1   **Supplemental Materials**

2   **Supplemental Tables**

3   **Table S1.** Families and tip labels from Combasch et al. (2017) used to evaluate the phylogenetic

4   distribution of doubly uniparental inheritance.

Family	Tip Label
Anomiidae	Anomia_ephippium
Arcidae	Arca_noae
Arcticidae	Arctica_islandica
Astartidae	Astarte_sulcata
Bathyspinulidae	Bathyspinula_hilleri
Cardiidae	Tridacna_maxima
Carditidae	Cardita_calyculata
Chamidae	Chama_asperella
Clavagellidae	Clavagella_melitensis
Cleidothaeridae	Cleidothaerus_albidus
Condylocardiidae	Carditella_capensis
Corbulidae	Notocorbula_tunicata
Crassatellidae	Crassinella_lunulata
Cuspidariidae	Cuspidaria_cuspidata
Cyamiidae	Cyamiomactra_laminifera
Cyrenidae	Corbicula_fluminea
Cyrenoididae	Cyrenoida_floridana
Dimyidae	Dimya_lima
Donacidae	Donax_trunculus
Dreissenidae	Dreissena_polymorpha
Etheriidae	Etheria_elliptica
Gaimardiidae	Gaimardia_trapezina
Galeommatidae	Galeomma_turtoni
Gastrochaenidae	Gastrochaena_dubia
Glauconomidae	Glauconome_rugosa
Glossidae	Glossus_humanus
Glycymerididae	Glycymeris_glycymeris
Gryphaeidae	Hyotissa_hyotis
Hemidonacidae	Hemidonax_pictus
Hiatellidae	Hiatella_arctica
Hyriidae	Hyridella_australis
Iridinidae	Aspatharia_pfeifferiana
Isognomonidae	Isognomon_alatus
Kelliellidae	Kelliella_cfnitida
Kelliidae	Bornia_sebetia
Lasaeidae	Lasaea_adansoni

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Laternulidae	Laternula_elliptica
Limidae	Lima_lima
Limopsidae	Limopsis_sp
Lucinidae	Lucina_pensylvanica
Lyonsiidae	Lyonsia_floridana
Mactridae	Mactra_violacea
Malleidae	Malleus_albus
Malletiidae	Malletia_johnsoni
Margaritiferidae	Margaritifera_margaritifera
Mesodesmatidae	Donacilla_cornea
Montacutidae	Mysella_charcoti
Mycetopodidae	Anodontites_elongata
Myidae	Mya_arenaria
Myochamidae	Myadora_brevis
Mytilidae	Mytilus_edulis
Neilonellidae	Neilonella_whoii
Noetiidae	Eontia_ponderosa
Nucinellidae	Nucinella_giribeti
Nuculanidae	Nuculana_pernula
Nuculidae	Nucula_atacellana
Ostreidae	Ostrea_edulis
Pandoridae	Pandora_pinna
Pectinidae	Pecten_maximus
Periplomatidae	Cochlodesma_praetenue
Pharidae	Phaxas_pellucidus
Phaseolidae	Lametila_abyssorum
Philobryidae	Philobrya_sublaevis
Pholadidae	Pholas_dactylus
Pinnidae	Pinna_carnea
Placunidae	Placuna_placenta
Plicatulidae	Plicatula_plicata
Poromyidae	Poromya_illevis
Propeamussiidae	Propeamussium_sp
Psammobiidae	Gari_maculosa
Pteriidae	Pteria_hirundo
Pulvinitidae	Pulvinites_exempla
Sareptidae	Pristigloma_nitens
Semelidae	Abra_alba
Siliculidae	Silicula_sp
Solecurtidae	Tagelus_plebeius
Solemyidae	Solemya_velesiana
Solenidae	Solen_vaginoides
Sphaeriidae	Sphaerium_nucleus

Spondylidae	Spondylus_ambiguus
Tellinidae	Angulus_versicolor
Teredinidae	Teredo_clappi
Thraciidae	Thracia_villosiuscula
Thyasiridae	Thyasira_equalis
Tindariidae	Tindaria_kennerlyi
Trapezidae	Trapezium_sublaevigatum
Trigoniidae	Neotrigonia_margaritacea
Ungulinidae	Cycladicama_cumingi
Unionidae	Unio_pictorum
Veneridae	Venus_verrucosa
Verticordiidae	Haliris_fischeriana
Vesicomyidae	Calyptogena_magnifica
Yoldiidae	Yoldia_limatula

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6 **Table S2.** GenBank accessions for female (F) and male (M) mitochondrial genomes used in

7 phylogenetic analyses of 12 OXPHOS genes.

Order	Taxon	Accession F	Accession M
Adapedonta	*Solen grandis*	HQ703012	-
Anomalodesmata	*Myadora brevis*	KX815961	-
Arcida	*Arca navicularis*	MG641752	-
Cardiida	*Limecola balthica*	MN528028	MN528029
	Scrobicularia plana	MN528026	MN528027
Lucinida	*Lucinella divaricata*	EF043342	-
Myida	*Dreissena rostriformis*	MW080914	-
Mytilida	*Arcualata senhousia*	GU001953	GU001954
	Geukensia demissa	MN449487	MN449488
	Mytilus californianus	JX486124	JX486123
	Mytilus edulis	MF407676	AY823623
	Mytilus galloprovincialis	FJ890849	FJ890850
	Mytilus trossulus	GU936625	GQ438250
	Perumytilus purpuratus	MH330333	MH330330
	Semimytilus algosus	MT026712	MT026713
Octopoda	*Octopus bimaculatus*	KT581981	-
Ostreida	*Crassostrea gigas*	EU672831	-
Pectinida	*Pecten maximus*	KP900975	-
Pteriida	*Pinctada margaritifera*	HM467838	-
Solemyida	*Petrasma pervernicosa*	KY244080	-

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Trigoniida	*Neotrigonia margaritacea*	KU873118	-
Unionida	*Anodonta anatina*	KF030964	KF030963
	Arconaia lanceolata	KJ144818	KJ775864
	Chamberlainia hainesiana	MK994770	MK994771
	Cumberlandia monodonta	KU873123	KU873124
	Echyridella menziesii	KU873121	KU873122
	Lampsilis siliquoidea	MF326973	MF326974
	Lampsilis powelli	MF326971	MF326972
	Margaritifera margaritifera	MK421956	MK421959
	Microcondylaea bonellii	MK994772	MK994773
	Monodontina vondembuschiana	MK994774	MK994775
	Pilsbryoconcha exilis	MK994776	MK994777
	Potamida littoralis	KT247374	KT247375
	Potamilus alatus	KU559011	KU559010
	Pyganodon grandis	FJ809754	FJ809755
	Quadrula quadrula	FJ809750	FJ809751
	Sinanodonta woodiana	HQ283346	MH349356
	Solenaia carinata	KC848654	KC848655
	Solenaia oleivora	KF296320	KY007143
	Unio crassus	KY290447	KY290450
	Unio delphinus	KT326917	KT326918
	Unio pictorum	HM014134	MH349358
	Unio tumidus	KY021076	KY021075
	Utterbackia peninsularis	HM856636	HM856635
	Venustaconcha ellipsiformis	FJ809753	FJ809752
Venerida	*Meretrix larmarckii*	KP244451	KP244452
	Ruditapes philippinarum	AB065375	AB065374

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9 **Table S3.** GenBank accession numbers for male mitochondrial genomes used in RELAX

10 analyses.

Order	Taxon	Accession
Cardiida	*Limecola balthica*	MN528029
	Scrobicularia plana	MN528027
Mytilida	*Arcualata senhousia*	GU001954
	Geukensia demissa	MN449488
	Mytilus californianus	JX486123

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	Mytilus edulis	AY823623	
	Mytilus galloprovincialis	FJ890850	
	Mytilus trossulus	GQ438250	
	Perumytilus purpuratus	MH330330	
	Semimytilus algosus	MT026713	
Unionida	*Anodonta anatina*	KF030963	
	Arconaia lanceolata	KJ775864	
	Chamberlainia hainesiana	MK994771	
	Cumberlandia monodonta	KU873124	
	Echyridella menziesii	KU873122	
	Lampsilis powelli	MF326972	
	Lampsilis siliquoidea	MF326974	
	Margaritifera margaritifera	MK421959	
	Microcondylaea bonellii	MK994773	
	Monodontina vondembuschiana	MK994775	
	Pilsbryoconcha exilis	MK994777	
	Potamida littoralis	KT247375	
	Potamilus alatus	KU559010	
	Pyganodon grandis	FJ809755	
	Quadrula quadrula	FJ809751	
	Sinanodonta woodiana	MH349356	
	Solenaia carinata	KC848655	
	Solenaia oleivora	KY007143	
	Unio crassus	KY290450	
	Unio delphinus	KT326918	
	Unio pictorum	MH349358	
	Unio tumidus	KY021075	
	Utterbackia peninsularis	HM856635	
	Venustaconcha ellipsiformis	FJ809752	
Venerida	*Meretrix larmarckii*	KP244452	
	Ruditapes philippinarum	AB065374	

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Table S4. GenBank accession numbers for female (F) and male (M) mitochondrial genomes used in the divergence time analysis of Mytilida.

Order	Taxon	Accession F	Accession M

Mytilida	*Arcualata senhousia*	GU001953	GU001954
	Geukensia demissa	MN449487	MN449488
	Mytilus californianus	JX486124	JX486123
	Mytilus edulis	MF407676	AY823623
	Mytilus galloprovincialis	FJ890849	FJ890850
	Mytilus trossulus	GU936625	GQ438250
	Perumytilus purpuratus	MH330333	MH330330
	Semimytilus algosus	MT026712	MT026713

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Table S5. Omega (ω) values for Cardiida+Unionida and Mytilida+Venerida based on alternative models generated by RELAX from a concatenated alignment of 12 male mitochondrial genes and the male mitochondrial gene *COX2*. Percentages represent the proportion of each alignment assigned to ω values. Both analyses strongly supported (p ≤ 0.0001) relaxed selection in Cardiida+Unionida.

Dataset	Group	ω_1	ω_2	ω_3
Concatenated	Cardiida+Unionida	0.0008 (66.69%)	0.349 (27.07%)	2.558 (6.24%)
	Mytilida+Venerida	$8.66e^{-8}$ (66.69%)	0.089 (27.07%)	8.636 (6.24%)
COX2	Cardiida+Unionida	0.015 (82.88%)	0.618 (16.77%)	11932049 (0.3%)
	Mytilida+Venerida	0.003 (82.88%)	0.506 (16.77%)	9999999171 (0.3%)

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Table S6. Empirical average site concordance factor and mean and standard deviation (SD) for simulated average site concordance factors (sCFs) for modeling a single origination of doubly uniparental inheritance. Statistics for 12 genes concatenated and each gene, as well as associated p-values for one-tailed tests are reported. Bolded values were determined to be statically significant.

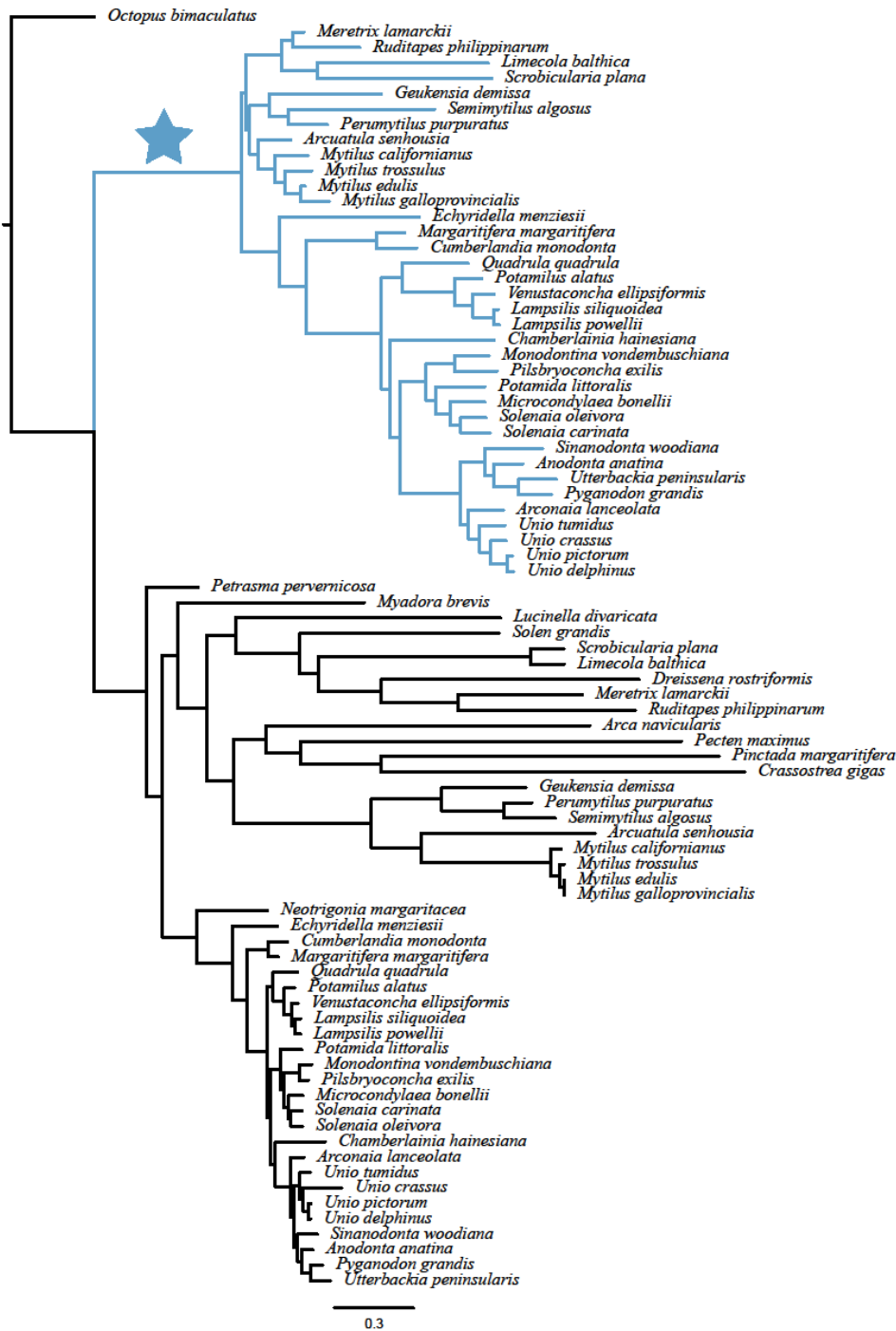| Dataset	Empirical	Mean	SD	p
Concatenated	**32.06**	**25.72**	**1.59**	**< 0.001**
ATP6	27.51	25.66	4.79	0.70
COX1	37.84	29.91	4.55	0.08
COX2	38.18	28.23	5.53	0.07
COX3	43.97	40.01	5.64	0.48

CYTB	29.21	24.97	4.09	0.30
ND1	33.64	24.76	4.09	0.03
ND2	23.91	24.20	4.37	1
ND3	31.36	24.50	6.09	0.26
ND4	42.48	23.02	3.07	0.28
ND4L	36.92	31.83	8.15	0.53
ND5	32.07	29.35	3.64	0.45
ND6	20.79	18.49	5.22	0.66

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29   **Figure S1.** Constraint tree enforcing monophyly of male mitochondria used for phylogenetic

30   modeling. Blue branches correspond the male mitochondrial lineages, and the blue star

31   corresponds to the branch for origination testing using site concordance factors.
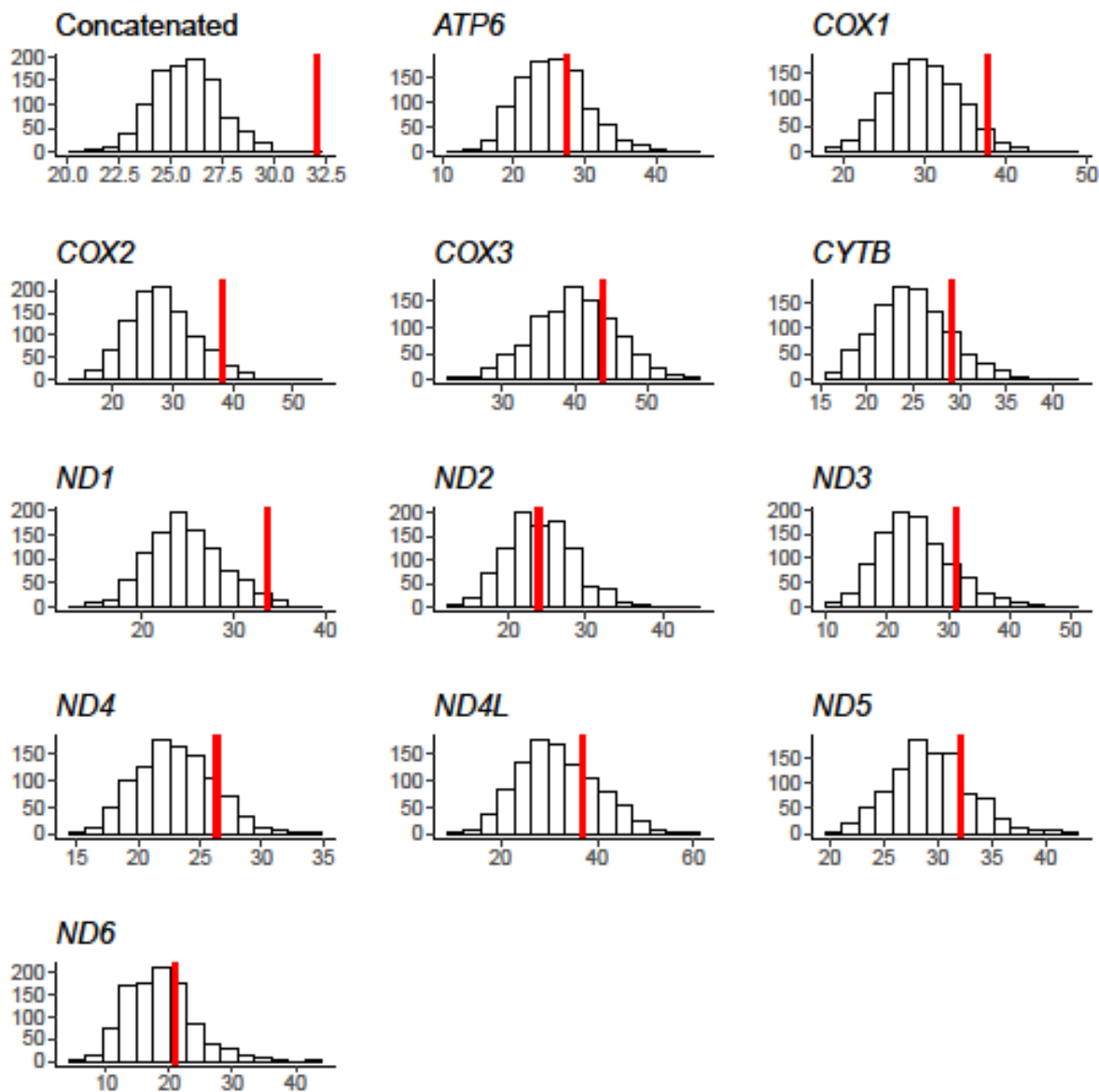
8
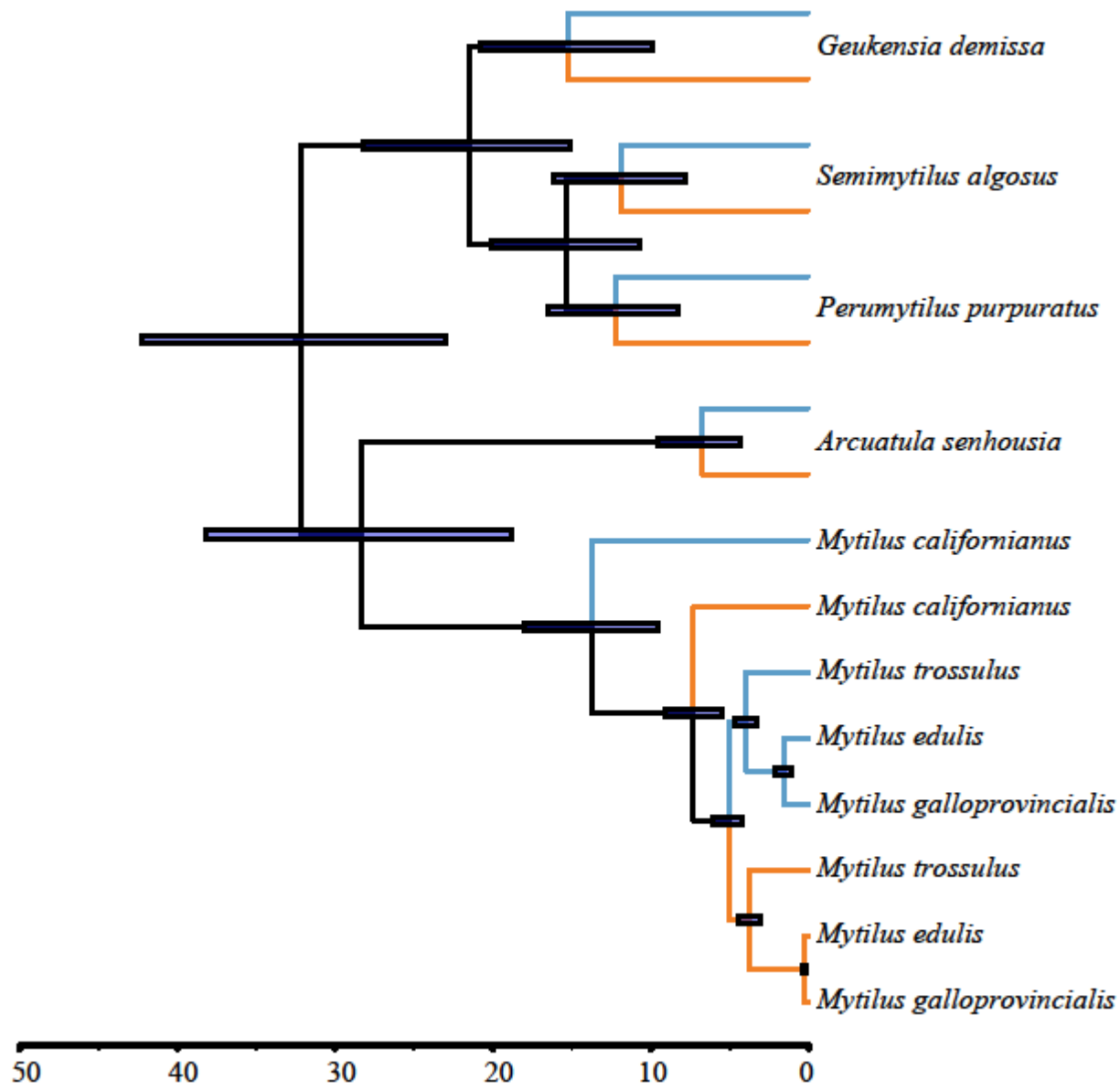
Figure S2. Maximum clade credible tree generated from the calibrated phylogenetic analysis of Mytilida in BEAST. Divergence time is scaled to million years before present and node bars represent the 95% CI. Branches representing female and male specific mitochondria are highlighted orange and blue, respectively.

Figure S3. Null distribution and observed site concordance factors used to assess support for a single origination of male mitochondrial DNA using a concatenated alignment of 12 gene and each gene independently. In each plot, white bars represent the null distribution based on 1000 simulated amino acid datasets and the red line represents the observed value based on empirical data.

Supplemental Files

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45 **File S1.** Concatenated amino acid alignment of 12 mitochondrial genes used in this study in fasta
46 format.

47 **File S2.** Constraint tree enforcing monophyly of male mitochondria used for phylogenetic
48 modeling.

49 **File S3.** Concatenated nucleotide alignment of 12 male mitochondrial genes used in the RELAX
50 analysis in fasta format.

51 **File S4.** Nucleotide alignment of the male mitochondrial gene *COX2* used in the RELAX
52 analysis in fasta format.

53 **File S5.** Concatenated nucleotide alignment of 12 mitochondrial genes for all Mytilida used in
54 the BEAST analysis in nexus format.

55 **File S6.** Phylogram generated from a concatenated amino acid alignment of 12 genes in IQ-
56 TREE. Node labels are support values from 10^3 ultrafast bootstrap replicates.

57