Molecular Phylogenetics and Evolution 127 (2018) 1020–1033

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

# Multilocus phylogeny of the zebra mussel family Dreissenidae (Mollusca: Bivalvia) reveals a fourth Neotropical genus sister to all other genera

Susan R. Geda[a,b,*,1], Nathan K. Lujan[c,1], Michael Perkins[d], Erin Abernethy[e], Mark H. Sabaj[f], Michael Gangloff[a]

[a] Biology Department, Appalachian State University, Boone, NC, USA
[b] Florida Fish and Wildlife Conservation Commission, Milton, FL, USA
[c] University of Toronto Scarborough, Ontario, Canada
[d] North Carolina Wildlife Resources Commission, Marion, NC, USA
[e] Integrative Biology Department, Oregon State University, Corvallis, OR, USA
[f] Academy of Natural Sciences, Philadelphia, PA, USA

ARTICLE INFO

*Keywords:*
Neotropical
Amazon basin
Guiana shield
Brazilian shield
Xingu river
*Rheodreissena*

ABSTRACT

Dreissenidae is one of the most economically and ecologically important families of freshwater and estuarine mollusks. Fourteen extant species and three genera are currently recognized: *Congeria* contains three species from karst caves along the eastern Adriatic coast and one from the Orinoco River of Venezuela, *Dreissena* contains six species native to Eastern European rivers and estuaries, and *Mytilopsis* contains three species from the Gulf of Mexico, Caribbean, and northwestern coast of South America and one from the Tocantins River of Brazil. Previous molecular phylogenetic studies have examined all species except those from South American rivers, and found each genus to be monophyletic with *Congeria* and *Mytilopsis* forming a clade sister to *Dreissena*. We present the first multilocus phylogeny of Dreissenidae inclusive of South American riverine species. Bayesian and maximum likelihood analyses of a 3085 bp alignment consisting of mitochondrial (COI and 16S) and nuclear (18S and 28S) gene regions found Neotropical species to be consistently and strongly supported as sister to all other dreissenids, although incomplete sequencing of the single Orinoco specimen obscured Neotropical monophyly. Our intergeneric relationships are inconsistent with an extensive fossil record suggesting that dreissenids originated in Europe approximately 30 My before dispersing to the Western Hemisphere. Fossil-calibrated analyses indicated that Neotropical dreissenids diverged from European lineages in the mid to late Eocene (~39.3 Ma), and Brazilian and Guiana shield populations diversified during the Oligocene to Miocene. We erect the new genus *Rheodreissena* for all Neotropical freshwater dreissenids and present haplotype data indicative of at least three species. Widespread anthropogenic alteration of the middle Xingu River and lower Amazon threatens the persistence of these endemic, poorly studied mussels and may facilitate introduction beyond their native range.

## 1. Introduction

### 1.1. Extant Dreissenidae systematics, diversity and distributions

Dreissenidae is the only extant family of the superfamily Dreissenoidea and contains 14 extant species in three genera (*Congeria* Partsch 1835, *Dreissena* van Beneden 1835 and *Mytilopsis* Conrad 1858). Although intergeneric relationships among bivalves are poorly resolved in general, relationships among dreissenid genera are well-established, with *Congeria* and *Mytilopsis* forming a strongly supported clade that is sister to *Dreissena* (Bilandžija et al., 2013). At a broader phylogenetic scale, there is also strong molecular support for Dreissenidae being sister to one or all members of the superfamily Myoidea, a predominantly marine, globally distributed group containing extant families Corbulidae, Erodonidae and Myidae (Taylor et al., 2009; Bieler et al., 2014; WoRMS Editorial Board, 2017).

The dreissenid genus *Congeria* was first erected for four fossil species from the Hungarian Miocene (Partsch, 1836). The first extant *Congeria*, *C. kusceri* (Bole, 1962), was discovered more than a century later from caves in the Dinaric Karst region along the eastern Adriatic coast in

* Corresponding author at: Florida Fish and Wildlife Conservation Commission, 1600 Garcon Point Rd., Milton, FL 32583, USA.
*E-mail address:* Susan.Geda@myfwc.com (S.R. Geda).
[1] First two authors contributed equally.

https://doi.org/10.1016/j.ympev.2018.07.009
Received 2 March 2018; Received in revised form 5 July 2018; Accepted 11 July 2018
Available online 20 July 2018

southern Europe. Bilandžija et al. (2013) recently described two additional extant species (*C. mulaomerovici* and *C. jalzici*) from the same region based on combinations of molecular and morphological data. In an anomalous exception to the otherwise subterranean, Baltic distribution of recent *Congeria*, Schütt (1991) described *Congeria hoeblichi* from the Caroní River, a tributary to the Orinoco in Venezuela, making it one of only two extant dreissenids to be described from the interior of South America.

*Dreissena* is the most species rich dreissenid genus and Stepien et al. (2013) divided living *Dreissena* into three subgenera: *Pontodreissena* Logvinenko & Starobogatov 1966, *Carinodreissena* L'vova & Starobogatov 1982, and *Dreissena*. According to those authors, subgenus *Carinodreissena* contains *Dreissena blanci* (Westerlund, 1890) and *D. carinata* (Dunker, 1853); subgenus *Dreissena* contains the zebra mussel *D. polymorpha* (Pallas, 1771; type species for the genus) and *D. anatolica* Locard, 1893; and subgenus *Pontodreissena* contains the quagga mussel *D. rostriformis* (Deshayes, 1838; Orlova et al., 2005) and *D. caputlacus* Schütt, 1993. Subgenera *Dreissena* and *Pontodreissena* are naturally distributed across estuarine and fresh waters of the Ponto-Caspian region in western Europe, whereas subgenus *Carinodreissena* is more narrowly restricted to ancient lakes in the Balkan Peninsula. After being introduced to the Laurentian Great Lakes during the late 20th century, *D. polymorpha* and *D. rostriformis* became economically significant nuisance species in lakes and rivers throughout eastern North America (Karayatev et al., 2015).

Extant members of the genus *Mytilopsis* are mostly native to brackish and freshwater coastal habitats of the subtropical Western Hemisphere. Four species are recognized: *M. leucophaeata* (Conrad, 1831; type species) is distributed throughout the Gulf of Mexico, *M. sallei* (Récluz, 1849) throughout the Caribbean, and *M. trautwineana* (Tryon, 1866) along the Pacific Coast of northwestern South America from Ecuador to Panama. *Mytilopsis lopesi* (Alvarenga and Ricci, 1989) is the most recently described species and is exceptional in that it was described from the Tucuruí rapids of the middle Tocantins River, Brazil, relatively far inland from the coast and from the ranges of all congeners. *Mytilopsis lopesi* is thus the second dreissenid species to be described from inland freshwaters of South America and the only species to be described from the Southern Hemisphere. Two *Mytilopsis* species (*M. leucophaeata* and *M. sallei*) are highly invasive in subtropical and tropical estuarine systems globally and have also become economically significant nuisances (Therriault et al., 2004; Tan and Brian, 2006).

A majority of dreissenid species were originally described and assigned to genera based entirely on phenotypic traits, such as shell morphology and larval brooding characteristics, which are known to be highly plastic in bivalve lineages (Claxton et al., 1998; Ouellette-Plante, 2013; Bilandžija et al., 2013; Morton and Puljas, 2013). However, phylogenetic examination of relationships within and among dreissenids is becoming increasingly common, utilizing molecular data paired with previously described morphological patterns. To date, molecular analyses have tested the generic assignment of all recent dreissenid species (Stepien et al., 2001, 2003, 2013; Bilandžija et al., 2013) except the two atypically distributed species from tropical freshwaters of South America (*Congeria hoeblichi* and *Mytilopsis lopesi*). A molecular phylogenetic reappraisal of the Dreissenidae that includes Neotropical freshwater species is therefore needed to confirm their generic placement and to test fossil-based biogeographical scenarios.

### 1.2. Dreissenid fossil record and biogeographic history

Based on modern distributions and an extensive Lower Eocene to recent fossil record throughout Europe, Dreissenidae has traditionally been hypothesized to have originated there and begun diversifying within shallow marine to freshwater environments of the Oligocene to Pliocene Paratethys Sea (Nuttall, 1990a; Bilandžija et al., 2013). Prior to the description of *Congeria hoeblichi* from Venezuela, the only extant dreissenid genus recognized as native to the Western Hemisphere was *Mytilopsis*. *Mytilopsis* first appears in the Western Hemisphere fossil record in late Oligocene deposits of western Panama and Peru, but the genus has a more extensive European fossil record spanning the Eocene to latest Miocene (Nuttall, 1990a, 1990b). Based on fossil evidence, therefore, *Mytilopsis* is thought to have originated in Europe, expanded its distribution across the Atlantic in the Oligocene, then became extinct in Europe around the Miocene–Pliocene transition (Nuttall, 1990a, 1990b; Van der Velde et al., 2010).

Both the extant *Mytilopsis sallei* and fossil †*Mytilopsis scripta* (Conrad, 1874) have been documented throughout southeastern Colombian and northeastern Peruvian fossil beds formed by Lake Pebas – an enormous fluvio-lacustrine wetland that dominated western Amazonia in the early to middle Miocene and drained northward to what is now western Venezuela (Nuttall, 1990b; Hoorn et al., 2010). However, these inland South American dreissenids are thought to have either gone extinct (†*Mytilopsis scripta*) or been locally extirpated (*Mytilopsis sallei*) as Andean uplift drove the transition of Lake Pebas to a more strictly fluvial network in the Late Miocene (Nuttall, 1990b; Hoorn et al., 2010). *Congeria* has no fossil record in the Western Hemisphere or anywhere outside the European Paratethys Sea (Harzhauser and Mandic, 2010), raising doubts about the generic placement of Venezuela's recent *Congeria hoeblichi*.

Molecular clock analyses of Dreissenidae, which have necessarily been calibrated using the same fossil evidence just described, have supported conclusions based on the fossil record alone, with Dreissenidae being hypothesized to have first diversified in the middle to late Eocene, *Mytilopsis* separating from *Congeria* in the Oligocene to early Miocene, and extant *Dreissena* and *Mytilopsis* beginning to diversify in the middle Miocene (Bilandžija et al., 2013). The current classification of Dreissenidae suggests that two lineages invaded South American freshwaters with one (*Mytilopsis lopesi*) entering the Tocantins River, which drains into the mouth of the Amazon, and the second (*Congeria hoeblichi*) entering the Caroní River, which drains into the lower Orinoco.

### 1.3. Goals

In this study, we use a multilocus mitochondrial and nuclear dataset to reevaluate dreissenid intergeneric relationships, test the generic assignments of South American freshwater dreissenids, and examine genetic diversity as a means of estimating potential species-level diversity among South American freshwater dreissenids. We also reevaluate the timing of dreissenid evolutionary divergence and biogeographical dispersal using a fossil-calibrated relaxed molecular clock analysis, and describe a new genus-level lineage distributed across rivers draining the Brazilian and Guiana shields.

## 2. Materials and methods

### 2.1. Tissue collection and DNA extraction

Tissue samples were collected between 2005 and 2016 from multiple dreissenid populations and taxa within the Amazon and Orinoco basins. DNA was obtainable from only a single specimen among dozens collected in 2005 and 2010 from the Ventuari River near its confluence with the Orinoco River in southern Venezuela. The 2005 collections from the Ventuari were all dry relicts and only two live individuals were found during a return visit to the same locality in 2010. The two live individuals were preserved in 95% ethanol; one (AUM 9703) provided a basis for this study's DNA sequencing and the second (INPA 1646) was retained for morphological analysis. All specimens from the Orinoco are tentatively identified as *Congeria hoeblichi*, although they were collected > 750 river km upstream from the type locality of *C. hoeblichi*, which is in the lower Caroní River at Ciudad Guayana, Venezuela (Schütt, 1991). Xingu River specimens were collected from five sites in 2012 (n = 26 individuals) and six sites (one previously

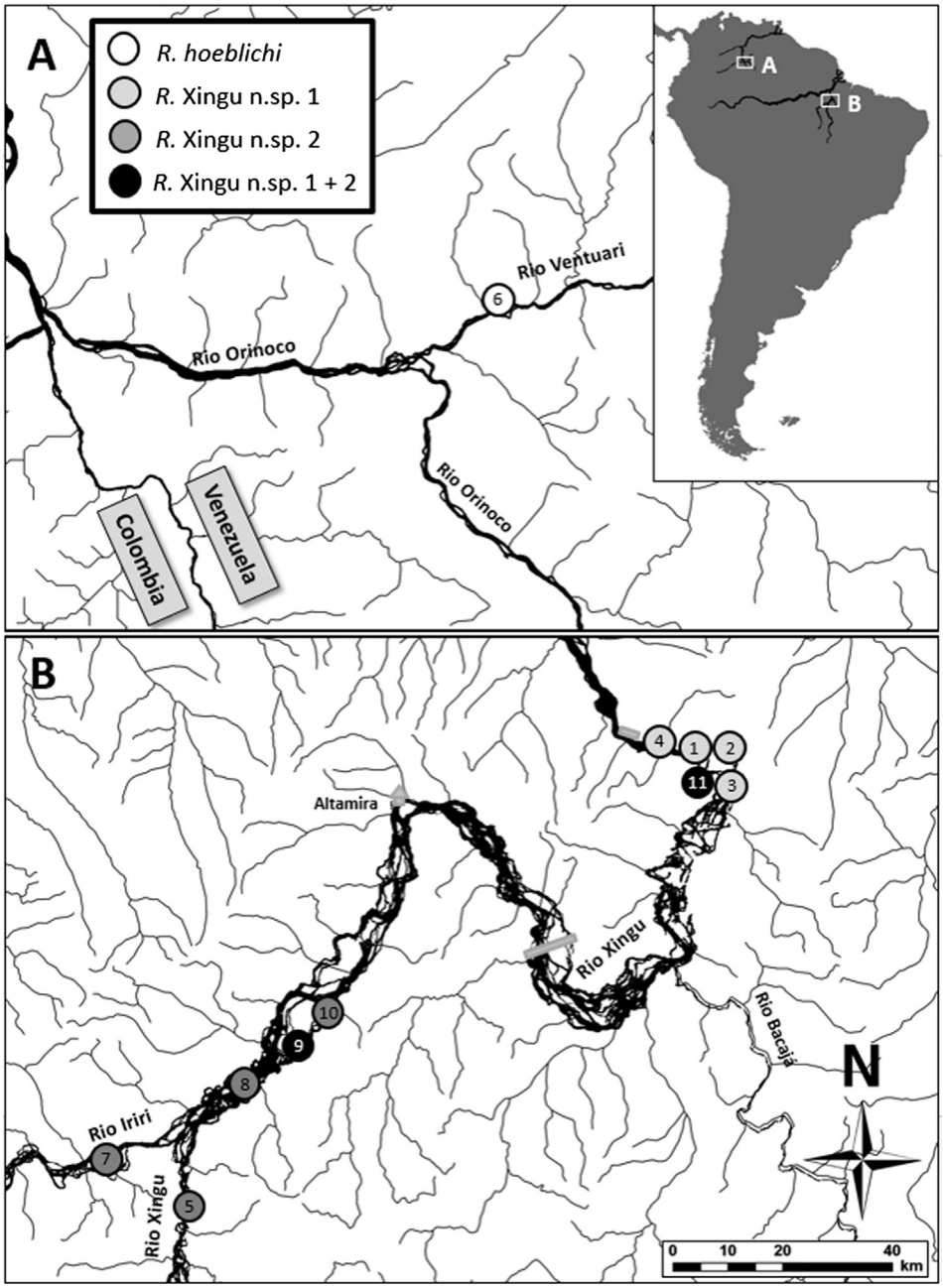


**Fig. 1.** Map of sampling localities in the Xingu and Iriri rivers in Para, Brazil, and a map of localities from the Ventuari River in Venezuela. Sites denoted with a white dot indicate collection localities of *Rheodreissena hoeblichi*. Sites denoted with a light gray dot indicate collection localities of *Rheodreissena* Xingu Species 1. Dark gray dots indicate collection localities of *Rheodreissena* Xingu Species 2, and black dots indicate collection localities where both Species 1 and Species 2 were present.

sampled, five new) in 2016 (n = 28 individuals, Figs. 1–4; Table 1). All 2012 Xingu River samples were stored in 95% ethanol at room temperature; samples collected in 2016 were stored in 70% ethanol at room temperature until samples could be transported to Appalachian State University and transferred to 95% ethanol. Morphological observations were made from all specimens used in this study.

DNA from tissue collected in 2005 and 2012 was extracted using a Qiagen DNeasy Extraction kit (Hilden, Germany) following manufacturer protocols. For the 2016 Xingu samples, DNA was extracted from adductor muscles using a MoBio Cell and Tissue DNA Extraction Kit (Carlsbad, CA) following manufacturer protocols. DNA yields were quantified using a NanoDrop 2000 UV–Vis Spectrophotometer (ThermoFisher, Inc., Waltham, MA). Xingu Species 1 and 2 samples that respectively yielded 40–160 ng/μL and 20–80 ng/μL of gDNA were used for PCR amplification.

### 2.2. PCR amplification

A region of the mitochondrial COI gene was amplified and sequenced from all Neotropical samples, whereas 16S mitochondrial, 18S and 28S nuclear ribosomal RNA gene regions were obtainable from only the Xingu River specimens. Primers were adapted from Therriault et al. (2004) and Frischer et al. (2002; Table 2). PCR amplifications for mtDNA were carried out under the following conditions: 10 μL GoTaq® Green Master Mix 2X per sample (manufacturer's concentration; Promega Corporation, Madison, WI), 1 μL each primer per sample (0.5 μM final concentration), 1 μL 20–160 ng/μL DNA template per sample, and nuclease-free water to a final volume of 20 μL per sample. PCR amplifications for nDNA were carried out under the following conditions: 10 μL GoTaq® Green Master Mix 2× per sample (manufacturer's concentration; Promega Corporation, Madison, WI), 0.5 μL each primer per

**Fig. 2.** *Rheodreissena hoeblichi*, AUM 9703, collected from the deltaic confluence of the Ventuari and Orinoco rivers, Amazonas State, Venezuela, 71.5 km east of San Fernando de Atabapo, 3°58′42″S, 67°03′38″W, 29 Mar 2010, N.K. Lujan et al. (field no. VEN10-19). Scale bar = 1 mm. Photos by NKL.

samples (0.5 μM final concentration), 2 μL 20–160 ng/μL DNA template per sample, and nuclease-free water to a final volume of 20 μL per sample.

Reactions were run on an Eppendorf Mastercycler Nexus thermal cycler (Hamburg, Germany) using a touchdown protocol. The thermal profile began with an initial denaturation cycle of 94 °C (5 min) followed by 24 cycles consisting of a denaturation period at 94 °C (45 s), an annealing cycle that started at 68 °C and decreased 1 °C per cycle (2 min), and an extension cycle at 72 °C (60 s). Additionally, 25 cycles were performed with a denaturation cycle at 94 °C (45 s), an annealing cycle at the appropriate annealing temperature depending on the gene (COI: 50 °C; 28S: 46.5 °C; 16S: 48.5 °C; 18S: 55 °C) (60 s), and an extension cycle at 72 °C (60 s). A final extension cycle at 72 °C (10 min) was performed. Reactions were held at 10 °C until product could be removed.

PCR products were run on 1.5% agarose gels using standard gel electrophoresis in standard 1x TBE buffer. Gels were run for 1–1.5 h at 100 V and then visualized using UV-transillumination of ethidium bromide stained gels. For 28S and 16S there were several samples that yielded multiple products for one individual. In this case, the appropriate band (band of the appropriate size for the expected product) was extracted from the 1.5% gel using a sterile X-ACTO© blade, placed in a 1.5 mL Eppendorf tube and then submersed in 1X TE buffer. The sample was then left overnight, and subsequently used as DNA template in a secondary PCR reaction with the same parameters as described above. When this secondary reaction did not yield PCR product, a DNA precipitation was performed on the excised gel band and 1XTE buffer solution. 1/10th the total volume of 3 M sodium acetate (pH 5.2) was added to the solution, along with 2X total volume of 100% molecular grade ethanol. The samples were then placed in a −80 °C freezer for 1 h. Samples were decanted, to leave the gel band behind, and centrifuged for 30 min at 14,000 RPM. The supernatant was removed, and the pellets were washed with 70% ethanol and centrifuged for an additional 5 min. The samples were then air dried for approximately 10–15 min and then rehydrated with 1X TE buffer. This solution was then used for a tertiary PCR reaction, using the parameters described above. These products were then confirmed on a gel and sent off with primary products to Retrogen Inc. (San Diego, CA) for sequencing.

### 2.3. Sequence preparation

Sequences were edited and assembled using Geneious R7. To confirm sequence identity, all contigs were entered into a BLAST search of the NCBI GenBank sequence database to identify closely-related taxa. To elucidate phylogenetic relationships throughout Dreissenidae, sequence data from Neotropical specimens were aligned with sequence data from other dreissenid taxa and several outgroups within the subclass Heterodonta that were downloaded from GenBank (Table 3).

All sequences were first automatically aligned using the ClustalW algorithm then visually adjusted and trimmed. Sequences for the COI alignment were checked for quality by determining the presence of stop codons through translation into amino acids. Sequences with HQ scores below 50% were discarded. HQ refers to the percentage of bases in a sequence that are considered of high quality.

Given the potential for spurious phylogenetic signal to arise from hypervariable, largely unalignable 'loop' regions in ribosomal DNA sequences, two sets of alignments were generated, one in which 'loop' regions remained and another in which these regions were excluded, reducing overall concatenated alignment length by ~230 bp. The entire concatenated alignment containing both nuclear and mitochondrial gene regions was 3085 bp in length (2853 bp with highly variable ribosomal 'loop' regions trimmed), with 484 bp of COI sequence, 404 bp of 16S fragments (345 bp trimmed), 1576 bp of 18S (1495 bp trimmed), 621 bp of 28S (529 bp trimmed). There were 293 (73%) variable sites in the COI alignment, 256 (63%) in the 16S alignment (201 trimmed), 298 (19%) in the 18S alignment (220 trimmed), and 296 (48%) in the 28S alignment (213 trimmed). For all analyses, the concatenated alignment was partitioned using codon positions (COI) and whole gene regions (16S, 18S, 28S). Separate best-fit nucleotide substitution models were determined for the maximum likelihood and Bayesian phylogenetic analyses using PartitionFinder v1.1.1 (Lanfear et al., 2012; Table 4).

Genetic divergence rates (uncorrected p-distances) among Dreissenidae taxa and appropriate outgroups were analyzed using maximum composite likelihood (Tamura et al., 2013) as implemented in MEGA v6. Haplotype diversity was calculated using DnaSP v5.10.1 (Librado and Rozas, 2009), and haplotype maps were created using PopART (Clement et al., 2002). The haplotype analysis was performed with all singleton haplotypes omitted so the resulting network does not include all sites or individuals sampled.

### 2.4. Phylogenetic analyses

All phylogenetic analyses were rooted using the freshwater mussel *Corbicula fluminea* (Cyrenidae) as the designated outgroup. RAxML v8.0.0 (Stamatakis, 2014) was used to conduct maximum likelihood

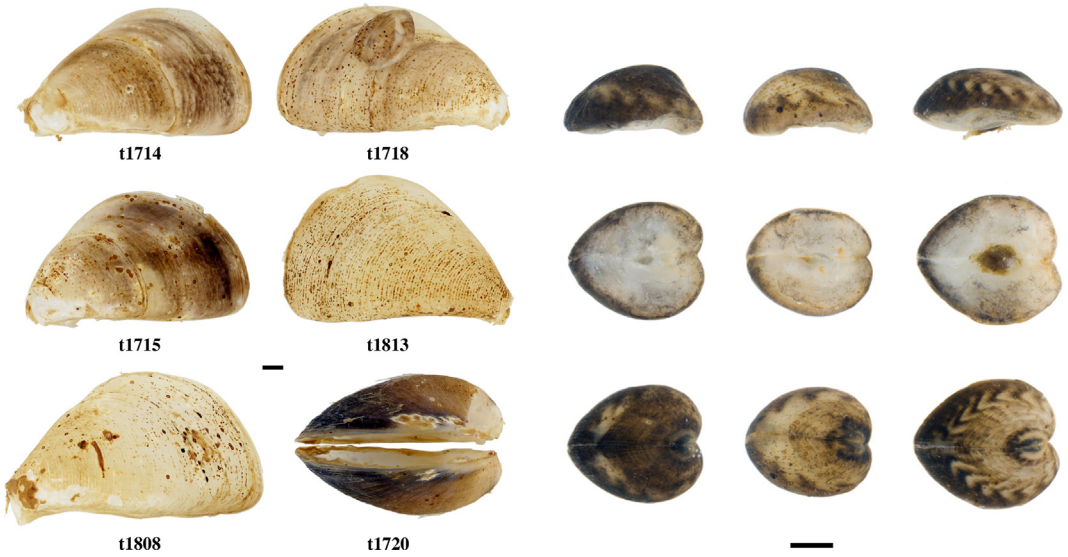


**Fig. 3.** *Rheodreissena* Xingu Species 1 and 2 from the Xingu River, Para State, Brazil. Left two columns show voucher specimens for sequenced individuals of Species 1 (t1714, t1715, t1718, t1720, t1808, t1813). Right three columns show representatives of Species 2 collected at 3°07′45″S, 51°39′55″W on 29 Aug 2016 by L.M. Sousa, et al. (field no. LMS2016082902). Note that specimen t1718 (Species 1) has a smaller individual of Species 2 attached. Scale bars = 1 mm. Photos by MHS.

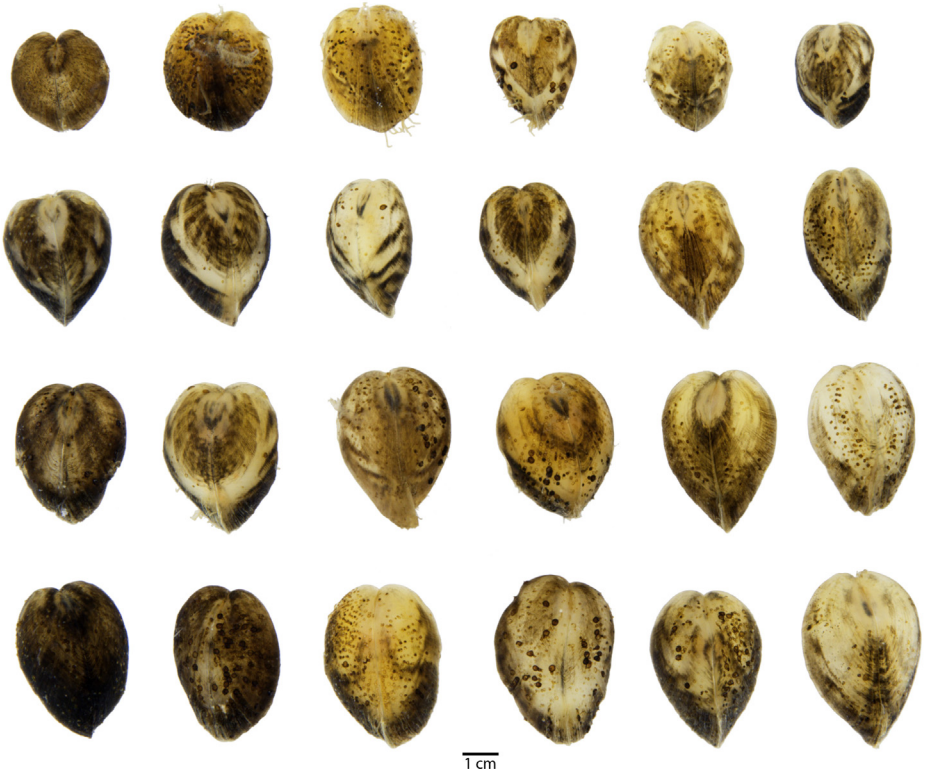


**Fig. 4.** Dorsal view of *Rheodreissena* Xingu Species 2, collected from Cachoeira do Espelho, Xingu River, Para, Brazil on 27 Aug 2016. Photo by MHS.

phylogenetic analyses of all individual gene alignments and the whole and trimmed concatenated alignments. For every analysis, RAxML was programed to conduct a 200-generation search for the best tree topology followed by a 2000 generation bootstrap. MrBayes v3.2 (Ronquist et al., 2012) was used to conduct a Bayesian Markov chain Monte Carlo phylogenetic analyses of the whole and trimmed concatenated alignments. For both analyses, we conducted a 500,000-generation search of tree space using two parallel searches of four chains each (nchains = 8; two cold chains, six hot) and default temperature parameters. All partitions were unlinked, rates were allowed to vary, and each partition was assigned to substitution models as determined above (Table 4). Convergence of the Bayesian analyses was checked using Tracer v1.6 (Bouckaert et al., 2014).

All maximum likelihood and Bayesian phylogenetic analyses were initially run without constraints (Supplemental Figs. 1–5). Analyses of the concatenated dataset yielded a non-monophyletic group of Neotropical freshwater taxa in which the Ventuari species (*Congeria hoeblichi*) and a strongly supported clade of Xingu species were sister to a strongly supported clade of all other dreissenid species. We believe that our inability to sequence any genes other than the COI gene region

**Table 1**
GenBank (GB) accession numbers, specimen numbers, haplotype numbers, GPS coordinates and locality data for Xingu specimens examined in this analysis. Dash marks indicate that no sequence data were obtained for that specimen at that locus.

| Taxon | Collection locality | Museum code | Specimen number | COI (Haplotype) | 28S | 16S | 18S |
|---|---|---|---|---|---|---|---|
| Xingu sp. 1 | 1. Lower Volta Grande a rocky outcrop in the main channel ~500 m upstream from a campsite (−3.1292, −51.6653) Collected: 2012 and 2016 | TBD | t1757 | KU513976 (6) | – | – | – |
| | | TBD | t1758 | KU513977 (10) | – | – | – |
| | | TBD | t1767 | KU513978 (9) | – | – | – |
| | | TBD | t1769 | KU13972 (1) | – | – | – |
| | | TBD | t1770 | KU13970 (4) | – | – | – |
| | | TBD | t1754 | KX027437 (12) | – | – | – |
| | | TBD | 29VIII16.2.4 | MF03758 (27) | – | MF037615 | MF037624 |
| | | TBD | 29VIII16.2.8 | MF037588 (30) | – | – | MF037623 |
| | | TBD | 29VIII16.2.11 | MF037602 (27) | MF037604 | MF037618 | MF037630 |
| | | TBD | 29VIII16.2.12 | MF037589 (28) | MF037605 | – | MF037619 |
| | | TBD | 29VIII16.2.13 | MF037590 (6) | – | – | – |
| | | TBD | 29VIII16.2.15 | MF037591 (24) | MF037606 | – | MF037622 |
| | | TBD | 29VIII16.2.61 | MF037592 (25) | MF037609 | – | MF037628 |
| | | TBD | 29VIII16.2.62 | MF037593 (29) | – | MF037617 | – |
| Xingu sp. 1 | 2. Lower Volta Grande downstream of Cachoeira Tamaracá, off the left bank of a large braid of river (−3.12820, −51.62143) Collected: 2012 | TBD | t1858 | KU513983 (11) | – | – | – |
| | | TBD | t1859 | KU513984 (16) | – | – | – |
| Xingu sp. 1 | 3. Lower Volta Grande a main straight channel running from south-southwest to north-northeast (−3.18427, −51.61735) Collected: 2012 | TBD | t1813 | KU513979 (15) | – | – | – |
| | | TBD | t1808 | KX027436 (2) | – | – | – |
| Xingu sp. 1 | 4. Deep channel along the right bank of the river ~38 km southeast of Vitória do Xingu (−3.09233, −51.73725) Collected: 2012 | TBD | t1714 | KU513973 (3) | – | – | – |
| | | TBD | t1715 | KU513974 (5) | – | – | – |
| | | TBD | t1718 | KU513975 (13) | – | – | – |
| | | TBD | t1720 | KU13971 (14) | – | – | – |
| Xingu sp. 1 | 5. Xingu River, along right bank ~14 km upstream of Rio Iriri confluence (−3.9392, −52.5790) Collected: 2012 | TBD | t1841 | KU513980 (7) | – | – | – |
| | | TBD | t1842 | KU513981 (8) | – | – | – |
| *R. hoeblichi* | 6. Ventuari River, Island in deltaic confluence with the Orinoco River, 71.5 km east of San Fernando do Atabapo (−3.97841, −67.06047) Collected: 2005 | AUM9703 | AUM9703 | KU13969 | – | – | – |
| | Collected: 2010; N: ~26 specimens (some damaged) | AUM 44764 | AUM 44764 | – | – | – | – |
| Xingu sp. 2 | 7. Iriri River, Cahoeira Grande, ~12 km upstream confluence with Xingu River, above and below rapids (−3.84196, 52.73487) Collected: 2016 | TBD | 26VIII16.1.12 | MF037603 (28) | MF037612 | MF037621 | MF037626 |
| | | TBD | 26VIII16.1.17 | MF037594 (19) | – | – | – |
| | | TBD | 26VIII16.1.21 | MF037595 (21) | – | – | – |
| Xingu sp. 2 | 8. Xingu River, Rebojo do Avelino, ~10 km downstream confluence with Iriri River, right descending bank (−3.75201, −52.51154) Collected: 2016 | TBD | 27VIII16.1.4 | MF037596 (22) | MF037611 | MF037614 | – |
| | | TBD | 27VIII16.1.5 | MF037597 (18) | MF037607 | – | – |
| | | TBD | 27VIII16.1.6 | MF037598 (23) | MF037608 | – | – |
| Xingu sp. 1 and Xingu sp. 2 | 9. Xingu River, Cachoeira do Espelho, ~26 km downstream confluence with Iriri River, and ~45 km upstream of Altamira. Right descending bank, below rapids (−3.64477, −52.37960) Collected: 2016 | TBD | 27VIII16.2.17 (sp. 2) | MF037599 (19) | – | – | – |
| Xingu sp. 2 | 10. Xingu River, Ja Bota, ~30 km downstream confluence with Iriri River, and ~40 km upstream of Altamira. Right descending bank (−3.62195, −52.36140) Collected: 2016 | TBD | 28VIII16.1.1 | MF037600 (20) | – | MF037620 | MF037625 |
| Xingu sp. 1 and Xingu sp. 2 | 11. Xingu River, Cachoeira Itamaraca, ~4 km upstream Belo Monte, below rapids (−3.14695, −51.65779) Collected: 2016 | TBD | 29VIII16.1.4 (sp. 1) | MF037601 (26) | MF037610 | – | MF037629 |

from *Congeria hoeblichi* was the primary reason why Neotropical freshwater taxa were found to be non-monophyletic. Thus, for our final analyses, we constrained all three Neotropical freshwater taxa to be monophyletic and these are the results presented herein.

### 2.5. Divergence time estimation

We estimated the timing of dreissenid evolutionary diversification using a node dating approach implemented in the program BEAST v2.4.7 (Bouckaert et al., 2014). Following the logic of Bilandžija et al. (2013), we applied a uniform prior distribution of 55.8–33.9 My for the basal-most node in Dreissenidae, which spans the Eocene and the ages of both the earliest confirmed dreissenid fossil (37.2–33.9 Ma) and an earlier record for which dreissenid identification is less certain (55.8–48.6 Ma; Benton, 1993). The origin of *Dreissena*, which is distinguished from other extant and fossil genera by lacking a shell

**Table 2**
Primers used to amplify and sequence each of the gene regions examined herein.

| Locus | Origin | Forward | Reverse | Expected product size | Authors | Annealing temperature |
|---|---|---|---|---|---|---|
| COI | Mitochondrial | GTTCCACAAATCATAAGGATATTGG | TACACCTCAGGGTGACCAAAAAACCA | 700 bp | Therriault et al. (2004) | 56.5 °C |
| 16S | Mitochondrial | CCGTTCTGAACTCAGCTCATGT | CGACTGTTTAACAAAAACAT | 460 bp | Therriault et al. (2004) | 48.5 °C |
| 28S | Nuclear | TCCGATAGCGCACAAGTAC | TTGCACGTCAGAATCGCTA | 600 bp | Therriault et al. (2004) | 46.5 °C |
| 18S | Nuclear | CTGCCAGTAGTCATATGC | ACCTTGTTACGACTTTAC | 1800 bp | Frischer et al. (2002) | 55.0 °C |

**Table 3**
Taxa, accession numbers, and localities for sequences downloaded from GenBank that were utilized for outgroups in our analyses. Dashes indicate that no data were available for that species at that locus.

| Taxon | COI | 28S | 16S | 18S | Locality |
|---|---|---|---|---|---|
| *Dreissena presbensis* | EF414478.1[1] | EF414469.1[1] | EF414449.1[1] | – | [1]Greece: Lake Dojran |
| | EF414479.1[2] | EF414470.1[2] | EF414450.1[2] | – | [2]Greece: Lake Vegoritis |
| | – | EF414474.1[3] | EF414455.1[3] | – | [3]Macedonia: Lake Ohrid |
| | – | EF414475.1[4] | EF414460.1[4] | – | [4]Montenegro: Lake Skutari |
| | – | EF414476.1[4] | EF414461.1[4] | – | |
| *Dreissena stankovici* | DQ840108.1 | DQ333768.1 | DQ333703.1 | – | Macedonia: Lake Ohrid |
| *Dreissena blanci* | EF414481.1 | EF414471.1 | EF414452.1 | – | Greece: Lake Trichonis |
| *Dreissena bugensis* | – | – | JQ348913.1[5] | – | [5]USA: Lake Erie, OH |
| | JX099436.1[6] | – | JX099457.1[6] | JX099479.1[6] | [6]Netherlands: Ijsselmeer, Lelystadt |
| | DQ840132.1[7] | FJ455425.1[8] | AF038996.1[9] | – | [7]Mediterannean, Black sea |
| | | | | | [8]USA: Lake Mead |
| | | | | | [9]Croatia: Jama u Predolcu, Metkovic |
| *Dreissena polymorpha* | JX099437.1[10] | JX099499.1[10] | JX099458.1[10] | JX099478.1[10] | [10]Croatia: Jarun Lake, Zagreb |
| | EF414493.1[11] | – | – | – | [11]Turkey: Lake Buyukcekmece |
| | EF414494.1[12] | – | EF414465.1[12] | AM774543.1[13] | [12]Germany: Lake Tressow |
| | EF414495.1[14] | – | EF414466.1[14] | AF120552.1[15] | [13]Netherlands: Amsterdam |
| | KC429149.1[15] | – | DQ280038.1[15] | – | [14]Romania: Lake Razim |
| | – | – | AF507049.1[16] | – | [15]Unknown |
| | – | – | AF038997.1[15] | – | [16]Ukraine: Dniester Liman |
| *Dreissena rostriformis* | KP057252.1[17] | JQ700562.1[18] | AF507048.1[19] | – | [17]United Kingdom: Great Britian |
| | – | JQ700563.1[18] | AY302247.1[15] | – | [18]Caspian Sea: near Azerbaijan |
| | – | JQ700564.1[18] | – | – | [19]Ukraine: Dniester Liman, Black Sea |
| *Mytilopsis leucophaeata* | HM100251.1[20] | EF414468.1[20] | EF414448.1[20] | KX713323.1[21] | [20]Belgium: Antwerp Harbour |
| | | | | | [21]USA: Florida Keys |
| *Mytilopsis sallei* | JX099435.1 | JX099497.1 | JX099455.1 | JX099476.1 | China: Hong Kong |
| *Congeria kusceri* | JX099430.1 | – | JX099450.1 | JX099471.1 | Croatia: Pukotina e Tunelu Polje Jezero–Peracko Blato, Ploce, S. Dalamatia |
| *Congeria kusceri* | JX099419.1 | JX099481.1 | JX099439.1 | JX099460.1 | Bosnia and Herzegovina: Doljasnica, Popovo Polje |
| *Congeria kusceri* | JX099420.1 | JX099482.1 | JX099440.1 | JX099461.1 | Bosnia and Herzegovina: Gradnica, Neum |
| *Congeria kusceri* | JX099423.1 | JX099485.1 | JX099443.1 | JX099464.1 | Croatia: Jama u Predolcu, Metkovic, S. Dalmatia |
| *Congeria kusceri* | JX099424.1 | JX099486.1 | JX099444.1 | JX099465.1 | Croatia: Jasena Ponor, Vrgorac, S. Dalmatia |
| *Congeria kusceri* | JX099429.1 | JX099491.1 | JX099449.1 | JX099470.1 | Croatia: Pukotina e Tunelu Polje Jezero-Peracko Blato, Ploce, S. Dalamatia |
| *Congeria jalzici* | JX099421.1 | JX099483.1 | JX099441.1 | JX099462.1 | Slovenia: Izvir Jamske Skoljke, Metlika, Bela Krajina |
| *Congeria mulaomerovici* | JX099418.1 | JX099480.1 | JX099438.1 | JX099459.1 | Bosnia and Herzegovina: Dabarska Pecina, Sanski Most, N. Bosnia |
| *Corbicula fluminea* | KU905760.1[22] | AM779732.1[23] | AF152024.1[24] | AM774558.1[23] | [22]USA: MA, Choptank River |
| | | | | | [23]United Kingdom: Norfolk |
| | | | | | [24]USA: MI. Huron River, Ann Arbor |
| *Corbula amurensis* | KJ028746.1 | – | – | – | China |
| *Glauconome rugosa* | KC429140.1[15] | DQ184799.1[28] | KC429302.1[15] | KC429392.1[15] | [26]Vietnam: Market, Ho Chi Minh City, Ho Chi Minh Province |
| *Moerella iridescens* | JN859967.1[15] | – | AB751330.1[27] | EF613237.1[15] | [27]Japan: Yamaguchi, Estuary of Kiya River |
| *Mya arenaria* | KX576717.1[15] | FM999792.1[28] | KT959487.1[29] | FM999791.1[28] | [28]Poland: Gydnia |
| | | | | | [29]USA: MA, Chesapeake Bay, West River |
| *Rangia cuneata* | – | KC429509.1[15] | KT959495.1[30] | KC429401.1[15] | [30]USA: MA, Rhode River, Canning House Bay |
| *Sinonovacula constricta* | – | AF131005.1[15] | AB751361.1[31] | AY695800.2[15] | [31]South Korea: Suguru Ujino |

**Table 4**
Partitions and molecular substitution models applied to all concatenated phylogenetic analyses.

| Partition | Bayesian substitution model | Maximum likelihood model |
|---|---|---|
| COI_1 | GTR + Gamma | GTR + Gamma |
| COI_2 | F81 + I | GTR + Gamma |
| COI_3 | HKY + I + G | GTR + Gamma |
| 16S | GTR + Gamma | GTR + Gamma |
| 18S | K80 + Gamma | GTR + Gamma |
| 28S | GTR + Gamma | GTR + Gamma |

apophysis for attachment of the anterior byssal retractor muscle, was assigned a log normal prior offset to 11.6 My corresponding to its first appearance in the fossil record of eastern Europe (Harzhauser and Mandic, 2010). Additionally, the following three groups were constrained as monophyletic using purely topological priors: all samples other than *Corbicula fluminea* (the root), *Congeria* + *Mytilopsis*, and all Neotropical freshwater samples.

The entire concatenated dataset was used for the BEAST analysis and was partitioned according to codon position (COI) and whole gene regions (16S, 18S, 28S). Partitions had unlinked generalized time reversible (GTR) site substitution models but linked clock and tree models. We applied a relaxed log-normal clock and a Yule tree prior. Four independent iterations of this analysis were run with each having a chain length of $1 \times 10^{-9}$ generations and tree space sampled every 66,666 generations. Tracer v1.6 was used to visualize and compare results of each analysis. No significant differences were found, so results of all four analyses were combined after removal of 30% burnin from each using LogCombiner v2.4.7. This yielded approximately 40,000 trees which were then downsampled using LogCombiner to generate a final set of 10,000 trees from which the maximum clade credibility tree was generated using TreeAnotator v2.4.7 (Bouckaert et al., 2014).

## 3. Results

### 3.1. Gene-tree analyses

Maximum likelihood analyses of the mitochondrial COI gene yielded a non-monophyletic Dreissenidae in which the outgroup *Mya arenaria* was nested among dreissenid genera and only the genus *Dreissena* (ML: 72) and a group containing all three Neotropical freshwater dreissenids (ML: 81) were strongly monophyletic (Supplemental Fig. 1). COI was the only gene-tree analysis that included *Congeria hoeblichi* from the Orinoco Basin because we were unable to obtain additional sequence data from our single DNA extraction of this species. Neotropical freshwater dreissenids were therefore exclusively represented by two Xingu River species in all other gene analyses.

Analysis of the mitochondrial 16S gene region yielded a respectively strongly monophyletic Dreissenidae (ML: 79), *Congeria* (ML: 84) and Xingu River species (ML: 92; Supplemental Fig. 2). Analysis of the nuclear 18S gene region yielded a strongly monophyletic Dreissenidae (ML: 100) in which a monophyletic group of Xingu River species (ML: 91) was sister to a strongly supported clade of all other dreissenids (ML: 71). Within this latter clade, all three genera were strongly monophyletic (ML: > 90) and *Dreissena* formed a well-supported clade with *Congeria* (ML: 60; Supplemental Fig. 3). Analysis of the 28S gene region yielded a strongly monophyletic Dreissenidae (ML: 100) in which the two Xingu River species were successively sister to a weakly supported clade of all other dreissenids (ML: 45). In this latter clade, both *Congeria* and *Dreissena* were strongly monophyletic (ML: > 85; Supplemental Fig. 4).

### 3.2. Concatenated analyses, Fig. 5

Our initial maximum likelihood and Bayesian analyses of the entire concatenated dataset yielded a strongly monophyletic Dreissenidae in which the Orinoco and Xingu river taxa were successively sister to a well-supported clade of all other dreissenids. Because our COI dataset contained all three Neotropical freshwater samples in our analysis, and these samples were strongly monophyletic in that gene-tree analysis (ML = 81), we re-ran our concatenated analyses so that Neotropical samples were constrained to be monophyletic. In these constrained analyses, the group of all non-Neotropical dreissenids was strongly monophyletic (ML: 100, BI: 1), as was each genus (*Congeria*, ML: 100, BI: 1.0; *Dreissena*, ML: 100, BI: 1.0; *Mytilopsis*, ML: 86, BI: 1.0). Relationships among these genera were poorly resolved, though, with only the maximum likelihood analysis yielding a weakly supported clade of *Congeria* + *Mytilopsis* (ML: 30).

Given the potential for unalignable 'loop' regions in ribosomal DNA sequences to arbitrarily bias phylogenetic analysis, we also trimmed our 16S, 18S, and 28S alignments of these regions, reconcatenated them with the COI alignment, and conducted a maximum likelihood analysis of the resulting trimmed, concatenated alignment. This analysis yielded the same topology as did our analysis of the untrimmed alignment, but node support values decreased slightly (Supplemental Fig. 5).

### 3.3. Genetic diversity of Neotropical freshwater dreissenids, Fig. 6

Fifty-eight novel sequences were generated in this study; however, only 28 represented unique haplotypes. The COI dataset was represented by 33 sequences (Table 1). Due to a lack of high-quality DNA, only the COI gene region could be amplified from a single *Congeria hoeblichi* individual from the Orinoco Basin. The COI haplotype map (Fig. 6) revealed two major haplogroups among Xingu River individuals, representing two highly divergent lineages. We refer to these throughout the manuscript as Xingu Species 1 and 2, represented by 22 and six haplotypes respectively. Populations of Species 1 below the Volta Grande at Xingu River Site 1 had the greatest genetic diversity, with seven haplotypes observed across only 11 individuals; three haplotypes were unique and four were shared across four other sites (Fig. 6). This distinctiveness was despite Site 1 being close to sites 2 and 11 (Fig. 1), each of which had only two haplotypes. Sites 2, 5, 10, and 11 each had two haplotypes, and site 4 had only one haplotype (Fig. 6).

Interspecific genetic distances between Xingu River species and other dreissenid genera ranged from 12.9 to 17.8% for COI, 4.2 to 10.9% for 16S, 0.5 to 0.8% for 18S, and 3.2 to 9.2% for 28S. Interspecific genetic distances between Xingu Species 1, Xingu Species 2, and *Congeria hoeblichi* were 1.4–8.1% for COI. Distance between Xingu Species 1 and 2 was 0.9% for 16S, 0.2% for 18S, and 1.2% for 28S (Tables 5–8).

### 3.4. Divergence time estimates, Fig. 7

Molecular clock analyses indicated that Neotropical freshwater mussels separated from the primarily-European clade containing *Congeria*, *Dreissena* and *Mytilopsis* in the middle to late Eocene ~39.3 Ma (95% HPD: 49.6–33.9 My), and that the Neotropical lineage began diversifying in a split between Guiana and Brazilian shield lineages in the early to middle Miocene ~18.6 Ma, although the confidence interval for this estimate was quite broad (95% HPD: 36.2–4.3 My; Fig. 7) likely due to only COI data being available for *Congeria hoeblichi*. Within the Xingu River, populations of Xingu Species 1 and 2 separated in the late Miocene to Pliocene ~4.2 Ma (95% HPD: 10.5–0.4 My).

The predominantly European clade began diversifying in the Oligocene ~30.4 Ma (95% HPD: 41.4–20.1 My) when *Dreissena* separated from a clade containing *Congeria* + *Mytilopsis*. *Congeria* and *Mytilopsis* separated in the Oligocene to early Miocene ~24.7 Ma (95% HPD: 36.8–14.3 My).

## 4. Discussion

### 4.1. Overview

Our inclusion for the first time of Neotropical freshwater species in a phylogenetic analysis of Dreissenidae has implications for the classification and biogeographic understanding of this ecologically and economically important clade. Results indicate that a new genus should be erected for Neotropical freshwater dreissenids, that at least one (possibly more) new species should be recognized, and that our understanding of when and where dreissenids originated should be reconsidered. We discuss each of these conclusions below.

### 4.2. New genus for Neotropical freshwater dreissenids

#### 4.2.1. Rheodreissena, gen. nov.

*4.2.1.1. Type species. Congeria hoeblichi Schütt, 1991*. Literature cited: Schütt, 1991. Eine neue, nearktische *Congeria* (Bivalvia: Dreissenidae). Archiv für Molluskenkunde der Senckenbergischen Naturforschenden Gesellschaft, 120, 183–185.

*4.2.1.2. Etymology*. *Rheodreissena* is from the Greek word *rheos*, meaning river or stream, and the family name Dreissenidae, in reference to the distinctively riverine, and often fast-flowing habitat these species are found in. The known members of this genus resemble other dreissenids by their small size, attachment to substrate via byssal threads, and formation of large local aggregations.

*4.2.1.3. Diagnosis*. *Rheodreissena* can be diagnosed from all other dreissenid genera by being generally much smaller, taller and more ovate than all other species in the family at maturity, and can be further diagnosed from *Dreissena* by the presence of an apophysis, which is smaller in *Rheodreissena* than in *Congeria* and *Mytilopsis*. *Rheodreissena* can also be diagnosed by habitat selection, specifically its restriction to clear, fast-flowing freshwaters in large inland river channels. Extant species of *Congeria* are restricted to hypogean freshwaters (lentic and lotic habitats). Extant species of *Mytilopsis* typically inhabit brackish coastal waters including lowland rivers, canals, lagoons and estuaries. Extant species of *Dreissena* typically inhabit freshwater lakes and rivers, but can tolerate brackish conditions such as estuarine deltas (e.g., *D. polymorpha*).

*4.2.1.4. Shell morphology*. Shell (Figs. 2–4) thin and very small, typically < 15 mm in length. Subovate in outline with sharply angled and flattened ventral surface; beaks/umbos slightly inflated and positioned strongly anterior, more acute than other members of Dreissenidae; byssal threads originating at anteroventral surface.

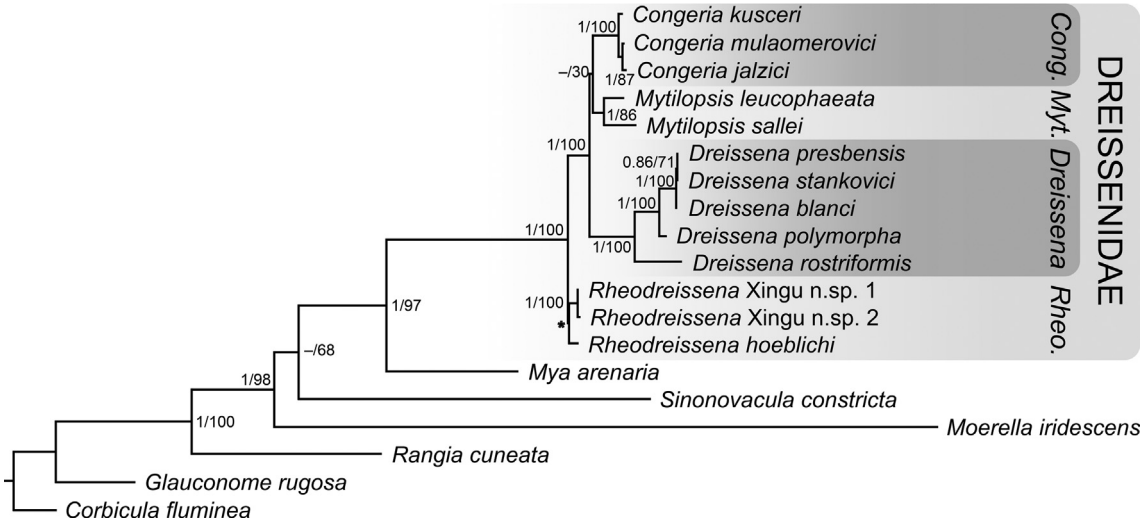


**Fig. 5.** Phylogenetic relationships among the taxa examined in this study based on Bayesian and maximum likelihood analyses of a 3085 bp alignment of mitochondrial (COI and 16S) and nuclear (18S and 28S) gene regions. Node labels are posterior probabilities before the slash, and maximum likelihood bootstrap proportions after. Asterisk indicates node that was constrained (see text).

*Rheodreissena* is typically more dorsally inflated and ventrally flattened than *Mytilopsis* and *Dreissena*. Compared to *Congeria*, *Rheodreissena* is anteriorly more rounded, and the greatest width of the shell (anterior view) is entirely ventral. *Congeria* has a more acute dorsolateral border than does *Rheodreissena*. Larger specimens of *Rheodreissena* have corrugations parallel to the posterior ridge, with one obvious corrugation halfway to two thirds down the shell. Smaller individuals lack this feature. Apophysis present in shell interior. Inner shells lack adductor scars and teeth.

*4.2.1.5. Coloration.* Periostracum color highly variable, from black to white, some specimens have dark stripes or spots. Individuals from the

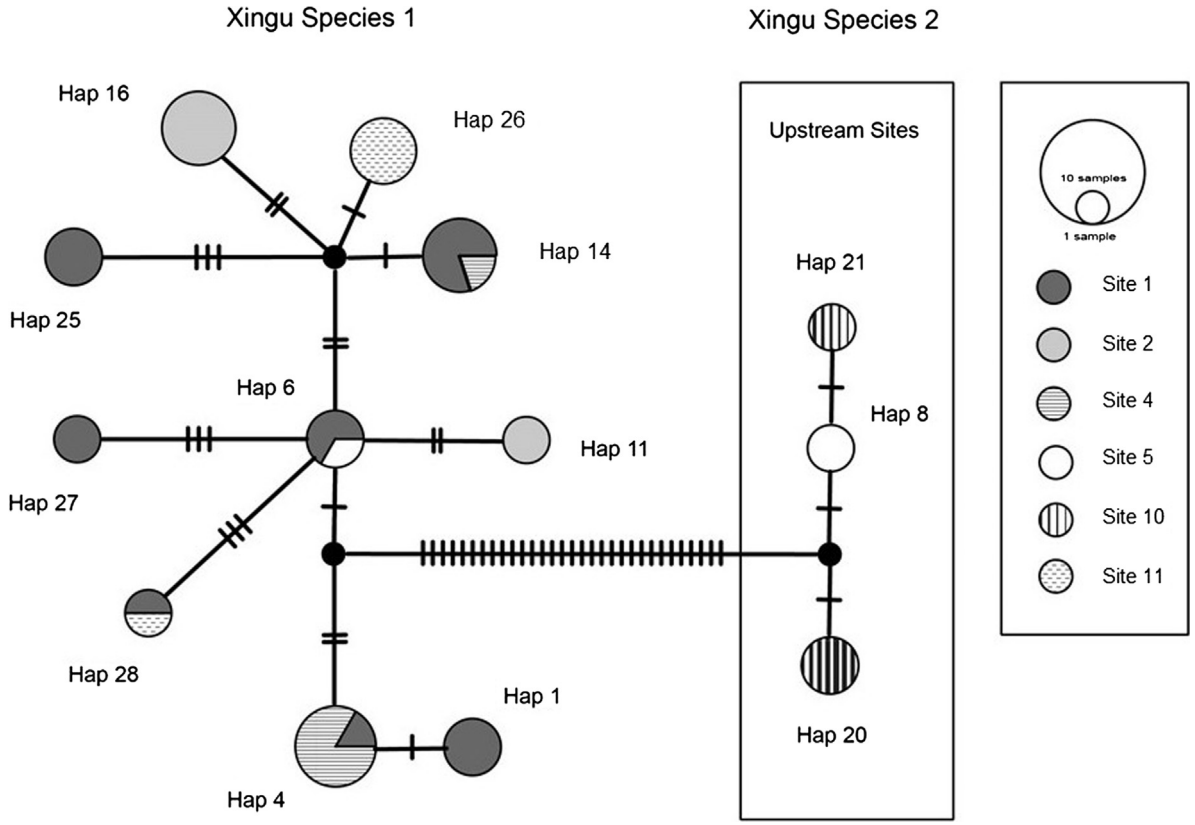


**Fig. 6.** COI haplotype network illustrating relationships between and within *Rheodreissena* Xingu Species 1 and 2. Circles represent haplotypes. Shading and fill patterns indicate collection site. Circle size corresponds to number of individuals in each haplotype. Each hash mark indicates a single nucleotide difference. Small black circles represent implied or unsampled haplotypes. Haplotypes represented by only a single individual were omitted from the figure. *Rheodreissena* Xingu Species 2 was morphologically identified at Site 11, but due to sequencing difficulties, there is no representation of this haplogroup. Site numbers correspond to the site numeration in Table 1. Length of lines between haplotypes correspond to number of nucleotide differences. An exception is the line between the two species, as it has too many nucleotide substitutions to be represented to scale.

**Table 5**
Uncorrected p-distances (x100) for COI data used in our phylogenetic analyses. Column number corresponds to taxon row numbers. Intrageneric comparisons are shaded.

| Taxon | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. *M. iridescens* | --- | | | | | | | | | | | | | | | | |
| 2. *C. fluminea* | 35.9 | --- | | | | | | | | | | | | | | | |
| 3. *G. rugosa* | 38.1 | 19.1 | --- | | | | | | | | | | | | | | |
| 4. *M. arenaria* | 40.6 | 37.0 | 35.0 | --- | | | | | | | | | | | | | |
| 5. *C. kusceri* | 36.9 | 33.0 | 31.2 | 26.7 | --- | | | | | | | | | | | | |
| 6. *C. mulaomerovici* | 35.4 | 31.4 | 32.1 | 25.7 | 7.0 | --- | | | | | | | | | | | |
| 7. *C. jalzici* | 35.4 | 31.2 | 32.3 | 26.2 | 8.2 | 5.0 | --- | | | | | | | | | | |
| 8. *D. polymorpha* | 37.0 | 33.4 | 33.6 | 26.6 | 18.5 | 17.6 | 18.3 | --- | | | | | | | | | |
| 9. *D. rostriformis* | 38.8 | 34.5 | 31.8 | 24.6 | 19.1 | 18.5 | 19.4 | 15.6 | --- | | | | | | | | |
| 10. *D. presbensis* | 37.5 | 33.8 | 34.4 | 28.6 | 19.8 | 19.0 | 20.2 | 10.5 | 17.0 | --- | | | | | | | |
| 11. *D. stankovici* | 37.7 | 34.1 | 34.3 | 28.4 | 19.8 | 19.0 | 20.3 | 10.5 | 16.7 | 0.6 | --- | | | | | | |
| 12. *D. blanci* | 37.2 | 33.2 | 33.0 | 26.6 | 19.3 | 17.2 | 19.0 | 10.8 | 16.5 | 3.5 | 2.9 | --- | | | | | |
| 13. *M. leucophaeata* | 33.6 | 32.7 | 31.4 | 24.6 | 16.3 | 15.8 | 15.3 | 17.4 | 18.2 | 19.0 | 19.2 | 18.5 | --- | | | | |
| 14. *M. sallei* | 37.2 | 32.5 | 30.9 | 26.0 | 14.4 | 13.3 | 14.4 | 16.9 | 16.7 | 17.0 | 16.7 | 16.0 | 13.5 | --- | | | |
| 15. *R. hoeblichi* | 35.9 | 31.4 | 29.3 | 25.7 | 15.2 | 15.6 | 16.5 | 15.6 | 16.5 | 18.3 | 18.5 | 17.2 | 14.2 | 12.9 | --- | | |
| 16. *R.* Xingu sp. 1 | 35.7 | 30.7 | 28.7 | 23.9 | 14.1 | 13.5 | 13.1 | 16.7 | 17.8 | 17.4 | 17.4 | 16.3 | 12.9 | 14.2 | 8.1 | --- | |
| 17. *R.* Xingu sp. 2 | 35.9 | 30.7 | 28.9 | 24.4 | 14.1 | 13.5 | 12.9 | 15.6 | 17.4 | 17.2 | 17.2 | 16.0 | 13.3 | 13.5 | 8.1 | 1.4 | --- |
| Intra-taxon distance | n/a | n/a | n/a | n/a | 0.9 | n/a | n/a | 0.0 | n/a | 0.8 | n/a | n/a | n/a | n/a | n/a | n/a | n/a |

Xingu River were largely grey, red, tan or black whereas individuals from the Ventuari River exhibited more of a mottled brown and black color. The nacre in all individuals examined was white to blueish-white. Preserved tissues white to brown in color.

*4.2.1.6. Included species.* *Rheodreissena* includes at least three species-level taxa, with one nominal species: *Rheodreissena hoeblichi*, new combination, was described from the Caroní River, a tributary to the lower Orinoco in Bolivar State, eastern Venezuela, and is considered herein to include specimens from the Ventuari River, a tributary to the upper Orinoco in Amazonas State, southern Venezuela. Two undescribed species, Xingu Species 1 and 2 from the Xingu River in Pará State, Brazil, also are referred here to *Rheodreissena*. *Mytilopsis lopesi*, a species described from the Tocantins River, was not examined in this study, but will be formally transferred to *Rheodreissena* in a morphological study by Maria Cristina Dreher Mansur et al. (MHS, pers. comm. 2018). Additional populations and perhaps species of *Rheodreissena* are likely present but undocumented in other ecologically similar South American rivers draining geologically ancient highlands of the Brazilian and Guiana shields, such as the

**Table 6**
Uncorrected p-distances (x100) for the 28S data used in our phylogenetic analyses. Column number corresponds to taxon row numbers. Intrageneric comparisons are shaded.

| Taxon | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. *S. constricta* | --- | | | | | | | | | | | | | | | | |
| 2. *R. cuneata* | 21.7 | --- | | | | | | | | | | | | | | | |
| 3. *G. rugosa* | 21.1 | 13.3 | --- | | | | | | | | | | | | | | |
| 4. *C. fluminea* | 21.7 | 16.8 | 7.2 | --- | | | | | | | | | | | | | |
| 5. *M. arenaria* | 20.3 | 16.4 | 15.8 | 17.4 | --- | | | | | | | | | | | | |
| 6. *C. kusceri* | 23.0 | 19.7 | 19.5 | 20.2 | 11.8 | --- | | | | | | | | | | | |
| 7. *C. mulaomerovici* | 23.0 | 19.5 | 19.1 | 20.3 | 11.7 | 0.3 | --- | | | | | | | | | | |
| 8. *C. jalzici* | 23.2 | 19.7 | 19.3 | 20.1 | 11.5 | 0.9 | 0.6 | --- | | | | | | | | | |
| 9. *D. polymorpha* | 24.6 | 21.5 | 20.9 | 21.9 | 13.5 | 7.1 | 7.0 | 7.1 | --- | | | | | | | | |
| 10. *D. rostriformis* | 24.2 | 21.1 | 22.5 | 22.7 | 13.9 | 7.4 | 7.4 | 7.2 | 5.1 | --- | | | | | | | |
| 11. *D. presbensis* | 25.4 | 21.5 | 21.3 | 22.3 | 13.7 | 8.1 | 8.0 | 8.4 | 1.6 | 5.9 | --- | | | | | | |
| 12. *D. stankovici* | 25.6 | 21.7 | 21.5 | 22.5 | 13.9 | 8.3 | 8.2 | 8.6 | 1.8 | 6.1 | 0.4 | --- | | | | | |
| 13. *D. blanci* | 25.4 | 21.9 | 21.3 | 22.3 | 14.1 | 8.1 | 8.0 | 8.4 | 1.6 | 6.3 | 0.4 | 0.8 | --- | | | | |
| 14. *M. leucophaeata* | 24.2 | 19.9 | 20.7 | 21.3 | 11.5 | 4.2 | 4.7 | 4.9 | 8.9 | 10.1 | 10.5 | 10.1 | 10.4 | --- | | | |
| 15. *M. sallei* | 24.0 | 19.9 | 20.3 | 21.3 | 11.9 | 4.6 | 4.5 | 4.7 | 8.6 | 9.2 | 9.2 | 9.4 | 9.2 | 2.2 | --- | | |
| 16. *R.* Xingu sp. 1 | 23.4 | 19.7 | 20.1 | 20.9 | 10.7 | 3.2 | 3.3 | 3.5 | 7.2 | 8.4 | 7.4 | 7.6 | 7.8 | 5.5 | 4.5 | --- | |
| 17. *R.* Xingu sp. 2 | 23.6 | 20.5 | 20.1 | 20.9 | 11.9 | 3.6 | 3.7 | 3.9 | 7.6 | 9.2 | 8.2 | 8.4 | 8.2 | 5.2 | 4.5 | 1.2 | --- |
| Intra-taxon distance | n/a | n/a | n/a | n/a | n/a | 0.1 | n/a | n/a | n/a | n/a | 0.0 | n/a | n/a | n/a | n/a | n/a | |

**Table 7**
Uncorrected p-distances (x100) for the 16S data set used in our phylogenetic analyses. Column number corresponds to taxon row numbers. Intrageneric comparisons are shaded.

| Taxon | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. *S. constricta* | --- | | | | | | | | | | | | | | | | | |
| 2. *M. iridescens* | 31.7 | --- | | | | | | | | | | | | | | | | |
| 3. *R. cuneata* | 26.9 | 29.9 | --- | | | | | | | | | | | | | | | |
| 4. *C. fluminea* | 27.8 | 32.9 | 25.7 | --- | | | | | | | | | | | | | | |
| 5. *G. rugosa* | 29.0 | 33.5 | 30.8 | 18.4 | --- | | | | | | | | | | | | | |
| 6. *M. arenaria* | 28.1 | 33.2 | 30.5 | 29.0 | 30.2 | --- | | | | | | | | | | | | |
| 7. *C. kusceri* | 21.1 | 31.7 | 27.5 | 31.2 | 31.7 | 25.7 | --- | | | | | | | | | | | |
| 8. *C. mulaomerovici* | 22.1 | 32.0 | 28.7 | 31.1 | 32.3 | 25.1 | 1.8 | --- | | | | | | | | | | |
| 9. *C. jalzici* | 21.5 | 31.7 | 28.1 | 30.5 | 31.7 | 25.1 | 1.2 | 0.6 | --- | | | | | | | | | |
| 10. *D. polymorpha* | 22.4 | 30.8 | 28.1 | 30.2 | 32.6 | 26.0 | 8.2 | 7.9 | 7.9 | --- | | | | | | | | |
| 11. *D. rostriformis* | 25.1 | 32.0 | 28.7 | 29.6 | 31.7 | 26.0 | 8.8 | 10.0 | 8.4 | 6.6 | --- | | | | | | | |
| 12. *D. presbensis* | 21.5 | 29.9 | 27.5 | 29.9 | 31.7 | 25.7 | 6.3 | 6.9 | 6.9 | 2.7 | 5.4 | --- | | | | | | |
| 13. *D. stankovici* | 21.5 | 29.9 | 27.5 | 29.9 | 31.7 | 25.7 | 6.3 | 6.9 | 6.9 | 2.7 | 5.4 | 0.0 | --- | | | | | |
| 14. *D. blanci* | 21.4 | 29.9 | 27.5 | 29.9 | 31.7 | 25.7 | 6.3 | 6.9 | 5.9 | 2.7 | 5.4 | 0.0 | 0.0 | --- | | | | |
| 15. *M. leucophaeata* | 23.3 | 33.2 | 28.4 | 30.5 | 33.5 | 25.1 | 5.7 | 6.3 | 5.7 | 8.8 | 10.0 | 7.3 | 7.3 | 7.3 | --- | | | |
| 16. *M. sallei* | 22.7 | 31.7 | 27.8 | 31.1 | 30.8 | 25.1 | 7.3 | 7.9 | 7.3 | 10.9 | 11.2 | 8.5 | 8.5 | 8.5 | 7.3 | --- | | |
| 17. *R.* Xingu sp. 1 | 21.5 | 32.0 | 28.1 | 31.1 | 31.1 | 25.7 | 5.1 | 5.1 | 4.5 | 8.8 | 10.9 | 7.3 | 7.3 | 7.3 | 6.0 | 7.6 | --- | |
| 18. R. Xingu sp. 2 | 21.8 | 31.1 | 28.1 | 30.8 | 31.4 | 25.4 | 4.8 | 4.8 | 4.2 | 7.9 | 10.0 | 6.3 | 6.3 | 6.3 | 5.1 | 6.6 | 0.9 | --- |
| Intra-taxon distance | n/a | n/a | n/a | n/a | n/a | n/a | 0.0 | n/a | n/a | 0.0 | n/a | 0.0 | n/a | n/a | n/a | n/a | n/a | n/a |

Tocantins, Trombetas, and Uraricoera rivers in Brazil, and the Cuchivero, Ocamo and Padamo rivers in Venezuela.

*4.2.1.7. Distribution*. Large main channels of clearwater rivers draining granitic basement rocks of the Brazilian and Guiana shields in northern South America. *Rheodreissena hoeblichi* appears to be widely, albeit patchily, distributed across southern tributaries of the Orinoco River that drain the Guiana Shield highlands. It is currently only known from two localities, respectively in the lower Caroní and Ventuari rivers. Xingu Species 1 and 2 are distributed in the Middle Xingu River, with Xingu Species 1 being most abundant in the lower Volta Grande rapids, and Species 2 occupying those rapids and extending further upstream to at least the mouth of the Iriri River, a major left-bank tributary (Fig. 1). The two Xingu species were only found to co-occur at Sites 5, 9 and 11.

*4.2.1.8. Ecology*. Species of *Rheodreissena* likely play an important ecological role in local ecosystems, as do other dreissenids (Caraco et al., 1997; Higgins and Vander Zanden, 2010). *Rheodreissena* shares interstitial habitats with a wide range of benthic surface-scraping and picking invertivorous fishes (e.g., Anostomidae, Doradidae, Loricariidae) that likely feed on them to varying degrees. Gonçalves (2011) found that *Rheodreissena* in the Xingu River (identified therein as *Mytilopsis*) formed a regular part of the diet of the highly valuable ornamental loricariid species *Hypancistrus zebra* (Isbrücker & Nijssen, 1991).

In the Xingu River, *Rheodreissena* occurred in aggregations of ~10–40 individuals, often on the undersides of large cobbles (0.3–0.6 m diameter) in laminar flow above or below large high-gradient shoals and rapids. Individuals occurred on these rocks in a linear fashion where the rock and surrounding substrate met. Some specimens

**Table 8**
Uncorrected p-distances (X100) for the 18S data set used in our phylogenetic analyses. Column number corresponds to taxon row numbers. Intrageneric comparisons are shaded.

| Taxon | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. *M. iridescens* | --- | | | | | | | | | | | | |
| 2. *C. fluminea* | 8.0 | --- | | | | | | | | | | | |
| 3. *R. cuneata* | 9.1 | 3.3 | --- | | | | | | | | | | |
| 4. *G. rugosa* | 8.1 | 1.2 | 3.5 | --- | | | | | | | | | |
| 5. *M. arenaria* | 8.0 | 2.9 | 4.0 | 2.9 | --- | | | | | | | | |
| 6. *C. kusceri* | 10.1 | 5.3 | 6.5 | 5.2 | 4.1 | --- | | | | | | | |
| 7. *C. mulaomerovici* | 10.1 | 5.3 | 6.5 | 5.2 | 1.1 | 0.0 | --- | | | | | | |
| 8. *C. jalzici* | 10.1 | 5.3 | 6.5 | 5.2 | 4.1 | 0.0 | 0.0 | --- | | | | | |
| 9. *D. polymorpha* | 9.9 | 5.1 | 6.4 | 5.1 | 4.0 | 0.2 | 0.2 | 0.2 | --- | | | | |
| 10. *M. leucophaeata* | 10.1 | 5.2 | 6.6 | 5.2 | 5.1 | 0.3 | 0.3 | 0.3 | 0.3 | --- | | | |
| 11. *M. sallei* | 10.1 | 5.2 | 6.6 | 5.2 | 4.1 | 0.3 | 0.3 | 0.3 | 0.3 | 0.0 | --- | | |
| 12. *R.* Xingu sp. 1 | 10.1 | 5.1 | 6.5 | 5.3 | 3.9 | 0.8 | 0.8 | 0.8 | 0.7 | 0.6 | 0.6 | --- | |
| 13. *R.* Xingu sp. 2 | 10.0 | 5.1 | 6.5 | 5.2 | 3.9 | 0.7 | 0.7 | 0.7 | 0.7 | 0.5 | 0.5 | 0.2 | --- |
| Intra-taxon distance | n/a | n/a | n/a | n/a | n/a | 0.0 | n/a | n/a | 0.0 | n/a | n/a | n/a | n/a |

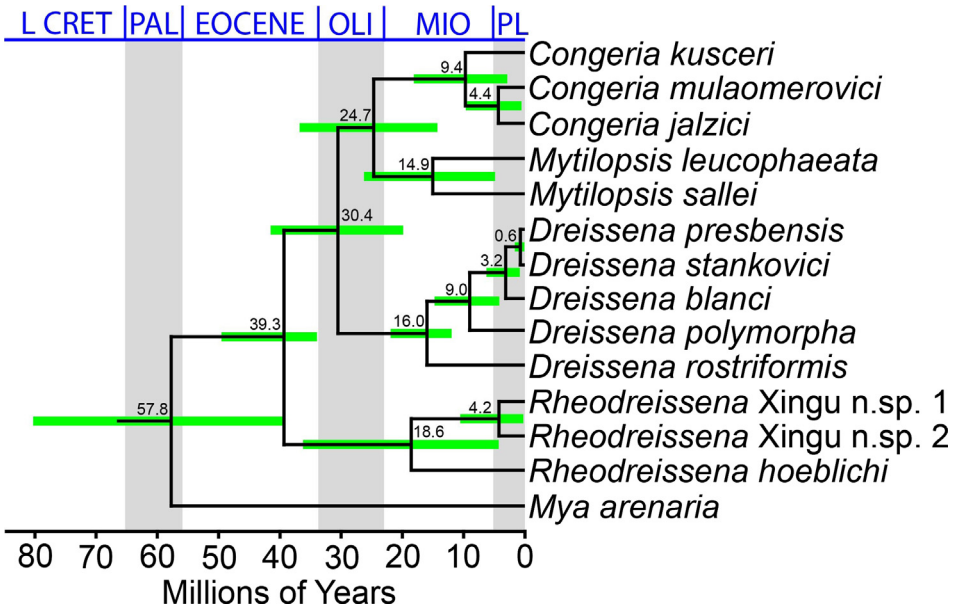


**Fig. 7.** Maximum clade credibility tree created by a BEAST v2.4.7 analysis of 3085 bp alignment of mitochondrial (COI and 16S) and nuclear (18S and 28S) gene regions using a relaxed log-normal clock model. Mean divergence ages are shown above the nodes and the 95% highest posterior density intervals are denoted with green bars. Geological time periods are abbreviated at the top in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were located in dimples in the rocks, possibly providing crypsis and refuge from flow and predation. Xingu species were typically found at depths of 1–8 m, but professional ornamental fishermen collected Species 1 as deep as 23 m using surface-supplied diving equipment. The two species were somewhat segregated by depth. Species 2 mostly occurred within a meter of the river's surface during the low-water season, particularly near shore on small interstitial cobble, whereas Species 1 was typically found at greater depths in mid-channel. That said, individuals of the small-sized Species 2 were occasionally found attached to Species 1 (Fig. 3). Habitat below ~25 m depth was not searched due to dangerous sampling conditions.

In the Orinoco, *Rheodreissena hoeblichi* was observed on the undersides of stones in aggregations of dozens to hundreds of individuals embedded among unidentified freshwater sponges. The rocks with which they were associated formed large 'reefs' that were shallow and experienced slow current at the end of the dry season, but were likely exposed to greater currents during the wet season. At the time and location where *R. hoeblichi* specimens were collected for this study, water physicochemical values were: temperature 31.1 °C, pH 5.9, specific conductivity 12.1 µS/cm, salinity 0.0 ppt, and dissolved oxygen 78.2% saturation (5.8 mg/L).

### 4.3. Intrageneric diversity and population structure

Genetic distances between *Rheodreissena* Xingu Species 1 and 2, and *R. hoeblichi* at loci for which comparative data are available indicate that all three lineages are at least as divergent from one another as are species within other dreissenid genera. At the COI locus, Xingu Species 1 and 2 were 1.4% different from each other and 8.1% different from *R. hoeblichi*, which is either within or well-above the range of COI interspecific distances reported for other genera (i.e., 0.27–3.9%; Wong et al., 2011). At the 16S locus, Xingu Species 1 and 2 were 0.9% different from each other, which is only slightly less than the previously reported range (i.e., 1.0–8.0%; Molloy et al., 2011). At the 28S locus, Xingu Species 1 and 2 were 1.2% different from each other, which is near the middle of the range reported for other genera (i.e., 0–2.0%; Molloy et al., 2011). Xingu Species 1 and 2 differ by 0.2% for 18S, but comparative data are unavailable.

Morphological evidence further supports the hypothesis that the two Xingu haplogroups represent distinct species. Species 1 is larger and more compressed than Species 2, whereas Species 2 is more inflated, ovate, and ventrally flattened. Species 1 also has less variation in the color of its periostracum – being mostly tan with slight variation in brown mottling, whereas Species 2 exhibits a wide range of colors, including white, tan, brown, red, and black. This color polymorphism may be due to differences in how individuals incorporate minerals from the environment during shell growth, adaptive evolution towards greater crypsis (as has been reported for a number of substrate-associated mollusks), or the result of apostatic selection by predators (Cook, 2017; Williams, 2017). Finally, as described above (Section 4.3.1.8), the species appear to prefer different depths, with Species 1 occurring at greater depths than Species 2.

Xingu Species 1 exhibited the greatest haplotype diversity at Site 1 (seven haplotypes), which is surprising given the proximity of Site 1 to sites 2, 3, 4, and 11, with no more than two haplotypes each. The downstream biased distribution and downstream position of the most genetically diverse population of Species 1 is consistent with a global trend in rivers towards downstream increases in genetic diversity (Paz-Vinas et al., 2015). Such trends are observed most strongly in organisms that rely primarily on water currents to passively transport their gametes or propagules, and are thought to be driven by the distinctive physical attributes of rivers (branching dendritic architectures, longitudinal habitat corridors, unidirectional downstream flow) and combinations of related demographic processes (downstream-biased dispersal, increased downstream habitat availability, upstream-directed colonization; Paz-Vinas et al., 2015). However, Site 1 was more heavily sampled than any other site, which could have biased the number of local haplotypes relative to other sites.

We also observed a possible instance of interspecific hybridization between Xingu Species 1 and 2, as indicated by a single Xingu Species 1 individual grouping with Xingu Species 2 haplotypes. This individual was collected from a locality where Xingu Species 2 was not detected (Site 5; Table 1) but may nonetheless occur. Interspecific hybridization has been previously documented between species within Dreissenidae (Voroshilova et al., 2010) and has been found to play a role in mediating displacement mechanisms among dreissenids (Ram et al., 2012).

### 4.4. Biogeographical implications

The discovery that an entirely Neotropical freshwater clade is sister to all other dreissenids invites consideration of the possibility that the family originated somewhere other than Europe. However, our results cannot reject the traditional hypothesis of European origin, which remains supported by a substantial body of fossil evidence (e.g., Nuttall,

1990a, 1990b). European and Neotropical origins for Dreissenidae are equally parsimonious alternatives – each requiring at least two trans-Atlantic dispersal events, one at the origin of the family and another within the genus *Mytilopsis*. Moreover, under either scenario, the first of these dispersals must have occurred without leaving any fossil evidence. However, in the absence of supplementary data, our results could also be explained by a geographically intermediate ancestral range that spanned both Europe and the Neotropics.

Without knowing more precisely the identity and historical geographic distribution of the sister group to Dreissenidae, modern comparative methods for inferring ancestral ranges are unable to provide insights beyond those already available from fossils. Available molecular phylogenetic data (Taylor et al., 2009) suggest that Dreissenidae (represented by *Dreissena polymorpha*) is sister to the superfamily Myoidea (represented by the corbulid *Corbula sinensis* and myids *Mya arenaria* and *Sphenia perversa*). All major lineages in the superfamily Myoidea are globally distributed throughout various coastal marine habitats and biogeographical realms, making them largely uninformative as to the distribution of the most recent common ancestor of all Dreissenidae.

Regardless of the exact biogeographical scenario, our phylogenetic results indicate that Dreissenidae was present in the Neotropics early in their evolutionary history, and there are compelling reasons why fossil evidence supporting such a hypothesis might be scarce. Upland habitats of the Brazilian and Guiana shields where *Rheodreissena* occur had already undergone at least three phases of non-deformational geologic uplift by the Paleocene (Lujan and Armbruster, 2011), tens of millions of years before the first dreissenid fossils appear in European deposits from the Eocene. Thus, riverine upland habitats similar to those observed today likely existed in these shield regions throughout the known history of Dreissenidae. If *Rheodreissena* occupied these regions since the inception of the family, a possible explanation for their absence from the early fossil record is the general absence of fossils of any kind from these upland, non-depositional habitats. Due to selective taphonomic processes alone, the Dreissenidae fossil record may be entirely limited to occurrences in lowland habitats, such as estuaries, lakes and wetlands, where depositional settings make preservation and fossilization more likely. It should also be noted that an early origin in upland, non-depositional environments, and a resulting absence of early upland lineages from the fossil record, would bias not only biogeographical inferences but also the inferred timing of Dreissenidae diversification, raising the possibility that the family originated earlier than their first Lower Eocene appearance in the European fossil record. Finally, the shells of Recent *Rheodreissena* are thin and those of dead individuals easily disintegrate, further reducing the likelihood that individuals might become intact fossils.

### 4.5. Conservation

The Volta Grande Rapids are already heavily impacted by the recently completed construction and operation of the Belo Monte hydroelectric complex, consisting of dams, dykes and reservoirs. Spanning the main channel of the Xingu River, the Pimental impoundment dam has flooded rapids upstream and severely dewatered habitats downstream, thereby directly impacting a 170 km stretch of the Middle Xingu River (Sabaj, 2015). Those impacts will negatively impact the Xingu River's many endemic rheophilic fishes and invertebrates (Sabaj, 2015). This is cause for concern because in addition to dams, other human activities such as deforestation, agriculture, mining and urban development are especially impacting the benthic fauna of South American river systems (Lujan et al., 2013), contributing to the extinction of taxa before they can be discovered (Finer and Jenkins, 2012; Stickler et al., 2013). Few studies have examined the effects of dam-mediated habitat alteration and fragmentation on South American freshwater mollusks, but conservationists are increasingly concerned that the profusion of large hydroelectric projects may radically impact important hotspots of freshwater biodiversity (Winemiller et al., 2016).

## Acknowledgements

Special thanks to Leandro Sousa (Universidade Federal do Pará–Altamira), Maria Cristina Dreher Mansur (Laboratório de Análises e Pesquisas Ambientais), and other members of the iXingu Project team for help with fieldwork and logistical support, Katherine A. Roach for water physicochemical values associated with *Rheodreissena hoeblichi*, Kirk O. Winemiller for supporting and encouraging field work in Venezuela, and Thiago Mahlmann at the National Institute of Amazonian Research and the University of Florida for providing outgroup specimens. Funding for this work was provided by the Appalachian State University Department of Biology, the ASU Office of Student Research, the iXingu Project (NSF DEB-1257813), and the Coypu Foundation. Salary support for NKL provided by the iXingu Project, Canada Department of Fisheries and Oceans, and the estate of George and Carolyn Kelso via the International Sportfish Fund at Texas A&M University. Members of the Gangloff and Siefferman labs at ASU reviewed and provided helpful comments on earlier drafts of this manuscript.

## Declarations of interest

None.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.07.009.

## References

Alvarenga, L.C.F., Ricci, C.N., 1989. A new species of *Mytilopsis*, Conrad, 1857, from Toncantins River, Tucurui, Para, Brazil (Mollusca, Bivalvia, Dreissenidae). Mem. Inst. Oswaldo Cruz. 84, 27–33. https://doi.org/10.1590/S0074-02761989000800010.

Benton, M.J., 1993. Fossil Record 2. Chapman & Hall, London.

Bole, J., 1962. Congeria Kuščeri sp. n. (Bivalvia, Dreissenidae). Bioloski Vestik 10, 55–61.

Bieler, R., Mikkelsen, P.M., Collins, T.M., Glover, E.A., Gonzalez, V.L., Graf, D.L., Harper, E.M., Healy, J., Kawauchi, G.Y., Sharma, P.P., Staubach, S., Strong, E.E., Taylor, J.D., Temken, I., Zardus, J.D., Clark, S., Guzman, A., McIntyre, E., Sharp, P., Giribet, G., 2014. Investigating the bivalve tree of life – an exemplar-based approach combining molecular and novel morphological characters. Invertebr. Systemat. 28, 32–115.

Bilandžija, H.B., Morton, M. Podnar, Ćetković, H., 2013. Evolutionary history of relict Congeria (Bivalvia: Dreissenidae): unearthing the subterranean biodiversity of the Dinaric Karst. Front. Zool. 10, 5. https://doi.org/10.1186/1742-9994-10-5.

Bouckaert, R., Heled, J., Kuhnert, D., Vaughan, T., Wu, C-H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10, 4. https://doi.org/10.1371/journal.pcbi.1003537.

Caraco, N.F., Cole, J.F., Raymond, P.A., Strayer, D.L., Pace, M.L., Findlay, S.E.G., Fischer, D.T., 1997. Zebra mussel invasion in a large, turbid river: phytoplankton response to increased grazing. Ecology 78, 588–602.

Claxton, W.T., Wilson, A.B., Mackie, G.L., Boulding, E.G., 1998. A genetic and morphological comparison of shallow and deep-water populations of the introduced dreissenid bivalve *Dreissena bugensis*. Can. Jour. Zool. 76 (7), 1269–1276. https://doi.org/10.1139/z98-064.

Clement, M., Snell, Q., Walker, P., 2002. TCS: estimating gene genealogies. In: Proceedings 16th International Parallel and Distributed Processing Symposium.1530-2075/02. http://www.hicomb.org/HiCOMB2003/papers/HICOMB2002-03.pdf.

Cook, L.M., 2017. Reflections on molluscan shell polymorphisms. Biol. J. Linnean Soc. 121, 717–730.

Finer, M., Jenkins, C.N., 2012. Proliferation of hydroelectric dams in the Andean Amazon and implications for Andes-Amazon connectivity. PloS ONE 7 (4), e35126. https://doi.org/10.1371/journal.pone.0035126.

Frischer, M.E., Handsen, A.S., Wyllie, J.A., Wimbush, J., Murray, J., Nierzwicki-Bauer, S.A., 2002. Specific amplification of the 18S rRNA gene as a method to detect zebra mussel (*Dreissena polymorpha*) larvae in plankton samples. Hydrobiologia 487, 33–44. https://doi.org/10.1023/A:1022971918673.

Gonçalves, A.P., 2011. Ecology and ethnoecology of *Hypancistrus zebra* (Siluriformes: Loricariidae) on the Xingu River, Brazilian Amazon. Dissertation –. Federal University of Pará, Institute of Biological Sciences, Belém.

Harzhauser, M., Mandic, O., 2010. Neogene dreissenids in Central Europe: evolutionary shifts and diversity changes. In: van der Velde, G., Rajagopal, S., Bij de Vaate, A. (Eds.), The Zebra Mussel in Europe. Backhuys Publishers, Leiden/Margraf Publishers, Weikersheim, pp. 11–29.

Higgins, S.N., Vander Zanden, M.J., 2010. What a difference a species makes: a meta-analysis of dreissenid mussel impacts on freshwater ecosystems. Ecol. Mono. 80 (2),

179–196. https://doi.org/10.1890/09-1249.1.
Hoorn, C., Wesselingh, F.P., Hovikoski, J., Guerrero, J., 2010. The development of the Amazonian mega-wetland (Miocene; Brazil, Colombia, Peru, Bolivia). In: Hoorn, Carina, Wesselingh, Frank P. (Eds.), Amazonia, Landscape and Species Evolution: A Look into the Past. Wiley-Blackwell Publishing, pp. 123–142.
Isbrücker, I.J.H., Nijssen, H., 1991. *Hypancistrus zebra*, a new genus and species of uniquely pigmented ancistrine loricariid fish from the Rio Xingu, Brazil (Pisces: Siluriformes: Loricariidae). Icthyol. Explor. Freshwaters.
Karayatev, A.Y., Burkalova, L.E., Padilla, D.K., 2015. Zebra versus quagga mussels: a review of their population dynamics, and ecosystem impacts. Hydrobiologia 746 (1), 97–112. https://doi.org/10.1007/s10750-014-1901-x.
Lanfear, R., Calcott, B., Ho, S.Y.M., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29 (6), 1695–1701. https://doi.org/10.1093/molbev/mss020.
Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187.
Lujan, N.K., Armbruster, J.W., 2011. The guiana shield. In: Albert, James, Reis, Robert (Eds.), Historical Biogeography of Neotropical Freshwater Fishes. University of California Press, Berkeley, pp. 211–224.
Lujan, N.K., Roach, K.A., Jacobsen, D., Winemiller, K.O., Vargas, V.M., Ching, V.R., Maestre, J.A., 2013. Aquatic community structure across an Andes-to-Amazon fluvial gradient. J. Biogeogr. 40 (9), 1715–1728. https://doi.org/10.1111/jbi.12131.
Molloy, D.P., Giamberini, L., Burlakova, L.E., Karatayev, A.Y., Cryan, J.R., Trajanovski, S.L., Trajanovska, S.P., 2011. Investigation of the endosymbionts of *Dreissena stankovici* with morphological and molecular confirmation of host species. The Zebra Mussels of Europe. Ch 3.
Morton, B., Puljas, S., 2013. Life history strategy, with ctenidial and pallial larval brooding, of the troglodytic 'living fossil' *Congeria kusceri* (Bivalvia: Dreissenidae) from the subterranean alpine karst of Croatia. Linn. Soc. Lon. 108, 294–314. https://doi.org/10.1111/j.1095-8312.2012.02020.x.
Nuttall, C.P., 1990a. Review of the Cenozoic heterodont bivalve superfamily Dreissenacea. Paleontology 33, 707–737.
Nuttall, C.P., 1990b. A review of the Tertiary non-marine molluscan faunas of the Pebasian and other inland basins of north-western South America. Bulletin of the British Museum (Natural History). Geol. Ser. 45, 164–353.
Orlova, M.I., Therriault, T.W., Antonov, P.I., Shcherbina, G.K., 2005. Invasion ecology of quagga mussels (*Dreissena rostriformis bugensis)*: a review of evolutionary and phylogenetic impacts. Aqu. Ecol. 39, 401–418.
Ouellette-Plante, J., 2013. Spatial and temporal variation in the internal morphology of dreissenid mussels in relation to turbidity. Master's Thesis. McGill University, Montréal, pp. 84 p..
Paz-Vinas, I., Loot, G., Stevens, V.M., Blanchet, S., 2015. Evolutionary processes driving spatial patterns of intraspecific genetic diversity in river ecosystems. Mol. Ecol. 24, 4586–4604.
Ram, J.L., Karim, A.S., Banno, F., Kashian, D.R., 2012. Invading the invaders: reproductive and other mechanisms mediating the displacement of zebra mussels by quagga mussels. Invert. Reprod. Dev. 56, 21–32.
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., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542. https://doi.org/10.1093/sysbio/sys029.
Sabaj, M., 2015. Where the Xingu bends and will soon break. Am. Sci. 395–403.
Schütt, H., 1991. Eine neue, nearktische *Congeria* (Bivalvia: Dreissenidae). Archiv für Molluskenkunde der Senckenbergischen Naturforschenden Gesellschaft 120, 183–185.
Stamatakis, A., 2014. RAxML Version 8: a Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics, published online: January 21, 2014. https://doi.org/10.1093/bioinformatics/btu033.
Stepien, C.A., Grigorovich, I.A., Gray, M.A., Sullivan, T.J., Yerga-Woolwine, S., Kalayci, G., 2013. Evolutionary, biogeographic, and population genetic relationships of dreissenid mussels, with revision of component taxa. Quagga and Zebra Mussels: Biology, Impacts, and Control. In: Nalepa, T.F., Schloesser, D.W. (Eds.), Quagga and Zebra Mussels: Biology, Impacts, and Control. CRC Press, pp. 403–444.
Stepien, C.A., Morton, B., Dabrowska, K., Guarnera, R., Radja, R., Radja, B., 2001. Genetic diversity and evolutionary relationships of the troglodytic "living fossil" *Congeria kusceri* (Bivalvia: Dreissenidae). Mol. Ecol. 10, 1873–1879. https://doi.org/10.1046/j.0962-1083.2001.01329.x.
Stepien, C.A., Taylor, C.D., Grigorovich, I.A., Shirman, S.V., Wei, R., Korniushin, A.V., Dabrowska, K.A., 2003. DNA and systematic analysis of invasive and native dreissenid mussels: Is *Dreissena bugensis* really *D. rostriformis*? Aquat. Invaders. 14, 8–18.
Stickler, C.M., Coe, M.T., Costa, M.H., Nepstad, D.C., McGrath, D.G., Dias, L.C.P., Rodrigues, H.O., Soares-Filho, B.S., 2013. Dependence of hydropower energy generation on forests in the Amazon Basin at local and regional scales. PNAS 110 (23), 9601–9606. https://doi.org/10.1073/pnas.1215331110.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. https://doi.org/10.1093/molbev/mst197.
Tan, K.S., Brian, M., 2006. The invasive Caribbean bivalve *Mytilopsis sallei* (Dreissenidae) introduced to Singapore and Johor Bahru, Malaysia. Raffles Bull. Zool. 54, 429–434. https://doi.org/10.1111/j.1463-6409.2007.00299.x.
Taylor, J.D., Glover, E.A., Williams, S.T., 2009. Phylogenetic position of the bivalve family Cyrenoididae – removal from (and further dismantling of) the superfamily Lucinoidea. Nautilus 123, 9–13.
Therriault, T.W., Docker, M.F., Orlova, M.I., Heath, D.D., MacIsaac, H.J., 2004. Molecular resolution of the family Dreissenidae (Mollusca: Bivalvia) with emphasis on Ponto-Caspian species, including first report of *Mytilopsis leucophaeata* in the Black Sea basin. Mol. Gen. Evol. 30, 479–489. https://doi.org/10.1016/S1055-7903(03)00240-9.
Van der Velde, G., 2010. The Zebra Mussel in Europe. In: Rajagopal, S., Bij de Vaate, A. (Eds.), Backhuys Publishers, Leiden/Margraf Publishers, Weikersheim, pp. 11–29.
Voroshilova, I.S., Artamonova, V.S., Makhrov, A.A., Slyn'ko, Y.V., 2010. Natural hybridization of two mussel species *Dreissena polymorpha* (Pallas, 1771) and *Dreissena bugensis* (Andrusov, 1897). Biol. Bull. Russ. Acad. Sci. 37, 542.
Williams, S.T., 2017. Molluscan shell colour. Biol. Rev. 92, 1039–1058. https://doi.org/10.1111/brv.12268.
Winemiller, K.O., McIntyre, P.B., Castello, L., Fluet-Chouinard, E., Giarrizzo, T., Nam, S., Baird, I.G., Darwall, W., Lujan, N.K., Harrison, I., Stiassny, M.L.J., Silvano, R.A.M., Fitzgerald, D.B., Pelicice, F.M., Agostinho, A.A., Gomes, L.C., Albert, J.S., Baran, E., Petrere Jr., M., Zarfl, C., Mulligan, M., Sullivan, J.P., Arantes, C.C., Sousa, L.M., Koning, A.A., Hoeinghaus, D.J., Sabaj, M., Lundberg, J.G., Armbruster, J., Thieme, M.L., Petry, P., Zuanon, J., Torrente-Vilara, G., Snoeks, J., Ou, C., Rainboth, W., Pavanelli, C.S., Akama, A., van Soesbergen, A., Saenz, L., 2016. Balancing hydropower and biodiversity in the Amazon, Congo, and Mekong. Science 351, 128–129. https://doi.org/10.1126/science.aac7082.
Wong, Y.T., Meier, R., Tan, K.S., 2011. High haplotype variability in established Asian populations of the invasive Caribbean bivalve *Mytilopsis sallei* (Dreissenidae). Biol. Invas. 13, 341–348. https://doi.org/10.1007/s10530-010-9825-5.
WoRMs Editorial Board, 2017. World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. https://doi.org/10.14284/170.