Evolution of a key enzyme of aerobic metabolism reveals Proterozoic functional subunit duplication events and an ancient origin of animals

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### Supplementary Table S1 – List of all taxa analyzed in this study, including sequence name, species name, taxonomic group and GenBank accession number.

| Sequence Name                                      | Species          | Taxonomic Group | Accession number |
|----------------------------------------------------|------------------|-----------------|------------------|
| A._planci_echinodermataXP_022080645.1              | Acanthaster planci | Echinodermata    | XP_022080645.1   |
| A._planci_echinodermataXP_022096040.1              | Acanthaster planci | Echinodermata    | XP_022096040.1   |
| A._planci_echinodermataXP_022108231.1              | Acanthaster planci | Echinodermata    | XP_022108231.1   |
| A._millepora_cnidariaXP_029191641.1                | Acropora millepora | Cnidaria        | XP_029191641.1   |
| A._millepora_cnidariaXP_029193850.1                | Acropora millepora | Cnidaria        | XP_029193850.1   |
| A._millepora_cnidariaXP_029194161.1                | Acropora millepora | Cnidaria        | XP_029194161.1   |
| A._pisum_insectsXP_008188230.1                     | Acyrthosiphon pisum | Arthropoda     | XP_008188230.1   |
| A._sinensis_ReptilaAlligatorXP_014377070.2         | Alligator sinensis | Vertebrata     | XP_014377070.2   |
| A._sinensis_ReptilaAlligatorXP_025069507.1         | Alligator sinensis | Vertebrata     | XP_025069507.1   |
| A._macrogynus_FungiKNE71753.1                      | Allomyces macrogynus | Fungi          | KNE71753.1       |
| A._caninum_nematodaRCN36703.1                      | Ancylostoma caninum | Nematoda       | RCN36703.1       |
| A._caninum_nematodaRCN50722.1                      | Ancylostoma caninum | Nematoda       | RCN50722.1       |
| A._deanei_EuglenozoaEPY39061.1                     | Angomonas deanei  | Euglenozoa     | EPY39061.1       |
| A._gossypii_insectsAJQ30122.1                      | Aphis gossypii    | Arthropoda     | AJQ30122.1       |
| A._cerana_insectsXP_028525762.1                    | Apis cerana       | Arthropoda     | XP_028525762.1   |
| A._cerana_insectsPBC30104.1                        | Apis cerana cerana | Arthropoda   | PBC30104.1       |
| A._suum_nematodaADY42482.1                         | Ascaris suum      | Nematoda       | ADY42482.1       |
| A._suum_nematodaADY46393.1                         | Ascaris suum      | Nematoda       | ADY46393.1       |
| B._dorsalis_insectsXP_011197565.1                  | Bactrocera dorsalis | Arthropoda   | XP_011197565.1   |
| B._scammoni_mamalsXP_028018055.1                   | Balaenoptera      | acutorostrata  | XP_028018055.1   |
| B._mandarina_insectsXP_028031821.1                 | Bombyx mandarina  | Arthropoda     | XP_028031821.1   |
| B._belcheri_cephalochordataXP_019626395.1          | Branchiostoma     | belcheri       | XP_019626395.1   |
| B._belcheri_cephalochordataXP_019628290.1          | Branchiostoma     | belcheri       | XP_019628290.1   |
| B._belcheri_cephalochordataXP_019631903.1          | Branchiostoma     | belcheri       | XP_019631903.1   |
| B._belcheri_cephalochordataXP_019631904.1          | Branchiostoma     | belcheri       | XP_019631904.1   |
| B._belcheri_cephalochordataXP_019631905.1          | Branchiostoma     | belcheri       | XP_019631905.1   |
| Species                                    | Domain         | Strain          | Accession    |
|--------------------------------------------|----------------|-----------------|--------------|
| Branchiostoma belcheri                    | Cephalochordata| XP_019631906.1  |              |
| Branchiostoma belcheri                    | Cephalochordata| XP_019631907.1  |              |
| Caenorhabditis elegans                    | Nematoda       | CAA86325.2      |              |
| Caenorhabditis elegans                    | Nematoda       | CCD66235.1      |              |
| Callorrhinchus milii                      | Vertebrata     | AFP04321.1      |              |
| Callorrhinchus milii                      | Vertebrata     | AFP04347.1      |              |
| Candida auris                             | Fungi          | GBL52436.1      |              |
| Capsaspora owczarzaki                     | Filasterea     | XP_004343741.1  |              |
| Capsaspora owczarzaki                     | Filasterea     | XP_004363625.1  |              |
| Cavenderia fasciculata                    | Amoebozoa      | XP_004361360.1  |              |
| Centruroides sculptratus                  | Arthropoda     | XP_023232345.1  |              |
| Centruroides sculptratus                  | Arthropoda     | XP_023235488.1  |              |
| Chelonia mydas                             | Vertebrata     | XP_007068405.2  |              |
| Ciona intestinalis                         | Urochordata    | XP_002122423.1  |              |
| Ciona intestinalis                         | Urochordata    | XP_002129499.1  |              |
| Ciona intestinalis                         | Urochordata    | XP_002130367.1  |              |
| Clonorchis sinensis                       | Platyhelminthes| GAA34041.2      |              |
| Clonorchis sinensis                       | Platyhelminthes| GAA40293.2      |              |
| Cordyceps militaris                       | Fungi          | ATY62353.1      |              |
| Cottoperca gobio                          | Vertebrata     | XP_029291799.1  |              |
| Crassostrea gigas                         | Mollusca       | EKC30015.1      |              |
| Crassostrea gigas                         | Mollusca       | EKC33392.1      |              |
| Crassostrea virginica                     | Mollusca       | XP_022301966.1  |              |
| Crassostrea virginica                     | Mollusca       | XP_022321339.1  |              |
| Crassostrea virginica                     | Mollusca       | XP_022334803.1  |              |
| Danio rerio                               | Vertebrata     | NP_001230101.1  |              |
| Daphnia magna                             | Arthropoda     | JAJ79593.1      |              |
| Daphnia magna                             | Arthropoda     | JAK11924.1      |              |
| Daphnia magna                             | Arthropoda     | JAN68172.1      |              |
| Daphnia magna                             | Arthropoda     | KZS14245.1      |              |
| Daphnia magna                             | Arthropoda     | KZS16963.1      |              |
| *D._gigantea_cnidariaXP_028391286.1* | *Dendronephthya gigantea* | Cnidaria | XP_028391286.1 |
| *D._gigantea_cnidariaXP_028398962.1* | *Dendronephthya gigantea* | Cnidaria | XP_028398962.1 |
| *D._alloeum_insectsXP_015117129.1* | *Diachasma alloeum* | Arthropoda | XP_015117129.1 |
| *D._discoideum_AmoebozoaXP_645630.1* | *Dictostylium discoideum* | Amoebozoa | XP_645630.1 |
| *D._purpureum_AmoebozoaXP_003289977.1* | *Dictostylium purpureum* | Amoebozoa | XP_003289977.1 |
| *E._naucrates_bonyfishesXP_029357416.1* | *Echeneis naucrates* | Vertebrata | XP_029357416.1 |
| *E._naucrates_bonyfishesXP_029363301.1* | *Echeneis naucrates* | Vertebrata | XP_029363301.1 |
| *E._granulosus_platyhelminthesCDS21006.1* | *Echinococcus granulosus* | Platyhelminthes | CDS21006.1 |
| *E._granulosus_platyhelminthesCDS22227.1* | *Echinococcus granulosus* | Platyhelminthes | CDS22227.1 |
| *E._multilocularis_platyhelminthesCDS36355.1* | *Echinococcus multilocularis* | Platyhelminthes | CDS36355.1 |
| *E._traillii_birdXP_027757377.1* | *Empidonax traillii* | Vertebrata | XP_027757377.1 |
| *E._jubatus_mamalsXP_027950171.1* | *Eumetopias jubatus* | Vertebrata | XP_027950171.1 |
| *E._affinis_crustaceaXP_023325125.1* | *Eurytemora affinis* | Arthropoda | XP_023325125.1 |
| *E._affinis_crustaceaXP_023342071.1* | *Eurytemora affinis* | Arthropoda | XP_023342071.1 |
| *E._pallida_cnidariaKXJ19304.1* | *Exaipptasia pallida* | Cnidaria | KXJ19304.1 |
| *E._pallida_cnidariaKXJ28853.1* | *Exaipptasia pallida* | Cnidaria | KXJ28853.1 |
| *E._pallida_cnidariaXP_020908935.1* | *Exaipptasia pallida* | Cnidaria | XP_020908935.1 |
| *F._cherrug_birdXP_014132353.2* | *Falco cherrug* | Vertebrata | XP_014132353.2 |
| *F._peregrinus_birdXP_027647222.1* | *Falco peregrinus* | Vertebrata | XP_027647222.1 |
| *F._hepatica_platyhelminthesTHD21245.1* | *Fasciola hepatica* | Platyhelminthes | THD21245.1 |
| *F._exsecta_insectsXP_029665054.1* | *Formica exsecta* | Arthropoda | XP_029665054.1 |
| *G._occidentalis_arachnidsXP_003738563.1* | *Galandromus occidentalis* | Arthropoda | XP_003738563.1 |
| *G._occidentalis_arachnidsXP_018497754.1* | *Galandromus occidentalis* | Arthropoda | XP_018497754.1 |
| *H._album_AmoebozoaXP_020430426.1* | *Heterostelium album* | Amoebozoa | XP_020430426.1 |
| *H._vulgaris_cnidariaCDG71929.1* | *Hydra vulgaris* | Cnidaria | CDG71929.1 |
| *H._microstoma_platyhelminthesCDS29409.1* | *Hymenolepis microstoma* | Platyhelminthes | CDS29409.1 |
| *H._microstoma_platyhelminthesCDS33069.1* | *Hymenolepis microstoma* | Platyhelminthes | CDS33069.1 |
| *H._dujardini_tardigradesOQV14700.1* | *Hypsibius dujardini* | Tardigrada | OQV14700.1 |
| *H._dujardini_tardigradesOQV25778.1* | *Hypsibius dujardini* | Tardigrada | OQV25778.1 |
| Species                          | Phylum               | Genus                  | Family                | Species         | Gene Accession | Position | Location |
|---------------------------------|----------------------|------------------------|-----------------------|-----------------|----------------|----------|----------|
| N._fulva_insects                | Arthropoda           | Nylanderia             |          | fulva          | XP_029166884.1 |          |          |
| O._vulgaris_mollusca            | Mollusca             | Octopus                |          | vulgaris       | XP_029632996.1 |          |          |
| O._vulgaris_mollusca            | Mollusca             | Octopus                |          | vulgaris       | XP_029654384.1 |          |          |
| O._vulgaris_mollusca            | Mollusca             | Octopus                |          | vulgaris       | XP_029654670.1 |          |          |
| O._flexuosa_nematoda            | Nematoda             | Onchocerca              |          | flexuosa       | OZC08304.1     |          |          |
| O._flexuosa_nematoda            | Nematoda             | Onchocerca              |          | flexuosa       | OZC10327.1     |          |          |
| O._nerka_bonyfishes             | Vertebrata           | Ophiophagus             |          | hannah         | XP_029478737.1 |          |          |
| O._viverrini_platyhelminthes    | Platyhelminthes      | Opisthochirus           |          | viverrini      | OON15529.1     |          |          |
| O._faveolata_cnidaria           | Cnidaria             | Orbicella               |          | faveolata      | XP_020611790.1 |          |          |
| O._faveolata_cnidaria           | Cnidaria             | Orbicella               |          | faveolata      | XP_020612083.1 |          |          |
| O._faveolata_cnidaria           | Cnidaria             | Orbicella               |          | faveolata      | XP_020628871.1 |          |          |
| O._anatinus_mamals              | Vertebrata           | Ornithorhynchus         |          | anatinus       | XP_028921678.1 |          |          |
| O._bicornis_insects             | Arthropoda           | Osmia                  |          | bicornis       | XP_029050935.1 |          |          |
| O._furnacalis_insects           | Arthropoda           | Ostrinia                |          | furnacalis     | XP_028165010.1 |          |          |
| P._tepideriorum_arachnids       | Arthropoda           | Parasteatoda           |          | tepideriorum   | LAA03863.1     |          |          |
| P._tepideriorum_arachnids       | Arthropoda           | Parasteatoda           |          | tepideriorum   | XP_015909429.1 |          |          |
| P._sinensis_ReptilaTurtles      | Vertebrata           | Pelodiscus              |          | sinensis       | XP_006117027.2 |          |          |
| P._vannamei_crustacea           | Arthropoda           | Penaeus                 |          | vannamei       | XP_027209903.1 |          |          |
| P._vannamei_crustacea           | Arthropoda           | Penaeus                 |          | vannamei       | XP_027220003.1 |          |          |
| P._catodon_mamals               | Vertebrata           | Physeter                |          | catodon        | XP_023984594.1 |          |          |
| P._fungivorum_Amoebozoa         | Amoebozoa            | Planoprotostelium       |          | fungivorum     | PRP86732.1     |          |          |
| P._damicornis_cnidaria          | Cnidaria             | Pocillopora             |          | damicornis     | XP_027035685.1 |          |          |
| P._damicornis_cnidaria          | Cnidaria             | Pocillopora             |          | damicornis     | XP_027040785.1 |          |          |
| P._damicornis_cnidaria          | Cnidaria             | Pocillopora             |          | damicornis     | XP_027040786.1 |          |          |
| P._damicornis_cnidaria          | Cnidaria             | Pocillopora             |          | damicornis     | XP_027053611.1 |          |          |
| P._damicornis_cnidaria          | Cnidaria             | Pocillopora             |          | damicornis     | XP_027053612.1 |          |          |
| P._muralis_ReptilaLizard        | Vertebrata           | Podarcis                |          | muralis        | XP_028567561.1 |          |          |
| P._canaliculata_mollusca        | Mollusca             | Pomacea                 |          | canaliculata   | XP_025077031.1 |          |          |
| P._canaliculata_mollusca        | Mollusca             | Pomacea                 |          | canaliculata   | XP_025106368.1 |          |          |
| Species                      | Domain     | Phylum         | Genus           | Accession Number |
|------------------------------|------------|----------------|-----------------|------------------|
| _P._canaliculata_            | Mollusca   | Pomaecia       | _canaliculata_  | XP_025113260.1   |
| _P._abelii_mamals_           | Vertebrata | Pongo          | _abelii_        | NP_001125436.1   |
| _P._mucrosquamatus_Reptilia_ | Vertebrata | Protobothrops  | _mucrosquamatus_| XP_015684429.1   |
| _P._oleovorans_Bacteria      | Bacteria   | Pseudomonas    | _oleovorans_    | CDR91070.1       |
| _R._varieornatus_tardigrades | Tardigrada | Ramazzottius   | _varieornatus_  | GAU88467.1       |
| _R._varieornatus_tardigrades | Tardigrada | Ramazzottius   | _varieornatus_  | GAU93339.1       |
| _R._varieornatus_tardigrades | Tardigrada | Ramazzottius   | _varieornatus_  | GAV07616.1       |
| _R._bivittatum_amphibia      | Vertebrata | Rhinatrema     | _bivittatum_    | XP_029451037.1   |
| _R._bivittatum_amphibia      | Vertebrata | Rhinatrema     | _bivittatum_    | XP_029465296.1   |
| _R._bivittatum_amphibia      | Vertebrata | Rhinatrema     | _bivittatum_    | XP_029465306.1   |
| _R._appendiculatus_arachnids | Arthropoda | Rhipicephalus  | _appendiculatus_| JAP79177.1       |
| _R._appendiculatus_arachnids | Arthropoda | Rhipicephalus  | _appendiculatus_| JAP86015.1       |
| _R._irregularis_Fungi        | Fungi      | Rhizophagus    | _irregularis_   | PKY46143.1       |
| _S._trutta_bonyfishes        | Vertebrata | Salmonella     | _trutta_        | XP_029632067.1   |
| _S._enterica_Bacteria        | Bacteria   | Salmonella     | enterica_subsp.| CBY96094.1       |
| _S._rosetta_Chaoanoflagellate| Choanoflagellata | Salpingoeca | _rosetta_       | EGD76470.1       |
| _S._harrisi_mamals_          | Vertebrata | Sarcophilus    | harrisi_        | X023363049.1     |
| _S._scabiei_arachnids       | Arthropoda | Sarcoptes      | scabiei_        | KPM07743.1       |
| _S._stipitis_Fungi           | Fungi      | Scheffersomyces| stipitis_       | XP_001386057.1   |
| _S._bovis_platyhelminthes    | Platyhelminthes | Schistosoma | _bovis_         | RTG84748.1       |
| _S._haematobium_platyhelminthes| Platyhelminthes | Schistosoma | haematobium_    | KGB38128.1       |
| _S._japonicum_platyhelminthes| Platyhelminthes | Schistosoma | japonicum_      | CAX73753.1       |
| _S._japonicum_platyhelminthes| Platyhelminthes | Schistosoma | japonicum_      | CAX75389.1       |
| _S._japonicum_platyhelminthes| Platyhelminthes | Schistosoma | japonicum_      | TNN15762.1       |
| _S._culicis_Euglenozoa       | Euglenozoa | Strigomonas    | culicis_        | EPY30744.1       |
| _S._purpuratus_echinodermata | Echinodermata | Strongilocentrotus | purpuratus_     | XP_783413.2      |
| Taxon Name                  | Scientific Name         | Kingdom        | Species ID  |
|-----------------------------|-------------------------|----------------|-------------|
| _S._purpuratus_echinodermata| Strongylocentrotus purpuratus | Echinodermata   | XP_011664062.1 |
| _S._ratti_nematoda          | Strongyloides ratti     | Nematoda       | CEF67094.1   |
| _S._ratti_nematoda          | Strongyloides ratti     | Nematoda       | XP_024507139.1 |
| _S._pistillata_cnidaria     | Stylophora pistillata   | Cnidaria       | PFX23565.1   |
| _S._pistillata_cnidaria     | Stylophora pistillata   | Cnidaria       | XP_022795520.1 |
| _S._pistillata_cnidaria     | Stylophora pistillata   | Cnidaria       | XP_022801276.1 |
| _S._suricatta_mamals        | Suricata suricatta      | Vertebrata     | XP_029786927.1 |
| _T._guttata_bird            | Taeniopygia guttata     | Vertebrata     | NP_001232659.1 |
| _T._rubripes_bonyfishes     | Takifugu rubripes       | Vertebrata     | XP_011620203.1 |
| _T._triunguis_ReptilaTurtles| Terrapene carolina      | Vertebrata     | XP_026514188.1 |
| _T._thermophila_Ciliates    | Tetrahymena thermophila | Ciliophora     | EAR98203.3   |
| _T._thermophila_Ciliates    | Tetrahymena thermophila | Ciliophora     | XP_001031817.3 |
| _T._urticae_arachnids       | Tetranychus urticae     | Arthropoda     | XP_015787653.1 |
| _T._lacteum_Amoebozoa       | Tieghemostelium lacteum| Amoebozoa      | KYR00648.1   |
| _T._canis_nematoda          | Toxocara canis          | Nematoda       | KHN84477.1   |
| _T._nativa_nematoda         | Trichinella nativa      | Nematoda       | OUC47719.1   |
| _V._destructor_arachnids    | Varroa destructor        | Arthropoda     | XP_022654745.1 |
| _V._ursinus_mamals          | Vombatus ursinus        | Vertebrata     | XP_027701613.1 |
| _X._tropicalis_amphibia     | Xenopus tropicalis      | Vertebrata     | NP_989352.1  |
| _X._tropicalis_amphibia     | Xenopus tropicalis      | Vertebrata     | NP_001119993.1 |
| _Z._californianus_mamals    | Zalophus californianus  | Vertebrata     | XP_027425207.1 |
| _Z._tritici_Fungi           | Zymoseptoria tritici    | Fungi          | EGP83856.1   |
Supplementary Figure S2 – Bioinformatic pipeline. Ovals represent software or scripts, and rounded rectangles represent input/output files. The dataset was formed using 195 previously published NAD-IDH sequences distributed as: 193 eukaryotic sequences and two bacterial.
Supplementary Figure S3 – Credibility intervals and node ages estimated based on a mixture model in PhyloBayes and ones inferred by BEAST.