

A time-calibrated multi-gene phylogeny provides insights into the evolution, taxonomy and DNA barcoding of the *Pinnularia gibba* group (Bacillariophyta)

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Table S1. The primer sequences used in this study.

Marker	Primer code	Primer sequence (5' - 3')	Dir.	Reference
<i>cox1</i> -5P	GazF2	CAACCAYAAAGATATWGGTAC	>	Saunders 2005
	KEdtmR	AAACTTCWGGRTGACCAAAAA	<	Evans et al. 2007
	PBORcox2F	ATGGTNATGCCNKCTYTHATWGGNGG	>	Pinseel 2019
	PBORcox2R	CCCCAHARNGTGDCYAACCARCT	<	Pinseel 2019
<i>rbcl</i>	DPrbcl1F	AAGGAGAAATHAATGTCT	>	Daugbjerg and Andersen 1997
	DPrbcl7	AARCAACCTTGTAAGTCT	<	Daugbjerg and Andersen 1997
	NDrbcl13F	CGTTTAGAAGATATGCGTATTC	>	Daugbjerg and Andersen 1997
	NDrbcl6F	GTAAATGGATGCGTA	>	Daugbjerg and Andersen 1997
	NDrbcl12R	GCACCTAATAATGG	<	Daugbjerg and Andersen 1997
	15R	ACACCAGACATACGCATCCA	<	Jones et al. 2005
	17R	TGACCAATTGTACCACC	<	Jones et al. 2005
<i>rbcl</i> -3P	CfD	CCRTTYATGCGTTGGAGAGA	>	Hamsher et al. 2011
	DPrbcl7	AARCAACCTTGTAAGTCT	<	Daugbjerg and Andersen 1997
312 bp <i>rbcl</i>	rbcl708F_2	AGGTGAAGTTAAAGGTTTCWTAYTTAAA	>	Vasselon et al. 2017
	R3_2	CCTTCTAATTTACCWACAACA	<	Vasselon et al. 2017
331 bp <i>rbcl</i>	rbcl-646F	ATGCGTTGGAGAGARCGTTTC	>	Kelly et al. 2018
	rbcl-998R	GATCACCTTCTAATTTACCWACAACCTG	<	Kelly et al. 2018
D1-D2 LSU rDNA	DIR-F	ACCCGCTGAATTTAAGCATA	>	Scholin et al. 1994
	D2C-R	CCTTGGTCCGTGTTTCAAGA	<	Scholin et al. 1994
D2-D3 LSU rDNA	T16N-F	AMAAGTACCRYGAGGGAAAG	>	Hamsher et al. 2011
	T24U	SCWCTAATCATTCGCTTTACC	<	Hamsher et al. 2011
SSU rDNA	P2F	CTGGTTGATTCTGCCAGT	>	Van Hannen et al. 1999
	P4R	TGATCCTTCYGCAGGTTTAC	<	Guillou et al. 1999
	P12R	CGGCCATGCACCACC	<	Elwood et al. 1985
	P14F	CGGTAATTCCAGCTCC	>	Gunderson et al. 1986
	P15F	TTGACTCAACACGGG	>	Gunderson et al. 1986
	P16R	GWATTACCGCGGCKGCTG	<	Gunderson et al. 1986
V4 SSU rDNA	D512-F	ATTCCAGCTCCAATAGCG	>	Zimmermann et al. 2011
	D978-R	GACTACGATGGTATCTAATC	<	Zimmermann et al. 2011

Table S2. List of 36 outgroup strains used in the molecular clocks analysis with Genbank identifiers.

Strain code	Taxon	LSU rDNA	<i>rbcl</i>	<i>cox1</i>
AM710427	<i>Eolimna minima</i>	AM710516	AM710427	n.a.
Bfp04_02	<i>Sellaphora blackfordensis</i>	JN418637	JN418674	n.a.
Bfp5x8_F1_3	<i>Sellaphora blackfordensis</i>	JN418632	JN418669	JN418699
AM710435	<i>Mayamaea aromus</i> var. <i>permitis</i>	AM710524	AM710435	n.a.
Wes2f	<i>Mayamaea aromus</i> var. <i>permitis</i>	JN418633	JN418670	JN418700
CAL890TM	<i>Caloneis silicula</i>	JN418626	JN418663	n.a.
Tor1b	<i>Pinnularia</i> sp. (<i>divergens</i> group)	JN418605	JN418642	n.a.
Tor7c	<i>Pinnularia</i> sp. (<i>divergens</i> group)	JN418717	JN418652	n.a.
AM710559	<i>Caloneis budensis</i>	AM710559	AM710470	n.a.
Tor11b	<i>Pinnularia</i> cf. <i>altiplanensis</i>	JN418606	JN418643	JN418678
Alka1	<i>Pinnularia borealis</i>	JN418625	JN418662	n.a.
Tor3a	<i>Pinnularia borealis</i>	JN418608	JN418645	n.a.
Tor12d	<i>Pinnularia borealis</i>	JN418603	JN418640	n.a.
Ecrins7a	<i>Pinnularia borealis</i> var. <i>borealis</i>	JN418634	JN418736	n.a.
B2c	<i>Pinnularia</i> cf. <i>microstauron</i>	JN418601	JN418638	JN418675
Wiea	<i>Pinnularia</i> sp.	JN418611	JN418648	n.a.
PIN873TM	<i>Pinnularia</i> sp.	JN418623	JN418660	JN418692
CAL878TM	<i>Pinnularia</i> cf. <i>isselana</i>	JN418627	JN418664	JN418694
Corsea10	<i>P. subcommutata</i> var. <i>nonfasciata</i>	JN418617	JN418654	JN418686
AM710592	<i>Pinnularia substreptoraphe</i>	AM710592	AM710503	n.a.
PIN876TM	<i>Pinnularia acuminata</i>	JN418630	JN418667	JN418697
AM710579	<i>Pinnularia viridis</i>	AM710579	AM710490	n.a.
PIN883TM	<i>Pinnularia</i> sp.	JN418619	JN418656	JN418688
PIN706F	<i>Pinnularia neglectiformis</i>	JN418629	JN418666	JN418696
PIN649K	<i>Pinnularia</i> sp.	JN418628	JN418665	JN418695
PIN870MG	<i>Pinnularia viridiformis</i>	JN418622	JN418659	JN418691
Corsea2	<i>Pinnularia neomajor</i>	JN418618	JN418655	JN418687
Tor1a	<i>Pinnularia neomajor</i>	JN418604	JN418641	JN418677
Enc2a	<i>Pinnularia viridiformis</i>	JN418607	JN418644	JN418679
Enc2b	<i>Pinnularia acrosphaeria</i>	JN418635	JN418672	JN418701
PIN885TM	<i>Pinnularia nodosa</i>	JN418620	JN418657	JN418689
PIN650K	<i>Pinnularia subanglica</i>	JN418631	JN418668	JN418698
Tor4i	<i>Pinnularia</i> sp.	JN418613	JN418650	JN418683
Ecrins4a	<i>Pinnularia</i> cf. <i>marchica</i>	JN418602	JN418639	JN418676
PIN889MG	<i>Pinnularia grunowii</i>	JN418621	JN418658	JN418690
AM710461	<i>Pinnularia mesolepta</i>	AM710550	AM710461	n.a.

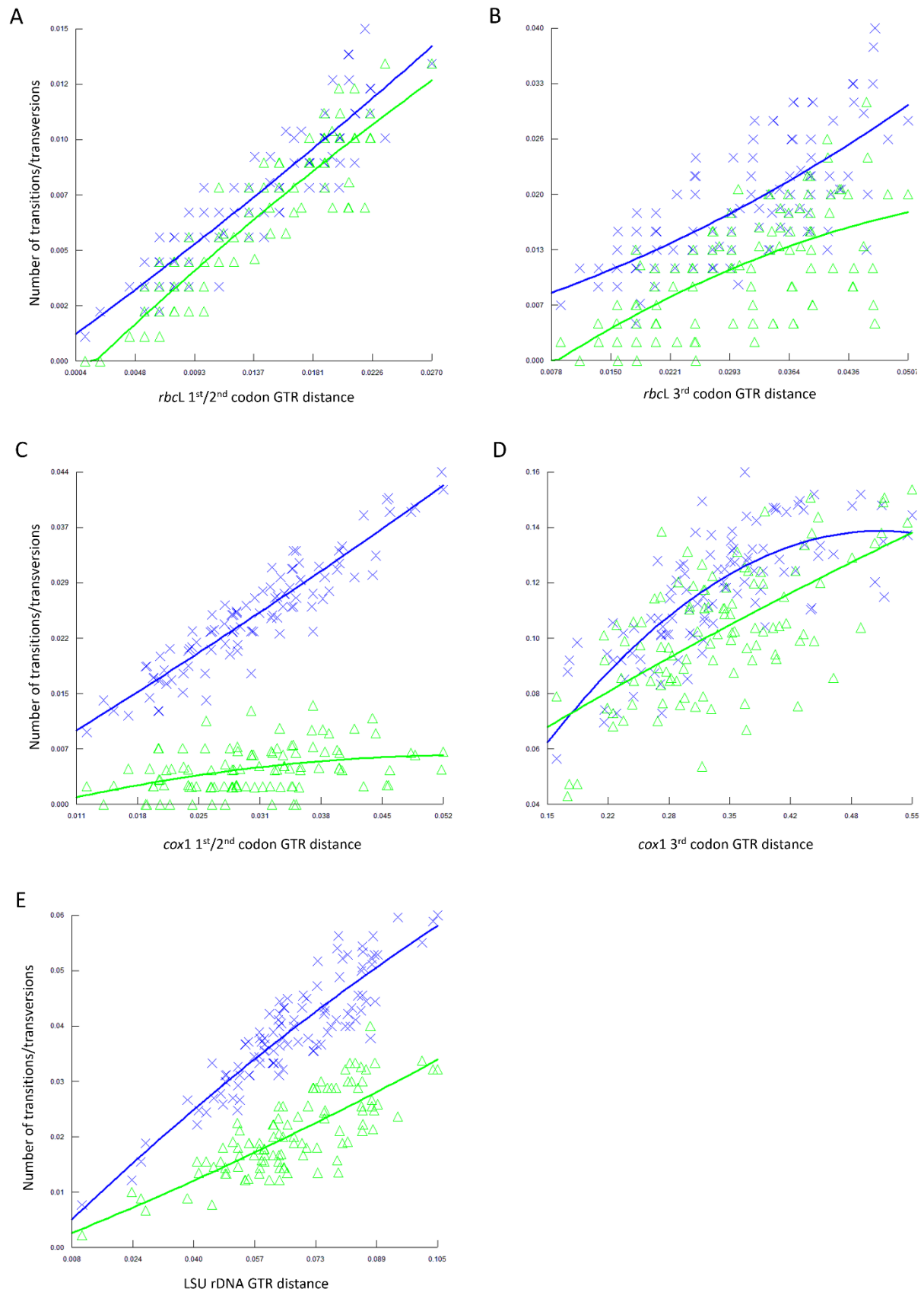


Figure S1. Saturation curves for protein-coding (A-D) and ribosomal (E) markers. Vertical axis represents estimated number of transitions (crosses) and transversions (triangles), and horizontal axis represents GTR distance.

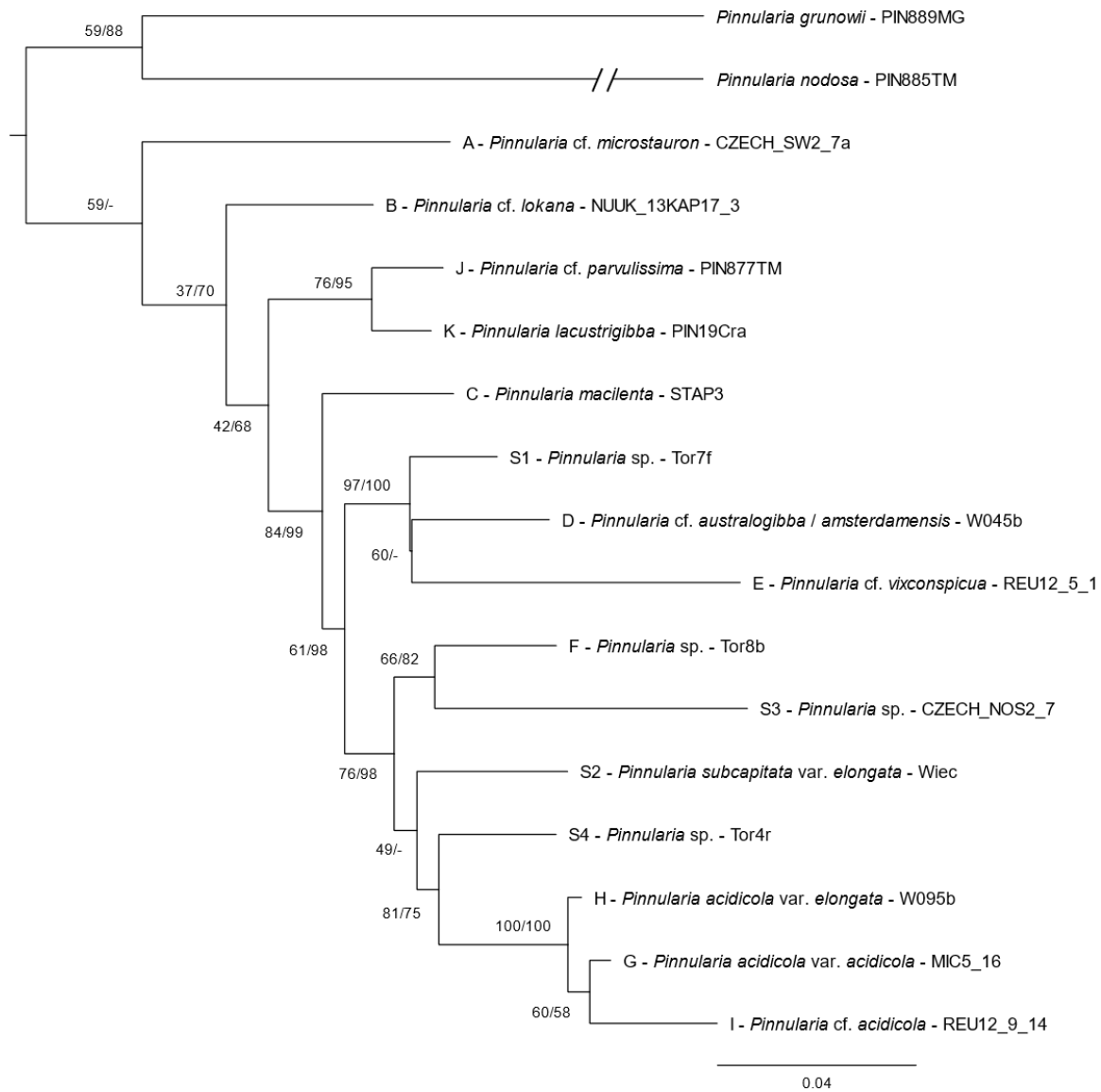


Figure S2. Rooted phylogeny of the *Pinnularia gibba* group inferred from a LSU rDNA alignment using ML under TIM3+F+I+G4 substitution model. Values at nodes indicate statistical supports given as percentages, ML ultrafast bootstrap approximation / BI posterior probabilities. Whenever the ML and BI topologies disagreed, a '-' is given for the BI posterior probability support. The tip labels correspond to the delimited species sensu Kollár et al. (2019): species code - taxon - strain.

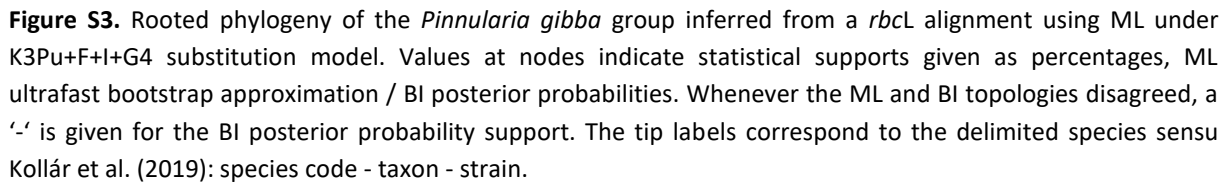


Figure S3. Rooted phylogeny of the *Pinnularia gibba* group inferred from a *rbcl* alignment using ML under K3Pu+F+I+G4 substitution model. Values at nodes indicate statistical supports given as percentages, ML ultrafast bootstrap approximation / BI posterior probabilities. Whenever the ML and BI topologies disagreed, a ‘-’ is given for the BI posterior probability support. The tip labels correspond to the delimited species sensu Kollár et al. (2019): species code - taxon - strain.

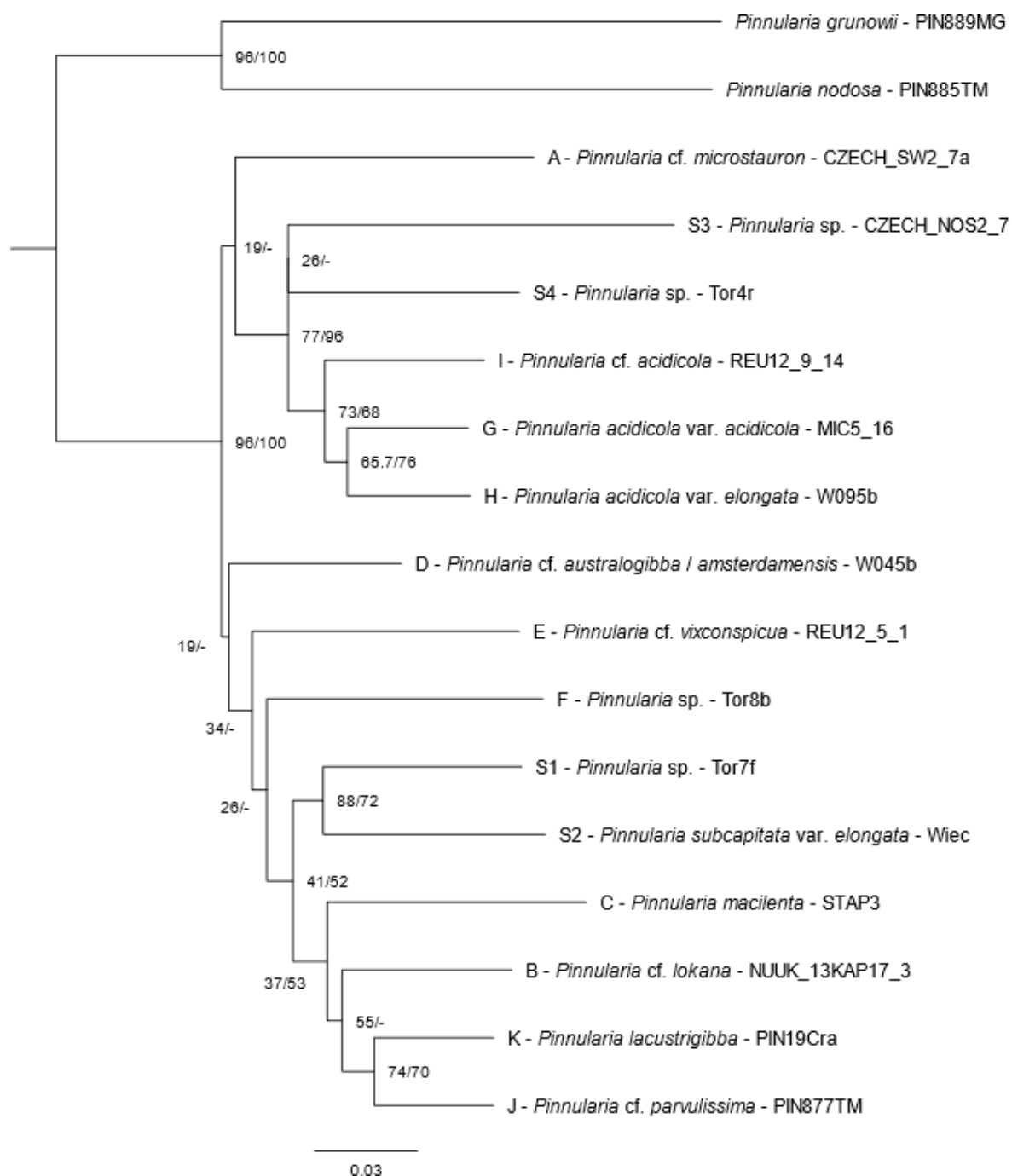


Figure S4. Rooted phylogeny of the *Pinnularia gibba* group inferred from a *cox1* alignment using ML under GTR+F+I+G4 substitution model. Values at nodes indicate statistical supports given as percentages, ML ultrafast bootstrap approximation / BI posterior probabilities. Whenever the ML and BI topologies disagreed, a '-' is given for the BI posterior probability support. The tip labels correspond to the delimited species sensu Kollár et al. (2019): species code - taxon - strain.

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