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Original Article

First record of the genus *Hildenbrandia* (Florideophyceae: Hildenbrandiales) from French Polynesia and description of *H. tahitiensis* sp. nov.

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ABSTRACT

Freshwater specimens of an encrusting red alga were collected from three rivers in Tahiti, French Polynesia. Observed morphological features corresponded to those of the genus *Hildenbrandia* (Florideophyceae: Hildenbrandriales), confirmed by genetic data. *rbc*L and 18S rDNA sequences of the Tahitian specimens were 4.95–5.15% and 0.94%, respectively, identical to those of a specimen from the Philippines. The Tahitian and Philippine lineages formed a well-supported monophyletic clade, which positioned basally to the rest of the freshwater *Hildenbrandia* clade. Tahitian specimen cells were 7.6–16.2 μm in diameter in surface view, were rectangular in cross section, were 11.6–16.4 μm long and 2–3 μm high, and presented filaments 37–240 μm long, occasionally branching. Although the cells were overall wider than in other freshwater differences were observed between the Tahitian and other species of *Hildenbrandia*. The genetic divergences and longer cell sizes between the Tahitian and Philippine lineages strongly indicated they were not conspecific. A formal description of the new species, *Hildenbrandia tahitiensis* sp. nov., is provided. This is the first report of the genus *Hildenbrandia* from French Polynesia. The present documentation of *Hildenbrandia* in a south central Polynesian locality extends our knowledge of the geographical range for this genus.

Introduction

Freshwater members of the genus *Hildenbrandia* represent a total of five taxonomically accepted species distributed worldwide (Guiry and Guiry 2021): *H. angolensis* Welwitsch ex West and G.S.West (global distribution), *H. cuprea* (Hansgirg) Caisová and J.Kopecký (Austria and the Czech Republic), *H. jigongshanensis* F.Nan et al. (China and Japan), *H. ramanaginae* M.Khan (India), and *H. rivularis* (Liebmann) J.Agardh (global distribution). In accordance with the literature, two species present a widespread distribution on a global scale, *H. angolensis* (Europe, Africa, and Pacific Islands) and *H. rivularis* (Europe, Caribbean Islands, South America, Africa, Middle East, South-East Asia, and Asia), whereas the remaining four were only reported locally from the type locality and nearby (Sherwood and Sheath 2003; Vieira et al. 2021).

Phylogenetic data, however, indicate that populations labeled under either of these two epithets, *H. angolensis* and *H. rivularis*, constitute polyphyletic lineages, which are far less widespread than presently presumed based on these two names. Recent DNA-based studies described as new species some of these lineages previously labeled under the epithet *H. rivularis* based on morphological data (Caisová and Kopecký 2008; Nan et al. 2017; Nan et al. 2019; Vieira et al. 2021). *Hildenbrandia* has a rather simple morphology with few diagnostic features and expresses large morphological variation (Sherwood and Sheath 2003; Vieira et al. 2021). Molecular approaches are therefore a needed tool in *Hildenbrandia* taxonomy.

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Table 1. Morphological features of *Hildenbrandia tahitiensis* sp. nov. (Florideophyceae: Hildenbrandiales) and other freshwater species of *Hildenbrandia*.

Species	Cell diameter	Cell length	Filament size	Country	Reference
***H. tahitiensis* sp. nov.**	**7.6–16.2**	**11.6–16.4**	**37–340**	**Tahiti**	**This study**
H. angolensis	5.5	4.6	44.4	Philippines	Sherwood and Sheath (2003)
H. angolensis	3.5–5	-	-	Angola	West and West (1897)
H. cuprea	4–6	-	-	Czech Rep.	Caisová and Kopecký (2008)
H. jigongshanensis	8.4	10.5	308–491	Japan	Nan et al. (2019)
H. jigongshanensis	3–6.4	5–6.3	275–325	Japan	Vieira et al. (2021)
H. jigongshanensis	9.8–19.6	9.9–10.4	364–409	China	Nan et al. (2017)
H. ramanaginae	4–6	7.5–16	80	India	Kahn (1974)
H. rivularis	4.5–7	4.5–9.5	-	China	Shi (2006)
H. rivularis	5.2	5.7	59.1	Europe	Sherwood and Sheath (2000)
H. rivularis	-	-	3.5–4	Angola	West and West (1897)

In bold, data for *H. tahitiensis* sp. nov. from Tahiti, French Polynesia. Cell and filament measurements are given in μm.

Two markers of reference are now being used for this genus, namely the nuclear small subunit 18S ribosomal RNA (abbreviated 18S rDNA) and the chloroplast ribulose-1,5-biphosphate carboxylase large subunit (*rbc*L) gene. Phylogenies based on these two markers (Sherwood and Sheath 2003) indicate that *Hildenbrandia* may encompass a greater diversity than presently known. So far, only two accepted species were described using molecular data, *H. cupreana* and *H. jigongshanensis* (=*H. japananensis*). International nucleotide sequence databases (GenBank, DDBK, and ENA) include three names among the five currently accepted freshwater species: *H. angolensis, H. rivularis*, and *H. jigongshanensis.* Nevertheless, the polyphyletic positions of *H. angolensis* and *H. rivularis* sequences in *Hildenbrandia* phylogenetic trees (Sherwood and Sheath 2003) raise uncertainty on the genetic identity of the genuine *H. angolensis* and *H. rivularis*. Further molecular work coupled with morphological analyses is needed to reassess the diversity of freshwater *Hildenbrandia.*

Based on the literature, *H. angolensis* is the only species reported so far from the Pacific Islands (the Hawaiian Islands only) (Carlile and Sherwood 2013), but no molecular data were yet generated to confirm this taxonomic identification and conspecificity with the species, *H. angolensis*, originally described from Angola. No reports of freshwater *Hildenbrandia* were made elsewhere in the Pacific Islands including French Polynesia. Marine species of *Hildenbrandia* have neither been reported from French Polynesian islands.

A crustose red alga was collected in 2019 and 2021 from three rivers in Tahiti (the Society Islands, French Polynesia). The morphology and phylogenetic position of these specimens are addressed in this study.

Material and methods

Taxon sampling

Freshwater *Hildenbrandia* specimens were collected from three different rivers in Tahiti, French Polynesia: Tuauru river (PF1507; Māhina; 17°31’31.8"S 149°29’17.7"W) on August 30th, 2019, Fautaua river (PF1508; Pīra’e; 17°33’53.3"S 149°32’17.5"W) on August 16th, 2019, and Mahateaho river (PF1767; Vallée Paraura; 17°37’02.6"S 149°19’56.0"W) on April 5th, 2021. Algal specimens were preserved in silica gel and RNA later for molecular analyses and kept in fresh

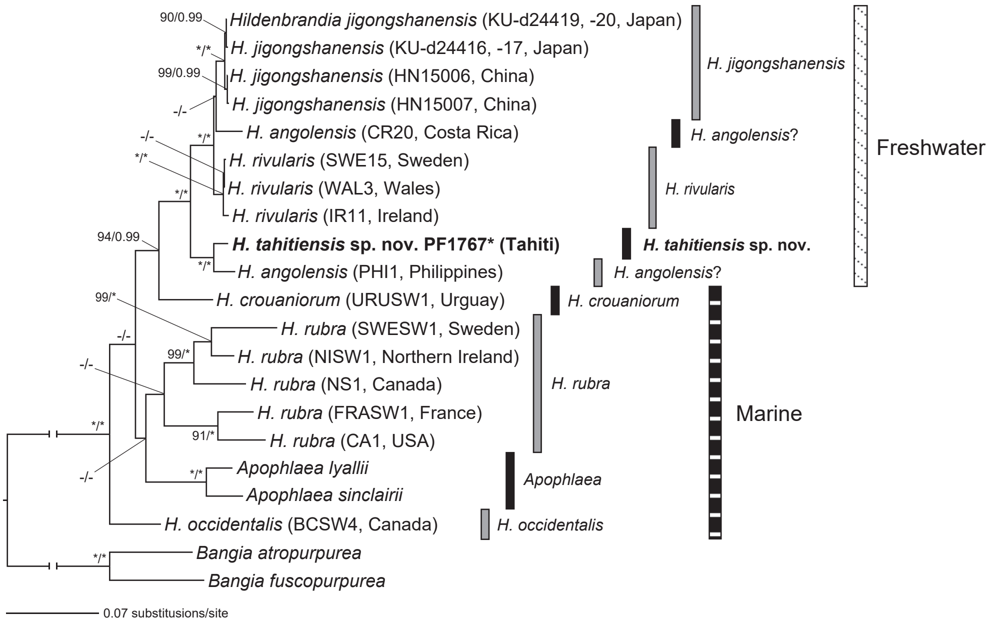

Figure 1. Phylogenetic tree of the red algal genus *Hildenbrandia* (Florideophyceae: Hildenbrandiales). The tree is the maximum likelihood phylogenetic tree obtained from a RAxML analysis of the concatenated alignment of *rbc*L+18S rDNA sequences (2474 bp) from 22 specimens. Numbers at nodes indicate bootstrap values (BP; right numbers) and Bayesian posterior probabilities (PP; left numbers) obtained from an MrBayes analysis. The asterisk (*) indicates 100% BP and 1.00 PP in maximum likelihood and Bayesian analyses, respectively. Only the BP ≥70% and PP ≥0.90 are shown.

water for morphological analyses at the laboratory. Herbarium preserved specimens were deposited at the University of French Polynesia Herbarium (UPF), registered in the Index Herbariorum (Thiers 2021).

Molecular phylogenetic reconstruction

Genomic DNA was extracted from the material using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The *rbc*L gene and 18S rDNA were amplified from the Tahitian samples using the same conditions and primer sets described in Sherwood and Sheath (2003) and Vieira et al. (2021). The PCR products were purified by ExoSAP-IT (Applied Biosystems) following the manufacturer's protocol and were sequenced using the BigDye Terminator v.3.1 Sequencing Kit (Applied Biosystems) and the 3130xl DNA Analyzer (Applied Biosystems) at Ochanomizu University. The obtained DNA sequences were manually aligned using AliView (Larsson 2014) and deposited in GenBank (Table S1).

To infer phylogenetic position of the Tahitian *Hildenbrandia* samples, 20 specimens, representative of the genetic diversity of

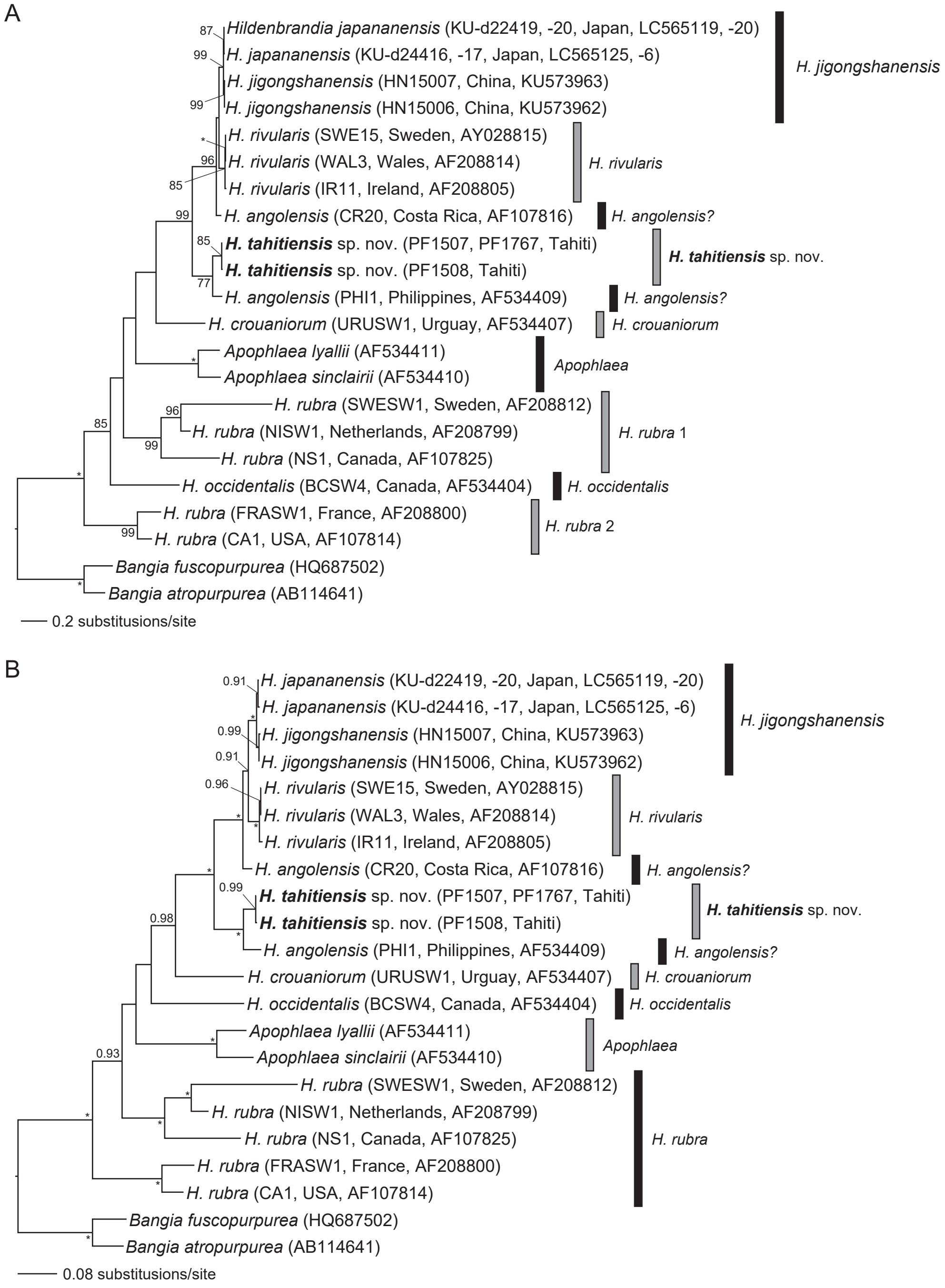

Figure 2. Phylogenetic trees of the red algal genus *Hildenbrandia* (Florideophyceae: Hildenbrandiales): A, maximum likelihood; B, Bayesian phylogenetic trees of *Hildenbrandia* inferred based on *rbc*L sequences (1010 bp) from 22 specimens, using RAxML and MrBayes analyses, respectively. Numbers at nodes indicate bootstrap values (BP; in A) and posterior probabilities (PP; in B). The asterisk (*) indicates 100% BP and 1.00 PP in ML and BI analysis. Only the BP ≥70% and PP ≥0.90 are shown. BI = Bayesian; ML = maximum likelihood.

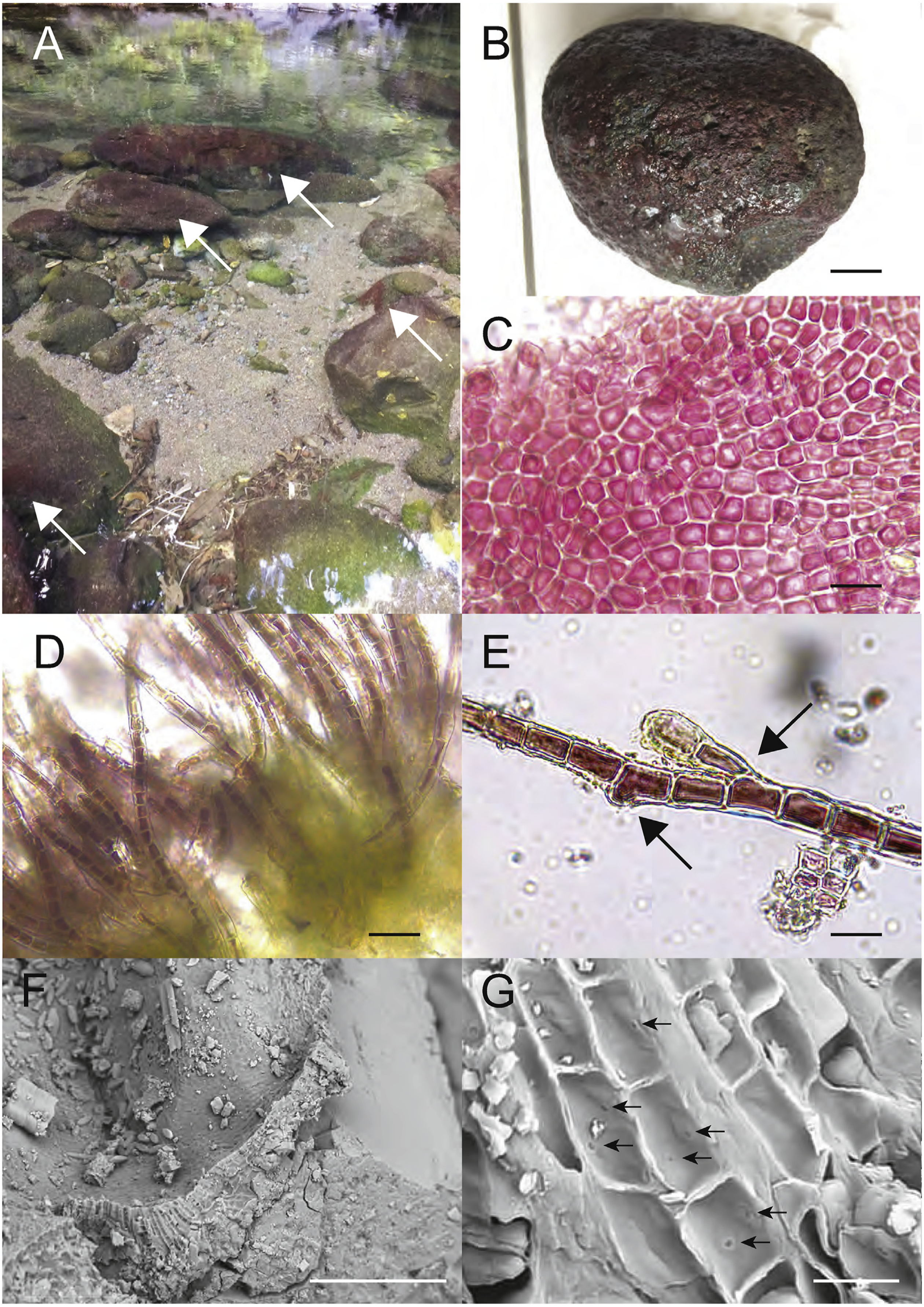

Figure 3. *Hildenbrandia tahitiensis* sp. nov. ecological habits, gross morphology, and anatomy. Voucher PF1507 from Tahiti, the Society Islands, French Polynesia: A, ecological habits growing on rocks in clean rivers (indicated by white arrows); B, external morphology; C, thallus surface view observed under the microscope; D–E, filaments observed under the microscope (branching indicated by black arrows); F–G, scanning electron micrographs of cross section through thallus showing lithophytic habit and multiple layer structure (F) and primary and secondary pit-plug structures (indicated by black arrows; G). <scale bar: 2 cm (B); 40 μm (C–D); 10 μm (E); 100 μm (F); 5 μm (G).>

Hildenbrandiales, were selected from our previous data set (Table 1) (Vieira et al. 2021). *Bangia atropurpurea* (Mertens ex Roth) C.Agardh and *B. fuscopurpurea* (Dillwyn) Lyngbye were selected as out-groups based on previous phylogenetic analysis on freshwater Rhodophyta (Nan et al. 2017). Phylogenetic analyses of the aligned sequences (22 specimens, 2474 bp) were subjected to maximum likelihood (ML) and Bayesian (BI) analyses. The GTR + I + G nucleotide substitution model, identified as the best-fit evolutionary model in Kakusan4 (Tanabe 2011), was used in both, ML and BI, analyses. The ML phylogeny was inferred using RAxML v.8.2.9 (Stamatakis 2014) using 10,000 bootstrap replicates. The BI phylogeny was inferred using MrBayes v.3.2.2 (Ronquist et al. 2012). The Bayesian analysis was initiated with a random starting tree and ran four chains of Markov chain Monte Carlo iterations simultaneously for 10,000,000 generations, sampling one tree every 1000 generations. The first 10,000 trees sampled were discarded as burn-in, based on the stationarity of log-likelihood as assessed in Tracer v.1.7.1 (Rambaut et al. 2018). A consensus topology and posterior probability values were calculated from the remaining trees.

Morphological analyses

Morphological observations of *Hildenbrandia* specimens consisted in measurements of cell size and filament height. Fresh epilithic *Hildenbrandia* material was manually scraped off stone surfaces with the help of scalpel blades. Microscopic observations were made under a BX51 Olympus microscope (Olympus, Tokyo, Japan) or a Leica MZ6 light microscope (Leica Microsystems, Wetzlar, Germany). In addition, scanning electron microscope photographs were taken using a Hitachi TM 3030 desktop microscope (Hitachi Ltd. Tokyo, Japan), operated at a voltage of 15 kV (in charge-up reduction mode), at the University of French Polynesia, to observe cells and pit-plug structures in cross section. Dried specimens were air-dried and mounted on aluminum stubs with conductive silver paste, sputter coated with gold-palladium with the eMSCoP SC 500 sputter coater.

Results and discussion

Trees' inference and species diversity

Among *Hildenbrandia* specimens (PF1507, PF1508, and PF1767) from Tahiti, PF1507 and PF1767 had identical sequences in 1010 bp of *rbc*L, and the sequence of PF1508 was 3 bp different from PF1507 and PF1767. We were only able to obtain 18S rDNA sequence from PF1767 (Table S1). Consistent with previous studies (e.g. Sherwood and Sheath 2003; Vieira et al. 2021), freshwater lineages formed a monophyletic clade, which included the Tahitian lineage. In the *rbc*L+18S rDNA concatenated-based trees (Figure 1), as well as in the *rbc*L-based trees (Figure 2), the Tahitian lineage formed a well-supported monophyletic group with a freshwater lineage from the Philippines, labeled as *H. angolensis* (Sherwood and Sheath, 2003), and positioned basally to the rest of the freshwater clade composed of *H. jigongshanensis*, *H. angolensis*, and *H. rivularis* (Figures 1, 2). The genetic divergences for *rbc*L (1010 bp) and 18S rDNA (1387 bp) sequences, between the Tahitian and Philippine specimens, were 4.95–5.15% and 0.94%, respectively. When we compared the divergence between other species in Hildenbrandiales using the available overlapping region, the range was 5.08–18.88% in 964 bp of *rbc*L and 0.28–11.76% in 1387 bp of 18S rDNA.

Ecological and morphological traits

The external and internal morphologies of algal specimens collected in this study presented the generic criteria of *Hildenbrandia*. The specimens consisted of epilithic, thin rose-red to brown-red, smooth thalli with irregular borders firmly attached onto the surface of stones (Figure 3A–B and F). The thalli consisted of rectangular to oval cells in surface view, 7.6–16.2 μm in diameter (Figure 3C), and rectangular cells in cross section (Figure 3G), 11.6–16.4 μm long and 2–3 μm high. Filaments, occasionally branching (Figure 3D–E), were 37–240 μm long, composed of 4–26 cells, 7–15 μm in length, and 5–8 μm in width. Cell lengths of specimens collected in this study were considerably longer than in any other species of freshwater *Hildenbrandia* (Table 1). Scanning electron microscope photographs clearly showed primary and secondary pit-plugs (Figure 3G; black arrows). In spite of dedicated search efforts in numerous samples, no reproductive structures were observed.

Taxonomic results

The genetic divergence between *rbc*L and 18S rDNA sequences of *Hildenbrandia* sp. (labeled as *H. angolensis*) from the Philippines and *Hildenbrandia* specimens from Tahiti and the substantially longer cell sizes indicate that the specimens from Tahiti represent a new species. A formal description of the new species is provided.

Hildenbrandia tahitiensis C.W.Vieira, Yi S.Kim, and M.Zubia, sp. nov.
LSID: http://phycobank.org/102820
(Figure 3)

Type. Holotype: UPF 5509 (PF1507), 17°31'31.8"S 149°29'17.7"W; Tuauru river, Māhina, Tahiti, French Polynesia 20 viii 2019 (C Vieira and YS Kim). UPF. Paratypes: UPF 5510 (PF1508), 17°33'53.3"S 149°32'17.5"W; Fautaua river, Pīra'e, Tahiti, the Society Islands, French Polynesia, 16 viii 2019 (C Vieira and YS Kim). UPF 5681 (PF1767), 17°37'02.6"S 149°19'56.0"W; Mahateaho river, Lieu Hitiaa, Vallée Paraura, 5 iv 2021 (M Zubia). UPF.

Diagnosis. Differing from other *Hildenbrandia* species and sister taxa from the Philippines by its longer cells (7.6–16.2 μm in diameter in surface view, 11.6–16.4 μm long and 2–3 μm high in cross section, with filaments 37–240 μm long and occasionally branching), the 5.08–18.88% difference in *rbc*L and 0.28–11.76% in 18S rDNA nucleotide sequences.

Description. Thallus epilithic, smooth, thin rose-red to brown-red in color, with irregular borders firmly attached onto the surface of stones; distinct rose-red to rust color underwater; thallus monostromatic to polystromatic; oval to rectangular cells in surface view, 7.6–16.2 μm in diameter, and rectangular in cross section, 11.6–16.4 μm long and 2–3 μm high; filaments 37–240 μm long, occasionally branching; reproductive structures unknown; chloroplast-encoded *rbc*L sequence [GenBank accessions LC631948 (PF1507), LC632058 (PF1508), LC631949 (PF1767)]; nuclear-encoded 18S rDNA sequences [GenBank accession LC631950 (PF1767)].

Habitat. Grows on rocks in clean fast-flowing rivers in shaded areas and particularly under the shade of trees.

Distribution. French Polynesia (the Society Islands, Tahiti).

Etymology. The specific epithet *tahitiensis* refers to the type locality of the species, in Tahiti (French Polynesia, the Society Islands).

Conclusions

This is the first report of the genus *Hildenbrandia* from Tahiti and more broadly from French Polynesia. Documentation of freshwater *Hildenbrandia* from a south central Polynesian locality extends our knowledge of the geographical range for the freshwater members of this genus. Although previously freshwater species of *Hildenbrandia* have only been reported from the Hawaiian Islands (Sherwood 2004; Carlile and Sherwood 2013), marine species have been documented from several localities in Pacific Islands including Central Polynesia (Tsuda and Walsh 2013), Fiji (N'Yeurt et al. 1996; South and Skelton 2003), the Federated States of Micronesia (Lobban and Tsuda 2003), Samoa (Skelton and South 2007), and the Solomon Islands (Womersley and Bailey 1970), under different names, that is, *H. crouaniorum* and *H. rubra*. Taxonomic reexaminations of these previous records are needed and further investigations are wanted to determine if marine species of *Hildenbrandia* also occur in French Polynesia.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.japb.2021.07.008.

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