

Supplement 1

Nitric oxide signaling in Placozoa: Microchemical characterization and the lineage-specific diversification

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Supplementary Data: NOS alignments (see Fig 1 and Supplementary Dataset Excel Table)

PDZ

nHomo : MEDHMFVQQIQPNVISVRLFKRKRVGGLFLVKERVSKPPVVISDLIRGGAAEQSLIQAGDIILAVNGRPLVDLSYDSA : 80
eHomo : -----
iHomo : -----
Trichoplax_H1-1 : MEELRDT--TIQVTKDNISENYGFQVKNYDAMTYCTVSHIVDNNKNNK--ANPDISELQLGDLLIANDISLADQSVEDM : 76
Trichoplax_H1-2 : -----
Trichoplax_H1-3 : -----
Hoiilungia_13-2 : -----
Hoiilungia_13-1 : MEELYDNGMTIEVVKDIVNGNYGFQVKKNDMPYCSVSHIVDNHADVKNINGSQKRLQLGDLLLAVNDISLADKSAEDI : 80

nHomo : LEVLRGIASETHVVLLIRGPEGFTTHLETTFTGDGTPKTIIRVTPQLPPTKAVDLSHQPPAGKEQPLAVDVGASGPGNGPQ : 160
eHomo : -----MGN-----LKSVAQEPGPPCG-----LGLGLG-- : 22
iHomo : -----MACP-----WKFLFKTKFHQYAMNGE-----KDINNN-- : 27
Trichoplax_H1-1 : YDIIDQNSKNDMVNLTIRSKHQLLN-----SNNHNGGENITKLNNGNSRIKIKRKIGLGRDPSLDGTFESNNNQD : 145
Trichoplax_H1-2 : -----
Trichoplax_H1-3 : -----
Hoiilungia_13-2 : -----
Hoiilungia_13-1 : YEIIDKNTKNNVNLTIIRPNESNEN-----PGQLQT-DSYRQLNGTSRAKVKKIGLSKDPDLDNNIYENN--- : 145

nHomo : HAYDDGQEAGSLPHANGLAPRPPGQDPAKATRVSLQGRGENNELLKEIEFVLSLLTSGSRGVKGGAPAKAEMKDMGIQV : 240
eHomo : -----LGLCGKQGPATPAPEPSR----- : 40
iHomo : -----VEKAPCATSSPVTQDDLQYHN----- : 48
Trichoplax_H1-1 : HGQDSNNGKKASPIAQINIDELMASDDDAIGSQNDCEQPVDGVNLR----- : 192
Trichoplax_H1-2 : -----
Trichoplax_H1-3 : -----
Hoiilungia_13-2 : -----
Hoiilungia_13-1 : TGHQDVTTKNTLPIGQININELMASDDGDENNLDSSQPIDNVKLR----- : 192

nHomo : DRDLDGKSHKPLPLGVENDRVFNLDLWKGKGNPVVLLNPNY[EKEQPPTSGKQSPTKNGSPSKCP----- : 303
eHomo : -----APASLLPAPPEHSPSSPLTQ-----PPEGP----- : 66
iHomo : -----LSKQONESPOPLVETGKKSPELVKLDATPLSSP----- : 82
Trichoplax_H1-1 : -----VKKNSEVDKIPAKKWTKEAVLEDLTDDK[KINKLLYTRRRCQSDGSGSQKHLTKLLINGKEIPGAI : 261
Trichoplax_H1-2 : -----MDGK[QEWLWSPATVSSPNSDSNGP----- : 26
Trichoplax_H1-3 : -----MDSQ[-----AVPNLEGQ----- : 13
Hoiilungia_13-2 : -----MDSQ[QEWEWSPSPMILT-KADSNGP----- : 25
Hoiilungia_13-1 : -----VKQHSNIDKITPKQSTKQVALEDMITDK[KINKLLYTRRRCQNSIQVQKHQTKITINGKEIPGSI : 261

HPL --Zn-- Pterin

nHomo : -----RSLKVKNNWETEVVLTDTL[LKSTLETGTEYI[SGSIMHPSQHARRPE-DVRT[GOLEPFLAKBFIDQYYSI : 374
eHomo : -----KSPRVKNWEVGSITYDTLSAQ[QDDGPTPRRC[SGSLVFPKRLQGRPSGPPAP[OLIS[ARDFINQYYSI : 138
iHomo : -----RHVRIKNNWGSMTFODTL[HK[KGILT[RSKSG[SGSIMTPKSLTRGPRDKPTPDEL[PAIBFVNOYYSI : 154
Trichoplax_H1-1 : EED-TSKNG[IRLKNWSNSSLHDTL[KK[KCPMT[SGSQV[SGSSMNFLEQKPEYTTESYR[YLID[AVNFIREYYLNL : 340
Trichoplax_H1-2 : -----SFR[ONLLTGEQNTDTF[QQ[ATTPG[TFRR[IG-SLMQVPSQTKRHNGLKPKEVVK[AKDFLMEYYASL : 97
Trichoplax_H1-3 : -----TMR[ONLLITTEQNTDTF[QR[TTIP[S[TGKR[IG-SLMQIPEKIKRENGLKPKELVK[ATF[FLOEYYASI : 84
Hoiilungia_13-2 : -----SFR[ONLLTNGQNTDTF[QQ[ITTS[TSKR[IG-SLMQIPNQTKRHNGLKPKELVK[AKBFLEYYASL : 96
Hoiilungia_13-1 : EEDDASKNN[IRLKNWSNSSLHDTL[TK[KCPMT[ASQV[SGSSMNYIPEKKPECNTERN[RYVID[AVSFIREYYHHL : 341

HEME

nHomo : KRFGS-----KAHMERLEEVNKEIDTTSYTIQKDTEL[YGAKHAWRNA[RCVGRIQWSKLOVFDARDCTTAHGMENYI : 447
eHomo : KRSGS-----QAHECRLQVEAEVAAT[TYQURESELVFGAKQAWRNA[RCVGRIQWGLQVFDARDCRSAQEMTYI : 211
iHomo : KEAKI-----EEL[ARVEAVTKET[ETT[TYQITGDEL[FAKQAWRNA[RCVGRIQWSNLOVFDARSCSTAREMEHI : 227
Trichoplax_H1-1 : RNENPNNSNIDFEKEELARLEQITEDVNTQYELTYSELVFGAKLAWRNA[RCVGRIQWSRLKVFDAHVKT[DEMEQAI : 420
Trichoplax_H1-2 : RKENT-----EAH[KRQADVDEI[AK[LYL[TEQELTYGRLAWRNA[RCVGRIQWGNLHVFDAVATPEDMENAI : 170
Trichoplax_H1-3 : RKEGS-----EAH[KRKADVIEE[IAK[LYL[TEQELTYGRLAWRNA[RCVGRIQWSNLHIFDAVATATMDYDAI : 157
Hoiilungia_13-2 : RKENT-----EAH[KRETDVDEI[TKRIYLDTEQELTYGRLAWRNA[RCVGRIQWANLHIFDAVATPEDMNVNI : 169
Hoiilungia_13-1 : MSNENPNDDIYEKEELARLEQITEDVNTQYELTYSELVFGAKLAWRNA[RCVGRIQWSRLKVFDAHVKT[DEMEQAI : 421

PF02898: NO synthase

nHomo : CNHVKYATNKCGLNLSA[ITVEPQ[---TDGKHDFRVWNS[QLIRYAGYKQPDGSGT[LGDPANVQFTEICTQCGW[PPR[RFVDVLP : 526
eHomo : CNHIKYATNKCGLNLSA[ITVEPQ[---CPGRGDFRIWNS[QLVRYAGYRQDDGSGV[RGDPANVETTELCTQHGWT[PGNR[RFVDVLP : 290
iHomo : CRHVRYSTNKCGLNLSA[ITVEPQ[---SDGKHDFRVWNV[-----AQLCIDIGW[PP[--- : 272
Trichoplax_H1-1 : CEHLKYATNKCGLNLSV[ITVEPQ[---NGSKQDFRIWNA[QLIRYAGYKMDGSGI[IGR[ATVEFTELCLSFGR[SGSR[RFVDVLP : 500
Trichoplax_H1-2 : TDHVKYATNKCGLNIS[-----MHDFRVWNV[QLIRYAGYEQADGSGVIGD[PSNIEFTKIQSYGWR[GNG[MFVDVLP : 237
Trichoplax_H1-3 : ISHLKYATNKCGLNLSA[ITVEPP[---ANDSHDFRVWNI[QLVRYAGYEQPDGSGVIGD[PANVEFTKICESYGW[KGK[MFVDVLP : 236
Hoiilungia_13-2 : IDHLKYATNKCGLNLSA[ITVEPP[---SDGLHDFRVWNV[QLIRYAGYEQPDGSGVIGD[PANVEFTQICQSYGWR[KGK[MFVDVLP : 248
Hoiilungia_13-1 : CEHLKYATNKCGLNLSV[ITVEPQ[---NGTKQDFRIWNA[QLIRYAGYKMDGSGVIGD[RA[TEFTELCLSL[GR[SGSR[RFVDVLP : 501

Cav

nHomo : LLLQANGDEPEFEIPELVLVEVIRHPK[EWFEKDL[GLK[MYALPAV[NMLLEIGGLEFSACPF[SGWYM[TEIGVRDYODN : 606
eHomo : LLLQAPDDPEPELLELVLVEVLEHPTLEWFAAL[GLR[MYALPAV[NMLLEIGGLEFPAAP[SGWYM[STEIGVRNLDOP : 370
iHomo : -----NGRDEPEFEIPELVLVEVAMHBP[EWFEREL[GLK[MYALPAV[NMLLEVGGLEFPAGPF[GWYM[TEIGVRDFODV : 347
Trichoplax_H1-1 : LLLQANGDEPEFEIPELVLVEVCLRHPK[PWFEENL[GLK[MYALPAV[NMMEFDVGGIEEFCAPF[GWYM[TEIGSRDLGDT : 580
Trichoplax_H1-2 : LLLQANGTEPEHEIPELELVEVISHPE[EWFEEL[GLK[MYALPAV[CLLDIGGVEFPAGPF[GWYM[TEIGSRDLGDT : 317
Trichoplax_H1-3 : LLLQANGDEPEFEIPELVLVEVISHPE[EWFEEL[GLK[MYALPAV[CLLDIGGVEFPAGPF[GWYM[TEIGARDFGDE : 316
Hoiilungia_13-2 : LLLQANGSEPEFEIPELVLVEVISHPE[EWFEKLC[GLK[MYALPAV[CLLDIGGIEEFPAGPF[GWYM[TEIGSRDFGDI : 327
Hoiilungia_13-1 : LLLQANGGEPQVYDISELVLVEIQIRHPK[PWFEENL[GLK[MYALPAV[NMMEFDVGGIEEFCAPF[GWYM[TEIGSRDLGDDV : 581

PF02898: NO synthase

nHomo : SRYNILEEVAKKNNL[PKTKT[SLWKDCA[VEIN[AVLYSFQSDKVTIVDHHSA[ESF[KH[ME[RYR[GGCPADW[WLVP : 686
eHomo : HRYNILEEVAVCM[DLDT[RTTS[SLWKDCA[AVEIN[AVLYSHYQLAKVTIVDHHAA[ASFMK[HE[BE[Q[RGGCPADW[WLVP : 450
iHomo : QRYNILEEVYGRRM[GLE[HKLASLWKDCA[VEIN[AVLYSHFQKQNTIMDHH[SAESFMK[MY[ME[RYR[GGCPADW[WLVP : 427
Trichoplax_H1-1 : YRNVYCEYVQ[QHM[GLD[IKNNS[SLWKDCA[VELN[AVLYSFQEDNVTIVDHH[SV[ESFMN[HE[HT[SI[RGGCPADW[WLVP : 660

Trichoplax_H1-2 : NRYNLCKPVAQYIKLDTREITLWRDAAVVELNRAVMHSEFLKHKVTLVDHHDASNTFMQHLDBOKLRGGCPADWVWVVP : 397
Trichoplax_H1-3 : NRYNLCKPVAQRMKLDTRTEITLWRDAAVVEINCAVMHSEFLKHKVTLVDHHDSTNSFMQHLDBOKLRGGCPADWVWVVP : 396
Hoiilungia_13-2 : NRYNLCKPVAQYIKLDTREITLWRDAAVVELNRAVMHSEFLKHKVTLVDHHDASNTFMQHLDBOKLRGGCPADWVWVVP : 407
Hoiilungia_13-1 : YRYNLCEYVQGMGLDTRKNSLWKKDAAVVELNRAVMHSEFLKHKVTLVDHHDSEFSFMQHLDBOKLRGGCPADWVWVVP : 661

CaM

nHomo : PMSGSTPVPFHQEMLYVRLKPSFEYQDPPVNTHWKGTNGTPTKERRAIGPKKAEAVKFSAKLMGQAMAKRVRKATILYAT : 766
eHomo : PISGSTPVPFHQEMVYVFLSPAARYQDPPKGSAAKGTGTRRKT---PKKAVANAVKISASLMGTVMARVRKATILYVGS : 526
iHomo : PMSGSTPVPFHQEMLYVRLKPSFEYQDPPVNTHWKGTNGTPTKERRAIGPKKAEAVKFSAKLMGQAMAKRVRKATILYAT : 506
Trichoplax_H1-1 : PISGSAEVYHQELLSYRLKPSFEYQDPAIDYKRR--KPKRRK---PKNAGNALFAHYLTKNTLAKRKKVITILYAT : 734
Trichoplax_H1-2 : PISGSAKVVYHQEMLYVRLKPSFEYQDPPKIH---TKGSRK---PKDLAKLVVSTDLMGKVLAKRRTATILYAT : 468
Trichoplax_H1-3 : PISGSAKVVYHQEMLYVRLKPSFEYQDPAKIHRRKTPIITKEGK---PKDLAKMVRSLTELMAKALAKRVRKATILYST : 472
Hoiilungia_13-2 : PISGSAKVVYHQEMLYVRLKPSFEYQDPPKIP---IKGTQRK---PKDLAKLVVSTDLMGKALAKRRTATILYAT : 478
Hoiilungia_13-1 : PMSGSAEVYHQELLSYRLKPSFEYQDPAIDYKRR--KPKRRK---PKDAGNALFAHYLTKNTLAKRKKVITILYAT : 735

PF00258: Flavodoxin 1

nHomo : ETGKSAQYAKTLCEIFKHAFDAKVMSEMEYIVHLEHETLVLVVSTFGNGDPEFENGKKGCAALMEMRHNVSQ---EER : 843
eHomo : ETGRAQSYAQQGLRERKAFDPRVLCMDEYIVVSLHEHETLVLVVSTFGNGDPEFENGKSGAAAALMEMSGYNSSPRPEQH : 606
iHomo : ETGKSAQYAKTLCEIFKHAFDAKVMSEMEYIVHLEHETLVLVVSTFGNGDPEFENGKKGCAALMEMRHNVSQ---EER : 578
Trichoplax_H1-1 : ETGKSEYAKIVNIIFSYGFDVKLVKMKDYIPSNLSQEKLLLVSTFGNGDPEFENGKKGCAALMEMRHNVSQ---EER : 809
Trichoplax_H1-2 : ETGTSERYAKMLKLLDIKFDKAVVMADYVFSNLDYKLVVETVASTFGNAEAPHNGEFGRRLHFRAHID--- : 540
Trichoplax_H1-3 : ETGTSERYAKMLKLLDIKFDKAVVMADYVFSNLDYKLVVETVASTFGNAEAPHNGEFGRRLHFRAHID--- : 539
Hoiilungia_13-2 : ETGTSERYAKMLKLLDIKFDKAVVMADYVFSNLDYKLVVETVASTFGNAEAPHNGEFGRRLHFRAHID--- : 554
Hoiilungia_13-1 : ETGKSEYAKIVNIIFSYGFDVKLVKMKDYIPSNLSQEKLLLVSTFGNGDPEFENGKKGCAALMEMRHNVSQ---EER : 810

AIL

FMN

nHomo : KSYKVRFNSVSSYSQKSSGGDPLDRDNFESAGPLANVRSVDFGLGSRAY-PHFCAFAVAVDTRLEELGGFRLLKMRREG : 922
eHomo : KSYKIRFNSISCSDDPVSSWRKRKRESSNTDSAGALTRFRCVDFGLGSRAY-PHFCAFAVAVDTRLEELGGFRLLKMRREG : 685
iHomo : KSYKVRFNSVSSYSQKSSGGDPLDRDNFESAGPLANVRSVDFGLGSRAY-PHFCAFAVAVDTRLEELGGFRLLKMRREG : 620
Trichoplax_H1-1 : -----ENRRSIHLPRGSKNPM--LSRIASSNYSVMKYAVDFGLGSRAY-PHFCAFAVAVDTRLEELGGFRLLKMRREG : 877
Trichoplax_H1-2 : -----LNASRTGSLHLPRRKSILRANETDRTSSFMNPKYSVDFGLGSRAYGENFCFAFGSLDTRLDGFRICPLGEG : 615
Trichoplax_H1-3 : -----LHSYHVQNEEDYNALNDYTRILNPKYSVDFGLGSRAYGENFCFAFGSLDTRLDGFRICPLGEG : 606
Hoiilungia_13-2 : -----LNASRTGSLHLPRRKTIRSDPDRASTSMRNPKYSVDFGLGSRAYGENFCFAFGSLDTRLDGFRICPLGEG : 629
Hoiilungia_13-1 : -----ENRRSIHLPRSSNRPP--ISRTSSNYSVMKYAVDFGLGSRAY-PHFCAFAVAVDTRLEELGGFRLLKMRREG : 878

nHomo : DELCGQBEAERFWAKKVEKACDVFQVGGDDVNIKANNSTIISNDRSKRNRKFRITFVA---EAPLETQGLNVEKRRVSA : 999
eHomo : DELCGQBEAERFQAQAQAACETFCVGDGDA--KAAARDVFSPKRSKRRQRYRISQAQ---EGLQLPLGLHVRKRMVFT : 760
iHomo : DELSGQDEAERFWAVQTEKACDVFQVGGDDVNIKANNSTIISNDRSKRNRKFRITFVA---EAPLETQGLNVEKRRVSA : 695
Trichoplax_H1-1 : DELFGQEDSFRKWTETEFHISCSDFHKSIDKDALSTIYKNSNHWPYKGFQMEESLDIEKTDICIGLKVHGVGEVIV : 956
Trichoplax_H1-2 : DELCGQBEAERFWAKKVEKACDVFQVGGDDVNIKANNSTIISNDRSKRNRKFRITFVA---EAPLETQGLNVEKRRVSA : 692
Trichoplax_H1-3 : DELCGQBEAERFWAKKVEKACDVFQVGGDDVNIKANNSTIISNDRSKRNRKFRITFVA---EAPLETQGLNVEKRRVSA : 684
Hoiilungia_13-2 : DELCGQBEAERFWAKKVEKACDVFQVGGDDVNIKANNSTIISNDRSKRNRKFRITFVA---EAPLETQGLNVEKRRVSA : 706
Hoiilungia_13-1 : DELFGQEDSFRKWTETEFHISCSDFHKSIDKDALSTIYKNSNHWPYKGFQMEESLDIEKTDICIGLKVHGVGEVIV : 957

FAD-PPi

nHomo : ARLLSRQLQSPKSSSTIIVVHHTNGSQEFLVQVGGHGLVFGNHDLVNALTETEDAPPVQMKVELFEERNTALG : 1079
eHomo : ATIRSVENLQSSKSTIATIIIVVLDTGGQEQGLVQVGGHGLVFGNHDLVNALTETEDAPPVQMKVELFEERNTALG : 839
iHomo : MRLLSRQLQSPKSSSTIIVVHHTNGSQEFLVQVGGHGLVFGNHDLVNALTETEDAPPVQMKVELFEERNTALG : 773
Trichoplax_H1-1 : GKLISRQLQSPKSSSTIIVVHHTNGSQEFLVQVGGHGLVFGNHDLVNALTETEDAPPVQMKVELFEERNTALG : 1032
Trichoplax_H1-2 : LTVLSVTNLQSSSSTIIVVHHTNGSQEFLVQVGGHGLVFGNHDLVNALTETEDAPPVQMKVELFEERNTALG : 766
Trichoplax_H1-3 : ATVLSVTNLQSSSSTIIVVHHTNGSQEFLVQVGGHGLVFGNHDLVNALTETEDAPPVQMKVELFEERNTALG : 758
Hoiilungia_13-2 : LTVLSVTNLQSPKSSSTIIVVHHTNGSQEFLVQVGGHGLVFGNHDLVNALTETEDAPPVQMKVELFEERNTALG : 780
Hoiilungia_13-1 : GKLISRQLQSPKSSSTIIVVHHTNGSQEFLVQVGGHGLVFGNHDLVNALTETEDAPPVQMKVELFEERNTALG : 1033

PF00667: FAD_binding 1

nHomo : VISNWTDELRLPP-CTTFQAFKYLDITTPPTLQLQFASLATESEKEKQVLLVLSKGLQBYEHWKGRNETTVEVLEEF : 1158
eHomo : PPGQWVRDRLPP-CTLRQALTFELDITSPSPQLRLRLSTLAEPEPQQELEALSQDPRRYEHWKFRCPCTILEVLEEF : 918
iHomo : ---WVSDKRLPP-CSIQSALTYFLDITTPPTQLLKLQAVATEEPERQLEALCO-PSEYSKKKEFNSPTFLVLEEF : 847
Trichoplax_H1-1 : GQKIWQVDFRLPLPCTLRDAFTYLLDITTPPTCMLEHATEAARDTKQVLELLGRGSKQYEDWKFEKCEHITVEVLEEF : 1112
Trichoplax_H1-2 : -EGIWVVDTHMPATFPLRAFMRFIDITSPPTCFGLHCTLSIAMQDNDNLABELAKCSKEYEDWKFEKCEHITVEVLEEF : 845
Trichoplax_H1-3 : -EKNWVVDNHIPPFLRAFMRFIDITSPPTCFGLHCTLSIAMQDNDNLABELAKCSKEYEDWKFEKCEHITVEVLEEF : 837
Hoiilungia_13-2 : -EGNWVVDTYIPTGFTLRFAFRFVDITSPPTCFGLHCTLSIAMQDNDNLABELAKCSKEYEDWKFEKCEHITVEVLEEF : 859
Hoiilungia_13-1 : GQKIWQVDFRLPLPCTLRDAFTYLLDITTPPTCMLEHATEAARDTKQVLELLGRGSKQYEDWKFEKCEHITVEVLEEF : 1113

FAD-Iso

nHomo : ESTQMPATLLQLLSLQPRYSISSPDPYVPEVHTVAIVSVYRTRDGECPHHGVCSSWLNRTQADELVCFVTRGAPS : 1238
eHomo : PSVALPAPLLQLLSLQPRYSISSAPSTHPGEIHTVAIVLAMRTQDGLCPHHGVCSTWLSQKPPDPVFCFTRGAPS : 998
iHomo : ESLRVSAGFLLSCLPLKPRFYSISSRDHTTPEIHTVAIVVYHTRDGGCPHHGVCSTWLSQKPPDPVFCFTRGAPS : 927
Trichoplax_H1-1 : ESLKVSPLLLQLLSLQPRYSISSPKHKVEIHTVAVVSVYRTRDGGCPHHGVCSSWLNRTQADELVCFVTRGAPS : 1192
Trichoplax_H1-2 : BSVRLPATLLKLRRLKORYYSISSMAVTPDEIHTVAVVSVYRTRDGGCPHHGVCSSWLNRTQADELVCFVTRGAPS : 923
Trichoplax_H1-3 : ESLIQSTLLKLRRLKORYYSISSMAVTPDEIHTVAVVSVYRTRDGGCPHHGVCSSWLNRTQADELVCFVTRGAPS : 915
Hoiilungia_13-2 : SSLRLPATLLKLRRLKORYYSISSMAVTPDEIHTVAVVSVYRTRDGGCPHHGVCSSWLNRTQADELVCFVTRGAPS : 937
Hoiilungia_13-1 : ESLKVSPLLLQLLSLQPRYSISSPKHKVEIHTVAVVSVYRTRDGGCPHHGVCSSWLNRTQADELVCFVTRGAPS : 1193

NADPH-Ribose

nHomo : FHLPRNPQVPCILVGPGTGIAPFRSFWQQRQFPIQH---KGMNP----- : 1279
eHomo : FRLPPEPSLPCILVGPGTGIAPFRSFWQQRRLHIES---KGLQP----- : 1039
iHomo : FHLPEDESHPCILIGPGTGIAPFRSFWQQRRLHIES---KGVGR----- : 968
Trichoplax_H1-1 : FHMPEVSVPTIILIGPGTGIAPFRSFWQQRRLHIES---MNLSTHRPVEQDIAISTHKSFAANI EKKRSHLRRQTSDASDYPPTGT : 1272
Trichoplax_H1-2 : FRMPPEVSVPTIILIGPGTGIAPFRSFWQQRRLHIES---EAAFVTVTKDYEDTEIPTGIDTPPLTP : 987
Trichoplax_H1-3 : FQMPSSNSPIIIMVGPGTGIAPFRSFWQQRRLHIES---AATAGSDAQKEEDNSNN--- : 968
Hoiilungia_13-2 : FHLPEVSHSPTIILIGPGTGIAPFRSFWQQRRLHIES---EANTIFSDTKEHENAEISNGNDTPPLTP : 1001
Hoiilungia_13-1 : FHMPEVSVPTIILIGPGTGIAPFRSFWQQRRLHIES---MNLSTHRPVEQDIAVPHKSKFTASLDRKSHLRRQTSDAADYPSRA : 1273

PF00175: NAD_binding_1

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nHomo      : -----CFMVLVFGCRQSKIDIYEETLQAKNKGVFRELYTAYSREPDKPKKYVQDILQE : 1334
eHomo      : -----TFMTLVFGCRCSQLDHLYDEVQNAQQRGVFGRVLTAFSREPDNPKTYVQDILRT : 1094
iHomo      : -----RFMTLVFGCRRPDEDIYQEMLEMAQKGVHAVHTAYSRLEGKPKKYVQDILRQ : 1023
Trichoplax_H1-1 : RSKDRGETDVSGSKNHCSVAKSVFEMILYFGCRNSKTDIYDETRKALTDHVLTHVQTAYSREEHKPKKYVQDILKK : 1352
Trichoplax_H1-2 : TENEDLETR-----FCKMTLFFGCRESTKDIYMEIKDCLTNGVLDVVHKAYSREPGQQRRYVQDLMKD : 1052
Trichoplax_H1-3 : -----FCDMVLYFGCRTPKDIYEELEDCVNKKVKEVYKAYSRADEPKKYVQDILSQ : 1024
Hoilungia_13-2 : TGNEGLATR-----FCKMLLFGCRESTKDIYKETEECLNNGVLDEVVYKAYSRELNQQRRYVQDLMKE : 1066
Hoilungia_13-1 : RSKDREESDNIESS--NSSVVKGVFEMILYFGCRNAKIDDIYDDTRKALTDHVLTHVQTAYSREEHKPKKYVQDILKK : 1351

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NADPH-A

NADPH

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nHomo      : QLAESVYRALKEQGCHIYVCGDVMAADVLKATRIMTQCKLSAEDAGVFSRMRDDNRYHEDIFGTLRLTYEVTNELR : 1414
eHomo      : ELAAEVHRLCLERGHMFVCGDVRMTRVLQTRILATEDMELDEAGDVGVLRDQQRYHEDIFGTLRLTYEVTNELR : 1174
iHomo      : QLASEVLRLHKEPGHIYVCGDVRMTRVVAHTLQLVAAKLKLNEEQVEDYFFOLKSQKRYHEDIFGAVFPYEAKDVA : 1103
Trichoplax_H1-1 : D-AFELCKLLDHGAHIYVCGDVSMSADVGRTIONVLEDYCAMSSTESQECTRLRDSGRYHEDIFGTLRLTYEVTNELR : 1431
Trichoplax_H1-2 : H-GGDLLHTLLEDGHIYVCGDVSMAADVSRTIONLLIDYCFLDQEDAIEYVKTKMERGRYHEDIFGTLRLTYEVTNELR : 1131
Trichoplax_H1-3 : N-GNEVLANLLDDDGHIYVCGDVSMAAGVTRAIONTLLIDYQFSQEDALEMTKNLKNGRYHEDIFGTLRLTYEVTNELR : 1103
Hoilungia_13-2 : H-GGDLLHLLDGDGHIYVCGDVSMAADVFRLONLLIDYCFLNQEDAIEYVKNMKETGRYHEDIFGTLRLTYEVTNELR : 1145
Hoilungia_13-1 : D-AFELCKLLDHGAHIYVCGDVSMSADVGRTIONVLEDYCAMSNTESQECTRLRDSGRYHEDIFGTLRLTYEVTNELR : 1430

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nHomo      : SESIAFIEESKDTDEGFQLLTGPSCPAGCKFCKRGQTLLNLSSGTPCGPRSASCPCRCALVSLLGLLAPQWFPRPSWVY : 1494
eHomo      : TQSFSLQERQLRG----- : 1187
iHomo      : VQPSSEMSAL----- : 1114
Trichoplax_H1-1 : KAAQRAWILVSGDDKTVKASASGSSIIPRIFPKAGGVSAGSPKLRHKVRSRRARHSPTVVLNPDEQQQLFNSNTET- : 1510
Trichoplax_H1-2 : KAAQRAWVMIRKHEFRKSNKGDGNGIAN-----LVDKVRARQKLDTSPLERGISVDVMRTEDEVPEQSQLSR : 1201
Trichoplax_H1-3 : RASQRTWTMTISKRLKGETGKG--TEMAN-----LIQKLKRKSGSDVRGRYM----- : 1149
Hoilungia_13-2 : KAAQRAWVMIRKYNFGKSHKANDNRVAD-----LVNKVQANQTLDKFLVDRGISVDVMRTEDEIPEQKPRSFSR : 1215
Hoilungia_13-1 : KAAQRAWILVSGDDKTVKASASGSSIIPRIFPKAGGVSAGSPKLRHKFRARRGRHSPTVVLNPDEQQQLFNNNTTEAN : 1510

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nHomo      : SLSFPAMQCFSNLQWLLQNSVPTPSLADKGNSRVHETTGTWPSLWGFFSLGFPWKGCRN : 1554
eHomo      : -----AVPWAFD-----PPGSDTNSP----- : 1203
iHomo      : ----- : -
Trichoplax_H1-1 : QTSTESNFKRLLERRHSVHAAKPDECDTEDSDDEF----- : 1545
Trichoplax_H1-2 : IFSMPGSDGPTMSISASFSGEQLNISSIESYEDE----- : 1235
Trichoplax_H1-3 : ----- : -
Hoilungia_13-2 : VFSMPGSDGPTMSISTSFSGEHLTISSIESQEDQ----- : 1250
Hoilungia_13-1 : TTTAESKFKRLLERRHSVHAAKPDDMDTEDSDDCL----- : 1545

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Abbreviations:

- PDZ domain
- HPL: N-terminal hairpin loop region
- Zn: Zinc binding site
- Pterin-binding site
- Heme: any compound of iron complexed in a porphyrin (tetrapyrrole) ring
- Cav: Caveolin binding consensus sequence
- CaM: Calmodulin binding domain
- AIL: Auto-Inhibitory loop
- FMN: Flavodoxin binding domain
- FAD-PPi: Flavin adenine dinucleotide pyrophosphate binding site
- FAD-Iso: Flavin adenine dinucleotide isoalloxazine binding site
- NADPH-Ribose: Nicotinamide adenine dinucleotide phosphate-oxidase ribose binding site
- NADPH-A: Nicotinamide adenine dinucleotide phosphate-oxidase adenine binding site

Of note, *Trichoplax* NOS1 (*Trichoplax_H1-1*) and NOS3 (*Trichoplax_H1-3*) are constitutively expressed, as shown in Fig. 6 (main text) and from reported transcriptomes (Sebe-Pedros et al. 2018; Moroz, 2018).