Supplementary Materials

Table S1. List of samples used in this study and GenBank accession numbers.

Species	Code	18S rRNA	28S rRNA	EF
Superfamily Planarioidea				
Family Planariidae				
Crenobia alpina	*C.alpina*	M58345	DQ665960	AJ250912
Phagocata vitta	*P. vitta*	DQ665998	DQ665989	KJ599702 *
Phagocata sp.	*Phagocata*	AF013150	DQ665990	-
Polycelis felina	*P.felina*	DQ665996	DQ665984	KJ599698 *
Polycelis nigra	*P.nigra*	AF013151	-	KJ599700 *
Polycelis tenuis	*P.tenuis*	Z99949	AF022762	KJ599701 *
Family Dendrocoelidae				
Subfamily Dendrocoelinae				
Dendrocoelum lacteum	*D.lacteum*	AJ312271	DQ665967	KJ599686*
Family Dugesiidae				
Cura pinguis	*C.pinguis*	AF033049	DQ665963	KJ599684 *
Dugesia notogaea	*D.notogaea*	KJ599713 *	KJ599720 *	KJ599687 *
Dugesia ryukyuensis	*D. ryukyuensis*	AF050433	DQ665968	KJ599688 *
Dugesia sicula	*D.sicula*	KF308693	DQ665969	KJ599689 *
Dugesia subtentaculata	*D.subtentaculata*	AF013155	DQ665970	KJ599690 *
Girardia anderlani	*G.anderlani*	DQ666013	DQ665972	-
Girardia schubarti	*G.schubarti*	DQ666015	DQ665976	KJ599691 *
Girardia tigrina	*G.tigrina*	AF013156	DQ665977	AJ250913
Neppia sp.	*Neppia*	DQ665999	DQ665982	KJ599695 *
Reynoldsonia reynoldsoni	*R.reynoldsoni*	-	KJ599726 *	KJ599704 *
Reynoldsonia sp.	*Reynoldsonia*	KJ599714 *	KJ599727 *	KJ599705 *
Romankenkius kenki	*R.kenki*	KJ599717 *	KJ599725 *	KJ599703 *
Romankenkius sp.	*Romankenkius*	KJ599716 *	KJ599728 *	-
Schmidtea mediterranea	*S.mediterranea*	AF047854	DQ665992	KJ599709 *
Schmidtea polychroa	*S.polychroa*	AF013154	DQ665993	AJ250914
Spathula alba	*S.alba*	DQ666006	DQ665991	KJ599708 *
Spathula agelaea	*S.agelaea*	KJ599718 *	KJ599729 *	KJ599707 *
Spathula ochyra	*S.ochyra*	KJ599719 *	KJ599730 *	KJ599710 *
Spathula sp.	*Spathula*	DQ666007	DQ665994	KJ599711 *
Family Geoplanidae				
Subfamily Bipaliinae				
Bipalium adventitium	*B.adventitium*	DQ666000	DQ665956	KJ599681 *
Bipalium kewense	*B.kewense*	AF033039	KJ599731 *	-
Bipalium multilineatum	*B.multilineatum*	DQ666012	DQ665957	KJ599682 *
Bipalium nobile	*B.nobile*	DQ666001	DQ665958	KJ599683 *
Novibipalium venosum	*N.venosum*	DQ666019	DQ665981	KJ599694 *
Subfamily Geoplaninae				
Cephaloflexa bergi	*C.bergi*	KJ599712 *	KC608355	KC614474
Choeradoplana gladismariae	*C.gladismariae*	KC608560	KC608443	KC614528
Cratera crioula	*C.crioula*	KC608558	KC608441	KC614544
Geoplana vaginuloides	*G.vaginuloides*	KC608494	KC608377	KC614492
Imbira marcusi	*I.marcusi*	KC608523	KC608406	KC614516

Table S1. *Cont.*

Species	Code	18S rRNA	28S rRNA	EF
Superfamily Planarioidea				
Family Geoplanidae				
Subfamily Geoplaninae				
Luteostriata abundans	*L.abundans*	KC631622	KC631625	KC614556
Luteostriata ernesti	*L.ernesti*	KC631623	KC631626	KC614555
Luteostriata muelleri	*L.muelleri*	KC631624	KC631627	KC614554
Obama burmeisteri	*O.burmeisteri*	DQ666004	DQ665973	KJ599696 *
Obama ladislavii	*O.ladislavii*	DQ666005	DQ665975	KJ599697 *
Paraba franciscana	*P.franciscana*	KC608569	KC608452	KC614552
Pasipha tapetilla	*P.tapetilla*	KC608570	KC608453	KC614553
Subfamily Microplaninae				
Microplana nana	*M.nana*	FJ969981 $	KJ599722 *	-
Microplana groga	*M.groga*	FJ969992 $	KJ599721 *	KJ599693 *
Microplana robusta	*M.robusta*	FJ969989 $	KJ599723 *	-
Microplana terrestris	*M.terrestris*	FJ969983 $	KJ599724 *	-
Subfamily Rhynchodeminae				
Tribe Rhynchodemini				
Dolichoplana striata	*D.striata*	KC608458	KC608341	KC614465
Platydemus manokwari	*P.manokwari*	AF048766	DQ665986	KJ599699 *
Rhynchodemus sp.	*Rhynchodemus*	KJ599715 *	KJ599733 *	KJ599706 *
Tribe Caenoplanini				
Artioposthia testacea	*A.testacea*	DQ666010	DQ665952	KC614560
Artioposthia sp.	*Artioposthia*	DQ666011	DQ665954	KJ634147 *
Arthurdendyus triangulatus	*A. triangulatus*	AF033044	DQ665953	KC614559
Australoplana sp.	*Australoplana*	AF050434	DQ665955	KC614558
Caenoplana coerulea	*C.coerulea*	AF033046	DQ665961	-
Caenoplana sp.	*Caenoplana*	AF048765	DQ665964	KJ599685 *
Endeavouria septemlineata	*E.septemlineata*	KC608454	KC608337	KC614461
Kontikia ventrolineata	*K.ventrolineata*	-	KJ599732 *	KJ599692 *

* New sequences obtained in this study. $ Sequences that have been completed.

Methods

DNA Extraction, Gene Amplification and Sequencing

High molecular weight DNA was purified from absolute ethanol fixed specimens according to Álvarez-Presas *et al.* 2011 [1]. Specific primers were used to amplify the entire length of the 18S

rDNA (as in [2]) and partial sequences for EF [3] and 28S rDNA genes [2]. The same amplification conditions as in the original articles were used. Amplification products were purified with a vacuum manifold (Multiscreen®HTS Vacuum Manifold, Millipore Corporation, Billerica, MA, USA). Both chains were sequenced with the same primers as in PCR amplifications, using Big-Dye (3.1, Applied Biosystems: Foster City, CA, USA) and an automated sequencer ABI Prism 3730 (Unitat de Genòmica dels Serveis Científico-Tècnics of the Universitat de Barcelona, Barcelona, Spain) or in Macrogen Europe (Amsterdam, The Netherlands).

Data Analysis

Coding EF sequences were translated into amino acids to guide the nucleotide alignment using Clustal W, as implemented in Bioedit v.7.0.9.0 program [4]. All sequences were unambiguously aligned. For ribosomal sequences, the alignment was obtained using MAFFT version 7 [5] using the G-INS-i iterative refinement method. Those positions that could not be unambiguously aligned were subsequently excluded from the analyses by applying GBlocks v.0.91b [6]. We estimated the DNA sequence evolution model that best fits the data using jModelTest 2 [7], applying the Akaike information criterion (AIC). Phylogenetic relationships were estimated by Maximum Likelihood (ML) (using RAxML 7.0.0 software [8]) and Bayesian inference (BI) (using MrBayes v. 3.2. [9]). Bootstrap support (BS) values were obtained for ML trees from 10,000 replicates. In the Bayesian analyses, we used four chains to allow heating and default priors. Five million generations were run using the Markov Chain Monte Carlo (MCMC) analysis in two independent runs for the BI. Sampling was every 1,000 generations. The stationarity and convergence of the runs were checked by plotting Log likelihood values *vs.* number of generations and inspecting when the standard deviation of split frequencies had reached <0.01, respectively. A consensus tree was determined after the first 25% of the saved trees had been removed, *i.e.*, by applying the default burn-in. GTR model was used in all the inferences, estimating the gamma distribution and the invariable sites. We constructed a concatenated data matrix by merging the individual alignments and used partitions in all the analyses.

References

1. Álvarez-Presas, M.; Carbayo, F.; Rozas, J.; Riutort, M. Land planarians (Platyhelminthes) as a model organism for fine-scale phylogeographic studies: understanding patterns of biodiversity in the Brazilian Atlantic Forest hotspot. *J. Evol. Biol.* **2011**, *24*, 887–896.
2. Álvarez-Presas, M.; Baguñà, J.; Riutort, M. Molecular phylogeny of land and freshwater planarians (Tricladida, Platyhelminthes): From freshwater to land and back. *Mol. Phylogenet. Evol.* **2008**, *47*, 555–568.
3. Carbayo, F.; Álvarez-Presas, M.; Olivares, C.T.; Marques, F.P.L.; Froehlich, E.M.; Riutort, M. Molecular phylogeny of Geoplaninae (Platyhelminthes) challenges current classification: Proposal of taxonomic actions. *Zool. Scr.* **2013**, *42*, 508–528.
4. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* **1999**, *41*, 95–98.
5. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780.

6. Talavera, G.; Castresana, J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol*. **2007**, *56*, 564–577.

7. Darriba, D.; Taboada, G.L.; Doallo R.; Posada, D. jModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **2012**, *9*, 772.

8. Stamatakis, A.P. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **2006**, *22*, 2688–2690.

9. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Syst. Biol.* **2012**, *61*, 539–542.