

**Supplementary Table 1:** List of strains, associated sequences and metadata used in this study. For the molecular markers (nSSU, nmSSU and ITS2) GenBank accession numbers (#) are listed (sequences in bold were obtained in this work). Phycobiliprotein (PBP) type, PC for phycocyanin and PE for phycoerythrin. Temperature, country, habitat and locality.

Name	Strain	nSSU #	nmSSU #	ITS2 #	PBP	Temp.	Country	Habitat	Locality
<i>Hemiselmis amylosa</i> <sup>(4)</sup>	Cowl- CSU	AF143944			PC 615 <sup>(5)</sup>		USA	freshwater	North America, Cowdry Lake/ Colorado
<i>Hemiselmis andersenii</i> *	CCMP1180	AM901353	AM901022		PE 545/ 555 <sup>(2)</sup>	20°C	MEX	marine	Gulf of Mexico
<i>Hemiselmis andersenii</i> *	CCMP441	AM901350	AM901019		PE 555 <sup>(2)</sup>	20°C	MEX	marine	Gulf of Mexico, Gulf Stream
<i>Hemiselmis andersenii</i> *	CCMP644	AM901351	DQ519365		PE 555 <sup>(2)</sup>	20°C	USA	marine	Gulf of Mexico, Gulf Stream
<i>Hemiselmis andersenii</i> *	CCMP439	AJ007283	AJ420690		PE 555 <sup>(2)</sup>	24°C	USA	marine	Gulf of Mexico, Cape San Blas
<i>Hemiselmis cf. andersenii</i>	RCC4116	MT628036	MT628035	MT628029	PE 551/ 553	20°C	JPN	marine	North Pacific Ocean
<i>Hemiselmis cf. andersenii</i>	RCC2614	MF179473	MF179479	MT628034		15°C	GBR	marine	North Atlantic Ocean, North Sea
<i>Hemiselmis aquamarina</i>	RCC4102	MF179476	MT605191- MT605193	MT628026- MT628028	PC 564	20°C	JPN	marine	North Pacific Ocean
<i>Hemiselmis aquamarina</i>	BMAK265 (RCC5634)	MT605165 MT605166	MT605187- MT605190	MT628030- MT628033	PC 564	20°C	BRA	marine	South Atlantic Ocean, Ubatuba, SP
<i>Hemiselmis cf. aquamarina</i>	UTEX2000 <sup>(6)</sup>	AM901367	AM901034				USA	marine	North Atlantic Ocean, Virginia, York River
<i>Hemiselmis cryptochromatica</i> *	CCMP1181	AM901354	AM901023		PC 659 <sup>(4)</sup>	14°C	USA	marine	North Atlantic Ocean, Boothbay Harbor, Maine
<i>Hemiselmis pacifica</i> *	CCMP706	AM901352	AM901020		PC 576 <sup>(1)</sup>	14°C	USA	marine	North Atlantic Ocean, Washington, San Juan Island

<i>Hemiselmis rufescens</i>	RCC659	MF179475	MF179481	MT628025	PE 554/ 555	15°C	NOR	marine	North Atlantic Ocean, North Sea
<i>Hemiselmis rufescens</i>	RCC4216	MF179474	MF179480		PE 554/ 555	15°C	FRA	marine	North Atlantic Ocean, English Channel
<i>Hemiselmis rufescens</i> *	CCAP 984/2	AJ007282	AM901016		PE 554 <sup>(2)</sup>		GBR	marine	North Atlantic Ocean, English Channel
<i>Hemiselmis rufescens</i> *	CCMP440	AM901349	AM901018		PE 555 <sup>(3)</sup>	20°C	USA	marine	North Atlantic Ocean, Maine, West Boothbay Harbor
<i>Hemiselmis tepida</i> *	CCMP443	AJ007284	AJ420691		PC 612 <sup>(3)</sup>	20°C	USA	marine	North Atlantic Ocean, Galveston Channel, Texas
<i>Hemiselmis tepida</i> *	CCMP442	HM126533	EF594307		PC 612 <sup>(3)</sup>	20°C	USA	marine	North Atlantic Ocean, Galveston Channel, Texas
<i>Hemiselmis cf. virescens</i>	RCC3575, (M1635, CACC1635 B)	MF179477	MF179484	MT628024	PC 612 <sup>(3)</sup>	17°C	SWE	marine	Kristineborg, Baltic Sea
<i>Hemiselmis virescens</i> *	UTEX 2002	AM901368	AM901035		PC 614 <sup>(3)</sup>		USA	marine	North Atlantic Ocean, Virginia, York River
<i>Chroomonas africana</i>		HG328376	HG328384		PC 645 <sup>(3)</sup>		ZAF	marine	South Atlantic Ocean, Cape Province, Yzerfontein region
<i>Chroomonas coerulea</i>	NIES-0714	HG328381	HG328389				JPN	freshwater	Asia, Honshu, Nagano, Sugadaira
<i>Chroomonas debatzensis</i> *	CCAC 5074 B	MG387973	MG387972				FRA	marine	North Atlantic Ocean, English Channel, Roscoff
<i>Chroomonas debatzensis</i> *	CCAC 0173 B (M1318)	AJ007279	AJ420679				FRA	marine	North Atlantic Ocean, English Channel, Île de Batz

<i>Chroomonas debatzensis</i> *	M1703	AJ420699	AJ420681		PC 630 <sup>(3)</sup>	DNK		Jutland, Hjerting
<b><i>Chroomonas cf. debatzensis</i></b>	<b>RCC3436</b>	<b>MF589232</b>		<b>MT628020,</b> <b>MT628021</b>		<b>17°C</b>	Unknown	<b>marine</b> <b>Unknown</b>
<b><i>Chroomonas cf. debatzensis</i></b>	<b>RCC1504</b>	<b>MT628016</b>		<b>MT628022,</b> <b>MT628023</b>		<b>17°C</b>	<b>FRA</b>	<b>marine</b> <b>North Atlantic Ocean,</b> <b>English Channel, Roscoff</b>
<i>Chroomonas gentoftensis</i> *	CCAC 1627 B (M1627)	AM901360	AM901029		PC 630 <sup>(3)</sup>	DNK	marine	Baltic Sea, Sjaelland, Bellevue Strandbad
<i>Chroomonas mesostigmatica</i>	CCMP1168	AF508268	AM901021		PC 645 <sup>(3)</sup>	Unknown	marine	Unknown
<i>Chroomonas mesostigmatica</i>	CCMP0269	AM901347	AM901017		PC 645 <sup>(3)</sup>	USA	Marine	North Atlantic Ocean, Maryland, Assateague Island
<i>Chroomonas nordstedtii</i> *	NIES-0708	HG328378	HG328386			JPN	freshwater	Asia, Hokkaido, Sapporo, Hokkaido University
<i>Chroomonas nordstedtii</i> *	UTEX 2779	AM901369	AM901036		PC 630 <sup>(3)</sup>	USA	freshwater	North America, Colorado, Wellington Reservoir
<i>Chroomonas pauciplastida</i>	CCMP0268	AM901346	DQ519363		PC 645 <sup>(3)</sup>	USA	marine	North Atlantic, Nantucket Sound
<i>Chroomonas placoidea</i>	CCAP 978/08	AM901345	AM901015		PC 645 <sup>(3)</sup>	GBR	marine	North Atlantic, Irish Sea, Yorkshire
<i>Chroomonas</i> sp.	CCAC 0060 (M0874)	AM901357	AM901026		PC 630 <sup>(3)</sup>	DEU	freshwater	Europe, Griether Ort
<i>Chroomonas</i> sp.	SAG 980-1	AJ420698	AJ420677		PC 645 <sup>(3)</sup>	GBR		Europe, Wales
<i>Chroomonas</i> sp.	CCAC 2291 (M2291/1)	AM901366	AM901033		PC 645 <sup>(3)</sup>	DEU	freshwater	Europe, Cologne, Wahner Heide
<i>Chroomonas</i> sp.	CCAC 1481 B (M1481)	AJ007278	AJ420680		PC 645 <sup>(3)</sup>	DEU	freshwater	Europe, Spessart, Biebergemuend

<i>Komma caudata</i>	MUCC Cr#10	U53122	U53121	PC 645 <sup>(3)</sup>	AUS	freshwater	Oceania, Wimmera river
<i>Cryptomonas curvata</i>	CCAC 0080	AM051189	AJ715462		DEU	freshwater	Europe, Muenster
<i>Cryptomonas marssonii</i>	CCAC 0086	AM051191	AJ566173		DEU	freshwater	Muenster
<i>Cryptomonas pyrenoidifera</i>	CCAC 0179 B (M1077)	AM051197	AJ566180		DEU	freshwater	Europe, Cologne
<i>Falcomonas daucooides</i>	Fada ShP- CSUCC	AF143943	AJ420689	PC 569 <sup>(3)</sup>	USA	marine	North Pacific Ocean, Shannon Point, Washington
<i>Geminigera cryophila</i>	CCMP2564	DQ452091	DQ452092		ATA	marine	Southern Ocean, McMurdo Sound
<i>Guillardia theta</i>	CCMP327	X57162	AJ010592	PE 545 <sup>(3)</sup>	USA	marine	North Atlantic, Long Island Sound
<i>Hanusia phi</i>	CCMP325	U53126	U53125		USA	marine	North Atlantic, Milford, Connecticut USA, Long Island Sound
<i>Plagioselmis nannoplanctica</i>	HB2011-4pn	KC928320	KC928321		Unknown		Unknown
<i>Proteomonas sulcata</i>	CCMP704	AJ007285	AJ420692		Unknown		Unknown
<i>Rhinomonas pauca</i>	MUCC Cr#47	U53132	U53131	PE546 <sup>(3)</sup>	AUS	marine	Bass Strait, Hobsons Bay
<i>Rhinomonas reticulata</i> var. <i>reticulata</i>	CCAP 979/15	HF952562	HF952608		GBR	marine	English Channel, Plymouth Sound
<i>Rhodomonas duplex</i>	NIES-765	HF952604	HF952620		JPN	marine	East China Sea, Okinawa, Yaga
<i>Rhodomonas lens</i>	CCMP739	HF952574	HF952611			marine	Gulf of Mexico
<i>Storeatula major</i>	CCMP320	U53130	U53129		Unknown		Unknown
<i>Teleaulax amphioxeia</i>	SCCAP K- 0434	AJ007287	AJ421146		Unknown		Unknown

Names in bold represents the strains sequenced in this study.

\* Names revised in the study of Lane and Archibald (2008) and Hoef-Emden (2018).

<sup>(1)</sup> Value of pigments according to Lane and Archibald (2008).

<sup>(2)</sup> Value of biliprotein maximum absorption according to Hill and Rowan (1989) and references therein.

<sup>(3)</sup> Value of biliprotein maximum absorption according to Hoef-Emden (2008) and references therein.

<sup>(4)</sup> Value of biliprotein maximum absorption according to Cunningham et al. 2019.

<sup>(5)</sup> Species name and phycobiliprotein characterization are according to Clay and Kugrens (1999).

<sup>(6)</sup> Strain is deposited in UTEX culture collection as *Chroomonas* sp. (see <https://utex.org/products/utex-lb-2000>)

**Supplementary Table 2:** Description of *Hemiselmis* species from the literature.

<i>Hemiselmis</i> species	Cell shape			Cell measurements		Chromatophore description	Biliprotein	Authentic strain	Habitat	Type locality
	lateral view	dorsal/ventral view	cell end	length (μ)	width (μ)					
<i>H. amylosa</i> Clay & Kugrens, 1999	bean-shaped	cylindrical	rounded	5-6	3-3.5	parietal	Cr-PC 615	CowL CSU	freshwater	Cowdry Lake, Colorado (USA)
<i>H. aquamarina</i> Magalhães & Oliveira, 2020	bean-shaped	ovoid	rounded	4.5-7.5	2.7-4.4	parietal, lobate, light green	Cr-PC 564	BMAK265 (syn. RCC5634)	marine, coastal	Ubatuba, SP (BRA)
<i>H. andersenii</i> Lane & Archibald, 2008	reniform	ovoid	acute	5.5-8.5	3-5	parietal, orange to dark red	Cr-PE 555	CCMP644	marine, oceanic	Gulf Stream
<i>H. cryptochromatica</i> Lane & Archibald, 2008	reniform	obovoid to pyriform	rounded	4.5-6.5	3-4.5	faint gray	Cr PC 630	CCMP1181	marine, coastal	Boothbay Harbor, Maine (USA)
<i>H. pacifica</i> Lane & Archibald, 2008	reniform	ovate	acute	7-8.5	4-6.0	parietal, grass-green to grey-green	Cr PC 615	CCMP706	marine, coastal	Friday Harbor, San Juan Island, Washington (USA)
<i>H. rufescens</i> Parke, 1949	bean-shaped		acute	4-8.5	3.5-5	parietal, lobated, French Rose	Cr-PE 555	PCC563	marine, coastal	Port Erin, Isle of Man, (GBR)
<i>H. tepida</i> Lane & Archibald, 2008	reniform	ovate	rounded	5.5-7	3.5-4.5	parietal, absinthe-green to emerald	Cr-PC 612	CCMP443	marine, coastal	Galveston Channel, Texas (USA)
<i>H. virescens</i> Droop, 1955	bean-shaped/falcate	cylindrical		5-7	2.5-3	brilliant green, turquoise to bottle-green	Cr-PC 612	no. 64	marine, coastal	Cumrae, Scotland (GBR)
<i>H. amyliifera</i> Butcher nom. inval. 1967	oblong, ovoid	slightly compressed	rounded	7.5	2.5-3	single, olive-yellow (CC 235)	unknown	unavailable	marine	Lowestoft, Suffolk (GBR)
<i>H. anomala</i> Butcher nom. inval. 1967	bean-shaped	ovoid	rounded	5-6.5	3-3.5	parietal, Paris green or Neptune green	unknown	unavailable	marine	Carmathenshire, Caernarvonshire (GBR)

<i>H. brunnescens</i> Butcher, nom. inval. 1967	ovate, bean-shaped	slightly compressed/elliptical	obtuse-rounded	5- 5.5	3	salmon-pink/ tangerine- orange	Cr-PE 555	PCC14 (syn. CCAP984/2)	marine	English Channel
<i>H. cyclopea</i> Butcher nom. inval. 1967	ovate, bean-shaped	slightly compressed/elliptical	obtuse	4.5- 5.5	4.5- 5	poppy red, parietal	unknown	unavailable	marine	Fishguard, Lowestoft and Conway (GBR)
<i>H. oculata</i> Butcher nom. inval. 1967	ovate, bean-shaped	elliptical, uncompressed	rounded	5-8	4-4.5	two, poppy red parietal	unknown	unavailable	marine	Knap Buoy, English Channel
<i>H. simplex</i> Butcher, nom. inval. 1967	bean-shaped	ovate, lanceolate or cylindrical	rounded	5-6.5	3.5-4	Paris green	unknown	unavailable	marine, coastal	Yorkshire, Lowestoft, Southend, and Conway (GBR)
<i>H. rotunda</i> Butcher nom. inval. 1967	ovate	uncompressed	very rounded	4.-5	2.5-3	parietal, lobated, Neptune green, absinthe	unknown	unavailable	brackish, marine	Bembridge, Isle of Wight (GBR)

### Supplementary Table 3

PCRs cycles for nmSSU of initial denaturation at 94°C during 5min. Followed by 35 cycles of: (i) 94°C for 30sec; (ii) 60°C for 1min and (iii) 72°C for 2min. Final extension step at 72°C for 7 minutes. PCRs for nSSU were performed as indicated in (Majaneva et al. 2014) and ITS2 as described in Hoef-Emden & Melkonian 2003.

#### Primers used for PCRs and sequencing reactions

Primers for nmSSU PCRs		
Sequences of oligonucleotides (5'- 3')		
CrNM1F	CAG TAG TCA TAT GCT TGT CTT AAG	(Hoef-Emden and Melkonian 2003)
SSUBR	TTG ATC CTT CTG CAG GTT CAC CTA C	(Hoef-Emden and Melkonian 2003)
18S 5'	CCA CCT GGT TGA TCC TGC CAG T	(Sogin 1990)
18S 3'	GAT CCT TCT GCA GGT TCA CCT ACG GAA	(Sogin 1990)
Primers for nSSU PCRs		
18SNF2	TGA TGG TCC CTT ACT ACA	(Majaneva et al. 2014)
SSUR	CTT GTT ACG ACT TCT CCT	(Majaneva et al. 2012)
Primers for nSSU and nmSSU sequencing reactions		
18S 5'	CCA CCT GGT TGA TCC TGC CAG T	(Sogin 1990)
CrNM1F	CAG TAG TCA TAT GCT TGT CTT AAG	(Hoef-Emden and Melkonian 2003)
528F	CGG TAA TTC CAG CTC C	(Sogin 1990)
1055F	GGT GGT GCA TGG CCG	(Bellorin et al. 2002)
18S 3'	GAT CCT TCT GCA GGT TCA CCT ACG GAA	(Sogin 1990)
536R	GAA TTA CCG CGG CTG CTG	(Bird et al. 1992)
1055R	CGG CCA TGC ACC ACC	(Bird et al. 1992)
18SNF2	TGATGGTCCCTTACTACA	(Majaneva et al. 2014)
SSUBR	TTG ATC CTT CTG CAG GTT CAC CTA C	(Hoef-Emden and Melkonian 2003)
SSUR	CTTGTTACGACTTCTCCT	(Majaneva et al. 2012)
Primers for ITS PCR and sequencing reaction		
ITS03F-800	CGA TGA AGA ACG YAG CGA	(Hoef-Emden and Melkonian 2003)
ITS05R-700	TAC TTG TTC GCT ATC GGT CTC T	(Hoef-Emden and Melkonian 2003)

### References

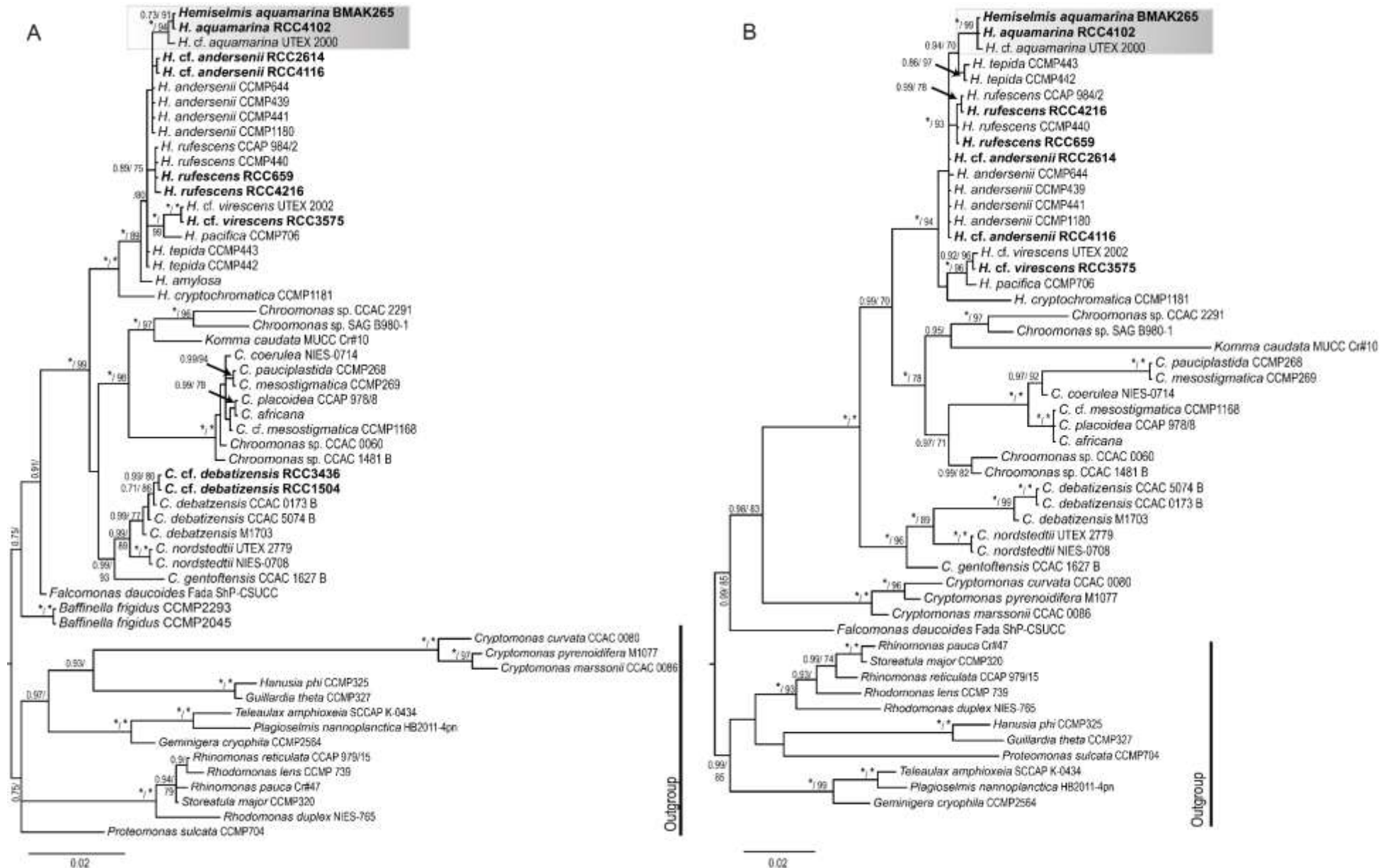
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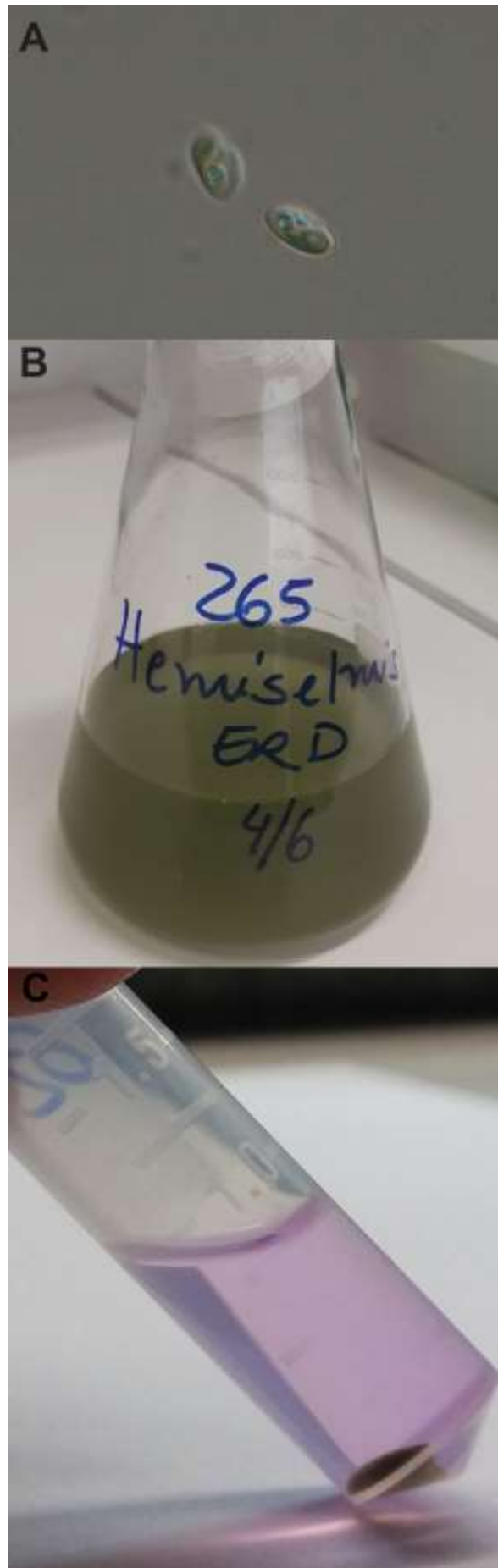
### Supplementary Table 3

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**Supplementary Figure 1:** Molecular phylogeny trees of *Hemiselmis* estimated by BA supported by PP and BS. Strains in bold were sequenced in the present study. (A) Phylogeny inference of nSSU rRNA gene using HKY+G+I as nucleotide substitution model. (B) Phylogeny inference based on sequences of nmSSU rRNA gene, using the nucleotide substitution model GTR+G+I. Nodes with \* are fully supported by PP or BS. Supports below 0.75 PP or 70% of BS are omitted. Scale bar indicates the rate of nucleotide substitution per site.



**Supplementary Figure 2:** *Hemiselmis aquamarina* colour images. A) Color picture of ells in light microscopy. B) Dense cultures aspect. C) Cr-PC 564 pigment after extraction.



Supplementary Figure 3: *Hemiselmis aquamarina* consensus secondary structure of nuclear ITS2.

