Title: Towards a phylogenetic classification of Leptothecata (Cnidaria, Hydrozoa)

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We use this section to present additional information, results (figures and tables) and the "Rogue taxa analysis".

Main text and Results:

> Outgroups

Overall, the support for the node defining the sister group was low, with the exception of the lineage Filifera IV+*Hydractinia* sp., in the 16S18S28S_Nw4 analysis[1] (Fig. 3; see also results of the 28S_N and 28S_Nrw4 analyses: Figs. S10, S11). The phylogenies for the 16S data had an artifact caused by long-branch attraction, causing Leptothecata to appear non-monophyletic (e.g., Fig. S3).

> Statocysta

Campanulinida *sensu novum* is also retrieved as a sister group of Eirenida *taxon novum* + Proboscoida *sensu novum* in the analyses of the unfiltered data matrix (16S18S28S), but it becomes sister clade to Eirenida *taxon novum* under the high filtering approach (16S18S28S_Nrw4) (Figs. 2-3).

> Macrocolonia

>> The nodal support of Staurothecidae fam. nov., Sertulariida *taxon novum*, Thyroscyphidae Stechow, 1920, and Sertulariidae Lamouroux, 1812 decreases with filtering (Figs. 1-3, Figs. S6-8).

>> In all the combined results, the basal lineages of Plumulariidae have high support values (Figs. 1-3; Table 2).

>> In the transition:transversion (ts:tv) frequency analysis for 16S and 18S, data matrices are similar, although the types of changes vary. In 16S, the relative frequencies of the nucleotides and the ts:tv relationship is very similar in filtered and unfiltered matrices (Fig. S12; Table 1), but frequencies change and bias in nucleotide frequencies decreases (Fig. S10; Table 1). In the 18S dataset, nucleotide frequencies are essentially constant (regardless of filtering level) in all matrices. The ts:tv ratio is similar for all but the highest filtering level (Fig. S10; Table 2). The 28S ts:tv relationships could not be calculated using DAMBE[153] due to buffer limitations.

Supplemental Figures

Figure 1 – Flow chart illustrating the strategy employed to acquire the genetic data information, emphasizing those changes we had to make (red dotted lines) and subsequent procedures after positive results (solid green lines). See Supplementary Tables 3 and 4.

Figure 2 – Map with the global distribution of the analyzed specimens. Red dots indicate the approximate geographic locations of specimens whose sequences were obtained from GenBank, and black dots indicate the geographic location of samples collected during this study (see Table S1). Map created using QGIS 2.10.1 http://qgis.org/ (Data source from DIVA-GIS website http://www.diva-gis.org/Data).

Figure 3 – Maximum-likelihood cladogram (unfiltered, 16S) for the Leptothecata. Three support values (two parametric, above the branch -aBAYES, aLRT-; one non-parametric, beneath the branch -SH-aLRT-) for each branch. Support values are integral numbers (1, 0) or decimals (no leading zroes). Basic taxonomy (species and genera) from WoRMS[2].

Figure 4 - Maximum-likelihood cladogram (low filtering, 16S) for the Leptothecata. Notations as in Fig. 3.

Figure 5 - Maximum-likelihood cladogram (high filtering, 16S) for the Leptothecata. Notations as in Fig. 3.

Figure 6 - Maximum-likelihood cladogram (unfiltered, 18S) for the Leptothecata. Notations as in Fig. 3.

Figure 7 - Maximum-likelihood cladogram (low filtering, 18S) for the Leptothecata. Notations as in Fig. 3.

Figure 8 - Maximum-likelihood cladogram (high filtering, 18S) for the Leptothecata. Notations as in Fig. 3.

Figure 9 - Maximum-likelihood cladogram (unfiltered, 28S) for the Leptothecata. Notations as in Fig. 3.

Figure 10 - Maximum-likelihood cladogram (low filtering, 28S) for the Leptothecata. Notations as in Fig. 3.

Figure 11 - Maximum-likelihood cladogram (high filtering, 28S) for the Leptothecata. Notations as in Fig. 3.

Figure 12 – Susbstitution patterns of the 16S and 18S markers. Matrices were unfiltered (16S, 18S, 28S), minimally filtered (16S_N, 18S_N, 28S_N) and high filtering (16S_Nrw4, 18S_Nrw4, 28S_Nrw4). Transitions (s) and transversions (v) are plotted against genetic distance calculated using the GTR model of evolution, including all alignment sites. (A) 16S, (B) 16S_N, (C) 16S_Nrw4, (D) 18S, (E) 18S_N, (F) 18S_Nrw4. Substitution patterns for the 28S marker could not be computed in the program DAMBE[153] because of buffer limitations.

Figure 13 - Maximum-likelihood cladogram from rogue-pruned, unfiltered, concatenated matrix (16S18S28S). Notations as in Fig. 3.

Figure 14 - Maximum-likelihood cladogram from rogue-pruned, lightly filtered, concatenated matrix (16S18S28S_N). Notations as in Fig. 3.

Figure 15 - Maximum-likelihood cladogram from rogue-pruned, highly filtered, concatenated matrix (16S18S28S_Nrw4). Notations as in Fig. 3.

Supplementary Table 1 – Species analyzed in the Leptothecata study. "Taxa/Category" refers to the groups, lineages and rogue taxa emphasized in the Results (Figure 1, 16S18S28S_N topology); order of genera and species does not imply monophyly. "Family (WoRMS)" indicates the family to which the genus or species belongs according to WoRMS[2]. The column "nr" indicates total number of analyzed samples. The column "S_s" refers to the number of species considered by WoRMS for that genus ("S") and number of species analyzed in our study for that genus ("s"). "16S ", "18S " and "28S" present GenBank accession numbers for each molecular marker; sequences generated in this study are in bold face. Molecular markers for a certain species obtained from different vouchers are highlited using a gray background (GenBank code, voucher and Country/Region). Code "no ID" refers to those sequences where information is missing (i.e., voucher and/or country/region). Species cells with symbol * are those species considered as potential taxonomic errors (see main text for details). Species cells with symbol ** are those species with different taxonomic identification with GenBank or WoRMS (September 2015).

Taxa / Category	Family (WoRMS)	nr	Species	S_s	16S info	18S info	28S info	Voucher	Country/Region
Subclass Leptothecata									
Order Lafoeida *sensu novum*									
	Syntheciidae	1	*Hincksella* sp.	11_1	**KT757182**			**KU:570**	**Japan**
	Lafoeidae	2	*Lafoea dumosa* (Fleming, 1820)	11_1	FN424137	**KT757183-4**	EU305520	**lem_27A**	Antarctica
								MHNG:INVE29952	Iceland
		3	*Acryptolaria conferta* (Allman, 1877)	39_1	AM887981			no ID	Gulf of Cadiz
Lineage 1 (L 1)	Melicertidae	4	*Melicertum octocostatum* M. Sars, 1835	2_1	FJ550510	FJ550595	FJ550451	MHNG:INVE48744	Norway
	Campanulinidae	5	*Stegella lobata* (Vanhöffen, 1910)	1_1	FN424119	AY789778		no ID	Antarctica
								641AN	Antarctica
Order Laodiceida *taxon novum*									
	Laodiceidae	6	*Laodicea undulata* (Forbes & Goodsir, 1853)	10_1	FJ550471		FJ550390	MHNG:INVE31753	France
		7	*Melicertissa* sp.	7_1	AY512515	AF358075	AY920798	no ID	Guam Iceland
								no ID	no ID
	Tiarannidae	8	*Modeeria rotunda* (Quoy & Gaimard, 1827)	2_1	FJ550476	FJ550540	FJ550396	MHNG:INVE32967	France
		9	*Stegopoma plicatile* (Sars, 1863)	5_1	FJ550513	FJ550598	FJ550454	MHNG:INVE48755	Norway
Lineage 2 (L 2)	Hebellidae	10	*Anthohebella parasitica* (Ciamician, 1880)	6_1	AY787918	EU272603	EU272545	MHNG:INVE29762	Spain
		11	*Hebella scandens* (Bale, 1888)	14_2	**KM822777**			**lem_S44**	Brazil
		12	*Hebella venusta* (Allman, 1877)		FJ550496	FJ550574	FJ550431	MHNG:INVE35476	Honduras
	Laodiceidae	13	*Staurodiscus gotoi* (Uchida, 1927)	16_1	FJ550472	FJ550535	FJ550391	MHNG:INVE33467	New Zealand
Order Statocysta									
Suborder Campanulinida *sensu novum*									
	Campanulinidae	14	*Calycella syringa* (Linnaeus, 1767)	5_1	AY789833	AY789776	FJ550372	664MA	USA
								no ID	France
		15	*Campanulina panicula* G.O. Sars, 1874	7_2	FJ550511	FJ550596	FJ550452	MHNG:INVE48748	Norway
		16	*Campanulina pumila*** (Clark, 1875)		AY789834	AY789777		781MA	USA
	Mitrocomidae	17	*Mitrocomella brownei* (Kramp, 1930)	9_2		FJ550521	FJ550374	no ID	France
		18	*Mitrocomella niwai* Bouillon & Barnett, 1999		FJ550473	FJ550536	FJ550392	no ID	New Zealand
	Phialellidae	19	*Phialella quadrata* (Forbes, 1848)	11_1	FJ550474	FJ550537	FJ550393	MHNG:INVE33466	New Zealand
	Tiaropsidae	20	*Tiaropsidium kelseyi* Torrey, 1909	7_1	AY512517	AF358079	EU305537	AGC1033	USA
		21	*Tiaropsis multicirrata* M. Sars, 1835	2_1	FJ550468	FJ550531	FJ550386	no ID	Iceland
Suborder Eirenida *taxon novum*									
Eirenids I									
	Aequoreidae	22	*Aequorea coerulescens* (Brandt, 1835)	23_5	**KT266599**			no ID	no ID
		23	*Aequorea conica* Browne, 1905		HM053552			IOCAS:36021	no ID
		24	*Aequorea floridana* (Agassiz, 1862)				EU305506	AGC333	Bahamas
		25	*Aequorea forskalea*** Péron & Lesueur, 1810		AY512518	AF358076	EU305505	AGC1075	USA
								no ID	no ID
		26	*Aequorea victoria* (Murbach & Shearer, 1902)		EU305469	AF358077	AY920799	no ID	no ID
								KUNHM2867	no ID
		27	*Rhacostoma atlanticum*** L. Agassiz, 1851	1_1	**KT266638**	**KT722425**	**KT757181**	**lem_S65**	Brazil
	Blackfordiidae	28	*Blackfordia virginica* Mayer, 1910	3_1	**KT266605**	**KT722387**	**KT757145**	**lem_S42**	Brazil
	Eirenidae	29	*Eirene hexanemalis* (Goette, 1886)	23_5	FJ418648			LMZ-200612-2	no ID
		30	*Eirene kambara* (Goette, 1886)		FJ418649	FJ418670		LMZ-200704	no ID
		31	*Eirene lacteoides* Kubota & Horita, 1992		FJ418650	FJ418671		LMZ-200712	no ID
		32	*Eirene pyramidalis* (Agassiz, 1862)		FJ418652	FJ418673		LMZ-200705	no ID
		33	*Eirene viridula* (Péron & Lesueur, 1809)		FJ550502	FJ550588	FJ550445	no ID	France

		34	*Eutonina indicans* (Romanes, 1876)	2_1	**KT266615**		**KT757189-90**	**lem_S131**	Brazil
	Malagazziidae	35	*Octophialucium indicum* Kramp, 1958	12_1	AY787897	EU272626	EU272577	MHNG:INVE29970	New Zealand
	Sugiuridae	36	*Sugiura chengshanense* (Ling, 1937)	2_1	HM053546			IOCAS:36023	no ID
Eirenids II									
	Eirenidae	37	*Eugymnanthea japonica* (Yamada, 1950)	3_1	AY285162	FJ418674		no ID	no ID
								no ID	Japan
		38	*Eutima curva* Browne, 1905	21_5	FJ550514	FJ550599	FJ550455	MHNG:INVE33468	New Zealand
		39	*Eutima gegenbauri* (Haeckel, 1864)		FJ550515	FJ550600	FJ550456	MHNG INVE31748	France
		40	*Eutima krampi* Guo, Xu & Huang, 2008		FJ418653	FJ418675		LMZ-200606-1	no ID
		41	*Eutima levuka* (Agassiz & Mayer, 1899)		FJ418654	FJ418676		LMZ-200707	no ID
		42	*Eutima sapinhoa* Narchi & Hebling, 1975			EU305493	EU305515	AL548	no ID
		43	*Helgicirrha brevistyla* Xu & Huang, 1983	11_2	FJ418655	FJ418677		LMZ-200606-2	no ID
		44	*Helgicirrha malayensis* (Stiasny, 1928)		FJ418645	FJ418668		LMZ-200612-3	no ID
	Lovenellidae	45	*Eucheilota maculata* Hartlaub, 1894	20_2	**KT266613**	**KT722394**	**KT757148**	**lem_S132**	Brazil
		46	*Eucheilota menoni* A. Agassiz, 1862		FJ550493	FJ550570	FJ550427	MHNG:INVE33457	New Zealand
		47	*Hydranthea margarica* (Hincks, 1862)	1_1	DQ855932	FJ550567	FJ550424	no ID	Spain
Suborder Proboscoida *sensu novum*									
Infraorder Campanulariida *sensu novum*									
	Bonneviellidae	48	*Bonneviella regia* (Nutting, 1901)	10_4	AY789805	AY789740		818AS	USA
		49	*Bonneviella* sp. 2 819AS		AY789806	AY789741		819AS	USA
		50	*Bonneviella* sp. 3 830AS		AY789807	AY789742		830AS	USA
		51	*Bonneviella* sp. 4 839AS		AY789808	AY789743		839AS	USA
	Campanulariidae	52	*Campanularia hincksii* Alder, 1856	35_3	AY789794	AY789729		37IT	Italy
		53	*Campanularia* sp.		FN424118	**KT722389**	**KT757187-8**	**lem_17A**	Antarctica
		54	*Campanularia volubilis* (Linnaeus, 1758)		AY789804	AY789739		USNM:1106166	USA
		55	*Orthopyxis crenata*** (Hartlaub, 1901)	19_4	FJ550466		FJ550383	no ID	New Zealand
		56	*Orthopyxis everta* (Clark, 1876)		AY789793	AY789728		66IT	Italy
		57	*Orthopyxis mianzani* Cunha, Genzano & Marques, 2015		**KT266632**	**KT722415**	**KT757151**	**MZUSP:4107**	Brazil
		58	*Orthopyxis sargassicola* (Nutting, 1915)		**KT266633**	**KT722416**	**KT757155**	**MZUSP:4247**	Brazil
		59	*Rhizocaulus verticillatus* (Linnaeus, 1758)	2_1	AY789803	AY789738		USNM:1106183	USA
		60	*Silicularia rosea* Meyen, 1834	4_1	FJ550482	FJ550549	FJ550406	MHNG:INVE25072	New Zealand
Lineage 3 (L 3)	Eirenidae	61	*Eirene ceylonensis* Browne, 1905	23_2	FJ418647	FJ418669		LMZ-200612-1	no ID
		62	*Eirene menoni* Kramp, 1953		FJ418651	FJ418672		LMZ-200706	no ID
	Lovenellidae	63	*Eucheilota bakeri** (Torrey, 1904)	20_1	AY789831	AY789774		704CA	USA
		64	*Lovenella gracilis* Clarke, 1882	12_1	AY789830	AY789773		848NJ	USA
Infraorder Obeliida *taxon novum*									
	Campanulariidae	65	*Clytia gracilis* (Sars, 1850)	58_6	DQ068062	DQ068052		USNM:1078723	Brazil
		66	*Clytia hemisphaerica* (Linnaeus, 1767)		AY789814	AY789753	FJ550457	826NS	Denmark
								no ID	France
		67	*Clytia hemisphaerica* 2** (Linnaeus, 1767)		AY346365			no ID	no ID
		68	*Clytia hummelincki* (Leloup, 1935)		AY789809	AY789744		856SA	South Africa
		69	*Clytia linearis* (Thorneley, 1900)		AY789810	AY789748		USNM:1078729	USA
		70	*Clytia noliformis* (McCrady, 1859)		DQ064792	EU272611	EU272554	AL1405	Brazil
								no ID	Brazil
		71	*Clytia paulensis* (Vanhöffen, 1910)		AY346361	AY789746		USNM:1106158	Italy
		72	*Gonothyraea inornata*** Nutting, 1901	2_2	AY789822	AY789761		564WA	USA
		73	*Gonothyraea loveni* (Allman, 1859)		**KT266616**	**KT722398**	**KT757149**	**MZUSP:1845**	USA
		74	*Laomedea calceolifera* (Hincks, 1871)	7_2	AY789829	AY789768	FJ550447	721MA	USA
								MHNG:INVE37296	France
		75	*Laomedea flexuosa* Alder, 1857		AY789824	AY789763		864IC	Iceland
		76	*Obelia bidentata* Clark, 1875	9_4	AY789815	AY789754	FJ550446	76NC	USA
								MHNG:INVE37294	France
		77	*Obelia dichotoma* (Linnaeus, 1758)		KM603472	**KT722411**	**KT757168**	**MZUSP:1776**	USA
		78	*Obelia geniculata* (Linnaeus, 1758)		**KT266629**	**KT722412**	**KT757167**	**MZUSP:1866**	USA
		79	*Obelia longissima* (Pallas, 1766)		KM603468	KM603469	**KT757159**	**MZUSP:1807**	USA
	Eirenidae	80	*Eirene brevistylus** ** Huang & Xu, 1994	23_1	FJ418646			LMZ-200703	no ID

Order Macrocolonia										
Suborder Staurothecida *taxon novum*										
Staurothecidae *família nova*										
	Sertulariidae	81	*Staurotheca antarctica* Hartlaub, 1904	24_4	FN424142	**KT722438**	**KT757203**	**lem_S155**	Antarctica
		82	*Staurotheca compressa* Briggs, 1938		FN424143			no ID	Antarctica
		83	*Staurotheca nonscripta Peña Cantero, Svoboda & Vervoort, 1997*		FN424144			no ID	Antarctica
		84	*Staurotheca pachyclada* (Jäderholm, 1904)		FN424145			no ID	Antarctica
Symplectoscyphidae *família nova*									
	Sertulariidae	85	*Antarctoscyphus elongatus* (Jäderholm, 1904)	10_3	FN424138			**lem_S154**	Antarctica
		86	*Antarctoscyphus grandis* (Blanco, 1977)		FN424139	**KT722383**	**KT757185-6**	**lem_46A**	Antarctica
		87	*Antarctoscyphus spiralis (Hickson & Gravely, 1907)*		FN424140	**KT722384**	**KT757141**	**lem_S151**	Antarctica
		88	*Symplectoscyphus curvatus* (Jäderholm, 1917)	105_6	FN424146	**KT722439**	**KT757144**	**lem_S156**	Antarctica
		89	*Symplectoscyphus exochus* Blanco, 1982		FN424147	**KT722440**	**KT757172**	**lem_S123**	Antarctica
		90	*Symplectoscyphus glacialis* (Jäderholm, 1904)		FN424148	**KT722441**	**KT757178**	**lem_S153**	Antarctica
		91	*Symplectoscyphus liouvillei* (Billard, 1914)		FN424149			no ID	Antarctica
		92	*Symplectoscyphus plectilis* (Hickson & Gravely, 1907		FN424150			no ID	Antarctica
		93	*Symplectoscyphus tricuspidatus* (Alder, 1856)		AY787907			MHNG:INVE29953	Iceland
Suborder Haleciida *sensu novum*									
Haleciidae *sensu novum*									
	Haleciidae	94	*Halecium beanii* (Johnston, 1838)	128_13	FJ550488	FJ550560	FJ550417	MHNG:INVE34009	South Africa
		95	*Halecium delicatulum* Coughtrey, 1876		FN424123			no ID	Antarctica
		96	*Halecium halecinum* (Linnaeus, 1758)		**KT266617**	**KT722399**	**KT757152**	**MZUSP:1782**	USA
		97	*Halecium incertus* Naumov & Stepanjants, 1962		FN424124	**KT722400**	**KT757191**	**lem_150**	Antarctica
		98	*Halecium labrosum* Alder, 1859		AY787916	FJ550550	FJ550407	MHNG:INVE29030	Iceland
		99	*Halecium lankesterii*** (Bourne, 1890)		AM888316			no ID	United Kingdom
		100	*Halecium lenticulare* Trebilcock, 1928		FJ550469	FJ550532	FJ550387	MHNG:INVE33461	New Zealand
		101	*Halecium mediterraneum* Weismann, 1883		FJ550492	FJ550566	FJ550423	MHNG:INVE34437	France
		102	*Halecium mirabile* Schydlowsky, 1902		**KT266618**			no ID	no ID
		103	*Halecium muricatum* (Ellis & Solander, 1786)		AY787915	EU272619	FJ550408	MHNG:INVE29028	Iceland
		104	*Halecium petrosum* Stechow, 1919		AY787893			MHNG:INVE29763	Spain
		105	*Halecium pusillum* Sars, 1856		FJ550499	FJ550580	FJ550437	MHNG:INVE36295	France
		106	*Halecium tenellum* Hincks, 1861		AM888322			no ID	United Kingdom
Lineage 4 (L 4)	Lafoeidae	107	*Abietinella operculata* (Jäderholm, 1903)	1_1	FN424136			no ID	Antarctica
		108	*Cryptolaria pectinata* (Allman, 1888)	5_1	AM887994			DBUA 1280.01	Gulf of Cádiz
		109	*Zygophylax biarmata* Billard, 1905	47_1	AM888343			DBUA 919.02	Gulf of Cádiz
Suborder Sertulariida *taxon novum*									
Sertularellidae *família nova*									
	Sertulariidae	110	*Sertularella africana* Stechow, 1919	131_10	FJ550490	FJ550563	FJ550420	MHNG:INVE34017	South Africa
		111	*Sertularella diaphana* (Allman, 1885)		**KT266639**	**KT722430**	**KT757131**	**MZUSP:1725**	Brazil
		112	*Sertularella ellisii (Deshayes & Milne Edwards, 1836)*		FJ550478	FJ550545	FJ550402	MHNG:INVE32156	Spain
		113	*Sertularella gayi* (Lamouroux, 1821)			FJ550579	FJ550436	MHNG:INVE36302	France
		114	*Sertularella mediterranea* Hartlaub, 1901		FJ550479	FJ550546	FJ550403	MHNG:INVE32948	France
		115	*Sertularella polyzonias*** (Linnaeus, 1758)		AM888340			DBUA 1186.01	Gulf of Cádiz
		116	*Sertularella robusta* Coughtrey, 1876		AM888339			no ID	Gulf of Cádiz
		117	*Sertularella rugosa* (Linnaeus, 1758)		AY787906			MHNG:INVE29032	Iceland
		118	*Sertularella sanmatiasensis* El Beshbeeshy, 2011		FN424141	**KT722431**	**KT757202**	**lem_S158**	Antarctica
		119	*Sertularella tenella* (Alder, 1857)			**KT722432**	**KT757179**	**MZUSP:4466**	Brazil
		120	*Symplectoscyphus turgidus** (Trask, 1857)	105_1	FJ550462	FJ550524	FJ550377	MHNG:INVE29467	USA
Thyroscyphidae	Thyroscyphidae	121	*Thyroscyphus ramosus* Allman, 1877	10_2	**KM822774**	**KM822775**	**KM822776**	**MZUSP:1664**	Brazil
		122	*Thyroscyphus marginatus*** (Allman, 1877)		FJ550495	FJ550573	FJ550430	MHNG:INVE35477	Honduras
		123	*Sertularelloides cylindritheca*** (Allman, 1888)	1_1	**KT266640**	**KT722433**		**MZUSP:1677**	Brazil
Sertulariidae *sensu novum*									
	Sertulariidae	124	*Abietinaria abietina* (Linnaeus, 1758)	37_2	FJ550484	FJ550554	FJ550411	MHNG:INVE29446	Iceland

		125	*Abietinaria filicula* (Ellis & Solander, 1786)		FJ550485	FJ550555	FJ550412	MHNG:INVE29947	Iceland
		126	*Amphisbetia minima* (Thompson, 1879)	29_2	AY787903	EU272602	EU272544	MHNG:INVE25071	New Zealand
		127	*Amphisbetia operculata* (Linnaeus, 1758)		FJ550489	FJ550561	FJ550418	MHNG:INVE34014	South Africa
		128	*Diphasia fallax* (Johnston, 1847)	28_2	AY787901	EU305491	FJ550414	MHNG:INVE29950	Iceland
		129	*Diphasia rosacea* (Linnaeus, 1758)		AM888306			DBUA 1172.02	United Kingdom
		130	*Dynamena crisioides* Lamouroux, 1824	19_5	**KT266609**	**KT722391**	**KT757177**	**MZUSP:1679**	Brazil
		131	*Dynamena disticha* (Bosc, 1802)		AY787909			MHNG:INVE29754	Spain
		132	*Dynamena moluccana*** (Pictet, 1893)		FJ550494	FJ550572	FJ550429	no ID	Thailand
		133	*Dynamena obliqua* Lamouroux, 1816		**KT266610**			no ID	no ID
		134	*Dynamena pumila* (Linnaeus, 1758)		AY787902	FJ550558	FJ550415	MHNG:INVE29026	Iceland
		135	*Hydrallmania falcata* (Linnaeus, 1758)	3_1	FJ550487	FJ550559	EU305519	MHNG:INVE29948	Iceland
		136	*Idiellana pristis* (Lamouroux, 1816)	2_1	**KT266622**	**KT722404**	**KT757192-3**	**MZUSP:1651**	Brazil
		137	*Salacia desmoides* (Torrey, 1902)	22_1	FJ550464	FJ550528	FJ550381	no ID	France
		138	*Sertularia argentea* Linnaeus, 1758	61_8	FJ550461	FJ550520	FJ550373	no ID	France
		139	*Sertularia cupressina* Linnaeus, 1758		FJ550475	FJ550539	FJ550395	MHNG:INVE29949	Iceland
		140	*Sertularia distans* (Lamouroux, 1816)		**KT266641**	**KT722434**	**KT757163**	**MZUSP:1705**	Brazil
		141	*Sertularia marginata* (Kirchenpauer, 1864)		**KT266642**	**KT722435**	**KT757150**	**MZUSP:1656**	Brazil
		142	*Sertularia perpusilla* Stechow, 1919		AY787894		EU305532	MHNG:INVE29765	Spain
		143	*Sertularia tumida* Allman, 1877		**KT266643**	**KT722436**	**KT757154**	**lem_S54**	Brazil
		144	*Sertularia turbinata* (Lamouroux, 1816)		**KT266644**	**KT722437**	**KT757147**	**MZUSP:1704**	Brazil
		145	*Sertularia unguiculata* Busk, 1852		AY787904			MHNG:INVE29969	New Zealand
		146	*Thuiaria cornigera*** Kudelin, 1914	50_2		Z92899		no ID	no ID
		147	*Thuiaria thuja* (Linnaeus, 1758)		AY787908	EU305503	EU305536	MHNG:INVE29951	Iceland
Suborder Plumupheniida *taxon novum*									
Lineage 5 (L 5)	Haleciidae	148	*Hydrodendron mirabile* (Hincks, 1866)	25_1	DQ855933	FJ550568	FJ550425	MHNG:INVE34779	Spain
		149	*Nemalecium lighti* (Hargitt, 1924)	2_1	**KT266628**	**KT722410**	**KT757146**	**lem_S17**	Brazil
Infraorder Aglaopheniida *taxon novum*									
Aglaopheniidae	Aglaopheniidae	150	*Aglaophenia acacia* Allman, 1883	73_14	FJ550507			MHNG:INVE37535	Italy
		151	*Aglaophenia elongata* Meneghini, 1845		FJ550508	FJ550593	FJ550450	MHNG:INVE37539	Italy
		152	*Aglaophenia harpago* Schenck, 1965		FJ550506	FJ550592	FJ550449	MHNG:INVE37531	Italy
		153	*Aglaophenia kirchenpaueri* (Heller, 1868)		AM887983			no ID	United Kingdom
		154	*Aglaophenia latecarinata* Allman, 1877		**KT266600**			**lem_S48**	Brazil
		155	*Aglaophenia lophocarpa* Allman, 1877		AM887988			no ID	Gulf of Cádiz
		156	*Aglaophenia octodonta* Heller, 1868		AM887989	FJ550541	FJ550397	MHNG:INVE32875	France
		157	*Aglaophenia parvula* Bale, 1882		DQ855914			MHNG:INVE34013	South Africa
		158	*Aglaophenia picardi* Svoboda, 1979		AY787891			MHNG:INVE29758	Spain
		159	*Aglaophenia pluma* (Linnaeus, 1758)		DQ855916	FJ550542	FJ550398	MHNG:INVE38220	France
		160	*Aglaophenia rhynchocarpa* Allman, 1877		**KT266601**	**KT722382**	**KT757157**	**MZUSP:1687**	Brazil
		161	*Aglaophenia struthionides* (Murray, 1860)		**KT266602**			no ID	no ID
		162	*Aglaophenia tubiformis* Marktanner-Turneretscher, 1890		DQ855917	EU272601	FJ550399	MHNG:INVE32960	France
								MHNG:INVE29967	France
		163	*Aglaophenia tubulifera* (Hincks, 1861)		AM887992			no ID	United Kingdom
		164	*Cladocarpus integer* (Sars, 1873)	52_1	FJ550512	FJ550597	FJ550453	MHNG:INVE48754	Norway
		165	*Gymnangium gracilicaule* (Jäderholm, 1903)	38_3	DQ855934	FJ550585	FJ550442	MHNG:INVE36839	Madagascar
		166	*Gymnangium hians* (Busk, 1852)		AY787922	Z86122		MHNG:INVE32586	Thailand
								no ID	no ID
		167	*Gymnangium montagui* (Billard, 1912)		AM888313			no ID	United Kingdom
		168	*Lytocarpia phyteuma* (Kirchenpauer, 1876)	40_3	AY787921			MHNG:INVE32597	Thailand
		169	*Lytocarpia canepa* (Blanco & Bellusci de Miralles, 1971)		**KT266645**	**KT722406**	**KT757196-7**	**lem_S139**	Argentina
		170	*Lytocarpia* sp.		FJ550505	FJ550591	FJ550448	MHNG:INVE36828	Madagascar
		171	*Macrorhynchia philippina* Kirchenpauer, 1872	25_2	**KT266625**	**KT722407**	**KT757153**	**MZUSP:1728**	Brazil
		172	*Macrorhynchia phoenicea* (Busk, 1852)		DQ855935	FJ550584	FJ550441	MHNG:INVE36813	Madagascar
		173	*Macrorhynchia phoenicea* 2** (Busk, 1852)		FJ550500	FJ550586	FJ550443	MHNG:INVE36832	Madagascar
Infraorder Plumulariida *sensu novum*									
Schizotrichidae	Halopteriditidae	174	*Schizotricha crassa* Peña Cantero & Vervoort, 2004	21_5	FN424125	**KT722426**	**KT757136**	**lem_S162**	Antarctica

		175	*Schizotricha falcata* Peña Cantero, 1998		FN424126	**KT722427**	**KT757135**	**lem_S157**	Antarctica
		176	*Schizotricha nana* Peña Cantero, Svoboda & Vervoort, 1996		FN424127	**KT722428**	**KT757132**	**lem_S127**	Antarctica
		177	*Schizotricha trinematotheca* Peña Cantero & Vervoort, 2005		FN424128			no ID	Antarctica
		178	*Schizotricha turqueti* Billard, 1906		FN424129	**KT722429**	**KT757142**	**lem_S159**	Antarctica
Kirchenpaueriidae	Kirchenpaueriidae	179	*Kirchenpaueria pinnata* (Linnaeus, 1758)	14_1	AY787911	FJ550578	FJ550435	MHNG:INVE29965	France
								MHNG:INVE36294	France
		180	*Kirchenpaueria pinnata* 2** (Linnaeus, 1758)		DQ855923	FJ550581	FJ550438	MHNG:INVE36296	France
		181	*Oswaldella grandis* Peña Cantero, Svoboda & Vervoort, 1997	26_5	FN424132	**KT722417**	**KT757140**	**lem_13A**	Antarctica
		182	*Oswaldella incognita* Peña Cantero, Svoboda & Vervoort, 1997		FN424133			no ID	Antarctica
		183	*Oswaldella laertesi* Peña Cantero, 2007		FN424134	**KT722418**	**KT757137**	**lem_S129**	Antarctica
		184	*Oswaldella shetlandica* Stepanjants, 1979		**KT266634**	**KT722419**	**KT757134**	**lem_S128**	Antarctica
		185	*Oswaldella stepanjantsae* Peña Cantero, Svoboda & Vervoort, 1997		FN424135	**KT722420**	**KT757133**	**lem_S130**	Antarctica
		186	*Pycnotheca mirabilis* (Allman, 1883)	3_1	FJ550465	FJ550529	FJ550382	MHNG:INVE25847	New Zealand
		187	*Ventromma halecioides*** (Alder, 1859)	3_1	AY787895	FJ550530	FJ550385	MHNG:INVE29766	France
		188	*Ventromma halecioides* 2** (Alder, 1859)		**KT266623**		**KT757180**	**lem_S106**	Brazil
Halopteridiidae	Halopteridiidae	189	*Antennella ansini* Peña Cantero & García Carrascosa, 2002	23_3	FJ550470	FJ550533	FJ550388	MHNG:INVE32157	Spain
		190	*Antennella kiwiana* Schuchert, 1997		DQ855918	FJ550534	FJ550389	MHNG:INVE33623	New Zealand
		191	*Antennella secundaria* (Gmelin, 1791)		DQ883445	FJ550575	FJ550432	MHNG:INVE32969	France
		192	*Halopteris alternata* (Nutting, 1900)	35_8	DQ855939			AL517	Brazil
		193	*Halopteris catharina* (Johnston, 1833)		DQ855920	FJ550517	FJ550370	no ID	France
		194	*Halopteris carinata* Allman, 1877		**KT266619**	**KT722401**	**KT757139**	**MZUSP:1681**	Brazil
		195	*Halopteris diaphana* (Heller, 1868)		**KT266620**	**KT722402**	**KT757138**	**MZUSP:1662**	Brazil
		196	*Halopteris liechtensternii* (Marktanner-Turneretscher, 1890)		AY787888	FJ550526	FJ550379	MHNG:INVE30116	Spain
								MHNG:INVE29751	Spain
		197	*Halopteris minuta* (Trebilcock, 1928)		AY787912	EU272620	EU272567	MHNG:INVE25073	New Zealand
		198	*Halopteris schucherti* Galea, 2006			FJ550577	FJ550434	no ID	Chile
		199	*Halopteris tenella* (Verrill, 1874)		DQ855938			AL575	Brazil
		200	*Monostaechas quadridens* (McCrady, 1859)	5_1	**KT266627**	**KT722409**	**KT757171**	**MZUSP:4632**	Brazil
		201	*Polyplumaria flabellata* Sars, 1874	8_1	AM888338			no ID	Gulf of Cadiz
Plumulariidae	Plumulariidae	202	*Dentitheca bidentata* (Jäderholm, 1905)	4_1	**KT266608**	**KT722390**	**KT757143**	**MZUSP1731**	Brazil
		203	*Nemertesia antennina* (Linnaeus, 1758)	43_2	AY787910	EU305498	EU305523	MHNG:INVE29954	France
								MHNG:INVE34437	France
		204	*Nemertesia ventriculiformis* (Marktanner-Turneretscher, 1890)		AM888336			no ID	Gulf of Cadiz
		205	*Plumularia filicaulis* Kirchenpauer, 1876	93_10	DQ855926	FJ550565	FJ550422	MHNG:INVE34020	South Africa
		206	*Plumularia habereri* Stechow, 1909			FJ550571	FJ550428	no ID	Thailand
		207	*Plumularia habereri* 2** Stechow, 1909		DQ855927			no ID	Thailand
		208	*Plumularia hyalina* (Bale, 1882)		AY787913	FJ550552	FJ550409	MHNG:INVE25333	New Zealand
		209	*Plumularia insignis* Allman, 1883			**KT722422**	**KT757200-1**	**lem_S134**	Argentina
		210	*Plumularia lagenifera* Allman, 1885		DQ855928	FJ550527	FJ550380	MHNG:INVE25120	USA
		211	*Plumularia margaretta* (Nutting, 1900)		FJ550483	FJ550553	FJ550410	MHNG:INVE29760	Spain
		212	*Plumularia margaretta* 2 (Nutting, 1900)		**KT266636**	**KT722423**	**KT757166**	**MZUSP:1734**	Brazil
		213	*Plumularia setacea* (Linnaeus, 1758)		**KT266637**	**KT722424**	**KT757170**	**lem_S144**	Argentina
		214	*Plumularia obliqua* (Johnston, 1847)		DQ855929	FJ550544	FJ550401	no ID	France
		215	*Plumularia pulchella* Bale, 1882		DQ855930	FJ550562	FJ550419	MHNG:INVE34016	South Africa
		216	*Plumularia spiralis* Billard, 1911		AY787920	FJ550569	FJ550426	MHNG:INVE32600	Thailand
Rogue taxa 1 (R 1)	Haleciidae	217	*Hydrodendron gardineri** (Jarvis, 1922)	25_1	AY787923			MHNG:INVE32618	Thailand
Rogue taxa 2 (R 2)	Campanulinidae	218	*Opercularella lacerata* (Johnston, 1847)	4_1	**KT266631**	**KT722414**	**KT757198-9**	**MZUSP:1848**	USA
Rogue taxa 3 (R 3)	Lafoeidae	219	*Billardia subrufa** (Jäderholm, 1904)	4_1	**KT266603**	**KT722385**	**KT757130**	**lem_S124**	Antarctica
Rogue taxa 3 (R 4)	Hebellidae	220	*Scandia gigas* (Pieper, 1884)	6_1	AY787919			MHNG:INVE29764	Spain

Taxa / Category	G/Sp G//Sp	nr	Species	S_s	16S info	18S info	28S info	Voucher	Country/Region
Order “Anthoathecata”									
Suborder Capitata									
Corynidae	13/56 3//3	221	*Coryne pintneri* Schneider, 1897	19_1	AJ878717	GQ424330	GQ424303	MHNG:INVE31976	France
								Sch251	France
		222	*Sarsia lovenii* (M. Sars, 1846)	11_1	GQ395329	GQ424337	GQ424310	MHNG:INVE48736	Norway
		223	*Stauridiosarsia ophiogaster*** (Haeckel, 1879)	9_1	EU305473	EU272615	EU272560	KUNHM 2803	no ID
Cladocorynidae	2/7 1//1	224	*Cladocoryne floccosa* Rotch, 1871	5_1	**KT266607**	EU272608	**KT757173**	**lem_S40**	Brazil
								AL1407	Brazil
Moerisiidae	3/10 1//1	225	*Moerisia inkermanica* Paltschikowa-Osroumowa, 1925	7_1	**KT266626**	**KT722408**	**KT757161**	**lem_S41**	Brazil
Pennariidae	1/5 1//1	226	*Pennaria disticha* Goldfuss, 1820	5_1	**KT266635**	**KT722421**	**KT757129**	**MZUSP:1670**	Brazil
Porpitidae	2/4 1//1	227	*Porpita porpita* (Linnaeus, 1758)	2_1	AY935322	GQ424319	EU883551	no ID	Mexico
								CWD147	no ID
								no ID	no ID
Zancleidae	3/40 1//1	228	*Zanclea prolifera* Uchida & Sugiura, 1976	33_1	EU305488	EU272639	EU272598	KUNHM:2793	no ID
Suborder Aplanulata									
Candelabridae	3/19 1//1	229	*Candelabrum cocksii* (Cocks, 1854)	14_1	AY512520	AY920758	AY920796	MHNG:INVE29591	France
								no ID	France
Corymorphidae	11/77 1//1	230	*Corymorpha pendula* L. Agassiz, 1862	44_1	EU876538	EU876565	EU879936	KUNHM:2962	USA
Hydridae	1/37 1//1	231	*Hydra circumcincta* Schulze, 1914	37_1	GU722769	EU876568	EU879939	DMAK13c	USA
								no ID	USA
Tubulariidae	7/69 1//2	232	*Ectopleura obypa* Migotto & Marques, 1999	33_2	**KT266612**	**KT722393**	**KT757156**	**MZUSP:0257**	Brazil
		233	*Ectopleura larynx* (Ellis & Solander, 1786)		**KT266611**	**KT722392**	**KT757164**	**MZUSP:1839**	USA
Subordem Filifera I									
Eudendriidae	2/75 1//3	234	*Eudendrium caraiuru* Marques & Oliveira, 2003	73_3		**KT722395**	**KT757160**	**lem_S105**	Brazil
		235	*Eudendrium carneum* Clarke, 1882		**KT266614**	**KT722396**	**KT757158**	**MZUSP:1700**	Brazil
		236	*Eudendrium glomeratum* Picard, 1952		AM991301	**KT722397**	**KT757162**	**lem_S58**	Italy
								MHNG:INVE39717	France
Suborder Filifera II									
Ptilocodiidae	5/9 1//1	237	*Hydrichthella epigorgia* Stechow, 1909	2_1	EU305478	EU272622	EU272569	KUNHM:2665	no ID
Proboscidactylidae	1/9 1//1	238	*Proboscidactyla ornata* (McCrady, 1859)	9_1	EU305481	EU272631	EU272587	KUNHM:2767	no ID
Magapiidae	3/5 1//1	239	*Fabienna sphaerica* Schuchert, 1996	2_1	AM183133	AY920767	AY920797	MHNG:INVE33453	New Zealand
Suborder Filifera III									
Hydractiniidae	13/111 1//1	240	*Hydractinia* sp.	73_1	**KT266621**	**KT722403**	**KT757165**	**lem_S104**	Chile
Stylasteridae	30/260 1//1	241	*Adelopora crassilabrum* Cairns, 1991	4_1	EU645356	EU272642	EU272541	USNM:1027760	New Caledonia
Suborder Filifera IV									
Bougainvilliidae	15/102 2//2	242	*Bimeria vestita* Wright, 1859	7_1	**KT266604**	**KT722386**	**KT757175**	**MZUSP:1653**	Brazil
		243	*Bougainvillia muscus* (Allman, 1863)	31_1	**KT266606**	**KT722388**	**KT757176**	**MZUSP:4104**	Brazil
Cytaeididae	4/17 1//1	244	*Perarella schneideri* (Motz-Kossowska, 1905)	5_1	AM411414	HM357626	HM357628	MHNG:INVE49086	Italy
Pandeidae	21/87 1//1	245	*Leuckartiara octona* (Fleming, 1823)	19_1	AM411421	EU272624	EU272573	no ID	France
								Sch354	France
Oceaniidae	7/27 1//1	246	*Turritopsis nutricula* McCrady, 1857	9_1	**KT266646**	**KT722442**	**KT757169**	**MZUSP:4093**	Brazil
Rathkeidae	5/14 1//1	247	*Lizzia blondina* Forbes, 1848	5_1	AM411417	EU272625	EU272574	Sch493	France
								MHNG:INVE48742	Norway

Taxa / Category	G/Sp G//Sp	nr	Species	S_s	16S info	18S info	28S info	Voucher	Country/Region
Order Siphonophorae									
Suborder Calycophorae									
Prayidae	13/27 1//1	248	*Praya dubia* (Quoy & Gaimard, 1833)	2_1	AY935285	AY937326	EU305526	YPM:35346	USA
Suborder Physonectae									
Apolemiidae	1/5 1//1	249	*Apolemia* sp. CWD-2005	5_1	AY935290	AY937331	EU272546	YPM:35090	W Pacific
Physophoridae	1/2 1//1	250	*Physophora hydrostatica* Forsskål, 1775	2_1	AY935300	AY937342	EU272582	YPM:35046	NW Atlantic
Agalmatidae	11/23 1//1	252	*Cordagalma ordinata*** (Haeckel, 1888)	2_1	AY935275	AY937317	EU272555	YPM:35032	NW Atlantic
Forskaliidae	1/6 1//1	253	*Forskalia edwardsii*** Kölliker, 1853	6_1	AY935278	AY937320	EU305516	YPM:35036	NW Atlantic

Rhodaliidae	9/14 1//1	254	*Stephalia dilata* (Bigelow, 1911)	3_1	AY935315	AY937357	EU305534	YPM:35358	Mexico
Suborder Cystonectae									
Physaliidae	1/1 1//1	251	*Physalia physalis* (Linnaeus, 1758)	1_1	AY935284	AF358065	EU448095	YPM:35345	USA
								no ID	no ID
Taxa / Category	**G/Sp G//Sp**	**nr**	**Species**	**SP_sp**	**16S info**	**18S info**	**28S info**	**Voucher**	**Country/Region**
Subclass Trachylinae									
Order Narcomedusae									
Aeginidae	8/8 1//1	255	*Solmundella bitentaculata* (Quoy & Gaimard, 1833)	1_1	EU293998	EU247812	EU247795	MHNG:INVE31746	no ID
								USNM:1107456	Antarctica
Order Trachymedusae									
Geryoniidae	4/4 2//2	256	*Geryonia proboscidalis* (Forsskål, 1775)	1_1	EU293979	EU247816	EU247807	JAM RB-BWD-4	Japan
		257	*Liriope tetraphylla* (Chamisso & Eysenhardt, 1821)	1_1	**KT266624**	**KT722405**	**KT757194-5**	**lem_S10**	Brazil
Rhopalonematidae	15/36 3//3	258	*Aglantha digitale* (O. F. Müller, 1776)	4_1	EU293985	EU247821	AY920791	USNM:1073329	USA
								no ID	no ID
		259	*Crossota rufobrunnea* (Kramp, 1913)	5_1	EU293986	EU247824	EU247800	JAM I060319b-N4	Japan
		260	*Pantachogon haeckeli* Maas, 1893	3_1	EU293988	AF358062	AY920792	MBARI T981 SS1	no ID
								no ID	no ID
Halicreatidae	6/9 1//1	261	*Botrynema brucei* Browne, 1908	2_1	EU293982	EU247822	EU247798	no ID	Japan
Order Limnomedusae									
Olindiidae	17/45 2//2	262	*Maeotias marginata* (Modeer, 1791)	1_1	AY512508	AF358056	EU247810	no ID	USA
		263	*Olindias sambaquiensis* Müller, 1861	6_1	**KT266630**	**KT722413**	**KT757174**	**lem_S01**	Brazil

Taxa / Category	**Nr**	**16S info**	**18S info**	**28S info**
TOTAL Leptothecata	**220**	211	165	139
TOTAL "Anthoathecata"	27	27	27	27
TOTAL Siphonophorae	7	7	7	7
TOTAL Trachylina	9	9	9	9
TOTAL HYDROZOA	**263**	**254**	**208**	**182**

Supplementary Table 2 – Information about the primers used to generate molecular markers using PCR. In bold we indicate the primers developed by us in the Marine Evolution Laboratory (*Laboratório de Evolução Marinha* – LEM-IB-USP). Primers developed by the Cnidaria Tree of Life are indicated by CNIDToL, GB indicates GenBank for the reference sequences used to map the relative position of the primer pairing with the DNA regions of interest in the PCR reactions.

primers Nuclear Markers					
Gene: 28S **Name of primer**	**Sequence primer 5´-3´**	**f/r**	**tm – base pair**	**Reference**	**Map of *primers* (GB: GU722663)** ***Hydra circumcincta* (Hydrozoa:Medusozoa)**
F63 mod	ACCCGCTGAAYTTAAGCATATHANTMAG	f	55°C – 28bp	Medina et al., 2001	ITS-2 --- 28S start
F15	**CTAACAAGGATTCCCCTAGTAACGGCGAGT**	**f**	**62°C – 30bp**	**LEM lab**	**15-44**
F97	CCYYAGTAACGGCGAGT	f	50ºC - 17bp	CNIDToL lab	28-44
F798	CCGTCTTGAAACACGGACC	f	56°C - 19bp	Medina et al., 2001	785-803
R798	GGTCCGTGTTTCAAGACGG	r	56°C - 19bp	Medina et al., 2001	785-803
28SRD4.8A-F	ACCTATTCTCAAACTTTAAATGG	f	46°C - 26bp	Schwendinger & Giribet. 2005	1,207-1,229
F1414	GACAGCAGGACGGTGGYCATGG	f	59°C - 22bp	Medina et al., 2001	1,391-1,412
R1446	GTTGTTACACACTCCTTAGCGG	r	55°C - 22bp	Medina et al., 2001	1,444-1,423
F1586	GTGCAGATCTTGGTDGNAGTAGCAAATATTC	f	60ºC - 31bp	Medina et al., 2001	1,598-1,628
F1689	CTAAGMSRYAGGGAAAYTC	f	52°C - 19bp	CNIDToL lab	1,701-1,719
F2103	**GATCCGTAACTTCGGGAAAAGGATTGGCTC**	**f**	**62°C - 30bp**	**LEM lab**	**2,103-2,132**
F2076sq	TAACYTCGGGAWAAGGATTGGCTC	f	55ºC - 24bp	Medina et al., 2001	2,109-2,132
R2144	**GAGCCAATCCTTTTCCCGAAGTT**	**r**	**57°C - 23bp**	**LEM lab**	**2,110-2,132**
R2084	AGAGCCAATCCTTTTCC	r	45ºC - 17bp	CNIDToL lab	2,130-2,146
R2813	CAGRTGTRCCGCCCCAGCCAAACT	r	66°C - 24bp	Medina et al., 2001	2,813-2,836
F2813	AGTTTGGCTGGGGCGGYACA	f	65°C - 20bp	Medina et al., 2001	2,813-2,836
F2800	GCAGGTGTCCTAAGGYRAGCTC	f	59°C - 22bp	Voigt et al., 2004	2,849-2,870
R3214	**GTGAATTCTGCTTCACAATGATAGGAAGAGCC**	**r**	**60°C - 32bp**	**LEM lab**	**3,081-3,112**
R3264	TTCYGACTTAGAGGCGTTCAG	r	51°C - 21bp	Medina et al., 2001	3,260-3,281
Gene: 18S	**Sequence primer 5´-3´**	**f/r**	**tm – base pair**	**Reference**	**Map of primers (GB: Z86108)**

Name of primer					***Obelia* sp. (Hydrozoa:Medusozoa)**
A	AACCTGGTTGATCCTGCCAGT	f	54°C - 21bp	Medlin et al., 1988	1-21
18Sini	ATCCTGCCAGTAGTCATA	f	45°C - 18bp	Turbeville et al., 1991	11-28
554f (GBF)	AAGTCTGGTGCCAGCAGCCGC	f	62°C - 21bp	Turbeville et al., 1991	568-588
18S-C	CGGTAATTCCAGCTCCAATAG	f	50°C - 21bp	Apakupakul et al., 1999	588-608
18S-L	CCAACTACGAGCTTTTTAACTG	r	50°C - 22bp	Apakupakul et al., 1999	627-648
18S inter (Rv)	**GCGAAGAAGAACAGACCGATCAG**	**r**	**52°C - 23bp**	**LEM lab**	**689-711**
18S-O	AAGGGCACCACCAGGAGTGGAG	f	60°C - 22bp	Apakupakul et al., 1999	1,166-1,187
18J-5	GCCTGCGGCTTAATTTGACTCAACACGGG	f	63°C - 29bp	Hillis & Dixon. 1991	1,187-1,215
1282r (GBR)	TCACTCCACCAACTAAGAACGGC	r	56°C - 23bp	Turbeville et al., 1991	1,293-1,315
18S-Y	CAGACAAATCGCTCCACCAAC	r	54°C - 21bp	Apakupakul et al., 1999	1,303-1,323
Rh-18S9R	GATCCTTCCGCAGGTTCACCTAC	r	56°C - 23bp	Turbeville et al., 1991	1,811-1,833
B	TGATCCTTCCGCAGGTTCACCT	r	57°C - 22bp	Medlin et al., 1988	1,814-1,835
primers Mitochondrial Markers					
Gene: 16S	**Sequence primer 5´-3´**	**f/r**	**tm – base pair**	**Reference**	**Map of primers (GB: DQ787873)**
Name of primer					***Aurelia aurita* (Scyphozoa:Medusozoa)**
CB1	TCGACTGTTTACCAAAAACATA	f	60°C - 32bp	Cunningham & Buss. 1993	1,028-1,049
16Sar	CGCCTGTTTATCAAAAACAT	f	49°C - 20bp	Simon et al., 1991	1,029-1,048
SHB	GACTGTTTACCAAAAACATA	f	44°C - 20bp	Schroth et al 2002	1,030-1,049
1 Hyd-Scyph	**TGACCGTGDTAADGTAGC**	**f**	**50°C -18bp**	**LEM lab**	**1,134-1,151**
2 Scyhom	**CTGTTATCCCTACGGTAAC**	**r**	**49°C - 19bp**	**LEM lab**	**1,519-1,537**
2 Hydrom	**CTGTTATCCCTAAGGTAGC**	**r**	**49°C - 19bp**	**LEM lab**	**1,519-1,537**
16S-R-BR	**CATAATTCAACATCGAGG**	**r**	**45°C - 18bp**	**LEM lab**	**1,581-1,598**
CB2	ACGGAATGAACTCAAATCATGTAAG	r	52°C - 25bp	Cunningham & Buss. 1993	1,652-1,676

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Supplementary Table 3 - Protocols to generate nucleotide sequences for this study along with the optimized volumes for the PCR and BigDye® reactions. For specific time (Tm) for every primer for PCR, Supplementary Table 3.

A – DNA extraction	**Protocol summary**	
Chaotropic resin Chelex INSTAGENE® (BIO-RAD #732-6030)	1. Incubate tissue sample [*Tm* = 56ºC / T = 30´] + [Tm = 99ºC / T = 8´]	
	2. Extraction quality control via NANODROP®	
	3. Store at -4ºC	
B - Amplification by PCR	**Protocol summary**	
Basic program of the thermocycler for the PCR (expected fragment length ~600pb)	1. Initial cycle: *Tm* = 94ºC / T = 5´	
	2. Cycle repetition (35 or 40 Cycles): [*Tm* = 94ºC / T = 50´´]+[*Tm* = primer / T = 50´´]+[*Tm* = 72ºC / T = 50´´]	
	3. Final cycle: *Tm* = 72ºC / T = 7´	
	4. PCR quality control via NANODROP®	
	5. Store at -4ºC	
	Reagents and concentrations of PCR mixture	Volume
	Ultra pure H_2O	14.5ul
	Buffer *Taq*: 10X	2.5ul
	dNTPs (combined): 100 mM	1.33ul
	$MgCl_2$: 3.5 mM	3.5ul
	Primers (*forward* and *reverse*: 0.6 mM each)	1.5ul
	Taq polymerase: 0.8 units (5U/ml)	0.17ul
	DNA (~30 ng)	1.5ul
	TOTAL volume for PCR reaction	**25ul**
C – Double-stranded DNA purification (PCR product)	**Protocol summary**	
Magnetic particles AMPure XP® (Agencourt #A63880)	1. Add 1.8x of the volume of the PCR of AMPure XP solution. Remove supernatant in the magnetic bed	
	2. Two ethanol 70% washes	
	3. Final dilution in 15ul ulta pure H_2O	
	4. PCR prufication control: gel agarose 1.25%. stain with Gel-Red®	
	5. Store at -4ºC	
D – BigDye® Reaction	**Protocol summary**	
Thermocycler Program for the ABI BigDye® (Life Technologies #4336935).	1. Initial cycle: *Tm* = 96ºC / T = 1´	
	2. Cycle repetition (40 Cycles): [*Tm* = 96ºC / T = 10´´]+[*Tm* = *primer* / T = 5´´]+[*Tm* = 60ºC / T = 4´]	
	3. Store at -4ºC	
	Reagentes and concentrations for the BigDye® reaction.	Volume

	Buffer BigDye® Terminator v3.1	2ul
	BigDye® Terminator v3.1	1ul
	*Primer (*F *or* R*)*	0.5ul
	PCR purification (final DNA concentration: 50-70ng per reaction. *e.g.*: 50ng/ul)	1ul
	ulta pure H2O	5.5ul
	TOTAL reaction volume BigDye®	**10ul**
E – Analysis of the obtained sequences from the Sequencer ABI® 3730 DNA Analyser	**Software**	
Analysis of the .abi. files and generation of contigs (final format: genbank .gb)	Geneious® 5.1.6	

Rogue taxa analysis

To evaluate potential phylogenetic artifacts from unstable taxa, full rogue taxa analyses were perfomed in combined datasets. Original and filtered data matrices, together with optimal tree and (non parametric) bootraped trees were submitted to the online version of RogueNaRok server (http://rnr.h-its.org) using default parameters. We pruned the top-ten rogue taxa for every data matrix (Supplementary Table S4). Pruned datasets were analyzed and like complete datamatrices (Figs. S13-S15). Major clades represented in main results are also presented in these analyses with high support (Table S5). So, we concluded that i) those Leptothecata unstable terminals identified with RogueNaRok would be treated as *incertae sedis* in main results, ii) they do not affect relationships between major clades in most of the cases, and iii) stable clades with high support were used to propose a new phylogenetic classification of Leptothecata.

Bibliography

1. Collins, A. G. Towards understanding the phylogenetic history of Hydrozoa: Hypothesis testing with 18S gene sequence data. *Sci. Mar.* **64,** 5–22 (2000).
2. Schuchert, P. Hydrozoa. Accessed through: World Register of Marine Species at http://www.marinespecies.org/.

Supplementary Table S4 – Top-ten rogue taxa identified by RogueNaRok. Species in bold face and grey background cells were rogues in all concatenated matrices, species in bold face are rogues in two out of the three datamatrices.

	RogueNaRok (Mayoriry Rule MR)		
	16S18S28S_N	16S18S28S	16S18S28S_Nrw4
1	***Hydrodendron gardineri***	***Hydrodendron gardineri***	***Hydrodendron gardineri***
2	***Scandia gigas***	***Scandia gigas***	***Aequorea conica***
3	***Aequorea conica***	***Aequorea conica***	***Scandia gigas***
4	***Symplectoscyphus turgidus***	***Symplectoscyphus turgidus***	***Symplectoscyphus tricuspidatus***
5	*Halecium delicatulum*	***Schizotricha trinematotheca***	***Schizotricha trinematotheca***
6	***Turritopsis nutricula***	*Physalia physalis*	*Aglaophenia latecarinata*
7	*Cordagalma ordinata*	***Sertularia unguiculata***	*Dynamena obliqua*
8	***Sertularia unguiculata***	*Turritopsis nutricula*	*Dynamena disticha*
9	***Schizotricha trinematotheca***	***Billardia subrufa***	*Physophora hydrostatica*
10	***Billardia subrufa***	*Symplectoscyphus tricuspidatus*	*Praya dubia*

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Supplementary Table S5 - Results of nodal support for the main groups of Leptothecata pruned-of-rogues and complete matrices. Black cells indicate nodes with significant support according to all or most support methods (3 of 4 support methods for multilocus, and 3 of 3 for single locus analyses); grey cells with significant supports for one or two methods (ab = aBAYES; sh = SH_aLRT, bs = Bootstrap; see Fig. 1 for details); white cells reprent clades with non-significant support in all methods. Asterisk indicates monophyletic taxon absent in the topology but present in a para- or polyphyletic composition (i.e., small patristic distance between terminals); number in parenthesis () indicates number of figure in main text.

Taxon	16S18S28S_N		16S18S28S		16S18S28S_Nrw4	
	all taxa (1)	rogue taxa	all taxa (2)	rogue taxa	all taxa (3)	rogue taxa
Lafoeida						
Laodiceida		ab / bs		ab / sh	ab	ab
Statocysta						
Campanulinida						
Eirenida						
Eirenids I						
Eirenids II						
Proboscoida					ab	
Campanulariida						
Obeliida	ab / sh					
Macrolonia						
Staurothecida		no*	no*	no*	no*	no*
Staurothecidae						
Symplectoscyphidae						
Haleciida						
Sertulariida						
Sertularellidae					no*	
Thyroscyphidae						
Sertulariidae						bs / sh
Plumupheniida						
Aglaopheniida						
Plumulariida			ab / bs			
Schizotrichidae						
Kirchenpaueriidae						
Halopteridae						
Plumulariidae						

Sample

STEP

micro-tissue or sample with high mesoglea/chitin concentration

TOTAL DNA EXTRACTION

① Chelex [Instagene]

② new specimen´s sample

③ new specimen´s sample or new specimen from same sample

Magnetic beads [DNAdvance]

worked? worked?

YES = extraction > 5ng/ul [NanoDrop lecture]

GENIC DNA AMPLIFICTION

① PCR (Polymerase Chain Reaction)

② new PCR reaction with Taq High Fidelity

worked?

YES = product PCR > 15ng/ul [agarose gel 1.5%]

PCR PRODUCT PURIFICATION

Magnetic beads [AMPURE]

worked?

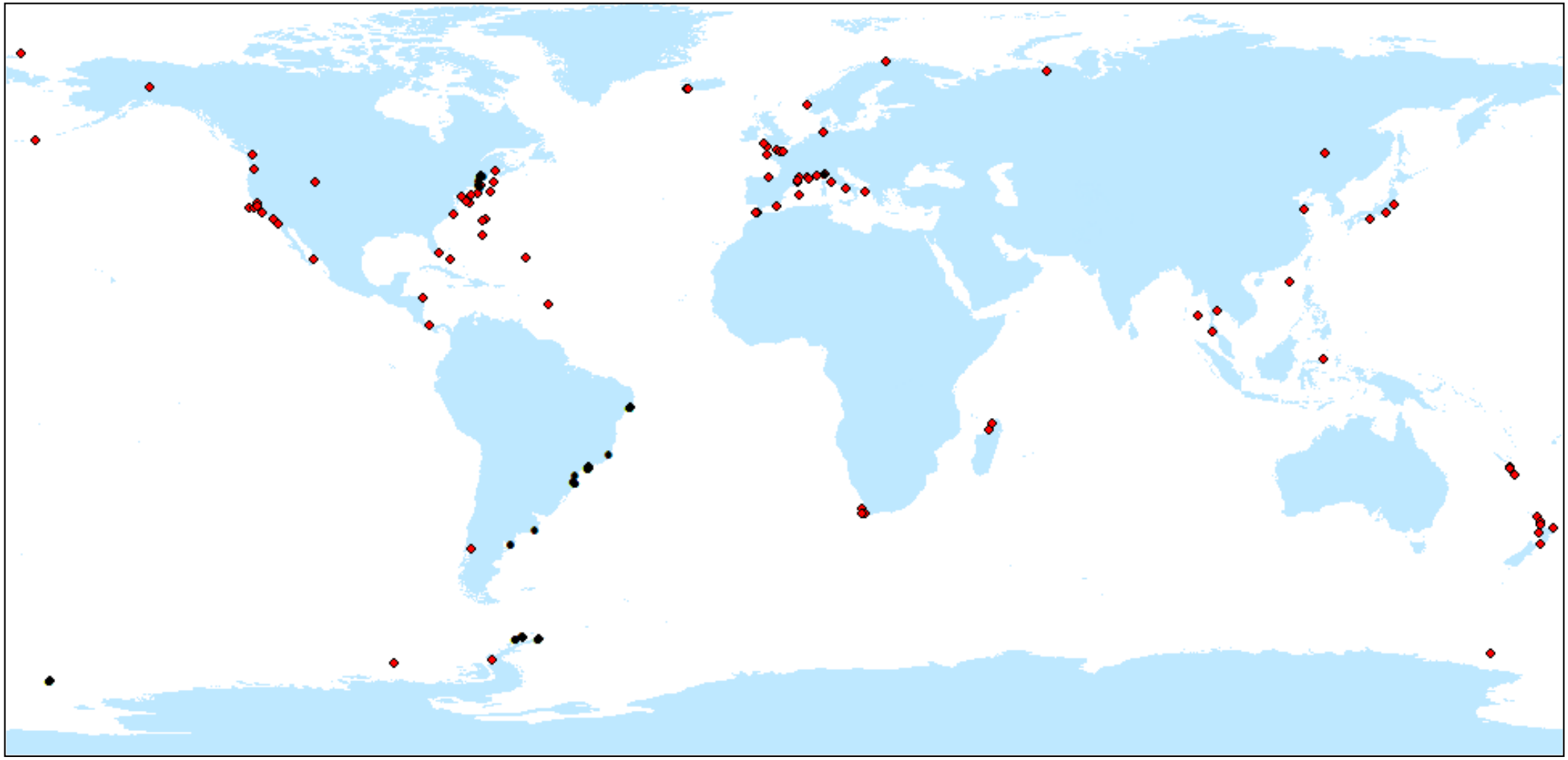
YES = PCR purification > 10ng/ul [NanoDrop and/or agarose gel1,5%]

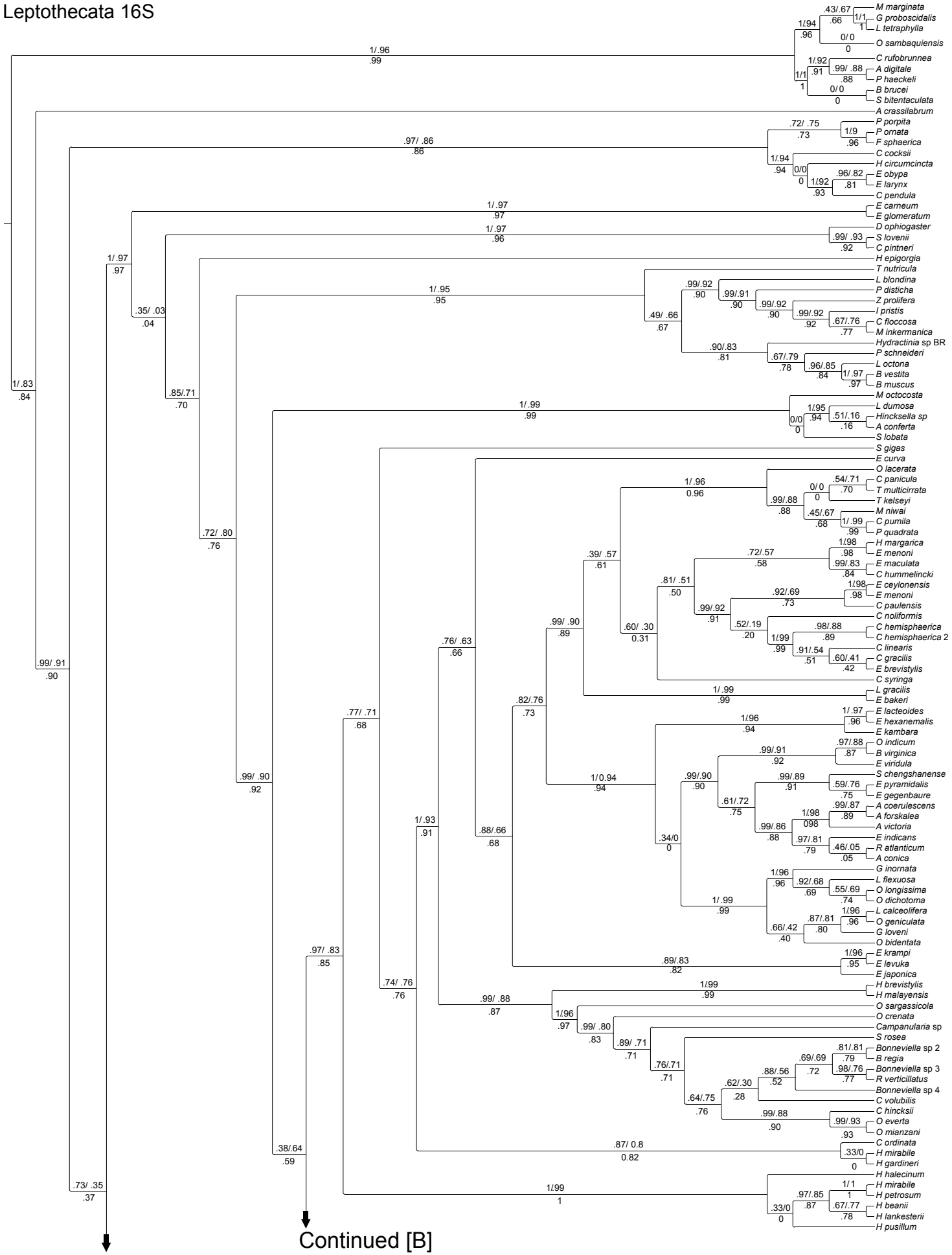
BIG DYE SEQUENCING ASSEMBLY

BIG DYE Reaction

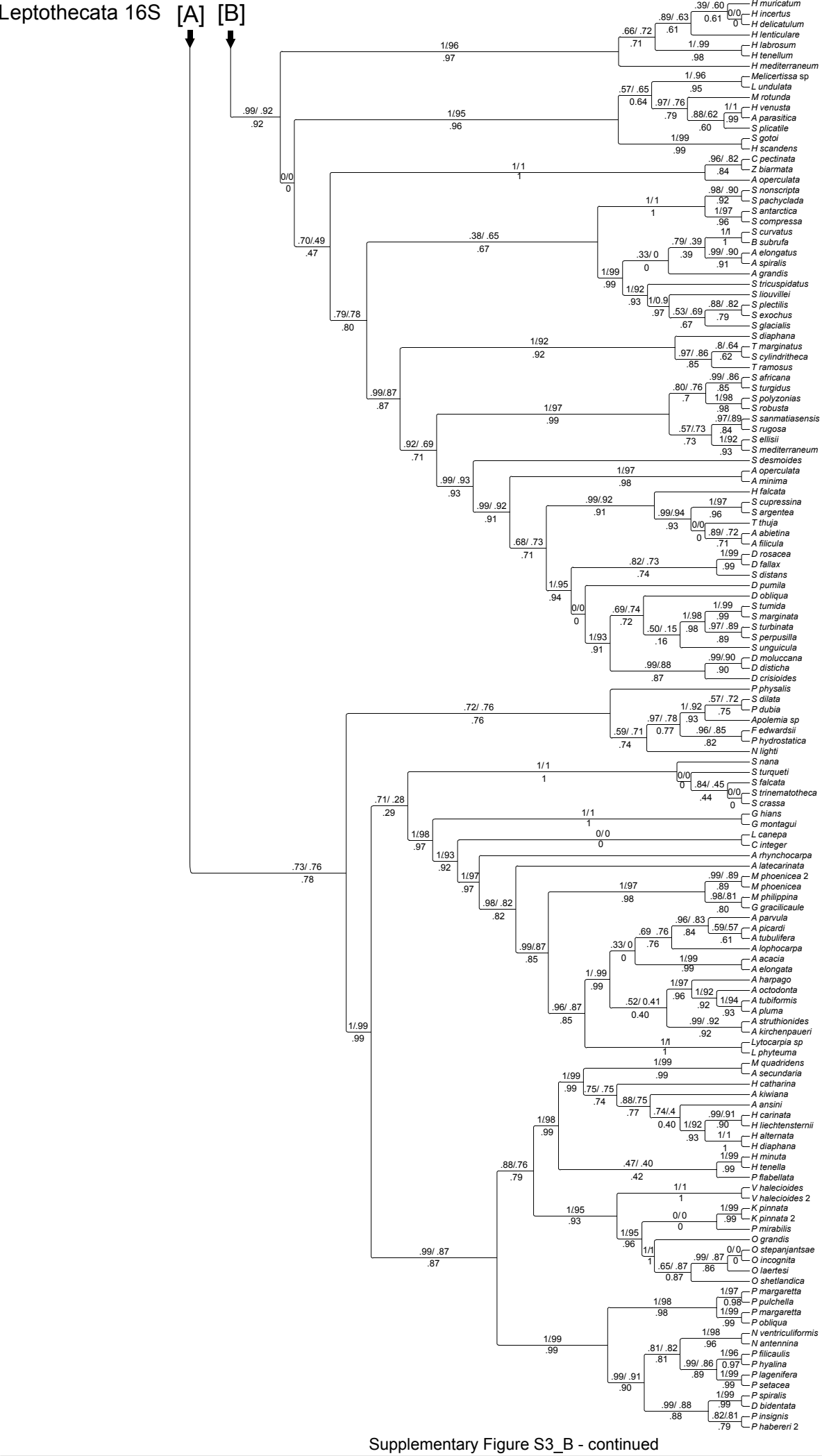
Sequencing [ABI PRISM 3100]

Sequence assembly [GENEIOUS or SEQUENCER]





Supplementary Figure S3_A



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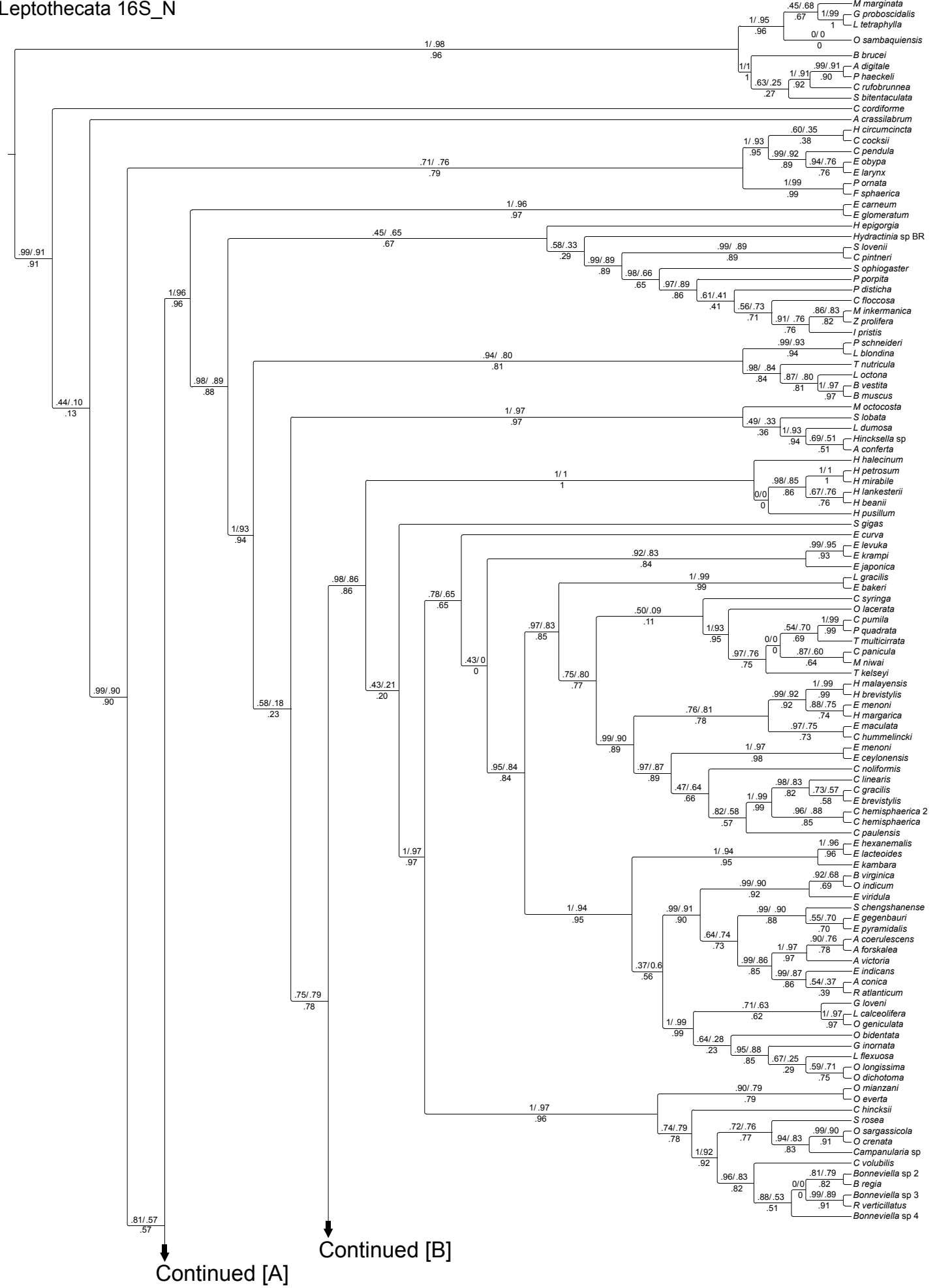
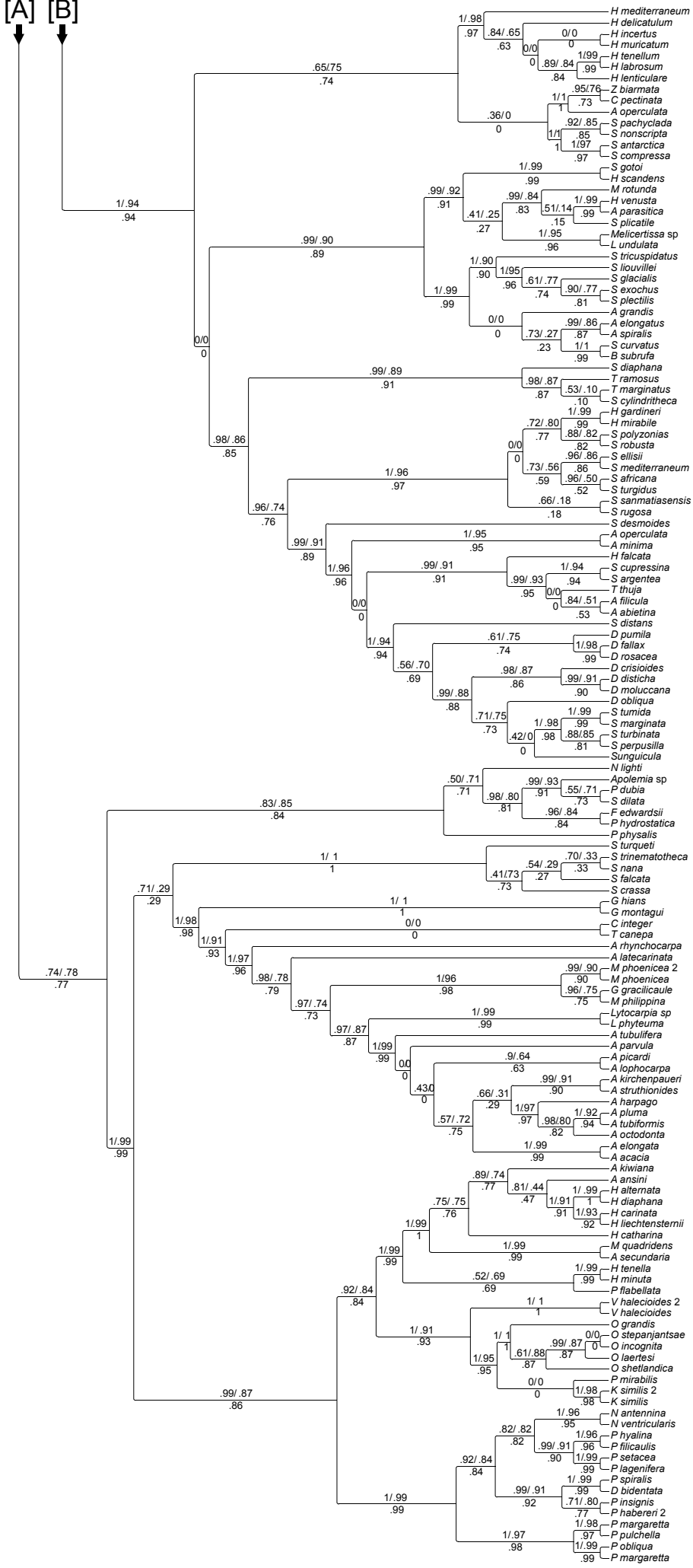


Supplementary Figure S4_A



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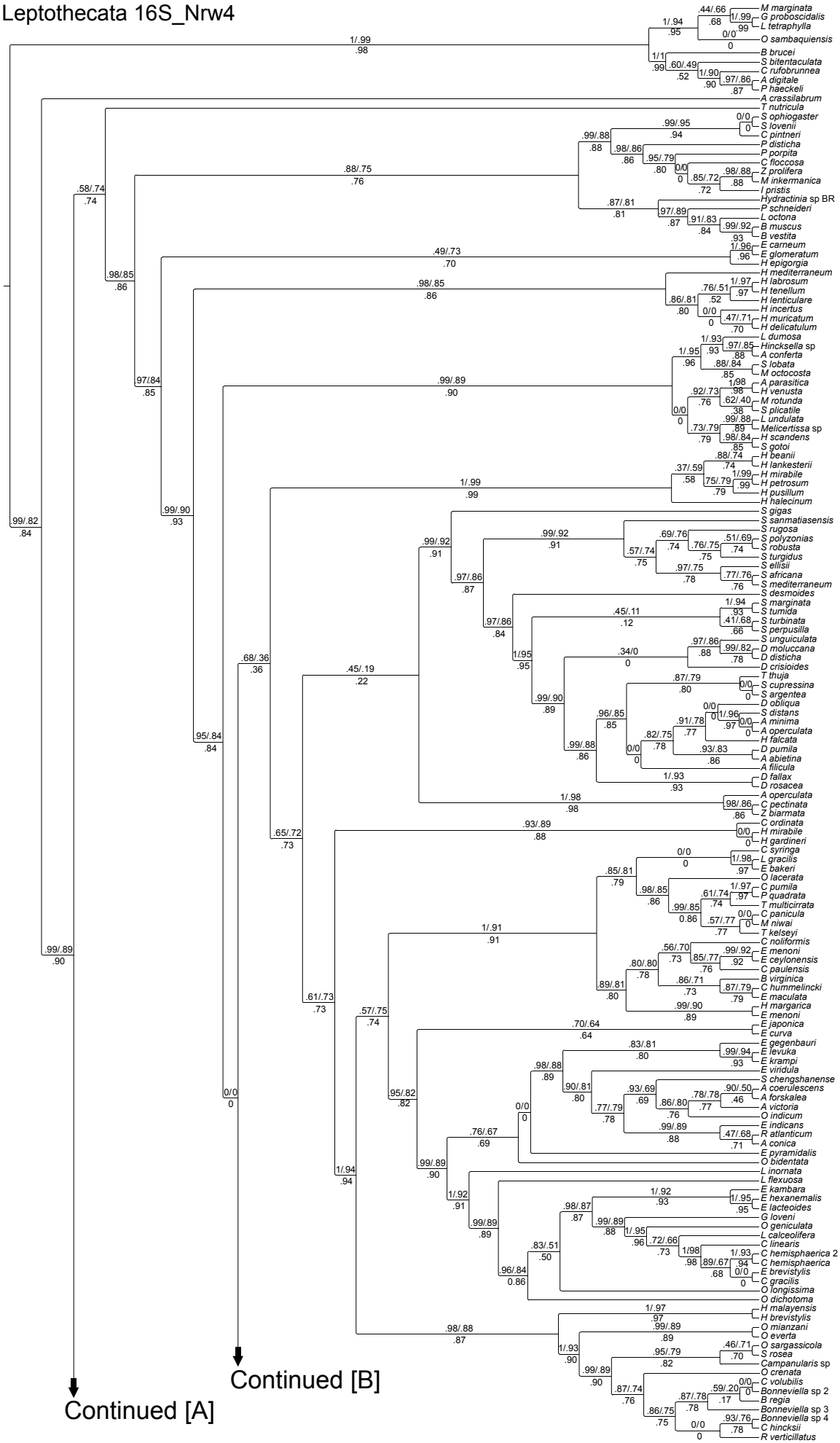
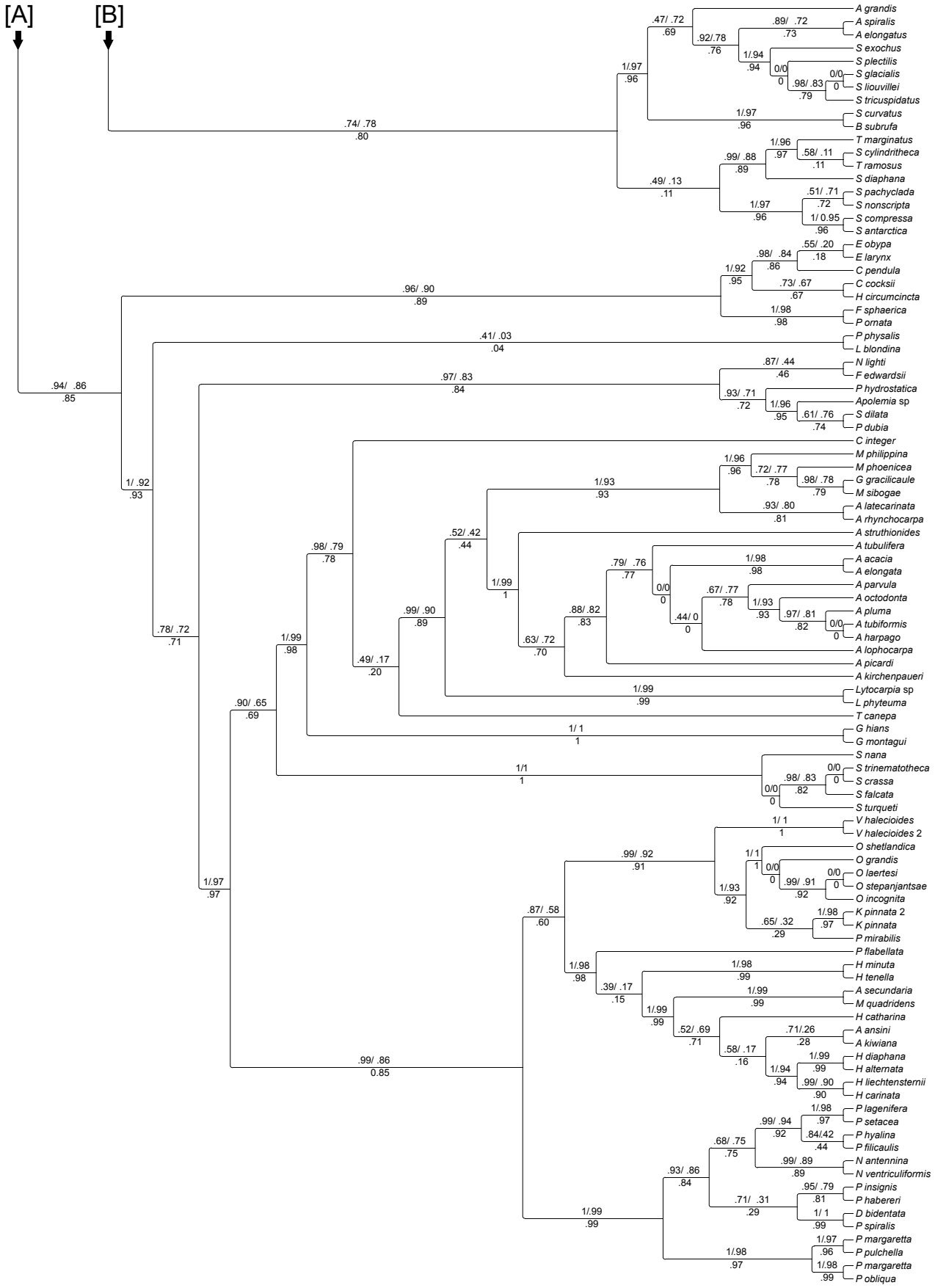
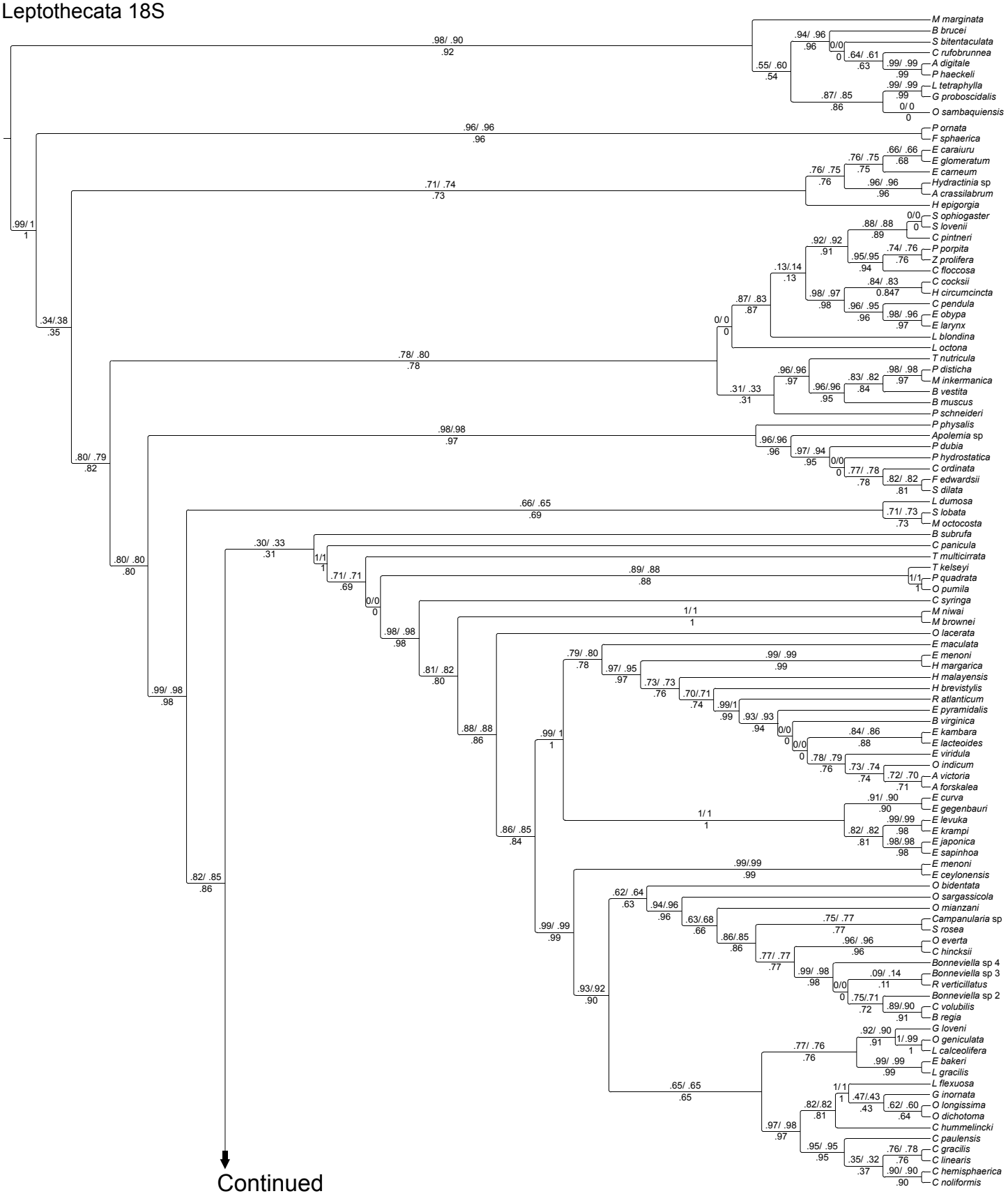


Supplementary Figure S5_A

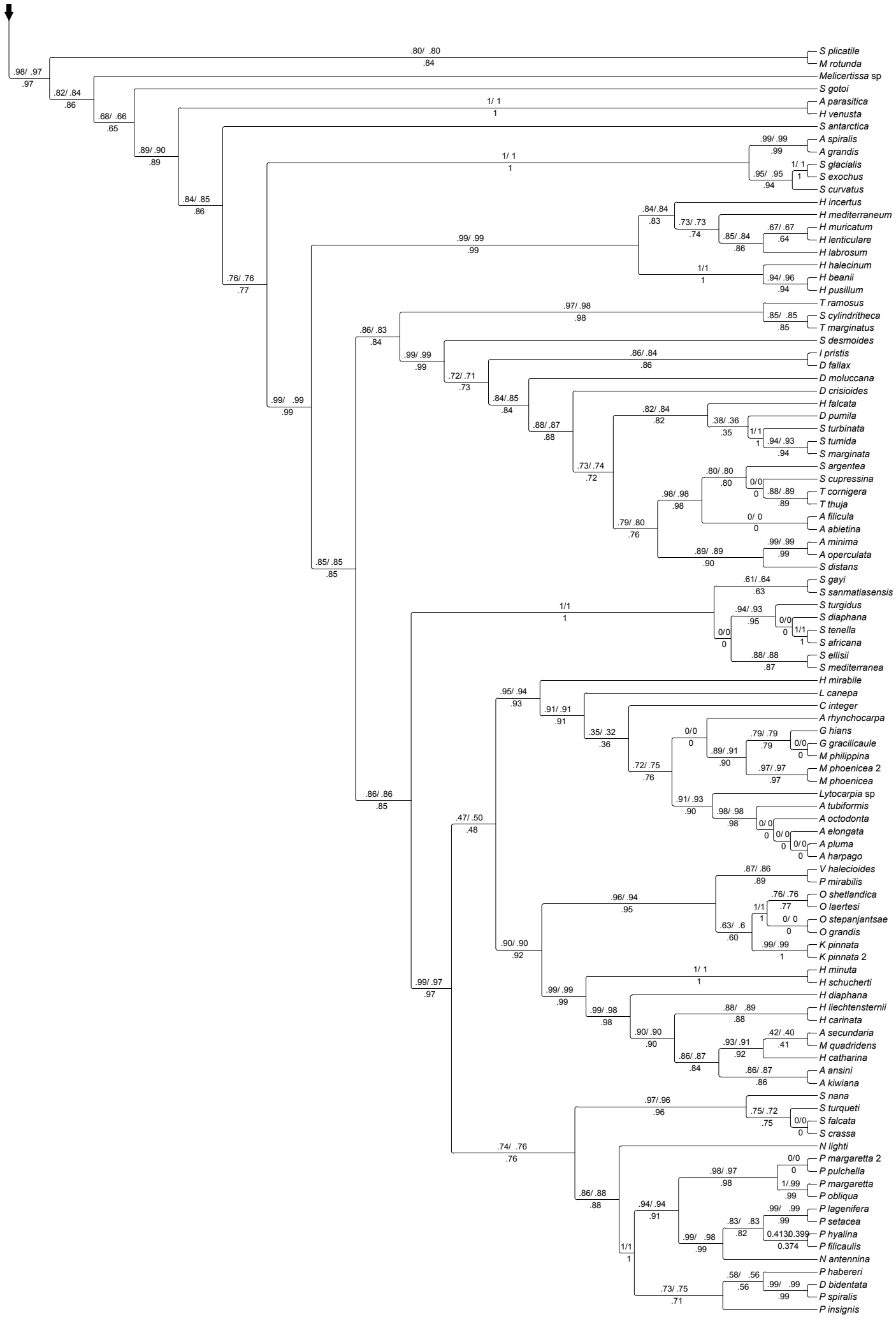


Supplementary Figure S5_B

Leptothecata 18S

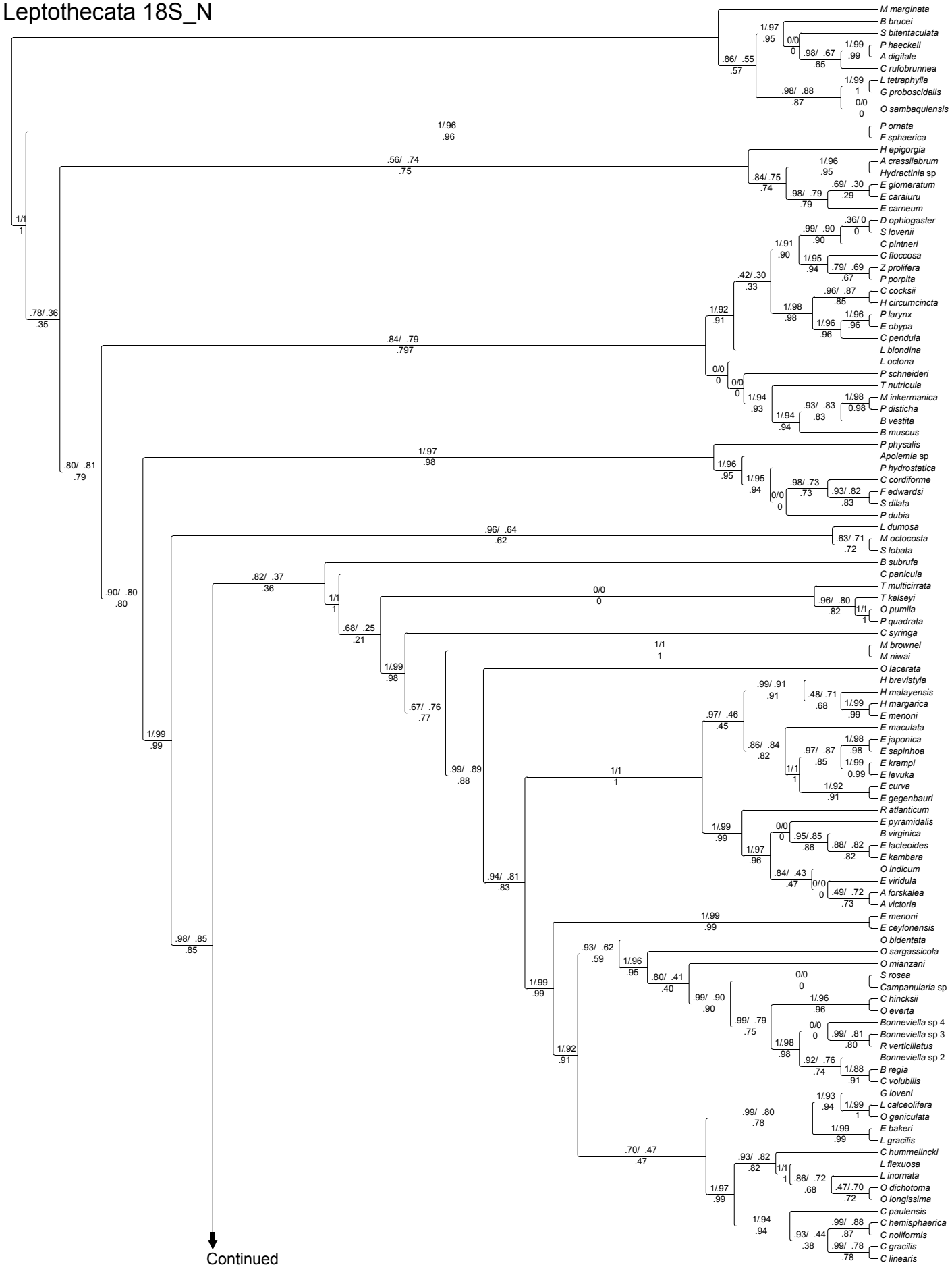


Supplementary Figure S6_A

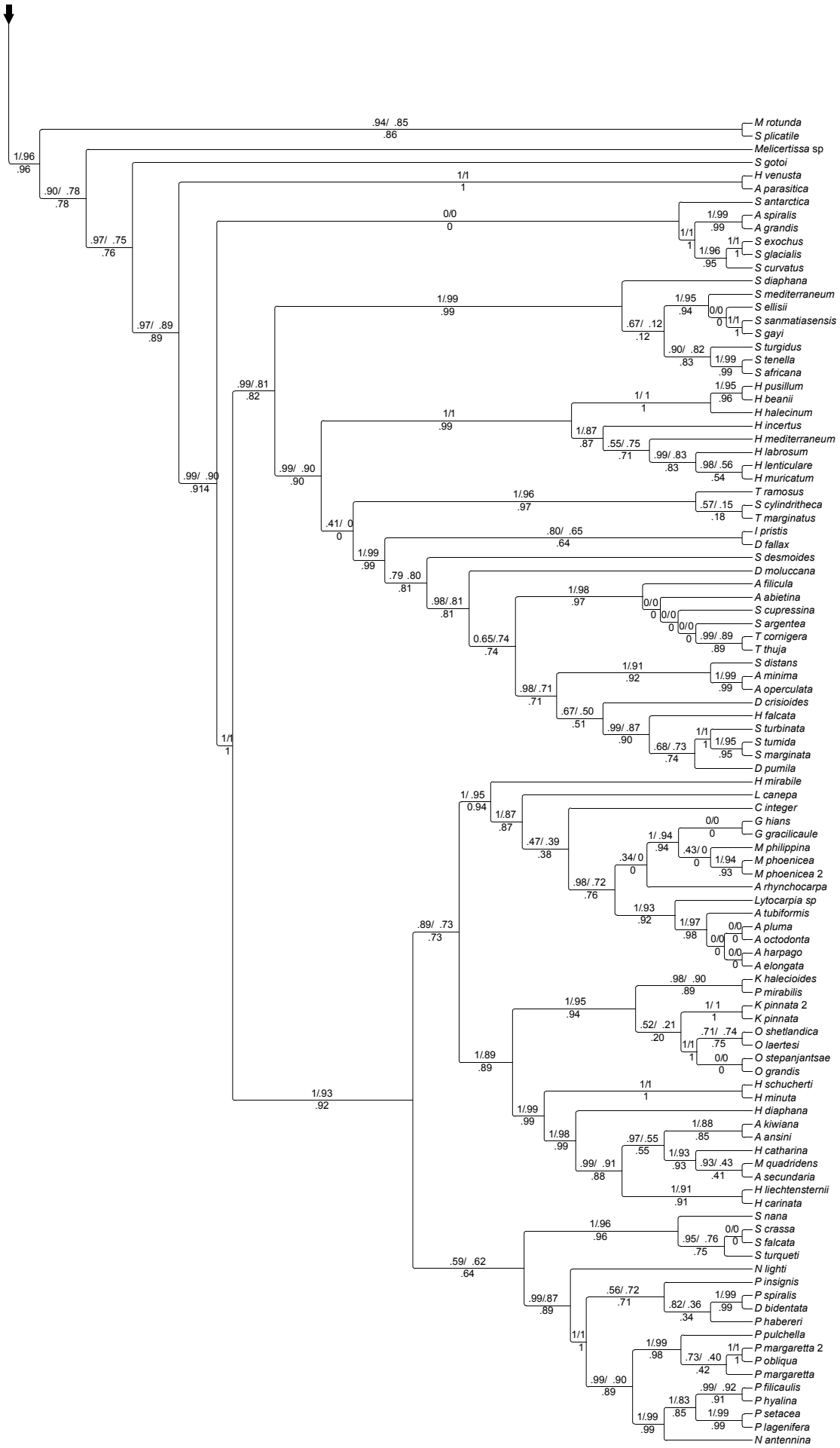


Supplementary Figure S6_B - continued

Leptothecata 18S_N

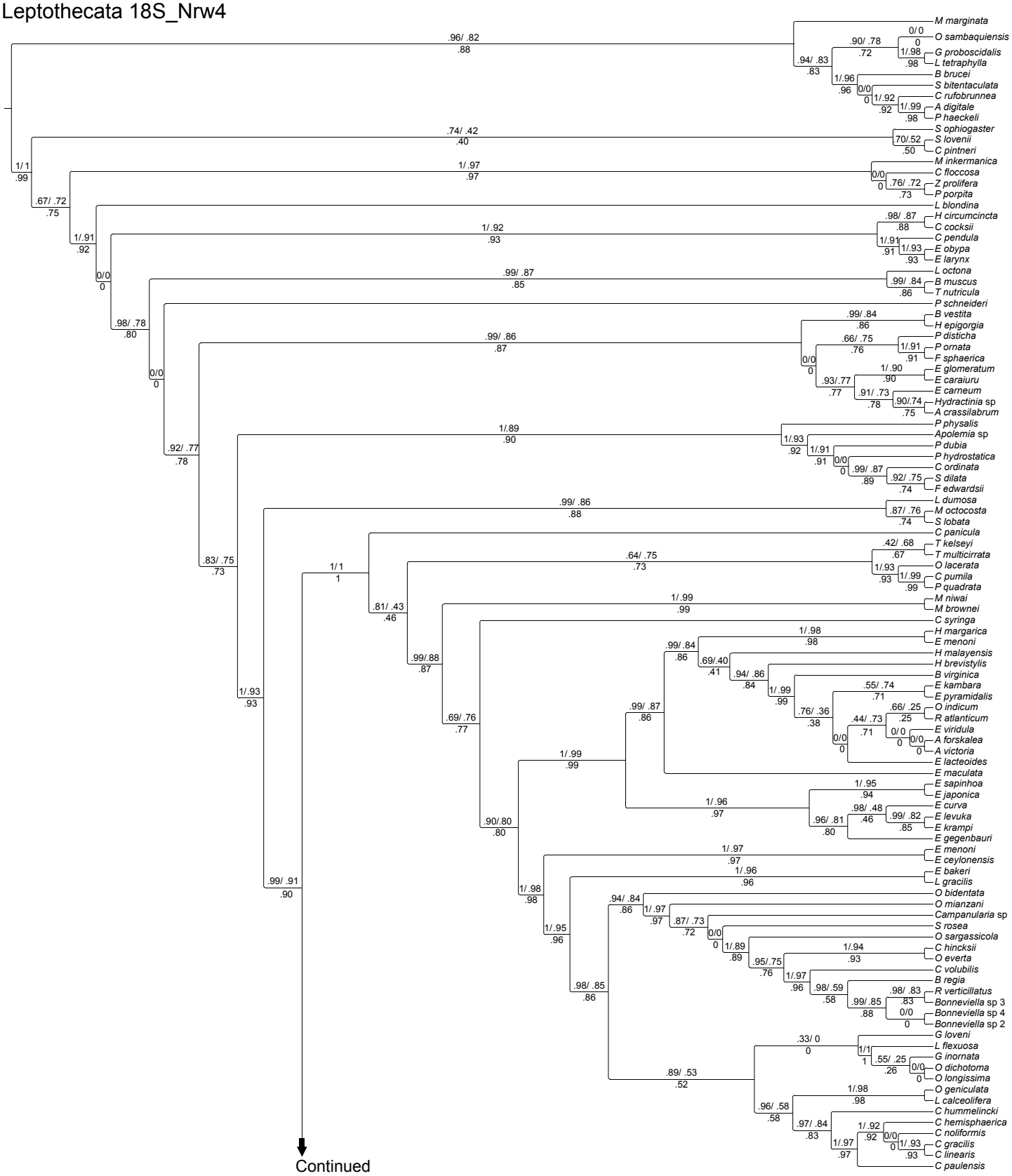


Supplementary Figure S7_A

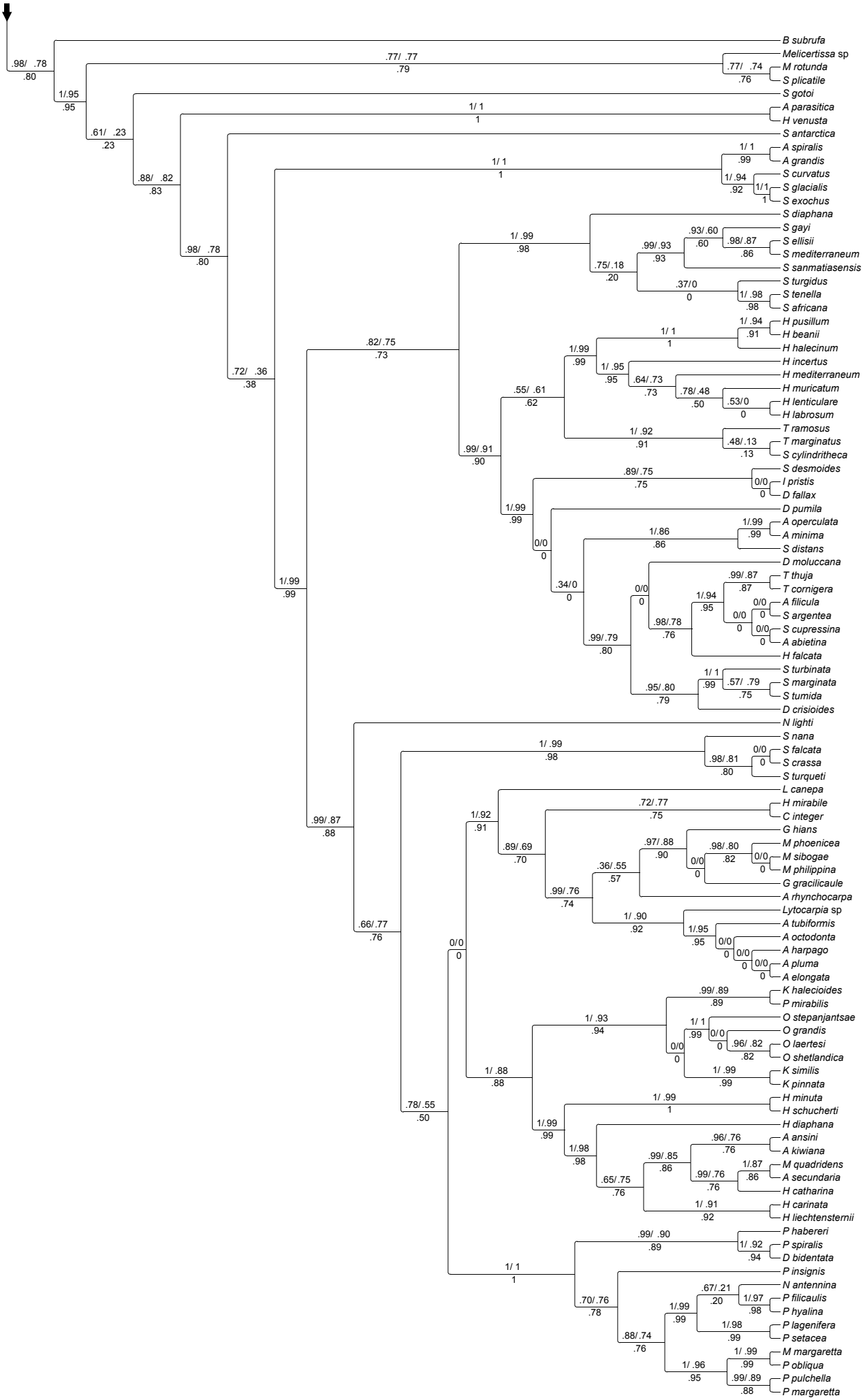


Supplementary Figure S7_B - continued

Leptothecata 18S_Nrw4



Supplementary Figure S8_A



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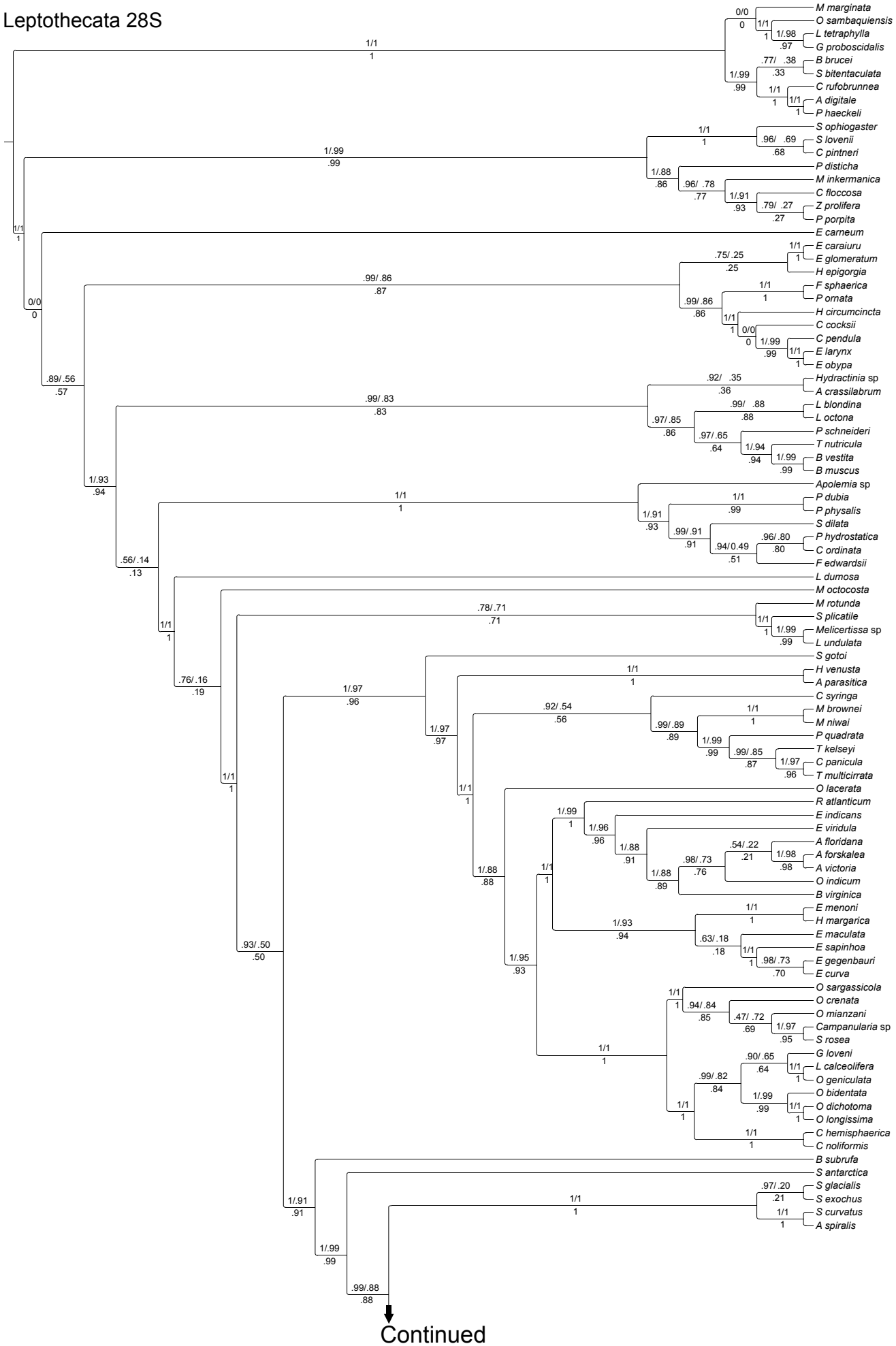
Leptothecata 28S



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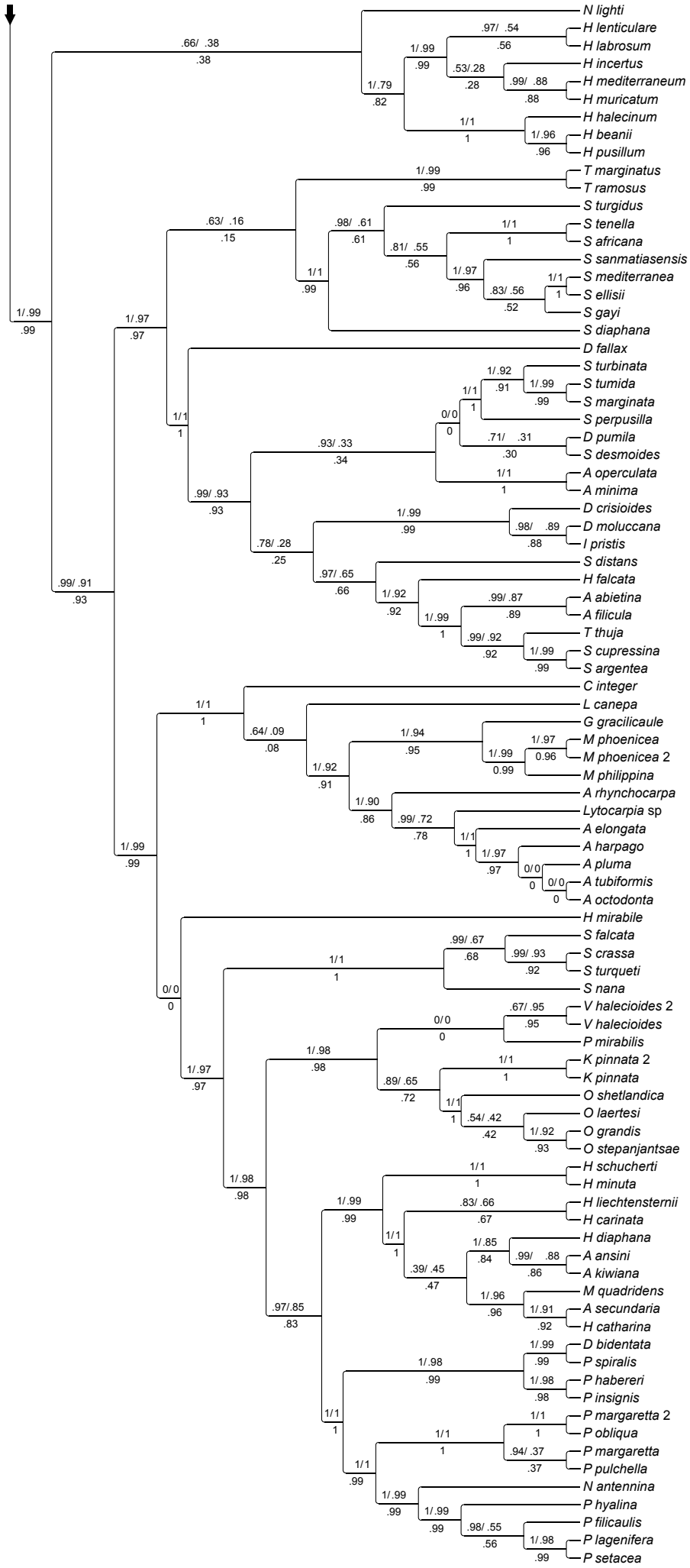
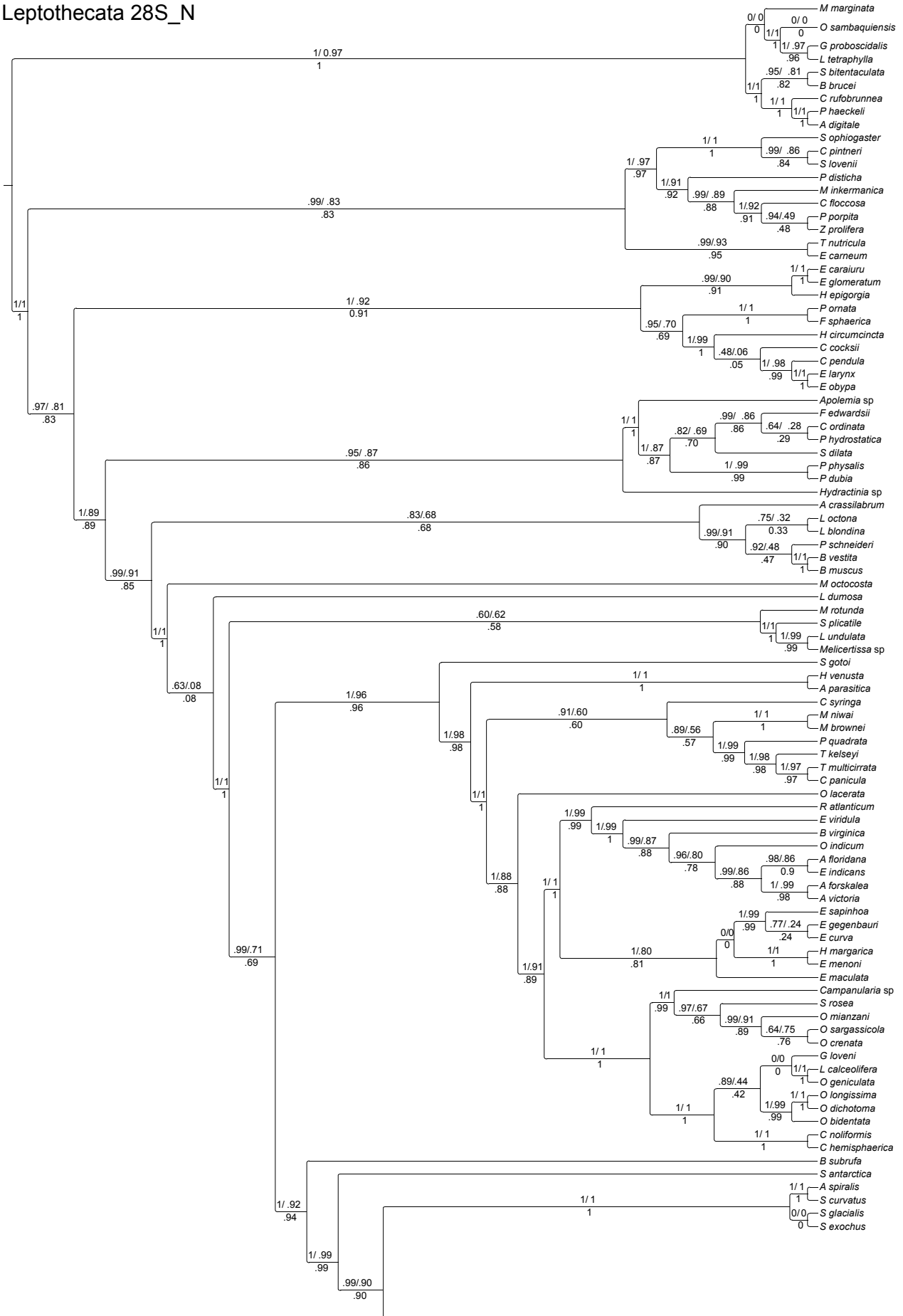


Supplementary Figure S9_B - continued

Leptothecata 28S_N



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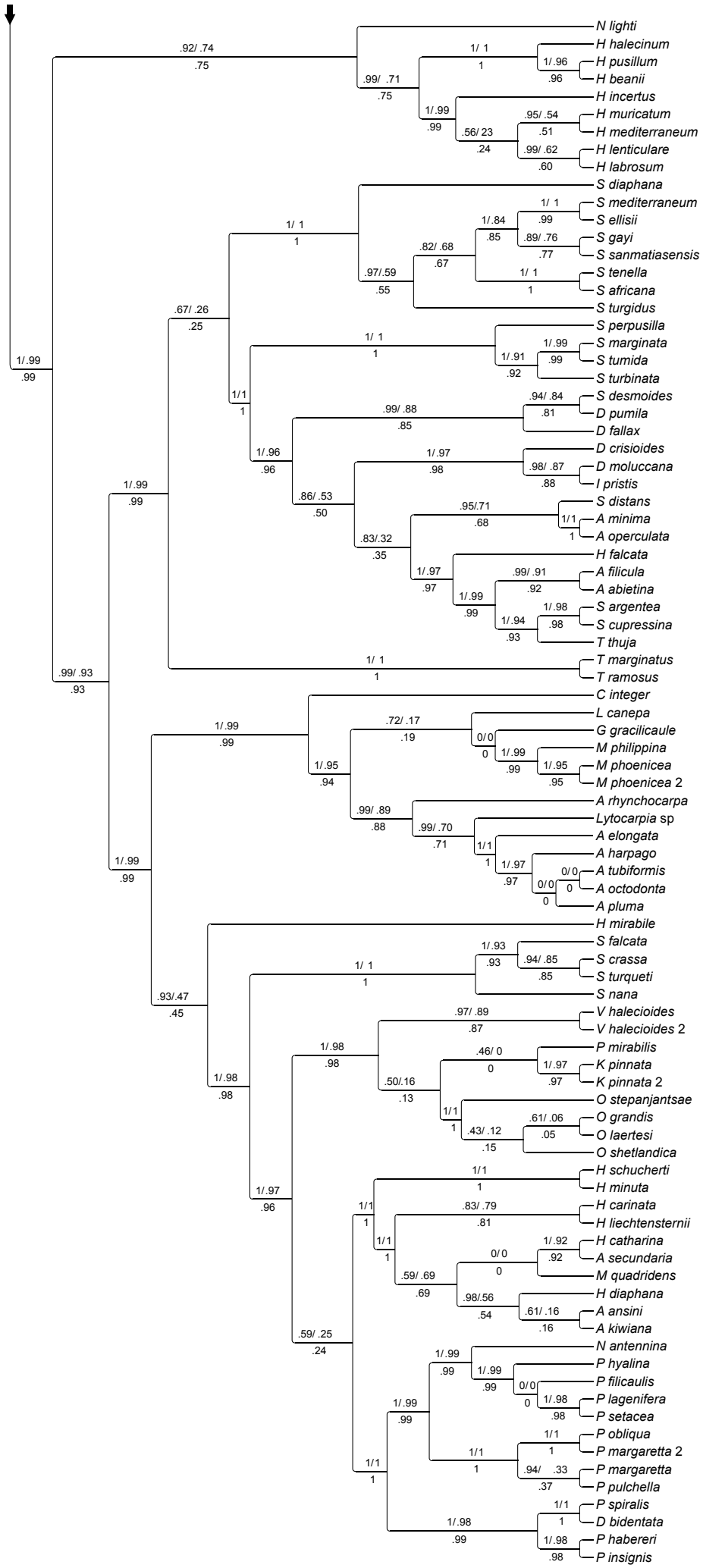
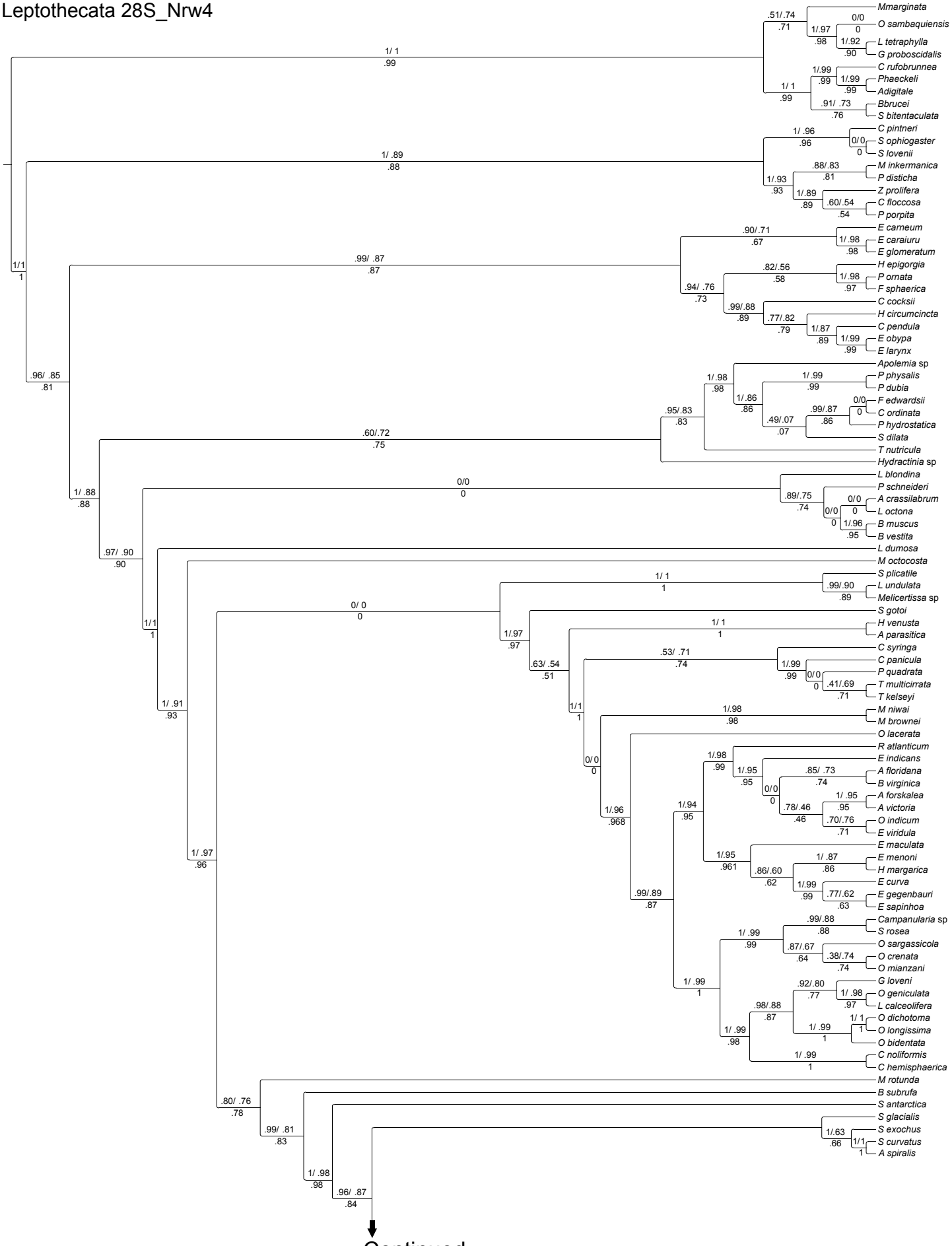


Supplementary Figure S10_B - continued

Leptothecata 28S_Nrw4



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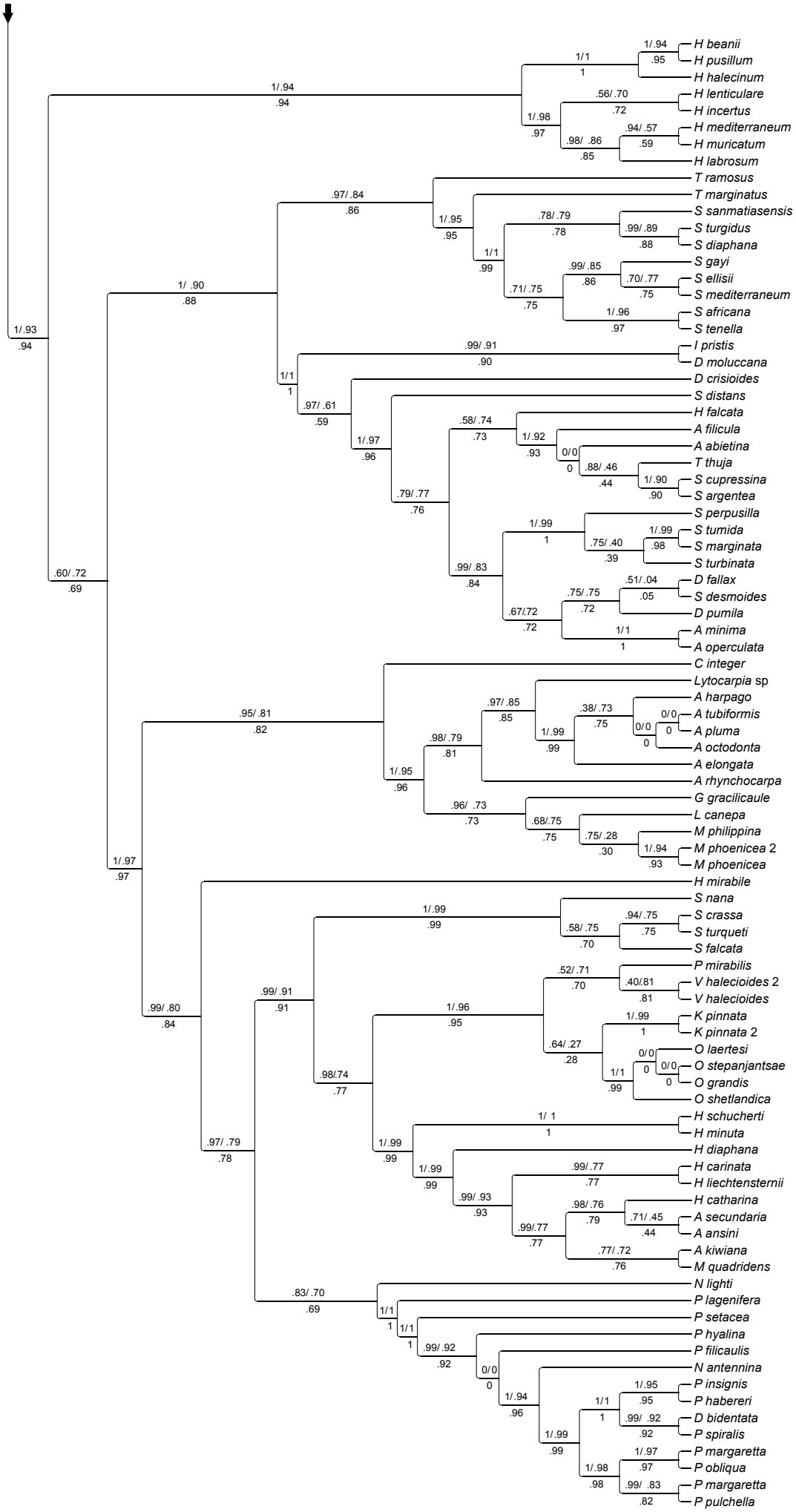

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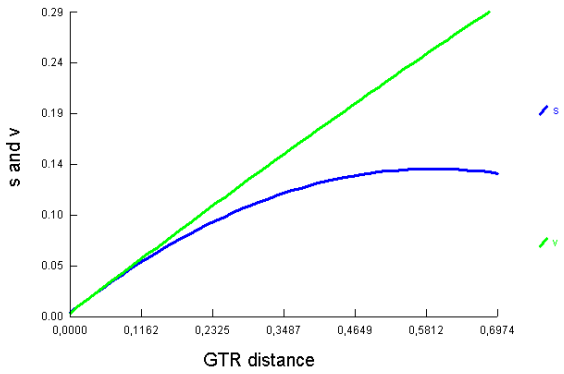

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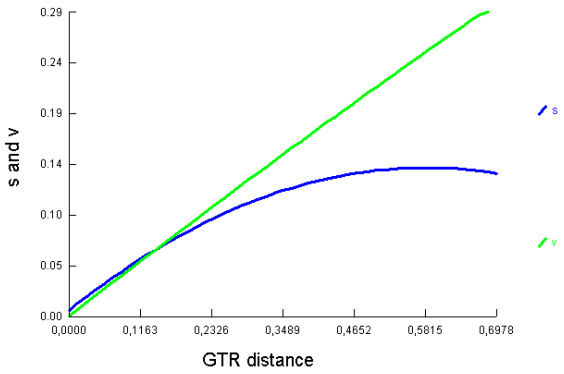

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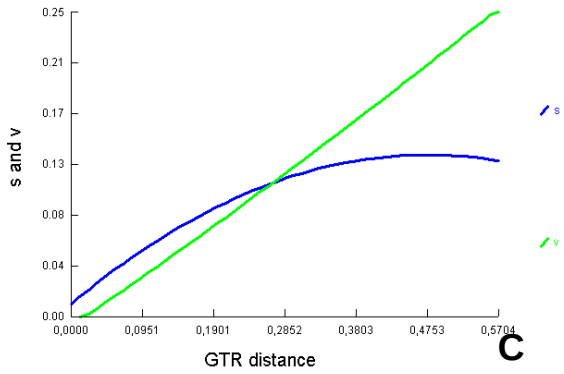

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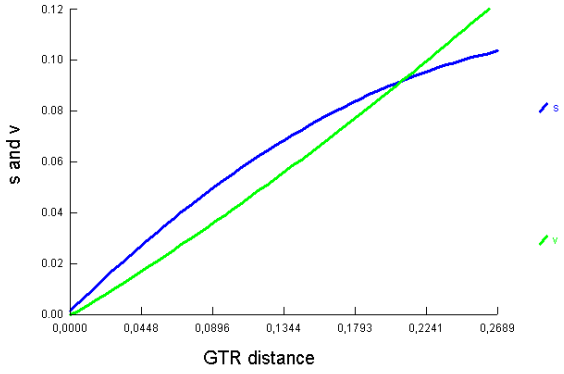

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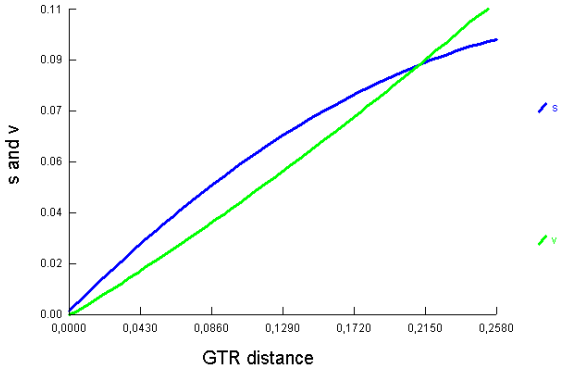

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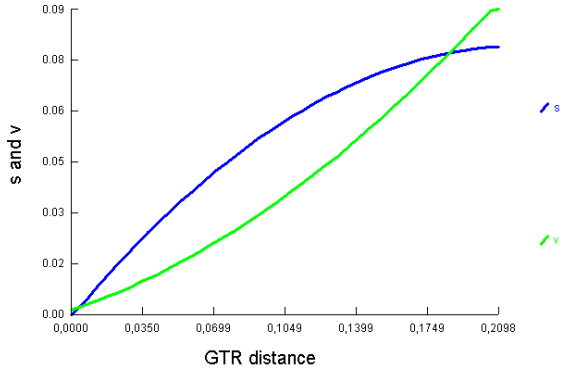

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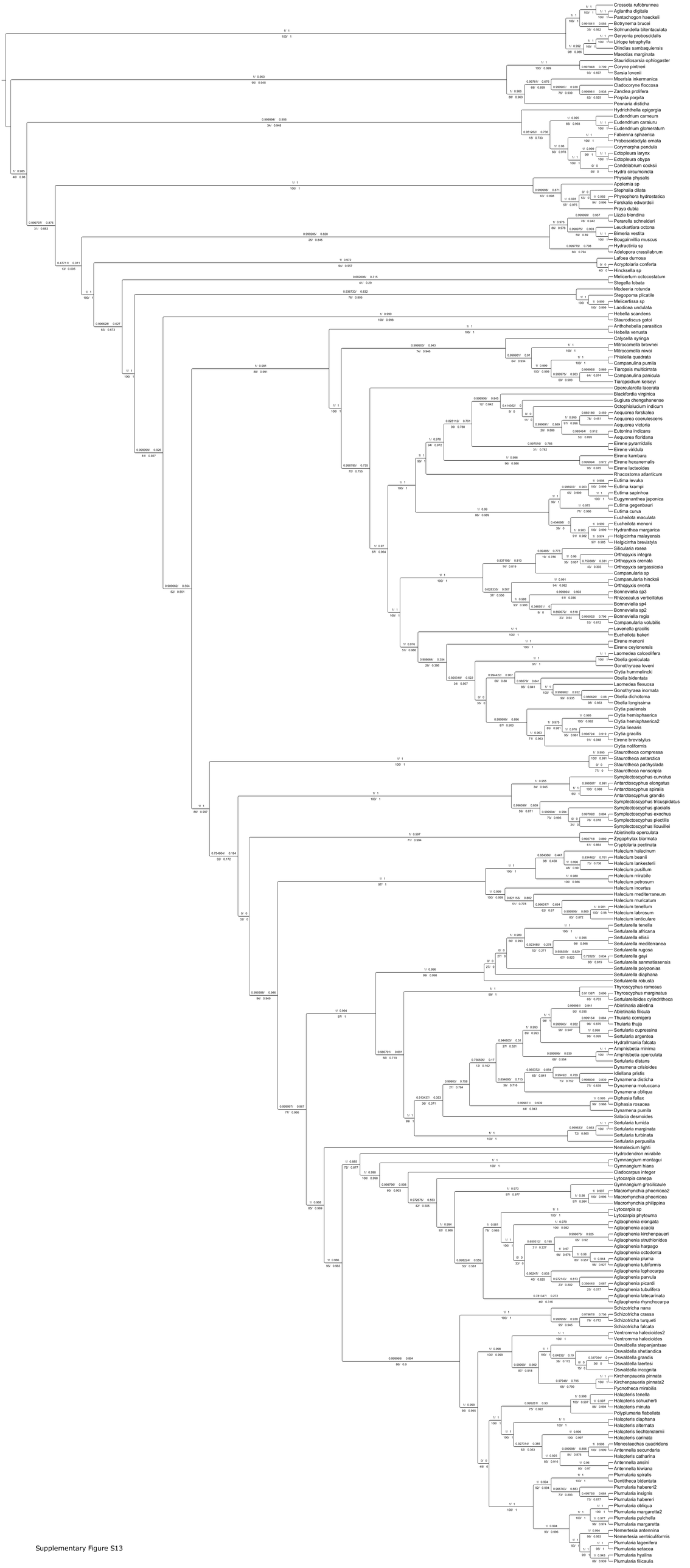
Supplementary Figure 12



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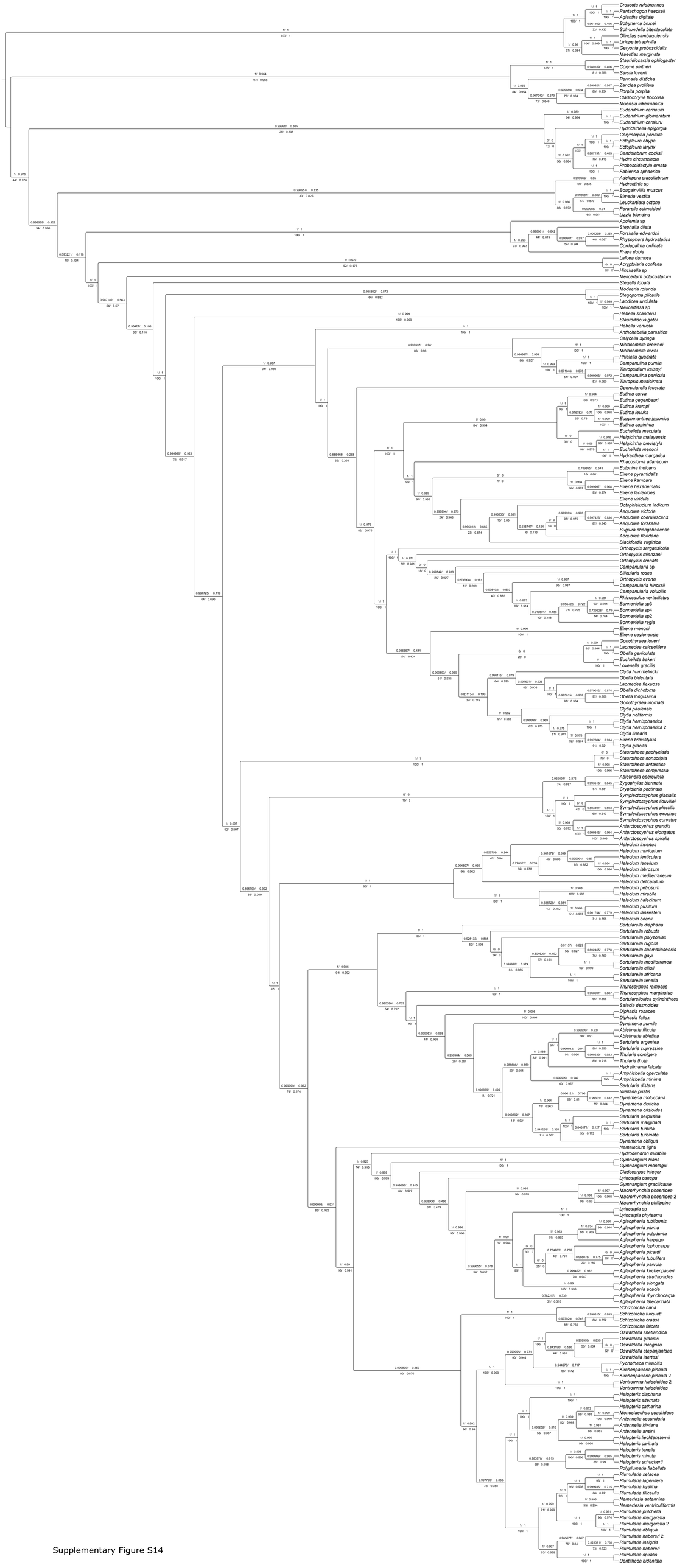
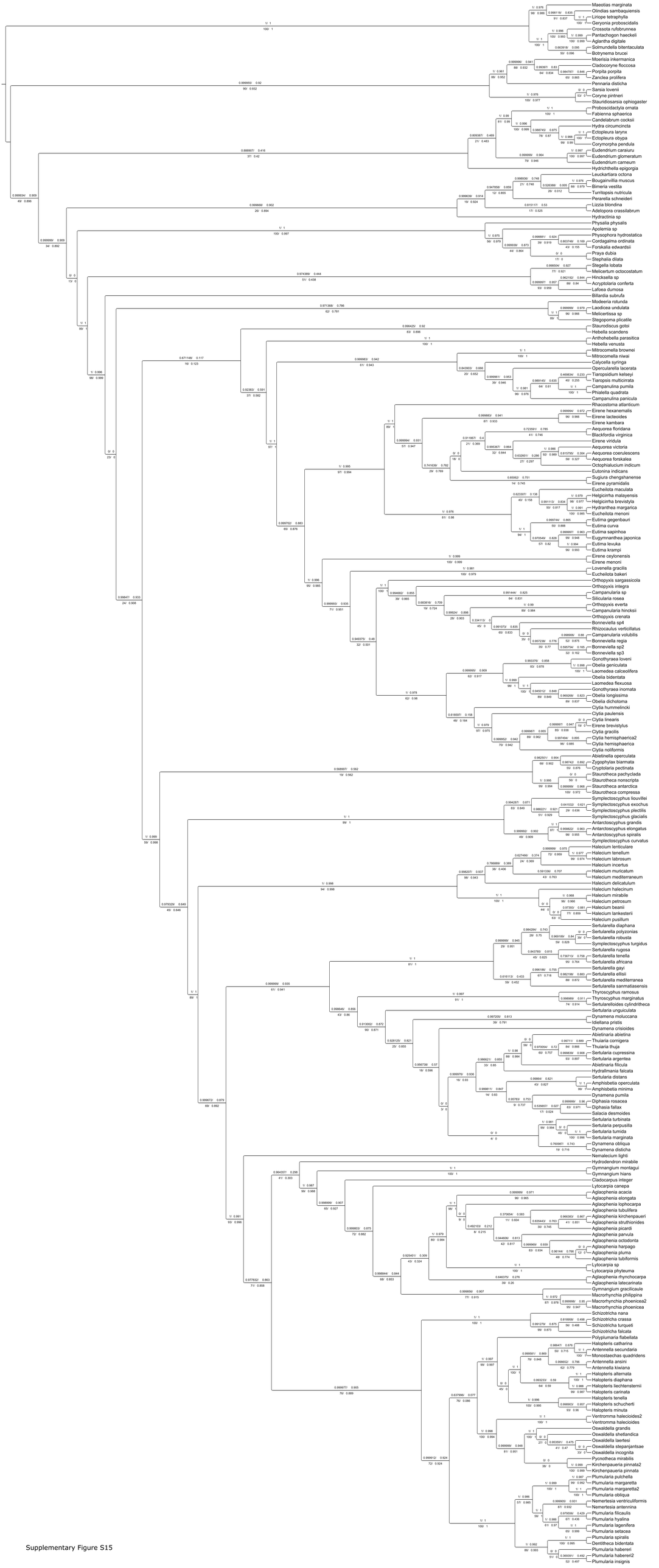


Supplementary Figure S14



Supplementary Figure S15