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Botryozyma nematodophila gen. nov., spec. nov. (Candidaceae)

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Abstract

The new genus *Botryozyma* with a single species, *B. nematodophila* is proposed for two isolates from nematodes (*Panagrellus zymosiphilus*) occurring in grapes with sour-rot. The new genus has typical ascomycetous characteristics and, being unable to produce ascospores, is placed in the family Candidaceae.

Introduction

Sour-rot is a serious disease of grapes which frequently occurs in the north and central region of Italy during periods of prolonged rain (Bisiach et al. 1981) and causes large losses of harvest. Dense bunches close to harvesting are mainly affected. The final stage of the disease is characterized by disintegration of the bunch and by a sour odour caused by the presence of acetic acid.

Various authors have analyzed the etiology and/or the chemical disease markers associated with sour-rot of grapes (Bisiach et al. 1986; Farris et al. 1988; Guerzoni & Marchetti 1982, 1987; Marchetti et al. 1984). Responsable agents are mainly yeasts, sometimes associated with acetic acid bacteria (Bisiach et al. 1986). Various *Drosophila* species can also be found around and on the affected grapes. These insects are considered the most important vectors of the sour-rot agents (Cantoni 1984).

Shann (1987a) reported that in a vineyard in Verona (Italy) affected by sour-rot disease, *Drosophila* species transported not only the usual yeast flora but also nematodes identified as *Panagrellus zymosiphilus* (Brunhold) Brunhold. The presence of a yeast-like fungus was observed on these nematodes (Shann 1987b). According Shann's description, this yeast-like fungus, designated S7, was able to reproduce asexually by blastoconidia. In addition, pseudohyphae were produced, which were attached to the cuticle of the nematodes by 'appressoria'. Sexually, the yeast-like fungus was said to produce "arthroasci'. However, careful examination revealed that the original S7 culture was a mixture of two taxa, one representing the species *Arthroascus schoenii* (Smith et al. 1990) and an unknown taxon characterized by the production of branch-like structures.

In the present communication the taxonomic position of the latter taxon is discussed and it is described.

Materials and methods

Two strains held by the Yeast Division of the Cen-

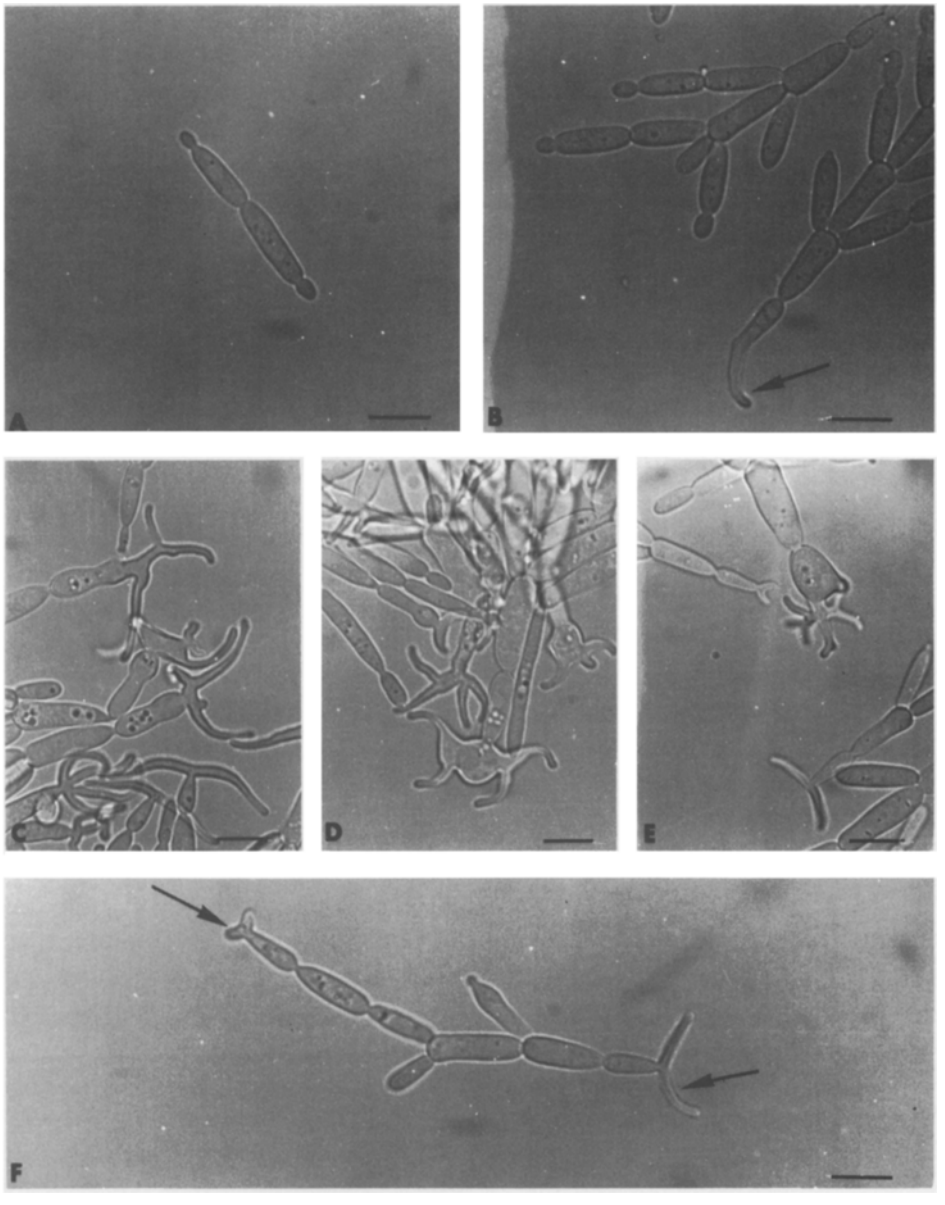

Fig. 1. Botryozyma nematodophila CBS 7426. (A). Budding yeast cells in YPG. (B). Pseudohyphal terminal cells with a simple branch-like structure (arrow). (C–E). Pseudohyphal terminal cells with complex branch-like structures. (F). Pseudohyphal terminal cells showing two, symmetrical branch-like structures (arrows). (Bar represents 5 μm).

traalbureau voor Schimmelcultures were examined:

- CBS 7426, from sour rot of grapes, Pescantina (Verona, Italy), C. Shann, 1986.
- CBS 7442, from sour rot of grapes, Pedemonte (Verona, Italy), C. Shann and R. Schmitt, 1988.

The morphological and physiological characters were determined by the conventional techniques applied in yeast classification (Van der Walt & Yarrow 1984). For growth tests on carbon compounds, cultures were incubated on a shaker at 28 cycles/min for 28 days at 25° C. Growth tests were done in triplicate. Growth on nitrogen sources was tested on agar plates for 7 days at 25° C.

The extraction and purification of the nDNA and the determination of the base composition were performed as described by Golubev et al. (1989). DNA-DNA hybridization experiments were carried out spectrophotometrically using the procedures described by Seidler & Mandel (1971) as modified by Kurtzman et al. (1980).

For ultra-thin sectioning, material was prepared according to methods previously described (Smith & Batenburg-van der Vegte, 1986). Freeze-fracturing was done in a Balzers freeze-etching unit at − 150° C according to Müller et al. (1980). For scanning electron microscopy (SEM) material was fixed in 2% glutaraldehyde, freeze dried at − 85° C and rotary shadowed with Platinum-Carbon.

The carbohydrate whole-cell analyses were performed according to Roeijmans et al. (1989).

Results

The two isolates are characterized by budding of cylindrical cells (Fig. 1A, 1B), occurring in pairs or short chains, but predominantly in pseudohyphal clusters. Terminal cells are able to form branch-like structures, simple (Fig. 1B) or complex (Fig. 1C–E); generally two symmetrical branches are formed (Fig. 1F; Fig. 2).

By transmission electron microscopy it was found that budding is holoblastic and that cell walls are of the ascomycetous type, consisting of a rather thin, dark outer layer and a broader, light inner layer (Fig. 3A). Cell walls of the branch-like structures were covered with an additional outer layer of fibrillar structure (Fig. 3B) similar to the fibrillar outer layer observed in infecting conidia of the endoparasitic nematophagous fungus *Drechmeria coniospora* (Drechsler) Gams & Jansson (Saikawa 1982; Dijksterhuis et al. 1990). Freeze-etching as well as ultra-thin sectioning of the branch-like structures revealed the presence of intrusions of the cytoplasma membrane into the cells (Fig. 4).

An ascomycetous teleomorph was not detected on the common sporulation media V8, MacClary acetate, YM, Gorodkowa agars and DMA (van der Walt & Yarrow 1984).

The physiological capacities of both strains were rather limited. Visible CO_2 production from any carbon source was not observed and only 10 out of 38 carbon compounds tested and only 2 out of 7 nitrogen compounds tested could be utilized (Table 1).

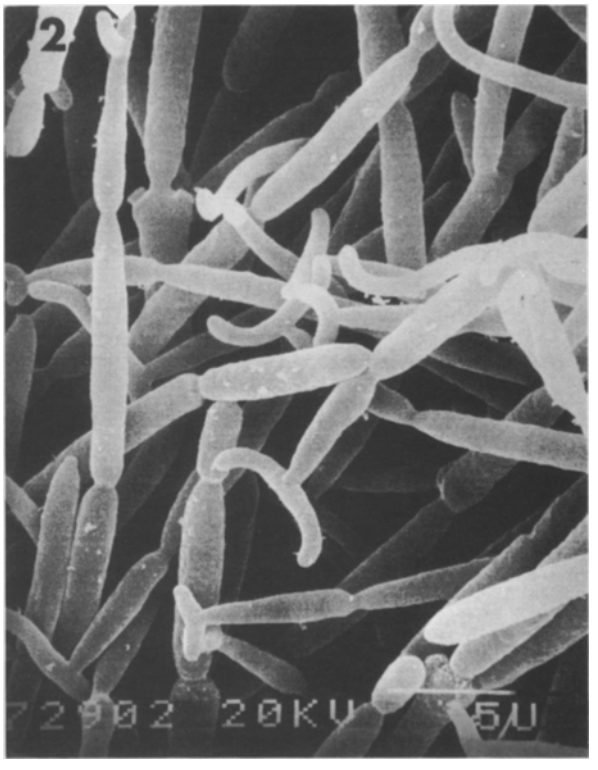

Fig. 2. Botryozyma nematodophila CBS 7426. SEM micrograph of pseudohyphal terminal cells showing symmetrical branch-like structures. (Bar represents 5 μm).

The average calculated mol% G + C is 29.6 ± 0.4 (three determinations).

By DNA-DNA reassociation it was demonstrated that CBS 7426 had a low homology of 14.9 ± 6.0% with CBS 7425, an isolate supposed to be its teleomorph, but identified as *Arthroascus schoenii* (Smith et al. 1990).

The carbohydrate profile of whole-cell hydrolysates is typical of the ascomycetes, consisting of the components glucose, mannose, galactose, mannitol, inositol and arabitol (Fig. 5).

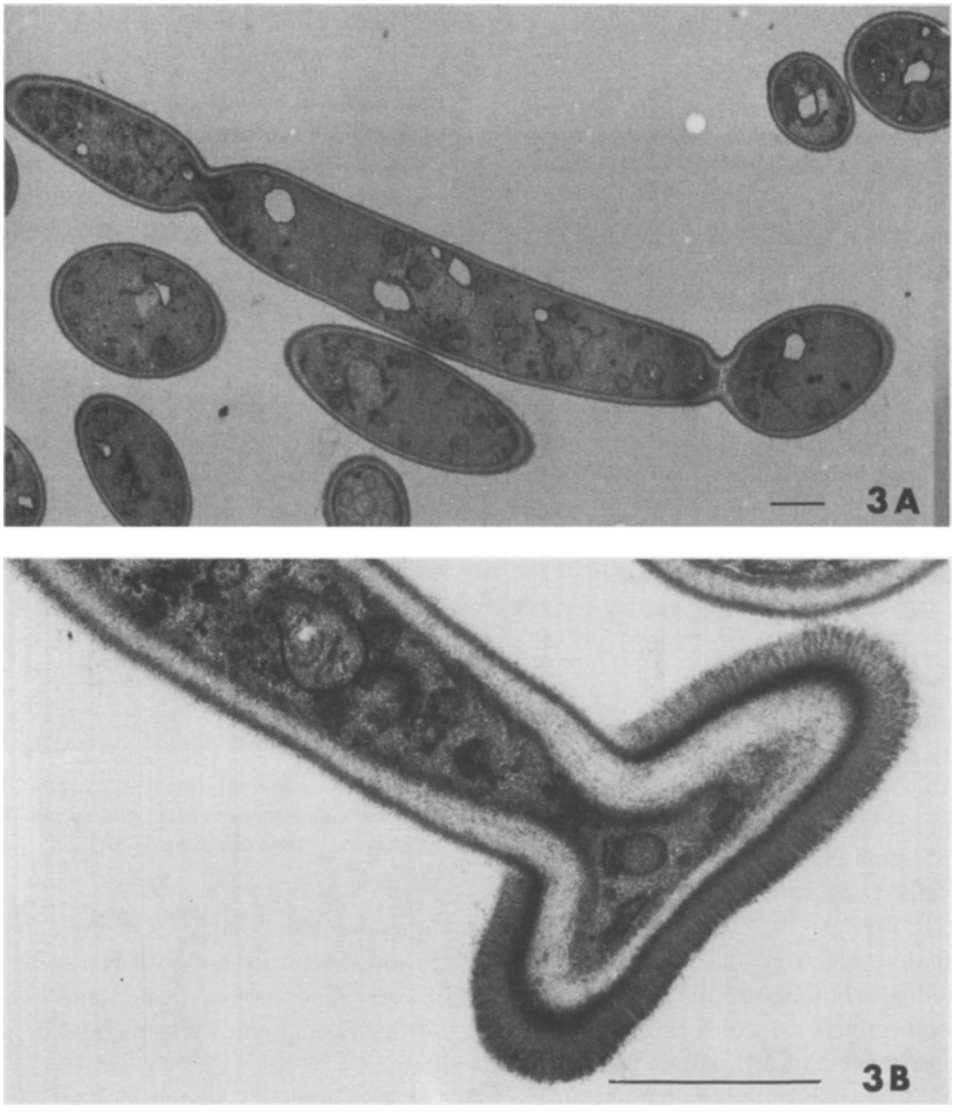

Fig. 3. Botryozyma nematodophila CBS 7426. TEM micrographs. (A). Budding cell showing characteristic holoblastic conidiogenesis. (B). Branch-like structure with fibrillar outer layer. (Bar represents 1 μm).

Discussion

The conidiation process, the ultrastructure of the cell wall, the carbohydrate composition, the negative result in the DBB test and the absence of hydrolysis of urea point to an ascomycetous character of the two isolates. Because they lack a teleomorph state, these isolates must be placed in the Candidaceae (von Arx 1981). This family includes at present 12 genera which are differentiated mainly by morphological features (Kreger-van Rij 1984; Barnett et al. 1990). The two isolates cannot be assigned to any of the presently accepted anamorphic yeast genera on the basis of the peculiar morphology of terminal cells, viz. the formation of branch-like structures. Therefore, the two strains are placed in a new genus.

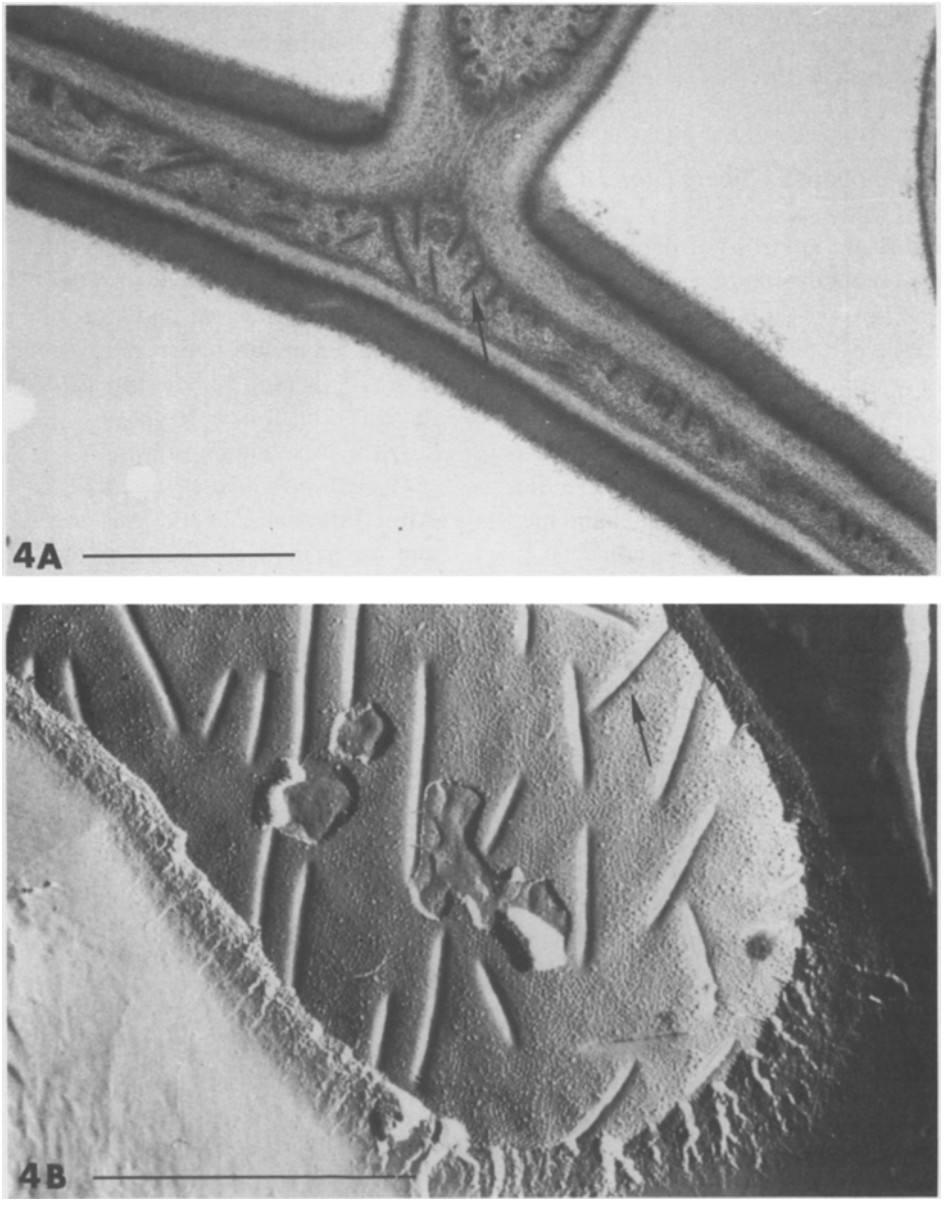

Fig. 4. Botryozyma nematodophila CBS 7426. Electron micrograph of ultra-thin sectioned (A) and freeze-fractured (B) branch-like structures with intrusions of the cytoplasma membrane (arrows). (Bar represents 1 μm).

Taxonomy

Botryozyma *Shann & M.Th. Smith gen. nov. (Candidaceae)*

Cellulae zymoticae, holoblastice gemmantes. Pseudohyphae adsunt. Structurae ramoidae formantur in cellulis terminalibus, singulae vel complicatae. Parietes cellularum ascomycetoidei, sine D-xyloso vel L-fucoso. Ascosporae nullae.

Yeast cells reproducing by multilateral, holoblastic budding. Pseudohyphae formed.

Branch-like structures produced by pseudohyphal terminal cells. Ultrastructure of the cell walls of the ascomycetous type without D-xylose and L-fucose. Ascospores not formed.

Type species: *Botryozyma nematodophila* Shann & M.Th. Smith sp. nov. (Figs. 1–6).

Botryozyma from the Gr. βοτρυς (grape) and ζυμη (yeast).

Botryozyma nematodophila *Shann & M.Th. Smith, sp. nov.*

In medio liquido cum dextroso et peptono et extracto levedinis post 3 dies 25° C cellulae cylindricae (8.0–22.5) × (2.0–5.5) μm, raro fusiformes, binae, sed praecipue catenis vel pseudomycelio connexae. Cellulae terminales saepe structuras ramosas producentes (2.0–20.0) × (1.0–2.0) μm. Sedimentum formatur. Cultura in agaro cum dextroso et peptono et extracto levedinis post 3 dies 25° C cremeo-alba vel cremea, butyrosa, haud lucida, margo pauce lobata. Ascosporae nullae.

Fermentatio et crescentia in variis substratis carbonis et nitrogeni et reliqui characteres in tabula 1 monstrantur.

Typus CBS 7426, isolatus ex uvis infectis, exsiccatus in CBS Baarn, et vivus in collectione zymotica Delphis Batavorum praeservatur.

Growth in glucose-yeast extract-peptone water: After 3 days at 25° C cells cylindrical (8.0–22.5) × (2.0–5.5) μm, reproducing predominantly by budding in the apical zone and occurring in pairs, short chains, but mainly in pseudomycelial elements; fusiform cells may be present. End cells often producing branch-like structures, (2.0–20.0 × 1.0–2.0) μm. A sediment is present.

Growth on glucose-yeast extract-peptone agar: After 3 days at 25° C the cells are similar in shape and size as in glucose-yeast extract-peptone water.

Table 1. Characteristics examined in *Botryozyma nematodophila* sp. nov.

Fermentation: Gas formation not detectable					
Growth on					
D-Glucose	+	Cellobiose	−	L-Arabinitol	−
D-Galactose	−	Salicin	−	D-Glucitol	+
L-Sorbose	+	Arbutin	−	D-Mannitol	+
D-Glucosamine	−	Melibiose	−	Galactitol	−
D-Ribose	−	Lactose	−	myo-Inositol	−
D-Xylose	−	Raffinose	−	D-Glucono-δ-lactone	−
L-Arabinose	−	Melezitose	−	2-Keto-D-gluconate	+
D-Arabinose	−	Inulin	−	D-Gluconate	−
L-Rhamnose	−	Soluble starch	−	DL-Lactate	+
Sucrose	−	Glycerol	+	Succinate	+
Maltose	−	Erythritol	−	Citrate	−
α, α-Trehalose	+	Ribitol	−	Ethanol	+
Me-α-D-glucoside	−	Xylitol	−		
Nitrate	−	Vitamin free medium	−		
Nitrite	−	37° C	+		
Ethylamine	+	0.1% Cycloheximide	+		
L-Lysine	+	50% glucose	−		
Cadaverine	−	0.1% acetic acid resistance	v		
Creatine	−				
Creatinine	−				
Additional characteristics					
Urease		−			
Starch formation		−			
Acetic acid production on Custers medium		−			
Diazonium Blue B Test		−			
G + C%		29.5 ± 0.6 (CBS 7426) 29.7 ± 0.1 (CBS 7442)			

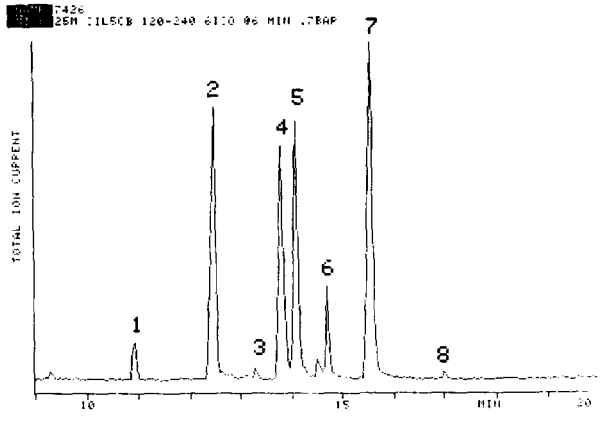

Fig. 5. Total ion gas chromatogram of a whole cell hydrolysate of *Botryozyma nematodophila;* 1 = arabitol, 2 = α-mannose, 3 = α-galactose, 4 = α-glucose, 5 = β-mannose + β-galactose, 6 = mannitol, 7 = β-glucose and 8 = inositol.

Streak culture cream-coloured, dull, delicately wrinkled, butyrous, margin slightly lobed.

Growth on Dalmau plate cultures on glucose-yeast extract-peptone agar: After 3 days at 25° C a well-developed pseudomycelium is formed. Fusiform cells may be formed, branch-like structures are present.

Ascospore formation: Absent.

Physiological characteristics: Fermentation and growth on various carbon compounds, growth on nitrogen sources and some additional properties are summarized in Table 1.

Nematodophila *referring to the nematodes as the first isolation locality of the species*

Type strain: CBS 7426 isolated from sour rot of grapes, deposited living in the collection of the Centraalbureau voor Schimmelcultures Delft, and dried in Baarn.

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