

# Contamination in the model species *Cyanidioschyzon merolae* NIES-3377

Shigekatsu Suzuki<sup>1,2)\*</sup>, Shuhei Ota<sup>2)</sup> and Yuuhiko Tanabe<sup>2)</sup>

<sup>1)</sup>Institute of Life and Environmental Sciences, University of Tsukuba  
1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan

<sup>2)</sup>Biodiversity Division, National Institute for Environmental Studies  
16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

*Cyanidioschyzon merolae* is a thermophilic, unicellular red alga that thrives in acidic hot-spring habitats worldwide. This species possesses simple cell components such as a single plastid, mitochondrion, and Golgi apparatus. The first sequenced eukaryotic algal nuclear genome was that of *C. merolae*, and the gapless telomere-to-telomere genome subsequently became available. Methods for synchronizing culture and genetic transformations have also been developed. These characteristics indicate that *C. merolae* is an advantageous experimental resource in molecular cell biology. Specifically, the 10D strain, for which the genome is available, is widely used in many laboratories. However, purification of these strains has received little attention. This study revealed that the NIES-3377 strain, an axenic culture of the 10D strain, was contaminated with another unicellular red alga (*Galdieria partita*). Furthermore, phylogenetic analysis suggested that the *Galdieria* strain may not have originated from the same sampling locality as the original 10D strain. The strain was probably contaminated during subculture before being deposited into the culture collection. These results strongly highlight the importance of quality checks for strain establishment and maintenance, particularly in unicellular red algae with similar morphological traits.

Key words: *Cyanidioschyzon merolae*, Cyanidiophyceae, contamination, amplicon analysis

## INTRODUCTION

The family Cyanidiophyceae consists of blue-green, unicellular red algae that occupy acidic hot springs and volcanic caldera habitats worldwide. Moreover, these algal species are resistant to low pH (at least pH 0.5) (Pinto *et al.*, 2003) and high temperature (up to 64°C) conditions (Huang *et al.*, 2024). Furthermore, mesophilic groups were observed in dark caves (Park *et al.*, 2023). To date, Cyanidiophyceae comprises the following eight genera: *Cyanidioschyzon* De Luca, Taddei & Varano, *Cyanidium* Geitler, *Galdieria* (Galdieri) Merola, *Cyanidiococcus* S.-L. Liu, Y.-R. Chiang, H.S. Yoon, & H.-Y. Fu, *Gronococcus* H.S. Yoon, S.I. Park, & C. Ciniglia, *Cavernulicola* (Schwabe) H.S. Yoon, S.I. Park, D.E. Bustamante, M.S. Calderon, Del Guacchio, & A. Mansilla, *Sciadococcus* H.S. Yoon, S.I. Park, T.-Y. Huang, &

S.-L. Liu, and *Cyanidiofrigus* T.-Y. Huang, H.S. Yoon, Y.-R. Chiang, H.-Y. Fu, T. Watanabe, G.S. Gerung, P.J.L. Geraldino & S.-L. Liu (Huang *et al.*, 2024; Liu *et al.*, 2020; Merola *et al.*, 1981; Park *et al.*, 2023). Additionally, several *Galdieria* species, including *G. daedala* Sentsova ex Pollio, De Natale & Del Guacchio, *G. maxima* Sentsova ex Pollio, De Natale & Del Guacchio, and *G. partita* Sentsova ex Pollio, De Natale & Del Guacchio, have long been taxonomically invalid; however, their descriptions have been corrected (Del Guacchio *et al.*, 2023). Considering that *Galdieria* species form small round cells and possess poor morphological characteristics for clear identification (Ciniglia *et al.*, 2004), species of this genus were mainly taxonomically classified based on nuclear and organellar genomes (Liu *et al.*, 2020; Park *et al.*, 2023) and thermal tolerance (Huang *et al.*, 2024). Difficulties in species identification have led to problems with strain purification. For example, *Galdieria javensis* H.S. Yoon, S.I. Park, & R.A. Andersen (formerly *G. sulphuraria* [Galdieri] Merola)

\*Corresponding author

E-mail: suzuki.shigekatsu.gw@u.tsukuba.ac.jp

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strain 074 consists of the two strains 074W and 074G (Gross & Schnarrenberger, 1995). Neither species was morphologically distinguishable under autotrophic conditions; however, they exhibited different preferences for sugars under heterotrophic conditions. For an authentic strain of *Galdieria maxima* IPPAS P507, two different 18S rRNA sequences were deposited in GenBank (Gross *et al.*, 2001; Kondo *et al.*, 2004). However, this heterotrophic species (Gross, 1999) contained the non-heterotrophic species *Cyanidiococcus* sp. (Liu *et al.*, 2020). This contamination may have resulted from the isolation procedure.

Among the cyanidiphyceans, *Cyanidioschyzon merolae* De Luca, Taddei & Varano is the only species that lacked rigid cell walls (Merola *et al.*, 1981), except at the haploid stage of *Galdieria* (Hirooka *et al.*, 2022). Additionally, this species possesses useful traits for molecular and cellular biology studies (Miyagishima & Tanaka, 2021). These traits include a small size (1.5–3.0  $\mu\text{m}$  in diameter) and a simple organellar composition of a single nucleus, mitochondrion, plastid, Golgi apparatus, and peroxisome. Furthermore, the growth rate is relatively fast, with a doubling time of 9.2 h (Minoda *et al.*, 2004), and the cell cycle was controlled synchronously during the diel cycle (Suzuki *et al.*, 1994). *Cyanidioschyzon merolae* is the first eukaryotic alga to be sequenced. The nuclear, plastid, and mitochondrial genomes are 16.5 Mbp, 150.0 kbp, and 32.2 kbp in size, respectively (Ohta *et al.*, 1998, 2003; Matsuzaki *et al.*, 2004). Subsequently, the complete (gapless and telomere-to-telomere) nuclear genome comprises 20 chromosomes (Nozaki *et al.*, 2007), whereas the nuclear genome contains 4,775 protein-coding genes with a small amount of repeat sequences and introns (Matsuzaki *et al.*, 2004; Misumi *et al.*, 2005; Nozaki *et al.*, 2007). Moreover, basic molecular genetic techniques such as efficient homologous recombination (Fujiwara *et al.*, 2013; Minoda *et al.*, 2004; Watanabe *et al.*, 2014), gene expression regulating systems (Fujiwara *et al.*, 2015; Sumiya *et al.*, 2014), and the CRISPR-Cas9 system (Tanaka *et al.*, 2021) have been developed. Therefore, *C. merolae* is used worldwide as a model organism.

The *C. merolae* 10D strain and its derivatives are

widely used in molecular cell biology studies. The original 10D strain was established from an algal mixture in hot spring water in Italy (Toda *et al.*, 1995), which was sampled by Gabriele Pinto (University of Naples Federico II) (Matsuzaki *et al.*, 2004). Initially, this strain was xenic, deposited in the Microbial Culture Collection at the National Institute for Environmental Studies (MCC-NIES) in 2004, and was available as the NIES-1332 strain. In 2013, NIES-1332 was replaced with an axenic strain of 10D by the same depositor and distributed as the NIES-3377 strain. NIES-3377 was cryopreserved in 2015.

This study reports on the contamination of NIES-3377. The contaminant was an acidophilic unicellular red alga (*Galdieria partita*) that is genetically different from all the other cyanidiphycean strains of MCC-NIES. Although this contamination may have occurred before strain deposition, only a few sequencing analyses previously conducted with NIES-3377 have been contaminated with *G. partita*. These findings highlight the importance of quality checks during the establishment and maintenance of algal strains.

## MATERIALS AND METHODS

### Cultivation

*Cyanidioschyzon merolae* NIES-3377, *G. partita* NIES-3892, and *G. javensis* NIES-3638 were obtained from MCC-NIES. NIES-3377 was thawed from a cryopreserved sample in 2015. This strain was maintained in modified Allen medium (Minoda *et al.*, 2004) at 42°C under 16:8 h (L:D) white fluorescence light at  $\approx 50 \mu\text{mol photons/m}^2/\text{s}$ . Prior to use, the medium was autoclaved, and filter-sterilized  $(\text{NH}_4)_2\text{SO}_4$  was added to a final concentration of 2.62 g/L.

To test the mixotrophy of NIES-3377, cells were cultivated for three days in 1 mL of modified Allen medium or 10% bacterial standard medium (BSM; [https://www.uni-due.de/biology/ccac/growth\\_media\\_bsm.php](https://www.uni-due.de/biology/ccac/growth_media_bsm.php)) in a 24-well plate. The cell width (short side of the cells or diameter) was manually measured using an inverted light microscope with the Fiji package (Schindelin *et al.*, 2012).

### Strain establishment

A single cell of *C. merolae* was isolated from NIES-3377 in modified Allen medium in glass test tubes using the micropipette method. After approximately one month of cultivation, cell growth was examined using light microscopy. Microphotographs were obtained using a Nikon ECLIPSE Ni-U microscope (Nikon, Tokyo, Japan) with a 100× objective lens containing differential interference contrast optics, and images were captured using a DS-Fi3 digital camera (Nikon, Tokyo, Japan).

### Detection of contamination in NIES-3377

DNA of the strains was extracted using a Quick-DNA Fungal/Bacterial Kit (Zymo Research, Irvine, CA, USA) or a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) with an SK mill (Tokken, Chiba, Japan) following the manufacturer's protocols.

Owing to the difficulty of distinguishing between unicellular red algae species, specific forward primers for *Cyanidioschyzon* (*Cyanidioschyzon*\_F: 5'-GTCTTTCGGCTGGTGAGCGCGC-3') and *Galdieria* (*Galdieria*\_F: 5'-AGCGTTTGGCAAGGATATACC-3') and a universal reverse primer (*Rhodophyte*\_R: 5'-CGCGTGCAGCCCAGGACATC-3') were developed.

PCR was performed for amplicon sequencing using a universal primer set (*Cya*\_Gal\_F: 5'-GGTTCGATTCCGGAGAGGGAGCC-3') and SR12 (Nakayama *et al.*, 1998). PCR products were purified using AMPure XP (Beckman Coulter, Brea, CA, USA), and DNA libraries were constructed using a Native Barcoding Kit 24 V14 (SQK-NBD114.24; Oxford Nanopore Technologies, Oxford, UK) following the manufacturer's protocol. Libraries were pooled and sequenced using Flongle (Oxford Nanopore Technologies). The raw reads were base-called using Dorado version 0.9.0 (<https://github.com/nanoporetech/dorado>) in super accuracy mode. Reads with < Q15, < 1 kbp, and > 2 kbp were eliminated using chopper version 0.9.0 (De Coster & Rademakers, 2023). Taxonomic classification of the reads was performed using Emu version 3.5.1 (Curry *et al.*, 2022) with the pr2 database version 5.0.0 (Guillou *et al.*, 2013).

### Phylogenetic analysis

The consensus sequences of *Cyanidioschyzon merolae* and *Galdieria* sp. in NIES-3377 were extracted from the amplicon sequences of NIES-3377 using amplicon\_sorter version 2024-10-16 with default options (Vierstraete & Braeckman, 2022). The sequences were deposited in GenBank/DBJ/ENA under accession numbers LC868824 and LC868825, respectively. Most of the available 18S rRNA sequences of *Galdieria* spp. were downloaded from the NCBI GenBank on January 29, 2025. A single variant (XR\_009833484.1) was selected from the 18S rRNA gene sequence of *G. javensis* 074W (NIES-3638). For *Galdieria phlegrea* DEV009, raw reads of the genome sequence (SRR953992) were downloaded, and the initial four base pairs were trimmed using chopper. The trimmed reads were assembled using SPAdes version 4.0.0 (Prjibelski *et al.*, 2020) with iontorrent and careful selection options. The 18S rRNA sequences were extracted from the assembly using SSU-align version 0.1.1 (Nawrocki *et al.*, 2009). For *G. partita* THAL043, the raw reads of the transcriptome sequence (SRR8259111) were downloaded, and the reads with < Q20 or < 15 bp were trimmed using fastp version 0.24.0 (Chen *et al.*, 2018) with default options. Trimmed reads were assembled using SPAdes with the RNA option, and 18S rRNA sequences were extracted from the assembly using SSU-Align. Nine 18S rRNA sequences were extracted from the genomes (Rossoni *et al.*, 2019) using SSU-align and added to the dataset. Thereafter, the sequences were aligned using Mafft version 7.525 (Nakamura *et al.*, 2018) with the G-INS-i option. Subsequently, maximum likelihood analysis was performed using IQ-TREE version 2.2.6 (Nguyen *et al.*, 2015) using the TVM + F + G4 model with non-parametric bootstrapping of 200 replicates.

### Detection of contamination in the previous sequencing experiments

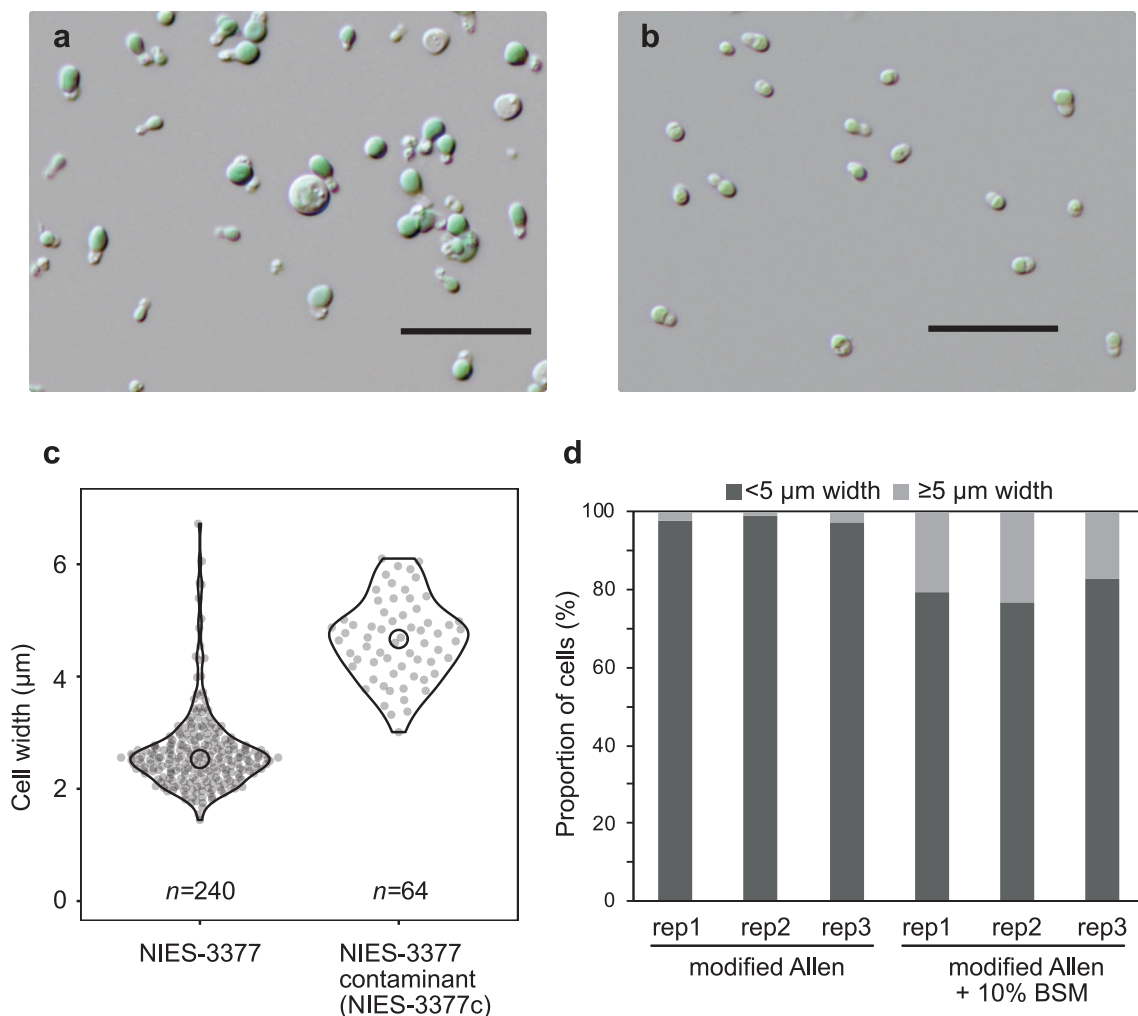
One genome, 58 transcriptomes, and one bisulfite sequence for *C. merolae* 10D and its derivatives were downloaded from the SRA database of NCBI. Short-read data were assembled using SPAdes with

a meta-option for paired-end libraries and default options for single libraries. The 18S rRNA sequences were extracted from the assemblies using SSU-align. For PacBio RNA sequencing (RNA-seq) reads, 18S rRNA sequences were directly extracted from the reads using SSU-align. The 18S rRNA sequences were taxonomically assigned using the Emu with the pr2 database with minimum abundance > 0.01 and the sr (for short reads) and map-pb (for long reads) options.

## RESULTS AND DISCUSSION

### Discovery of contamination in NIES-3377

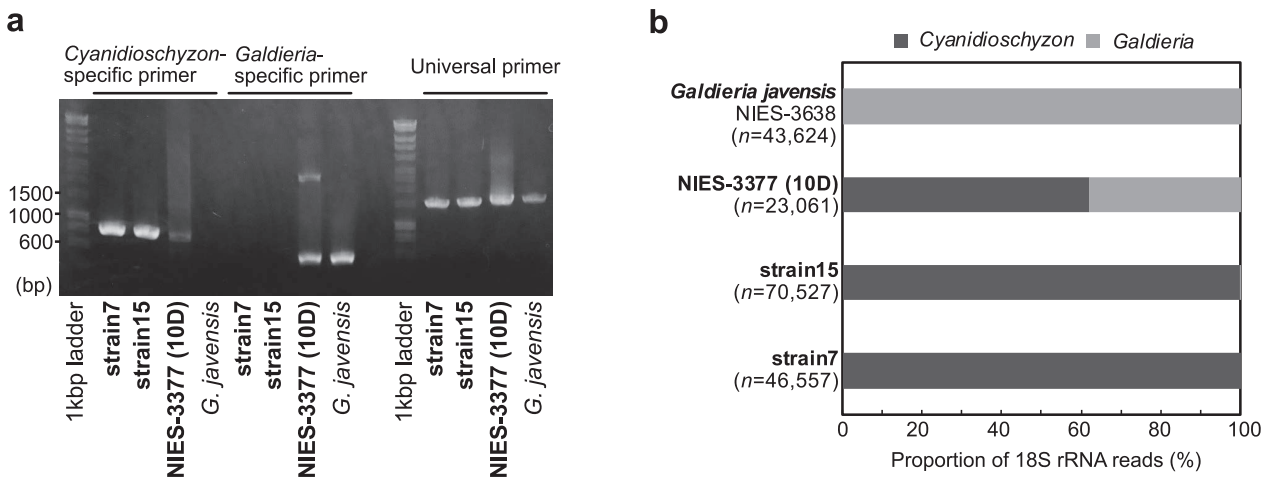
The NIES-3377 strain maintained in MCC-NIES originated from the 10D strain. Initially, the xenic 10D strain was deposited in the MCC-NIES collection as NIES-1332. Subsequently, the strain was purified in the depositor's laboratory, and axenic 10D replaced NIES-1332 with NIES-3377. To assess the conditions of NIES-3377, light microscopic observation was performed and revealed round cells larger than typical *C. merolae* cells in NIES-3377 (Fig. 1a). Smaller cells were isolated as strain7 and



**Fig. 1** Heterogeneity in *Cyanidioschyzon merolae* NIES-3377 (10D). (a) Light microscopic images of NIES-3377 and (b) the re-isolated and purified strain7. The cell form of NIES-3377 is heterogeneous and contains large round cells. Scale bars = 20 µm. (c) Distribution of the cell width (diameter) of NIES-3377 and its contaminant. The contaminant strain (NIES-3377c) was established from NIES-3377. (d) Proportion of cells with < 5 µm and ≥ 5 µm width in NIES-3377. Three replicates are presented under autotrophic and heterotrophic conditions.

strain15 (Fig. 1b). The short side (width or diameter) of the cells of the typical NIES-3377 population was approximately  $2.5\ \mu\text{m}$  (Fig. 1c), and these cells exhibited dumbbell-like cell forms, which is typical for *C. merolae* (De Luca *et al.*, 1978). The cell size was larger than those in the species description of *C. merolae* ( $1.0\text{--}1.5\ \mu\text{m}$ ) (De Luca *et al.*, 1978; Merola *et al.*, 1981) or those in previous studies examining strain 10D ( $1.5\text{--}2.0\ \mu\text{m}$ ) (Matsuzaki *et al.*, 2004). This size discrepancy may be caused by differences in culture conditions, and the species description should be addressed in future studies. In contrast, a small proportion (0.8–2.8%) of NIES-3377 cells were round cells with  $\geq 5\ \mu\text{m}$  width (Fig. 1c, d and Supplementary Fig. S1), and a larger cell was isolated as NIES-3377c. The proportion of large round cells significantly increased to 17.1–23.5% ( $p < 0.05$ , *t*-test) for three days after the addition of an organic compound mix that included glucose (BSM) (Fig. 1d, Supplementary Fig. S1, Supplementary Fig. S2, and Supplementary Table S1). These results suggest that large round cells absorb and use glucose more effectively than the typical *C. merolae* cells. Among the unicellular Cyanidiphyceae species, *C. merolae* and *Cyanidium caldarium* (Tilden) Geitler lack heterotrophy (glucose utilization), whereas *Galdieria* spp. can metabolize glucose and grow in the dark

(Huang *et al.*, 2024; Liu *et al.*, 2020). Moreover, the morphological characteristics of the large round cells are similar to those of *Galdieria* spp. (Huang *et al.*, 2024). Generally, morphological distinction between Cyanidiphyceae species is difficult. Thus, additional evidence was obtained using molecular information. Based on the available 18S rRNA sequence information, primers specific to *Cyanidioschyzon* (Cyanidioschyzon\_F) and *Galdieria* (Galdieria\_F) were designed (Supplementary Fig. S3). These primers amplified 776 bp and 527 bp PCR products (Fig. 2a). PCR products were obtained using Cyanidioschyzon\_F for strain7 and strain15 but not for the authentic strain of *Galdieria javensis* (NIES-3638). However, Galdieria\_F amplified the PCR product from NIES-3638 but not from strain7 and strain15. Both primers amplified the PCR products of NIES-3377, suggesting that NIES-3377 contained at least two different 18S rRNA sequences assigned to *Cyanidioschyzon* and *Galdieria*. PCR was performed using universal primers that could amplify the 18S rRNAs of both *Cyanidioschyzon* and *Galdieria* (Fig. 2a), and amplicon analyses were performed using nanopore sequencing (Fig. 2b, Supplementary Table S2). For *G. javensis*, 99.7% of raw reads were assigned to *Galdieria*. For strain7 and strain15, 99.97 and 99.96% of the raw reads,



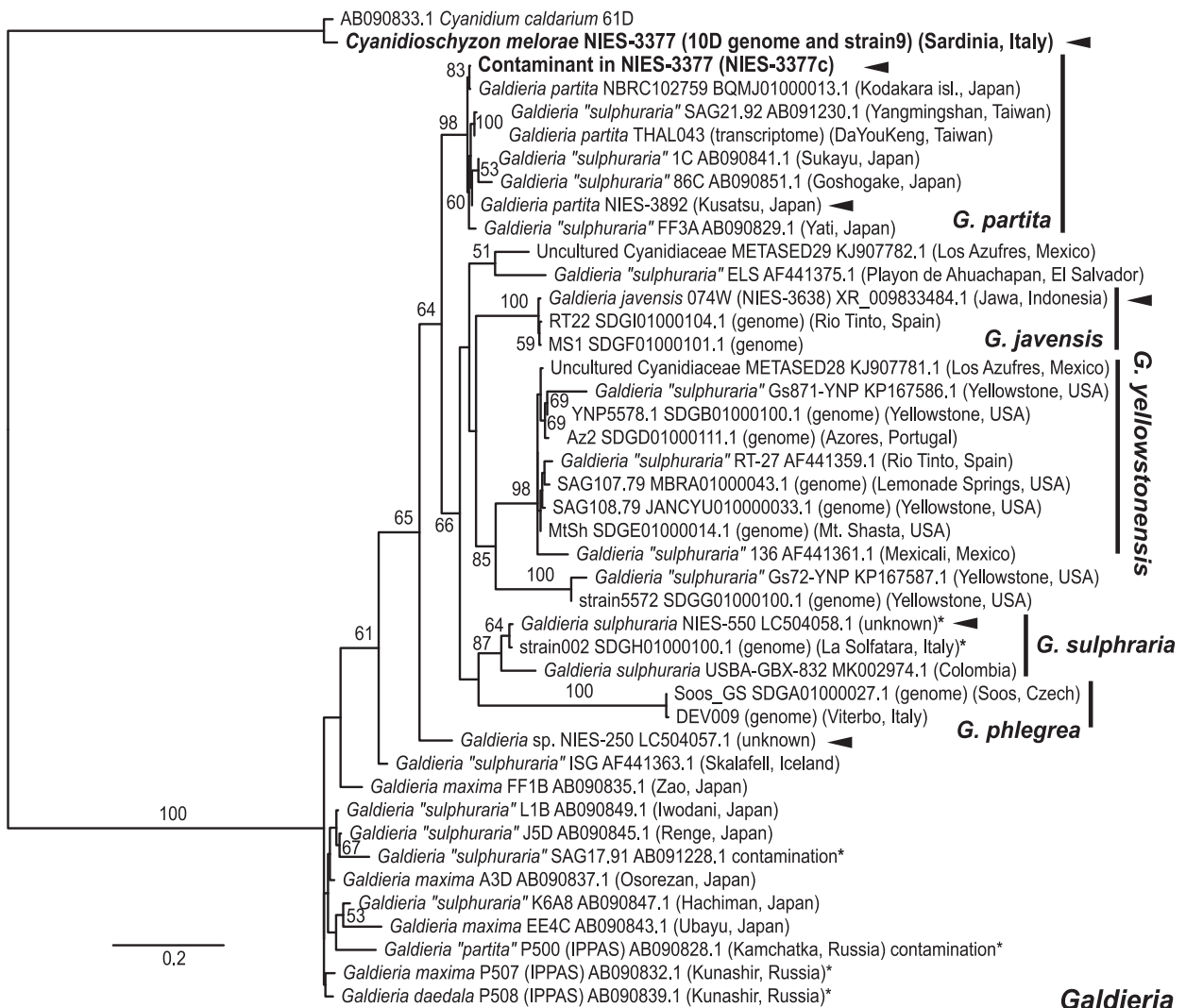
**Fig. 2** Detection of contamination in *Cyanidioschyzon merolae* NIES-3377. **(a)** Gel image of polymerase chain reaction (PCR) amplification of NIES-3377 and the purified strains (strain7 and 15) from NIES-3377 with specific primers for *Cyanidioschyzon* and *Galdieria*. An authentic strain of *Galdieria javensis* (NIES-3638) is used as a positive/negative control. **(b)** Proportion of 18S rRNA reads assigned as *Cyanidioschyzon* and *Galdieria* using amplicon sequencing.

respectively, were assigned to *Cyanidioschyzon*. These analyses revealed an extremely small number of contaminated reads (*Cyanidioschyzon* or *Galdieria*), possibly caused by misclassification during the demultiplexing process. The reads aligned to one consensus sequence for each amplicon library, strongly indicating that the strains were cloned without contamination. For NIES-3377, the reads were assigned to both *Cyanidioschyzon* (62.02%) and *Galdieria* (37.98%) and aligned to two consensus sequences, indicating that this strain was

not unialgal.

### Identification and putative origin of the contamination in NIES-3377 (NIES-3377c)

Of the two consensus sequences of NIES-3377, *Cyanidioschyzon* was identical to the 18S rRNA sequence of the available genome of *C. merolae* 10D. To elucidate the phylogenetic position of the contaminant in NIES-3377 (NIES-3377c), a phylogenetic analysis was performed using 18S rRNA sequences (Fig. 3). Phylogenetic analysis revealed monophylet-



**Fig. 3** Phylogenetic tree of *Galdieria* based on 18S rRNA sequences. Maximum likelihood analysis was performed using 43 taxa and 1,955 nucleotides. Non-parametric bootstrapping values (BP) are indicated on each branch, and BPs < 50 are not shown. NIES-3377 and its contaminant are represented with bold type. Arrowheads indicate strains maintained in Microbial Culture Collection at the National Institute for Environmental Studies (MCC-NIES). Asterisks represent authentic strains; however, AB091228.1 and AB090828.1 may be derived from contamination.

ic clades for five species of *Galdieria* with strong bootstrap support values (BP): *G. partita* (BP = 98), *G. sulphuraria* (BP = 87), *G. phlegrea* (BP = 100), *G. yellowstonensis* complex (BP = 98), and *G. javensis* (BP = 100). The remaining two species of *Galdieria*, including *G. daedala* (IPPAS P508) and *G. maxima* (IPPAS P507), did not clearly form monophyletic groups with the other strains of each species. In terms of *G. partita*, a single sequence of *G. partita* IPPAS P500 that was re-isolated from the original strain and sequenced by Kondo *et al.* (2004) was separated from the other *G. partita* strains, including SAG21.92 and THAL043. However, based on some genes of the plastid genome, IPPAS P500 was monophyletic with the SAG21.92 and THAL043 strains (Ciniglia *et al.*, 2004; Huang *et al.*, 2024). Moreover, the re-isolated IPPAS P500 strain used for 18S rRNA sequencing exhibited a similar cell size and plastid number as that of the authentic strain of *G. maxima*, but not *G. partita* (Kondo *et al.*, 2004). Therefore, the 18S rRNA sequence of the re-isolated IPPAS P500 strain may have originated from contamination, and the clade, including SAG21.92 and THAL043, should be *G. partita*. Additionally, the NIES-3377c strain belongs to the *G. partita* clade. Notably, the cell size of NIES-3377c (4.41–4.87  $\mu\text{m}$ , 95% CI for a median) was consistent with that of *G. partita* strains (THAL001, THAL023, THAL025, THAL043, and THAL069), which ranged between 3.75–5.02  $\mu\text{m}$  (Fig. 1c) (Huang *et al.*, 2024). The *G. partita* clade consists of strains isolated only from the volcanic regions of the East Asian islands. These included strains from Japan (NBRC 102759, NIES-3892, FF3A, 1C, and 86C) and Taiwan (SAG 21.92, and THAL043). A previous study reported *G. partita* strains from the Philippines and Kunashir in Russia (Del Guacchio *et al.*, 2023; Hsieh *et al.*, 2018). These habitats suggest that *G. partita* may be specific to these regions as well as volcanic regions in the West Pacific Island chain (Hsieh *et al.*, 2018). In the *G. partita* clade, NIES-3377c was most closely related to NBRC102759 (BP = 83), with 99.65% similarity. However, NIES-3377c and NBRC 102759 were phylogenetically distinct from the other strains of *G. partita* and branched at the most basal position

in the *G. partita* clade. The NBRC 102759 strain was sampled from Kodakara Island in Kagoshima, Japan in 1990 (<https://www.nite.go.jp/nbrc/catalogue/NBRCCatalogueDetailServlet?ID=NBRC&CAT=00102759>). The habitat of *G. partita* was geographically isolated from the sampling location of *C. merolae* 10D in Sardinia Island, Italy. Moreover, the nuclear genome of strain 10D did not contain the genome of *G. partita* (Matsuzaki *et al.* 2004). This suggests that some putative sources of the contaminant (NIES-3377c) may originate from the NBRC 102759 strain or its close relatives but not from the original strain of *C. merolae* 10D, whose genome was sequenced. The following four *Galdieria* strains were maintained in the MCC-NIES: *G. sulphuraria* NIES-550 (authentic strain), *G. javensis* NIES-3638 (authentic strain), *G. partita* NIES-3892, and *Galdieria* sp. NIES-250. Of these, NIES-3892 that was deposited in MCC-NIES in 2016 was phylogenetically related to NIES-3377c with 98.76% similarity; however, it did not exhibit a sister relationship. Moreover, NIES-3377 that had been cryopreserved since 2015 was also contaminated. Therefore, contamination in strain 10D probably occurred before the deposition of this strain (in 2013) during subculturing, although we cannot completely exclude the possibility of cross-contamination from other acidophilic strains with any potential contaminants during subculturing in MCC-NIES.

#### Contamination in previous sequencing data of strain 10D and its derivative strains

*Cyanidioschyzon merolae* strain 10D is distributed worldwide, and its simple gene repertoire is particularly useful for elucidating metabolic processes using high-throughput sequencing technologies. To elucidate the effects of *Galdieria* contamination on the sequencing analyses, contamination was evaluated using most of the genome, transcriptome, and bisulfite sequencing data available from GenBank (Supplementary Table S3). Raw reads of one genome sequence, 58 RNA-seq reads (54 short and four PacBio long reads), and one bisulfite sequencing dataset from 10 research projects were downloaded. For long RNA-seq read datasets, 18S rRNA sequenc-

es were extracted from the raw reads. For short-read datasets, the reads were assembled into contigs, and 18S rRNA sequences were extracted from the contigs. The extracted 18S rRNA sequences were taxonomically assigned.

Two datasets (SRR5742238 and SRR847294) lacked the 18S rRNA sequences of *C. merolae* despite the existence of other species, which may have been confused in any experimental process. Only one dataset (DRR258824) contained an 18S rRNA gene sequence from *Galdieria*. This is the RNA-seq data for a *C. merolae* mutant (CMK212Cox) (Takahashi *et al.* 2021), which suggests that *G. partita* contaminants rarely remain after genetic transformation. Overall, these data demonstrate that contamination of *Galdieria* could be ignored in sequencing analyses. Cell destruction methods for DNA/RNA extraction in previous studies were assessed. All methods destroyed the cells using only lysis buffer without any physical procedures such as grinding or bead beating. *Galdieria* cells are typically surrounded by a rigid cell wall (Hirooka *et al.*, 2022; Lee *et al.*, 2015; Miyagishima & Tanaka, 2021), and the cell wall prevents DNA/RNA extraction under mild conditions. Hence, *Galdieria* may not have been detected in almost all of the sequencing datasets. However, the results of previous strain 10D studies, such as those of the growth rate measurements without microscopic observations, must be cautiously interpreted.

## CONCLUSION

This study identified a contamination of *C. merolae* NIES-3377 by *G. partita*. Moreover, the *G. partita* contaminant may not have originated from the same sampling location as the original 10D strain. This strain probably contaminated the subculture before deposition into the culture collection. To date, the derivative strains of 10D have been classified by MCC-NIES as NIES-3377 and ATCC as PRA-402 (<https://www.atcc.org/products/pr-402>). Furthermore, whether PRA-402 contains *G. partita* contaminant remains unclear. In this study, we re-isolated a single *C. merolae* cell from NIES-3377 and evaluated its purification using comprehensive amplicon analy-

sis. This clone-verified strain was cryopreserved and distributed as the newly designated NIES-4696 (strain7 in this study) from MCC-NIES. To prevent further confusion regarding strain purification, this study proposes that NIES-4696 should be used in future studies. Cases of contamination by cyanidiphycean species have been reported; hence, amplicon analyses are powerful tools for the purification of strains. This study highlights the importance of quality checks for algal strains. In the future, algal strains established using methods other than single-cell isolation will need to be checked using metagenomic analyses (amplicon and shotgun sequencing analyses) before deposition to culture collections to certify their clonality.

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## SUPPLEMENTARY INFORMATION

Supplementary information related to this article is available in the online version on J-STAGE.

<https://www.jstage.jst.go.jp/browse/microresys/list>

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モデル生物 *Cyanidioschyzon merolae* NIES-3377 におけるコンタミネーション

鈴木重勝<sup>1,2)</sup>, 大田修平<sup>2)</sup>, 田辺雄彦<sup>2)</sup>

<sup>1)</sup> 筑波大学生命環境系, <sup>2)</sup> 国立環境研究所生物多様性領域

*Cyanidioschyzon merolae* は世界中の酸性温泉に生息する好熱性単細胞紅藻である。本種は細胞当たり1つの色素体, ミトコンドリア, ゴルジ体をもつなど, 単純な細胞形態を有する。そのゲノムは真核藻類のなかで初めて解読され, ギャップのない完全長ゲノム (16.5 Mbp) が利用可能である。また, 同調培養系や形質転換系も開発されている。これらの特徴は, 本種が分子細胞生物学の実験生物として優れていることを示している。特に, 最初にゲノム解読が行われた 10D 株が, 多くの研究室で広く用いられている。しかしながら, これまでこれらの株の純化についてはほとんど注意されてこなかった。本研究では, 10D 株の無菌株である NIES-3377 株に異なる単細胞性紅藻の *Galdieria partita* が混入していたことを明らかとした。さらに, 系統解析により, この *Galdieria* 株はおそらく 10D 株の原産地に由来するものではないことが示唆された。本株は系統保存施設に寄託される以前の継代培養の過程で混入した可能性がある。このことは, 特に形態的に類似する単細胞性紅藻において, 培養株の確立やその維持における品質管理の重要性を強く示している。