

TABLE S1 | Accession numbers of COI from specimens of *Megalonema* analyzed for species delimitation, with localities of collection. Corrections were made to the Laranjinha and Taquara rivers, which are in Paraná State, not São Paulo, as catalogued in the databases. *Voucher specimen catalogued in the Museu de Zoologia da Universidade Estadual de Londrina (MZUEL 9474).

BOLD System	Gen bank	BOLD System species identification	Locality
<i>Megalonema tinga</i>			
ACG7093A	CAPV049-17	<i>Megalonema platanum</i>	Brazil, São Paulo, upper Paraná basin, Paranapanema River, Tibagi River
ACG7093B	LARI356-13	<i>Megalonema argentinum</i>	Argentina, Victoria, Paraná River, Canal Principal
ACG7093C	LARI445-16	<i>Megalonema platanum</i>	Argentina, Paraná River, Espinal Ecoregion
ACG7093D	LARI446-16	<i>Megalonema platanum</i>	Argentina, Paraná River, Espinal Ecoregion
ACG7093E	PEDII234-22	<i>Megalonema platanum</i> , egg	Brazil, São Paulo, upper Paraná basin, Paranapanema River, Rosana
ACG7093F	PEDII235-22	<i>Megalonema platanum</i> , egg	Brazil, São Paulo, upper Paraná basin, Paranapanema River, Rosana
ACG7093G	PEDII236-22	<i>Megalonema platanum</i> , egg	Brazil, São Paulo, upper Paraná basin, Paranapanema River, Rosana
ACG7093H	PEDII237-22	<i>Megalonema platanum</i> , egg	Brazil, São Paulo, upper Paraná basin, Paranapanema River, Rosana
ACG7093I	PEDII238-22	<i>Megalonema platanum</i> , egg	Brazil, São Paulo, upper Paraná basin, Paranapanema River, Rosana
ACG7093J	PEDII239-22	<i>Megalonema platanum</i> , egg	Brazil, São Paulo, upper Paraná basin, Paranapanema River, Rosana
ACG7093K	PEDII240-22	<i>Megalonema platanum</i> , egg	Brazil, São Paulo, upper Paraná basin, Paranapanema River, Rosana
ACG7093L	PRP311-16	<i>Megalonema platanum</i> , egg	Brazil, Paraná, upper Paraná basin, Paranapanema River, Laranjinha River
ACG7093M	PRP312-16	<i>Megalonema platanum</i> , egg	Brazil, Paraná, upper Paraná basin, Paranapanema River, Laranjinha River
ACG7093N	PRP313-16	<i>Megalonema platanum</i> , egg	Brazil, Paraná, upper Paraná basin, Paranapanema River, Laranjinha River
ACG7093O	PRP314-16	<i>Megalonema platanum</i> , egg	Brazil, Paraná, upper Paraná basin, Paranapanema River, Laranjinha River
ACG7093P	PRP315-16	<i>Megalonema platanum</i> , egg	Brazil, Paraná, upper Paraná basin, Paranapanema River, Taquara River
ACG7093Q	PRP316-16	<i>Megalonema platanum</i> , egg	Brazil, Paraná, upper Paraná basin, Paranapanema River, Taquara River
ACG7093R	PRP317-16	<i>Megalonema platanum</i> , egg	Brazil, Paraná, upper Paraná basin, Paranapanema River, Taquara River
ACG7093S	PRP960-16	<i>Megalonema platanum</i> , Morphology*	Brazil, Paraná, upper Paraná basin, Laranjinha River, Mouth
<i>Megalonema platanum</i>			
ACY8268A	FWFA1460-16	<i>Megalonema platanum</i>	Argentina, Salta, río Bermejo
ACY8268B	FWFA1461-16	<i>Megalonema platanum</i>	Argentina, Salta, río Bermejo
ACY8268C	FWFA1462-16	<i>Megalonema platanum</i>	Argentina, Salta, río Bermejo
ACY8268D	FWFA398-15	<i>Megalonema platanum</i>	Argentina, Formosa, río Pilaga, Paraguay River basin
ACY8268E	FWFA399-15	<i>Megalonema platanum</i>	Argentina, Formosa, Laguna Herradura, Paraguay River basin
ACY8268F	FWFA683-15	<i>Megalonema platanum</i>	Argentina, Formosa, río Paraguay
ACY8268G	LARI087-12	<i>Megalonema platanum</i>	Argentina, Entre Ríos, Rosario, Paraná River, Paranacito, canal lateral
<i>Megalonema platycephalum</i>			
ADK5242A	GBMIN95061-17	<i>Megalonema</i> sp.	Peru
ADK5242B	GBMND28115-21	<i>Megalonema platycephalum</i>	Peru, Iquitos varzea
ADK5242C	GBMND28116-21	<i>Megalonema platycephalum</i>	Peru, Iquitos varzea

TABLE S2 | Estimates of Evolutionary Divergence between Sequences. The number of base substitutions per site from between sequences is shown. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Kimura 2-parameter model (Kimura, 1980). The analysis involved 29 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There was a total of 603 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1																													
2	0.000																												
3	0.000	0.000																											
4	0.000	0.000	0.000																										
5	0.000	0.000	0.000	0.000																									
6	0.000	0.000	0.000	0.000	0.000																								
7	0.000	0.000	0.000	0.000	0.000	0.000																							
8	0.000	0.000	0.000	0.000	0.000	0.000	0.000																						
9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																					
10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																				
11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																			
12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																		
13	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																	
14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																
15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000															
16	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000														
17	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000													
18	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000												
19	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000											
20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000										
21	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000									
22	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000								
23	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000							
24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
26	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
27	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
28	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
29	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

REFERENCES

- Kimura M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980; 16:111-20.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013; 30:2725-29.

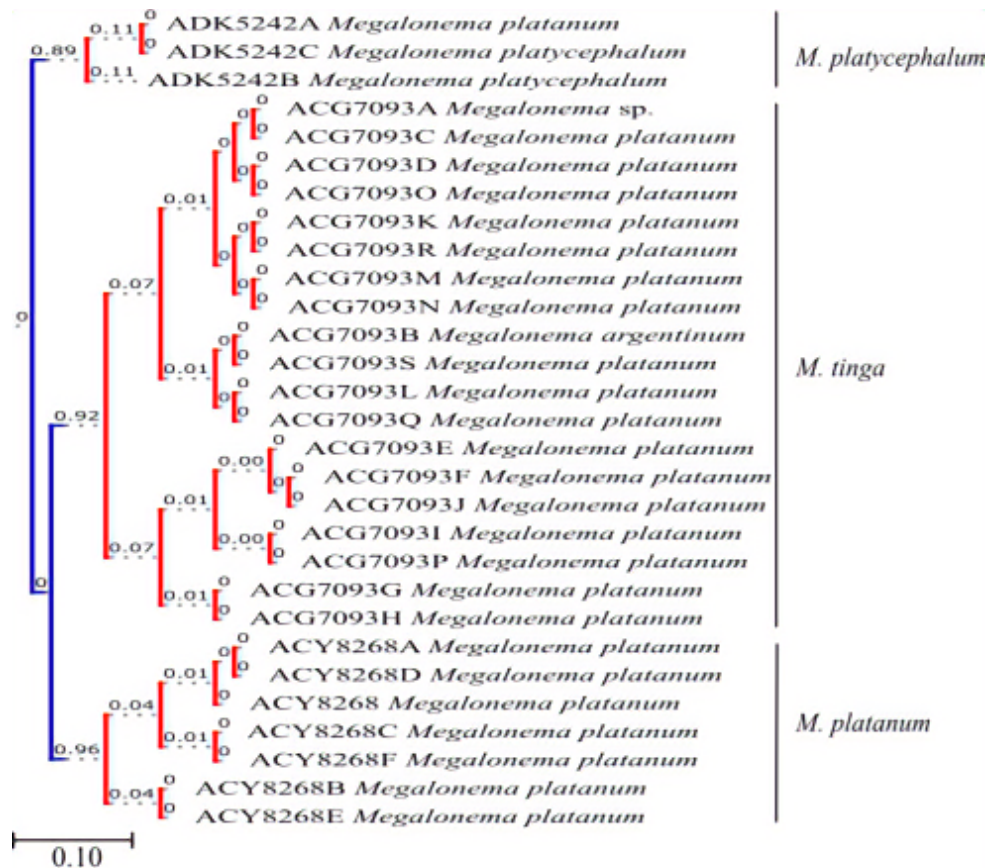


FIGURE S3 | PTP tree from barcode COI sequences of *Megalonema* species (code and species ID from Bold system code as terminal taxa), with bootstrap index on the nodes. New identification in front of the bars.

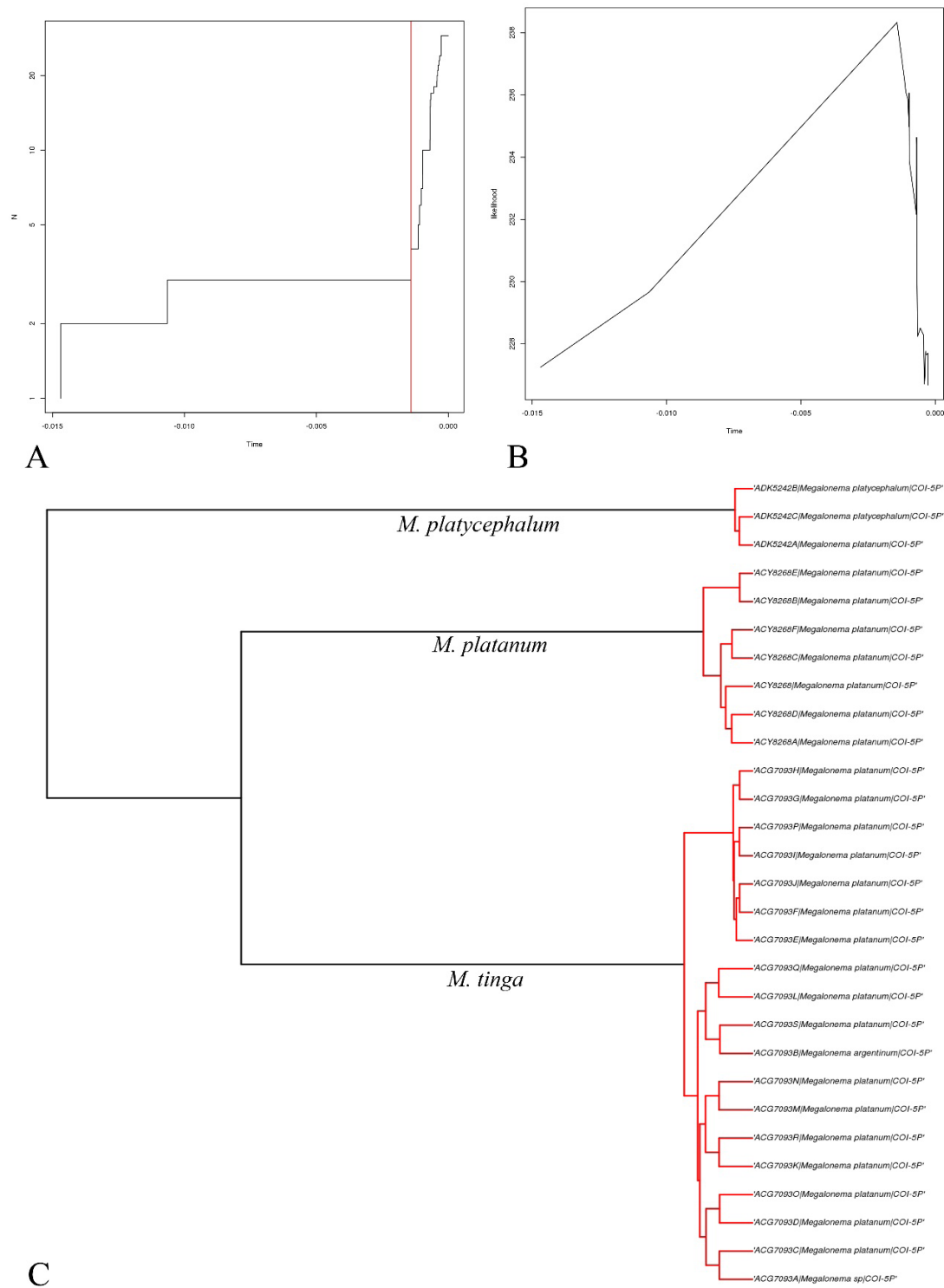


FIGURE S4 | GMYC results. **A.** Variation of the number of lineages over time; **B.** likelihood variation over time; **C.** tree showing the species discrimination.

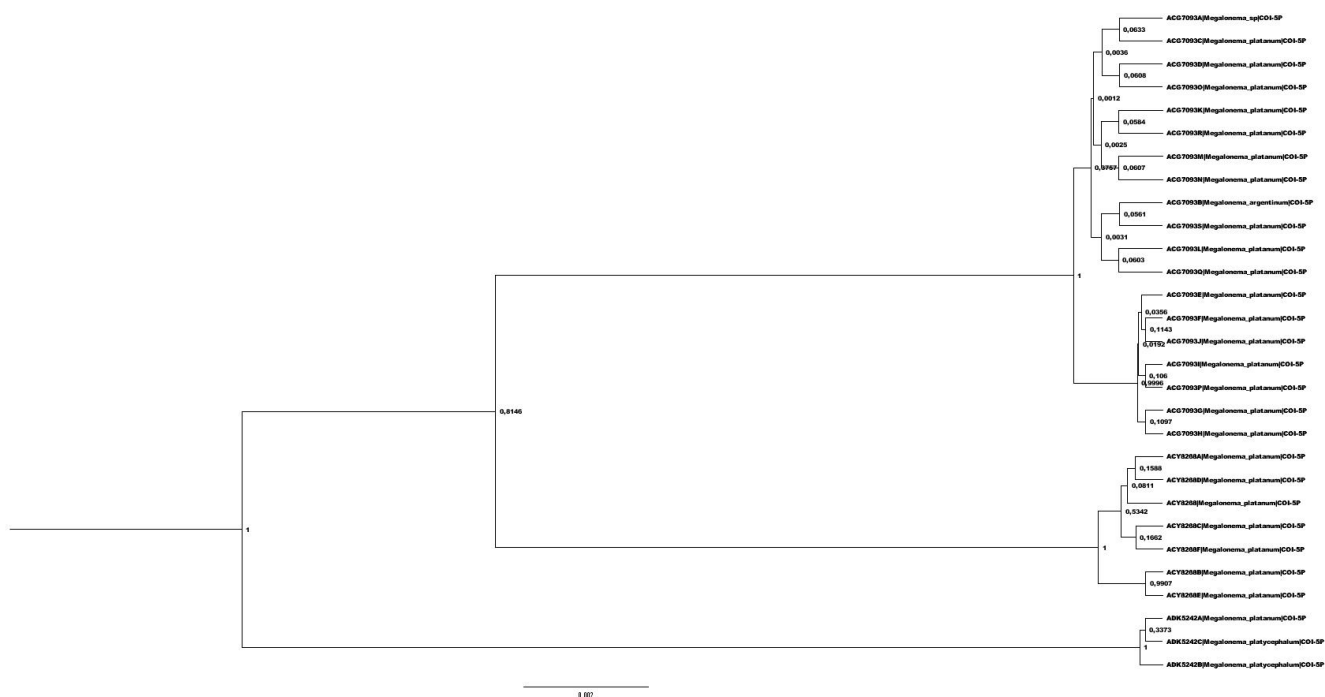


FIGURE S5 | GMYC from barcode COI sequence analysis, with posterior probabilities on the nodes.

Neotropical Ichthyology

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