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Citation	Journal of the Faculty of Agriculture, Hokkaido University, 63(2), 185-208
Issue Date	1987-03
Doc URL	http://hdl.handle.net/2115/13057
Type	bulletin (article)
File Information	63(2)_p185-208.pdf

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THE PRIMARY AND AUXILIARY AMBROSIA FUNGI ISOLATED FROM THE AMBROSIA BEETLES, *SCOLYTOPLATYPUS SHOGUN* BLANDFORD (COLEOPTERA : SCOLYTIDAE) AND *CROSSOTARSUS NIPONICUS* BLANDFORD (COLEOPTERA : PLATYPODIDAE)

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Received October 21, 1986

Abstract

An ambrosia fungus and several kinds of yeasts associated with the ambrosia beetles *Scolytoplatypus shogun* Blandford and *Crossotarsus niponicus* Blandford were isolated and identified. A specific ambrosia fungus, *Ambrosiella* sp., was isolated from both the mycetangia and the galleries of *S. shogun*. *Pichia* spp. were also isolated from the galleries of the beetles. It is considered that the fungus, *Ambrosiella* sp., is the primary ambrosia fungus and the *Pichia* spp. are the auxiliary ambrosia fungi of *S. shogun*, respectively. Two kinds of yeasts, *Endomycopsis platypodis* and *Torulopsis norvegica*, were commonly isolated from both mycetangia and galleries of *C. niponicus*. These fungi seem to be the primary ambrosia fungi of this beetle.

The morphological and physiological characteristics of these isolates were described. The range of the optimum temperature of *Ambrosiella* sp. was extremely narrow at approximately 23°C. For *E. platypodis* and *T. norvegica*, the range of the optimum temperature was relatively wide from 20°C to 30°C.

Introduction

It is well known that ambrosia beetles carry viable fungus spores within small organs called mycetangia in their bodies. When an ambrosia beetle

[J. Fac. Agr. Hokkaido Univ., Vol. 63, Pt. 2. 1987]

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tunnels into wood, these viable spores are dislodged from the mycetangia, and a mass of velvety fungus soon lines the interior of the tunnel. Until the present time, many species of fungi, more than 30 genera, have been reported as the fungi associated with ambrosia beetles. Only few species of these fungi, however, have been demonstrated as the true mutualistic symbiont or ambrosia fungi of these beetles.

In this paper, we have reported that one species of ambrosia fungus and several kinds of species of yeasts were isolated from mycetangia and galleries of *Scolytoplatypus shogun*, and several kinds of yeasts were isolated from the mycetangia and galleries of *Crossotarsus niponicus*.

The morphological and physiological characteristics of each species of these isolates have been described and identified.

Materials and Methods

Collection of beech logs infested with the beetles.

The ambrosia beetles used in the present study were *Scolytoplatypus shogun* as Scolytidae and *Crossotarsus niponicus* as Platypodidae.

Beech logs (*Fagus crenata* Blume) infested with the beetles were collected at the Hiyama Forest Experiment Station of Hokkaido University, Kaminokuni, Hiyama in September 1980 and July 1981, and immediately carried to the campus of the Faculty of Agriculture, Hokkaido University in Sapporo. The logs collected in the fall were overwintered under snow on the campus and were used in the next spring.

Isolation of microorganisms from galleries of the beetles.

The surface of the pinholed beech logs were washed by ethyl alcohol and burned. After that, the logs were cut into small pieces of about 2 cm^3, and the adults and larvae of the beetles were aseptically removed from the

TABLE 1. Composition of media.

Substance	YM broth (g)	CM broth (g)
Glucose	10	20
Peptone	5	10
Yeast extract	3	5
Malt extract	3	—
$MgSO_4$-$7H_2O$	—	2
K_2HPO_4	—	5
Distilled water	1000 ml	1000 ml

galleries. The inner surface of each gallery lined with velvety fungi was washed with TM and CM broth for culture. Components of both broth are shown on Table 1.

Collection of the adults.

In the overwintering period, the adults of *S. shogun* were collected from pinholed beech logs. In the flying period, the new adults of each species were collected within 12 hours after appearance.

Culture conditions for isolates.

i). The galleries were washed by YM and CM broth. After that, each broth was basically incubated by shaking culture at 25°C for five days, and then the cultures were incubated on YM and CM agar plates.

ii). Velvety fungus from the surface of the galleries was directly inoculated on both agar plates.

iii). The surface of each gallery was washed by sterile distilled water and drop of that water was incubated on both agar plates.

Isolation of microorganisms from the mycetangia of beetles.

The mycetangium of *S. shogun* is a small tissue located under the surface of the center of the pronotum of female adults. The shape of this tissue is like a jellyfish (Fig. 1)[8,9]. This tissue is easily separable from other tissues under microscope.

The mycetangium of *C. niponicus* is a bag around a sphere-shaped tissue located at the back of the preoral cavity of female adults (Fig. 2)[6,7,9]. This mycetangium is cleanly inseparabled, therefore, in the case of *C. niponicus*, the mycetangium was cut out with another tissues in the head attached.

The mycetangia of each species were aseptically placed in YM and CM

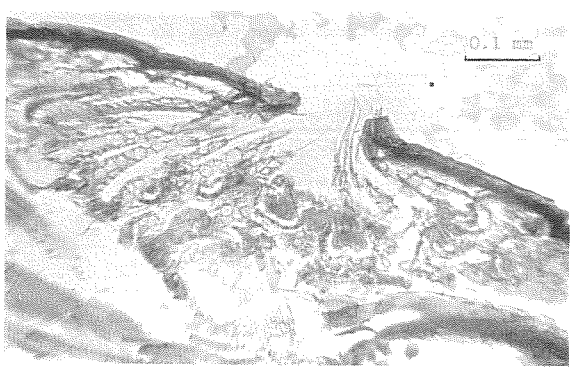

Fig. 1. The mycetangium of *S. shogun*.

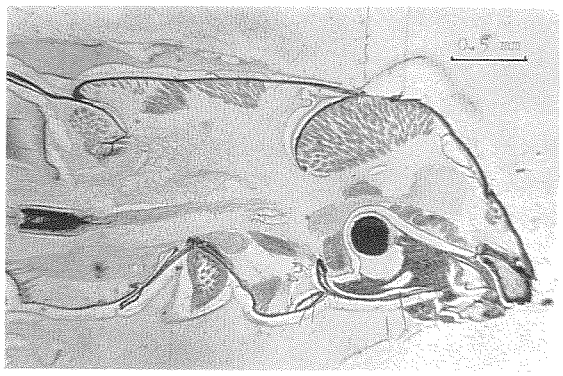

Fig. 2. The mycetangium of *C. niponicus*.

broth on slide glass and then were incubated on agar plates. Small amounts of the broth on slide glass were microscopically observed after incubation.

Isolation of microorganisms from the homogenates of larvae.

The larvae of each species were collected from pinholed beech logs onto cheese cloth and washed twice with distilled water. Each larva was homogenized gently in 1 ml of YM broth with sterile teflon homogenizer. The homogenates were spread over YM agar plates and incubated at 25°C up to form colonies.

Isolation of microorganisms from the digestive tracts of adult beetles in flight season.

The digestive tracts of new adults were dissected and incubated on YM agar plates.

Identification of the isolates.

Isolated yeasts were basically identified according to LODDER[5], and isolated ambrosia fungi were identified according to BATRA[2].

Scanning electron microscopy.

Scanning electron microscopy of the ambrosia fungi and the wall of the galleries of *S. shogun* was obtained by a 2% OsO_4 vapor fixation followed by coating with carbon and gold. Samples were observed by the scanning electron microscope Type JSM-SI.

Results

1. Fungi associated with *Scolytoplatypus shogun*.

Isolated fungi from mycetangia of adult beetles, homogenates of larvae,

TABLE 2. The fungi isolated from *S. shogun*.

Condition of isolation	Adult female				Larvae	Walls of galleries			
	New adults staying in gallery	Flying period	Breeding period		Homo-genates	Over-wintering period	New egg cradles	Breeding period	Empty gallery just after emergence
	Mycetangia		Digestive tracts	Body surface					
Season	May	June	June	Jul.-Aug.	July	Mar.-May	June	July	Aug.
Number of tested	20	27	10	10	4	5	2	3	1
Number of strains isolated	8	23	1	5	7	23	10	9	2
Ambrosiella sp.	###	### ###	—	—	—	—	###	###	—
Candida tenuis	—	—	—	—	—	###	—	—	—
Pichia sp. 1.	—	—	—	+	+	###	—	##	—
Pichia sp. 2.	—	—	—	##	##	##	—	##	+
Pichia sp. 3.	—	—	+	—	—	—	—	—	—
Endomycopsis sp.	—	—	—	—	—	+	—	—	—
unknown sp. 1.	+	—	—	—	—	—	—	—	—
unknown sp. 2.	+	—	—	—	—	—	—	—	—
white fungus	##	##	—	+	—	+	—	+	—

Numbers of (+) indicate numbers of strains isolated.

TABLE 3. Morphological characteristics of *Ambrosiella* sp. isolated from the new adults of *S. shogun*.

Ambrosiella sp.

Colony characteristics: Young colonies on YM agar at 25°C are white, 3 days old colonies are white to cream-colored, 1 cm in diameter, the center of the colony becomes elevated 1.5 mm high 2.0 mm in diameter; marginal mycelium hyaline to subhyaline, detached, becoming dendroid; superficially white aerial hyphae are formed. 7 days old colonies are greyish green, 4 cm in diameter, with a fruity odor, the center of the colony become elevated, reddish brown pigment oozing as small droplets on masses of aerial hyphae, aerial hyphae are white to dark grey, undersurface dark green. 14 days old colonies are dark grey, 5.5 cm in diameter, with a stimulative odor, a reddish brown zone of diffusion present, simmilar pigment also oozing in the form of small droplets on the mycelium; mycelium white to dark grey, superficially wooly aerial hyphae, at places sporodochia appear.

Microscopic characteristics: Hyphae hyaline to reddish brown, repeatedly branched, becoming dendroid sometimes irregular form. Conidia brastosporic, globose to subglobose, subhyaline to reddish brown, thick-walled, contain many granules, brone singly or in monilioid chains, smooth-walled, measure 11–28 μ in diameter.

Optimum growth temperature: 23°C.

TABLE 4. Morphological and physiological characteristics of *Candida tenuis* isolated from the gallery of *S. shogun*.

Candida tenuis Diddens et Lodder

Growth in YM broth: After 3 days at 25°C, the cells are oval, long-oval to elongate, 1.2~7.0 μ×1.2~18.0 μ, single, in pairs or in short chains, ring and sediment.

Streak culture on YM agar: After 1 month at 25°C, cream-colored, punctate, dull, mucoid, raised, border filamentations.

Dalmau plate culture on corn-meal agar: Pseudomycelium well developed. No true mycelium. Blastospore present.

Fermentation: Glucose + Sucrose − Lactose −
Galactose + Maltose − Raffinose −

Assimilation of carbon compounds:

Glucose + Sucrose + Melibiose −
Galactose + Maltose + Raffinose −
L-Sorbose + Trehalose + Inositol −

Assimilation of KNO_3: Negative.

TABLE 5. Morphological and physiological characteristics of *Pichia* sp. 1. isolated from the homogenates of the larvae of *S. shogun*.

Pichia sp. 1.

Growth in YM broth: After 3 days at 25°C, the cells are globose, 1.2~5.0 μ, single, in pairs or in clusters. After 1 month no pellicle but ring and mucoid sediment.

Streak culture on YM agar: After 1 month at 25°C, cream-colored, smooth, shiny, mucoid, cross-section convex, bordes entire.

Dalmau plate culture on corn-meal agar: No pseudomycelium formation.

Sporulation: Spores formed easily on YM agar. Asci are globose, contain 2 to 4, usually 4 ascospores. Ascospores are hat-shaped, spheroidal or hemicycle, which have short brim.

Fermentation: Absent.

Assimilation of carbon compounds:

Glucose	+	Maltose	−	Lactose	−	
Galactose	−	Cellobiose	+	Melibiose	−	
Sucrose	−	Trehalose	+	L-Arabinose	+	(weak)

Assimilation of KNO_3: Negative.

TABLE 6. Morphological and physiological characteristics of *Pichia* sp. 2. isolated from the homogenates of the larvae of *S. shogun*.

Pichia sp. 2.

Growth in YM broth: After 3 days at 25°C, the budding yeast cells are spherical, oval and cylindrical, 1.2~8.0×1.2~12.5 μ, and occur singly or in pairs. A sediment and a ring are present. After one month at 25°C, a sediment and a ring are present.

Growth in YM agar: After 3 days at 25°C, the budding yeast cells are spherical to oval, cylindrical, 1.5~5.0×1.5~10.0 μ, and occur singly or in branched chains. After one month at room temperature the streak culture is cream-colored, dull, butyrous, smooth or slightly wrinkled, with a undulate partly filamentous margin.

Dalmau plate culture on corn-meal agar: A primitive pseudomycelium is abundantly formed. It consists of oval chain cells and of a tree-like appearance.

Formation of ascospore: Asci are oval to long-oval. The spores are hat-shaped, usually four are formed per ascus. They are easily liberated from the ascus. Sporulation is good in YM broth, on YM agar and cornmeal agar.

Fermentation:

Glucose	+	Sucrose	−	Lactose	−
Galactose	−	Maltose	−	Raffinose	−
Trehalose	−				

Assimilation of carbon compounds:

Glucose	+	Maltose	+	Melibiose	−	Rhamnose	+
Galactose	+	Cellobiose	−	Raffinose	−	Ethanol	+
L-Sorbose	+	Trehalose	−	Xylose	+(weak)	Mannitol	+
Sucrose	+(weak)	Lactose	−	L-Arabinose	−	Inositol	−

Assimilation of potassium nitrate: Negative.

TABLE 7. Morphological and physiological characteristics of *Pichia* sp. 3 isolated from the digestive tracts of the adult female of *S. shogun*.

Pichia sp. 3.

Growth in YM broth: After 3 days at 25°C, the cells are oval, cylindrical to elongate, 1.5~5.0 μ×2.0~16.0 μ, single, in pairs or in short chains; heavy ring, islets and sediment are present. After 1 month at 25°C, ring, islets and flaky sediment are present.

Streak culture on YM agar: After 1 month at 25°C, cream-colored, wrinkled, dull, butyrous, raised, border filamentous.

Dalmau plate culture on corn-meal agar: Pseudomycelium well developed and blastospore present.

Formation of ascospores: Ascospores are hat-shaped. Formation of ascospores is poor on YM agar.

Fermentation:

Glucose	+	Maltose	+	Raffinose	−
Galactose	+	Trehalose	+		
Sucrose	+	Lactose	−		

Assimilation of carbon compounds:

Glucose	+	Cellobiose	+	Raffinose	−
Galactose	+	Trehalose	+	Xylose	+
Sucrose	+	Lactose	−	Inositol	−
Maltose	+	Melibiose	−		

Assimilation of KNO_3: Negative.

TABLE 8. Morphological and physiological characteristics of *Endomycopsis* sp. isolated from the gallery of *S. shogun*.

Endomycopsis sp.

Growth in YM broth: After 3 days at 25°C, the cells are ovar, cylindrical, elongate, 1.2~6.0 μ×2.5~28.0 μ, single or in pairs; no ring and no pellice but flaky sediment. After 1 month indistinct ring, incomplete thin pellicle and flocculent sediment. Budding is on a broad base.

Streak culture on YM agar: After 1 month at 25°C, beige colored with reddish tinge, verrucose, dull, leathery, raised, border filamentous.

Dalmau plate culture on YM agar: Psudomycelium and true mycelium well developed.

Formation of ascospore: The asci are situated terminally or laterally on the hyphae, long-oval or elongate, contain 4 ascospores. Ascospores are hat shaped.

Fermentation: Absent.

and their galleries of *S. shogun* are shown in Table 2. Morphological and physiological characteristics of these isolates were investigated and identified as shown from Table 3 to Table 8. Morphological characteristics are also indicated from Fig. 3 to Fig. 10.

Ambrosiella sp., which was classified by BATRA[2] as the specific ambrosia fungus, was isolated from 19 individuals out of 27 beetles tested, and they were isolated purely without another microorganisms except two individuals. The scanning electron microscopy of *Ambrosiella* sp. from mycetangia and galleries is shown in Fig. 11 and Fig. 12.

In addition to the isolation of *Ambrosiella* sp., numerous strains of

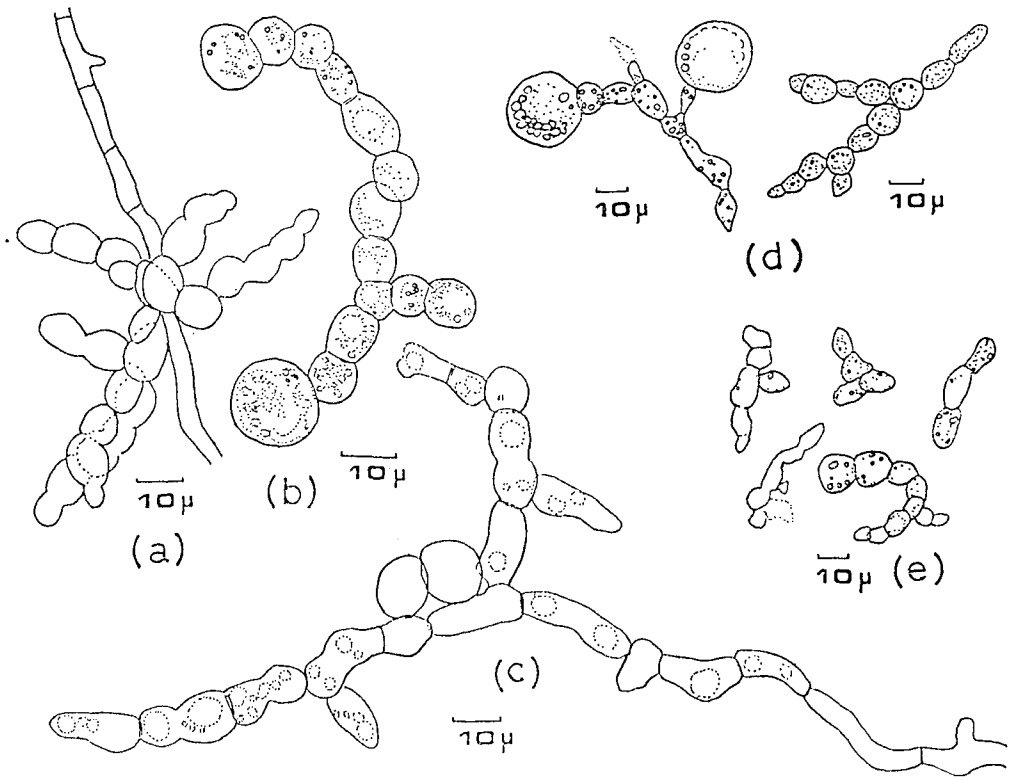

Fig. 3. *Ambrosiella* sp.

(a) After 2 days YM slant culture.
(b) After 5 days YM slant culture.
(c) After 3 days Corn meal slide culture.
(d) Chains of ambrosia cells and conidia around egg of *Scolytoplatypus shogun*.
(e) Chains of cells from mycetangia of *S. shogun*.

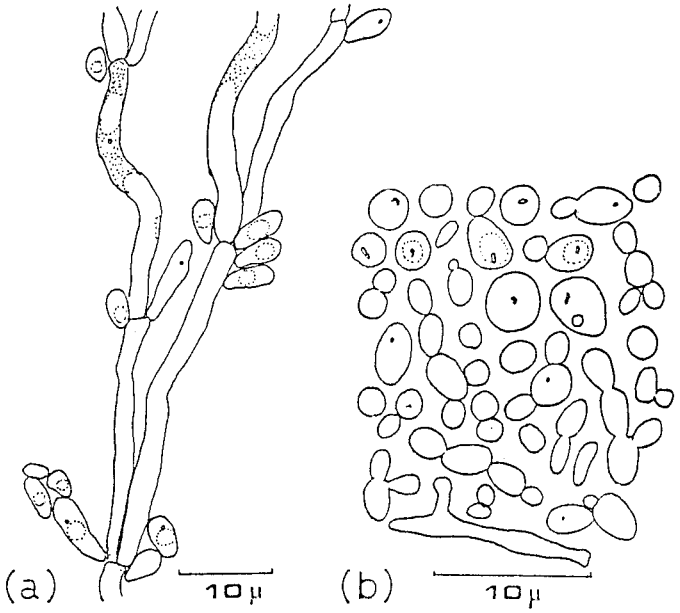

Fig. 4. *Candida tenuis.*
(a) After 6 days Dalmau Plate culture on corn meal agar.
(b) After 3 days in YM broth.

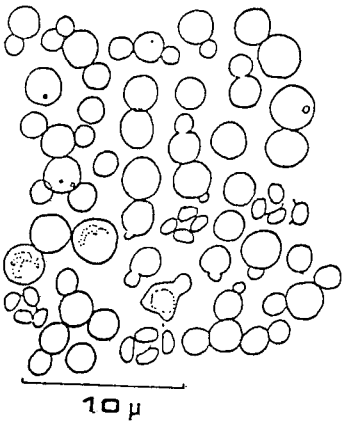

Fig. 5. *Pichia* sp. 1.
After 3 days in YM broth.

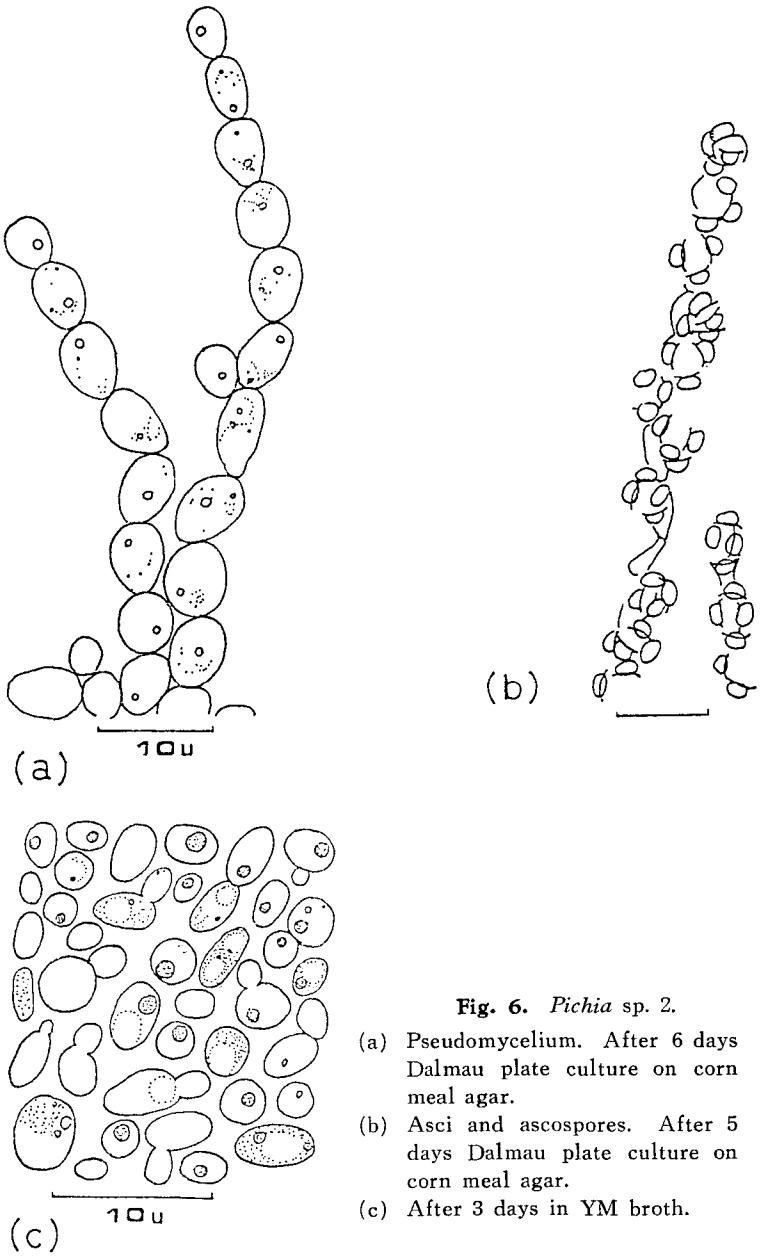

Fig. 6. *Pichia* sp. 2.

(a) Pseudomycelium. After 6 days Dalmau plate culture on corn meal agar.
(b) Asci and ascospores. After 5 days Dalmau plate culture on corn meal agar.
(c) After 3 days in YM broth.

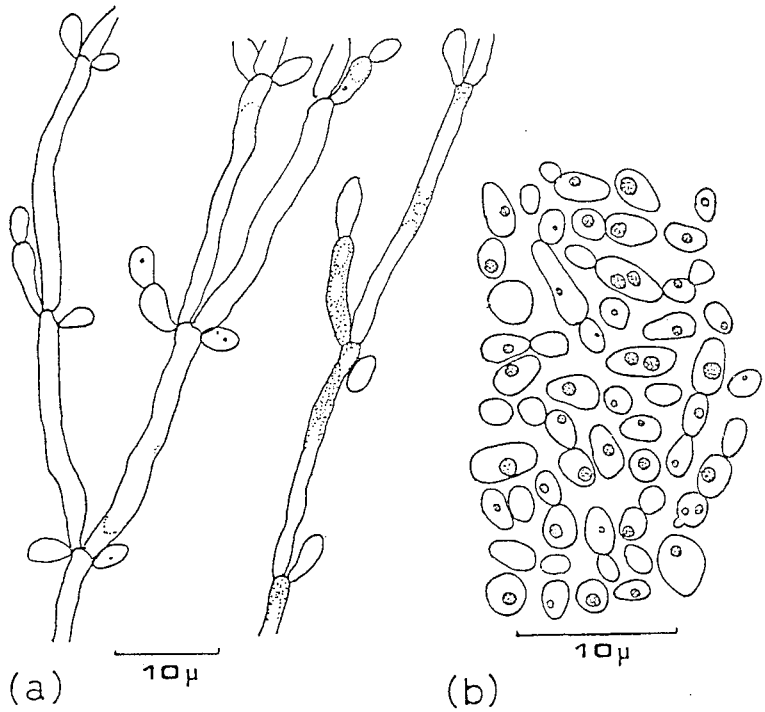

Fig. 7. *Pichia* sp. 3.
(a) After 5 days Dalmau plate culture on corn meal agar.
(b) After 3 days in YM broth.

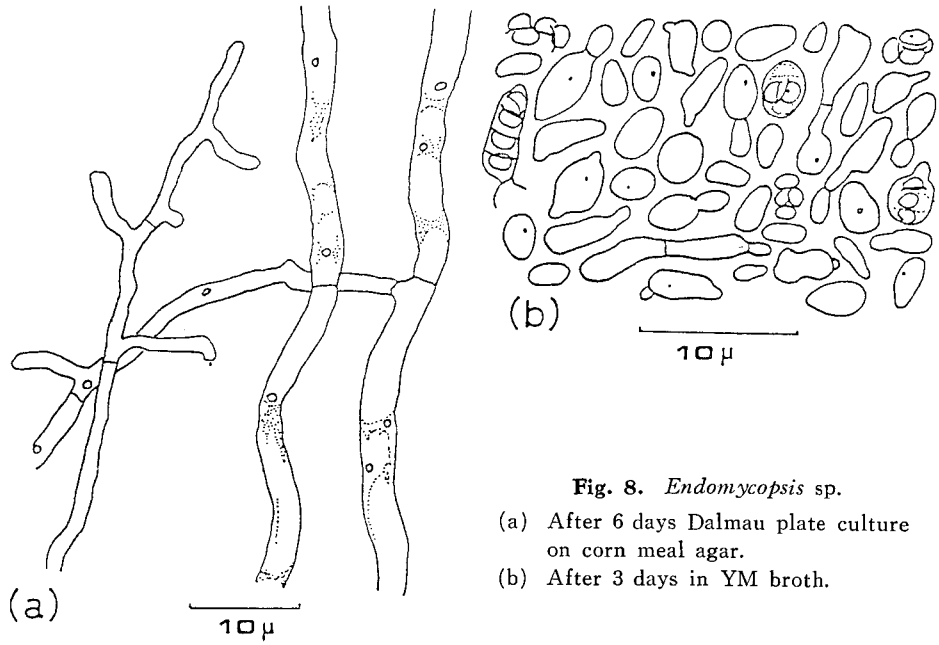

Fig. 8. *Endomycopsis* sp.
(a) After 6 days Dalmau plate culture on corn meal agar.
(b) After 3 days in YM broth.

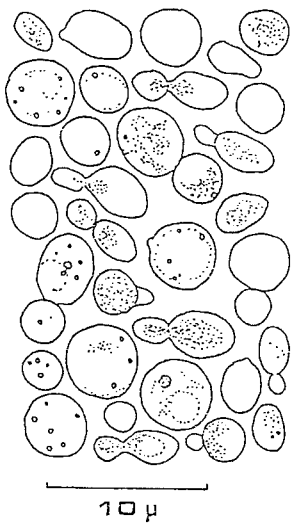

Fig. 9. Unknown species 1.
After 3 days in YM broth.

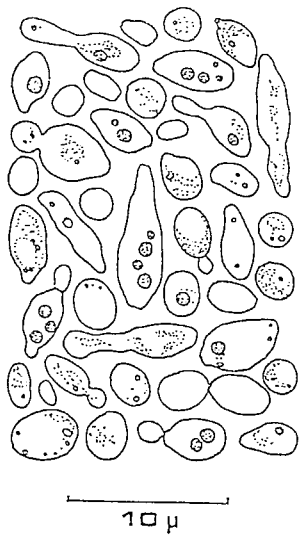

Fig. 10. Unknown species 2.
After 3 days in YM broth.

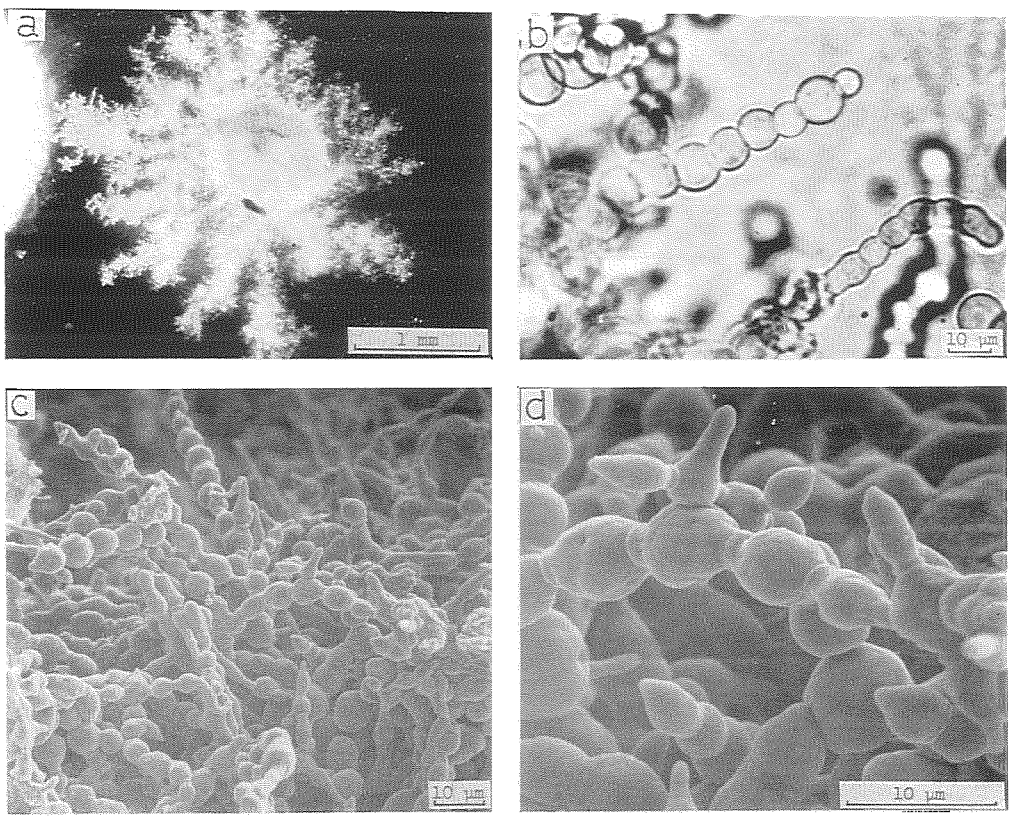

Fig. 11. *Ambrosiella* sp. isolated from the mycetangia of *S. shogun*.
a. *Ambrosiella* sp. growing from the mycetangia of *S. shogun* on an agar plate.
b. At higher magnification under the light microscope.
c. d. Like chains under a scanning electron microscope.

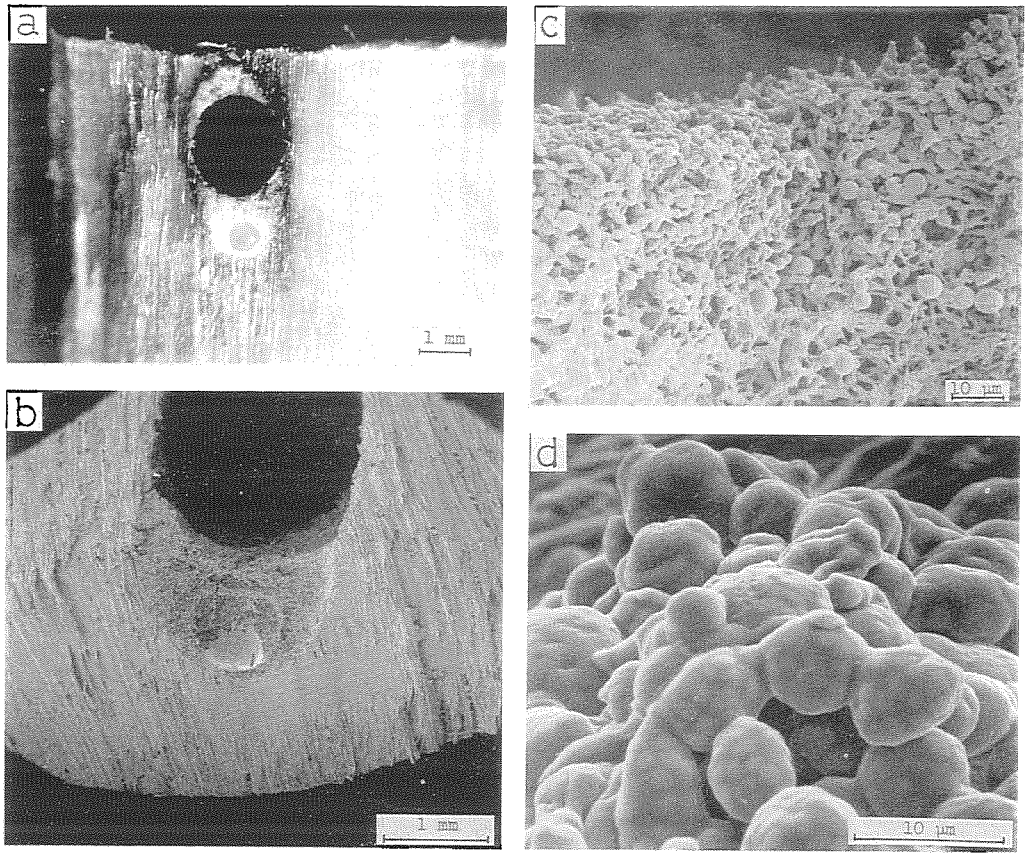

Fig. 12. *Ambrosiella* sp. growing in the galleries of *S. shogun*.

a. An egg of *S. shogun* in an egg cradle. The egg is enveloped in white fungus.

b. The egg cradle under a scanning electron microscope. (The egg had been removed.)

c. d. The white fungus under a scanning electron microscope. It is clear that this fungus is the same *Ambrosiella* sp. as shown in Fig. 11.

Candida tenuis, *Pichia* spp. and *Endomycopsis* sp. were mainly isolated from the galleries. From the two kinds of unknown species in Table 2, neither true nor pseudomycelium were observed, and ascospores were not observed either (Figs. 9, 10). Therefore, they were calssified as *Torulopsis* or *Cryptococcus*, but they were not identified, since assimilation of the inositol was not clarified.

The threshold of optimum temperature on the growth of the *Ambrosiella*

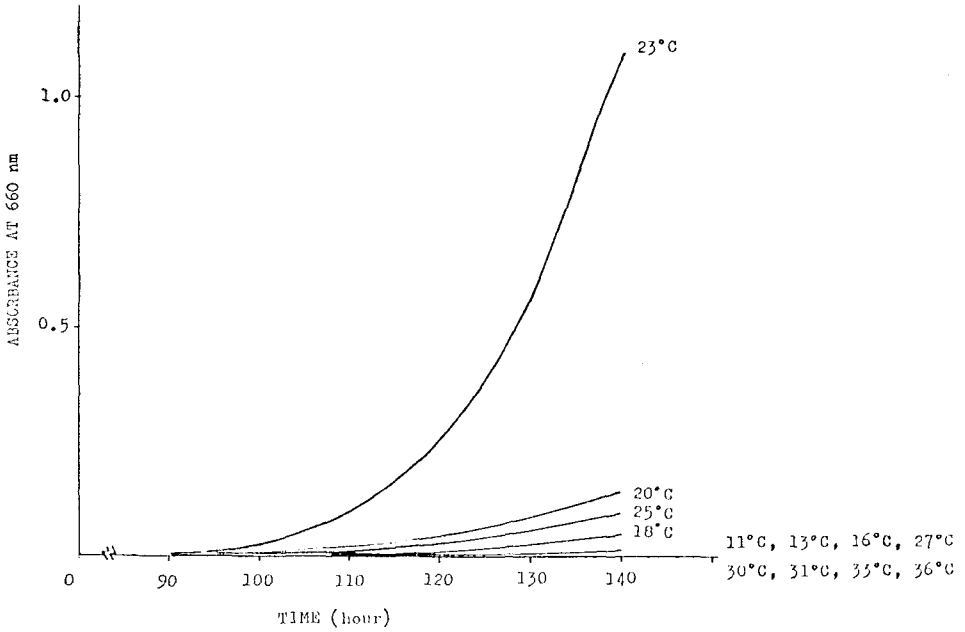

Fig. 13. Growth temperature of *Ambrosiella* sp. isolated from *Scolytoplatypus shogun*.

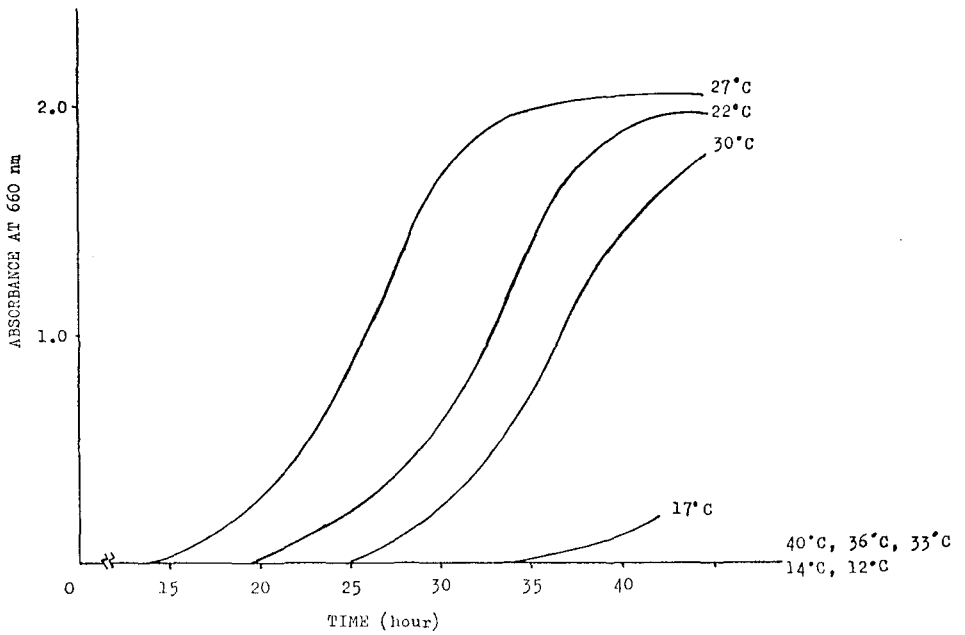

Fig. 14. Growth temperature of *Pichia* sp. 2.

sp. was almost 23°C, and showed extremely narrow range (Fig. 13). In the case of *Pichia* sp. 2, it was between 22°C and 30°C (Fig. 14).

2. Fungi associated with *Crossotarsus niponicus*.

The fungi isolated from the homogenates of the heads of adult beetles, homogenates of the larvae and their galleries of *C. niponicus* are shown in

TABLE 9. The fungi isolated from *Crossotarsus niponicus*.

	Adult female	Larvae	Walls of galleries
	Homogenates of heads	Homogenates	New adults were staying
Season	May-June	March	March
Number of tested	11	10	1
Number of strains isolated	12	8	10
Endomycopsis platypodis	++	+	+++
Endomycopsis sp.	−	+++	−
Torulopsis norvegica	+++	+	++
Torulopsis sp.	−	+	−
White fungus	+	−	−

Numbers of (+) indicate numbers of strains isolated.

TABLE 10. Morphological characteristics of *Endomycopsis platypodis* isolated from the homogenates of the heads of the female adults of *C. niponicus*.

Endomycopsis platypodis (Baker et Kreger-van Rij)

Synonym: *Hansenula platypodis* (Baker et Kreger-van Rij) Fiol 1967.

Growth in yeast extract-malt extract: After 3 days at 25°C, the cells are spherical and oval, 1.4~10.1 μ; single or in pair. Elongated budding cells and pseudomycelium are also present. Vegetative reproduction occurs by multilateral budding and by budding on broad base. A sediment and a ring are present. After one month at 25°C, a flocculent sediment and a ring are present.

Growth in yeast extract-malt extract agar: After one month at 25°C, the streak culture is cream-colored, convex butyrous, smooth dull; the middle part is slightly raised, punctate and cream-colored with a brown tinge; the edge is fringed with mycelium.

Dalmau plate culture on corn-meal agar: True mycelium and pseudomycelium bearing blastspores are abundantly produced. Single, distinct, centrally-located pore bodies of dolipore type present in the hyphal septa. Anastomoses may occur.

Formation of ascospore: The asci are situated terminally or laterally on the hyphae. They contain usually four, hat-shaped spores. Sporulation was good on corn-meal and YM agar.

Assimilation of potassium nitrate: Positive.

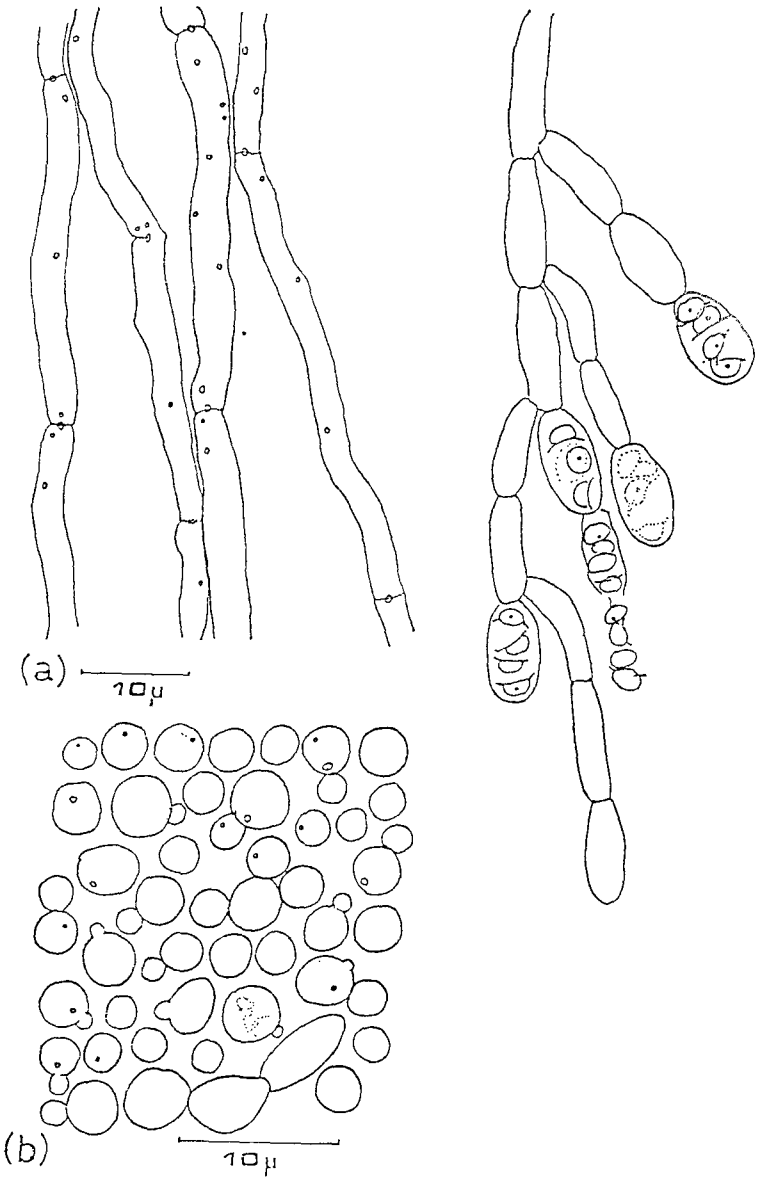

Fig. 15. *Endomycopsis platypodis.*

(a) Pseudomycelium, true mycelium, ascophoric hyphae and asci. After 4 days Dalmau plate culture on corn meal agar.
(b) After 3 days in YM broth.

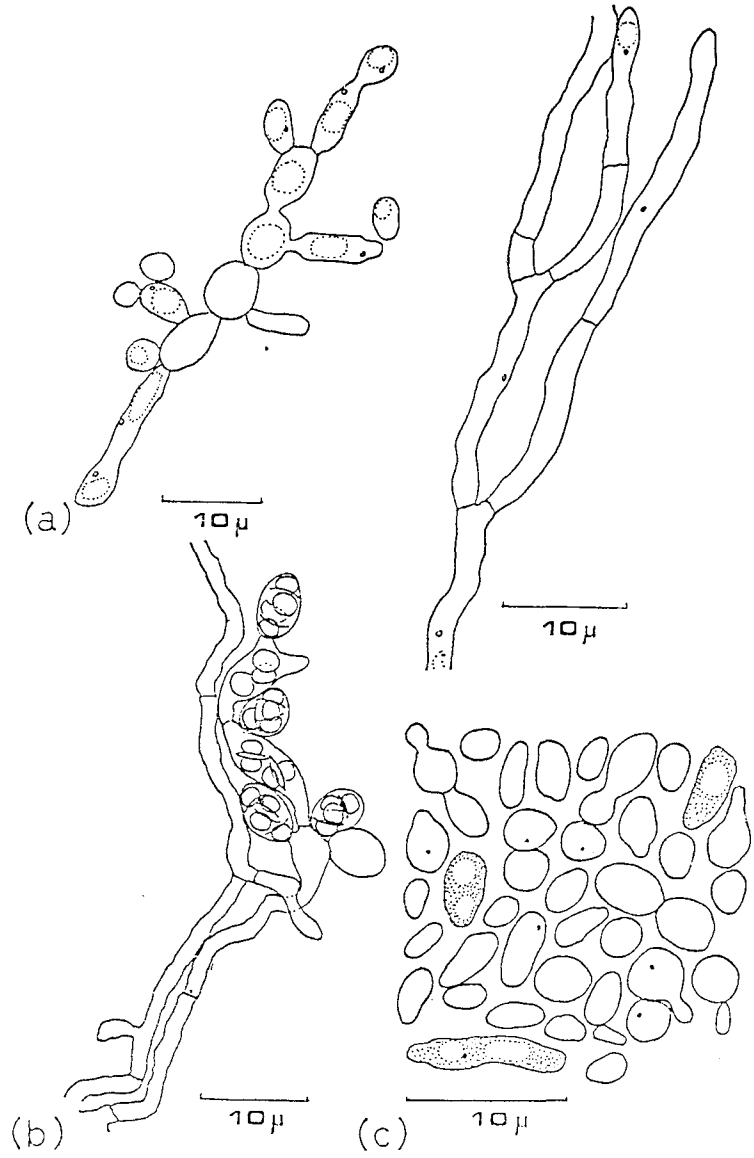

Fig. 16. *Endomycopsis* sp.

(a) Pseudomycelium and true mycelium. After 4 days Dalmau plate culture on corn meal agar.
(b) Ascopholic mycelium and asci. After 12 days Dalmau plate culture on corn meal agar.
(c) After 3 days in YM broth.

TABLE 11. Morphological and physiological characteristics of *Endomycopsis* sp. isolated from the homogenates of the larvae of *C. niponicus*.

Endomycopsis sp.

Growth in YM broth: After 3 days at 25°C, the cells are oval, lemonshape, bottle shape or elongate, single, in pairs or in chains. After 1 month at 25°C, no pellicle but a flocculent sediment are present. Budding is on a broad base.

Streak culture on YM agar: After 1 month at 25°C, cream-colored, partly reddish, wrinkled, dull, butyrous, raised, border erose.

Dalmau plate culture on corn-meal agar: True mycelium and pseudomycelium well developed.

Formation of ascospores: The asci are situated terminally or laterally on the hyphae, oval to long-oval, contain usually four hat shaped spores. Ascospores are easily libalated from asci.

Fermentation: Absent,

Assimilation of carbon compounds:

Glucose	+	Sucrose	−	Lactose	−
Galactose	−	Maltose	+	L-Arabinose	+ (weak)
L-Sorbose	+	Trehalose	−	Rhamnose	+

TABLE 12. Morphological and physiological characteristics of *Torulopsis norvegica* isolated from the homogenates of the heads of the female adults of *C. niponicus*.

Torulopsis norvegica Reiersöl

Growth in YM broth: After 3 days at 25°C, the cells are globose to short-oval, 1.2~4.6 μ, single or inpairs. After 1 month no pellicle but mucoid sediment.

Streak culture on YM agar: After 1 month at 25°C, cream-colored, smooth, shiny, mucoid, cross-section convex, border entire.

Dalmau plate culture on corn-meal agar: No pseudomycelium formation.

Fermentation: Absent.

Assimilation of carbon compounds:

Glucose	+	Trehalose	−	Rhamnose	−
Galactose	−	Lactose	−	Ethanol	+ (weak)
L-Sorbose	+	Melibiose	−	Mannitol	+
Sucrose	−	Raffinose	−	Inositol	−
Maltose	−	Xylose	+		
Cellobiose	+	L-Arabinose	−		

Assimilation of KNO_3: Positive.

TABLE 13. Morphological and physiological characteristics of *Torulopsis* sp. isolated from the homogenates of the larvae of *C. nipponicus*.

Torulopsis sp.

Growth in YM broth: After 3 days at 25°C, the cells are globose to oval, 1.5~6.5 μ, single or in pairs. After 1 month no pellicle, slightly ring formation but mucoid sediment.

Streak culture on YM agar: After 1 month at 25°C, light greyish yellow, smooth, mucoid, cross-section convex, border entire.

Dalmau plate culture on corn-meal agar: No pseudomycelium formation.

Formation of ascospores: No ascospore formation.

Assimilation of carbon compounds:

Glucose + Inositol −

Assimilation of KNO_3: Positive.

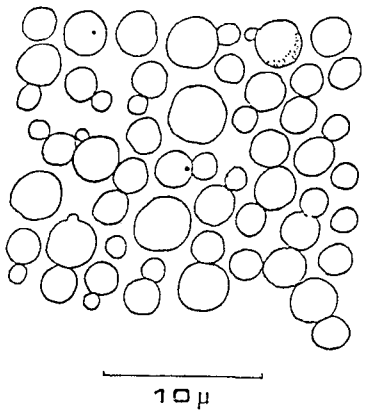

Fig. 17. *Torulopsis norvegica.* After 3 days in YM broth.

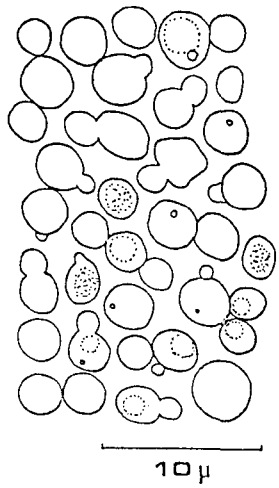

Fig. 18. *Torulopsis* sp. After 3 days in YM broth.

Table 9. Morphological and physiological characteristics of these isolates were investigated and identified as shown from Table 10 to Table 13. Morphological characteristics are also indicated in Figs. 15-18.

Almost all strains of isolates from both mycetangia and galleries of *C. niponicus* were either two species of yeasts, *Endomycopsis platypodis* or *Torulopsis norvegica*. No ambrosia-type fungus was isolated in this study.

The thresholds of optimum temperature on the growth of *E. platypodis* and *T. norvegica* were between 20°C and 30°C (Figs. 19, 20). The range of temperature shown for the growing of these yeasts was relatively wide.

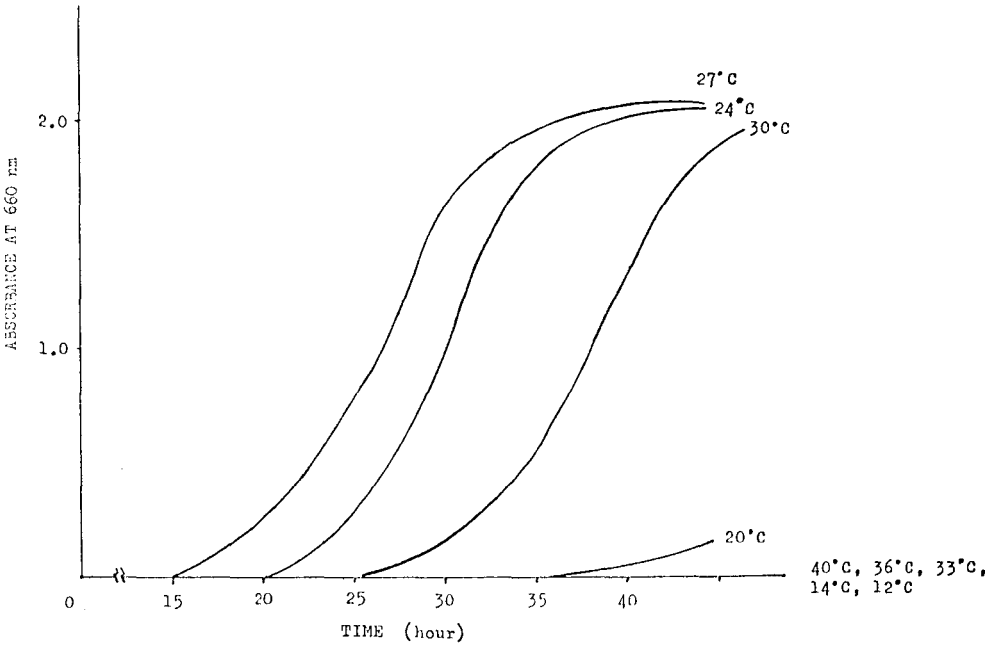

Fig. 19. Growth temperature of *Endomycopsis platypodis.*

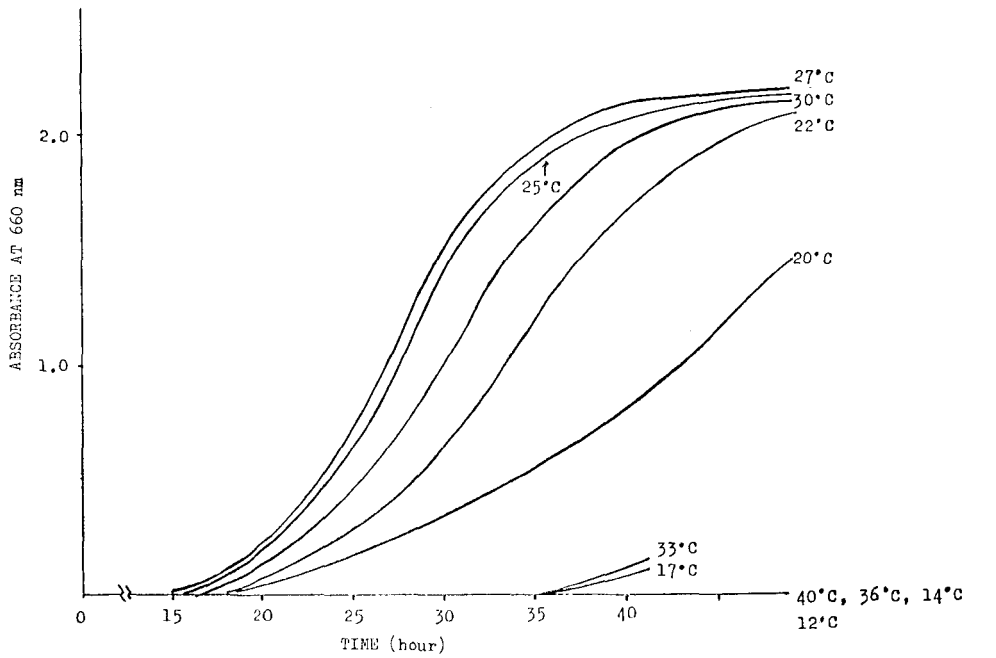

Fig. 20. Growth temperature of *Torulopsis noruvegica.*

Discussion

As shown in Table 2 and Table 9, the flora of microorganisms in the galleries of ambrosia beetles changes each season. During the periods when eggs and larvae are growing, the flora of fungi is relatively simple. Few species of ambrosia fungi appear to be predominant. These fungi seem to be deeply associated with ambrosia beetles. After pupation, however, many other species of fungi are found there.

BATRA[1], BATRA & BATRA[3,4] have suggested that while the young larvae of ambrosia beetles are usually associated with only one species of ambrosia fungi, adult beetles feed on more species of fungi. They have also suggested that the adult beetles contain their primary ambrosia fungus in their mycetangia predominantly even in the case of feeding on auxiliary or non-ambrosia fungi. They have also offered their opinion on primary and auxiliary ambrosia fungi, that is, each species of ambrosia beeltes is normally associated with only one species of fungus, namely the primary ambrosia fungus, and some primary ambrosia fungi of an ambrosia beetle play the role of auxiliary ambrosia fungi of another ambrosia beetles.

In the case of *S. shogun*, an *Ambrosiella* sp. which showed the shape of typical monilioid chains, was isolated from both mycetangia and galleries (Figs. 11, 12). Only this *Ambrosiella* sp. was isolated from the mycetangia of female adults at their flying period and from the walls of their galleries in the breeding period. It seems that this *Ambrosiella* sp. plays an important role in the life of *S. shogun* as a primary ambrosia fungus. This fungus has two forms, the ambrosial (=yeast) phase and the mycelial phase. In the galleries, monilioid chains were observed as shown in Fig. 12. BATRA & BATRA[3,4] have discovered that ambrosia fungi are pleomorphic. The fungi can easily change their form when their growth medium has been changed, and that ambrosia beetles can change the form of the fungi from a moldlike form to a yeastlike one, as in the case of some termites.

The threshold of optimum temperature for growing of this *Ambrosiella* sp. was near 23°C, and showed an extremely narrow range as shown in Fig. 13. It was anticipated that the *Ambrosiella* sp. would be isolated from the homogenates of the larvae. As shown in Table 2, however, no *Ambrosiella* sp. grew from the homogenates. In this examination, the growing temperature was 2°C higher than the optimum temperature. It may be the reason why no *Ambrosiella* sp. had grown from the homogenates.

The genus *Ambrosiella* was classified by BATRA[2] as a specific ambrosia fungus. He has reported that 11 species of the genus *Ambrosiella* were

isolated from 20 species of the beetles which belong to the genera *Anisandrus, Corthylus, Gnathotrichus, Monarthrum, Xyleborus, Xylosandrus, Trypodendron, Ips* and *Myelophilus* of Scolytidae and from 1 *Platypus* sp. of Platypodidae.

A large amount of *Pichia* sp. 1. and *Pichia* sp. 2. were isolated from the galleries in which larvae were growing, and from the homogenates of larvae. This suggests that the fungi, especially *Pichia* sp. 2., are eaten by the larvae. These fungi, however, were not isolated from the mycetangia. Therefore, these fungi may be included in the group of the auxiliary ambrosia fungi of *S. shogun.*

It seems that *Pichia* sp. 1. may be the same or very nearly related to *Pichia pini,* which was isolated from *Dendroctonus monticolae, D. brevicomis, D. ponderosae, Ips confusus* and *I. oregoni* by SHIFRINE & PHAFF[11].

Only in winter. *Candida tenuis* was isolated from the galleries as the dominant species. The role of this fungus is not clear. LODDER[5] reported that *Candida tenuis* was isolated from some beetles which belong to the genera *Harpium, Rhagium, Leptura* and *Mycroplophorus.*

In the case of *C. niponicus, Endomycopsis platypodis* and *Torulopsis norvegica* were regularly and dominantly isolated from the homogenates of the heads of female adults in their flying period, from the homogenates of larvae and from the walls of galleries. It seems that *E. platypodis* and *T. norvegica* are primary ambrosia fungi of *C. niponicus.* We have previously reported that *E. platypodis* is commonly isolated from homogenates of whole bodies of adult beetles and from galleries of *C. niponicus.*[10]

According to LODDER[5], *E. platypodis* was isolated from the beetles *Xyleborus aemulus* (from gallery), *X. xanthophus* (from gallery), *Platypus cylindrus* (from frass) and *Crossotarsus externedentatus* (from gallery).

Several strains of another fungus which belongs to the genus *Endomycopsis* were isolated from the homogenates of larvae. These fungi may be included in the group of auxiliary ambrosia fungi of *C. niponicus.*

Acknowledgements

We are greatful to all members of the Hiyama Forest Experiment Station of Hokkaido University for their aid in the collection of pinholed beech logs. We are also indebted to Dr. T. TANAKA, Laboratory of Applied Biology and Biotechnology, Suntory Limited for his technical cooperation in the measurement of the growing temperature for several isolates, and for his valuable advice.

This work was supported in part by a Grant-in-Aid for Special Project Research on Biological Aspects of Optimal Strategy and Social Structure

from the Japan Ministry of Education, Science and Culture.

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