Hanusia phi gen. et sp. nov. (Cryptophyceae): characterization of 'Cryptomonas sp. Φ'

James A. Deane a; David R. A. Hill b; Steven J. Brett c; Geoffrey I. McFadden a

a Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, 3052, Australia.
b School of Botany, The University of Melbourne, Parkville 3052, Australia.
c The Department of Ecology and Evolutionary Biology, Monash University, Clayton, 3168, Australia.

Online Publication Date: 01 May 1998

To cite this Article: Deane, James A., Hill, David R. A., Brett, Steven J. and McFadden, Geoffrey I. (1998) "Hanusia phi gen. et sp. nov. (Cryptophyceae): characterization of 'Cryptomonas sp. Φ'", European Journal of Phycology, 33:2, 149-154

To link to this article: DOI: 10.1080/09670269810001736643

URL: http://dx.doi.org/10.1080/09670269810001736643
Hanusia phi gen. et sp. nov. (Cryptophyceae): characterization of ‘Cryptomonas sp. Φ’

JAMES A. DEANE1, DAVID R. A. HILL2, STEVEN J. BRETT3 AND GEOFFREY I. McFADDEN3

1 Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, 3052, Australia
2 School of Botany, The University of Melbourne, Parkville 3052, Australia
3 The Department of Ecology and Evolutionary Biology, Monash University, Clayton, 3168, Australia

(Received 21 April 1997; accepted 8 October 1997)

Cryptomonas sp. Φ is an undescribed cryptomonad used for many studies into the endosymbiotic origin of plastids. Cryptomonas sp. Φ was characterized using electron microscopy, DNA sequencing and karyotyping. DNA sequence data show that Cryptomonas sp. Φ has been confused with the related cryptomonad, Guillardia theta, which is the organism actually used in most published studies on cryptomonad endosymbiosis to date. It is proposed that the strain Cryptomonas sp. Φ be recognized as a distinct species under the name Hanusia phi. Although Hanusia phi shares molecular and karyotypic features with G. theta, it cannot be assigned to Guillardia because it does not fit the morphological description of this genus. H. phi differs from G. theta in periplast structure, configuration of the furrow-gullet system, and cell size. Morphologically, however, H. phi is very similar to Teleaulax acuta, Teleaulax merimbula and the diplomorph of Protomonas sulcata.

Key words: cryptomonad, Cryptomonas sp. Φ, Hanusia phi, nucleomorph

Introduction

In 1963 Guillard reported the isolation of a cryptomonad he designated ‘flagellate Φ’ (Guillard, 1963). Gilloot & Gibbs (1983) regarded flagellate Φ as a species of Cryptomonas, and referred to it as Cryptomonas sp. Φ. Since then, Cryptomonas sp. Φ has become a model organism for molecular investigation of the endosymbiotic origin of the plastids in cryptomonads (e.g. Douglas, 1988, 1991; Douglas & Dunford, 1989, 1990a, b; Douglas & Turner, 1991; Douglas et al., 1990, 1991; Wang & Liu, 1991; McFadden et al., 1994a, b; Rensing et al., 1994; Reith & Douglas, 1990). Cryptomonads acquired plastids by secondary endosymbiosis, a process in which a eukaryotic alga (itself the product of primary endosymbiosis between a photosynthetic prokaryote and a eukaryotic host) was engulfed and retained by a eukaryotic phagotroph (see reviews by McFadden & Gilson, 1995, and McFadden et al., 1997).

Cryptomonas sp. Φ has not been formally described as a species, so we undertook a morphological and molecular characterization for its taxonomic description. During our investigations, it became apparent that Cryptomonas sp. Φ has been confused with Guillardia theta Hill et Wetherbee (Hill & Wetherbee, 1990). Many studies of endosymbiosis in cryptomonads have inadvertently used G. theta instead of Cryptomonas sp. Φ. Here, Cryptomonas sp. Φ is described and compared with G. theta and a formal name proposed.

Materials and methods

Cryptomonas sp. Φ (CCMP 325) and Guillardia theta (CCMP 327) were obtained from the Bigelow Culture Collection of Marine Phytoplankton and maintained in H/2 seawater medium (McLachlan, 1973) in a south-facing window at 20 °C.

For scanning electron microscopy, cells in culture medium were fixed for 2 h by addition of an equal volume of 2% osmium tetroxide in seawater and filtered onto a 2 μm pore size isopore filter (Millipore). Filters and attached cells were washed with distilled water, dehydrated in ethanol, critical-point-dried and coated with gold. For transmission electron microscopy, cells were harvested by centrifugation, fixed for 1 h with 2% glutaraldehyde in 0.05 M sodium cacodylate buffer pH 7.5 plus 0.4 M sucrose, post-fixed in 1% osmium tetroxide in 0.05 M sodium cacodylate buffer, dehydrated in ethanol, and embedded in Spurr’s resin (Spurr, 1969). Embedded cells were thin sectioned and stained with uranyl acetate and lead citrate. Cells were prepared for freeze fracture as reported by Hill & Wetherbee (1986).

To prepare cells for pulsed field gel electrophoresis (PFGE), they were harvested by centrifugation and resuspended in plug buffer (10 mM Tris/HCl pH 7.5, 100 mM Na2EDTA, and 100 mM NaCl). Resuspended cells were mixed with an equal volume of 1.2% low-gelling-temperature agarose in plug buffer at 37 °C andpipetted into chilled plug moulds. Once solid, plugs were digested for 48 h at 50 °C in two changes of 10 mM Tris/HCl pH 7.5, 400 mM Na2EDTA, and 10 mg ml−1.

* Correspondence to: J. Deane
  e-mail: jamesd@rubens.unimelb.edu.au
Pronase E (Sigma P-5147). Plugs were stored in 10 mM Tris/HCl pH 7.5 at 4 °C and electrophoresed in 1% agarose, 0.5 × TBE buffer using a BioRad CHEF DR III apparatus. The following electrophoresis conditions were used: 65 h run, 14 °C, 30 s decreasing to 10 s pulse change interval and 4.1 V cm⁻¹.

The 18S ribosomal RNA (rRNA) gene from the CCMP 325 host cell nucleus was amplified using the polymerase chain reaction (PCR) and the two universal eukaryotic 18S primers TACCTGGTGATCTGGCCAG (FAD3) and TGATCTTTGAGGTTCACTAC (FAD4). Cryptomonas sp. Φ and G. theta nucleomorph 18S rRNA genes were amplified using the FAD4 primer in conjunction with a primer specific for the cryptomonad nucleomorph 18S gene (Cavalier-Smith et al., 1996). PCR reactions were electrophoresed in 0.8% agarose, and 18S rRNA gene PCR products excised then purified using Prep-a-Gene® (BioRad). PCR products were restriction-digested and cloned using plasmid vectors. Cloned PCR products were sequenced using universal 18S rRNA and plasmid-specific primers in conjunction with dye-labelled dideoxy terminators (ABI) on an ABI 373A Automated Sequencer. Sequences were edited and assembled using Sequencher 3.0 (Gene Codes, Michigan) and aligned with ClustalW (Thompson et al., 1994).

The complete nuclear (X57162) and nucleomorph (X57008) 18S rRNA sequences attributed to Cryptomonas sp. Φ by Douglas et al. (1991) were obtained from GenBank.

Results

Morphology

The general morphology of Cryptomonas sp. Φ has been reported by Gillot & Gibbs (1983) and our results are in close agreement with their data. Cryptomonas sp. Φ (Fig. 1) is easily distinguished from Guillardia theta (Figs 2, 3) when viewed by scanning electron microscopy. Cells of Cryptomonas sp. Φ (CCMP 325) are approximately ovoid, 5–10 μm in length, 3–5 μm wide and have two flagella anchored at the anterior end of a furrow (Fig. 1). The furrow runs about half the length of the cell and is lined with rows of large ejectosomes (Figs 1, 4). Cells from a rapidly growing culture may have a narrow tail at the posterior end of the cell (Fig. 5). The periplast is of the sheet type and the surface component is covered with a dense mat of rosulate scales (Figs 6, 7). These scales are approximately 130 nm in diameter and are composed of densely packed granular material surrounding a slightly raised axis (Fig. 7). Many small ejectosomes are found in vesicles underneath the periplast, particularly at the posterior of the cell (Fig. 5). Each interphase cell has one orange-red plastid and a pyrenoid without traversing thylakoids that is surrounded by a starch sheath (Fig. 8). Cells sometimes have two plastids (not shown), a condition that is assumed to exist just prior to cell division. The predominant biliprotein in the plastid is Cr-phycocerythrin 545 (cryptomonad phycocerythrin type I). An ascending flagellar root complex (Gillot & Gibbs,
Figs 4–10. Ultrastructure of *Cryptomonas* sp. Φ (CCMP 325). c, plastid; n, nucleus. Fig. 4. Cross-section of cell with four large ejectisomes (small arrows) surrounding furrow (large arrow). Fig. 5. Posterior region of cell with many small ejectisomes (arrows) in vacuoles beneath the periplast. Fig. 6. Cross-section through the periplast showing the inner (arrow) and outer (open arrow) components. Fig. 7. Freeze-etch image of scales on the surface periplast component. Fig. 8. Longitudinal section showing internal architecture. The pyrenoid (p) is surrounded by a starch sheath. Fig. 9. The ascending microtubular flagellar root complex displaying 2 component roots. Arrows show individual microtubules. Fig. 10. Detail of the nucleomorph (nm) and its position within the cell. Note electron-dense particles and nucleolus. Scale bars represent: Figs 4 and 10, 500 nm; Figs 5 and 8, 1 μm; Figs 6, 7 and 9, 200 nm.

1983) consists of 2 microtubular roots (Fig. 9). Gillot & Gibbs (1983) provide a detailed description of the flagellar apparatus. The nucleomorph is free of the pyrenoid and contains a number of electron-dense granules together with a fibrillar granular nucleolus (Fig. 10). Cells usually exist as single, motile monads and do not form palmelloid colonies.

*Guillardia theta* (CCMP 327) (Figs 2, 3) was as described by Hill & Wetherbee (1990). In comparison with *Cryptomonas* sp. Φ, *G. theta* is shorter, rounder and has a gullet.
Nucleomorph karyotyping

The nucleomorph karyotype of *Cryptomonas* sp. Φ as resolved by PFGE is indistinguishable from that of *G. theta* (Fig. 11). In both species, nucleomorph chromosomes are approximately 175, 180 and 195 kb. A 130 kb band representing the plastid DNA (McFadden *et al.*, 1994a) is also resolved. Nuclear chromosomes are too large to be resolved under these conditions and migrate as a single band in both species (Fig. 11).

Molecular analysis

The 18S rRNA genes amplified from the nucleus (GenBank XU53126) and nucleomorph (GenBank XU531254) of *Cryptomonas* sp. Φ (CCMP 325) were 1728 bp and 2021 bp respectively excluding primers. When the sequences of these genes were aligned with the corresponding GenBank entries nominally for *Cryptomonas* sp. Φ (Douglas *et al.*, 1991), there were 14 mismatches along the length of the nuclear genes and 264 between the nucleomorph genes. To test whether Douglas *et al.* (1991) sequence was actually from *G. theta* rather than from *Cryptomonas* sp. Φ, we sequenced a variable region of the 18S rRNA from *G. theta* (CCMP 327). Comparison of the sequence from this 463 bp *PstI* nucleomorph fragment of *G. theta* (CCMP 327) (GenBank AF047376) with the corresponding nucleomorph sequence lodged in GenBank under the name *Cryptomonas* sp. Φ revealed near identity, with only three mismatches (Fig. 12). Conversely, within the same region, the real *Cryptomonas* sp. Φ (CCMP 325) and the supposed *Cryptomonas* sp. Φ (Douglas *et al.*, 1991) have 32 mismatches (Fig. 12).

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**Fig. 11.** Pulsed field gel of *Guillardia theta* and *Cryptomonas* sp. Φ (CCMP 325) showing nucleomorph chromosomes I, II and III, plastid DNA (cpDNA) and a bright band of nuclear chromosomes.

but no furrow (Fig. 3). The cell surface of *G. theta* consists of longitudinally orientated plates that form a raised rib where they meet (Fig. 2).

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**Fig. 12.** Alignment of a region of the nucleomorph 18S ribosomal RNA genes from *Guillardia theta* (CCMP 327), the cryptomonad used by Douglas *et al.* (1991) and *Cryptomonas* sp. Φ (CCMP 325). Bases are in bold positions where all three sequences are not the same. # indicates positions where the sequence of Douglas *et al.* (1991) does not match CCMP 325. * indicates a position where the sequences of Douglas *et al.* and *G. theta* do not match. Dashes show where gaps have been inserted to facilitate alignment.
Diagnoses

Hanusia, genus novum

Cryptomonadales Novarino et Lucas; monadibus perpetuo natantibus, marinis; vestibulo subapicali in sulcum ad medium retorsum ducenti; periplasto lamellis nullis, lamina interiore poris qui ejectisomati bus commodant regulatim interrupta, exterio r continuu cum squamis rosulatis imbricatis et fibrillis crassis immixtis, sine fascia midiventrali; chromatophoro unico, pigmento Cr-phycoerythrin 545, pyrenoide sine thylacoidibus perducentibus; nucleomorph unica dorsali inter pyrenioide nuc leumque: ut morphologia proxime Teleaulax Hill atque Proteomonas Hill et Wetherbee diplomorphem differt tamen XVIIIIs rRNA structura.

Species typica: Hanusia phi, sp. nov.

Cryptomonadales Novarino & Lucas; perpetually swimming, marine; vestibulum subapical, posteriorly drawn into a furrow running about halfway down the length of the cell; without periplast plates, an inner sheet interrupted regularly with pores to accommodate ejectisomes, the exterior a continuous layer of rosulate scales with some intermixed coarse fibrils, without a mid-ventral band; single chromatophore with Cr-phycoerythrin 545 pigment (cryptomonad phycoerythrin type I); single pyrenoid without traversing thylakoids; single nucleomorph dorsal between the pyrenoid and nucleus: similar in morphology to Teleaulax Hill and the Proteomonas Hill & Wetherbee diplomorph but differing in 18S ribosomal RNA gene sequence.

Type species: Hanusia phi, sp. nov.


Hanusia phi Deane, species nova

5–10 µm longis, 3–5 µm latis, leviter compressis, aspectu laterali ex elliptica obovatam; plerumque cum quattuor plusve seriebus ejectisomatum; pyrenoide vaginam amyli perfectam facienti.


5–10 µm long, 3–5 µm wide, slightly compressed, narrowly elliptic to obovate in lateral view; usually with 4 or more rows of ejectisomes; pyrenoid with a continuous starch sheath.

Holotype: Fig. 13.

Observations: Hanusia phi, previously known as Cryptomonas sp. Φ, is a new member of the Cryptomonadales (Novarino & Lucas, 1993), very close in morphology to Teleaulax and the diplomorph of Proteomonas, but distinct in its 18S rRNA gene sequence, which is similar to that of G. theta (Hill & Saunders, unpublished data); differing from G. theta, however, in periplast structure, configuration of the furrow-gullet system, and cell size.

Fig. 13. Drawing of Hanusia phi showing features visible by light microscopy.

Discussion

The external appearance and ultrastructure of CCMP 325 are as described for Hanusia phi (Cryptomonas sp. Φ) by Gillot & Gibbs (1983). Hanusia phi is easily distinguished from Guillardia theta on the basis of size, shape and periplast type.

18S rRNA phylogeny, using either nuclear or nucleomorph genes, shows that Hanusia phi is clearly the sister taxon to G. theta (Cavalier-Smith et al., 1996). A close relationship between Hanusia phi and G. theta is also supported by these species having identical nucleomorph karyotypes (Fig. 11). This seems particularly striking since Rensing et al. (1994) resolved the nucleomorph chromosomes of six cryptomonad species and none was found to share the same nucleomorph karyotype.

The circumscription of Cryptomonas has recently been revised and Hanusia phi, which had previously been referred to this genus, does not conform to the altered description (Hill, 1991). Although Hanusia phi shares molecular and karyotypic features with G. theta, it cannot be assigned to Guillardia because it does not fit the physical description of this genus (Hill & Wetherbee, 1990). Morphologically, Hanusia phi is very similar to Teleaulax acuta, Teleaulax meriminda and the diplomorph of Proteomonas sulcata (Hill, 1991; Hill & Wetherbee, 1986). However, phylogenetic trees constructed using nuclear 18S rRNA sequence from Teleaulax and Proteomonas (D. Hill & G. Saunders, unpublished data) suggest it would be wrong to assign Hanusia phi to either of these genera. For these reasons, we have proposed that a new genus,
Hanusia, be created to accommodate the species formerly known as Cryptomonas sp. Φ and that hereafter it be known as Hanusia phi.

Comparison of nucleomorph 18S rRNA genes (Fig. 12) clearly demonstrates that the organism studied by Douglas et al. (1991) was in fact G. theta rather than Hanusia phi. The three mismatches (Fig. 12) between the G. theta sequence and the sequence of Douglas et al. (1991) are most likely due to sequencing or Taq polymerase errors. G. theta was most probably used instead of Hanusia phi in other molecular studies such as those by Douglas (1988, 1991), Douglas & Dunford (1989, 1990a, b), Douglas & Turner (1991), Douglas et al. (1990, 1991), Wang & Liu (1991), McFadden et al. (1994b), Rensing et al. (1994) and Reith & Douglas (1990). Similarly, McFadden et al. (1994a) used the wrong culture for the in situ hybridization and karyotyping components of their study. It would appear that the cultures were switched at some stage after having left the Bigelow culture collection since the source cultures held there still match the original reports (Gillot & Gibbs, 1983; Hill & Wetherbee 1990). One can easily imagine a possible source of the mix-up when recalling that G. theta was originally known as Cryptomonas sp. θ (McKerracher & Gibbs, 1982). Indeed, the simple act of tilting the culture bottle on its side would convert θ to Φ. Whatever the case may be, this study serves to exemplify the importance of holding source cultures in carefully managed collections. It also serves as a reminder to avoid easily confused strain designations such as Φ and θ.

Acknowledgements

J.D. is supported by a Melbourne University Postgraduate Award. We thank Sue Douglas, Bob Andersen, Uwe Maier and Sally Gibbs for helping to unravel the mix-up. Thanks also to Peter Robins and George Scott for Latin translations.

References


