

Additional file 5: Alignment of predicted amino acid sequences of rubber tree aquaporins with structure determined Spinach SoPIP2;1.

SoPIP2;1	-----MSKEVSEEA-----QAHQH GKDYVDPPPAPFFDLGE-----	31 (60)
HbPIP1;1	-----MEGKEEDVRLGANKYRETQPIGTSASQSDDKDYTEPPAAPLFEPTE-----	46 (60)
HbPIP1;2	-----MEGKEEDVRLGANKYRETQPIGTAAQSLDDKDYTEPPAAPLFEPTE-----	46 (60)
HbPIP1;3	-----MEGKEEDVRLGANKFTERQPIGTSASQ--DKDYKEPPAPLFEPGE-----	44 (60)
HbPIP1;4	-----MEGKEEDVRLGANKFTERQPIGTSAQT--DKDYKEPPAPLFEPGE-----	44 (60)
HbPIP1;5	-----MQPLGTSAQT--DKDYRDPLPAPLFEPVE-----	27 (60)
HbPIP2;1	-----MAKDVEVGG---QGGEFQAKDYNDPPPAPLIDAE-----	32 (60)
HbPIP2;2	-----MAKDIEVGG---DGSEFRAKDYHDPAPSPLIDAE-----	32 (60)
HbPIP2;3	-----MAKDVEVAE---NPGEFSAKDYHDPAPPLIDVEE-----	32 (60)
HbPIP2;4	-----MAKDVEVAE---NPGEFSAKDYHDPAPPLIDVEE-----	32 (60)
HbPIP2;5	-----MVKDVTEQ-----GSFPAKDYHDPAPPLIDAVE-----	29 (60)
HbPIP2;6	-----MVKDITEQ-----GSFSAKDYHDPAPPLIDAVE-----	29 (60)
HbPIP2;7	-----MAKEVSEE---RQP---GKDYVDPPPAPPLIDMAE-----	28 (60)
HbPIP2;8	-----MAKEVSEE---TQPT--HGKDYVDPPPAPPLIDVAE-----	30 (60)
HbPIP2;9	-----MAKEVTEE---AGEASQQERDYVEPPAPPLFDPEE-----	32 (60)
HbPIP2;10	-----MVKEMGEEGSFEHEIHGQH GKDYVDPPPAPPLDMEE-----	36 (60)
HbTIP1;1	-----MPIRNI AVGHPQE-----	13 (60)
HbTIP1;2	-----MPIRNI AVGHPQE-----	13 (60)
HbTIP1;3	-----MPINRVAIGLPRED-----	14 (60)
HbTIP1;4	-----MPINRIEVLPRQD-----	14 (60)
HbTIP1;5	-----MPITRI AVGNPGE-----	13 (60)
HbTIP1;6	-----MPVARI AVGNPGE-----	13 (60)
HbTIP1;7	-----MPITSIAIGSPA-----	13 (60)
HbTIP1;8	-----MAITSIAIGSPA-----	13 (60)
HbTIP2;1	-----MARIAFGRFDD-----	11 (60)
HbTIP2;2	-----MARIAFGRFDD-----	11 (60)
HbTIP2;3	-----MPMIAVGSIGD-----	11 (60)
HbTIP2;4	-----MARMAFGSFGD-----	11 (60)
HbTIP3;1	-----MPRRYALGRAEE-----	12 (60)
HbTIP3;2	-----MSTRRYAFGNAEE-----	13 (60)
HbTIP4;1	-----MAKIALGSQRE-----	11 (60)
HbTIP5;1	-----MAPSSLNARFKQ-----	12 (60)
HbTIP5;2	-----MAPTSLNDRFKQ-----	12 (60)
HbNIP1;1	-----MADEISGTNGKHGVVLDVEGDNPCYSPPPCNDRPPCASKTKEDSI-----	45 (60)
HbNIP1;2	-----MAQAEISVANGRHGVVLDVKDDNPCNPAPAGNKPPPCASKTKEDSI-----	46 (60)
HbNIP2;1	-----MATIDPNPNNSASIGD---LVSVENPKSQLVESFG-----	33 (60)
HbNIP3;1	-----MASPNSITSVVSPKQLP---TKYSVAEAKASRSREWFLT-----	38 (60)
HbNIP4;1	-----MSGADDHE---IRDVEEG---QQEDSSD---SQNN SK-----	28 (60)
HbNIP4;2	-----MATAHADII EEEEE---VSKIEQG---LPPSTTADASANN TVGPCL-----	41 (60)
HbNIP5;1	-----MPESEAGTPTV--SAPATPGTPGGPLFSSLRVDSLSDYDRKSMR-CKCLPV	48 (60)
HbNIP6;1	-----MDNNNEEVPSAPSTPATPGTPGAPLFGGFRAERSG TNRKSL LGCKCF SV	50 (60)
HbNIP7;1	-----MKMKQLLEDQLPYPDISNNSSNGLSRDCPEMGSNAMSIDGVFAKYSVSRCL	53 (60)
HbXIP1;1	-----MDLVATQGDNNHQPF SKSVENCDAINDFKGTSKPKTSFLVFIGAHE-----	46 (60)
HbXIP1;2	-----MDSHNT-----KGSQYPMMTFLYRIGAYE-----	24 (60)
HbXIP1;3	-----MDLDLVL SHDAGNQAFPNQLDGQKNKINDSLES PKKTF LSCIGVHE-----	46 (60)
HbXIP1;4	-----MDLDLVL SHDAGNQAFPNQLDGQDKNIKSGLES PKKTF LSCIVVHE-----	46 (60)
HbXIP2;1	MADNASRVVEDEENG YGGRKVQPFAS TPRPDMDKTEGKKHHPTT LSRILGFED-----	53 (60)
HbXIP3;1	-----M-----	1 (60)
HbSIP1;1	-----	
HbSIP1;2	-----	
HbSIP1;3	-----	
HbSIP2;1	-----	

TM1

SoPIP2_1	-----LKLWSFWRAAIAEFITATLLFLYITVATVIGHSKETVV-----	68 (120)
HbPIP1;1	-----LTSWSFYRAGIAEFITATFLFLYISVLTVMGVVKAPTK-----	83 (120)
HbPIP1;2	-----LTSWSFYRAGIAEFITATFLFLYISILTVMGVVKAPTK-----	83 (120)
HbPIP1;3	-----LCSWSFYRAGIAEFITATFLFLYITVLTVMGFSKSTNK-----	81 (120)
HbPIP1;4	-----LSSWSFYRAGIAEFITATFLFLYITVLTVMGFSKPNNK-----	81 (120)
HbPIP1;5	-----LKSWSFWRAGIPEFFATFFFLYVTVLTVMCFSRSPNK-----	64 (120)
HbPIP2;1	-----FTQWSFYRAIIAEFIATLLFLYITVLTVIGYKSQTDPAKNAD	74 (120)
HbPIP2;2	-----LTKWSFYRAIIAEFIATLLFLYITVLTVIGYKSQTDPAKNAD	74 (120)
HbPIP2;3	-----LGKWSFYRALIAEFITATLLFLYITVLTVIGYKSQTDPAKNAD	74 (120)
HbPIP2;4	-----LGKWSLYRALIAEFITATLLFLYITVLTVIGYKSQTDPLKNAD	74 (120)
HbPIP2;5	-----LTKWSFYRALIAEFITATLLFLYITVLTVIGYKSQTDPAKNAD	71 (120)
HbPIP2;6	-----LTKWSFYRALIAEFITATLLFLYITVLTVIGYKSQTDPAKTHD	71 (120)
HbPIP2;7	-----IKLWSFYRALIAEFITATLLFLYITVATVIGYKKQADP-----	65 (120)
HbPIP2;8	-----LKLWSFYRALIAEFITATLLFLYITVATVIGYKKQADP-----	67 (120)
HbPIP2;9	-----LGLWSFYRAIIAEFIATLLFLYITVATVIGYKKQADP-----	69 (120)
HbPIP2;10	-----LRRWSFYRAL-AEFVATLLFLHVSATVIGYKSQADP-----	73 (120)
HbTIP1;1	-----ATHPDALKAALAEFISTLIFVFAGEGSGMAFSKLTNNG----	51 (120)
HbTIP1;2	-----ATHPDALRAALAEFISTLIFVFAGEGSGMAFSKLTNDNA----	51 (120)
HbTIP1;3	-----VVHPGALKAALAEFISTAI FVFAGQGSGMAFSKLTNDNA----	52 (120)
HbTIP1;4	-----VTHPSALKAALAEFISTLIFVFAEEGSGMAFSRLTNDNA----	52 (120)
HbTIP1;5	-----ASQPDALRAALAEFFSMIIFVFAGEGSGMAFNKLTNDNG----	51 (120)
HbTIP1;6	-----ASQPDALRAALAEFFSMIIFVFAGEGSGMAFSKLTNNG----	51 (120)
HbTIP1;7	-----ASQPDALKAALAEFISMLIFVFAGEGSGMAFNKLTNDNG----	51 (120)
HbTIP1;8	-----ASQPDALKAALAEFISMLIFVFAGEGSGMAFNKLTNDNG----	51 (120)
HbTIP2;1	-----SFSLGSFKAYLAEFISTLLFVFAGVGSIAIYNKLTNDNA----	49 (120)
HbTIP2;2	-----SFSLGSFKAYLAEFISTLLFVFAGVGSIAIYNKLTNDNA----	49 (120)
HbTIP2;3	-----SFSIGSIKAYLSEFIATLLFVFAGVGSIAIYSKLTADA----	49 (120)
HbTIP2;4	-----FFSIGSIKASLSEFIATLLFVFAGVGSIAIYSKLTADA----	49 (120)
HbTIP3;1	-----ATNPDSMRAALAEFVSTLIFVFAGEGSLALDKLYRET-----	50 (120)
HbTIP3;2	-----ATHPDMSKAALAEFVSTLIFVFAGEGSLALDKLYRET-----	51 (120)
HbTIP4;1	-----ATQPDICALIVEFITTTFFVFVGVGAAMAADKLVGG-----	48 (120)
HbTIP5;1	-----SVTPDALRSYLAEFISTFFYVFVVGVSAMAAARKL--MT----	48 (120)
HbTIP5;2	-----SVTPDALRSYLAEFISTIFYVVFVVGVSAMASDTEKLMP----	50 (120)
HbNIP1;1	-----LSISVPFIQKLI AEVVGTYFLIFAGCTAVAVNLNFDKE-----	83 (120)
HbNIP1;2	-----SSISVPFIQKLI AEVVGTYFLIFAGCTSVAAANLNVDKV-----	84 (120)
HbNIP2;1	-----KNYPDPFLKKVVAEVIATYLLVFVTCGAAAI STSDERR-----	71 (120)
HbNIP3;1	-----DDGSPSVLQKIIAELIGTYILIFVGC GAALTDKVQK-----	74 (120)
HbNIP4;1	-----RSGFCSSNATLIAEMVGTYMLIFCGCGSVAVNYIYG-S-----	65 (120)
HbNIP4;2	-----STSFVSITQKLI AEVIGTYFVVFAGCGVTVNKIYG-S-----	78 (120)
HbNIP5;1	N--APTGWQSHTCFTDFPSPDVSLTRKLGAEFVGTFILIFAATAGPIVNQKHNGV----	101 (120)
HbNIP6;1	EEWALEEGRLPVSCSIPPPVSLARKVGAEFITGLILIFAGTATAIVNQKTQGT-----	105 (120)
HbNIP7;1	P-----EGMDLNPARMVLAEMMGTFVLMFCVCGIIGTTQITRGQ-----	92 (120)
HbXIP1;1	-----FFSREMWRALVELVATACLLFTLTISIISCLESHVPE-----	84 (120)
HbXIP1;2	-----FFSPELCRAVTEMAATTCLLFMLTTTIIARLESHETE-----	62 (120)
HbXIP1;3	-----LLSPETWKAATTELVTACQLFTLITMTVACLESHVAE-----	84 (120)
HbXIP1;4	-----LLSPEKWKAATQVLVSTACLLFTLITMTVACLESHVAE-----	84 (120)
HbXIP2;1	-----LSSLHVWRASLAEALGTASLVFAMDTIVISSYETETKT-----	91 (120)
HbXIP3;1	-----VLTQVWRASFSEFLGTAVLVFVIDTVVISTIESETKI-----	39 (120)
HbSIP1;1	-----MGVIKAAIGDAILTSMWVFSMPLLGLILASAVAAYIG-VE-----	38 (120)
HbSIP1;2	-----MGAIKAAIGDAVLTFMWVFCSSMFGFLTSLIATALG-VQ-----	38 (120)
HbSIP1;3	-----MGAIKSAIGDAVLTFMWVFCSSMFGFLTSLIATALG-VH-----	38 (120)
HbSIP2;1	-----MSSAVTLRLIISDFVISFMWVWSGALIKMFVNRVLGVG-----	38 (120)

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	TM2	HB	TM3	
SoPIP2_1	-CGSVGLLGIAWAFGGMIFVLVYCTAGISGGHINPAVTFGLFLA	---	RKVSLLRALVYMI	124 (180)
HbPIP1;1	-CSTVGIQGIAWSFGGMIFALVYCTAGISGGHINPAVTFGLFLA	---	RKLSLTRLALYYMV	139 (180)
HbPIP1;2	-CSTVGIQGIAWAFGGMIFALVYCTAGISGGHINPAVTFGLFLA	---	RKLSLTRLALYYMV	139 (180)
HbPIP1;3	-CATVGTQGIAWAFGGMIFALVYCTAGISGGHINPAVTFGLFLA	---	RKLSLTRLALFYII	137 (180)
HbPIP1;4	-CTTVGTQGIAWAFGGMIFALVYCTAGISGGHINPAVTFGLFLA	---	RKLSLTRLALFYMI	137 (180)
HbPIP1;5	-CASVGVQGIAWAFGGMIFVLVYCTAGISGGHINPAVTFGLTLA	---	RKVS LTRALFYMV	120 (180)
HbPIP2;1	PCGGVGILGIAWAFGGMIFILVYCTAGISGGHINPAVTLGLFLA	---	RKVS LVRALYMA	131 (180)
HbPIP2;2	ACGGVGILGIAWAFGGMIFILVYCTAGISGGHINPAVTFGLFLA	---	RKVS LVRALYMA	131 (180)
HbPIP2;3	ACGGVGILGIAWAFGGMIFILVYCTAGISGGHINPAVTFGLFLG	---	RKVS LIRALLYMV	131 (180)
HbPIP2;4	ACGGVGILGIAWAFGGMIFILVYCTAGISGGHINPAVTFGLFLG	---	RKVS LIRALLYMV	131 (180)
HbPIP2;5	SCGGVGILGIAWAFGGMIFILVYCTAGISGGHINPAVTFGLFLA	---	RKVS LVRAMYMV	128 (180)
HbPIP2;6	ACGGVGILGIAWAFGGMIFILVYCTAGISGGHINPAVTFGLFLA	---	RKVS LVRAMYMV	128 (180)
HbPIP2;7	-CAGVLLGIAWAFGGMIFILVYCTAGISGGHINPAVSFGLFLA	---	RKVS LIRAVAYMV	121 (180)
HbPIP2;8	-CGGVLLGIAWAFGGMIFILVYCTAGISGGHINPAVTFGLFLA	---	RKVS LIRAVAYMV	123 (180)
HbPIP2;9	-CAGVFLGIAWSFGGMIFILVYCTAGISGGHINPAVTFGLFLA	---	RKVS LIRAVAYMV	125 (180)
HbPIP2;10	-CATVGLGVAWAFGGMIFILVYCTAGISGGHINPAVTFGLLLA	---	RKLS LVRAYMV	129 (180)
HbTIP1;1	ATTPAGLVAASIAHAFALFVAVSVGANISGGHVNPAVTFGAFVG	---	GNITLLRGILYWI	108 (180)
HbTIP1;2	ANTPAGLVAASIAHAFALFVAVSVGANISGGHVNPAVTFGAFVG	---	GNITLLRGILYWI	108 (180)
HbTIP1;3	SNTPAGIIMASLAHAFGLFVG VSTATNISGHVNPAVTFGAFVG	---	GNISLLRGILYWI	109 (180)
HbTIP1;4	SNTPAGIIMASLAHAFGLFVG VSTAFNISGHVNPAVTFGAFVG	---	GSISLIRGILYWI	109 (180)
HbTIP1;5	STTPAGLIAASLAHAFALFVAVSVGANISGGHVNPAVTFGAFVG	---	GNITLLRGILYWI	108 (180)
HbTIP1;6	STTPAGLIAASLAHAFALFVAVSVGANISGGHVNPAVTFGAFVG	---	GNITLLRGILYWI	108 (180)
HbTIP1;7	STTPAGLVAASLAHGFALFVAVSVGANISGGHVNPAVTFGAFVG	---	GHTIFIRSVLYWI	108 (180)
HbTIP1;8	STTPAGLVAASLAHGFALFVAVSVGANISGGHVNPAVTFGAFVG	---	GHTILIRSVLYWV	108 (180)
HbTIP2;1	ALDPAGLVAIAICHGFALFVAVSVGANISGGHVNPAVTFGLALG	---	GQITILTGFIFYWI	106 (180)
HbTIP2;2	ALDPAGLVAIAICHGFALFVAVAVGANISGGHVNPAVTFGLALG	---	GQITILTGVIFYWI	106 (180)
HbTIP2;3	ALDPPGLVAVAVAHAFALFVGVAIAANISGGHLNPAVTFGLAVG	---	GNITILTGFIFYWI	106 (180)
HbTIP2;4	ALDPPGPVAVAVAHAFGLFVGVAIAANISGGHLNPAVTFGLAVG	---	GNITILTGFIFYCI	106 (180)
HbTIP3;1	EPPASGLVMIALAHALALFSALSASINISGGHVNPAVTFGALVG	---	GRISVLQAFYYWV	107 (180)
HbTIP3;2	GPPASGLVMIALAHALALFSALSASINISGGHVNPAVTFGALVG	---	GRISVLRALYYWV	108 (180)
HbTIP4;1	--SLAGLLFVALAHALVVAVMISAG-HISGGHLNPAVTLGLLAG	---	GHTIVFRSILYWI	103 (180)
HbTIP5;1	GADPSSLVIVAIAANSFALSSAVYIAANISGGHVNPAVTFSLAVG	---	GHISVPTALFYWI	105 (180)
HbTIP5;2	GADPSSLVIVAIAANAFALSSAVYIAANVSSGHVNPAVTFSLAVG	---	GHINVPTAIFYWI	107 (180)
HbNIP1;1	----VTLPGISIVWGLAVMVLVYSVGHISGAHFNPAVTLAFATC	---	KRFPWKQVPAYIA	136 (180)
HbNIP1;2	----VTLPGISIVWGLAVMVLVYSVGHISGAHFNPAVTLAFATC	---	KRFPWKQVPAYIA	137 (180)
HbNIP2;1	----ISKLGASVAGGLIVTVMIVAVGHVSGAHMNPVTTAFAAF	---	RHFPWKQVPFYAA	124 (180)
HbNIP3;1	----LTIVGIAIAWGVVLMAAIYALGHVSGAHFNPAVSIALAAA	---	RKFSWKNVPMYIL	127 (180)
HbNIP4;1	----ITFPGVCVVWGLIVMVMIIYSVGHISGAHFNPAVSITFAIF	---	RQFPIKQLPLYIL	118 (180)
HbNIP4;2	----VTFPGISVTWGLIVMVMIIYTVGHISGAHFNPAVTITSAIF	---	RRFPFREVPLYIV	131 (180)
HbNIP5;1	----ESLIGNAACAGLAVMIIILSTGHISGAHLNPSLTIAFAAL	---	RHFPWMQVPAYIA	154 (180)
HbNIP6;1	----ETLIGLAASTGLAVMIVILSTGHISGAHLNPSVTIAFAAL	---	KHFPWKHVPHYIG	158 (180)
HbNIP7;1	----VALLEYASTAGLSVIVLVFALGPISGAHVNPAVTIAFAAF	---	GHFPWSRVPHYVL	145 (180)
HbXIP1;1	-----PKLLVPFAVFIIAFFFLLTTPVPLSGGHMSPVFTFIAALK	---	GVTILVRALLYVL	136 (180)
HbXIP1;2	-----PKLLIPIAVIVIAFLLLVTVPVPLSGGHMSPITTFISALR	---	GLITLVRALFNVL	114 (180)
HbXIP1;3	-----AKLLVPVVVFSTIFLLLVTIPVSGVHMNPTFTFIFALK	---	GVTIFVRALVYIL	136 (180)
HbXIP1;4	-----PKLLVPVVVFSTIFLLLVTIPVSGGHMNPFTTFIFALK	---	GAITFVRALVYIL	136 (180)
HbXIP2;1	-----PNLIMSALAITVTILLNATFPISGGHINPVITLSAAFT	---	GLVLSRAAIYIL	143 (180)
HbXIP3;1	-----PNLILSCLVAITVTIILLATYPISGGHINPLVTFSAAFT	---	GLISMTKAFIYIL	91 (180)
HbSIP1;1	AMSVAGLFITINVATCFVLTFSLIGAALGGASFNPATISFYAAGIKPDASLMSMAVRFP			98 (180)
HbSIP1;2	HLFWASMFITTVLFFIFFFLFGLIAEFFGGASFNP TGTASFYAAGFGGDN-LFSMALRFP			97 (180)
HbSIP1;3	HQFWASLFITTVIVFVFVFLFGLIAEFLGGASFNP TGTASFYAAGFGGDN-LFSMALRFP			97 (180)
HbSIP2;1	HHEPRGEAIKATLSIINMIFFAFLGKITKGAYNPLTVFSPAISG-DFS RFLLTVGARIP			97 (180)

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	TM3	TM4	
SoPIP2_1	AQCLGAICGVGLVKAFMK-GPYNQFGGGANSVAL-----	GYNKGTALGAEIIG	171 (240)
HbPIP1;1	MQCLGAICGAGVVKGFEGRHQYTLLGGGANSVNP-----	GYTKGDGLGAEIVG	187 (240)
HbPIP1;2	MQCLGAICGAGVVKGFEGRHQYTLLGGGANSVNP-----	GYTKGDGLGAEIVG	187 (240)
HbPIP1;3	MQCLGAICGAGVVKGFEGDRVYETLLGGGANVVAH-----	GYTKGDGLGAEIVG	185 (240)
HbPIP1;4	MQCLGAICGAGVVKGFEGNRAYETLLGGGANVVAH-----	GYTKGDGLGAEIVG	185 (240)
HbPIP1;5	MQCLGAIFGAGIVKGFQP-TPFETLGGGANVVNP-----	GYSKGDGLGAEIVE	167 (240)
HbPIP2;1	AQCLGAICGCGLVKAFQK-AYYNRYGGGANELAD-----	GYSKGTGLGAEIIG	178 (240)
HbPIP2;2	AQCLGAICGCGLVKAFQK-AYYNRYGGGANELAD-----	GYSKGTGLGAEIIG	178 (240)
HbPIP2;3	AQCLGAICGCGLVKAFQK-AYYTRYGGGANELSS-----	GYSKGTGLGAEIIG	178 (240)
HbPIP2;4	AQCLGAICGCGLVKAFQK-AYYNRYGGGANELSD-----	GYNKGTGLGAEIIG	178 (240)
HbPIP2;5	AQCLGAIAGVGLVKAFQS-SFYKRYGGGANSLAA-----	GYSKGVGLGAEIIG	175 (240)
HbPIP2;6	AQCLGAIAGVGLVKAFQS-SHYKRYGGGANSLAN-----	GYSTGVGLGAEIIG	175 (240)
HbPIP2;7	AQCLGAICGVGLVKAFMK-HPYNGLGGGANTVAP-----	GYNKGTALGAEIIG	168 (240)
HbPIP2;8	AQCLGAICGVGLVKAFMK-HPYNALGGGANSVAH-----	GYNKGTALGAEIIG	170 (240)
HbPIP2;9	AQCLGAICGVGIVKGIMK-DFYNAQGGGANTVAA-----	TYSKGTGLGAEIIG	172 (240)
HbPIP2;10	SQCLGAIAGAGLVKAVMK-DDYKSLGGGVNSVSS-----	GYSKGTALGAEIIG	176 (240)
HbTIP1;1	AQLLGSTVACLLLKFSBTG-----GLTTAGFALSS-----	GVGVWNAFVFEIVM	151 (240)
HbTIP1;2	AQLLGSTVACLLLKFSBTG-----GLTTSAFALSS-----	GVGVWNAFVLEIVM	151 (240)
HbTIP1;3	AQLLGSTVACLLLKFSBTH-----GMTTSAFALSS-----	GVNVWNAFVFEIVM	152 (240)
HbTIP1;4	AQLLGSTVACLLLKFSBTH-----GMTASAFSLSV-----	RGECEVCTCIRDCT	152 (240)
HbTIP1;5	AQLLGSVVACLLLKFBTGTG-----GLETSAFALSS-----	GVSSWNAFVFEIVM	151 (240)
HbTIP1;6	AQLLGSVVACLLLKFBTGTG-----GLETSAFALSS-----	GVSSWNAFVFEIVM	151 (240)
HbTIP1;7	AQLLGSVVACLLLKFBTGTG-----GLETSAFALSS-----	GVGAWNAFVFEIVM	151 (240)
HbTIP1;8	AQLLGSVVACLLLKFBTGTG-----GWETSAFALSS-----	GVGAGNAFVFEIVM	151 (240)
HbTIP2;1	AQLLGSIVACFLLKFVBTGTG-----DLPIPTHSVAA-----	GVGAIEGVVMEIVI	149 (240)
HbTIP2;2	AQLLGSIVACFLLKFVBTGTG-----GLAIPTHSVAA-----	GVGAIEGVVMEIVI	149 (240)
HbTIP2;3	AQCLGSIVACLLLQFVTN-----GKSVPTHGVAS-----	GMNAFEGVMEIII	149 (240)
HbTIP2;4	AQCLGSIVACPLLYFVAN-----GKSVPTHGVAS-----	GMNAFEGVMEIVL	149 (240)
HbTIP3;1	AQLLGAIVASLLRLVTN-----GMRPVGFYIAS-----	GVGEVHGLIMEVM	150 (240)
HbTIP3;2	AQLLGSIVASLLRLVTN-----RMRPVGFYVAS-----	GAAEVHGLILEVM	151 (240)
HbTIP4;1	DQLVASSAACLLLSYLTGTG-----GMATPVITLAS-----	GVGYVQGVVWEILL	146 (240)
HbTIP5;1	SQMLASVMACLLLRVIV-----GQSLPTYTIAE-----	EMTGFGASVIEGVL	148 (240)
HbTIP5;2	SQMLASVMACLLLRVAIV-----GQSLPTYTIAA-----	EMTGFGASVFEGVL	150 (240)
HbNIP1;1	CQVIGSTLAAGTIRLIIFBTG---KQDQFTGTMPAG-----	SDMQSFVVEFII	179 (240)
HbNIP1;2	CQVTGSTLAAGTIRLIIFBTG---KQDQFTGTLPAG-----	SDMQSFVVEFII	180 (240)
HbNIP2;1	AQLTGAIASFTLRVLLHP----IKQVGTSPSG-----	SDLQALIMEIVV	166 (240)
HbNIP3;1	AQVLGATLACLTCLKVLFHDQDDIQATMTQYKDST-----	SDLEAFIWEFII	173 (240)
HbNIP4;1	AQFVGSLLASGTLYILLDV---EDEDFFGTPKPVG-----	PHGRSFVIELIT	161 (240)
HbNIP4;2	AQVLGSILASGTALVFDV---TPNAYFGTVPVG-----	SNVQPLVIEIII	174 (240)
HbNIP5;1	AQVSASICASFALKGVFHP---FMSGGVTVPSV-----	STGQAFALEFLI	196 (240)
HbNIP6;1	AQVMASVSAAFALKGIFHP---IMGGGVTVPSG-----	GYGQAFALEFII	200 (240)
HbNIP7;1	AQIVGSILATYVGKCVYDI---KPELMVTQPLQ-----	DCNSAFWVEFIA	187 (240)
HbXIP1;1	AQCIGSIMAYMVIKNVMNNSAVEKYSLGCMID----GNEG----	GIASGTALVLEFSC	187 (240)
HbXIP1;2	AQCVGSIMAYLVIKSVMNNTAEKYSLGCMVN---GNRS----	GVNAGTALILEFTC	165 (240)
HbXIP1;3	AQCLGSTMAYLIVKRAMNPKIAEKYSLGCSMG---GNGE----	GISAGTALAIEFAC	187 (240)
HbXIP1;4	AQCLGSTMANLIVKRAMNPKIAEKYSLGCSVSG---GNGE----	GISAGTALAIEFAC	187 (240)
HbXIP2;1	AQCLGGILGALALKAVVNSTIEKTFSLGGCTLSIVAPGPHG-PIVIGLGTAAQALWLEIIC		202 (240)
HbXIP3;1	AQCAGGVGALALKAVVNSKIESTFSLGGCTLHIVAPGPDGRPTVIGLETGQALWLEIIC		151 (240)
HbSIP1;1	AQAAGGVGAKAILQAMP SKYKHLKGPSLKVDL-----	HTGAVAEGTL	142 (240)
HbSIP1;2	AQAAGAVGGALAILEVMPPQYKHMGLGGPTLKVDL-----	HTGAIAEGLL	141 (240)
HbSIP1;3	AQAAGAVGGALAILEVMPPQYKHMGLGGPTLKVDL-----	HTGAIAEGVL	141 (240)
HbSIP2;1	AQVIGSITGVRYIIIEYFPP-----EIGFGPRLNVD-----	IHHGALTEGLL	137 (240)

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	TM4	TM5	HE
SoPIP2_1	TFVLVYTVFSATDPKRSARDS-----HVPILAPLPIGFAVFMVHLATIPITGTGINPARS		226 (300)
HbPIP1;1	TFVLVYTVFSATDAKRNARDS-----HVPILAPLPIGFAVFLVHLATIPITGTGINPARS		242 (300)
HbPIP1;2	TFVLVYTVFSATDAKRNARDS-----HVPILAPLPIGFAVFLVHLATIPITGTGINPARS		242 (300)
HbPIP1;3	TFVLVYTVFSATDAKRNARDS-----HVPILAPLPIGFAVFLVHLATIPITGTGINPARS		240 (300)
HbPIP1;4	TFVLVYTVFSATDAKRNARDS-----HVPILAPLPIGFAVFLVHLATIPITGTGINPARS		240 (300)
HbPIP1;5	TFALVYTVLSATDAKRSARDS-----HVPILASLPIGFAVFLVHLATIPITGTGINPARS		222 (300)
HbPIP2;1	TFVLVYTVFSATDPKRNARDS-----HVPVLAPLPIGFAVFMVHLATIPVTGTGINPARS		233 (300)
HbPIP2;2	TFVLVYTVFSATDPKRNARDS-----HVPVLAPLPIGFAVFMVHLATIPVTGTGINPARS		233 (300)
HbPIP2;3	TFVLVYTVFSATDPKRNARDS-----HVPVLAPLPIGFAVFMVHLATIPITGTGINPARS		233 (300)
HbPIP2;4	TFVLVYTVFSATDPKRNARDS-----HVPVLAPLPIGFAVFMVHLATIPITGTGINPARS		233 (300)
HbPIP2;5	TFVLVYTVFSATDPKRNARDS-----HVPVLAPLPIGFAVFMVHLATIPITGTGINPARS		230 (300)
HbPIP2;6	TFVLVYTVFSATDPKRSARDS-----HVPVLAPLPIGFAVFMVHLATIPITGTGINPARS		230 (300)
HbPIP2;7	TFVLVYTVFSATDPKRSARDS-----HVPVLAPLPIGFAVFMVHLATIPITGTGINPARS		223 (300)
HbPIP2;8	TFVLVYTVFSATDPKRSARDS-----HVPVLAPLPIGFAVFMVHLATIPVTGTGINPARS		225 (300)
HbPIP2;9	TFVLVYTVFSATDPKRNARDS-----HVPVLAPLPIGFAVFMVHLATIPITGTGINPARS		227 (300)
HbPIP2;10	TFVLVYTVFSATDPKRNARDS-----FVPILVPLPIGFAVFMVHLATIPITGTGINPARS		231 (300)
HbTIP1;1	TFGLVYTVYATAIDPKKG-----NLGIIAPIAIGFIVGANILAGGAFDGAASMPAVS		203 (300)
HbTIP1;2	TFGLVYTVYATAIDPKKG-----NLGIIAPIAIGFIVGANILAGGAFDGAASMPAVS		203 (300)
HbTIP1;3	TFGLVYTVYATAFDRNKG-----DVGIIAPLAIGFVVGANILAGGAFEGASMPAVS		204 (300)
HbTIP1;4	DVCLVYTVYATALDPKKG-----EVGIIALLAIGFVVGANILAGGAFEGASMPAVS		204 (300)
HbTIP1;5	TFGLVYTVYATAVDPKKG-----NVGIVAPIAIGFIVGANILAGGAFDGAASMPAVS		203 (300)
HbTIP1;6	TFGLVYTVYATAVDPKKG-----NVGTVAPIAIGFIVGANILAGGAFDGAASMPAVS		203 (300)
HbTIP1;7	TFGLVYTVYATAVDPKKG-----NIGIIAPIAIGFIVGANILAGGAFDGAASMPAVS		203 (300)
HbTIP1;8	TFGLVYTVYATAVDPKKG-----DIGIIAPIAIGFIVGANILAGGAFDGAASMPAVS		203 (300)
HbTIP2;1	TFALVYTVYATAADPKKG-----SLGIIAPIAIGFIVGANILAAGPFSGGSMNPARS		201 (300)
HbTIP2;2	TFALVYTVYATAADPKKG-----SLGIIAPIAIGFIVGANILAAGPFSGGSMNPARS		201 (300)
HbTIP2;3	TFALVYTVYATAADPKKG-----NLGIIAPIAIGFIVGANILAAGPFSGGSMNPARS		201 (300)
HbTIP2;4	TFGLYTVYATAAEPEKG-----NLGIMAPLAIGFVFGANILATSPFSGGSMNPASS		201 (300)
HbTIP3;1	TFGLVYTVYATAVDPKRG-----SLGIIAPLAIGFIVGANILVGGPFDGASMPARA		202 (300)
HbTIP3;2	TFGLAYTVYATAVDTNRG-----SLGIIAPLAIGLIVGANILVGGPFDGASMPARA		203 (300)
HbTIP4;1	TFSLFLTIVYGTIVDPKKG-----SIDGLGPLLTGLVVGANILAGGSFSGAAMNPARS		198 (300)
HbTIP5;1	TFGLVYTVYAAAG-DPRRS-----LQGVLTGPLAIGLMAGANVLAAGPFSGGSMNPACA		199 (300)
HbTIP5;2	TFGLVYTVYAAAG-DPRCS-----LLGATGPLAIGLMAGANVLAAGPFSGGSMNPACA		201 (300)
HbNIP1;1	TFYLMFIISGVATDNRAIGE-----LAGLAVGSTVLLNVLFAGPISGASMPARS		229 (300)
HbNIP1;2	TFYLMFIISGVATDNRAIGE-----LAGLAVGATVLLNVMFAGAISGASMPARS		230 (300)
HbNIP2;1	TFSMFVTSAVATDTKAIGE-----LAGVAVGSAVCITSLAGPVS GGSMNPARS		216 (300)
HbNIP3;1	TFNLMFNICGVATDHRGSKD-----LSGVAIGGTLFNVLLAGPITGASMPARS		223 (300)
HbNIP4;1	SFLLMFVISGVATDNRAIGE-----LAGIAIGMTIMLNVFISGPVSGGSMNPVRS		211 (300)
HbNIP4;2	TFLLMFVISGTTTTHRAVGE-----LGGIGVGMTILLNVFVAGPVSGASMPARS		224 (300)
HbNIP5;1	TFNLLFVVTAVATDTRAVGE-----LAGIAVGATVMLNLVAGPSSGGSMNPVRT		246 (300)
HbNIP6;1	SFNLMFVVTAVATDTRAVGE-----LAGIAVGATVMLNLIAGQSTGASMPVRT		250 (300)
HbNIP7;1	TFIIMFLIASLTYQTS-VGH-----LSGFIVGLSIGLAVLITGPLSGGSLNPARS		236 (300)
HbXIP1;1	TFVVLVFGVTVAFDKRRFKELGLVMVCVILAATMGLAIFVSITVTGRGGYAGVGLNPARC		247 (300)
HbXIP1;2	SFLVLYVAITIAFNKKMCQELGFTMVCVIVAGVYALAVFASITVTGQAGYRGVGLNPARF		225 (300)
HbXIP1;3	TFLVLYFSVTVAFDKKRCKQLGLTMFCVMVSGILAVAYFISLITGQVGYGGPRLNPARC		247 (300)
HbXIP1;4	TFLVLYFSISVAFDKERCKQLGLTMFCVIVSGIFAVAYFISLITGQVGYGGARLNPARC		247 (300)
HbXIP2;1	TFVFLFSSIWVAFDKRQAKPLGRVIVCSIIGLVVGLLVFISTTATTATRGYAGVGMNPARC		262 (300)
HbXIP3;1	GFVFLFASVWMAFDHRQAKALGHVKIFMIVGIVLGLLVVSTSVTTAKGYAGAGLNPARC		211 (300)
HbSIP1;1	TFVFCLAMLLVMVKGPKN-----LLLKVWMAVAVTVGLVVSGRQYTGPSLNPARA		192 (300)
HbSIP1;2	TFLISFAVLIIIFLRGPCN-----SIVQNWLLAVVTVTLVVTGSKYTGPSMNPANA		191 (300)
HbSIP1;3	TFLISFAVLVIIILRGPRN-----SLVQNWLLAVVTVTLVVSGSKYTGPSMNPANA		191 (300)
HbSIP2;1	TFAIVTISLGLSRKIPGS-----FFMKTWISSVSKLALHILGSDLTGCGMNPASV		187 (300)

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TM6

SoPIP2_1 FGAAVIFNSNKVWDDQWI FWVGPFFIGA AVAAAYHQYVLRAAAIAKALGSFRSNPTN----- 281 (360)
HbPIP1;1 LGAAIIIFNKDKGWDDHWI FWVGPFFIGA ALAALYHQVVIRAI PFKK----- 287 (360)
HbPIP1;2 LGAAIIIFNKDKGWDDHWI FWVGPFFIGA ALAALYHQVVIRAI PFKK----- 287 (360)
HbPIP1;3 LGAAIIIFNKDHAWDDHWI FWVGPFFIGA ALAAYVHQIVIRAI PFKARA----- 287 (360)
HbPIP1;4 LGAAIIIFNKDHAWDDHWI FWVGPFFIGA ALAAYVHQIVIRAI PFKARA----- 287 (360)
HbPIP1;5 LGAAIVYNEDCAWYDHWI FWVRPFFIGA ALAALSNQIVIRAI PFMSKA----- 269 (360)
HbPIP2;1 FGAAVIYNQDKAWDDQWI FWVGPFFIGA AIAAFYHQYILRAAAVKALGSFRSTSNI----- 288 (360)
HbPIP2;2 FGAAVIYNQDKAWDDQWI FWVGPFFIGA AIAAFYHQYILRAAAKALGSFRSNSNI----- 288 (360)
HbPIP2;3 FGAAVIYNKDKAWDDQWI FWVGPFFIGA AIAAFYHQYILRAAAIAKALGSFRSNA----- 286 (360)
HbPIP2;4 FGAAVIYNKDKAWDDQWI FWVGPFFIGA AIAALYHQYILRAAAIAKALGSFRSNA----- 286 (360)
HbPIP2;5 LGAAVIYNQDKPWDDHWI FWVGPFFIGA AIAAFYHQFILRAGAVKALGSFRSNPTV----- 285 (360)
HbPIP2;6 LGAAVIYNQDKAWDDQWI FWVGPFAGAAIAAFYHQFILRAGAVKALGSFRSNPSTV----- 286 (360)
HbPIP2;7 FGAAVIYNNDKAWDDHWI FWVGPFVGA LAAAAYHQYILRAAAIAKALGSFRSNPTN----- 278 (360)
HbPIP2;8 FGAAVIYNNDKIWDDHWI FWVGPFIGA LAAAAYHQYILRAAAIAKALGSFRSNPTN----- 280 (360)
HbPIP2;9 FGAAVIYNNDKVWDDQWI FWVGPFVGA LAAAIYHQHILRGTAIKALGSFTTNN----- 280 (360)
HbPIP2;10 LGPAVIYNKKTIWDDHWI FWAGPFLGA LAAALYHQYVLRAGA KALGSFRSNRSI----- 285 (360)
HbTIP1;1 FGPAALVSWs---WDNHVWY WAGPLIGGGLAGV VYELLFIGNTHEQLPSTDY----- 252 (360)
HbTIP1;2 FGPAALVSWs---WDNHVWY WAGPLIGGGLAGLI YEFFFIGNTHEQLPTDY----- 252 (360)
HbTIP1;3 FGPAALVSWD---WTNHVWY WVGPLIGGGLAGI IYNLFITR THEPVPSTSEF----- 253 (360)
HbTIP1;4 FGPAALVSWD---WANHWVY GI-----FIKKV----- 227 (360)
HbTIP1;5 FGPAVVSWT---WTSHVWY WVGPLIGA AIALVYDNIYIGENAHEPLSTSDF----- 252 (360)
HbTIP1;6 FGPAVVSWT---WTSHVWY WVGPLIGA AIAIVYDNI FIGKNAHEPLSTNDF----- 252 (360)
HbTIP1;7 FGPAVVSWT---WDNHVWY WLGPFLLGAGIA AVVYEVFFISPS THEQLPSTEF----- 252 (360)
HbTIP1;8 FGPAVVSWT---WDNHVWY WLGPLLGA GIAAVVYEVFFISPS THEQFPSADF----- 252 (360)
HbTIP2;1 FGPAVASGD---FHDNWIY WVGPLIGGGLAGLVYGNLYIPG-DHAPLSNEF----- 248 (360)
HbTIP2;2 FGPAVASGD---FHDNWIY WVGPLIGGGLAGLVYGNLYIPS-DHAPLSSEY----- 248 (360)
HbTIP2;3 FGPAVVS GD---FSENWIY WVGPLIGGGLAGLVY GQIFIGSYVPAPSSSEDYA----- 250 (360)
HbTIP2;4 FGPAVVS GD---FSENCIY WVGPLIGGGLSGLQY GQVFIGSYSPVPSSSEDYA----- 250 (360)
HbTIP3;1 FGPAALVGWR---WRNHWIY WLGPFVGGGLAALI YEYTVIPTDPLPHH THQPLAPDDY-- 257 (360)
HbTIP3;2 FGPAALVGWR---WRNHWIY WLGPFIRGG LAAALIYR---I ISSAFGT----- 243 (360)
HbTIP4;1 FGPAALVSWD---WTHHWVY WVGPLIGGGLAGFIYENNFITRSHLPLPNDEETY LSTN--- 251 (360)
HbTIP5;1 FGS AVIAGR---FKNQAVY WVGPLIGGT FAGLLYDNAVFPNQVPDSIRGISDGVGA---- 252 (360)
HbTIP5;2 FGS AVIAGR---FKNQAVY WVGPLIGGT VAGLLYDNNVFP SQVPDSI-----GV----- 247 (360)
HbNIP1;1 LGPAIVSSQ---YKGIWVY LISPI LGAQAGAWAYNMIRYTDKPLREITKSA SFLKSTGRA 286 (360)
HbNIP1;2 LGPAIVSSQ---YKGLWIY IVPILGAQAGAWVYNMIRYTDKPLREITMSA SFLKNTGRA 287 (360)
HbNIP2;1 LGPAIASAY---YKGIWVY IGPVVG TLLGACSYNLIRVTDQPIQAI SYSLKLRIRISND 273 (360)
HbNIP3;1 LGPAIVSGV---YKNLWVY IVPILGALAAA LIYSMLRVPNPEKPEEKNKIVLNYLYSPA 280 (360)
HbNIP4;1 LGPAIVMHI---YTGIWIY IGPVIGAILGGFCYNLIRFTDKPLREISKSSSTLINSFRS- 267 (360)
HbNIP4;2 IGP AIVKHV---YTGLWVY IGPVIGAIAGAFAYNLLRTP EKPLDELANKGILKNSNSRN 281 (360)
HbNIP5;1 LGPAVAAGN---YRDLWIY LVAPT LGALAGAGTYTLVKLREDEADP---PRPVR SFR-- 298 (360)
HbNIP6;1 LGPAIAANN---YKGIWIY LTAPILGALCGAGTYS AVKLPEEDADTREKPSEARSFR-- 305 (360)
HbNIP7;1 LGPAIISWN---FKDIWVY ITAPVIGSLAGALMFHALRIQSRPCTSTD SSTNAGLLGHSI 293 (360)
HbXIP1;1 LGPALLHGG-PLWHGHVW FWVGPF LACIIYYCYTLTFG----- 284 (360)
HbXIP1;2 LGPALLLGG-SLWDGHVW FWVGPF LACIVYYGFTTLTL PKQGLVRAEKEHHITQLVLGSCY 284 (360)
HbXIP1;3 IGP AVL VGG-SLWESLWV FWVGPF CASIVL----- 276 (360)
HbXIP1;4 IGP AVL VGG-SLWQSLWV VWVGPF CASIVL----- 276 (360)
HbXIP2;1 FGPAIIRGG-HLWNGHWV FWVGPI IASIAFAVYTKIVPSAEVHA----- 305 (360)
HbXIP3;1 LGPAIVRGG-RLWDGHVW FWVGPAVSSVAFSLYTKLIPPQLSHTIF----- 256 (360)
HbSIP1;1 YGWA YINWHNTWELFYVY WICPFAGATLAAWVFRYLFKAPI-KDKQA----- 239 (360)
HbSIP1;2 FGWA YVNKWHDTWEQFYVY WICPFIGA ILAAWVFRLVFPP PAPKQKEA----- 239 (360)
HbSIP1;3 FGWA YINKRHD TWEQFYVY WICPFVGA ILAAWVFRLVFPP PAPKQKKA----- 239 (360)
HbSIP2;1 MGWA YARGDHITKEHILVY WLAPIEATLLAVWTFKLLVRPQKQEKEESKSKSD----- 240 (360)

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SoPIP2_1 -----
HbPIP1;1 -----
HbPIP1;2 -----
HbPIP1;3 -----
HbPIP1;4 -----
HbPIP1;5 -----

HbPIP2;1	-----	
HbPIP2;2	-----	
HbPIP2;3	-----	
HbPIP2;4	-----	
HbPIP2;5	-----	
HbPIP2;6	-----	
HbPIP2;7	-----	
HbPIP2;8	-----	
HbPIP2;9	-----	
HbPIP2;10	-----	
HbTIP1;1	-----	
HbTIP1;2	-----	
HbTIP1;3	-----	
HbTIP1;4	-----	
HbTIP1;5	-----	
HbTIP1;6	-----	
HbTIP1;7	-----	
HbTIP1;8	-----	
HbTIP2;1	-----	
HbTIP2;2	-----	
HbTIP2;3	-----	
HbTIP2;4	-----	
HbTIP3;1	-----	
HbTIP3;2	-----	
HbTIP4;1	-----	
HbTIP5;1	-----	
HbTIP5;2	-----	
HbNIP1;1	-----	
HbNIP1;2	-----	
HbNIP2;1	EQAHNKDPFDAL	285 (372)
HbNIP3;1	EP-----	282 (372)
HbNIP4;1	-----	
HbNIP4;2	-----	
HbNIP5;1	-----	
HbNIP6;1	-----	
HbNIP7;1	AIRRS-----	298 (372)
HbXIP1;1	-----SVDED-	289 (372)
HbXIP1;2	GAGFPSHVEEKV	296 (372)
HbXIP1;3	-----	
HbXIP1;4	-----	
HbXIP2;1	-----	
HbXIP3;1	-----	
HbSIP1;1	-----	
HbSIP1;2	-----	
HbSIP1;3	-----	
HbSIP2;1	-----	

Additional file 5: Alignment of predicted amino acid sequences of rubber tree aquaporins with structure determined Spinach SoPIP2;1.

Multiple alignments were performed using ClustalW2. Trans-membrane helices (TM1–TM6) and the two short helices forming the two NPAs (HB and HE) (shaded), P₁–P₅ residues (shown in blue), NPA motifs (shown in red), and ar/R selectivity filter residues (shown in green) are indicated. The highly conserved cysteine residues in XIPs are shown in **bold**. The positions corresponding to S115 and S274 in SoPIP2;1 (GenBank accession number 1Z98) are highlighted in bright green. The residues corresponding to S262 in GmNOD26 (GenBank accession number P08995) are shown in sky blue. The residues at the position corresponding to L197 from SoPIP2;1, determined to be the key residue involved in gating (occurring just before TM5) are underlined. The numbers at the end of each line

indicate amino acid position excluding the introduced gaps (number without parentheses) or including the introduced gaps (number in parentheses).