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A. aegypti Rh50 GP2             --------------------MSSLGSSVCGYVLLLLVQIVLIIVFGFFTNYSKELLPINTI---------NGTTV
M. sexta Rh-like                ---------------------MVLKYSAMKYSPVLVVQLILMVLFFIFVRYGDEKE-------------------
T. rubripes Rhcg2*              ----MGNFFGRQR---------NAYVRVSLPAVCFVWQIAMIILFGVFIRYNEESDAHWVE-------HKKTNNI
H. sapiens RhCG*                ----------MAW---------NTNLRWRLPLTCLLLQVIMVILFGVFVRYDFEADAHWWS-------ERTHKNL
C. intestinalis_Rh-like         MCLRCPIFPYMVRYILKHLFFAHSNTRGKATALLLVAQAILLVLFGLFVDYDVAAGPRNNS-------LTTHTTL
C. gigas Rh-like                ---------------------MATVKSMRLIIPLAIVQIVFLIIFGIKIEYHETAKP--------------VDTV
M. magister_Rh-like             ----------------------MKLSHVHGFLALG-VQVAFFILFCAFVRYHPDAHARYHPLVNGSELENELDHY
C. elegans Rhr-2                --------------------MWSVLHRRQFAIIAGLMQTVFIVLFAKYVKYIDP--LD--------------DSR
C. elegans Rhr-1*               --------------------MRSPLHQNQLTLILGLFQVVFLVIFALYGSYDASALPS--------------ETK


A. aegypti Rh50 GP2             EEMEEETPLAKYPHFQDIHVMIFIGFGFLMTFLKRYGFSATGLNLLVAALAIQWAIVMRGCYVMEGGK---IPLS
M. sexta Rh-like                ---------AHVRGFEGTHVMIFVGFGFLMTFLKKYCYSALGFNWLLAALVIQWALLCQSFFTMKDNT---IYIT
T. rubripes Rhcg2*              TSDIENDFYYRYPSFQDVHVMIFVGFGFLMTFLKRYSFGGVGFNFLIAAFGIQWALLMQGWFHFLDHSTGKIYIG
H. sapiens RhCG*                S-DMENEFYYRYPSFQDVHVMVFVGFGFLMTFLQRYGFSAVGFNFLLAAFGIQWALLMQGWFHFLQDRY--IVVG
C. intestinalis_Rh-like         A--------HYYPIYQDVHVMMLIGFGFLMTFLKRHGFGSVGFNFLLTCYVIEWSTLVNGWFGMIGSNEGRILID
C. gigas Rh-like                K---PSIVGGLYPSFQDVHVMIYIGFGFLMTFLKRYGYSAVSINLLLASFCAQWAIIVR---AAISGN---WTVG
M. magister_Rh-like             KSDDPWIHSKSYPMFQDVHVMIFIGFGFLMMFLKRYGLSAVGLNFMIAALCLQWAILVNGFFHLKKGT---IVVD
C. elegans Rhr-2                RVYSGT----DYPLFQDVHLMIFVGFGFLMAFLKRYGFSAVSVNLLLSAFVIQFAMLLRGFMTVAFQETGLFSIG
C. elegans Rhr-1*               NVEEAARMTNLYPLFQDTHVMIFIGFGFLMTFLKRYGFSAVSINMLLAVFTIQWGIIVRGMASAHHGFK--FTIS


A. aegypti Rh50 GP2             LDNLIGADIAAAAVLISMGALLGRTTPMQLLVMTIFEIAIFAANEFLQ----VDLMRIADVGGSITVHAFGAYFG
M. sexta Rh-like                KKSLLEADIMSATVLITFGALLGVATGLQLLFIAIVETVIACLNLWLV----TDVFKAADVGGSIAIHTFGAYFG
T. rubripes Rhcg2*              VENLINADFCVAGSLIAYGALLGKVSPVQLLVLTLFGVTLFAVEEYI----ILHLLHCRDAGGSMVIHAFGGYYG
H. sapiens RhCG*                VENLINADFCVASVCVAFGAVLGKVSPIQLLIMTFFQVTLFAVNEFI----LLNLLKVKDAGGSMTIHTFGAYFG
C. intestinalis_Rh-like         IKSLLEADFAVAAVLISFGAILGVASPVQLIVMATIEVVCYNVSIYVGIHPLLTYIQVTDVGGSMFIHAFGAYFG
C. gigas Rh-like                VMEMLTADFAAATILISFGAVLGKTGPLQLLIMACIEIVLAQVNEHIG----VHILHTADVGESMFIHAFGAYFG
M. magister_Rh-like             LNAMLGADFTAAAVLISFGVLLGKTTPTQLIVLALMEIPLFAVNEVIG----RSYFGAIDMGDSMFVHVFGAYFG
C. elegans Rhr-2                IPEMISAESSCAAVLITMGVLLGRLTPVQFLLLAFFETGINVLVEHYV----FNYLHVNDSGRSLSVHTFGAYFG
C. elegans Rhr-1*               LEQLLTADFAAAVILISMGAMLGKLSPSQYVIMAFFETPVALIVEHIC----VHNLQINDVGGSIIVHAFGAYFG
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A. aegypti Rh50 GP2             LAVSFVLRPNKENCKT----G------PMECSSYSSDITAMIGTVFLWIFWPSFNSALVDG-AEQERAIINTYLS
M. sexta Rh-like                LGVSMALRFRREDTTTPMNNGNPAPVVDLNGASYTSDVTAMIGSIFLWIYWPSFNSGLTNSDAEYQRAVINTYLS
T. rubripes Rhcg2*              LGISWVLYRPNLHQSK-----------RLHGSVYHSDVFAMIGTLFLWMFWPSFNSAITDHGDGQHRAAINTYLG
H. sapiens RhCG*                LTVTRILYRRNLEQSK-----------ERQNSVYQSDLFAMIGTLFLWMYWPSFNSAISYRGDSQHRAAINTYCS
C. intestinalis_Rh-like         LAVARVLYKKSQTLSK-----------N-EGSEYHSDIFAMIGTLFLWLYWPSFNAGPAS-GTERHRAVINTVLS
C. gigas Rh-like                LAVARVLYSEDIERAS-----------GKEGAVYHSDLFSMIGTVFLWLYWPSFNGGAVE-GDQQHRAFINTYLS
M. magister_Rh-like             LAVSLMLHRRDAS--T-----------EKEGSSYQSDLFAMIGTVFLWLYWPSFNAGAAP-GDDQHRGIINTYIS
C. elegans Rhr-2                LAAACVGHKKNVMEMD------------EHGGIHHSDLFSMIGTLLLWVFFPSFNAAIQEPEDARHRAIMNTYLA
C. elegans Rhr-1*               LACAKGFGKKEQRGHT------------NEGSTYHTDIFAMIGAIFLWIYWPSFNAAVAATDDARQRAVANTFLS
                                                                                     Ω   Δ

A. aegypti Rh50 GP2             LAGATVTTYIMSVMVS-HEKKFDMVHVQNSTLAGGVAVGSICNLLIHPFGAILVGVIAGVISVLGYRFLTPAMLS
M. sexta Rh-like                LAAATVTAFVMSSAVNKHPGRFDMVHIQNSTLAGGVAVGSVCNMHIGAGGAMAIGIGSGLLSVIGYRYLTP-RLT
T. rubripes Rhcg2*              LASCVLTTVALSSMHD-KRGRLDMVHIQNATLAGGVAMGTAAEFMISPYGALIVGFCCGIISTFGYLYVTPFLEK
H. sapiens RhCG*                LAACVLTSVAISSALH-KKGKLDMVHIQNATLAGGVAVGTAAEMMLMPYGALIIGFVCGIISTLGFVYLTPFLES
C. intestinalis_Rh-like         LSACTVVTFALSAVTD-KKNKLDMVHIQNATLAGGVAIGASADLIVQPFGALLVGSTAATVSTLGFRYLQPVLQR
C. gigas Rh-like                LLACTVTTFIVSSLVD-KRGKFDMVHIQNATLAGGVAVGTSAHMPIQPWGAMLLGTTAGIISVLGYKYLTPLMAS
M. magister_Rh-like             LAACCLTTFALSTLLD-KHKKFDMVHVQNSTLAGGVAVGTAADLMIHPWGAALIGILAALVSVCGYMFLTPLLAS
C. elegans Rhr-2                MASGTVTTFMISSCVD-TLGRFNMIHIQSSTLAGGVAIGSSANAVLHPYHAVIVGVIAALLSVIGHAWISPRLER
C. elegans Rhr-1*               LCACTMTTFLVSQAVD-KHKRFDMVHIANSTLAGGVAIGTTANVVLEPYHAMIIGVIAGAVSVIGYKYITPFLSE
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A. aegypti Rh50 GP2             KLRISDTCGVNNLHGMPALLSAVFSGIYASVATSEAYGNSLTSIFPAMKPNTTLTEEDMHEMVIGGYGRSAAM--
M. sexta Rh-like                KIGILDTCGVNNLHGMPGVFSGLLSVLFAGLASAQDYGTELSNVFEAVGPD----------------GRSAGS--
T. rubripes Rhcg2*              YLKLQDTCGVHNLHAVPGMLGGFVGAIVAAAATESVYSK--------------EGLINTFNFEGKYADRSVGT--
H. sapiens RhCG*                RLHIQDTCGINNLHGIPGIIGGIVGAVTAASASLEVYGK--------------EGLVHSFDFQGFNGDWTART--
C. intestinalis_Rh-like         NIKLHDTCGVHNLHGMPGILGAVISAIVATIATKDAYQ-------------------DSYHELFKDASRTSSQ--
C. gigas Rh-like                KLRIHDTCGVNNLHGMPGILAAIGGAVAAAMSSKETWGDSLYSIFTAMEPTMMNTTMTTNAMTANVTEVMGRSAM
M. magister_Rh-like             RLRIHDTCGVHNLHGLPGILAAIIGAVAAGVASEASYGLSLYEIFPARAP--LEDSLELQKLQEVLPDLEGGSGI
C. elegans Rhr-2                TFHLFDTCGVHNLHGMPGILAGLLSIGFAYFYEPESYGKTLYHIYP-----------YWIGG-ELHGDRENVS--
C. elegans Rhr-1*               KLGIHDTCGVNNLHGMPGLIAGFASIAFLFIYDETRYPAQYDKIYP-----------GMARGEDRTRMFDEKT--
                                     $       £
                                                                                                      G

Aedes_aegypti_Rh50-GP2          ----QGAYQLLAILLTVVIAIMGGLCTGLVLKSPTMRQLEQDEHHKDDVCWETPVELET----------------
M. sexta Rh-like                ----QALYQFIALAVTIGLSLVGGFVTGLLSKMPVFGSLKDAERYDDQINWELP---------------------
T. rubripes Rhcg2*              ----QGGYQAAGTCVAVAFGLVGGAIVGFILKFPIWGDPADDNCFDDEAYWEVPEDEET--IP--PVLEYNNHMI
H. sapiens RhCG*                ----QGKFQIYGLLVTLAMALMGGIIVGLILRLPFWGQPSDENCFEDAVYWEMPEGDSTVYIPEDPTFKPSGPSV
C. intestinalis_Rh-like         ----NGGYQIAALVCVLCIALLSGTLTGFLLKLPVWDNLSAEELFEDEVFWDVSGS-------------------
C. gigas Rh-like                E---QGGYQMAALCLTLAIAIVGGVITGFILKIPFLDNPTGDLLFDDKDQWNVADEGF-----------PNSGEL
M. magister_Rh-like             SGTSQALYQLLALLVTLVIAIAGGIVTGIVLRLKPLALLKTEELYED-EKWWIMEAEE-----------DEGHKG
C. elegans Rhr-2                ----QAQYQALGLLTILVTAVIGGLLTGCILKIKVWNQVDDPDFPHGEMNYYAQSDVN---------FLSKYKHA
C. elegans Rhr-1*               ----QALNQLMAIGLVFLASTVSGYLTGLLLKLKIWDQVRDDEYYADGDYFETPGDYD---------FTSRIVTS


A. aegypti Rh50 GP2             TVTTSLCQ----------
M. sexta Rh-like                ------------------
T. rubripes Rhcg2*              HKHQDIAETNFSVEQS---
H. sapiens RhCG*                PSVPMVSPLPMASSVPLVP
C. intestinalis_Rh-like         ------------------
C. gigas Rh-like                PLNGKSGDGKSRDDTQM--
M. magister_Rh-like             SVTIPMADNGAR-------
C. elegans Rhr-2                QEQERLREREQMQEIY---
C. elegans Rhr-1*               VKQIEVAEYNPLSQKEV--
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Supplemental figure S1: Clustal W amino acid alignment (Thompson et al., 1994) of CeRhr-1 with other Rh-glycoproteins including vertebrate Rh-proteins of verified ammonia transport capability (indicated by *). Conserved ammonia-conducting residues are highlighted with a grey background. Symbol Δ indicates ammonia-conducting residues in the external vestibule; symbol Ω indicates ammonia-conducting residues in the pore entrance; symbol £ indicates ammonia-conducting residues in the pore center; symbol $ indicates ammonia-conducting residues in the internal vestibule (Khademi et al., 2004; Wu et al., 2010; Zidi-Yahiaoui et al., 2009). G indicates the predicted N-glycosylation-site in CeRhr-1 (green underlaid) and CeRhr-2 (red underlaid). Yellow underlaid are the predicted 12 transmembrane domains of CeRhr-1 (Ji et al., 2006). GenBank accession numbers are given in brackets behind the species name. *Aedes aegypti* Rh50-GP2 (AY926464), *Manduca sexta* Rh-like protein (ABI20766), *Homo sapiens* RhCG (AAH30965), *Takifugu rubripes* Rhcg2 (AB218982), *Ciona intestinalis* Rh type B glycoprotein (NP_001027959), *Crassostrea gigas* Rh type B-A (EKC21768), *Metacarcinus magister* Rh-like protein (AEA41159), *Caenorhabditis elegans* Rhr-1 (NM_072035), *Caenorhabditis elegans* Rhr-2 (NP_505961). Asterisks indicate transporters with verified ammonia transport capabilities (Marini et al., 2000; Nawata et al., 2010b).

Table S1. Primers employed in real-time PCR targeting actin, rhesus (Rh)-like ammonia transporter (CeRhr-1, CeRhr-2), vesicular H$^+$-ATPase (VHA; subunit A), Na$^+$/K^{+-}ATPase (NKA; α-subunit) from the nematode *Caenorhabditis elegans*

Primer	Nucleotide sequence (5’ → 3’)	Annealing Temp. (ºC)	Product size (bp)	GenBank Acc. #
Actin				
CeActin-F	ATCGTCCTCGACTCTGGAGAT	60		
CeActin-R	TCACGTCCAGCCAAGTCAAG	60	100	NM_073417
Rhr-1				
CeRhr-1 F	TTCTTGTCTGAGAAACTCGGA	60		
CeRhr-1 R	GATTGCCATAAGCTGGTTCAA	60	210	NM_072035
CeRhr-1 SpeI F	GTATAACTAGTCAAAAAATGAG TCGCCTCT	60		
CeRhr-1 SmaI R	GTATACCCGGGTTATTAGACCTCT TTTTGGCTGAGTG	60	1422	NM_072035
Rhr-2				
CeRhr-2 F	ACAGTGGTAGATCTCTTTCC	60		
CeRhr-2 R	GCTGCATCTTCTGGTTCTTG	60	205	NM_073560
Na$^+$/K$^+$-ATPase				
CeNKA F	GACCTCGGAACTGACATGGT	60		
CeNKA R	CCCATAAGCAAGGGAGATCA	60	135	U18546
H$^+$-ATPase				
CeVHA F	CTTGAAGGCTCGTGAAGACC	60		
CeVHA R	ACGACTTCCTTTTCGAGCAA	60	150	NM_068639.5