**Supplementary File**

**5a**

|  |  |  |  |
| --- | --- | --- | --- |
| **Expanded Marker** | **uniprot\_id** | **Protein** | **Gene** |
| p0023 | D3R080 | Translation initiation factor IF-2 | infB |
| p0313 | Q2JK96 | Arginine--tRNA ligase (EC 6.1.1.19) (Arginyl-tRNA synthetase) (ArgRS) | argS |
| p0076 | D5SWY7 | tRNA-2-methylthio-N(6)-dimethylallyladenosine synthase (EC 2.8.4.3) ((Dimethylallyl)adenosine tRNA methylthiotransferase MiaB) (tRNA-i(6)A37 methylthiotransferase) | miaB |
| p0004 | P19486 | Elongation factor 1-alpha (EF-1-alpha) (Elongation factor Tu) (EF-Tu) | tuf |
| p0383 | E7H5S9 | Phosphoserine aminotransferase (EC 2.6.1.52) (Phosphohydroxythreonine aminotransferase) (PSAT) | serC |
| p0389 | P21469 | 30S ribosomal protein S7 (BS7) | rpsG |
| p0072 | Q2GDY4 | Phenylalanine--tRNA ligase beta subunit (EC 6.1.1.20) (Phenylalanyl-tRNA synthetase beta subunit) (PheRS) | pheT |
| p0010 | C9RQN5 | DNA-directed RNA polymerase subunit beta (RNAP subunit beta) (EC 2.7.7.6) (RNA polymerase subunit beta) (Transcriptase subunit beta) | rpoB |
| p0229 | E6SH25 | Arginine biosynthesis bifunctional protein ArgJ [Cleaved into: Arginine biosynthesis bifunctional protein ArgJ alpha chain; Arginine biosynthesis bifunctional protein ArgJ beta chain] [Includes: Amino-acid acetyltransferase (EC 2.3.1.1) (N-acetylglutamate synthase) (AGSase); Glutamate N-acetyltransferase (EC 2.3.1.35) (Ornithine transacetylase) (Ornithine acetyltransferase) (OATase)] | argJ |
| p0268 | E6SJH8 | Aspartate carbamoyltransferase (EC 2.1.3.2) (Aspartate transcarbamylase) (ATCase) | pyrB |
| p0027 | A9WK84 | Leucine--tRNA ligase (EC 6.1.1.4) (Leucyl-tRNA synthetase) (LeuRS) | leuS |
| p0182 | A0A0E8TCT0 | Glutamate synthase (EC 1.4.1.14) (EC 1.4.7.1) | gltB\_1 |
| p0109 | Q4FV41 | DNA mismatch repair protein MutS | mutS |
| p0346 | B0VFP4 | Thymidine phosphorylase (EC 2.4.2.4) | deoA |
| p0162 | D3R256 | Ribosome biogenesis GTPase Era | era |
| p0030 | D9PW62 | ATP-dependent DNA helicase (EC 3.6.1.-) | pcrA1 |
| p0138 | C6D886 | Lon protease (EC 3.4.21.53) (ATP-dependent protease La) | lon |
| p0241 | E8R3Q8 | Glutamate 5-kinase (EC 2.7.2.11) (Gamma-glutamyl kinase) (GK) | proB |
| p0071 | N2BS08 | DNA polymerase I (EC 2.7.7.7) | polA |
| p0046 | B5YFL1 | Holliday junction ATP-dependent DNA helicase RuvB (EC 3.6.4.12) | ruvB |

**5b**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Node | Fossil | Minimum | Soft Maximum | Reference |
| 1. LUCA
 | Strelley Pool Formation, Western Australia | 3347 Ma | 4520 Ma | Betts et al. 2018 |
| 1. Total group Cyanobacteria
 | Manzimnyama Banded Ironstone Formation,South Africa | 3225 Ma | 4520 Ma | Betts et al. 2018 |
| 1. Crown group Cyanobacteria
 | *Bangiomorpha pubescens* | 1033 Ma | 4520 Ma | Betts et al. 2018 |
| 1. Heterocystous/akinete-forming Cyanobacteria (Cyanobacteria sections IV+V)
 | *Anhuthruix magna* | 720 Ma | 4520 Ma | This paper |
| 1. Alphaproteobacteria
 | *Bangiomorpha pubescens* | 1033 Ma | 4520 Ma | Betts et al. 2018 |
| 1. Anoxychlamydiales
 | *Bangiomorpha pubescens* | 1033 Ma | 4520 Ma | This paper |
| 1. Lokiarchaeota
 | Changzhougou Formation, Northern China | 1619 Ma | 4520 Ma | Betts et al. 2018 |

**Calibration 4**

***Fossil taxon and specimen:*** *Anhuthruix magna* [Llb x4B, Institute of Geology and Paleontology, Technical University of Berlin,Berlin, Germany]from the Liulaobei Formation in the Huainan region of North China

***Phylogenetic justification:*** *A. magna* was originally described as a tubular organism(Steiner 1994), however Pang et al. (2018) interpreted it as a filamentous cyanobacteria based on new material.  Based on the presence of binary fission, hormogonia, akinetes, and probably heterocysts, an affinity with subsections IV+V of cyanobacteria was proposed.

***Minimum age:*** 720 Ma

***Maximum age:*** 4520 Ma

***Age justification:*** The Liulaobei Formation forms part of the Huainan Group in Anhui, Northern China. Geochronometric data from the Huainan Group proposed a Tonian age (840 +/- 72 Ma; Wang et al. 1984), but these measures have since been determined as unreliable (Dong et al. 2008). A pre-Cryogenian age was proposed based on stromatolite biostratigraphy and the presence of *Chuaria*, *Ellipsophysa*, and *Tawuia* in the Huainan Group (Cao et al. 1985; Fu 1989). This is further supported by the presence of the acanthomorphic acritarch *Trachyhystrichosphaera aimika* in the Liulaobei Formationand an overall acritarch assemblage that is distinct from those of the Ediacaran (Tang et al. 2013). Thus, a minimum age based on the upper boundary of the Tonian, 720 Ma (Van Kranendonk et al. 2012).

**Calibration 6**

**Crown Chlamydiia/Chlamydiae/Anoxychlamydiales**

***Fossil taxon and specimen***: *Bangiomorpha pubescens*. (Holotype) HUPC 62912, Slide HUST-1A, England Finder coordinates: O-35.

***Locality and Stratigraphy level***: Lower Hunting Formation, Somerset Island, Arctic Canada. ***Soft Minimum age***: 1033 Ma (1092 Ma ± 59 Myr )

***Soft Maximum age***: 4520 Ma (4510 Ma ± 10 Myr)

***Description***: Recent work [(Stairs et al. 2020)](https://paperpile.com/c/usFOmy/O0p2) suggests that members of the Anoxychlamydiales (including a lineage named Chlam. Bact SM23\_39), a clade within Chlamydiia (and Chlamydiae), donated at least three subunits of hydrogenase maturase (E, F, G) to stem eukaryotes prior to the radiation of crown eukaryotes. These phylogenies imply that a minimum age for Anoxychlamdiales is the age of the oldest crown eukaryote and thus a minimum age is derived following Betts et al. 2020

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