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A new subaerial alga, *Vischeria hainanensis sp. nov.* (Eustigmatophyceae), from Hainan Island, China

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Abstract

A new subaerial species of Eustigmatophyceae, *Vischeria hainanensis*, is described based on material collected from Hainan Island, China. This species resembles *V. stellata* but is distinguished by its smooth cell wall in early culture stages. Morphological characteristics of *V. hainanensis* are described in detail, and phylogenetic analyses of 18S rRNA and ITS2 sequences place it within a distinct clade, well-separated from other *Vischeria* species. Both morphological and molecular evidence support the recognition of this alga as a new species, thereby increasing the known diversity of subaerial Eustigmatophyceae in China.

Key words: eustigmatophyte algae, morphology, phylogeny, *Vischeria*

Introduction

The genus *Vischeria* was established by Pascher (1938: 553) and subsequently classified within the class Eustigmatophyceae (Hibberd & Leedale 1970). To date, 16 species have been formally described within this genus (Guiry & Guiry 2025), though the biodiversity of this group remains incompletely explored, with many taxa likely yet to be discovered. Morphologically, *Vischeria* species are characterized by cell walls with distinct projections or ridged ornamentations, distinguishing them from the genus *Eustigmatos* D.J. Hibberd (1981:101). However, similar ornamentations have been reported in aged cultures (>6 months) of *Eustigmatos magnus* (J.B. Petersen) D.J. Hibberd (1981: 103) (Temraleeva & Portnaya 2023). The most actively growing vegetative cells of *Vischeria stellata* (Chodat) Pascher (1938:559) in liquid culture develop completely smooth, unornamented walls over time (Gao *et al.* 2016).

The remarkably low level of 18S rRNA gene sequence divergence within the genera *Eustigmatos* and *Vischeria* became increasingly apparent as more strains and species of Eustigmataceae were studied (Přibyl *et al.* 2012, Fawley *et al.* 2014, Fawley & Fawley 2017, Temraleeva & Portnaya 2023). Recent taxonomic revisions, supported by morphological and molecular data, have proposed merging *Eustigmatos* with *Vischeria* (Kryvenda *et al.* 2018, Stoykova *et al.* 2019). Furthermore, Barcytė *et al.* (2022) redefined the family Chlorobotryaceae using multilocus phylogenetic analyses (*rbcL* and 18S rRNA sequences), resulting in the transfer of *Vischeria* from Eustigmatophyceae to Chlorobotryaceae.

Given these ongoing taxonomic uncertainties, integrative approaches combining morphological, ultrastructural, and molecular phylogenetic data are essential for clarifying species boundaries and evolutionary relationships within *Vischeria*.

In this study, we describe a new species of *Vischeria* isolated from tree bark of Hainan Island, China. Using light microscopy (LM), transmission electron microscopy (TEM), and molecular sequence data, we aim to: (1) provide a formal taxonomic description of the new species, and (2) elucidate its phylogenetic position within the updated classification system of Eustigmatophyceae. Our findings contribute to the understanding of the diversity and adaptive evolution of this ecologically and biotechnologically significant algal lineage.

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Material and methods

Sample collection, strain cultivation and examination

The algal strain was isolated from Arecaceae tree bark in Qiongtai Normal University Guilinyang Campus (19°58'46"N, 110°31'23"E), Hainan Island, China. Living specimens of *Vischeria* were isolated with capillary under the inverted microscope (NikonTs2R, Tokyo, Japan). All of the strains were cultured in BG-11 medium (Andersen *et al.* 2005) and were maintained at 23–25℃ under conditions of a 12:12 light: dark cycle at 3000 lux photons from 24-well culture plates. Two distinct strains (QTNU-B1 and QTNU-B3) were isolated, the morphology of cells from cultures was observed daily under a light microscope (NikonNi, Tokyo, Japan). Sample preparation for transmission electron microscope (TEM) analysis was based on the methods of Zhang *et al*. (2013).

DNA extraction, PCR, and sequencing

The total DNA of the strains was extracted using Chelex 100 resin (Beijing Solarbio Science & Technology Co., Ltd.). We amplified and sequenced the 18S small subunit ribosomal RNA (18S rRNA) and internal transcribed spacer2 (ITS2) regions from our strain. Polymerase chain reactions (PCR) were conducted using published primers, reaction mixtures, and amplification protocols (Olmos *et al*., 2000; McManus *et al*., 2011). The PCR products were purified using the TIANgel Purification Kit (TIANGEN BIOTECH (BEIJING) CO., LTD.) and sequenced at BGI Genomics (formerly Beijing Genomics Institute, Beijing, China).

Phylogenetic analysis

The sequences were submitted to the BLAST search program of the National Center for Biotechnology Information (NCBI) to identify closely related sequences. All sequences were downloaded from GenBank and aligned pairwise using the Clustal W algorithm (Thompson *et al.* 1997) implemented in the BioEdit v7.2.1 sequence analysis software (Hall 1999). Prior to alignment, very short sequences (<200 bp) were excluded to ensure data quality. All taxa used in this study, along with their corresponding GenBank accession numbers, are listed in Table S1. Sequences from our strain were submitted to GenBank with the following accession numbers: ITS2 (PV329995, PV329996) and 18S rRNA (PV329997, PV329998). For phylogenetic tree construction, trees based on 18S rRNA genes were rooted with sequences from *Thalassiosira pseudonana*; *Monodopsis unipapilla* served as the outgroup for ITS2—based trees.

In total, 86 1561-bp 18S rRNA gene sequences and 20 257-bp ITS2 gene sequences were included in the analysis. The optimal substitution models for each gene were selected using ModelFinder v1.6.8 (Kalyaanamoorthy *et al.* 2017) with all algorithms and the Bayesian Information Criterion (for ML: ITS2 gene =TNe; 18S rRNA gene = TN+F+R2). Maximum likelihood (ML) phylogenies were constructed with IQ-TREE v1.6.8 (Nguyen *et al.* 2015), incorporating 5000 ultrafast bootstraps and the Shimodaira-Hasegawa-like approximate likelihood-ratio test (SH-aLRT; Guindon *et al.* 2010). All resulting phylogenetic trees were visualized and edited using FigTree v1.4.2 (Rambaut 2014). Analyzing the secondary structure of ITS2 involves using SnapGene v8.2.1 for ITS2 folding and Adobe Illustrator CS6 for visualization operations.

Results

Taxonomy

Class Eustigmatophyceae
Family Chlorobotryaceae
Genus *Vischeria* Pascher

Vischeria hainanensis W. Zhu & X. Jiang ***sp. nov.*** (Fig. 1)

Cells spherical, 7−12 μm in diameter, mostly solitary, occasionally occurring in groups of two or three. Reproduction

occurs by the production of two autospores. Cell wall smooth. Chloroplast parietal, cup-shaped, with a single large pyrenoid.

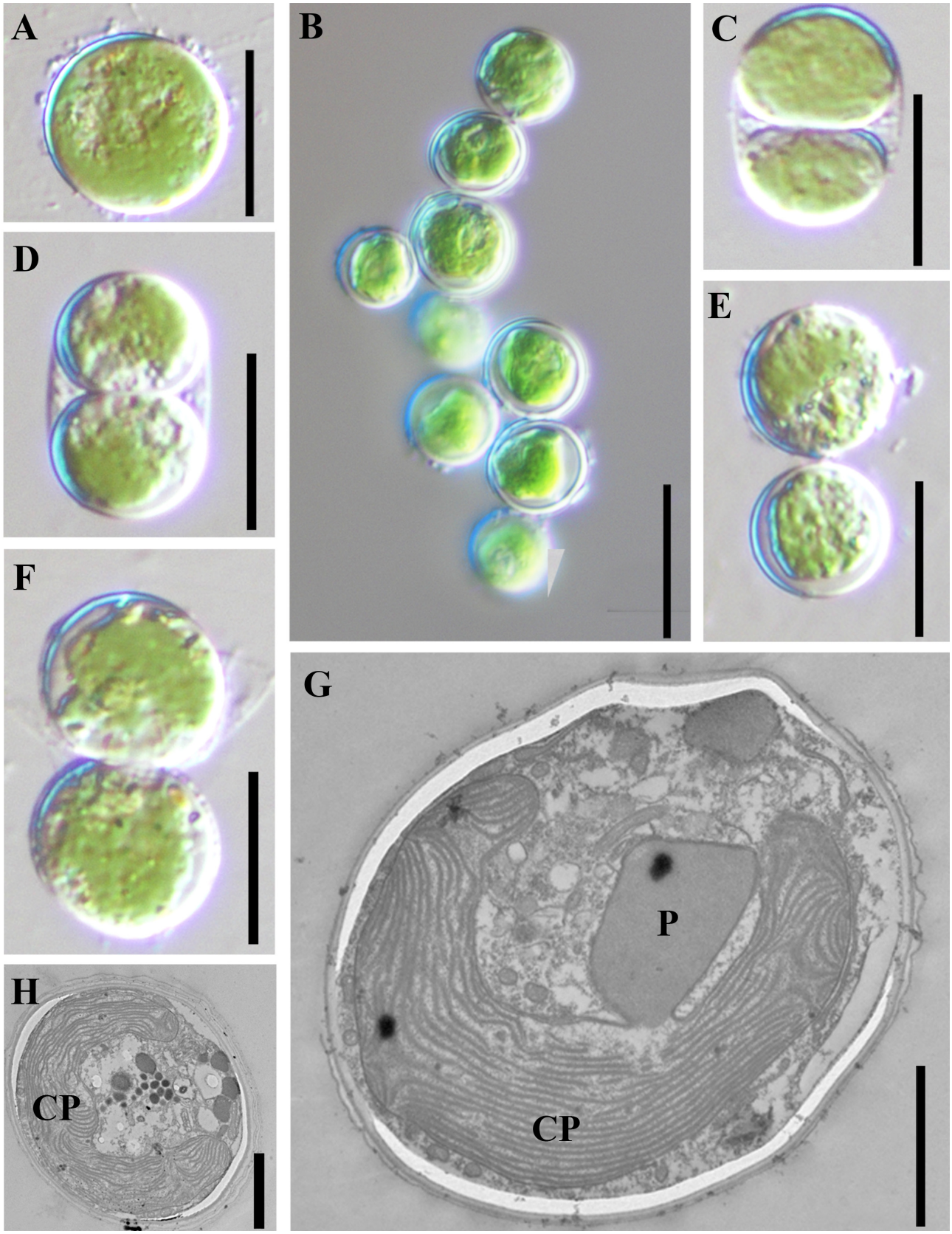

FIGURE 1. Morphology (A−F) and ultrastructure (G−H) of *Vischeria hainanensis*. Pyrenoid (P), chloroplast (CP). Scale bars: A−F—10 µm, G−H—2 µm.

Type:—CHINA. Hainan Island, Guilinyang Campus of Qiongtai Normal University(19°58’32”N, 110°30’44”E), collected from Arecaceae tree bark by Weiju Zhu & Xiaohan Zhu on 9 September 2023. (Holotype: QTNU-B1; isotype: QTNU-B3). Deposited as FACHB–3852 in the Freshwater Algae Culture Collection at the Institute of Hydrobiology Chinese Academy of Science, Wuhan, Hubei Province, China.

Authentic strain:—Deposited as FACHB-3852 in the Freshwater Algae Culture Collection at the Institute of Hydrobiology Chinese Academy of Science, Wuhan, Hubei Province, China.

Etymology:—The specific epithet refers to the location of the holotype, Hainan Island, China.

Morphological characteristics

Vegetative cells of *Vischeria hainanensis* are predominantly spherical in liquid culture (Fig. 1A), occurring singly or rarely in pairs or triplets (Fig. 1B). Cell diameter ranges from 7 to 12 μm. Reproduction occurs through the formation of 2 autospores (Fig. 1C−F). The cell wall is smooth throughout development. Newly released autospores measure approximately 3.5−5.0 μm in diameter.

TEM observations reveal a single large pyrenoid (P) protruding from the inner face of the chloroplast (CP) (Fig. 1G). The chloroplast is parietal and cup-shaped (Fig. 1H). We observed no consistent morphological or ultrastructural differences between strains QTNU-B1 and QTNU-B3 under the conditions examined.

Molecular phylogenetic analysis

Phylogenetic analysis of the 18S rRNA gene confirmed that strains QTNU-B1 and QTNU-B3 belong to the genus *Vischeria* with strong statistical support (100% bootstrap; Fig. 2). Similarly, ITS2-based phylogeny placed both strains within the *Vischeria* clade (Fig. 3). Both phylogenetic trees showed that the two strains we discovered were separated from other species and formed an independent clade.

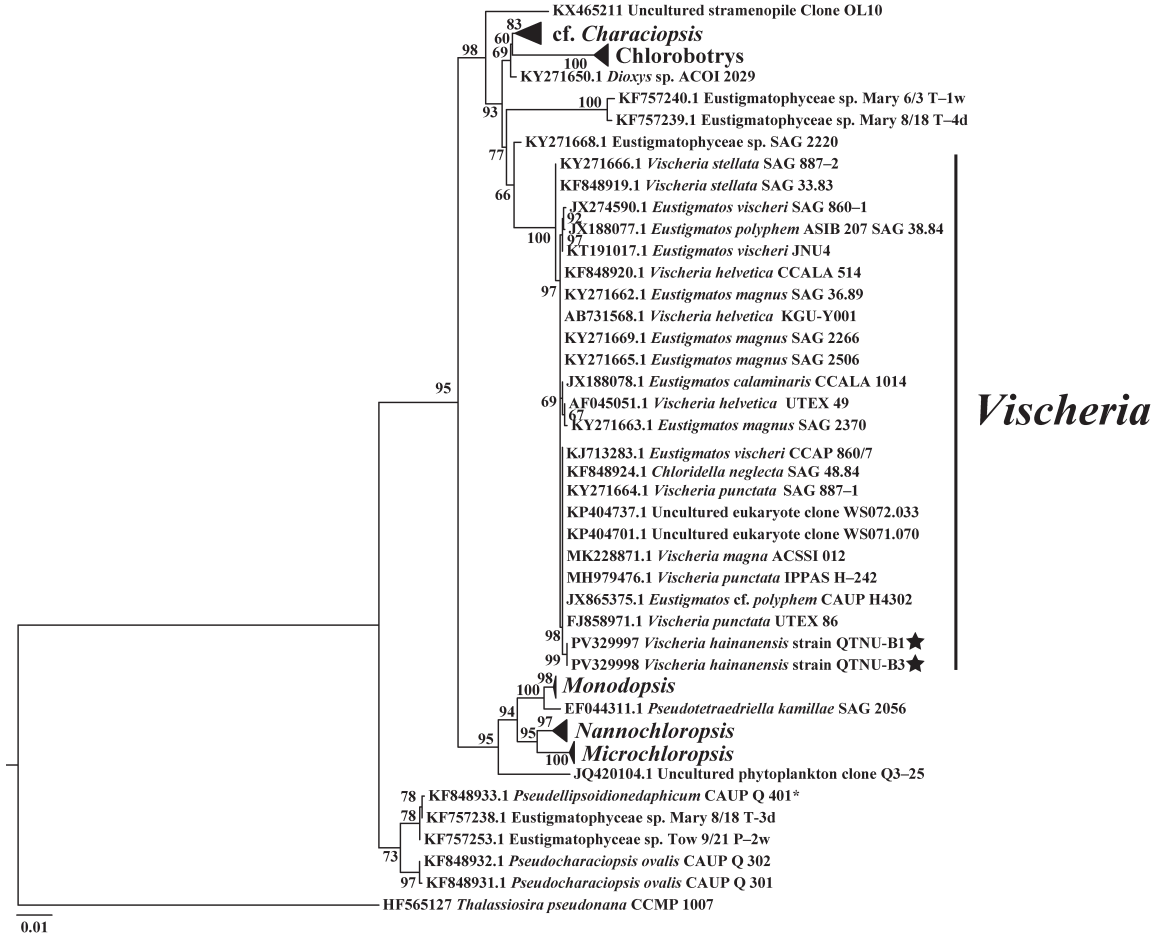

FIGURE 2. ML phylogenetic tree based on 18S rRNA gene. The support values of the analysis are displayed on the branches as the maximum likelihood bootstrap values. The support values <50% were marked with a hyphen (-). Our strains are marked with an asterisk.

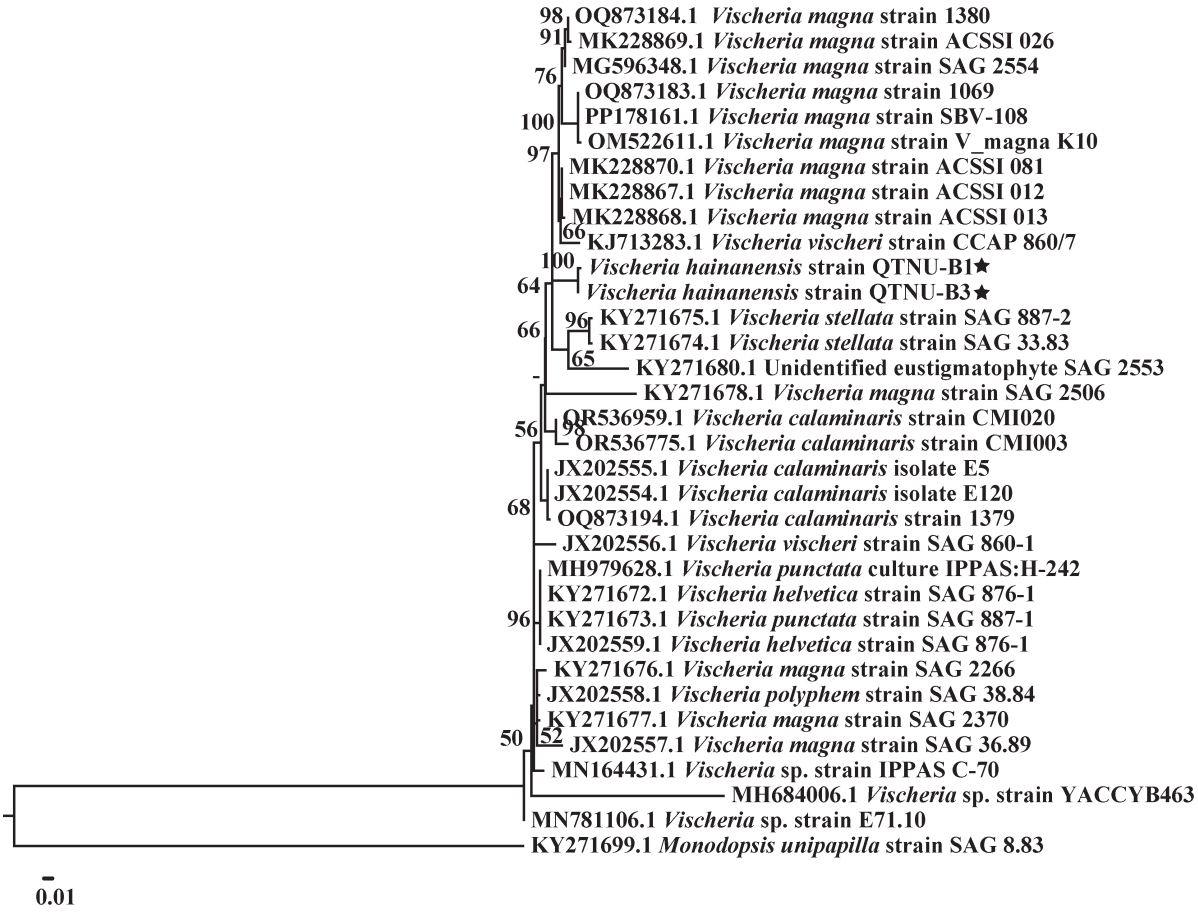

FIGURE 3. ML phylogenetic tree based on ITS2. The support values of the analysis are displayed on the branches as the maximum likelihood bootstrap values. The support values <50% were marked with a hyphen (-). Our strains are marked with an asterisk.

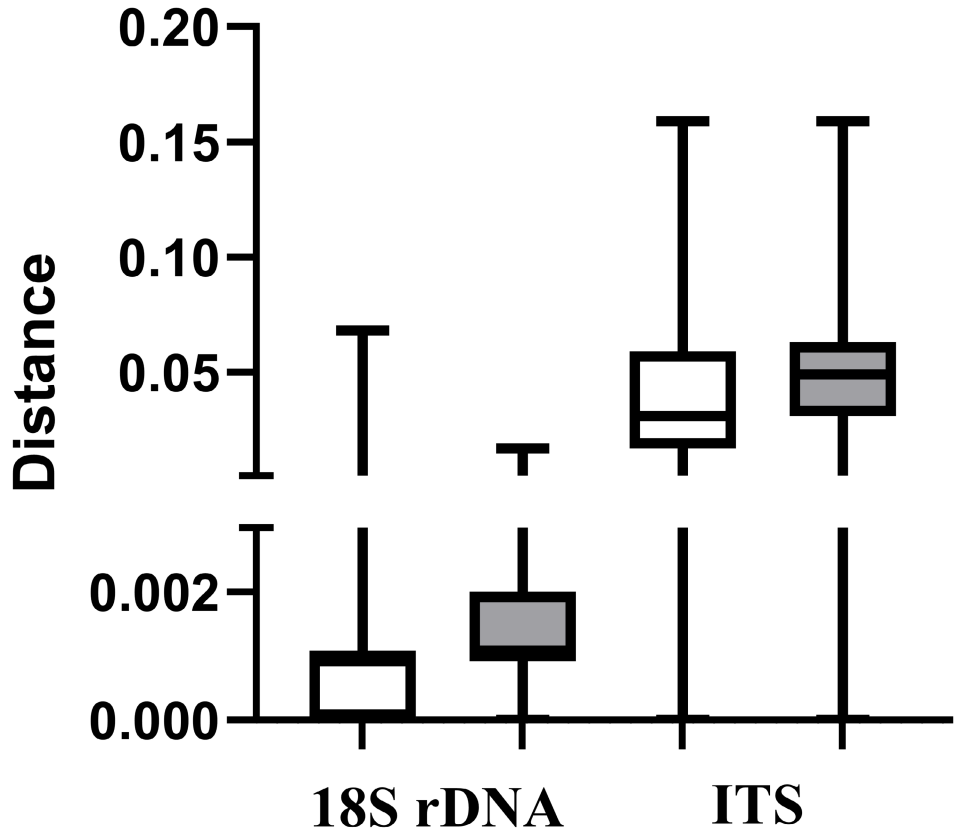

FIGURE 4. Intraspecific paired p-distances (white bars) and interspecific paired p-distances (grey bars) of *Vischeria* strains.

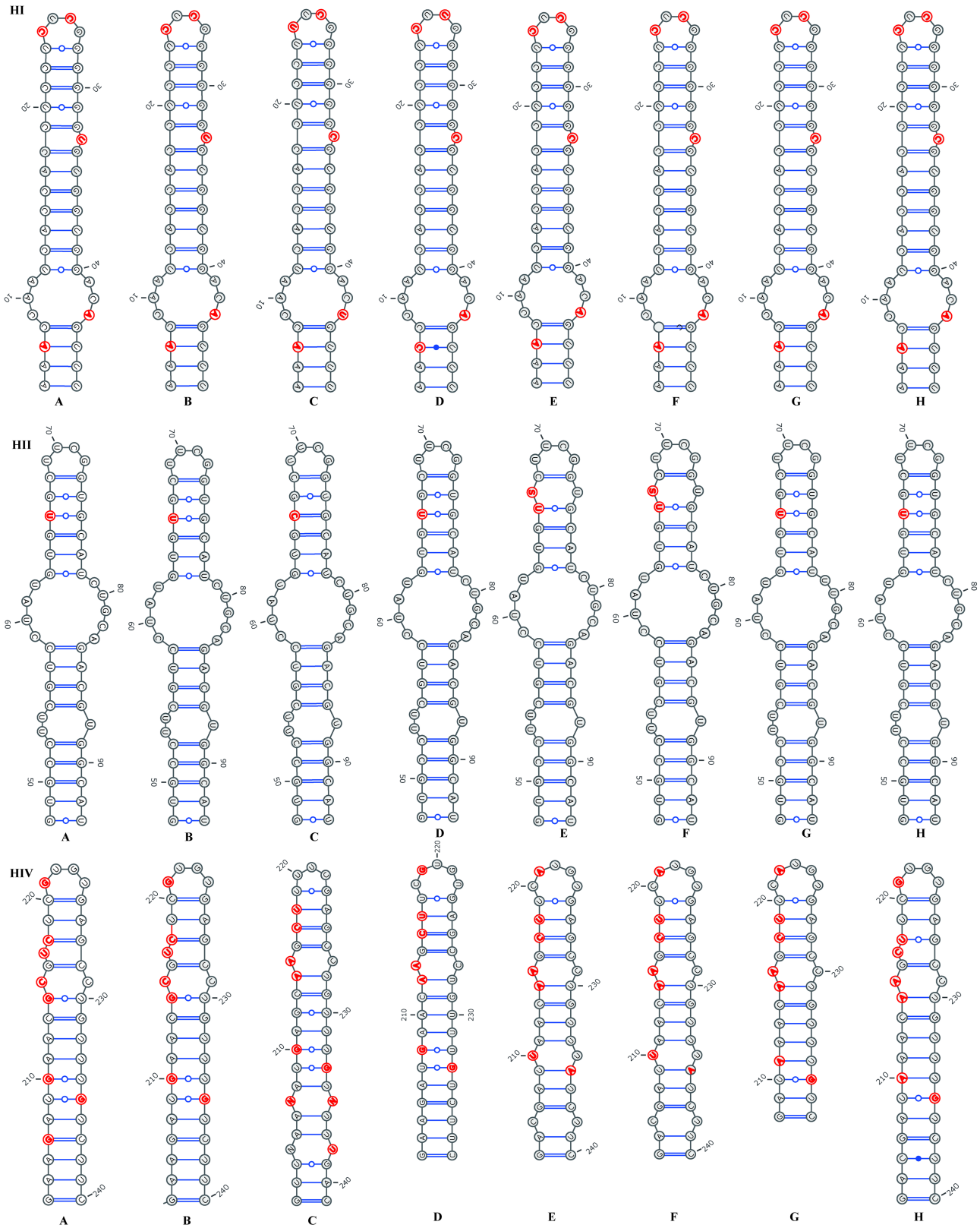

FIGURE 5. Comparison of the secondary structures of ITS2 hairpin structures I, II and IV among strains of the genus *Vischeria* (A: *V. hainanensis* strain QTNU—B1; B: *V. hainanensis* strain QTNU—B3; C: *V. stellata* strain SAG 887-2; D: *V. magna* strain SAG 2554; E: *V. helvetica* strain SAG 876-1; F: *V. punctata* strain SAG 887-1; G: *V. vischeri* strain SAG 860-1; H: *V. calaminaris* isolate E120).

The new species clustered into a distinct monophyletic clade, phylogenetically separated from all other examined *Vischeria* strains (Fig. 4). Sequence divergence analyses based on the 18S rRNA gene revealed intraspecific pairwise distances ranging from 0 to 0.001 and interspecific distances from 0 to 0.003 among *Vischeria* strains. For the ITS2

region, both intraspecific and interspecific pairwise distances exhibited broader ranges of 0–0.159. Specifically, the genetic divergence between *V. hainanensis* and other *Vischeria* species was 0.001–0.003 for the 18S rRNA gene, while ITS2 sequence distances ranged from 0.049 to 0.302. Notably, the lack of significant differentiation in pairwise distances between the new species and known congeners may primarily stem from the unresolved phylogenetic positions of many *Vischeria* species.

ITS secondary structure analysis

A comparative analysis was conducted on the ITS2 secondary structures of two new strains (QTNU-B1, QTNU-B3) and all *Vischeria* species (*Vischeria magna* strain SAG 2554, *Vischeria helvetica* strain SAG 876-1, *Vischeria punctata* strain SAG 887-1, *Vischeria stellata* strain SAG 887-2, *Vischeria vischeri* strain SAG 860-1, *Vischeria calaminaris* isolate E120). The ITS2 secondary structure conformations of the two new strains were the same. In contrast, when comparing the new strains with other *Vischeria* species, inter-specific differences were observed in all four helical domains (helices I–IV) (Figs.5, 6). Half-compensatory base changes (hCBC) were detected between the two new strains and other strains. Specific nucleotide polymorphisms included C→U, U→C, and A→C substitutions within helix I between the new strains and *V. stellata*, as well as a U→C substitution in helix II. There were C→U, U→C, and A→C substitutions within helix I between the new strains and *V. magna*, and a U→C substitution within helix I between the new strains and *V. helvetica*, *V. punctata*, *V. vischeri*, *V. calaminaris*. Mutations in the unpaired stem region and the apical loop of helix IV were observed between the two new strains and other strains (Fig. 5). A U→C substitution was found within the basal loop of helix III between the new strains and other strains (Fig. 6).

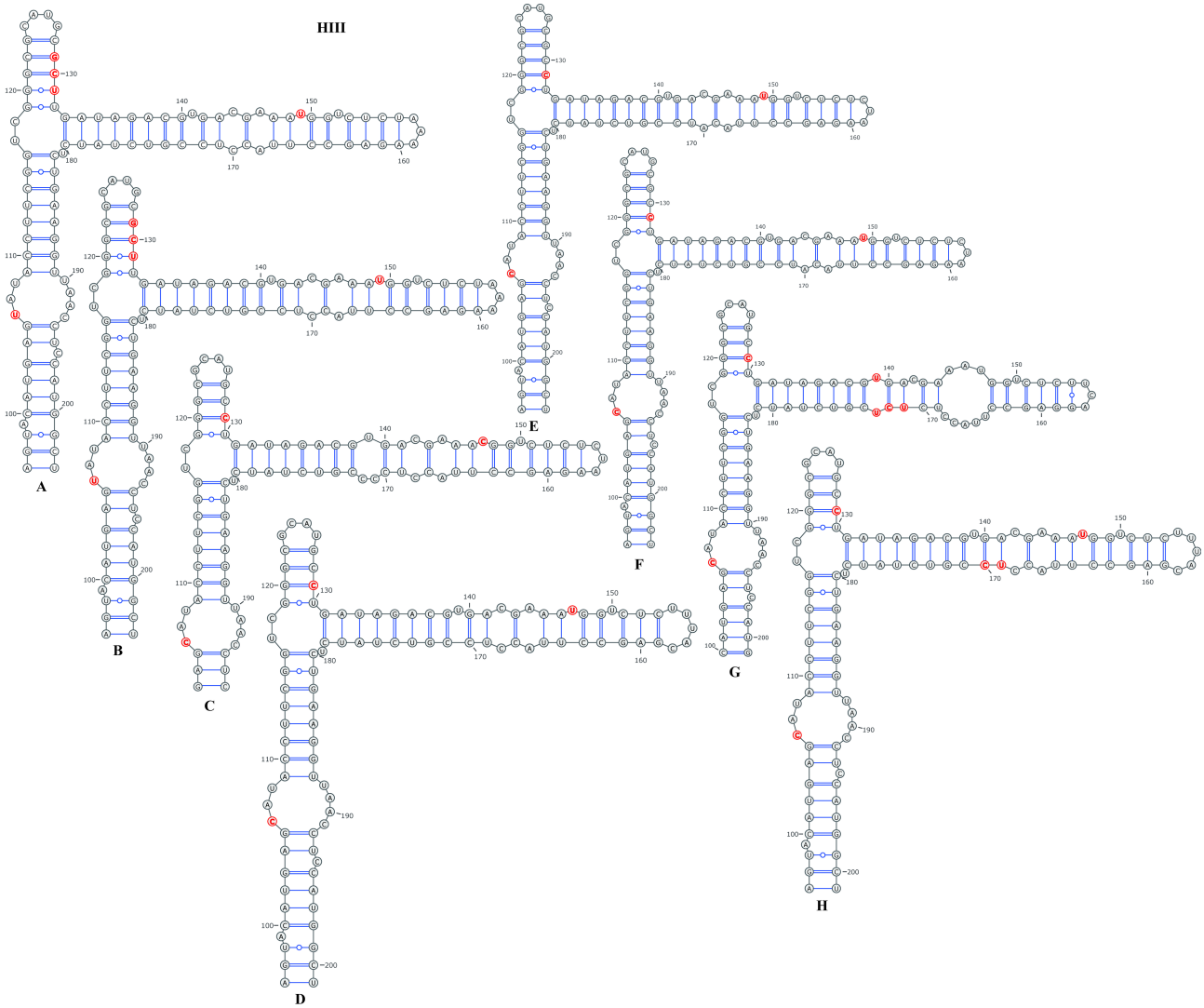

FIGURE 6. Comparison of the secondary structures of ITS2 hairpin structures III among strains of the genus *Vischeria* (A: *V. hainanensis* strain QTNU-B1; B: *V. hainanensis* strain QTNU-B3; C: *V. stellata* strain SAG 887-2; D: *V. magna* strain SAG 2554; E: *V. helvetica* strain SAG 876-1; F: *V. punctata* strain SAG 887-1; G: *V. vischeri* strain SAG 860-1; H: *V. calaminaris* isolate E120).

Discussion

Combined morphological and molecular evidence supports the recognition of strains QTNU-B1 and QTNU-B3 as a new species, herein described as *Vischeria hainanensis sp. nov.* This finding enhances the known diversity of *Vischeria*, a genus of increasing biotechnological significance due to its potential for producing high-value compounds, including eicosapentaenoic acid (EPA) and palmitoleic acid (POA) (Zhang *et al*., 2013, Gao *et al*., 2016, Huang *et al*., 2019, Stoykova *et al*., 2019, Gao *et al*., 2023, Krivina *et al*., 2024, Sidorov *et al*., 2024). *V. hainanensis* shares morphological similarities with *V. stellata*, but can be distinguished by its smooth cell wall in early culture stages-a notable deviation from the typical projections or ridges observed in other *Vischeria* species (Hibberd 1981, Gao *et al*. 2016). Additional distinguishing features include its cup-shaped chloroplast and stable pyrenoid morphology (Table 1).

TABLE 1. Comparison of the cell morphology of between the circular *Vischeria* species and *V. hainanensis*.

Species	*V. calaminaris*	*V. magna*	*V. polyphem*	*V. stellata*	*V. vischeri*	*V. hainanensis*
Cell shape	globose to oval	spherical	spherical	oval to spherical	spherical	mostly spherical
Cell wall	smooth	smooth	smooth	projections emerged during early culture	smooth	mostly smooth
Cell size	7−9(12) μm in diameter, large cells (18–30 μm) occasional	10−11 μm in diameter, with a maximum of 15 μm	9−18 μm in diameter	5−12 μm in diameter, maximum of 35 μm	7−9 μm in diameter	7−12 μm in diameter
Chloroplast	single, parietal, massive, cup-shaped and lobed	single lobed	single parietal and deeply lobed	single parietal, lobed	single parietal, lobed	single parietal and cup-shaped
Pyrenoid	polyhedral pyrenoid	polyhedral shape	polyhedral shape	single large pyrenoid protruding from the inner face of chloroplast by forming a small stalk	stalked polyhedral pyrenoid	single large pyrenoid protruding from the inner face of chloroplast

Phylogenetic analyses of both 18S rRNA and ITS2 sequences confirm the distinct phylogenetic position for recognizing strains QTNU-B1 and QTNU-B3 as a novel species, for which the name *V. hainanensis* is proposed. This finding adds to the understanding of biodiversity within the Eustigmatophyceae, particularly in under-explored geographical regions. The phylogenetic analysis places the newly identified *V. hainanensis* within a closely related group containing several established species, notably *V. stellata*, *V. calaminaris, V. vischeri*, and *V. magna*. A significant and consistent morphological trait uniting these species is their characteristic spherical (or globose to oval) cell shape (Table 1). This correlation between molecular phylogeny and conserved morphology is noteworthy. Analysis of the ITS2 secondary structure revealed that *V. hainanensis* shares a conserved four—helix structure with other *Vischeria* species, but specific base variations were detected in Helices I to IV, including multiple hCBCs and mutations in non—paired regions. These significant structural differences further support its independence as a new species and also reflect the diversity in the molecular structural evolution of species within the genus *Vischeria*. These results are consistent with recent studies suggesting a need for taxonomic re-evaluation of the genus using integrative approaches (Kryvenda *et al.* 2018, Temraleeva & Portnaya 2023).

Ecologically, *V. hainanensis* was isolated from tree bark, unlike many congeners typically found in soil (Temraleeva & Portnaya, 2023), suggesting adaptation to specific microhabitats. This ecological specificity may reflect niche differentiation and contribute to genetic and morphological divergence. Further studies on its physiology and ecology will provide deeper insights into the evolutionary mechanisms within this group.

Conclusions

The designation of *V. hainanensis* as a new species within the genus *Vischeria* is substantiated by both morphological characteristics and molecular phylogenetic analyses. Further studies incorporating a broader sampling of *Vischeria* taxa

are necessary to reconstruct a more comprehensive phylogenetic framework and elucidate the evolutionary history of the genus.

Acknowledgements

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