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The phylogenetic relationships of *Eeniella nana* Smith, Batenburg-van der Vegte et Scheffers based on the partial sequences of 18S and 26S ribosomal RNAs (Candidaceae)*

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SUMMARY

Two strains of *Eeniella nana* were examined for their partial base sequences of 18S and 26S rRNAs. In the partial base sequences of 18S rRNA (positions 1451 through 1618, 168 bases) the strains of *E. nana* have five, five, four and eleven base differences with those of *Dekkera bruxellensis* (type species), *D. anomala* (and *Brettanomyces anomalus*), *D. naardenensis* and *D. custersiana*, respectively. In the 26S rRNA partial base sequencings (positions 1611 through 1835, 225 bases and positions 493 through 622, 130 bases) the base differences were 46, 43, 34 and 40 and the percent similarities were 53–54, 51–54, 56–57 and 51–53, respectively. The sequence data obtained are discussed phylogenetically and taxonomically, especially on retention of the generic name *Eeniella*.

INTRODUCTION

The genus *Eeniella* Smith, Batenburg-van der Vegte et Scheffers was introduced with a single species, *E. nana* Smith, Batenburg-van der Vegte et Scheffers [9]. This species was once described invalidly as *Brettanomyces nanus* Scheffers [8]. *Eeniella nana* was characterized morphologically by its bipolar conidiogenesis [9], physiologically by the production of a large amount of acetic acid [8] and chemotaxonomically by the Q-9 system [22]. The morphological characteristics mentioned above differentiated *E. nana* from *Brettanomyces* sp. Kufferath et van Laer (and the genus *Dekkera* van der Walt) [9–12].

In a previous study [20] we analyzed the partial base sequences of the 18S and 26S rRNAs of the species of the teleomorphic genus *Dekkera* (and the anamorphic genus *Brettanomyces*) and reported that *D. custersiana* Lee et Jong (≡ *B. custersianus* van der Walt) and *D. naardenensis* Jong et Lee (≡ *Brettanomyces naardenensis* Kolfschoten et Yarrow) are phylogenetically separate from the other species of the genus *Dekkera* (and the genus *Brettanomyces*).

This paper deals with the phylogenetic relationships of the monotypic genus *Eeniella* on the basis of the sequence data obtained.

This paper is dedicated to Professor Herman Jan Phaff in honor of his 50 years of active research which still continues.

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* Significance of the coenzyme Q system in the classification of yeasts and yeast-like organisms. Part LVIII. For part LVII, see ref. [20].

MATERIALS AND METHODS

Two strains of *E. nana* (CBS 1945, type strain and CBS 1955) were used in this experiment. They were cultured, and their ribosomal RNAs were prepared as described previously [13,14].

Two partial base sequences of the 18S and 26S rRNAs of the yeast strains were sequenced by the method of Lane et al. [5] using reverse transcriptase with three oligonucleotide DNA primers [13,14]. The three primers used in this experiment were 5′-ACGGGCGGTGTGTAC-3′, which is complementary to the sequence in positions 1641 through 1627 (in *Saccharomyces cerevisiae* Meyen ex Hansen [6]) of 18S rRNA, and 5′-GGTCCGTGTTTCAAGACGG-3′ and 5′-TTGGAGACCTGCTGCGC-3′, which are complementary to the sequences in positions 654 through 636 and 1857 through 1841, respectively, (in *S. cerevisiae* [3]) of 26S rRNA. The partial base sequences analyzed were manually aligned.

The chemicals and reagents used in our study were the same as those described previously [14].

RESULTS AND DISCUSSION

The partial base sequences in positions 1451 through 1618 (168 bases) of 18S rRNAs of the two strains of *E. nana* are shown in Fig. 1(A). In the partial base sequence of *E. nana* CBS 1945 (type strain), the following base substitutions were observed compared with *S. cerevisiae* IFO 2376: in positions 1506 (U to C), 1509 (U to G), 1552 (U to G), 1590 (A to G) and 1602 (U to C). The base sequence on the fingerprint segment [14,16] was comprised of five bases (ACUAG). It was consistent in this respect with those of *Dekkera bruxellensis*

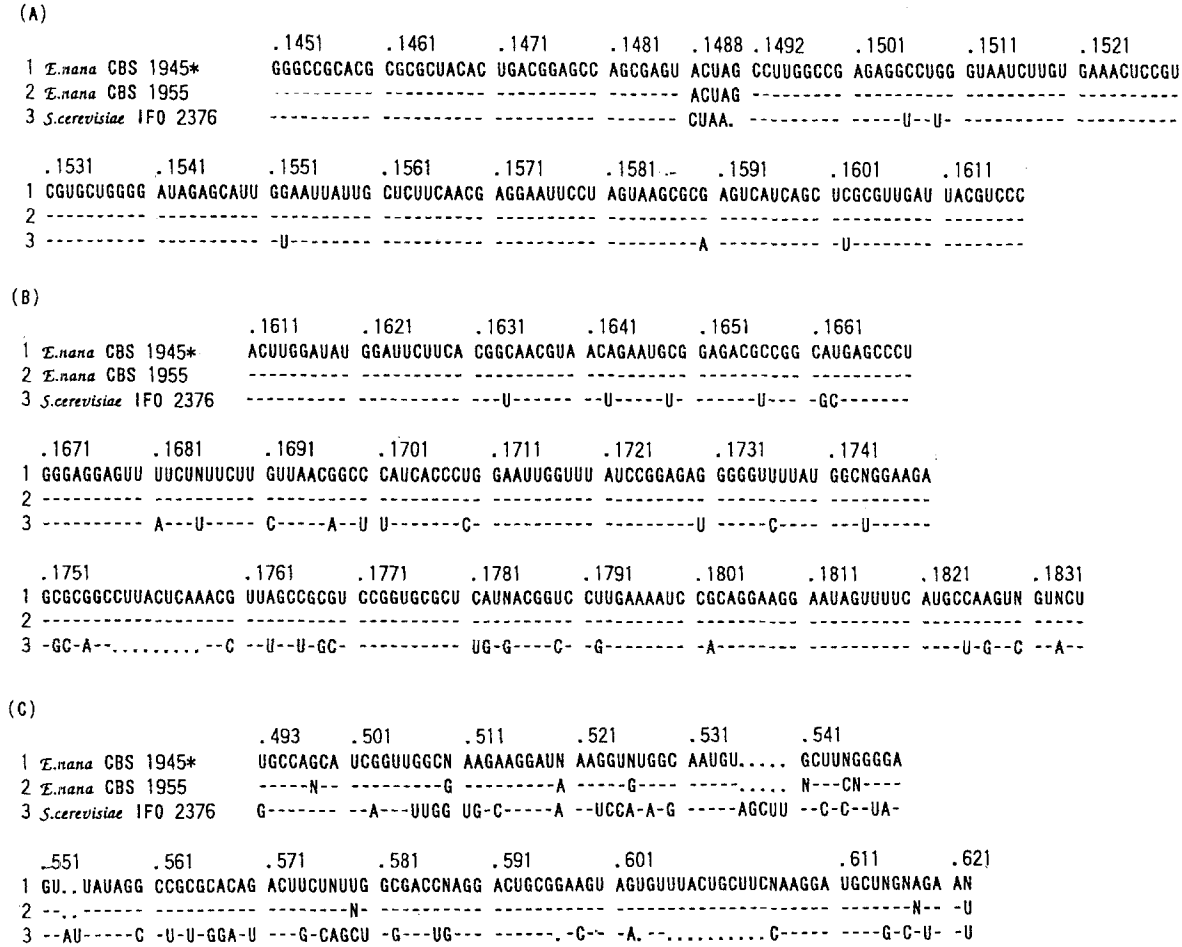

Fig. 1. The primary partial base sequences of 18S and 26S rRNAs in strains of *Eeniella nana*. The primary partial base sequences of 18S (A) and 26S (B and C) rRNAs were manually aligned. The Arabic numerals indicate positions in *Saccharomyces cerevisiae* [3,6,14]. The partial base sequence data reported here will appear in the DDBJ Nucleotide Sequence Database under accession numbers D31922–D31927. N represents A, G, C or U. *Type strain.

van der Walt (AAUAG), *Dekkera anomala* Smith et van Grinsven (AUUAG) and *D. naardenensis* (ACUAA), but inconsistent with that of *D. custersiana* (AUUUAA, six bases) [20].

The number of base differences was calculated among the strains examined. There were no base differences between the two strains of *E. nana*. The strains of *E. nana* had five, five, four, eleven and nine base differences compared with *D. bruxellensis* IFO 1590 (type strain) and *D. anomala* IFO 0627 (and *Brettanomyces anomalus* Custers IFO 0796, type strain), *D. naardenensis* IFO 1588 (type strain), *D. custersiana* IFO 1585 (type strain) and *S. cerevisiae* IFO 2376, respectively.

Based on the sequence data obtained, a phylogenetic tree was drawn by the Neighbor-Joining method [7]. As shown in Fig. 2, *E. nana* was located in a position near *D. naardenensis*. It is noted that *E. nana* was separated phylogenetically from the apiculate yeast species, e.g. *Wickerhamia fluorescens* Soneda (type species), *Kloeckeraspora osmophila* Niehaus (type species [17], ≡ *Hanseniaspora osmophila* (Niehaus) Phaff, Miller et Shifrine) and *H. valbyensis* Klöcker (type species) in spite of showing reproduction by bipolar budding [9].

The partial base sequences in positions 1611 through 1835 (225 bases) of the 26S rRNAs of the two strains of *E. nana* are shown in Fig. 1(B). The *E. nana* strains had an insertion sequence comprised of nine bases between positions 1757 and 1758. The number of base differences was calculated among the strains examined. There were no base differences between the two strains of *E. nana*. The strains of *E. nana* had 46, 43, 34, 40 and 40 base differences compared with *D. bruxellensis* IFO 1590 (type strain) and *D. anomala* IFO 0627 (and *B. anomalus* IFO 0796, type strain), *D. naardenensis* IFO 1588 (type strain), *D. custersiana* IFO 1585 (type strain) and *S. cerevisiae* IFO 2376, respectively.

Based on the sequence data obtained, a phylogenetic tree was drawn by the Neighbor-Joining method [7]. As shown in Fig. 3, *E. nana* was located far from *D. naardenensis* as well as *D. bruxellensis*, *D. anomala* (and *B. anomalus*) and *D. custersiana*. It is noted that *E. nana* was separated phylogenetically from the apiculate yeast species, e.g. *W. fluorescens* (type species), *K. osmophila* (type species [17]) and *H. valbyensis* (type species).

The partial base sequences in positions 493 through 622 (130 bases) of 26S rRNAs of the two strains of *E. nana* are

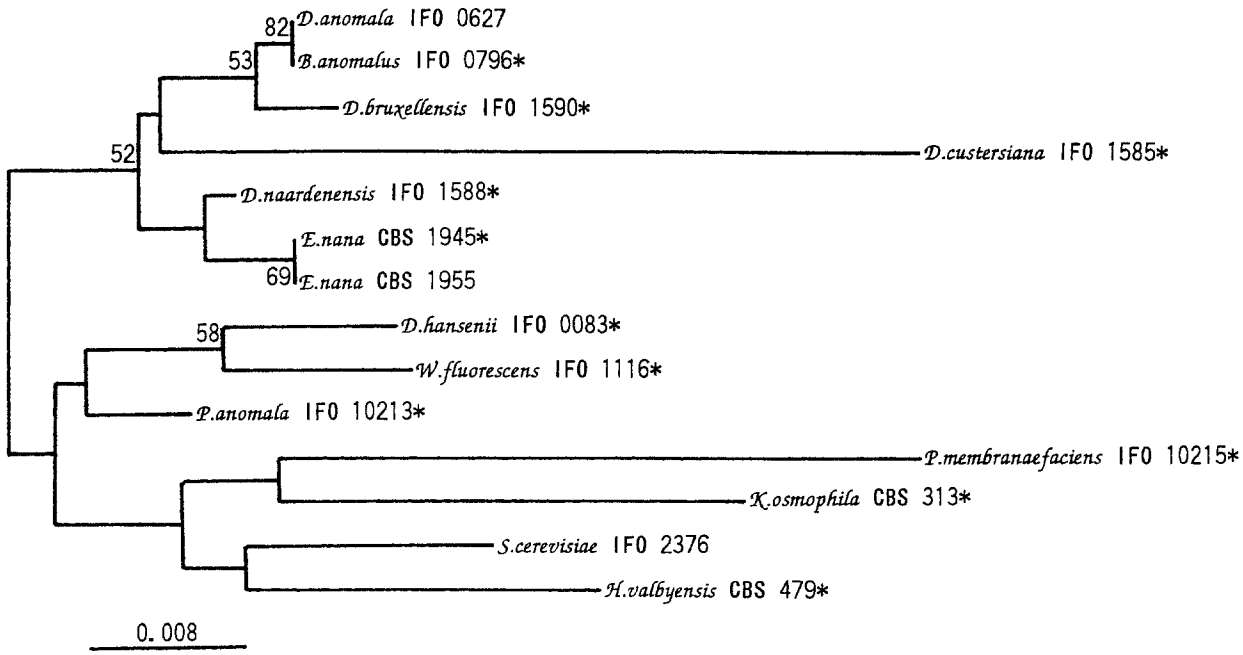

Fig. 2. A phylogenetic tree based on the partial base sequences in positions 1451 through 1618 (168 bases) of 18S rRNAs in *Eeniella nana* and related species. The phylogenetic tree was drawn by the Neighbor-Joining method [7] according to Kimura's two parameters [4]. The numerals indicate the percentages of bootstrap samplings, derived from 1000 samples, supporting the internal branches [2]. The numerals below 50% were omitted here. The partial base sequence data were cited from previous papers [17–21]. *Type strain.

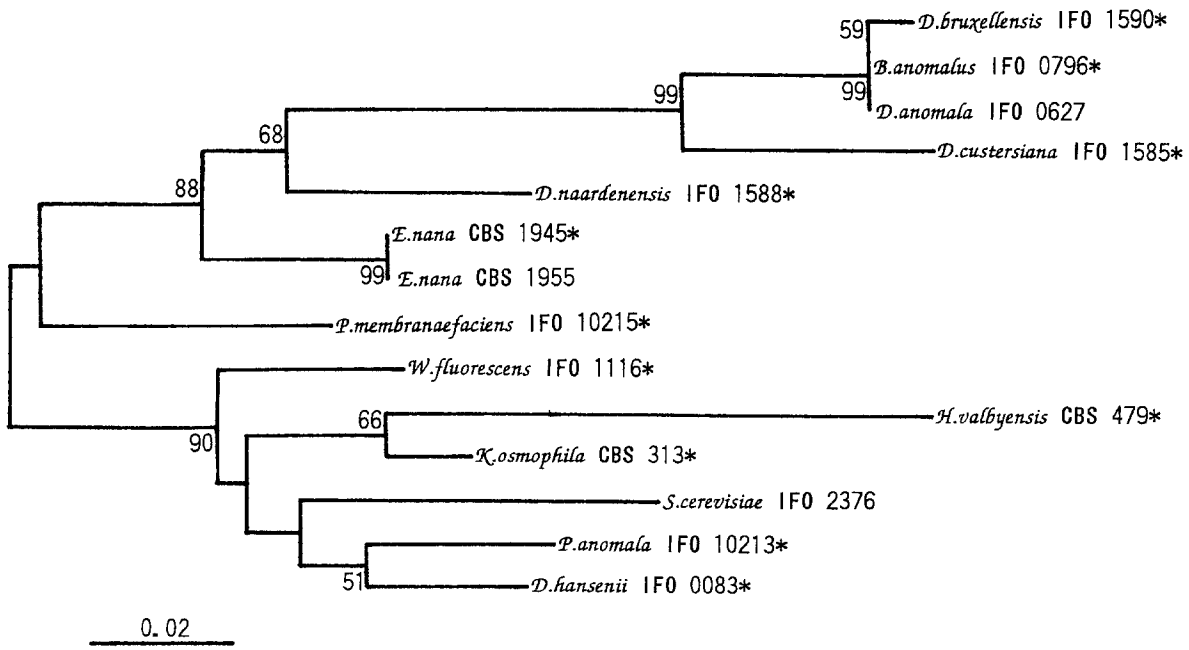

Fig. 3. A phylogenetic tree based on the partial base sequences in positions 1611 through 1835 (225 bases) of 26S rRNAs in *Eeniella nana* and related species. The phylogenetic tree was drawn by the Neighbor-Joining method [7] according to Kimura's two parameters [4]. The numerals indicate the percentages of bootstrap samplings, derived from 1000 samples, supporting the internal branches [2]. The numerals below 50% were omitted here. The partial base sequence data were cited from previous papers [17–21]. *Type strain.

shown in Fig. 1(C). Since base substitutions occurred at higher rates in this region, percent similarities (= maximum homology, %) were calculated among the strains examined by computer analysis using a Hitachi DNAsis (Ver. 7, Hitachi Software Engineering Co., Yokohama, Japan). There was a high percent similarity (92) between the two strains of *E. nana*. The percent similarities of the strains of *E. nana* were calculated to be 53–54, 51–54, 56–57, 51–53 and 60 with *D. bruxellensis* IFO 1590 (type strain), *D. anomala* IFO 0627 (and *B. anomalus* IFO 0796, type strain), *D. naardenensis* IFO 1588

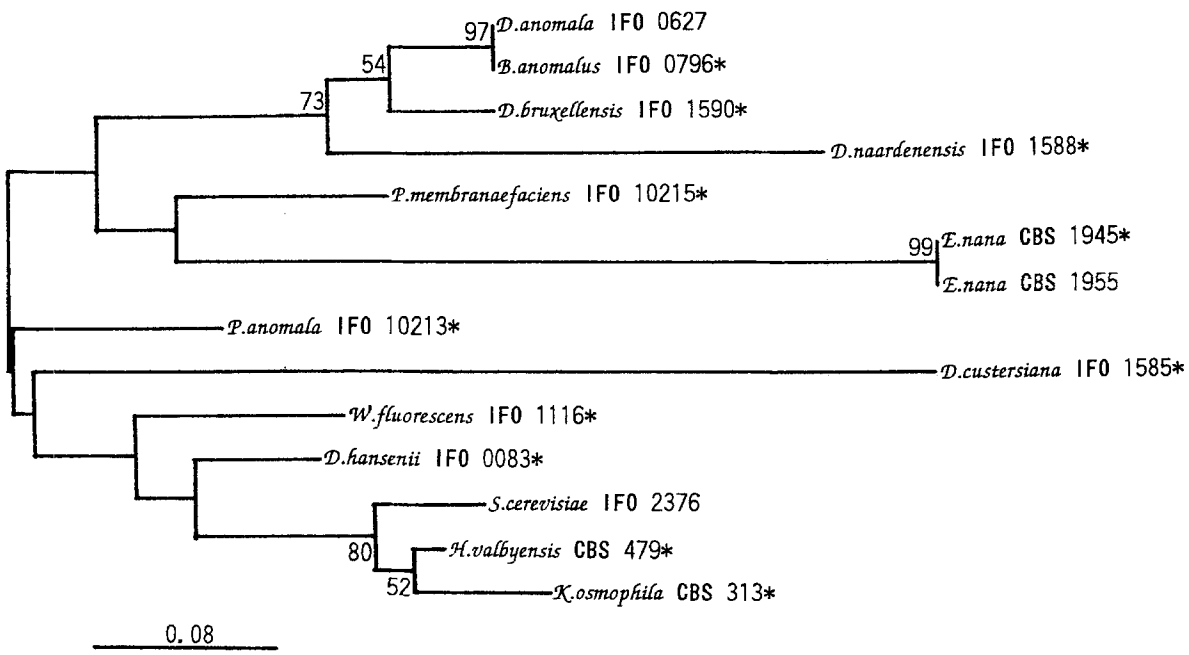

Fig. 4. A phylogenetic tree based on the partial base sequences in positions 493 through 622 (130 bases) of 26S rRNAs in *Eeniella nana* and related species. The phylogenetic tree was drawn by the Neighbor-Joining method [7] according to Kimura's two parameters [4]. The numerals indicate the percentages of bootstrap samplings, derived from 1000 samples, supporting the internal branches [2]. The numerals below 50% were omitted here. The partial base sequence data were cited from previous papers [17–21]. *Type strain.

(type strain), *D. custersiana* IFO 1585 (type strain) and *S. cerevisiae* IFO 2376, respectively.

Based on the sequence data obtained, a phylogenetic tree was drawn by the Neighbor-Joining method [7]. As shown in Fig. 4, *E. nana* was located in a distant position from the four species of the teleomorphic genus *Dekkera* (and the anamorphic genus *Brettanomyces*). The apiculate yeast species, *H. valbyensis* (type species), *K. osmophila* (type species [17]) and *W. fluorescens* (type species) were distributed far from the species of the genera *Eeniella* and *Dekkera* (and *Brettanomyces*).

Smith et al. [9] proposed the genus *Eeniella* for *Brettanomyces nanus* Scheffers nom. inval. [8] based on the differences in reproduction by bipolar budding and the ogival to apiculate form of the cells. The present study has demonstrated that the monotypic genus *Eeniella* (Q-9 [22]) is not so close but rather distant phylogenetically from the species of the apiculate yeast genera *Hanseniaspora* Zikes (Q-6 [15]), *Kloeckeraspora* Niehaus (Q-6 [15]) and *Wickerhamia* Soneda (Q-9 [15]).

Clark-Walker et al. [1] constructed a phylogenetic tree by the mitochondrial cytochrome oxidase subunit 2 sequence analysis of *Eeniella* and *Brettanomyces* (and *Dekkera*) species. *Eeniella nana* was more closely allied to the species of the genus *Brettanomyces* (and *Dekkera*) than *B. custersianus* (≡ *D. custersiana*). Our data gave similar results in the partial base sequence of 18S rRNA. However, *E. nana* was quite different phylogenetically in the 26S rRNA partial base sequencings. *Dekkera anomala* and *D. bruxellensis* (type species) were on the other hand closely related to one another in all the partial base sequencings of 18S and 26S rRNAs [20]. We consider that the genus *Dekkera* should be restricted to the two species mentioned above.

In the 18S rRNA partial base sequencing (positions 1451 through 1618, 168 bases), *E. nana* had five, five, four and eleven base differences compared with *D. bruxellensis* (type species), *D. anomala* (and *B. anomalus*), *D. naardenensis* and *D. custersiana*, respectively. Moreover, the species had great base differences in the 26S rRNA partial base sequencings (positions 1611 through 1835, 225 bases and 493 through 622, 130 bases): the base differences were 46, 43, 34 and 40 and the percent similarities were 53–54, 51–54, 56–57 and 51–53, respectively. These data indicate that *E. nana* is phylogenetically separate and the name of the genus can be retained [14,16] in accordance with the opinion of Smith et al. [10].

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