Supplementary material to: Molecular phylogenetic analysis of Punctoidea (Gastropoda: Stylommatophora), by Rodrigo B. Salvador, Fred J. Brook, Lara D. Shepherd, Martyn Kennedy. *Zoosystematics and Evolution*, 2020.

PART I. Species identification

All specimens sequenced for this work (Table 1) had their identities determined by one of us or by other experts. Species were identified by comparison with primary type material or photographs of type material where feasible, or by comparison with reference material in museum collections, and by consulting taxonomic literature (original descriptions, published catalogues, revisionary works).

The species are listed below accompanied by the initials of the identifier in square brackets, as follows: FJB = Fred J. Brook; GMB = Gary M. Barker; JG = Jochen Gerber; KB = Kevin Bonham; MM = Michal Maňas; RBS = Rodrigo B. Salvador; RGF = Robert G. Forsyth. Authorship of taxa has been omitted for clarity.

Allodiscus dimorphus [FJB]; *Alsolemia longstaffae* [FJB]; *Anguispira alternata* [JG & MM]; *Anguispira jessica* [JG]; *Anguispira kochi* [JG & RGF]; *Anguispira nimapuna* [JG]; *Anguispira strongyloides* [JG & MM]; *Chalcocystis aenea* [RBS]; *Charopa coma* [FJB]; *Cystopelta bicolor* [KB]; *Diemenoropa kingstonensis* [KB]; *Discus catskillensis* [RGF]; *Discus nigrimontanus* [JG]; *Discus patulus* [JG]; *Discus perspectivus* [MM]; *Discus ruderatus* [JG]; *Discus shimeki* [RGF]; *Discus whitneyi* [RGF]; *Fectola infecta* [FJB]; *Flammulina zebra* [FJB]; *Laoma leimonias* [FJB]; *Libera fratercula* [FJB]; *Lilloiconcha* cf. *gordurasensis* [RBS]; *Lilloiconcha gordurasensis* [RBS]; *Lilloiconcha superba* [RBS]; *Mitodon wairarapa* [FJB]; *Mocella eta* [FJB]; *Neophenacohelix giveni* [FJB]; *Oreohelix idahoensis* [JG]; *Oreohelix strigosa depressa* [JG]; *Oreohelix subrudis* [RGF]; *Oreohelix vortex* [JG]; *Otoconcha dimidiata* [FJB]; *Paralaoma servilis* [FJB]; *Phacussa helmsi* [FJB]; *Phenacohelix pilula* [FJB]; *Phrixgnathus celia* [FJB]; *Punctum californicum* [RGF]; *Punctum pygmaeum* [RBS]; *Punctum randolphii* [MM]; *Radioconus amoenus* [RBS]; *Radiodiscus* sp. [RBS]; *Radiodomus abietum* [JG]; *Ranfurlya constanceae* [GMB]; *Scelidoropa officeri* [KB]; *Sinployea atiensis* [FJB]; *Stenacapha hamiltoni* [KB]; *Succinea manaosensis* [RBS]; *Suteria ide* [FJB]; *Therasia thaisa* [FJB]; *Zilchogyra* sp. [RBS].

PART II. Stylommatophoran phylogeny

Herein is presented the information regarding the Stylommatophora phylogenies built to further corroborate the polyphyly of Punctoidea, as well as to assess the position of its three main component branches within the group of ‘pulmonate’ land snails.

METHODOLOGY

A total of 17 species from our Punctoidea ingroup (see main text), representing all families and subfamilies, was selected for this phylogenetic analysis (Table S1). To those were added 23 stylommatophoran species representing the main (most common and diverse) families in the group (Table S1). Two species of freshwater gastropods in superorder Hygrophila (*Acroloxus lacustris* and *Planorbis planorbis*) were chosen as outgroups. All additional sequence data were retrieved from GenBank (Table S1).

The resulting alignment of each marker (COI, 16S, and ITS+28S) was run through Gblocks (Talavera & Castresana, 2007), with the least restrictive settings, to eliminate poorly aligned and divergent positions. The sequences were then concatenated for a single phylogenetic analysis. Analyses were performed with MrBayes 3.2.6 (Bayesian Inference, BI; Ronquist et al., 2012) and PhyML 3.0 (Maximum Likelihood, ML; Guindon et al., 2010). Both BI and ML analysis were conducted the with the same parameters laid out in the main manuscript.

RESULTS

After selection through Gblocks, the concatenated alignment was 1563 bp long: 590 bp in the COI fragment, 228 bp in the 16S, and 845 bp in the IT2+28S. We were unable to obtain 16S sequence data for six species (Table S1).

Punctoidea as previously interpreted is polyphyletic. The BI and the ML analyses returned trees that differed in overall arrangement (Figs. S1 and S2, respectively), but the positioning of the three branches comprising Helicodiscidae, Discoidea and Punctoidea *stricto sensu* are very similar in both analyses. Support values are low for overall relationships, as expected from such a wide sample of land snails and a short resulting (post-Gblocks) DNA sequence. Even so, the relationships within Discoidea and Punctoidea *stricto sensu* were well-supported, and similar to those in the main phylogeny.

DISCUSSION

Our results (Figs. S1, S2) show that Punctoidea as previously recognized is markedly polyphyletic. The group actually comprises three main branches (all in suborder Helicina), which are only very distantly related to one another.

Helicodiscidae constitutes a branch on its own. It was recovered, albeit with low support, as a sister taxon to Arionidae in the ML tree (Fig. S2), with both together being sister to the 'limacoid clade' (now infraorder Limacoidei; Bouchet et al., 2017). The same is observed in the BI tree (Fig. S1), although the relationship between Arionidae and Helicodiscidae is not

resolved. Despite the low support, both trees recovered almost the same pattern, so we can expect that Helicodiscidae might be a part of either Limacoidei or Arionoidei.

Discoidea was recovered as closely related to Helicoidei and Succineidae, with moderate support (0.93 PP) in the BI tree (Fig. S1), but very weak support in the ML tree (Fig. S2). The relationship to Succineidae might be due to long-branch attraction, but that with Helicoidei may be real. The same internal arrangement within Discoidea as in our main phylogeny was recovered with good support: basal Oreohelicidae; paraphyletic *Discus*; and monophyletic *Anguispira*.

Punctoidea has a more ambiguous position within Stylommatophora. The ML tree (Fig. S2) recovered it as the basal-most group of suborder Helicina, albeit with very weak support, whereas the BI tree (Fig. S1) placed it in a more derived position within this suborder. Relationships within Punctoidea were in line with our main phylogeny, with the Endodontidae basal, Cystopeltidae comprising a distinct family, and a Punctidae + Charopidae clade, within which the Punctidae and Charopinae were distinct well-supported groups.

References

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Table S1. List of taxa used for Stylommatophora phylogenetic trees, with GenBank accession numbers. Taxa in blue are samples of species previously assigned to Punctoidea.

Species	COI	16S	ITS2+28S
Achatina fulica (Férussac, 1821)	MK858408	KP317640	KU992690
Acroloxus lacustris (Linnaeus, 1758)	AY282581	EF489311	EF489364
Anguispira alternata (Say, 1816)	MN792584	MN756711	MN782441
Anguispira strongyloides (Pfeiffer, 1854)	MN792589	MN756716	MN782446
Arion silvaticus Lohmander, 1937	AF513018	AJ715331	AY145392
Bradybaena similaris (Férussac, 1822)	MN022742	GQ851001	AY014138
Bulimulus tenuissimus (d'Orbigny, 1835)	JF514631	—	HM027507
Chalcocystis aenea (F. Krauss, 1848)	MN792590	MN756717	MN782447
Charopa coma (Gray, 1843)	MN792591	MN756718	MN782448
Clausilia bidentata (Strøm, 1765)	JX911288	AF012082	AY014051
Cochlicopa lubrica (O.F. Müller, 1774)	MN022720	GU331954	AY014019
Cornu aspersum (O.F. Müller, 1774)	MK883428	KF247035	AY014128
Cystopelta bicolor Petterd & Hedley, 1909	MN792592	MN756719	MN782449
Diemenoropa kingstonensis (Legrand, 1871)	MN792616	MN756740	MN782473
Discus rotundatus (O.F. Müller, 1774)	FJ917285	FJ917265	FJ917212
Discus ruderatus (Hartmann, 1821)	MN792597	MN756724	MN782454
Dorcasia alexandri Gray, 1838	MN022731	—	AY014079
Euconulus polygyratus (Pilsbry, 1899)	MG423330	MK266581	MK299747
Flammulina zebra (Le Guillou, 1842)	MN792601	MN756728	MN782458
Gulella caryatis (Melvill & Ponsonby, 1898)	HQ328133	HQ328323	GQ330510
Helicodiscus parallelus (Say, 1821)	KT707362	—	DQ256731
Helix pomatia Linnaeus, 1758	KX241543	KF247036	AY841333
Libera fratercula (Pease, 1867)	MN792603	MN756730	MN782460
Lilloiconcha gordurasensis (Thiele, 1927)	MN792604	MN756731	MN782461
Megalobulimus oblongus (O.F. Müller, 1774)	KJ546458	KJ546457	AY014078
Oreohelix strigosa depressa Pilsbry, 1904	MN792611	MN756735	MN782468
Orthalicus ponderosus (Strebel & Pfeffer, 1882)	JF514655	—	HM027506
Otoconcha dimidiata (L. Pfeiffer, 1853)	MN792614	MN756738	MN782471
Oxychilus alliarius (Miller, 1822)	MN022739	—	JF837183
Paralaoma servilis (Shuttleworth, 1852)	MN792615	MN756739	MN782472
Partula taeniata Mörch, 1850	HQ203060	AF311874	AF310637
Phacussa helmsi (Hutton, 1882)	MN792618	MN756742	MN782475
Placostylus ambagiosus Suter, 1906	HQ011482	—	MN567951
Planorbis planorbis (Linnaeus, 1758)	EF012175	EF489315	EF489369
Psadara peruviana (Haas, 1951)	MT080615	MT080823	MT080839
Punctum pygmaeum (Draparnaud, 1801)	MN812719	MN756747	MN782479
Rhytida greenwoodi (Gray, 1850)	KT970868	KT970900	KP230525
Subulina octona (Bruguière, 1789)	JX988066	JX988353	MF444887
Succinea manaosensis Pilsbry, 1926	MN186467	MN186468	MN186473
Tandonia budapestensis (Hazay, 1880)	KF894326	KU234276	AY014117
Vertigo antivertigo (Draparnaud, 1801)	KY512680	KY216596	AY014027
Vitrina pellucida (O.F. Müller, 1774)	MN022738	JN400647	AY014111

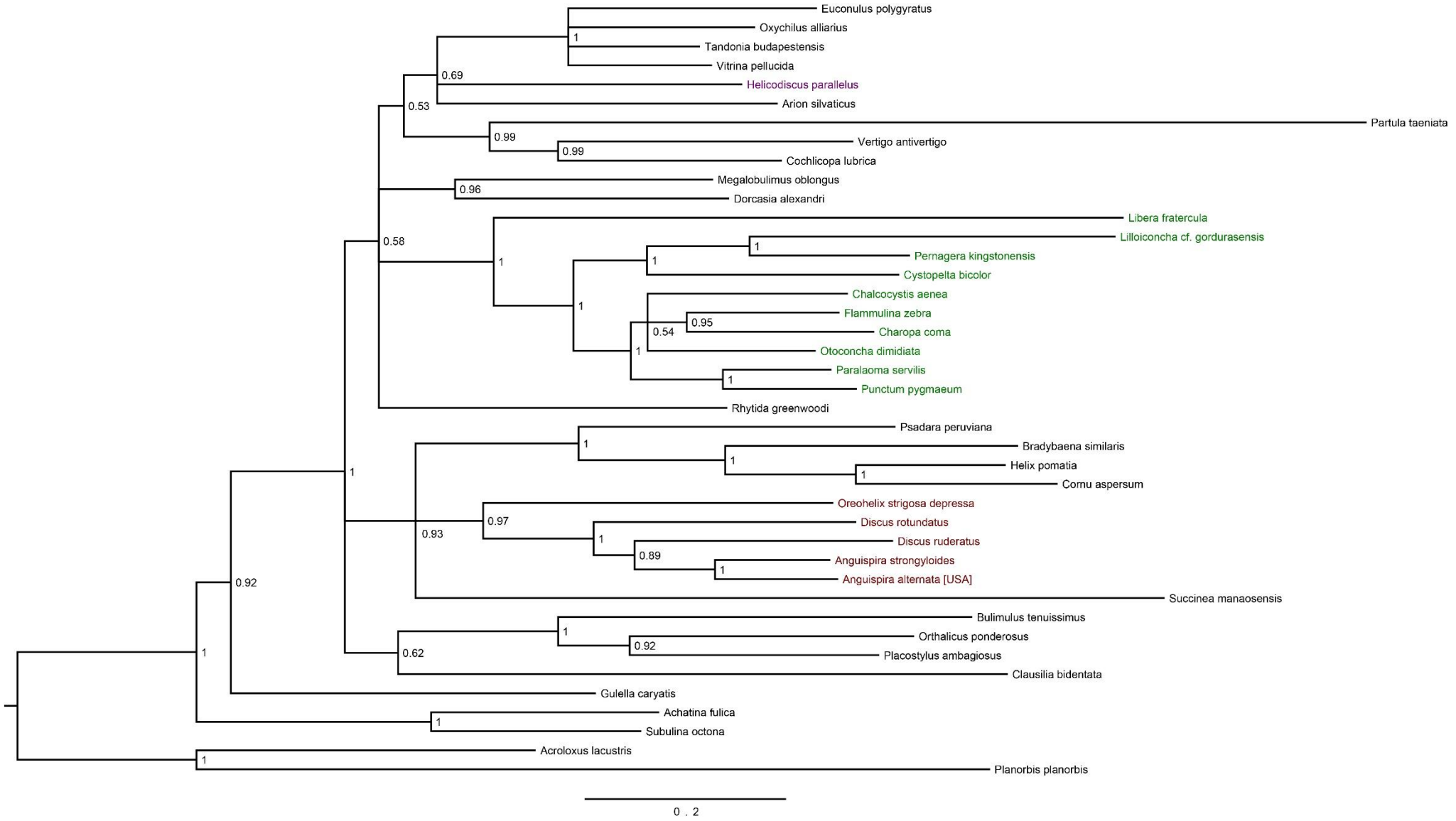

Figure S1. Bayesian Inference tree, rooted by the Hygrophila, with clades previously assigned to Punctoidea colored. Numbers shown on nodes are BI posterior probabilities (0 to 1). Scale bar is substitutions per site.

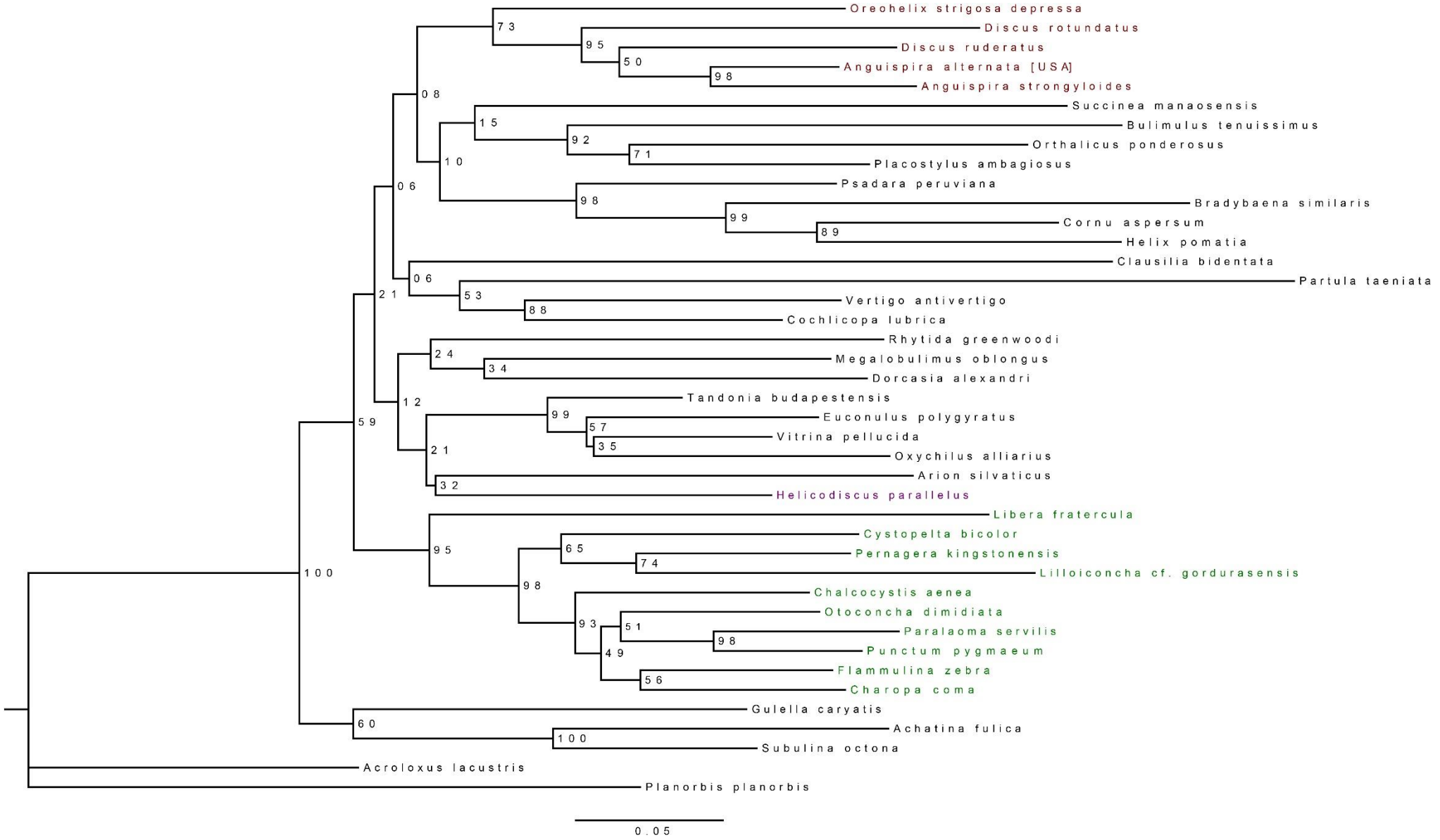

Figure S2. Maximum Likelihood tree, rooted by the Hygrophila, with clades previously assigned to Punctoidea colored. Numbers shown on nodes are bootstrap values (0 to 100%). Scale bar is substitutions per site.