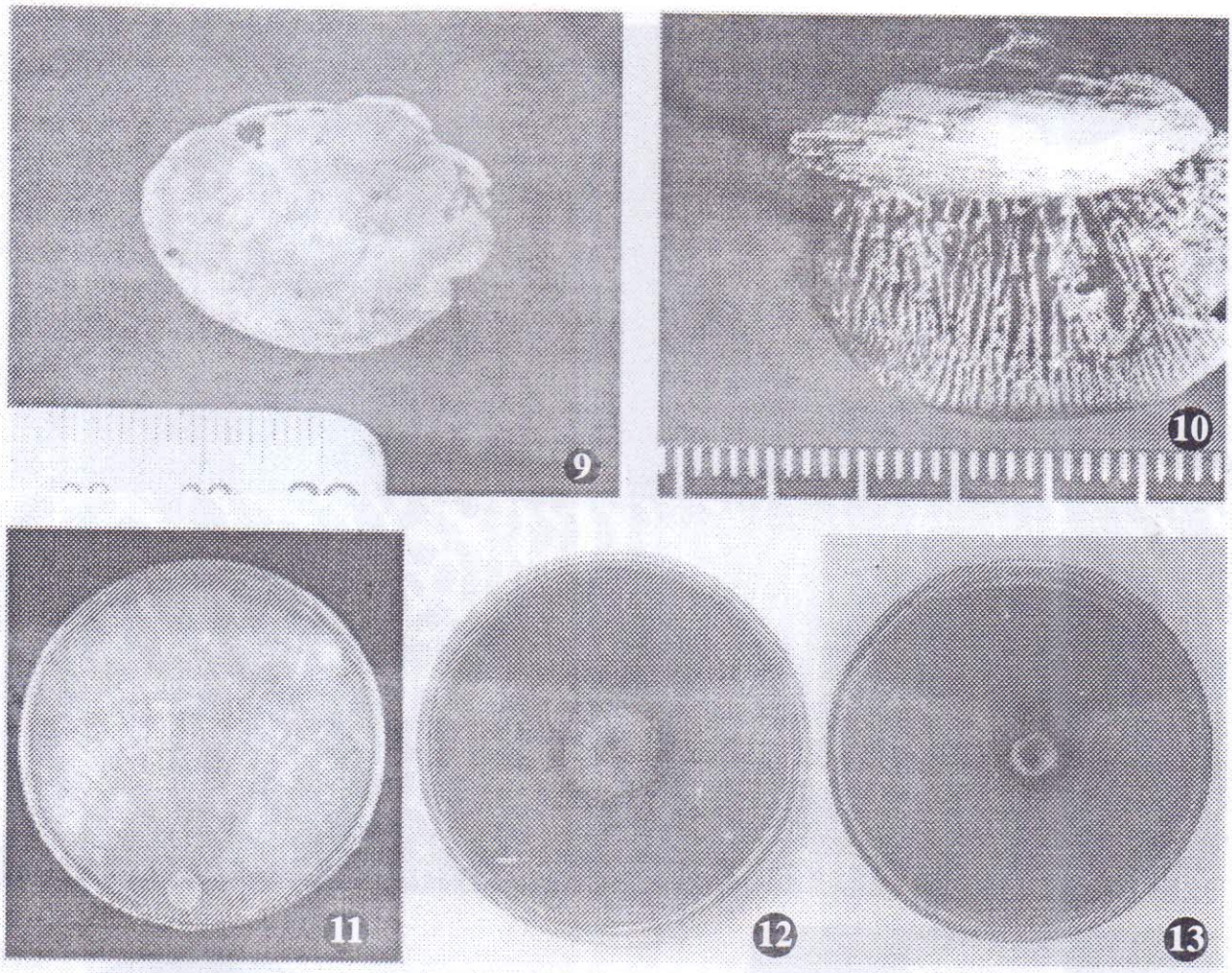
BASIDIOCARPS

Macroscopic characters: Basidiocarps annual, pileate, sessile to substipitate, with a thick, short , lateral or central stalk, 1-4 cm in length and 3.5-5.5 cm in diameter; pileus dimidiate, fan shaped to circular, usually solitary, occasionally imbricate, 2.5-20 x 3-27 x 0.3-1.5 cm; upper surface (Fig. 1) white to wood-brown, faintly zonate, velvety when fresh, glabrous on drying; thinly crustose towards the base in some basidiocarps; margin irregular, thin, sometimes thick and rounded; context coriaceous to corky, white to wood-brown, 0.1-0.6 cm thick; hymenial surface (Fig.2) white to wood-brown, may be poroid (Fig.5), daedaloid (Fig.4) or lamellate (Fig.3); poretubes concolourous with the context, 0.5-1.8 cm long.

Microscopic characters: Hyphal system trimitic; (a) generative hyphae hyaline, branched with conspicuous clamp connections, 1.3- 3.2 µm in diameter, thin-walled (Fig.22), some slightly thick-walled (Fig.23), a few pale brown, thick-walled (Fig.24), occasionally found among them cuticular cells in the crustose part of the pileus surface; (b) skeletal hyphae subhyaline to pale brown, thick-walled to solid, aseptate, occasionally encrusted, unbranched or apically branched with characteristic 2-3 tortuous branches, one often shorter than the others (Fig.25), apex tapering or blunt, 3-4.5 µm wide, abundant everywhere, many tape-like and twisted (Fig.26), 4.5-5.2 µm wide, occasionally found; (c) binding hyphae brownish, thick-walled to solid (Fig.27), much branched with long proliferating branches, somewhat tortuous, 1.3-2.2 µm wide, abundant, particularly in lower context and pore fields; (d) cuticular cells brownish, thick-walled, somewhat isodiametric (Fig.28) occurring occasionally in the crustose part of the pileus surface; (e) basidia (Fig.29) clavate, hyaline, thin-walled, tetrasterigmatic, 14.4-22 x 3.5-7 µm bearing clamp connection at the base; (f) basidiospores (Fig.30) hyaline, thin-walled, short cylindric, adaxially slightly concave, basally bent, 5.0-8.5 x 2.0-3.6 µm.



Figs. 1-8. *Daedalea flavida*. **Figs. I-5.** Basidiocarp: 1. Upper surface. 2. Hymenial surface. **Figs. 3-5.** Hymenial configurations: 3. Lamellate; 4.Daedaloid; 5.Poroid. **Figs.6-8.** Cultural characters: 6.Growth on malt extract agar after 6 weeks. 7. Growth on gallic acid agar. 8. Growth on tannic acid agar.

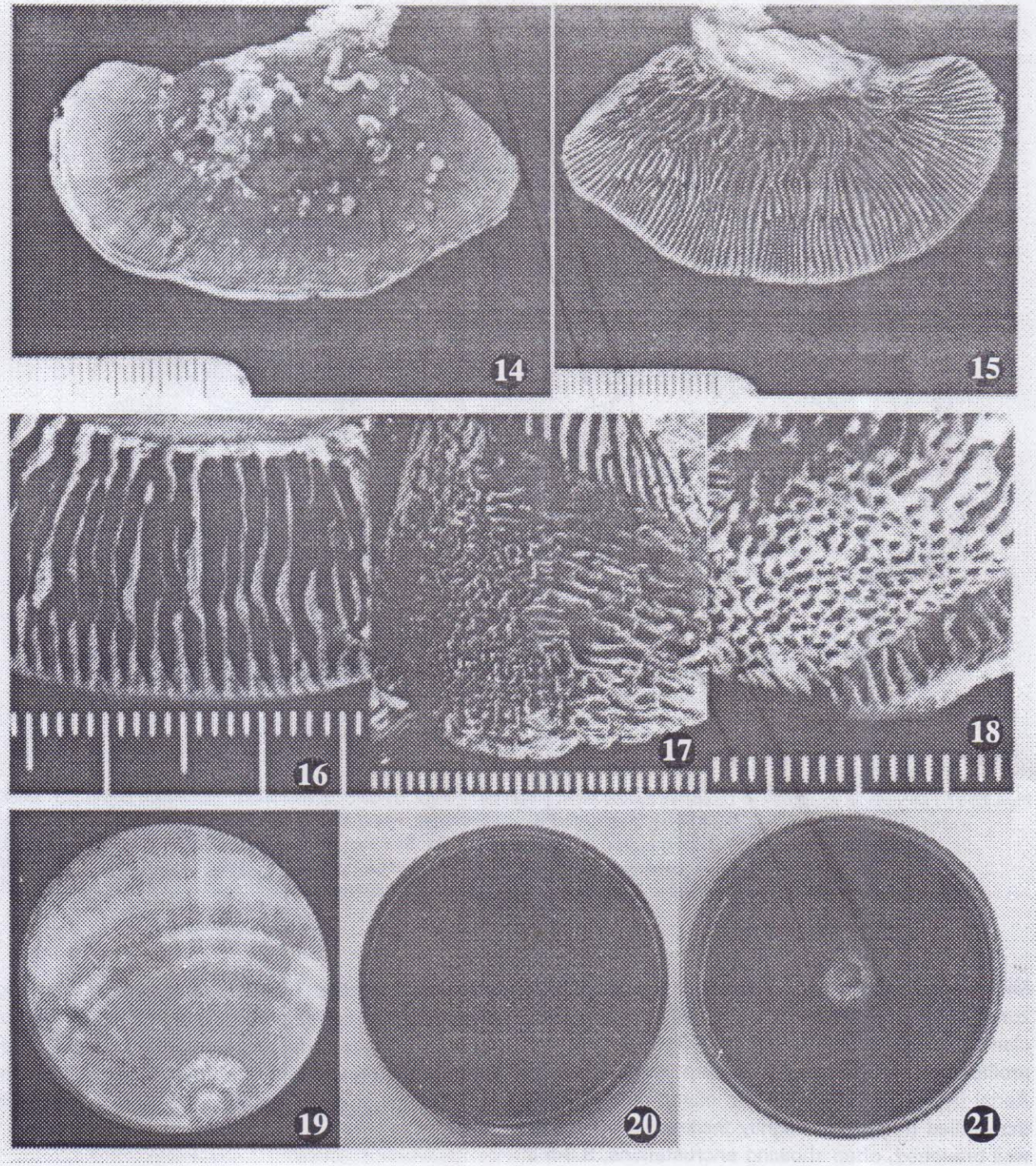


Figs.9-13. *Lenzites adusta*. **Figs.9-10.** Basidiocarp : 9. Upper surface. 10. Hynienial surface. **Figs. 11-13.** Cultural characters: 11 .Growth on malt extract agar after 6 weeks. 12. Growth on gallic acid agar. 13. Growth on tannic acid agar.

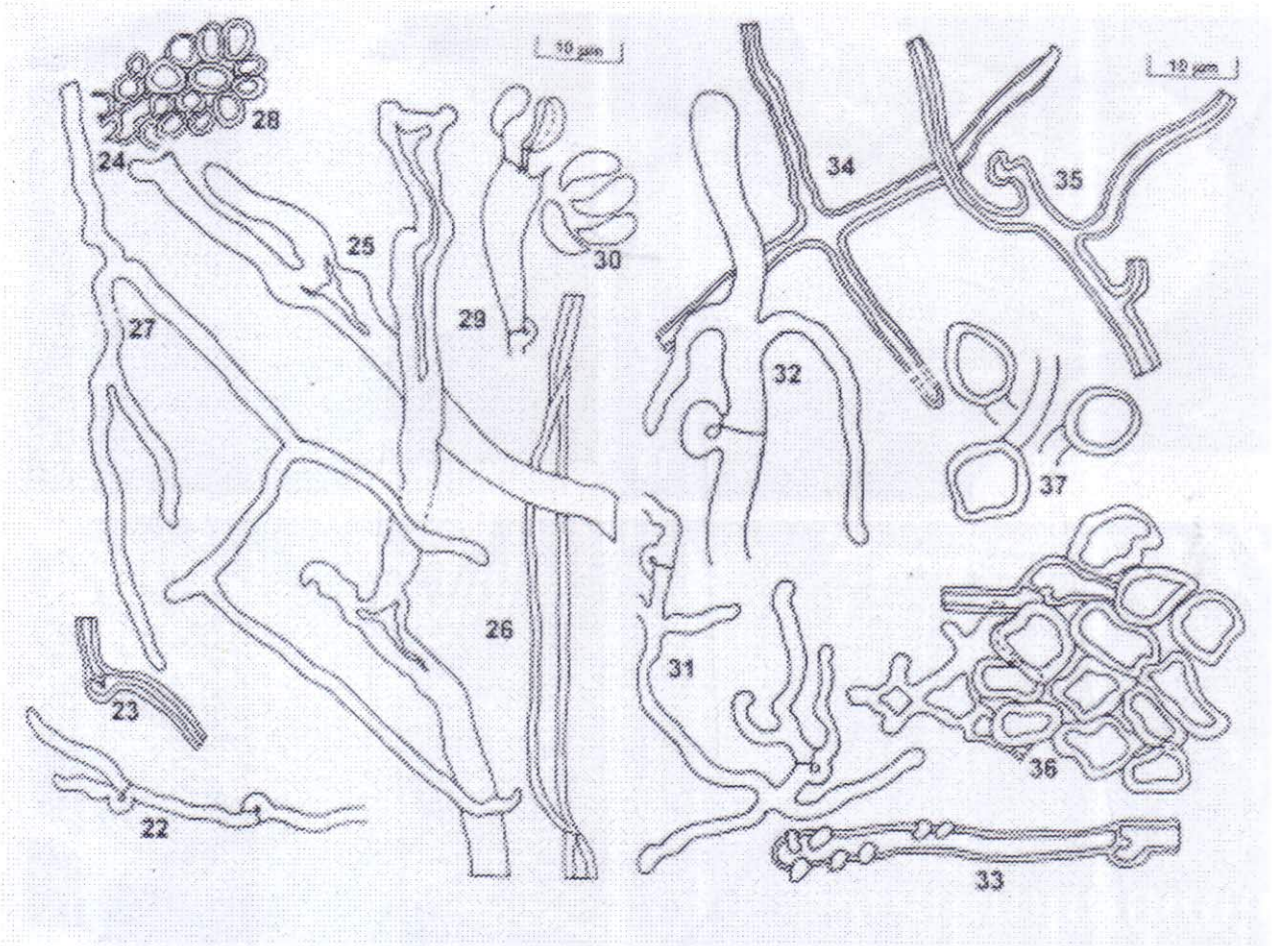
Specimens examined: VBMH 80108, on stump of *Shorea robusta* Gaertn.f, 25.07.2006, Bankura, W.B.; VBMH 811 10, on stump of *Shorea robusta* Gaertn.f, 27.07.2006. Santiniketan, W.B.; VBMH 81111, on stump of *Shorea robusta* Gaertn.f, 27.07.2006, Santiniketan, W.B.; VBMH 81112, on stump of *Ficus religiosa* L., 27.07,2006, Santiniketan, W.B.; VBMH 81113, on stump of *Ficus religiosa* L., 05.08.2006, Hooghly, W.B.; VBMH 82108, on dead log of *Shorea robusta* Gaertn.f, 13.08.2006, Simlipal Forest, Orissa; VBMH 82109, on dead log of *Shorea robusta* Gaertn.f, 13.08.2006, Simlipal Forest, Orissa; VBMH 82115, on stump of *Ficus religiosa* L., 14.08.2006, Dum Dum, W.B.; VBMH 851021, on dry unknown log, 20.08.2006, Bolpur,W.B.; VBMH 91 1022, on dry unknown log, 20.08.2006, Bolpur,W.B.

CULTURAL CHARACTERS

Growth characters: Growth (Fig.6) rapid, plate covered in one week. Advancing zone sharp, even, hyaline and appressed, 1-2 mm wide, in some plates mycelia extended to the margin. Aerial mycelium white, woolly-floccose to start with, gradually changing to woolly-felty surrounding the inoculum and showing fine radiations from inoculum to the margin, finally becoming subfelty to felty in older mat, dense white mycelium raising along the edges of the lower Petridish. Reverse bleached with fine, transparent, net-like ornamentation; thin, skin-like, hyaline layer formed on the agar below the aerial mycelium.



Figs.14-21.*Lenzites japonica.* **Figs. 14-18.** Basidiocarp: 14.Upper surface. 15. Hymenial surface. **Figs.l6-18.** Hymenial configurations: 16.Lenzitoid; 17. Daedaloid; 18. Poriod. **Figs. 19-*21.*** Cultural characters: 19. Growth on malt extract agar after 6 weeks. 20. Growth gallic acid agar. 21. Growth on tannic acid agar

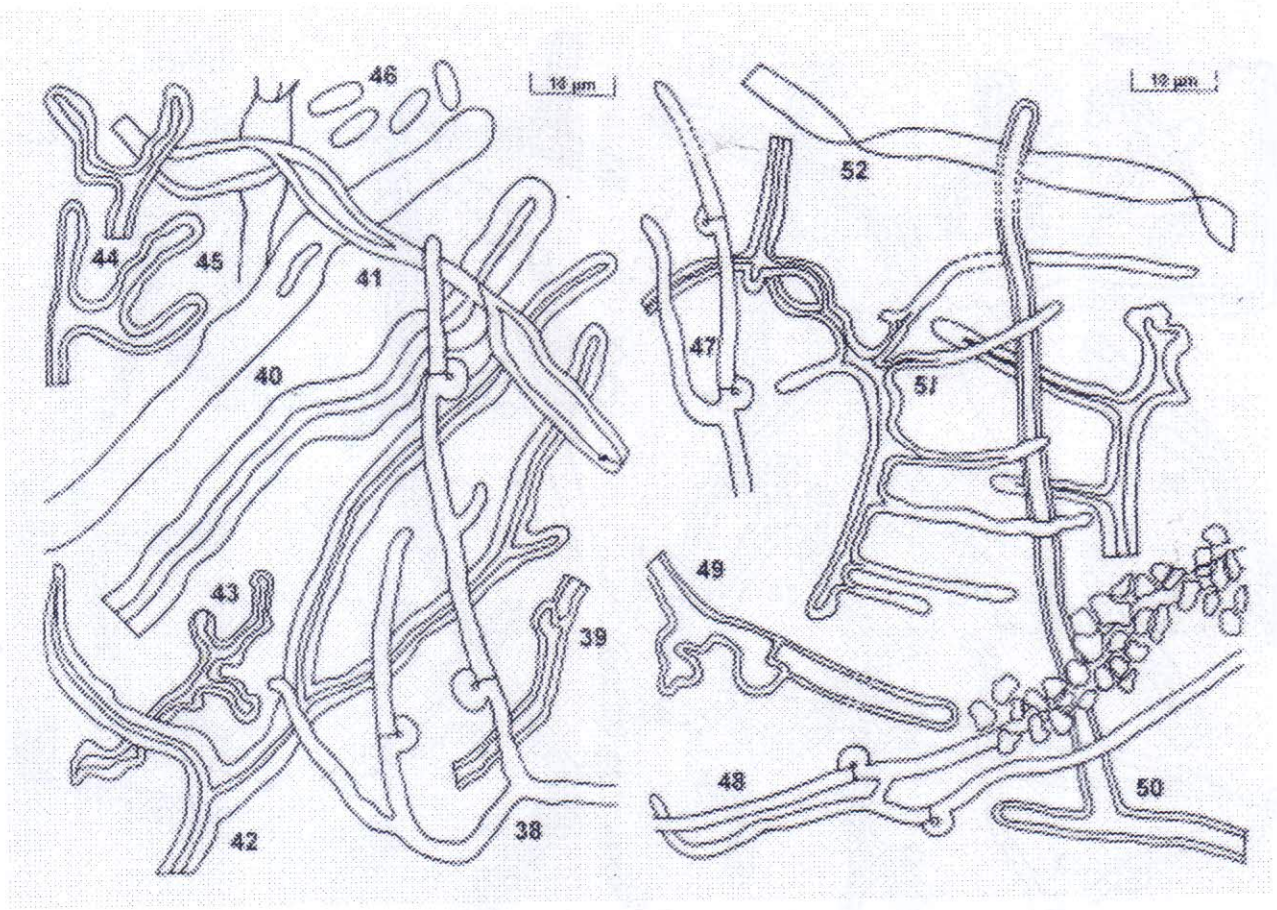


Figs.22-37 : Micrscopic structures of *Daedalea flavida* From basidiocarp. **Figs. 22-30 :** 22.Thin-walled generative hypha. 23. Thick-walled generative hypha. 24. Pale brown thick-walled generative hypha found among the cuticular cells.25.Skeletal hyphae. 26 Tape-like skeletal hypha. 27.Bindmg hypha. 28. Cuticular cells. 29. Basidium. 30. Basidiospores. From culture. **(Figs. 31-37):** 31. Thin-walled hypha from advancing zone 32. Wider thin-walled hypha. 33. Slightly thick-walled hypha with encrustations. 34. Branched fibre hypha. 35. Branched fibre hypha with thickened clamp at one end. 36.Cuticular cells. 37. Chlamydospores.

Microscopic characters: Hyphae in the advancing zone hyaline, thin-walled, branched, septate with clamp connections, branches often arising by proliferation of clamps, 1.3-2 urn wide (Fig.31). Aerial hyphae- (a) hyaline with clamp connections, thin-walled (Fig.32) or slightly thick-walled (Fig.33), well branched, often showing encrustations, 3.3-5.2 µm wide; (b) fibre hyphae hyaline, refractive, of two kinds-(i) thick-walled, showing wide or narrow lumina, branched, branches arising mainly at right angles to the stem, 2-4 µm wide (Fig.34), abundant; (ii) thick-walled, often with thickened clamps at one end (Fig.35), producing narrower side branches of different lengths, 1.5-4 µm wide, found in woolly part of the mat and not frequent; (c) thin- to thick-walled, hyaline cuticular cells formed by closely interwoven irregularly swelled hyphae, occasionally showing clamp connections (Fig.36), occur in the felty area of the mat; (d) chlamydospores abundant, thick-walled, intercalary and terminal, of various shapes, globose, subglobose or pear shaped, 6.5-10.4 x 5.2-9 µm (Fig.37).

Oxidase reactions: Positive in both gallic acid (Fig. 7) and tannic acid agar (Fig.8). Sexuality: Result of matings of 16 monosporous cultures obtained from a fresh basidiocarp (VBMH 911022) shows the species to be heterothallic and tetrapolar. The distribution of four mating groups is as follows:

A1B1: 1, 8, 10, 13, 14	A2B2: 3, 5, 6, 9
A1B2: 2, 4, 7, 16	A2B1: 11, 12, 15



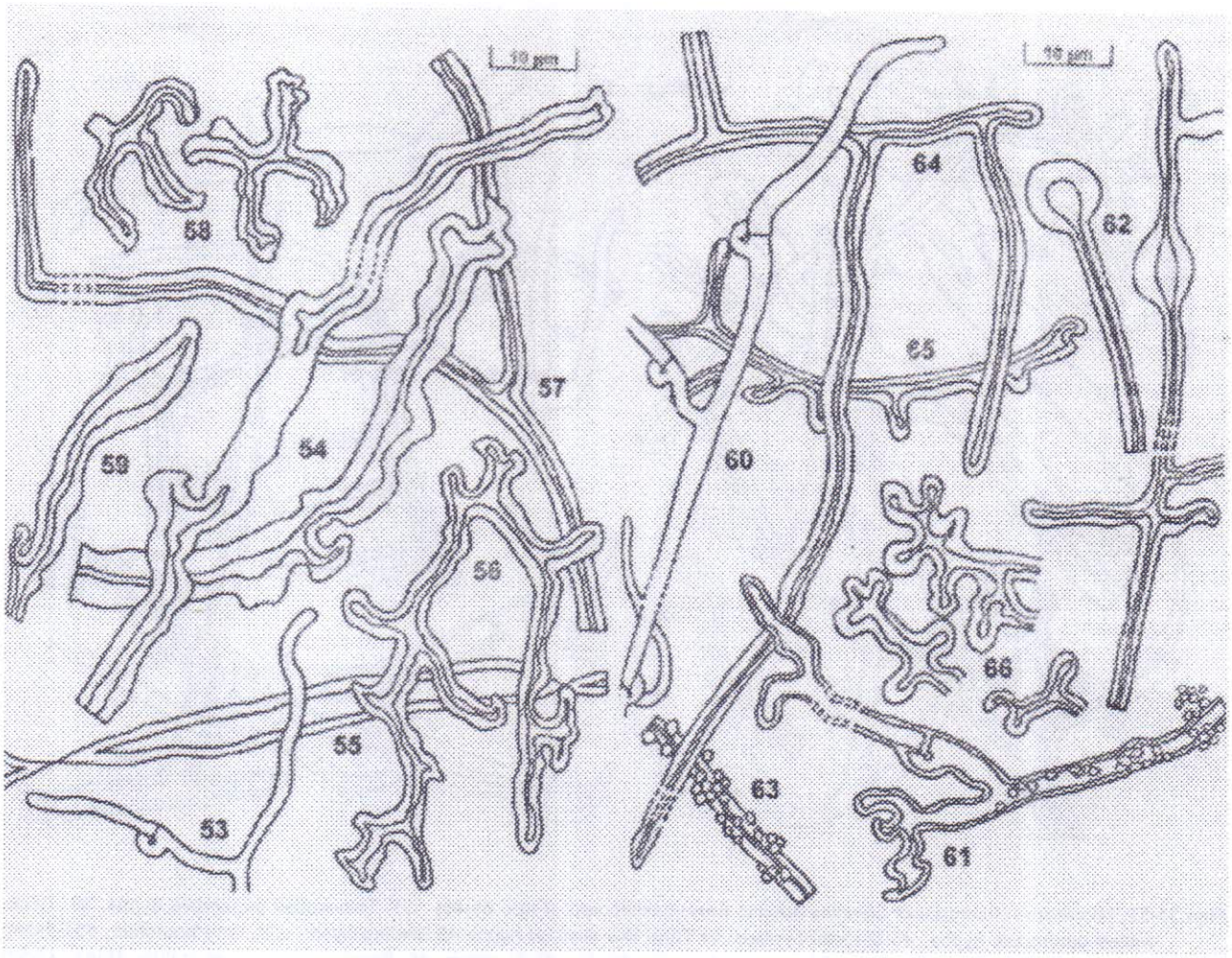
Figs.38-52. Micrscopic structures of *Lenzites adusta*. From basidiocarp. **(Figs. 38-46)** : 31. Thin-walled generative hypha. 39. Thick-walled generative hypha. 40.Skeletal hyphae. 41.Tape like skeletal hypha.42.Binding hypha with long branches. 43.Binding hypha; with short branches. Candelabra shaped binding hyphae.45.Basidium. 46. Basidiosporesi From culture. **(Figs. 47-52)** : 47.Thin-walled hypha from advancing zone. 48. Thin-walled hyphae with H-connection and encmstations.49.Thick-walled hypha with clamp connection.50. Fibre hypha with long branches.51 .Fibre hyphae with short branches arising on one side.52.Twisted, tape like hypha.

Cultures examined: VBMH 80108, VBMH 81111, VBMH 81112, VBMH 82115. VBMH 911022

***Lenzites adusta* Massee *Kew Bull.* 11: 250, 1910.**

BASIDIOCARPS

Macroscopic characters: Basidiocarps annual to perennial, pileate, pileus dimidiate or attached by a broad base, usually single, coriaceous when fresh, flexible when dry, 5-12 x 7-26 x 1.5-2.5 cm; margin sharp, bent downwards in thin specimens, white when fresh, cream coloured on drying; upper surface (Fig.9) concolourous with the margin, first very finely velutinate, with age becoming glabrous, azonate; context white when fresh and creamy white on drying, up to 7 mm thick, corky; hymenial surface (Fig. 10) creamish, lamellate, lamellae irregular towards the base, regular towards the margin , 10-12/cm. Microscopic characters: Hyphal system trimitic; (a) generative hyphae hyaline, with clamp connections, branched, mostly thin-walled (Fig.38), few thick-walled (Fig.39), 1.3-2.6 µm wide, quite abundant in all the regions; (b) skeletal hyphae hyaline, unbranched, somewhat tortuous, of two types- (i) mostly solid, rarely with narrow lamina, apex widely tapered, 4-6 µm wide, greatly abundant (Fig.40); (ii) slightly thick-walled, twisted and tape-like, with wide lamina, 4-6.5 (-7.8) µm wide, as abundant as the former kind of skeletal hyphae (Fig.41); (c) binding hyphae hyaline, mostly thick-walled showing narrow lamina. rarely solid, much branched, with long (Fig.42) as well as short (Fig.43) branches, most of the branches characteristically dichotomous, the former more common in the



Figs. 53-66 : Micrscopic structures of *Lenzites japonica* From basidiocarp. **(Figs.53-59) :** 53.Thin-walled generative hypha. 54.Skeletal hyphae.55.Tape like twisted skeletal hypha. 56. Binding hypha with short branches. 57.Binding hypha with long branches. 58. Candelabra shaped binding hyphae. 59. Skeleto-cystidia.

From culture (Figs.60-66) : 60.Thin-walled hypha from advancing zone. 61 Slightly thick-walled wider hypha with clamp connection and encrustations. 62. Fibre hyphae with characteristic terminal and intercalary swellings. 63. Encrusted unbranched fibre hypha. 64.Fibre hypha with long branches. 65. Fibre hypha with short branches and broken clamp at one end.66.Plectenchymatic layer.

context, while the latter in the pore field, some of the short branches penetrating into the poremouth like candelabra (Fig.44), 2.6-4.0 µm wide; (d) basidia clavate, hyaline, thin-walled, tetrasterigmatic, 11-13 x 3.5 µm (Fig.45); (e) basidiospores ellipsoid-cylindric, hyaline, thin-wailed, 5-8 x 2-3.5 µm (Fig.46); (f) cystidia proper not found but some branches of binding hyphae project into the hymenium and simulating cystidia-like structures (cystidioid hyphae).

Specimens examined: VBMH 6868, on unknown fallen log, 22.07.2005, Goyarkata. Jalpaiguri, W.B.; VBMH 7214, on stump of *Ficus benghalensis* L.,27.07.2006, Santiniketan, W.B.; VBMH 78301, on stump of *Shorea robusta* Gaertn.f, 18.08.2006, Burdwan W.B.; VBMH 87302, on stump of *Pinus roxburghii* Sargent, 22.08.2006, DehraDun, Uttarakhand; VBMH 88303, on log of *Shorea robusta* Gaertn.f, 02.09.2006, Bankura, W.B.; FR1 78, date of collection, host and locality not mentioned.

CULTURAL CHARACTERS

Growth characters: Growth (Fig. 11) moderately rapid, 4.2-5.7 cm in diameter in one week, plate covered in two weeks. Advancing zone even, hyaline and appressed in a narrow zone. Aerial mat

white, dense floccose to start with, later changing to woolly-felty in the younger part, thin and transparent around the inoculum, small, dense, ball-like areas appearing here and there oh the mat and wolly-felty mycelium raising along the edges of the lower Petridish, reverse unchanged.

Microscopic characters: Hyphae in the advancing zone hyaline, thin-walled, branched, septate with clamp connections, 1.3-3.2 µm wide (Fig.47). Aerial hyphae- (a) as in the advancing zone but wider, up to 3.3 µm wide, branches often found arising from opposite a clamp and H-connections as well as encrustations occasionally found (Fig.48); (b)hyphae hyaline, thick-walled, with clamp connections, branched, 2.2-4.5 µm wide, not abundant (Fig.49); (c) fibre hyphae hyaline, thick-walled, but showing lumina, branched, with short as well as long branches (Fig.50), frequently branches arising on one side of the main hyphae (Fig.51), 2.6-4.2(-7.0) µm wide, some twisted and tape-like (Fig.52), 5.2-7.8 µm wide, quite frequent in culture.

Oxidase reactions: Positive in both gallic acid (Fig. 12) and tannic acid agar (Fig. 13).

Sexuality: Result of matings of 20 monosporous cultures obtained from a fresh basidiocarp (VBMH 87302) shows the species to be heterothallic and tetrapolar. The distribution of four mating groups is as follows:

A1B1:1,2.3,6, 15, 19	A2B2: 5,9, 18
A1B2: 11, 12, 13, 14, 16	A2B1:4, 7, 8, 10, 17,20

Cultures examined : VBMH 87302, VBMH 87303, FRI 78.

***Lenzites japonica* Berk and Curt.,Proc. Amer. Acad. Arts. 4: 111 -130, I860.**

BAS1DIOCARPS

Macroscopic characters: Basidiocarps annual, pileate, sessile to substipitate, single, sometimes imbricate, pileus dimidiate, fan-shaped to circular, up to 10 x 7 x 1.5 cm; upper surface (Fig. 14) grayish-brown in the major portion with wood-brown margin, velvety when fresh, glabrous on drying, surface nodulose towards the base, finely zonate and smooth towards the margin; margin regular or irregular, thin, sterile in a narrow zone; context coriaceous to corky, wood-brown, up to 5 mm thick; hymenial surface (Fig. 15) wood-brown to grayish-brown, lenzitoid (Fig. 16) but also may be poroid (Fig. 18) and daedaloid (Fig. 17), up to 1 cm long, pores about 1/mm.

Microscopic characters: Hyphal system trimitic; (a) generative hyphae hyaline, thin -walled, with clamp connections, branched, 1-2 µm wide, not abundant (Fig.53); (b)skeletal hyphae hyaline, thick-walled to solid, tortuous (Fig.54), mostly unbranched, rarely with a notch towards the apex, apex pointed or blunt, some tape-like (Fig.55), 4-6.5 µm wide, abundant in all the regions of the basidiocarps; (c) binding hyphae hyaline, thick-walled with narrow lumina, somewhat tortuous, much branched, branches mostly short (Fig.56), rarely long (Fig.57), 2-3.5 µm wide, more abundant in the pore field than in the context, some short branches penetrating into the poremouth like candelabra (Fig.58); basidia and basidiospores not found; true cystidia lacking, but apex of some skeletal hyphae penetrate into the poremouth from pore field behaving as skeleto-cystidia (Fig.59).

Specimens examined: TAA 101168, on unknown deciduous wood, date of collection not mentioned, Chugujevski, Bulyga-Fadejevo, Kavalerovo, USSR; TAA 103836, on unknown deciduous wood, , date of collection not mentioned, Chugujevski, Bulyga-Fadejevo, Kavalerovo, USSR; TAA103837, on logs of *Ulmus laciniala,* date of collection not mentioned, Chugujevski, Bulyga-Fadejevo, Kavalerovo, USSR; TAA 105360, date of collection, host and locality not mentioned; TAA 105362, on unknown deciduous wood, date of collection not mentioned, Chugujevski, Bulyga-Fadejevo, Kavalerovo, USSR.

CULTURAL CHARACTERS

Growth characters: Rate of growth (Fig. 19) rapid, plate covered in one week. Advancing zone even, hyaline, thin and appressed in a narrow zone, 2-3 mm wide. Aerial mycelium white, slightly appressed to start with, gradually changing to woolly-floccose in younger mat and woolly-felty in older part, with dense mycelium raising along the edges of the lower Petridish and also below the upper lid. Reverse bleached.

Microscopic characters: Hyphae in the advancing zone hyaline, thin-walled, septate with clamp connections, much branched, 1.3- 3.3 μm wide (Fig.60). Aerial hyphae- (a) as in the adyancing zone but some wider, 2.6-4 μm wide; (b) hyphae hyaline, slightly thick-walled, branched, with clamp connections, often encrusted, 2.6-6.5 μm wide, few in number (Fig.61); (c) fibre hyphae hyaline, thick-walled, but always showing lumina, often with characteristic terminal and intercalary swellings (Fig. 62), occasionally encrusted (Fig. 63); (ii) branched, branches may be long (Fig.64) or short, often in the form of protuberances only, sometimes showing broken clamp at one end (Fig.65); (d) in felty areas occur fibre hyphae with numerous short branches or irregular protuberances, having thick refractive walls, all closely interwoven to form a plectenchyma layer(Fig.66).

Oxidase reactions: Positive in both gallic acid (Fig.20) and tannic acid agar (Fig.21). Sexuality: Result of matings of 15 monosporous cultures obtained from a basidiocarp (TAA 105360) shows the species to be heterothallic and tetrapolar. The distribution of four mating groups is as follows:

A1B1: 1, 5. 7,9, 10 A2B2: 3, 8
A1B2: 4, 14, 15 A2B1:2,6, 11, 12, 13

Culture examined: TAA 105360.

Table-1. Results obtained by pairing of monosporous mycelia from different sporophores of *Daedalea flavida* Lev., *Lenzites adusta* Massee *and Lenzites japonica* Berk, and *Curt.*

		Daedalea flavida					*Lenzites adustra*			*Lenzites japanica*
		VBMH 80108	VBMH 81111	VBMH 81112	VBMH 82115	VBMH 911022	VBMH 87302	VBMH 88303	FRI 78	TAA 105360
Daedalea flavida	VBMH 80108	+	+	+	+	+	–	–	–	–
	VBMH 81111	+	+	+	+	+	–	–	–	–
	VBMH 81112	+	+	+	+	+	–	–	–	–
	VBMH 82115	+	+	+	+	+	–	–	–	–
	VBMH 911022	+	+	+	+	+	–	–	–	–
Lenzites adusta	VBMH 87302	–	–	–	–	–	+	+	+	–
	VBMH 88303	–	–	–	–	–	+	+	+	–
	FRI 78	–	–	–	–	–	+	+	+	–
Lenzites japanic	TAA 105360	–	–	–	–	–	–	–	–	+

It is observed that the 9 isolates studied fall into three distinct groups, *Daedalea flavida* Lev., *Lenzites adusta* Massee and *Lenzites japonica* Berk and Curt in each of which the isolates are compatible among themselves, but incompatible with isolates in other groups.

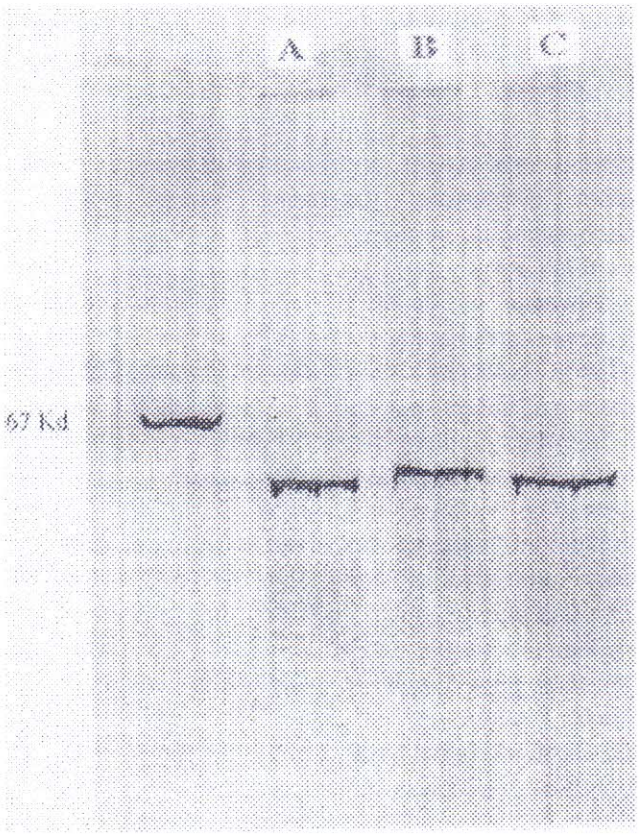


Fig.67 : Electrophoretic spectra of soluble proteins of *(A)Daedalea flavida,* (B) *Lenzites japonica* and (C) *Lenzites adusta.*

Result of conspecificity test

Result of electrophoretic analysis

A comparative electrophoretic study of soluble proteins of *Daedalea flavida* Lev., *Lenzites adusta* Massee and *Lenzites japonica* Berk, and Curt, have shown their individuality (Fig.67).

In all cases electrophoretic spectra of soluble proteins were obtained from 10 days old mycelial mat cultured under the same cultural condition. Thus, from electrophoregram of protein preprations obtained from these three species, it is possible to observe 17 electrophoretic bands in *Daedalea flavida* Lev., 21 in *Lenzites adusta* Massee and 19 in *Lenzites japonica* Berk and Curt. The protein fractions obtained from all these studied species differed in relative electrophoretic mobility (REM) and in the intensity of their staining (Table 2).

In *Daedalea flavida,* there are polypeptides with REM 0.064, 0.104, 0.112, 0.144, 0.152,' 0.168, 0.192, 0.200, 0.232, 0.248, 0.256, 0.280, 0.304, 0.312, 0.344, 0.464, 0.576 which are mostly not observed in *Lenzites adusta* where is noted the electrophoretic tractions with REM 0.064, 0.071, 0.078, 0.100, 0.107, 0.121, 0.150, 0.178, 0.200, 0.214, 0.235, 0.250, 0.264, 0.285, 0.300, 0.321, 0.335, 0.407, 0.507, 0.707, 0.714. The fractions with REM *of Lenzites japonica* are 0.008, 0.072, 0.080, 0.090, 0.112, 0.121, 0.168, 0.200, 0.224, 0.232, 0.256, 0.296, 0.328, 0.336, 0.352, 0.424, 0.488, 0.560, 0.576. In *Daedalea flavida* with REM 0.064, 0.200 polypeptides are only common with *Lenzites adusta ;* and REM 0.112, 0.168, 0.200, 0.232, 0.256, 0.576 polypeptides are common with *Lenzites japonica.* REM 0.121, 0.200 polypeptides are only common between *Lenzites adusta* and *Lenzites japonica.*

Fig.67 shows that all these three species have very few protein bands in common. It has been established that 2 out of 38 (5.3%) bands are common between *Daedalea flavida* and *Lenzites adusta,* 6 out of 36 (16.6%) bands are common between *Daedalea flavida* and *Lenzites japonica* and *2 out of 40 bands (5%) are* common between *Lenzites adusta* and *Lenzites japonica.*

The degree of dissimilarity of protein phenotypes of these 3 species is high and makes up to 94.7% (*Daedalea flavida* and *Lenzites adusta*), 83.4% (*Daedalea flavida* and *Lenzites japonica*) and 95% (*Lenzites adusta* and *Lenzites japonica*).

DISCUSSION

It is evident from the foregoing observations that there are more dissimilarities among *Daedalea flavida, Lenzites adusta* and *Lenzites japonica* than similarities. They are similar in showing poroid to lenzitoid hymenial surface in their cream-brown basidiocarps which are smooth on their abhymenial surface, constituting trimitic hyphal system with generative, skeletal and binding hyphae, producing white mat in culture, constituting hyaline hyphae, exhibiting positive oxidase test, causing white rot in woods in nature and having tetrapolar sexuality.

But according to modern taxonomists of fungi exomorphological characters have little value in fungal taxonomy (Kotlaba and Pouzar, 1957: Teixeira, 1962). Moreover, it is at present a common experience of the mycologists to come across to polypore fungi during field collection showing all ranges from poroid to daedaloid poremouths in their hymenial surface in the same collection and often in the same specimen (Ryvarden, 1974; Hjortstam and

Table 2 : Relative Electrophoretic Mobility (REM) of Soluble Proteins of *Daedalea flavida* Lev., *Lenzites adusta* Massee and *Lenzites japonica* Berk, and Curt.

Daedalea flavida	*Lenzites japonica*	*Lenzites adusta*
-	0.008	-
0.064	-	0.064
-	-	0.071
-	0.072	-
-	-	0.078
-	0.080	-
-	0.090	-
-	-	0.100
0.104	-	-
-	-	0.107
0.112	0.112	-
-	0.121	0.121
0.144	-	-
-	-	0.150
0 152	-	-
0.168	0.168	-
-	-	0.178
0.192	-	-
0.200	0.200	0"200
-	-	0.214
-	0.224	-
0.232	0.232	-
-	-	0.235
0. 248	-	-
-	-	0.250
0 256	-	-
	0.256	-
-	-	0.264
0.280	-	-
-	-	0.285
-	0.296	-
-	-	0.300
0.304	-	-
0 312	-	-
-	-	0.321
-	0.328	-
-	-	0.335
-	0.336	-
0.344	-	-
-	0.352	-
-	-	0.407
-	0.424	-
0.464	-	-
-	0.488	-
-	-	0.507
-	0.560	-
0.576	0.576	-
-	-	0.707
-	-	0.714
in all 17	*19*	21

Ryvarden, 1987). So, similarity in exomorphology of these 3 species are insignificant from taxonomical point of view.

Critical observations *on their hyphal systems* reveal great differences. *Daedalea flavida* produces subhyaline to yellowish skeletal hyphae while in *Lenzites adusta* and *Lenzites japonica* these hyphae are hyaline. Moreover, in both *Lenzites adusta* and *Lenzites japonica*, the skeletal hyphae are unbranched while in *Daedalea flavida*, they are unbranched in the stem part but characteristically branched at the apex. In *Lenzites japonica* skeletal hyphae are very much tortuous and characteristically notched towards the apex. It is not found in *Lenzites adusta*. The binding hyphae in *Daedalea flavida* are of one type only and they are yellowish, long and proliferating; but in *Lenzites adusta* as well as in *Lenzites japonica*, these hyphae are hyaline and of two types: (i) long type and (ii) short type, the latter being as abundant as the former. Moreover, in latter two species occur candelabra-shaped binding hyphae at the poremouths but these are lacking in *Daedalea flavida*.

Regarding the distribution of the binding hyphae there is a difference between *Lenzites japonica* and *Lenzites adusta*. In *Lenzites japonica*, binding hyphae with long branches are more common in the context while short branched ones are more abundant in the pore field. But in *Lenzites adusta* there are no such differences in distribution but the binding hyphae are wider (up to 7 μm) than other two species. The generative hyphae are always thin-walled in *Lenzites japonica* but they are sometimes thick-walled in basidiocarps of *Daedalea flavida* and *Lenzites adusta*. Besides, cuticular cells which are found in *Daedalea flavida* are absent in other two species. Similarly, skeleto-cystidia are only found in *Lenzites japonica,* while cystidioid hyphae were noticed in *Lenzites adusta*. Basidiospores are short cylindric, adaxially slightly concave, basally bent in *Daedalea flavida* and ellipsoid-cylindric in *Lenzites adusta*. Basidiocarps of *Lenzites japonica* were sterile and therefore nature of basidiospores could not be studied. As presence or absence of hyphal types as well as the different types of hyphae in a basidiocarp are regarded by modern taxonomists as characters of taxonomic value (Roy, 1982; Ryvarden 1989; Reid, 1976; Thind, 1980; De, 1984, Parmasto and Ryvarden, 1990), such dissimilarity in the hyphal characters of the three species are

taxonomically noteworthy. The three species further exhibit dissimilarities in the growth pattern of their cultures as well as in cultural anatomy. Texture of the old mat is subfelty to felty in *Daedalea flavida,* woolly-felty with dense ball-like areas here and there in *Lenzites adusta,* woolly-felty in *Lenzites japonica.* In thin-walled hyphae branches often arise by proliferation of clamps in *Daedale flavida* where as H-connections and encrustations are the characters of thin-walled hyphae in *Lenzites adusta.* Besides, fibre hyphae are branched in case of *Daedalea flavida* and *Lenzites adusta;* but are of two types in *Lenzites japonica:* (i) branched and (ii) unbranched. In *Daedalea flavida* fibre hyphae from woolly part of the mat often exhibit branches arising at right angles to the main stem and sometimes there are thickened clamp connections at one end. In *Lenzites adusta* branches of the fibre hyphae arise on one side of the main hyphae and wide up to 7 μm. In *Lenzites japonica* branches of the fibre hyphae are with characteristic terminal or intercalary swellings and encrustations. Cuticular cells found in felty mat of *Daedale flavida* are absent in other two species; but in *Lenzites japonica* plectenchyma layer is formed in the cultural felty mat constituting short branches or irregular protuberances of fibre hyphae. These structures are not produced by the other two species in culture. Chlamydospores appear in culture of *Daedalea flavida* only but not in *Lenzites japonica* and *Lenzites adusta.* The three species also proved to be incompatible in conspecificity tests.

So, it is evident that there are marked differences among these three species in their hyphal characters, cultural characters and genetical characters which indicate that the three species under study are not the same.

Finally, electrophoretic study shows that the three species produce strikingly different banding patterns in their soluble protein profile and this proves their strong unrelatedness.

So, all these evidences from anatomical , cultural, biological, genetical and biochenvcal studies indicate that each of the three fungi namely *Daedalea flavida* Lev., *Lenzites adusta* Massee and *Lenzites japonica* Berk and Curt is an individual species, one being different from the other.

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