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Ascobotryozyma americana gen. nov. et sp. nov. and its anamorph *Botryozyma americana*, an unusual yeast from the surface of nematodes

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Abstract

A new teleomorphic genus *Ascobotryozyma*, with a single species, *A. americana*, is proposed. Its anamorph is a *Botryozyma* that differs from the type species, *B. nematodophila*, on distributional, physiological, and molecular criteria; it is described as *Botryozyma americana*, anam. sp. nov. *Ascobotryozyma* is characterized by globose asci bearing four lunate ascospores. Fusion of thallus cells precedes ascus formation. *Ascobotryozyma americana* was isolated from the surface of nematodes (*Panagrellus dubius*) associated with galleries of the poplar borer (*Saperda calcarata*) in trembling aspen (*Populus tremuloides)* in eastern Washington, USA. The teleomorph has not been produced in pure culture.

Introduction

An unusual asexual yeast, *Botryozyma nematodophila* Shann & M. Th. Smith, was first described from nematodes associated with sour-rot of grapes in Verona, Italy (Smith et al. 1992). It was found on the nematode *Panagrellus zymosiphilus* (Brunhold) Brunhold vectored by *Drosophila* species (Smith et al. 1992). The yeast consists of unique thalli with determinate growth. The basal thallus cell is branched and acts as a holdfast, adhering the thallus to the nematode cuticle. A single cell buds from the apex of this cell, and from it multiple cells bud from each subsequent cell. *Botryozyma* was a monotypic genus until a second species was discovered on the surface of nematodes associated with beetle galleries in *Populus tremuloides* Michx. (trembling aspen) in eastern Washington, USA. by J.D. Rogers. The beetle involved is the poplar borer, *Saperda calcarata* Say (Cerambycidae). Wounds are created when the female cuts a slit in the bark in which she deposits one or two eggs, and galleries are carved out as the larvae feed on the sapwood and heartwood (Johnson & Lyon 1991). All isolates have been found on nematodes identified as *Panagrellus dubius* Sanwal (P. Stock, pers. comm.). This second species is similar in appearance to *B. nematodophila* but differs in distribution, nematode host, and physiological and molecular characteristics. Since little is known about this fungus, investigations have been directed towards elucidating its life cycle. More recent collections from beetle activity in *Populus* have yielded additional *Botryozyma* isolates and the ascigerous state, to be described herein.

Materials and methods

Strains examined in this study are listed in Table 1. The North American isolates were obtained from beetle wounds in *Populus tremuloides* in eastern Washington. Wood plugs were extracted using an increment borer from within and adjacent to the entrance sites. On several occasions trees with heavy infestations were felled and split, and wood and frass were collected from galleries. Samples were plated onto corn meal agar (CMA) (Difco) acidified with lactic acid, and incubated at room temperature (22–25 °C). Frass was collected and plated separately. Specimens

Table 1. Strains examined

Strain	Origin	mol% G+C±S.D.[a] Determined by first derivatives	
CBS 7426	nematodes associated with sour-rot grapes, 1986, Verona, Italy	31.1±0.4	(29.5)[b]
CBS 7442	nematodes associated with sour-rot grapes, 1988, Verona, Italy	30.8±0.5	(29.7)[b]
CBS 8461	nematodes associated with *Populus tremuloides*, 1992, Cheney, Washington, USA	29.6±0.2	
CBS 8462	nematodes associated with *Populus tremuloides*, 1992, Cheney, Washington, USA	27.6±0.4	
CBS 8463	nematodes associated with *Populus tremuloides*, 1996, Cheney, Washington, USA	29.6±0.3	
CBS 8560	nematodes associated with *Populus tremuloides*, 1998, Cheney, Washington, USA	29.8±0.5	
CBS 8751	nematodes associated with *Populus tremuloides*, 1998, Cheney, Washington, USA	nd	
CBS 8752	nematodes associated with *Populus tremuloides*, 1998, Cheney, Washington, USA	30.3±0.5	

CBS = Centraalbureau voor Schimmelcultures.
[a] = Standard Deviation, calculated from two determinations.
[b] = literature data (Smith et al. 1992).

were examined with a compound microscope for yeast colonies and yeast attached to nematodes. Nematodes bearing *Botryozyma* were serially transferred until cultures were free of contaminants except for organisms for the nematodes' nutrition. To obtain pure cultures, thalli which were not attached to nematodes were transferred to plates of potato dextrose agar (Difco) with 0.05% (w/v) yeast extract (PDYA).

Specimens were examined unstained using differential interference contrast (DIC) light microscopy and images were recorded onto Kodak Technical Pan film using an American Optical microscope equipped with a 35 mm photomicrographic camera. Detailed observations were made of thalli and asci via darkfield fluorescence microscopy, employing a Zeiss microscope. For observations of thallus or ascus walls material was mounted in 0.2% (w/v) aqueous Calcofluor White PMV (American Cyanamid) and examined through Zeiss exciter filter BG12 and barrier filters 47 and 65 in tandem. Nuclei were stained by mounting thalli or asci in Vectashield Mounting Medium with DAPI (Vector Laboratories) or staining with Hoechst 33258 dye (bisbenzimide) following the procedure of Mogford (1979). Material was examined via Zeiss exciter filter UG1 and barrier filter 41. Photographs were made with Kodak T-MAX 400 Professional film.

To compare thallus morphology, cells of two isolates from Italy, CBS 7426 and CBS 7442, and two isolates from North America, CBS 8560 and CBS 8752, were measured. Five individual subcultures were generated from each isolate by collecting cells from five different locations within the streak culture. Cells were grown on PDYA and maintained at room temperature for 5–10 days. From each subculture cells were removed from two different locations and each mounted separately in a drop of water on a glass microscope slide for examination. Five mature thalli were randomly selected from each location. A thallus was arbitrarily considered to be mature if at least five cells were present. The length and width of the basal cell and the next two apical cells, called the first and second cells, were measured from each thallus (Figure 4). These three cells were selected because they appear to comprise the minimal components of a thallus. They disarticulate together from a growing thallus and cannot be separated, and from this basic thallus subsequent budding and branching is initiated. Fisher's analysis of variance and a two-sample *t*-test were used to analyze statistical differences among isolates.

For scanning electron microscopy (SEM), *Ascobotryozyma* and nematodes were grown on acidified CMA for 7 days. Specimens were processed in a

Prep-Eze (Pelco) mesh tissue basket which allowed for chemicals to pass through while keeping samples together. Larger nematodes bearing thalli, typically adults and third- and fourth-stage juveniles, were collected and fixed in 3% glutaraldehyde buffered with 0.05 m potassium phosphate, pH 7.2 for 2 h at room temperature (Hussey & Mims 1990). Samples were rinsed three times in phosphate buffer, post-fixed in 1% osmium tetroxide and a similar buffer for 2 h at 4 °C, rinsed three times in distilled water, and dehydrated in an ethanol series. Specimens were critical point dried, affixed to mounts with adhesive tabs, coated with gold, and observed using a Hitachi S-570 scanning electron microscope.

The physiological characteristics of both *B. nematodophila* strains and six North American isolates were determined according to the methods of Yarrow (1998). Cultures were grown in tubes of liquid media for four weeks at 25 °C and shaken continuously at 30 rpm. Growth tests of each strain were performed at least three times. Utilization of nitrogen compounds were examined in duplicate after one week at 25 °C by the auxanographic method (Yarrow 1998). For mol% G+C calculations and DNA-DNA reassociations, strains were grown for 2 days at 25 °C on a rotary shaker at 125 rpm in 2 l YM broth (Wickerham 1951) using 1 l flat-bottom flasks. Isolation and purification of DNA and determination of DNA base composition were done according to the procedures cited before (Smith et al. 1995). DNA-DNA hybridization experiments were carried out according to the procedures described by Seidler & Mandel (1971) and modified by Kurtzman et al. (1980). The optimal reassociation temperature of 50 °C was determined following the methods of these authors. Reassociation experiments were performed at least twice.

Results

Botryozyma, the asexual state, was found on nematodes collected from beetle frass, entrance sites, and within galleries. The yeast can be grown in agar culture with or without nematodes. In pure culture it exhibits typical yeast-like growth. It can be maintained with nematodes provided there is a food source for the nematodes such as one or more yeasts other than *Botryozyma*. The fungus is able to attach to nematodes of any stage. Each thallus adheres to the cuticle by a branched basal cell that does not penetrate the cuticle (Figure 1a). It appears to have a commensal relationship, taking nutrition from the nematodes' environment rather than the nematode itself. Freshly-isolated nematodes typically bear small numbers of thalli which do not impede their activities. Observations of the two in culture together do not reveal any deleterious effects to the nematodes; nematodes are able to move to food sources and reproduce. Occasionally, a nematode becomes virtually immobilized by a heavy turf of thalli, but this phenomenon is probably a cultural artifact with little importance under natural conditions.

Six isolates were collected from beetle galleries in *Populus tremuloides* and the sexual state formed on three of these. Asci were typically not seen until seven or more days from the time samples were plated and were formed only on freshly-isolated nematodes. The teleomorph was never produced in pure agar cultures of the fungus and could not be maintained in nematode cultures. Globose asci developed on thallus cells (Figure 1b). Fusion between cells of two or more thalli was evident (Figure 1c), and all thallus cells except the basal cell appeared to be capable of fusing. Asci were borne from the side of a thallus cell (Figure 1c) or at or near the apex (Figures 1d and e). Cells often appeared irregular in shape with protuberances (Figures 1c and e) or angled extensions (Figures 1d and e). The apical region of empty asci appeared torn (Figures 1c and e), and indicated that ascospores were passively released. Four immature ascospores were visible in developing asci (Figures 1f and g). Ascospores are more or less lunate; the ascus in Figure 1h was crushed to release the ascospores for documentation.

Nuclear staining with DAPI and Hoechst 33258 stains appeared comparable. The thallus depicted in Figure 2a was first stained with Calcofluor, examined with appropriate filters (see Materials and methods), and photographed. The same thallus was then mounted in Vectashield-DAPI , examined with appropriate filters, and photographed (Figure 2b). Note that each thallus cell bears a single nucleus (Figure 2b). Cells are strictly uninucleate and divisions were noted only during new cell formation. The nucleus in the basal cell fluoresces dimly (Figure 2b) and mature basal cells become devoid of a nucleus owing to nuclear disintegration. Nuclei appeared slightly larger and brighter in cells giving rise to daughter cells (Figure 2b). Newly-formed asci contained a single, presumably diploid, nucleus (Figure 3a). The two divisions of putative meiosis apparently occurred rapidly; the four-nucleate state was seen much more frequently than the binucleate state (Figures 3b and

Table 2. Phenotypic characteristics of *Botryozyma* strains*

CBS	7426	7442	8461	8462	8463	8560	8751	8752
Fermentation: Gas formation not detectable								
Growth on								
D-Glucose	+	+	+	+	w	+	w	w
D-Galactose	–	–	–	–	–	–	–	–
L-Sorbose	+	+	w,–	v	–	–	–	v
D-Glucosamine	–	–	–	–	–	–	–	–
D-Ribose	–	–	–	–	–	–	–	–
D-Xylose	–	–	–	–	–	–	–	–
L-Arabinose	–	–	–	–	–	–	–	–
D-Arabinose	–	–	–	–	–	–	–	–
L-Rhamnose	–	–	–	–	–	–	–	–
Sucrose	–	–	–	–	–	–	–	–
Maltose	–	–	–	–	–	–	–	–
α,α-Trehalose	+	+	+,w	+	w	+,w	v	w,–
Me- α-D-glucoside	–	–	–	–	–	–	–	–
Cellobiose	–	–	–	–	–	–	–	–
Salicin	–	–	–	–	–	–	–	–
Arbutin	–	–	–	–	–	–	–	–
Melibiose	–	–	–	–	–	–	–	–
Lactose	–	–	–	–	–	–	–	–
Raffinose	–	–	–	–	–	–	–	–
Melezitose	–	–	–	–	–	–	–	–
Inulin	–	–	–	–	–	–	–	–
Soluble starch	–	–	–	–	–	–	–	–
Glycerol	+	+	+	+	+	+	+	+
Erythritol	–	–	–	–	–	–	–	–
Ribitol	–	–	–	–	–	–	–	–
Xylitol	–	–	–	–	–	–	–	–
L-Arabinitol	–	–	–	–	–	–	–	–
D-Glucitol	+	+	+	+	+,w	+	–,w	v
D-Mannitol	+	+	v	+,w	w	w	–,w	v
Galactitol	–	–	–	–	–	–	–	–
Myo-Inositol	–	–	–	–	–	–	–	–
D-Glucono-ä-lactone	–	–	–	–	–	–	–	–
2-Keto-D-gluconate	+	+	+	+	+	v	v	v
D-Gluconate	–	–	–	–	–	–	–	–
D-Glucoronate	–	–	–	–	–	–	–	–
D-Galacturonate	–	–	–	–	–	–	–	–
DL-Lactate	+	+	+	w,+	w,+	w,+	w,–	+,w
Succinate	+	+	+	+	+	+	+	+
Citrate	–	–	–	–	–	–	–	–
Methanol	–	–	–	–	–	–	–	–
Ethanol	+	+	v	v	+,w	+	v	+
Propane 1,2 diol	–	–	–	–	–	–	–	–
Butane 2,3 diol	–	–	–	–	–	–	–	–
Quinic acid	–	–	–	–	–	–	–	–
Saccharate	–	–	–	–	–	–	–	–
Galactonic acid	–	–	–	–	–	–	–	–
Nitrate	–	–	–	–	–	–	–	–

Table 2. Continued

CBS	7426	7442	8461	8462	8463	8560	8751	8752
Nitrite	–	–	–	–	–	–	–	–
Ethylamine	+	+	+	+	w	+	+	+
L-Lysine	+	+	w	+	+	+	w	w
Cadaverine	–	–	–	–	–	–	–	–
Creatine	–	–	–	–	–	–	–	–
Creatinine	–	–	–	–	–	–	–	–
Glucosamine	–	–	–	–	–	–	–	–
Imidazol	–	–	–	–	–	–	–	–
Growth w/o Vit	–	–	–	–	–	–	–	–
0.1% Cycloheximide	+	+	+	+	+	w	w	w
50% Glucose	–	–	–	–	–	–	–	–
Growth at								
25 °C	+	+	+	+	+	+	+	+
30 °C	+	+	+	+	+	+	+	+
35 °C	+	+	w	w	w	w	+	+
37 °C	+	+	–	–	–	–	–	–
40 °C	–	–	–	–	–	–	–	–

w = weak.
v = variable (Growth tests were run on each strain at least three times; utilization of nitrogen compounds was examined twice. Results that were inconsistent among tests are marked 'variable' (v).)
* Strains CBS 7426 and CBS 7442 are *Botryozyma nematodophila*. Remaining strains are *Ascobotryozyma americana* ≡ *Botryozyma americana*.

c). An ascospore was formed around each nucleus. Maturing ascospores were at first elongated and apparently tangled (Figure 1f), eventually becoming lunate and clearly separate (Figures 1g and h). Ascospores are apparently uninucleate, but too few were examined cytologically to be certain.

Thallus cells of the new species, described below as *A. americana*, were significantly shorter ($P < 0.001$) than those of *B. nematodophila* (Figure 4). The greatest difference was between the lengths of the second cell apical to the basal cell, the North American isolates ranging from 13.9–16.6 μm and *B. nematodophila* from 15.4–18.6 μm. Mean lengths of the basal cells ranged from 6.8–9.5 μm in the North American isolates and 9.0–10.3 μm in *B. nematodophila*. The first cell apical to the basal cell was most similar between the North American isolates and *B. nematodophila*, ranging, respectively, from 12.0–14.5 μm and 12.8–15.9 μm. These increments were generated through statistical analysis; sizes could not be measured at a precision greater than 0.5 μm.

Physiological capacities of the *B. nematodophila* strains and the six North American isolates are rather limited. Visible CO_2 production from glucose was not observed, and only 10 out of 46 tested carbon compounds and 2 out of 9 tested nitrogen compounds could be utilized (Table 2). One consistent difference between *B. nematodophila* and the North American isolates was the ability of the former to grow at 37 °C. The mol% G+C of all strains examined are presented in Table 1. The average values, as calculated in this study, are 31.0±0.2 mol% G+C for *B. nematodophila* and 29.4±0.9 mol% G+C for the North American isolates.

To settle the taxonomic status of the North American isolates and *B. nematodophila*, DNA-DNA genome comparisons were performed. DNA reassociation values of 100% were observed among representatives of the North American isolates and between both *B. nematodophila* strains (Table 3). Values ranging from 39 to 54% were observed between the North American strains and *B. nematodophila*.

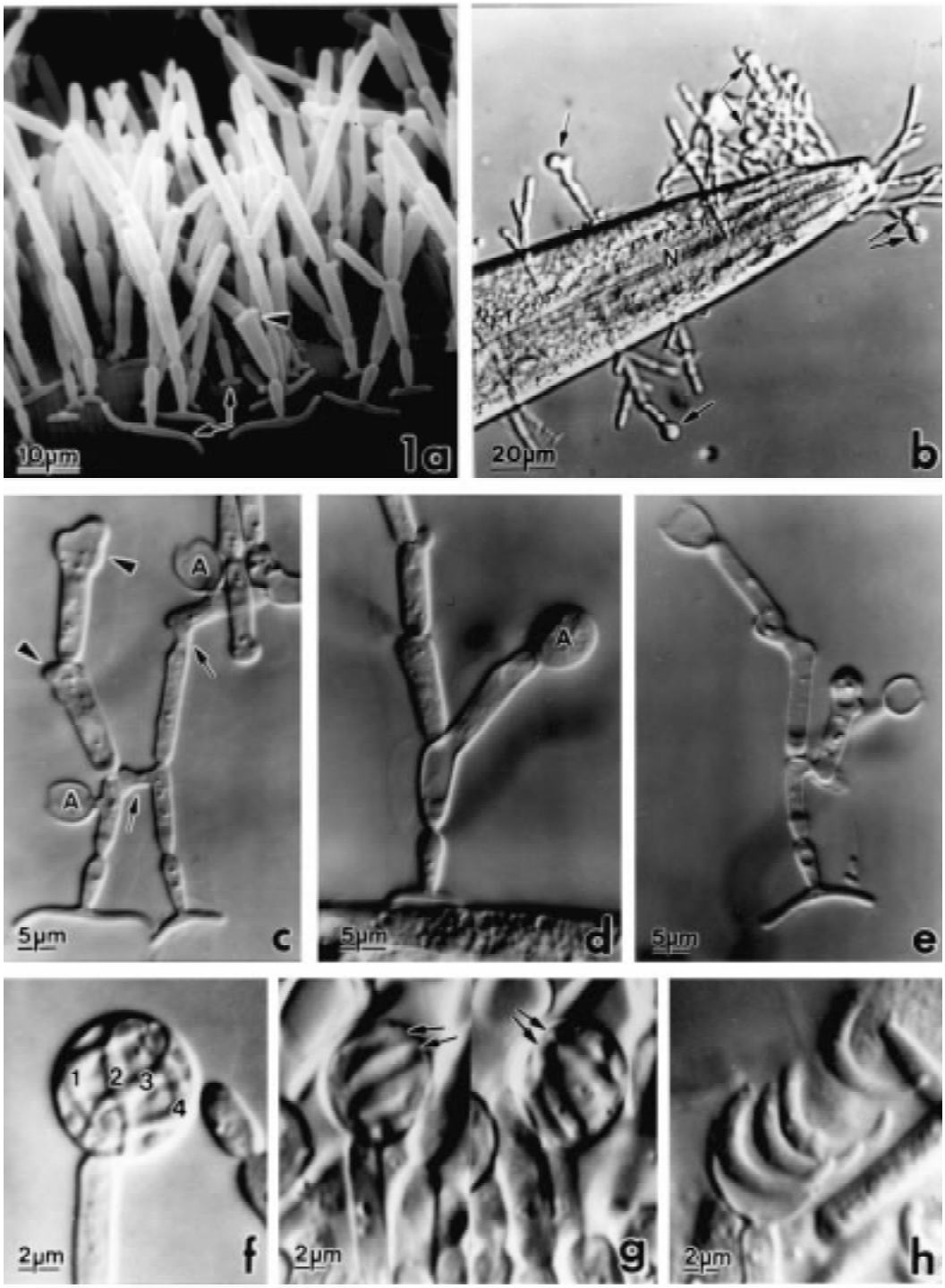
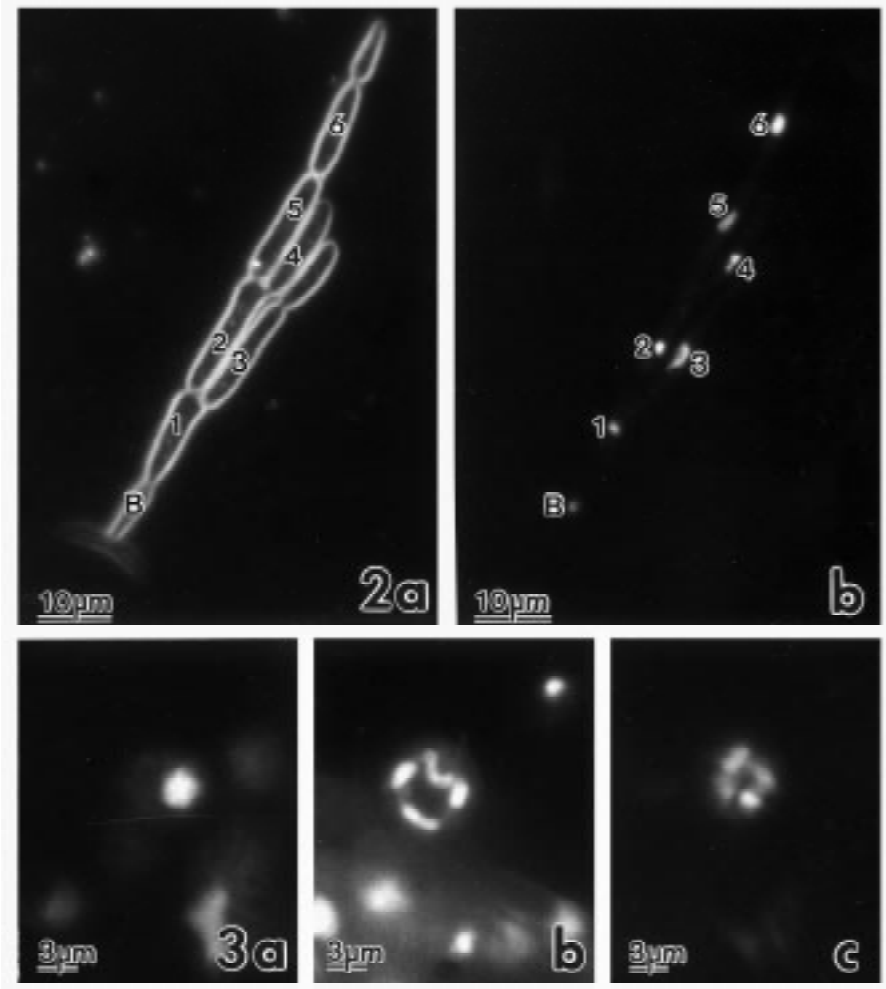


Figure 1. Ascobotryozyma americana. (a) SEM of a nematode cuticle covered with thalli which are attached by basal cells of various lengths (arrows). Arrowhead indicates a cell with an enlarged apical region which frequently arose due to multiple bud sites. (b) Nematode with thalli bearing globose asci (arrows). (c) Fused thalli with regions of fusion indicated by arrows. Note the dehisced asci (A) with torn apical regions. Irregular protuberances are also present (arrowheads). (d) Thallus attached to a nematode, forming an ascus (A) on a cell apex. (e) Thallus showing the typically irregular appearance after fusion. (f) Young ascus with four developing ascospores. (g) Two different foci of an ascus with four ascospores. Note ascospores (arrows) oriented in opposing directions. (h) Four lunate ascospores released from a crushed ascus.



Figures 2–3. Fluorescence micrographs. (2) (a) *Botryozyma* thallus mounted in Calcofluor. Cells are labeled to correlate with nuclei in the adjacent micrograph. (b) Same thallus mounted in DAPI. Note the nucleus in the basal (B) cell is fainter than the other nuclei, and no nuclei are present in the branched basal cell. Also note the nuclei giving rise to daughter cells (3 and 6) are slightly larger and brighter. (3) Asci with nuclear material stained with Hoechst 33258. (a) Young ascus with a single nucleus. (b) Ascus with four elongated nuclei. (c) Ascus with four nuclei.

Discussion

The discovery of the teleomorphic state confirms the ascomycetous affinity of *Botryozyma* as described by Smith et al. (1992). Because of its peculiar morphology it could not be assigned to a preexisting genus and it may eventually require a family of its own. *Botryozyma nematodophila* was separated into its own clade in a study examining the molecular phylogenetic relatedness of ascomycetous yeasts and yeast-like taxa based on the 5′ end of nuclear large subunit rDNA (Kurtzman & Robnett 1995). In a subsequent study including approximately 500 species of ascomycetous yeasts, *B. nematodophila* paired in a clade with *Candida caseinolytica* (Kurtzman & Robnett 1998); however, the bootstrap value was low and additional studies are needed to determine the phylogenetic relatedness of these species.

The delayed period between the time of plating and observation of the teleomorph (*A. americana*) is probably due to culture dynamics. Nematodes with thalli were found within 2–4 days after being plated, but typically few thalli on a small percentage of nematodes were present. Increases in the yeast population coincided with increases in nematode populations. Nematodes tend to aggregate in clusters and rub against each other as they move, thereby promoting dispersal. Asci were usually found when nematode and yeast

Table 3. DNA reassociations among strains of *Botryozyma* and *Ascobotryozyma*

DNA relatedness (%)*							
B. nematodophila			*A. americana*				
CBS	7426T	7442	8461	8462	8463	8560T	8752
7426T	–						
7442	98	–					
8461	42	45	–				
8462	48	51	100	–			
8463	51	54	100	100	–		
8560T	46	49	100	100		–	
8752	39	45	100	nd	nd	nd	–

* = Standard Deviation ≤ 5, calculated from three determinations.
T = Type strain.
nd= not determined.

populations were relatively high, probably because the greater number of cells increases the possibility of fusion among thalli.

Morphological variation in comparisons between asexual thalli and thalli bearing asci was evident. Asexual thalli not undergoing fusion or bearing asci were similar in appearance and cells were typically cylindrical. One exception occurred when many cells budded from one apex, causing the apex to be the widest part of the cell due to the numerous bud sites (Figure 1a). Morphological changes can occur in asexual thalli, however, when they are maintained in pure culture over a long period of time. Fusion between cells created irregular structures such as side branches, elongate fusion regions, and protuberances (Figures 1c and e). Thalli which did not have this unusual morphology were also present among fusing cells. In some isolates these irregular cell shapes continued to develop after the sexual state ceased forming. Preliminary attempts to make isolates reinstate or continue the formation of asci, such as by making crosses and changing media, were not successful.

As with many ascomycetous yeasts, asci are evanescent with passively discharged ascospores. Ascospores are released when the apical region of the ascus deliquesces (Figures 1c and e); therefore spores become mixed among thallus cells and are difficult to collect for germination studies. About 20 ascospores were isolated but germination was not observed.

The North American isolates are described as the new species *B. americana* based on distribution and physiological and molecular comparisons. Strains of *B. americana* differ from *B. nematodophila* in their inability to grow at 37 °C (Table 2). The average DNA reassociation value among *B. nematodophila* and the novel taxon is 47.0% with a Standard Deviation of 5.0%, a value considered low enough to distinguish species within a genus. Both species are similar in overall morphology but thalli of *A. americana* appear more stout and cells are significantly shorter (Figure 4). Although cell size is not a standard character used to differentiate yeast species, these data serve as one more difference in support of the designation of a new species.

The basal cell functions as a means of attachment to the nematode. The degenerated nucleus in the basal cell (Figure 2b) supports observations that the cell does not have an active role, such as penetrating the cuticle and obtaining nutrients. It is the branched structures of the basal cell which attach to the cuticle, providing a holdfast as the nematode moves throughout its environment. In the absence of nematodes, branches grow outward at an angle, as in Figures 1c and e. Basal cell branches varied in size and morphology (Figure 1a). Typically two branches of equal length were present, but occasionally one, three, or more branches of unequal length were present. The average length of two, equal-sized branches was from approximately 8–20 μm, but ranged from 3 μm up to 50 μm. Branched structures were more variable on isolates maintained in pure culture compared to those maintained with nematodes. In a few strains the branched structures decreased in size or were not produced at all as cultures were maintained without nematodes.

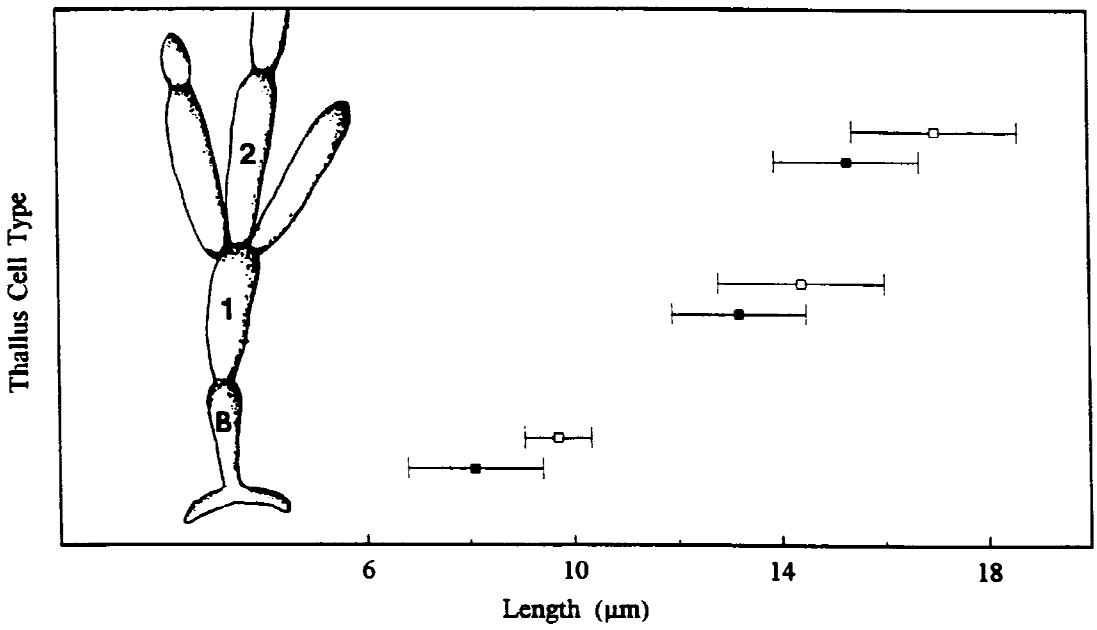


Figure 4. Comparison of thallus cell sizes between *B. americana* and *B. nematodophila*. Squares indicate the means and bars indicate standard deviations. ■ = *B. americana* □ = *B. nematodophila*.

Both *B. nematodophila* and *B. americana* were isolated from species of *Panagrellus. Panagrellus* are free-living nematodes originally known as the sour paste nematode. Species have been collected from a variety of habitats, including insect frass, slime flux of trees, pitchers of pitcher plants, beer mats, and spoiled cider (Hechler 1971).

Additional isolates have been collected from similar beetle wounds in *Populus* species in other locations, and from *Cryptorhynchus lapathi* (L.) wounds in *Salix* species (willow). These isolates display some morphological variation from *A. americana* and might represent a second new species. This topic is under investigation.

Taxonomy

Ascobotryozyma J. Kerrigan, M. Th. Smith, & J. D. Rogers gen. nov. Etym.: *Ascobotryozyma* for the ascomycetous state of *Botryozyma*. (Figures 1–3)

Asci e thallo *Botryozyma* orti. Asci globosi vel ellipsoidei evanescentes. Ascosporae hyalinae, lunatae, leves.

Asci originating from thallus of *Botryozyma*. Asci globose or broadly ellipsoid, evanescent. Ascospores hyaline, lunate, smooth.

Type species: **Ascobotryozyma americana** J. Kerrigan, M. Th. Smith, & J. D. Rogers sp. nov.

Ascobotryozyma americana J. Kerrigan, M. Th. Smith, & J. D. Rogers sp. nov. (Figures 1–3) Etym.: *americana* referring to the North American locality of the species.

Thalli ad paginam nematodorum ab cellulis basalibus affixi. Cellulae basales obovoideae, corporibus 5.0–13.0 × 2.0–4.0 μm, basibus furcatis. Cellulae ceterae subcylindricae vel subovoideae, 8.0–20.0 × 2.0–4.5 μm, ad apicem pullulantes. Asci globosi vel late ellipsoidei, tetraspori, 7.0–9.0 × 6.0–8.0 μm, uni vel duo e lateribus et apicibus cellularum pullulantes. Ascosporae hyalinae, lunatae, leves, 7.0–8.5 × 1.5–2.0 μm. Status anamorphosis *Botryozyma americana*.

Thalli attached to the nematode by basal cells. Basal cells obovoid with the body 5.0–13.0 × 2.0–4.0 μm, with bases branched. Other cells subcylindrical to subovoid, 8.0–20.0 × 2.0–4.5 μm, budding at the apex. Asci globose to broadly ellipsoid, four-spored, 7.0–9.0 × 6.0–8.0 μm, one or two budding from cell sides and apices. Ascospores hyaline, lunate, smooth, 7.0–8.5 × 1.5–2.0 μm. Anamorphic state *Botryozyma americana*.

Type strain: USA: Washington, Spokane Co., Rock Lake Road, 7 February 1998, Kerrigan, J. L., on *Panagrellus dubius* Sanwal from *Populus tremuloides*

Michx., *Saperda calcarata* Say galleries. Microscope slide of ascigerous state from above-cited location and date (HOLOTYPE, WSP 70152) (ISOTYPES, CBS 8560, WSP 70153, 70154).

Botryozyma americana J. Kerrigan, M. Th. Smith & J. D. Rogers, anamorph sp. nov. (Figures 1a and b, 2a and b)

A *Botryozyma nematodophila* differt in habitatione America boreali, in temperatura maxima minus quam 37 °C, et in structura DNA.

From *Botryozyma nematodophila* differs in habitation in North America, in its maximum temperature less than 37 °C, and in the structure of its DNA.

Type strain: Holotypes and isotypes same as teleomorph.

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