Phaffia rhodozyma (sexual form, Xanthophyllomyces dendrorhous) is a basidiomycetous yeast that has been found in tree exudates in the Northern Hemisphere at high altitudes and latitudes. This yeast produces astaxanthin, a carotenoid pigment with biotechnological importance because it is used in aquaculture for fish pigmentation. We isolated X. dendrorhous from the Southern Hemisphere (Patagonia, Argentina), where it was associated with fruiting bodies of Cyttaria hariotii, an ascomycetous parasite of Nothofagus trees. We compared internal transcribed spacer (ITS)-based phylogenies of P. rhodozyma and its tree host (Betulaceae, Corneaceae, Fagaceae, and Nothofagaceae) and found them to be generally concordant, suggesting that different yeast lineages colonize different trees and providing an explanation for the phylogenetic distance observed between the type strains of P. rhodozyma and X. dendrorhous. We hypothesize that the association of Xanthophyllomyces with Cyttaria derives from a previous association of the yeast with Nothofagus, and the sister relationship between Nothofagusceae and Betulaceae plus Fagaceae correlates with the phylogeny of X. dendrorhous strains originating from these three plant families. The two most basal strains of X. dendrorhous are those isolated from Corus, an ancestral genus in the phylogenetic analysis of the host trees. Thus, we question previous conclusions that P. rhodozyma and X. dendrorhous represent different species since the polymorphisms detected in the ITS and intergenic spacer sequences can be attributed to intraspecific variation associated with host specificity. Our study provides a deeper understanding of Phaffia biogeography, ecology, and molecular phylogeny. Such knowledge is essential for the comprehension of many aspects of the biology of this organism and will facilitate the study of astaxanthin production within an evolutionary and ecological framework.

Phaffia rhodozyma was originally isolated in the late 1960s, by Phaff and collaborators, who collected 10 strains from various broad-leaved trees in mountainous regions of Japan and Alaska (23). These strains had a red to orange color and could ferment glucose and produce amyloid compounds. Phaff et al. (23) referred to these strains as Rhodozyma montanae, but since a Latin diagnosis was not provided, the name was not valid. Later, this basidiomycetous yeast was described as Phaffia rhodozyma (20), and 67 additional strains were isolated from spring sap flows of Betula verrucosa in Russia (10).

The main carotenoid pigment produced by P. rhodozyma is astaxanthin, a compound that has not been found in other yeast species (3). Astaxanthin is economically important as an aquaculture feed component. This compound enhances pigmentation of fish and crustaceans (19) and is the most expensive feed ingredient in the aquaculture industry (12). Studies of the feasibility of P. rhodozyma as a dietary source of this pigment began almost 30 years ago (13). Such studies involved the development of methods for optimizing pigment production (24, 28), selection of hyperpigmented mutants (2), the use of chemical stimulants (11) or extracts of the filamentous fungus Epicoccum nigrum (5) in the growth medium, and genetic and metabolic engineering (21).

The sexual stage of P. rhodozyma was discovered in 1995, and its development is triggered when polyols are used as sole carbon sources (9). The teleomorphic state is characterized by the formation of a slender holobasidium with terminal basidiospores and was described as Xanthophyllomyces dendrorhous, with VKM Y-2786 (CBS 7918) as the type strain (9). Therefore, the name X. dendrorhous was used for the sexual form and P. rhodozyma was designated the asexual phase. The lack of sexual reproduction in the type strain of P. rhodozyma (CBS 5905) and differences in intergenic spacer (IGS) and internal transcribed spacer (ITS) sequences between CBS 5905 and VKM Y-2786 led Fell and Blatt (6) to propose that P. rhodozyma, represented solely by CBS 5905, was not conspecific with X. dendrorhous. The debate over this issue is not settled, and in another study isozyme analyses and restriction fragment length polymorphism and random amplified polymorphic DNA patterns suggested that CBS 5905 and five other strains of X. dendrorhous belong to a single species (27). The hypothesis that the anamorph P. rhodozyma is distinct, at the species level, from teleomorph X. dendrorhous also was favored by Kuesera et al. (14, 15), who based their arguments on the extensive chromosome length polymorphism seen in electrophoretic karyotypes (1, 22), the ability to produce respiration-
deficient petite mutants spontaneously or following brief exposure to ethidium bromide, and a reexamination of the sexual form. 

*P. rhodozyma* and *X. dendrorhous* have been found so far in tree exudates in the Northern Hemisphere at high altitudes and latitudes. No new strains of *P. rhodozyma/X. dendrorhous* have been isolated recently, and most of the current research is based on isolates first cultured in the 1960s and 1970s. This study is the first to document the occurrence of *Phaffia* yeasts in the Southern Hemisphere, and its main objective is to provide an adequate molecular and ecological characterization of the new isolates. Our working hypothesis is that *Phaffia* has a broader ecological distribution than the one presently known and that its occurrence in the Southern Hemisphere is linked to similar ecosystems in the Northern Hemisphere where *Phaffia* is known to occur. A deeper understanding of *Phaffia* biogeography, ecology, and molecular phylogeny is essential for the comprehension of many aspects of its biology and will facilitate the study of astaxanthin production within an evolutionary and ecological framework.

### MATERIALS AND METHODS

#### Yeast isolation and reference cultures. 
Water samples from Lake Ilon (71°56′S, 41°11′W), Nahuel Huapi National Park (Patagonia, Argentina), were collected during January 2003 in aseptic flasks and filtered in situ through a membrane with 0.45-μm pores with a sterile Nalgene (Rochester, NY) filtering apparatus. The filters were placed in sterile petri dishes and kept at 4°C for approximately 36 h, until arrival at the laboratory, where they were placed on MYP agar (components, g/liter: malt extract, 7; yeast extract, 0.5; peptone-soybean, 2.5; agar, 15 [pH 5]) plus 200 mg/liter chloramphenicol and incubated at 15 to 17°C until colonies emerged. 

Stromata of *Cyttaria hariotii*, an ascomycetous fungal parasite of *Nothofagus dombei* ("Cohuec"), were collected aseptically in November to December (spring season) of 2003 on the shore of Gutie´rrez Lake (71°43′S, 41°18′W). Fifteen *Cyttaria* fruiting bodies were collected and stored individually in sterile plastic bags with a known volume of distilled sterile water in a water-to-frUITING-body ratio of 1 g/ml. *Cyttaria* fruiting bodies were manually crushed inside the plastic bags, which were then transferred to Erlenmeyer flasks and agitated at 20°C for 30 min at 300 rpm. The extract was diluted (10^{-3} or 10^{-4}), and aliquots (100 to 200 μl) were spread on MYP agar plates. These plates were incubated at 15 to 17°C for 5 to 7 days, and every pigmented colony was subcultured on MYP agar for purification. The production of amyloid compounds and the ability to ferment glucose were evaluated by using the procedures of Yarrow (30). The two strains isolated from water samples plus 13 selected strains recovered from different *Cyttaria* fruiting bodies were used for further studies. The ability to form the sexual stage was investigated by using previously described procedures (14). All of the pigmented isolates were maintained on potato dextrose agar at 4°C. In addition, strains identified as *X. dendrorhous* were deep frozen in liquid nitrogen.

In addition to these newly collected cultures, reference cultures were obtained from the Centraalbureau voor Schimmelcultures (CBS); the Department of Soil Biology, Moscow State University (KBP); the Portuguese Yeast Culture Collection (PYCC); and the University of California at Davis (UCD) (Table 1).

#### Molecular characterization. 
We used the microsatellite primed-pCR technique (MSP-pCR) for PCR fingerprinting. DNA extraction, PCR and electrophoresis conditions, and gel image analysis procedures were those of Libkind et al. (17). The primer employed was the synthetic oligonucleotide (GTG), Measurements of the extent of DNA-DNA reassociation were made with a Gifilford Response UV-VIS spectrophotometer (Ciba Corning Diagnostics Corp., Oberlin, OH) as described by Sampaio et al. (25). For sequence analysis, ribosomal DNA (rDNA) was amplified using primers ITS5 (5′-GGAGGATTTGAGTCGT AACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). Sequences were obtained from GenBank. Phylogenetic analyses were made by using a heuristic maximum parsimony analysis (100 rounds of heuristic search with TBR branch swapping, starting from trees obtained by random addition of sequences with the MulTrees option on and the steepest descent option off). The molecular phylogenies were validated with 1,000 rounds of bootstrap analysis (7). Maximum parsimony analysis and bootstrap calculations were performed with PAUP* software (26).

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Other collection(s)</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. rhodozyma</em></td>
<td>CBS 5905&lt;sup&gt;T&lt;/sup&gt;</td>
<td>UCD 67-210, NRRL Y-10921</td>
<td>Exudate of <em>Fagus crenata</em>, Uchimiyama, Kyoto, Japan</td>
</tr>
<tr>
<td></td>
<td>PYCC 4172&lt;sup&gt;T&lt;/sup&gt;</td>
<td>UCD 67-210, NRRL Y-10921</td>
<td></td>
</tr>
<tr>
<td><em>X. dendrorhous</em></td>
<td>CBS 9090</td>
<td>UCD 67-210.2</td>
<td>Exudate of <em>Betula verrucosa</em>, Moscow, Russia</td>
</tr>
<tr>
<td></td>
<td>CBS 7918&lt;sup&gt;F&lt;/sup&gt;</td>
<td>VKM Y-2786</td>
<td>Exudate of <em>Alnus japonica</em>, Kiso, Japan</td>
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<td>CBS 5908</td>
<td>UCD 67-383</td>
<td>Sap of <em>Betula sp.</em> stumps, Finland</td>
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<tr>
<td></td>
<td>CBS 7919</td>
<td>ATCC 24230, UCD 67-385</td>
<td>Exudate of <em>Betula tauschii</em>, Shinkai, Kiso, Japan</td>
</tr>
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<td>ATCC 24228</td>
<td>UCD 68-653.3C</td>
<td>Exudate of <em>Cornus brachypoda</em>, Kario-yama, Hiroshima, Japan</td>
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<td>ATCC 24229</td>
<td>UCD 67-202</td>
<td>Exudate of <em>Cornus brachypoda</em>, Kario-yama, Hiroshima, Japan</td>
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<td>ATCC 24201</td>
<td>UCD 67-203</td>
<td>Exudate of <em>Betula maximowicziana</em> at Yatani, Yamagata, Japan</td>
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<td>ATCC 24261</td>
<td>UCD 67-484</td>
<td>Betula sp., Novgorod region, Russia</td>
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<td>VKM Y-1652</td>
<td>KBP 2607</td>
<td>Betula sp., Komi Republic, Russia</td>
</tr>
<tr>
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<td>VKM Y-1654</td>
<td>KBP 2607</td>
<td>Betula sp., Komi Republic, Russia</td>
</tr>
<tr>
<td></td>
<td>CRUB 0853&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>Stroma of <em>Cyttaria hariotii</em> on <em>Nothofagus dombei</em>, Gutierrez Lake, Patagonia, Argentina</td>
</tr>
<tr>
<td></td>
<td>CRUB 1149&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>Water from Lake Ilon, Patagonia, Argentina</td>
</tr>
</tbody>
</table>

<sup>a</sup> Same origin as strains CRUB 0924, CRUB 0919, and CRUB 1490 to CRUB 1495.

<sup>b</sup> Same origin as strain CRUB 1151.
Nucleotide sequence accession numbers. The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are DQ661021 to DQ661034.

RESULTS

Patagonian isolates. We isolated two orange-pigmented strains (CRUB 1149 and CRUB 1151) that formed amyloid compounds and gave positive results in the glucose fermentation test from high-altitude oligotrophic lakes of the Nahuel Huapi National Park (Patagonia). Based on sequences of the D1/D2 domains of large-subunit rDNA both strains were closely related to *P. rhodozyma*/ *X. dendrorhous*, i.e., there were no sequence mismatches in the comparison with the type strain of *Phaffia rhodozyma*. The *Phaffia*/ *Xanthophyllomyces* isolates from the Northern Hemisphere were found in slime fluxes of trees or tree stumps remaining after logging, so we hypothesized that the aquatic environment from which these two isolates were obtained was not their original habitat. Assuming that the yeast is primarily associated with ecological niches where fermentable sugars are present, we surveyed the plant biota surrounding the high-altitude lakes of the Nahuel Huapi National Park for the occurrence of slime fluxes. The main trees occurring in this region are *Nothofagus dombeyi* and *N. pumilio*, but trees of neither of these species had signs of active or dry slime fluxes.

While we were surveying the trees for slime fluxes, our attention was drawn to *Cyttaria hariotii*, an ascomycetous fungal parasite of *Nothofagus* spp., whose mycelium develops inside the trunk and the branches of the tree. As a reaction to the infection, persistent tumors are formed. Fructifications (stromata) are produced annually on the tumors. The stromata of *C. hariotii* are globose and yellow to orange, measure 2 to 6 cm in diameter, and mature in November-December (8). Yeasts were present at all maturation stages of the *Cyttaria* stromata.

Mature fruiting bodies yielded the highest yeast counts (above $9 \times 10^5$ CFU/g [wet weight]), while immature stromata and those in the first stages of maturation had 900 and 2,000 CFU/g, respectively. The proportion of pigmented yeasts in *C. hariotii* gradually declined with maturation. In late maturation stages, most of the yeast isolates were fermentative ascomycetes of the genera *Cândida* and *Saccharomyces* and only 1 to 5% of the colonies on the isolation plates were pigmented (red to orange cultures). Overall, we obtained 40 pigmented isolates, of which 36 produced amyloid compounds. Glucose was fermented by 33 of the strains that formed amyloid compounds.

Based on MSP-PCR, the Patagonian isolates belonged to a single species, as the profiles of the various strains were basically identical (Fig. 1). The sequence of the D1/D2 domains of large-subunit rDNA of CRUB 0853 (DQ661033) also was identical to that of the aquatic strain CRUB 1149 and that of the type strain of *Phaffia rhodozyma*.

Selected Patagonian (CRUB) isolates and the type strains of *P. rhodozyma* and *X. dendrorhous* were inoculated on ribitol agar and examined for the ability to develop basidia and basidiospores. All 15 South American isolates and the type strain of *X. dendrorhous* produced the sexual form, but the type strain of *P. rhodozyma* did not (14). Therefore, the Patagonian isolates were identified as *X. dendrorhous* rather than as *P. rhodozyma*.

The proportion of *X. dendrorhous* strains recovered depended on the maturity of the stroma. Mature stromata yielded the most isolates (27 strains), none were recovered from immature stromata, and six were isolated from stromata in the first stages of maturation. *N. dombeyi*, the host tree of *C. hariotii*, was surveyed for *Phaffia*/ *Xanthophyllomyces*, but no cultures were obtained from leaves, buds, or bark. Additional water samples from nearby lakes also were negative.

Molecular phylogeny and host specificity. In spite of their overall similarity, the fingerprints obtained with primer (GTG)$_5$ (Fig. 1) could be used to subdivide the isolates into three groups. The first group (A) contained the type strains of *P. rhodozyma* and *X. dendrorhous* and several other strains from different geographic areas. The second group (B) included two strains of *X. dendrorhous* isolated by Phaff et al. (23), and the third group (C) included the Patagonian isolates. The same set of strains also was evaluated with primer M13 (data not shown), and the same three groups were identified.

We used ITS sequence data and a maximum parsimony analysis to evaluate the relatedness of the *Phaffia*/ *Xanthophyllomyces* isolates (Fig. 2A). We expanded the ITS data set of Fell and Blatt (6) from 6 to 18 sequences. Four of the new sequences are from Patagonian isolates, and the remaining 
eight sequences are from Russian (two sequences) and Japanese (six sequences) strains isolated by W. I. Golubev and H. J. Phaff, respectively. The type strain of *X. dendrorhous*, which was isolated from *Betula verrucosa* in Russia, clustered with all of the other strains of this species originating from Betulaceae (*Alnus* and *Betula*) but not with the strains isolated from *Cornus* (Cornaceae) and *Nothofagus* (Nothofagaceae), the plant host of *Cyttaria* (Fig. 2A). The isolated position occupied by the type strain of *P. rhodozyma* CBS 5905 in the phylogenetic tree could be due to its association with a different tree (*Fagus crenata*, Fagaceae). Strains PYCC 4172 and CBS 9090 clustered together at the base of the clade composed of isolates from Betulaceae (Fig. 2A).

The original strain number of the *P. rhodozyma* type strain was UCD 67-210. This strain was sent in 1968 to the CBS culture collection (CBS 5905) (V. Robert, personal communication) and in 1976 to the NRRL culture collection (NRRL Y-10921) (C. Kurtzman, personal communication). From this last culture collection it was sent to the Portuguese Yeast Culture Collection (PYCC 4172) in 1982. Therefore, CBS 5905 and PYCC 4172 should have identical ITS sequences as they are subcultures of the same strain. The original number for CBS 9090 was UCD 67-210.2. According to the curator of the Phaff Yeast Culture Collection, University of California—Davis, subcultures of UCD 67-210 were lyophilized on several different occasions between 1968 and 1986 and UCD 67-210.2 was one of these stocks (K. Boundy-Mills, personal communication).

Given the apparent association of various ITS phylotypes of *X. dendrorhous* with different tree families, we tested the hypothesis that different populations of *X. dendrorhous* colonize different trees and analyzed the evolutionary relationships of the genera of trees that are known to harbor *Xanthophyllomyces* (Fig. 2B). We used complete ITS sequences, and in general, good agreement was observed between the fungal and the plant phylogenetic trees (Fig. 2).

Our ITS analysis was complemented with a phylogenetic analysis of the rDNA IGS region, a region previously evaluated by Fell and Blatt (6). They found that for *X. dendrorhous*, differences in sequences and the occurrence of gaps could be used to differentiate strains and for population-level studies. Our IGS alignment of 657 nucleotides differed substantially from the one presented by Fell and Blatt (6), with numerous short nucleotide repeats and gaps contributing to distinct alignment options. Similar difficulties also were reported by Fell and Blatt (6) for their IGS alignment. In spite of this we found, in our analysis, that the sequence of the Patagonian isolate was phylogenetically closer to that of CBS 5905 than to any of the already known sequences (Fig. 3).

**DISCUSSION**

**New habitat.** In this report we describe, for the first time, the isolation of *X. dendrorhous* in the Southern Hemisphere and we identify a new habitat for this yeast, the stromata of *C. hariotii*. The association of *X. dendrorhous* with stromata of *C. hariotii* was unexpected since all other reports of the occurrence of this yeast concerned tree exudates. The genus *Cyttaria* is associated exclusively with *Nothofagus* spp., and its 10 species occur solely in South America (Argentina and Chile) and Australasia (Australia and New Zealand) (8). It would be interesting to determine if *X. dendrorhous* is present on other species of *Cyttaria*, especially the Australasian taxa.

A peculiar characteristic of *X. dendrorhous* is its ability to ferment simple sugars. When mature, the fruiting bodies of *C. hariotii* have a sugar content of almost 10% (mainly D-glucose,
fructose, and sucrose) and contain polyols such as glycerol, D-mannitol, and D-arabinitol (16). Although we recovered two isolates from freshwater in a high-altitude lake, surveys of pigmented yeasts in 15 other Patagonian aquatic environments did not yield any additional isolates of this species (17). We hypothesize that the primary habitat of the two aquatic isolates also was C. hariotii because Nothofagus spp. infected with the fungus surround the lake from which the two strains were obtained. Mature stromata normally fall to the ground where they decompose, but stromata falling from trees on the lake shore may fall in the water, where they float for some time.

**South American populations.** MSP-PCR fingerprinting has been used to type fungal strains by several research groups. In most cases species-specific PCR patterns are obtained (17, 25, 29), but in some instances this approach results in intraspecific differentiation (4, 18). Our results suggest that primers (GTG)$_4$, and M13 are useful to discriminate populations of Phaffia/Xanthophyllomyces. The South American isolates formed a single, homogeneous cluster (group C) that did not include strains from other geographic areas. Thus, the Patagonian isolate and CBS 5905 challenges the expected identity of these three strains is not seen with the asexual form of X. dendrorhous.

**Host specificity model.** The comparative analysis of ITS sequence data from Xanthophyllomyces/Phaffia and their tree hosts suggests that different yeast lineages colonize different trees. Specific (insect?) vectors colonize different trees and could contribute to the observed separation. Since the two phylogenetic trees (Fig. 2) have similar topologies, we hypothesize that the observed phylogenetic pattern of X. dendrorhous follows the speciation of its plant hosts. However, two unanswered questions arise from these findings: (i) are the isolation mechanisms stronger or weaker than those that contribute to the unity of X. dendrorhous as a species, and (ii) if the forces contributing to the isolation of the several Xanthophyllomyces lineages are dominant, is speciation currently in progress? Further studies are needed to elucidate the degree of gene flow between representatives of the different Xanthophyllomyces lineages.

From the evolutionary point of view one can postulate that the present association of Xanthophyllomyces with Cyttaria derived from an earlier association of the yeast with Nothofagus. The sister relationship between Nothofagaceae and Betulaceae plus Fabaceae (Fig. 2B) was also observed in studies of angiosperm phylogeny (Angiosperm Phylogeny Website, version 7, May 2006, P. F. Stevens [http://www.mobot.org/MOBOT/research/APweb]) and corresponds very well with the phylogeny of X. dendrorhous strains originating from these three plant families (Fig. 2A). The two most divergent and basal strains of X. dendrorhous are those originating from Cornus (Fig. 2A), a divergent and ancestral genus in the phylogenetic analysis of the host trees (Fig. 2B).

In spite of the considerable number of strains that were analyzed, the type strain of P. rhodozyma remains phylogenetically isolated. According to our model this result is not connected with the number of strains employed in the analysis but is rather a direct consequence of the habitat (tree species) from which each isolate was recovered. We anticipate that the isolated placement of the type strain of P. rhodozyma will change only if new isolates from Fagus are obtained. Thus, the isolated position of the type strain of P. rhodozyma does not necessarily mean that CBS 5905 is not conspecific with (and the asexual form of) X. dendrorhous.

Interpretations of previous studies with IGS sequence data suggested that this approach was adequate to reflect the biogeography of X. dendrorhous since ATCC 24228 and ATCC 24230, the two Pacific Rim strains, were separated from CBS 6938 and CBS 7918, the two European strains (6). Moreover, the phylogenetic differences were regarded as indications that X. dendrorhous includes two varieties, and the isolated position of CBS 5905 was interpreted as additional evidence that P. rhodozyma and X. dendrorhous are not conspecific. Our IGS analysis partially supports the conclusions of Fell and Blatt (6). We think that the IGS data are promising for a biogeographic study of isolates from Betulaceae since the Pacific Rim strains are distinct from the European isolates. However the association between the Patagonian isolate and CBS 5905 challenges the two-species concept. We anticipate that IGS sequence typing might be a useful approach for the characterization of isolates within a given host group.

Was the type strain of P. rhodozyma mislabeled? The relationship between CBS 5905, PYCC 4172, and CBS 9090 remains unresolved. According to the records from several culture collections, all of these strains are derived from UCD 67-210, the original type strain of P. rhodozyma, but the expected identity of these three strains is not seen with the molecular characters. The hypothesis that one or more of these strains has been mislabeled cannot yet be discarded, although we could not identify the strain that might represent the source of such mislabeling (in terms of either identical ITS sequences or, for CBS 5905, the absence of the sexual stage). Golubev (9) reported that VKM Y-2274 (type strain of P. rhodozyma) could form basidia and basidiospores. The VKM strain was obtained from the UCD collection in 1975 (W. I. Golubev, personal communication). However, Kucsera et al. (14) reported that CBS 5905 could not form a sexual stage. We confirmed that
species is present. For CBS 5905 high, which is consistent with the hypothesis that only a single association experiments. These reassociation values all were among themselves and with Patagonian isolate of genetic isolation of the various lineages of tilocus sequence typing might be useful in assessing the degree of genetic isolation of the various lineages of asexual stage of the type strains of Phaffia rhodozyma. Further studies with additional molecular markers and the determination if there are multiple copies of the ITS region within each strain are needed to adequately clarify this situation.

**P. rhodozyma or X. dendrorhous?** Our results are not consistent with the hypothesis that *P. rhodozyma* and *X. dendrorhous* represent different species (6, 15). Most of the differences in the sequence data can be attributed to intraspecific variation associated with host specificity. The issue of the relatedness of the type strains of *P. rhodozyma* (CBS 5905) and *X. dendrorhous* (CBS 7918) among themselves and with Patagonian isolate CRUB 0853 also was evaluated with genomic DNA-DNA reassociation experiments. These reassociation values all were high, which is consistent with the hypothesis that only a single species is present. For CBS 5905 × CBS 7918, DNA-DNA reassociation values ranged from 96 to 100%, values for CBS 5905 × CRUB 0853 ranged from 79 to 100%, and values for CBS 7918 × CRUB 0853 ranged from 79 to 90%.

In conclusion, we think that nomenclatural changes are premature and support the position that *P. rhodozyma* is the asexual stage of *X. dendrorhous*. We think that a better understanding of the processes underlying the observed differences within *X. dendrorhous* might have relevance since they could shed new light on the evolution of the astaxanthin biosynthetic pathway and on the ecological conditions that selected for this trait. Finally, we do not reject the hypothesis that speciation is in progress in *X. dendrorhous*. The driving force underlying such a process could be isolation due to host specificity of the different lineages of *X. dendrorhous*. We anticipate that multilocus sequence typing might be useful in assessing the degree of genetic isolation of the various lineages of *X. dendrorhous* identified in this report.

**ACKNOWLEDGMENTS**

This work was partially funded by the Universidad Nacional del Comahue (project B121) and CONICET (project PIP6536) funds granted to M. van Brooock and by ANPCYT (project PICT 22200). D. Adrio, J. L., M. Lopez, J. Casqueiro, C. Fernández, and M. Veiga, 1995. Electrophoretic karyotype of the astaxanthin-producing yeast *Phaffia rhodozyma*. Curr. Genet. 27:447–450.


