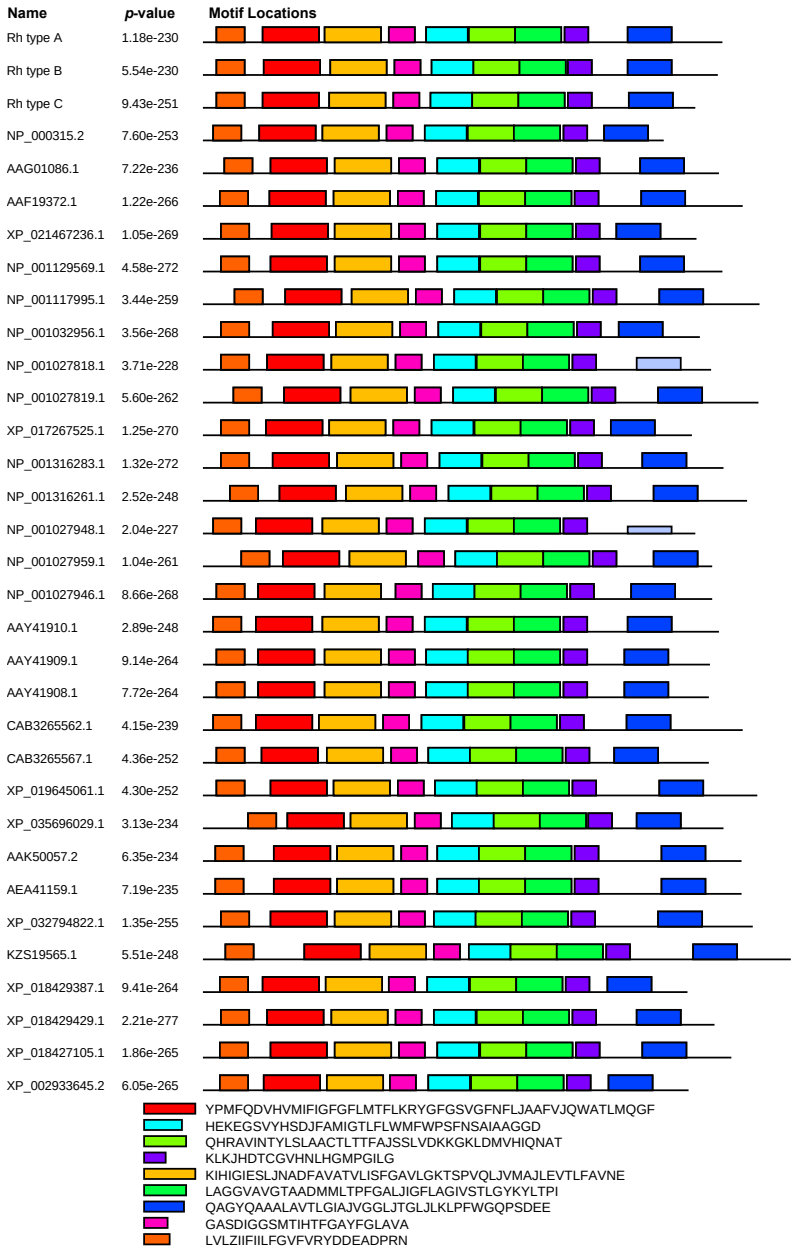
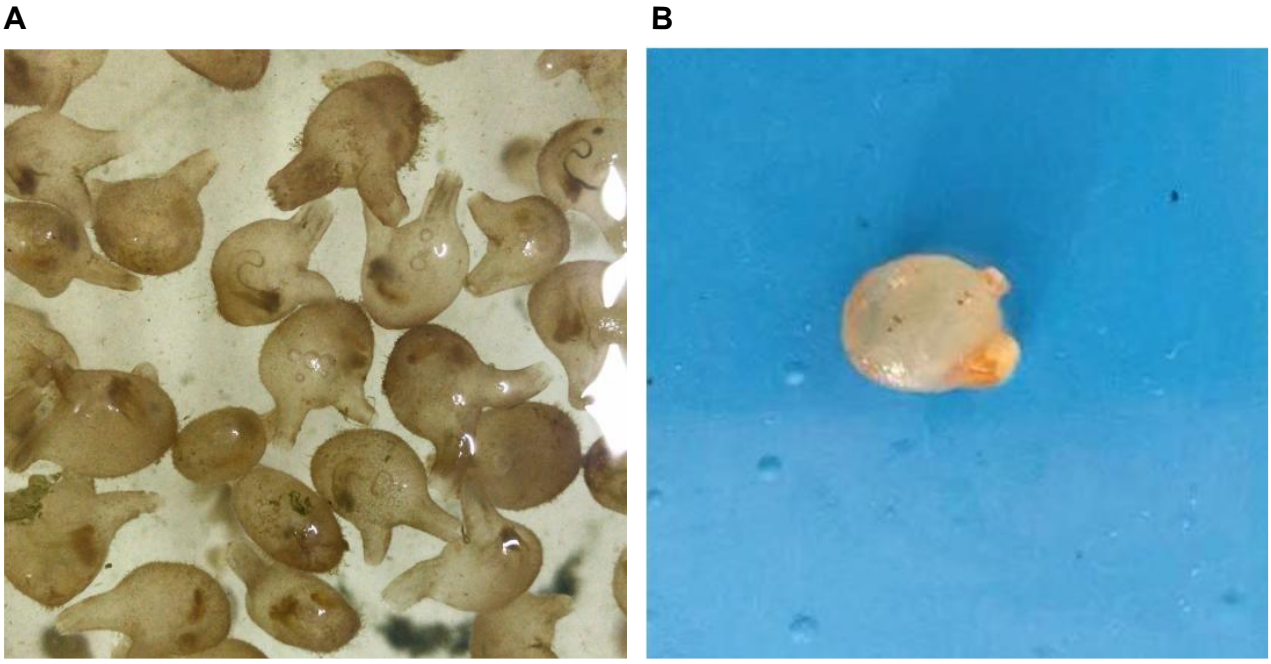
Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Figures



Supplementary Figure 1. Full block diagram of nine conserved motifs of complete 37 Rh protein sequences. Each color represents a different motif. The *p*-value represents the combined match *p*-value.



Supplementary Figure 2. (A) Healthy *Molgula manhattensis* at 0 h (control); **(B)** Dead *M. manhattensis* at 72 h in the acute ammonia toxicity test.

1.2 Supplementary Tables

Supplementary Table S1. List of selected Rh proteins, species names, and accession numbers used for analyses in the paper.

Accession number	Species NCBI blast name	Common name	Protein name
NP_001027948.1	Tunicates	Vase tunicate	*Ciona intestinalis* Rh type A
NP_001027959.1	Tunicates	Vase tunicate	*Ciona intestinalis* Rh type B
NP_001027946.1	Tunicates	Vase tunicate	*Ciona intestinalis* Rh type C
AAY41910.1	Tunicates	Pacific transparent sea squirt	*Ciona savignyi* Rh type A
AAY41909.1	Tunicates	Pacific transparent sea squirt	*Ciona savignyi* Rh type B
AAY41908.1	Tunicates	Pacific transparent sea squirt	*Ciona savignyi* Rh type C
CAB3265562.1	Tunicates	White warty sea squirt	*Phallusia mammillata* Rh type A
CAB3265567.1	Tunicates	White warty sea squirt	*Phallusia mammillata* Rh type B
XP_035696029.1	Lancelets	Florida lancelet	*Branchiostoma floridae* Rh type A
XP_019645061.1	Lancelets	Belcher's lancelet	*Branchiostoma belcheri* Rh type A
XP_019630876.1	Lancelets	Belcher's lancelet	*Branchiostoma belcheri* Rh type C
XP_021467236.1	Bony fishes	Rainbow trout	*Oncorhynchus mykiss* Rh type A
NP_001129569.1	Bony fishes	Rainbow trout	*Oncorhynchus mykiss* Rh type B
NP_001117995.1	Bony fishes	Rainbow trout	*Oncorhynchus mykiss* Rh type C
NP_001032956.1	Bony fishes	Pufferfish	*Takifugu rubripes* Rh type A
NP_001027818.1	Bony fishes	Pufferfish	*Takifugu rubripes* Rh type B
NP_001027819.1	Bony fishes	Pufferfish	*Takifugu rubripes* Rh type C
XP_017267525.1	Bony fishes	Mangrove killfish	*Kryptolebias marmoratus* Rh type A
NP_001316283.1	Bony fishes	Mangrove killfish	*Kryptolebias marmoratus* Rh type B
NP_001316261.1	Bony fishes	Mangrove killfish	*Kryptolebias marmoratus* Rh type C
XP_018429387.1	Anurans	Mountain slow frog	*Nanorana parkeri* Rh type A
XP_018429429.1	Anurans	Mountain slow frog	*Nanorana parkeri* Rh type B
XP_018427105.1	Anurans	Mountain slow frog	*Nanorana parkeri* Rh type C
XP_002933645.2	Anurans	Tropical clawed frog	*Xenopus tropicalis* Rh type A
AAU89493.1	Anurans	Tropical clawed frog	*Xenopus tropicalis* Rh type B
AAQ02688.1	Anurans	Tropical clawed frog	*Xenopus tropicalis* Rh type C
XP_032794822.1	Crustaceans	Water flea	*Daphnia magna* Rh type A
XP_032789004.1	Crustacean	Water flea	*Daphnia magna* Rh type B
KZS19565.1	Crustaceans	Water flea	*Daphnia magna* Rh type C
AAK50057.2	Crustaceans	Green crab	*Carcinus maenas* Rh-like
AEA41159.1	Crustaceans	Dungeness crab	*Metacarcinus magister* Rh-like
NP_000315.2	Primates	Human	*Homo sapiens* Rh type A
AAG01086.1	Primates	Human	*Homo sapiens* Rh type B
AAF19372.1	Primates	Human	*Homo sapiens* Rh type C
AAV40852.1	Flies	Fruit fly	*Drosophila pseudoobscura* Rh-like
AAF97864.1	Nematodes	Roundworm	*Caenorhabditis elegans* Rh-like
NP_414985.1	Enterobacteria	*N.A.*	*Escherichia coli* AmtB

Supplementary Table S2. Predicted NH_3 ligand-binding residues of Rh proteins in *M. manhattensis.* PDB:1U7G deposits the crystal structure of ammonia channel AmtB from *E. Coli*.

Protein name	Ligand name	Binding residues	Template
Rh type A	NH_3	F68 W223 H335	PDB: 1U7G_A
Rh type B	NH_3	M64 F69 A130 H182 W228	PDB: 1U7G_A
Rh type C	NH_3	F68 H181 W227 F230	PDB: 1U7G_A

Supplementary Table S3A. Identity percentages between deduced amino acid sequence of Rh type A of *M. manhattensis* and Rh proteins from other species and *E. coli* AmtB. Each identity was retrieved from the Percent Identity Matrix created by Clustal Omega 2.1.

Species NCBI blast name	Protein name	*M. manhattensis* Rh type A identity (%)
Tunicates	*C. savignyi* Rh type A	61.19
	C. intestinalis Rh type A	60.48
	P. mammillata Rh type A	59.60
Lancelets	*B. floridae* Rh type A	49.88
	B. belcheri Rh type A	49.56
Bony fishes	*T. rubripes* Rh type A	49.18
	O. mykiss Rh type A	49.18
	K. marmoratus Rh type A	48.82
Anurans	*X. tropicalis* Rh type A	47.74
	N. parkeri Rh type A	48.57
Crustaceans	*C. maenas* Rh-like	43.76
	D. magna Rh type A	48.12
	M. magister Rh-like	44.22
Primates	*H. sapiens* Rh type A	46.19
Flies	*D. pseudoobscura* Rh-like	44.03
Nematodes	*C. elegans* Rh-like	39.12
Enterobacteria	*E. coli* AmtB	22.57

Supplementary Table S3B. Identity percentages between deduced amino acid sequence of Rh type B of *M. manhattensis* and Rh proteins from other species and *E. coli* AmtB. Each identity was retrieved from the Percent Identity Matrix created by Clustal Omega 2.1.

Species NCBI blast name	Protein name	*M. manhattensis* Rh type B identity (%)
Tunicates	*C. intestinalis* Rh type B	61.79
	C. savignyi Rh type B	59.50
	P. mammillata Rh type B	59.14
Bony fishes	*O. mykiss* Rh type B	40.90
	K. marmoratus Rh type B	40.00
	T. rubripes Rh type B	37.90
Anurans	*N. parkeri* Rh type B	44.44
	X. tropicalis Rh type B	42.66
Crustaceans	*C. maenas* Rh-like	42.89
	M. magister Rh-like	42.44
Primates	*H. sapiens* Rh type B	42.89
Flies	*D. pseudoobscura* Rh-like	41.72
Nematodes	*C. elegans* Rh-like	33.86
Enterobacteria	*E. coli* AmtB	20.92

Supplementary Table S3C. Identity percentages between deduced amino acid sequence of Rh type C of *M. manhattensis* and Rh proteins from other species and *E. coli* AmtB. Each identity was retrieved from the Percent Identity Matrix created by Clustal Omega 2.1.

Species NCBI blast name	Protein name	*M. manhattensis* Rh type C identity (%)
Tunicates	*C. intestinalis* Rh type C	69.75
	C. savignyi Rh type C	68.52
Lancelets	*B. belcheri* Rh type C	57.89
Bony fishes	*K. marmoratus* Rh type C	44.52
	O. mykiss Rh type C	44.34
	T. rubripes Rh type C	42.26
Anurans	*X. tropicalis* Rh type C	47.66
	N. parkeri Rh type C	46.28
Crustaceans	*D. magna* Rh type C	49.88
	C. maenas Rh-like	47.56
	M. magister Rh-like	47.80
Primates	*H. sapiens* Rh type C	46.28
Flies	*D. pseudoobscura* Rh-like	45.09
Nematodes	*C. elegans* Rh-like	39.44
Enterobacteria	*E. coli* AmtB	22.75

Supplementary Table S4. Gene ontology (GO) enrichment analyses of Rh genes of *M. manhattensis*. MF: molecular function, BP: biological process, CC: cellular component. PPV (positive predictive value) is an estimate for the reliability of the predicted GO class being correct or approximately correct. It is normalized between 0 and 1.

GO ID	Ontology	Description	Estimated PPV	Query gene
GO:0015696	BP	ammonium transport	0.78	Rh type A, Rh type B, Rh type C
GO:0098662	BP	inorganic cation transmembrane transport	0.62	Rh type A, Rh type B, Rh type C
GO:0015670	BP	carbon dioxide transport	0.58	Rh type C
GO:0008519	MF	ammonium transmembrane transporter activity	0.79	Rh type A, Rh type B, Rh type C
GO:0030506	MF	ankyrin binding	0.56	Rh type C
GO:0022842	MF	narrow pore channel activity	0.56	Rh type C
GO:0005887	CC	integral component of plasma membrane	0.66	Rh type A, Rh type B, Rh type C