

Figure S1 Map showing sampling locations for *Pseudoplatystoma* species. 1- Cuiabazinho river, 2-Cuiabá river, 3- Paraguai river (MT), 4- Paraguai river (MS), 5- Miranda river, 6- Aquidauana river, 7-São Francisco river, 8- Ivinhema river, 9- Mogi-Guaçu river.



Figure S2 Neighbor Joining tree based on mitochondrial sequence ATPase 8 and 6 gene for *P. corruscans* (A) and *P. reticulatum* (B). Node numbers correspond to bootstrap values obtained in a 1,000 pseudoreplicates. Only values above 65% are shown.



Figure S3 Neighbor Joining tree based on mitochondrial sequence Cytochrome b gene for *P. corruscans* (A) and *P. reticulatum* (B). Node numbers correspond to bootstrap values obtained in a 1,000 pseudoreplicates. Only values above 65% are shown.



Figure S4 Neighbor Joining tree based on nuclear sequence RAG1Pse gene for *P. corruscans* (A) and *P. reticulatum* (B). Node numbers correspond to bootstrap values obtained in a 1,000 pseudoreplicates. Only values above 65% are shown.



Figure S5 Bayesian inference tree based on concatenated data set (ATPase + Cytb+ RAG1) for *P. corruscans* (A) and *P. reticulatum* (B). Node numbers correspond to Bayesian posterior probability (BI) support values. Only values above 65% are shown.



Figure S6 Phylogeny of *Pseudoplatystoma* genus based on *P. corruscans* and *P. reticulatum* obtained sequences (named in brackets) and publicly available Cytochrome b sequences. The tree was obtained by Bayesian inference and node numbers correspond to Bayesian posterior probability (BI) values. Only values above 65% are shown.