Supplementary materials

Title: Molecular evolution of gas cavity in [NiFeSe] hydrogenases resurrected *in silico*

Authors: Takashi Tamura[1,2,3]*, Naoki Tsunekawa[2], Michiko Nemoto[1], Kenji Inagaki[1], Toshiyuki Hirano[2], Fumitoshi Sato[2]

Affiliations:

[1]Graduate School of Life and Environmental Science, Okayama University, Okayama, 700-8530, Japan.

[2]Institute of Industrial Science, the University of Tokyo, Komaba 153-8505, Japan.

[3]PRESTO, Japan Science and Technology Agency.

*Correspondence to: tktamura@okayama-u.ac.jp.

This PDF file includes:

supplementary Figures S1 to S9

supplementary Table S1

Supplementary Fig. S1

>*D. vulgaris* Hildenborough

GACCCGTGActgggctgtgccgtgcacgtgctgcacgctgagtccggcaaggtcgccgtcatcg
aagtgaagtag

ΔG = -27.70 kJ/mol

```
          10        20
  |   CG  A       gu  c     cg
--GACC  UG cugggcu  gc gugca  \
  cugg  ac ggccuga  cg cacgu  u
\ ^   a-  -       gu  -     cg
     50          40         30
       60
  gccg-    c
       ucau g
       agug a
  gauga    a
      70
```

>*D. vulgaris* Miyazaki F

GACCCGTGActgggctgtgccgtgcacgtgctgcacgctgaatccggcaaggtctccgtcgtcg
aagtgaagtag

ΔG = -22.80 kJ/mol

```
                10         20           30
GACC-------  -    u-     g     ug--  -|   c
           CG UGAc  gggcu ugccg    ca cgug \
           gc gcug  ucugg acggc    gu gcac u
gaugaagugaa  u    cc     a     cuaa  c^   g
    70        60         50         40
```

>*D. alaskensis* G20

GACCCGTGActgggctgcgccgtgcacgtgctgcacgcagagtccggtaaggtttccgtagtcg
aattctag

ΔG = -23.90 kJ/mol

```
                          10        20
---------   --------| GUG       gc  c     cg
         GAC        CC   Acugggcu  gc gugca  \
         cug        gg   uggccuga  cg cacgu  u
gaucuuaag   augccuuu^ aa-       ga  -     cg
 70        60        50          40          30
```

>*D. piger*

GACCCGTGActgggctgtgccgtgcacgtgctgcacgctgagaccggcgaagaatccatcgtga
acctgggctag

ΔG = -20.80 kJ/mol

```
            10        20
GA--   .-GUGA| g   u    c   c
    CCC      cu ggc gugc gug a
    ggg      ga ucg cacg cgu c
gauc   \ ----^ g   -    u   g
    70        40         30
                 50
         accg--    aga
               gcga   a
               ugcu   u
         uccaag    acc
                60
```

> *B. wadsworthia* 3-1-6

GACCCGTGActgggttgtgccgtgcacgtgctgcacgccgaatccggcaaggttgccgtcatca
atatggattaa

ΔG = -23.50 kJ/mol

```
          10        20
  |   CG  A       g  c      cg
--GACC  UG cuggguu ug cgugca  \
  uugg  ac ggccuaa gc gcacgu  u
\ ^   a-  -       -  c      cg
     50           40         30
          60
  g----    cau
       ccgu   \
       ggua   c
  aauua    uaa
      70
```

>*D. baculatum*

GACCCGTGActgggctgtgccgtgcacgtgctgcacgctgagaccggtgaagaacacgttgtca
acattgactaa

ΔG = -21.50 kJ/mol

```
                           10        20
------------   C-    ------|    g  gu  c     cg
            GAC  CGUG      Acugg cu  gc gugca  \
            cug  gcac      uggcc ga  cg cacgu  u
aaucaguuacaa   uu    aagaag^    a  gu  -     cg
    70        60        50        40         30
```

>*D. salexigens*

GACCCG<u>TGA</u>ctgggttgtgccgtgcacgtgctgcacgcagagaccggtgaagaacatgttgttc
acgtaggcgaaggttgctaa

ΔG = -26.80 kJ/mol

```
        10           20
GACCC UG   .-g    g-|  c     cg
     G  Acu   gguu  ugc gugca  \
     c  ugg   ccag  acg cacgu  u
aau-- gu   \ -    ag^  -     cg
     80            40          30
                        50
             g--------     g  c
                      gugaa aa a
                      cacuu uu u
             aagcggaug     g  g
                 70         60
```

>*D. africanus* Walvis Bay

GATCCG<u>TGA</u>ctgggttgcgccgtgcacgtgttgcacgctgagactggcgaaaagaccgttgtca
ctgtcgagtag

ΔG = -22.40 kJ/mol

```
              10            20           30
------   CC     ug-    g---     -  g-  -|   u
      GAU  GUGAc   gguu    cgcc gu  ca cgug \
      cug  cacug   ccag    gcgg ca  gu gcac u
gaugag   u-     uug    aaaa    u  ga  c^   g
    70         60         50         40
```

>*D. hydrothermalis*

GATCCA<u>TGA</u>ctgggctgtgccgtgcacgtgctgcacgcagagaccggtgaagagcatgtttacc
acgtgggagaaggctgctag

ΔG = -26.40 kJ/mol

```
          10        20
.-GAUCCAUG|    g  g   c     cg
          Acugg cu ugc gugca  \
          uggcc ga acg cacgu  u
\ --------^    a  g   -     cg
                 40          30
  50         60
  ga  -   aug   a  a
    ag agc   uuu cc c
    uc ucg   aga gg g
  ga  g   ga-   g  u
     80         70
```

>*D. postgatei* 2ac9

GACCCCTGActgggctgcgccgtccacgtgctggacgcagataccggcaagcaaatcaaagtgg
aagttcccctgtaa

ΔG = -17.10 kJ/mol

```
          10          20
.-GACCCC  A    g--|   gcc     cg
        UG cugg   cugc   gucca  \
        ac ggcc   gacg   caggu  u
\ ------  -    aua^   ---     cg
                 40            30
 50        60
  agcaaaucaa  u  aa
            ag gg  \
            uc cc  g
  aaug------  c  uu
               70
```

>*D. phosphitoxidans*

GACCCCTGActgggttgcgccgtccacgtgctggacgtagaaaccggtcggaccgttaaagtag
acgtacccttataa

ΔG = -23.00 kJ/mol

```
          10        20
.-GACCC|       guugcgc      cg
       CUGAcugg       cgucca  \
       ggcuggcc       gcaggu  u
\ -----^       aaagau-      cg
       50        40         30
                  60
  ac---------    aa
             cguu  a
             gcag  g
  aauauucccau    au
         70
```

>*D. autrotrophicum* HRM2

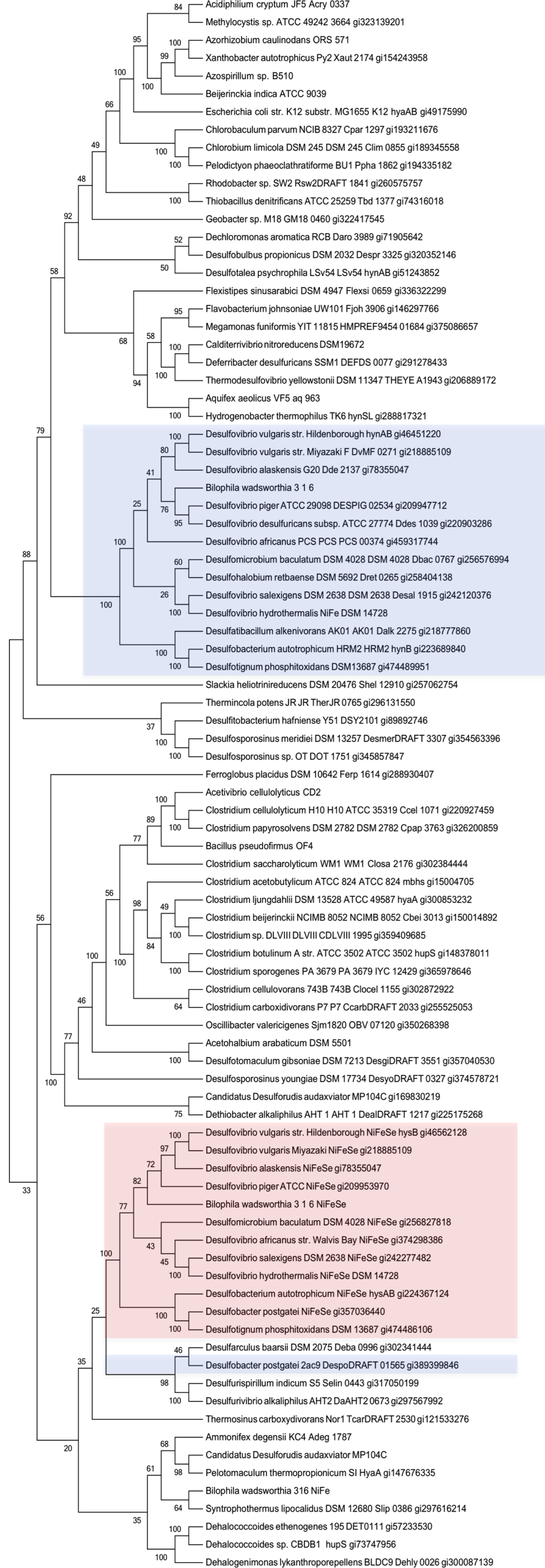
GACCCATGActgggctgtgccgtgcacgtgctgcacgcagagacaggcaaaacaaacgttgttg
agattccgctctag

ΔG = -18.70 kJ/mol

```
          10        20
.-GACCCA| A   gg  g   c     cg
        UG cug  cu ugc gugca  \
        ac gac  ga acg cacgu  u
\ ------^ g   a-  g   -     cg
                 40         30
 50        60
  aaacaaacguuguu   au
                gag  u
                cuc  c
  gau-----------   gc
                    70
```

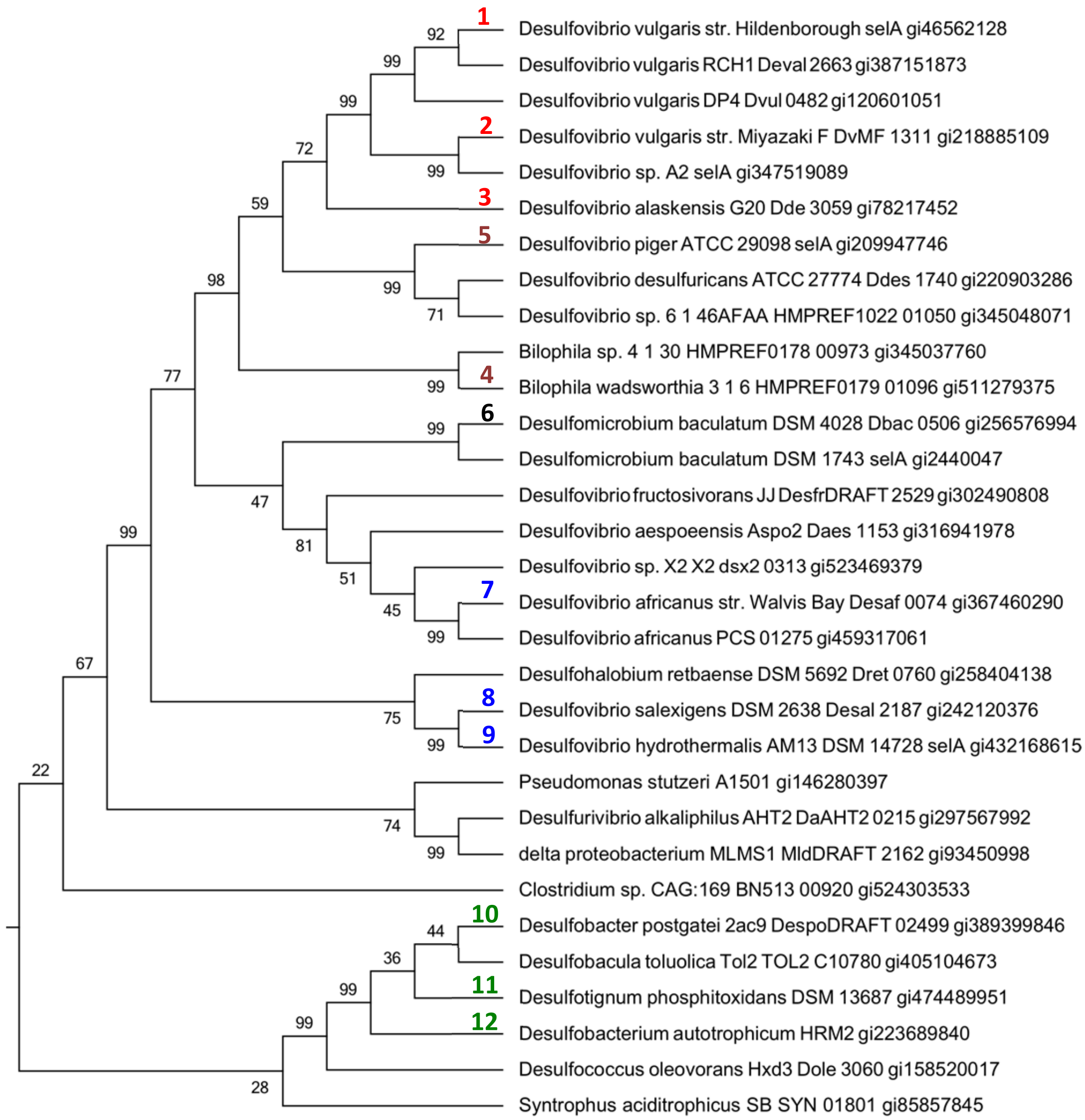
Supplementary Fig. S1: Structural models of the SECIS element predicted from the sequence immediately after the in-frame opal (UGA) codon. The stabilizing energy and secondary mRNA structure were computed on the mFold web server: http://mfold.rna.albany.edu/

supplementary Fig. S2

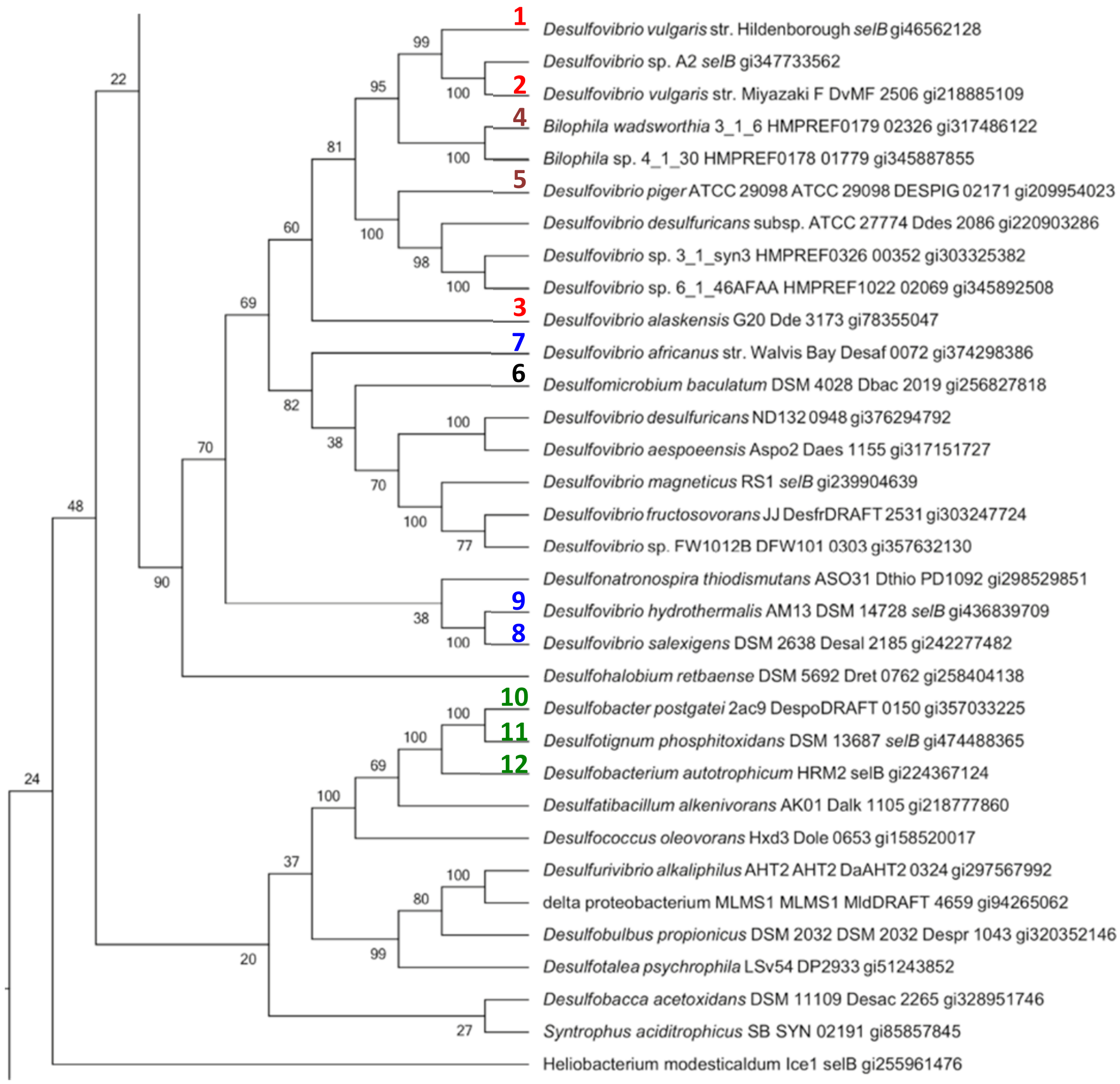


Supplementary Fig. S2: The phylogeny of 88 [NiFe(Se)] Hases inferred by the ML method. Below the branches are ML bootstrap percentages. Clades containing [NiFe] and [NiFeSe] Hases are shown in blue and red boxes, respectively.

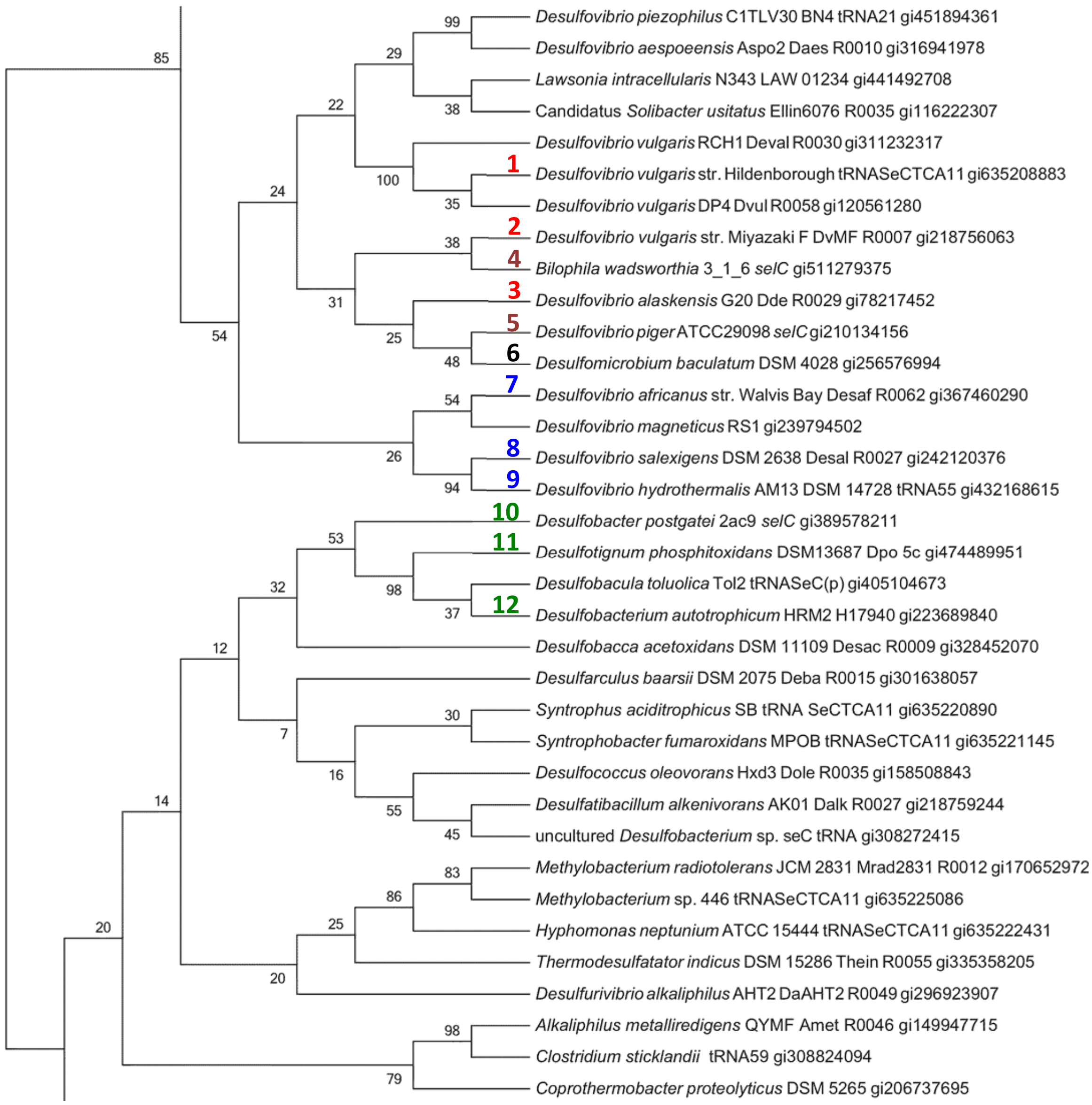
Supplementary Fig. S3



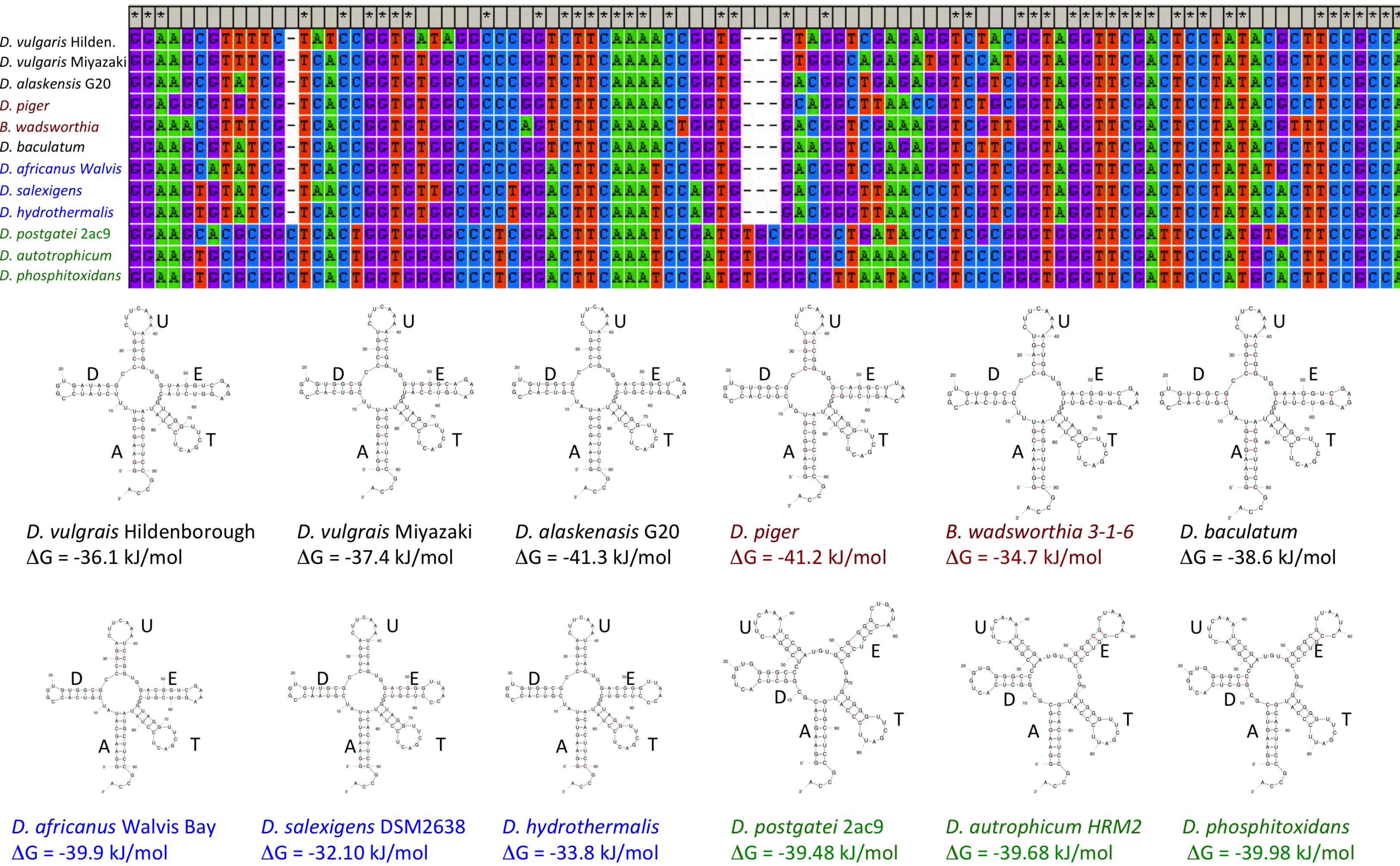
Supplementary Fig. S3: Phylogenetic relationships of *selA* genes in a maximum-likelihood phylogenetic tree made from 74 homologous lineages. Genes from sulphate-reducing bacteria of terrestrial origin (1-3), from clinical isolates from human faeces (4-5), of marine vent origin (7-9), and of sediment origin (10-12) are numbered in colors red, brown, blue, and green, respectively. Scores designated at each branch represent the percent of the 300-times Bootstrap test.



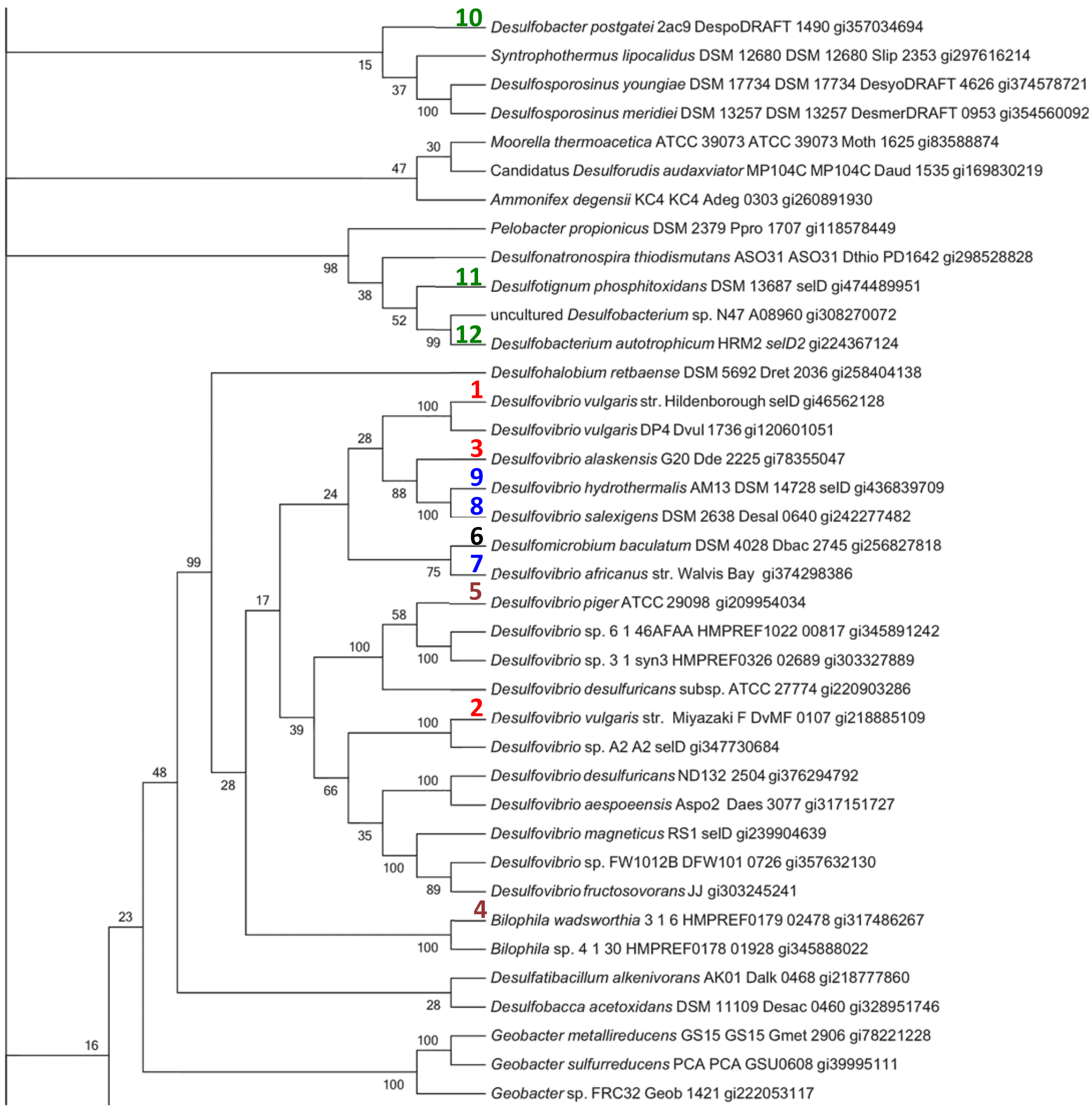
Supplementary Fig. S4: Phylogenetic relationships among the *selB* genes in a maximum-likelihood phylogenetic tree made from 88 homologous lineages. Genes from sulphate-reducing bacteria of terrestrial origin (1-3), from clinical isolates from human faeces (4-5), of marine vent origin (7-9), and of sediment origin (10-12) are numbered in colors red, brown, blue, and green, respectively. Scores designated at each branch represent the percent of the 300-times Bootstrap test.



Supplementary Fig. S5: Phylogenetic relationships of *selC* gene in the maximum-likelihood phylogenetic tree made from 48 homologous lineages. Genes from sulphate-reducing bacteria of terrestrial origin (1-3), from clinical isolates from human feaces (4-5), of marine vent origin (7-9), and of sediment origin (10-12) are numbered in colors red, brown, blue and green, respectively. Scores designated at each branch represent the percent of the 500-times Bootstrap test. The cut-off value for the consensus tree was set at a bootstrap value of 15%. Accordingly, sequences encoding *selC* were hard to discern their phylogenetic relationships except for the three linages (10-12) due to the extra 3 bases that they have between the E and T arms of their predicted cloverleaf model.



Supplementary Fig. S6: Sequence alignment and cloverleaf models for the tRNA^{UGA} sequences encoded in genomes harboring [NiFeSe] Hase genes.



Supplementary Fig. S7: Phylogenetic relationships of the *selD* genes in a maximum-likelihood phylogenetic tree made from 95 homologous lineages. Genes from sulphate-reducing bacteria of terrestrial origin (1-3), from clinical isolates from human faeces (4-5), of marine vent origin (6-9), and of sediment origin (10-12) are numbered in colors red, brown, blue, and green respectively. Scores designated at each branch represent the percent of the 300-times Bootstrap test.

Fig. S8

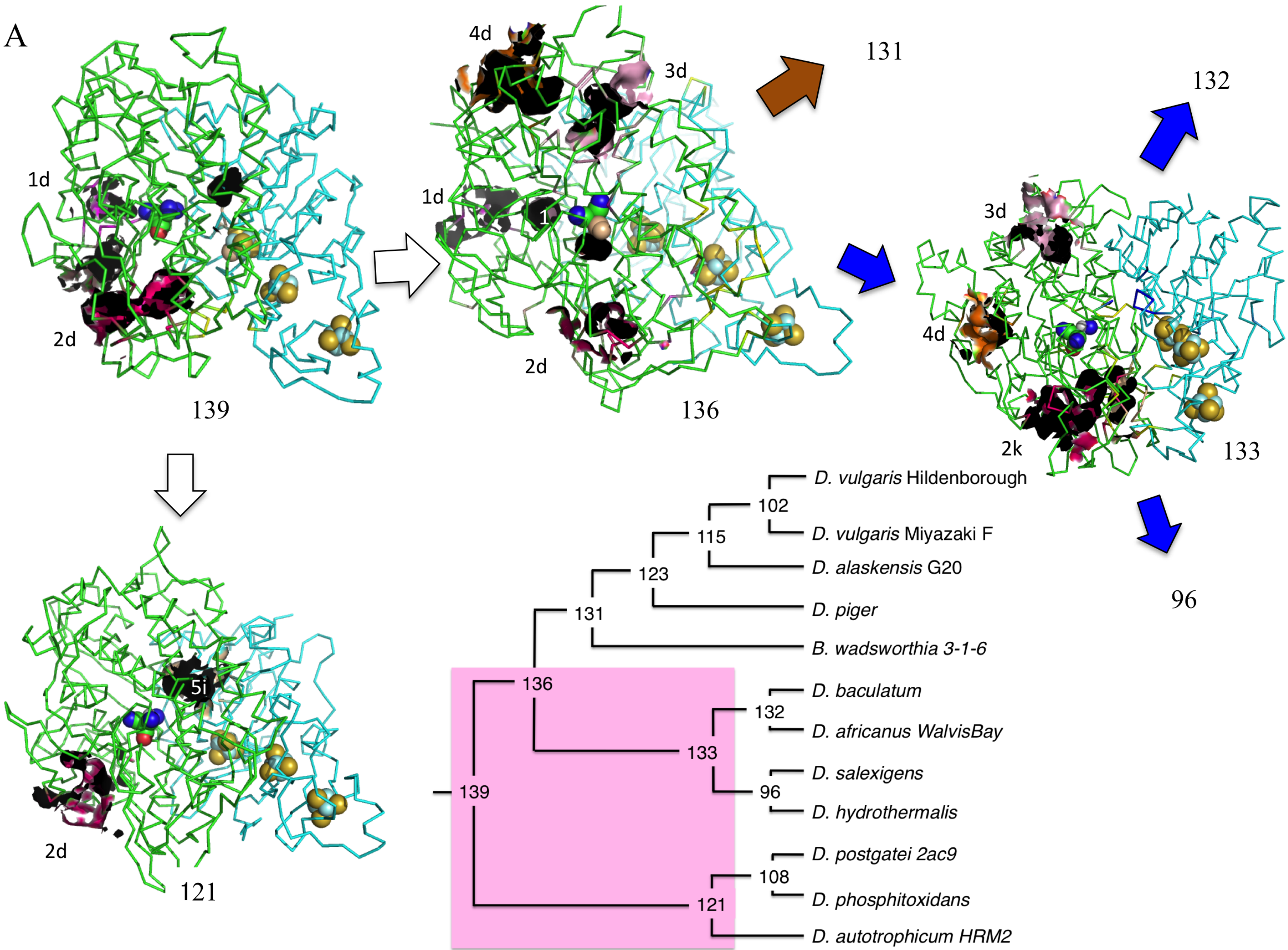

A
1d
2d
139
4d
3d
1d
2d
136
131
132
3d
4d
2k
133
96
5i
2d
121
D. vulgaris Hildenborough
102
D. vulgaris Miyazaki F
115
D. alaskensis G20
123
D. piger
131
B. wadsworthia 3-1-6
136
D. baculatum
132
D. africanus WalvisBay
133
D. salexigens
139
96
D. hydrothermalis
D. postgatei 2ac9
108
D. phosphitoxidans
121
D. autotrophicum HRM2

Fig. S8

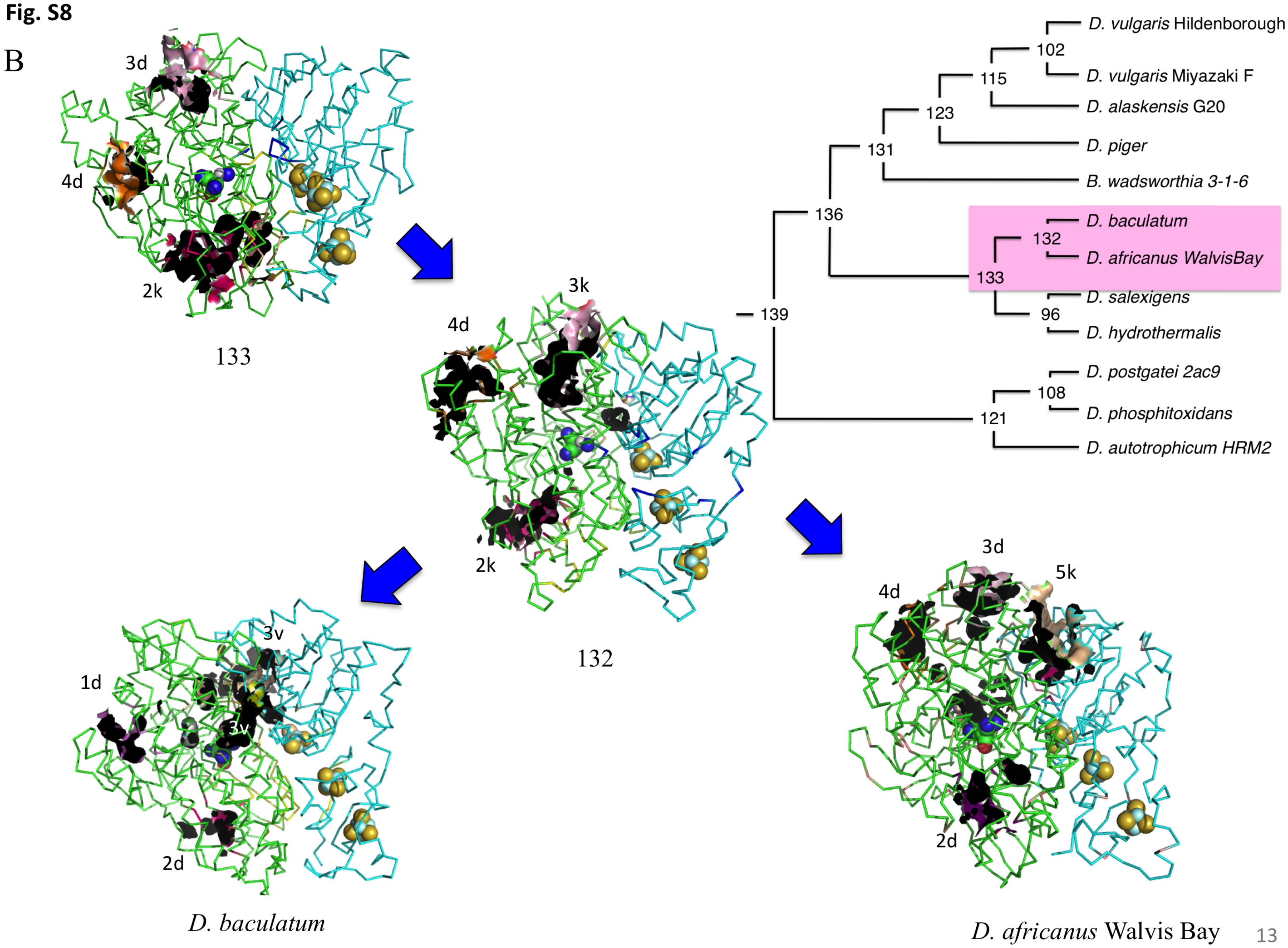

B
3d
4d
2k
133
3k
4d
2k
132
1d
3v
3v
2d
D. baculatum
3d
5k
4d
2d
D. africanus Walvis Bay
D. vulgaris Hildenborough
102
D. vulgaris Miyazaki F
115
D. alaskensis G20
123
D. piger
131
B. wadsworthia 3-1-6
136
D. baculatum
132
D. africanus WalvisBay
133
D. salexigens
139
96
D. hydrothermalis
D. postgatei 2ac9
108
D. phosphitoxidans
121
D. autotrophicum HRM2

Fig. S8

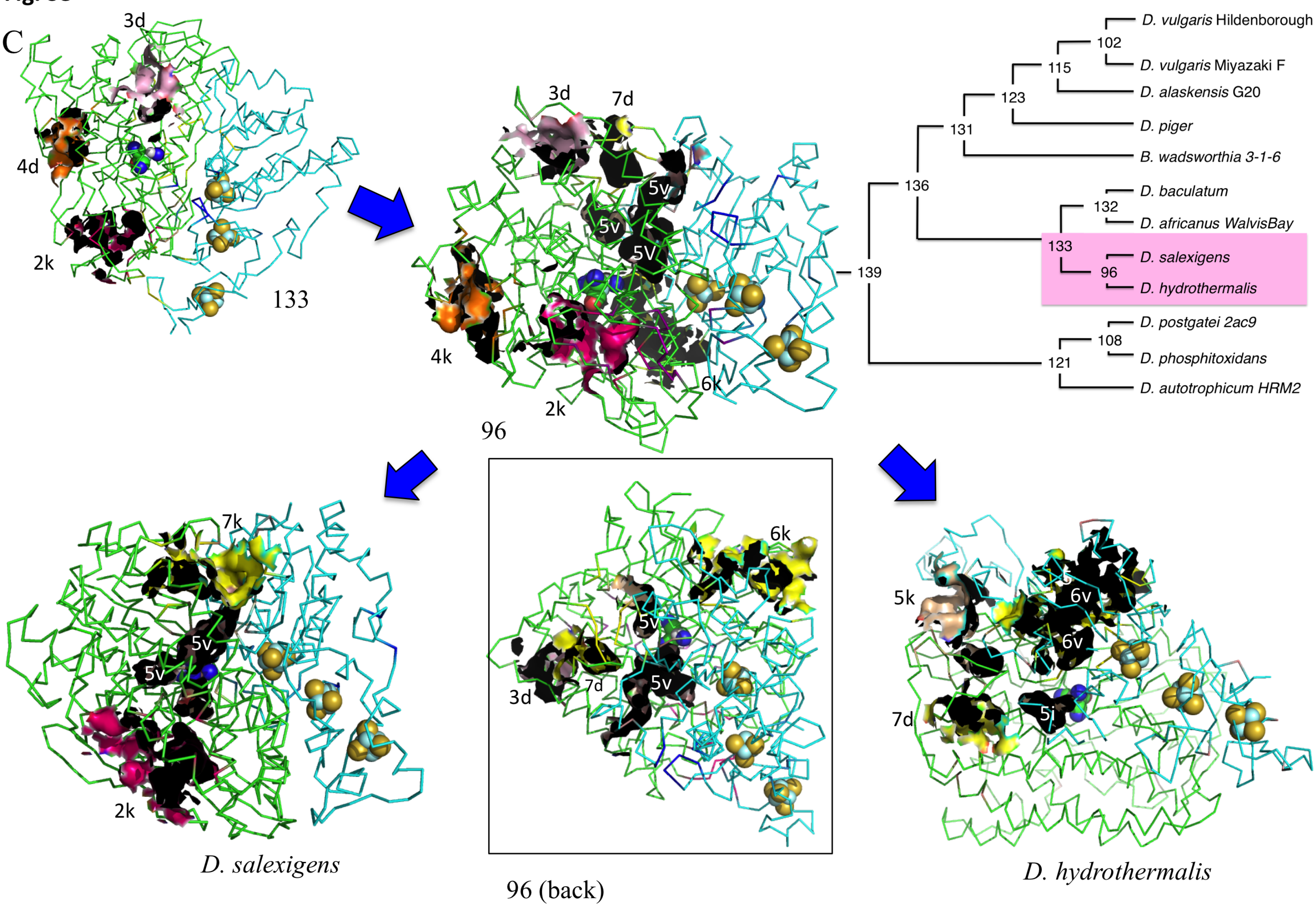

C
3d
4d
2k
133
3d
7d
5v
5v
5V
4k
2k
6k
96
D. vulgaris Hildenborough
102
D. vulgaris Miyazaki F
115
D. alaskensis G20
123
D. piger
131
B. wadsworthia 3-1-6
136
D. baculatum
132
D. africanus WalvisBay
133
D. salexigens
96
D. hydrothermalis
139
D. postgatei 2ac9
108
D. phosphitoxidans
121
D. autotrophicum HRM2
7k
5v
5v
2k
D. salexigens
6k
5v
3d
7d
5v
96 (back)
5k
6v
6v
7d
5i
D. hydrothermalis

Fig. S8

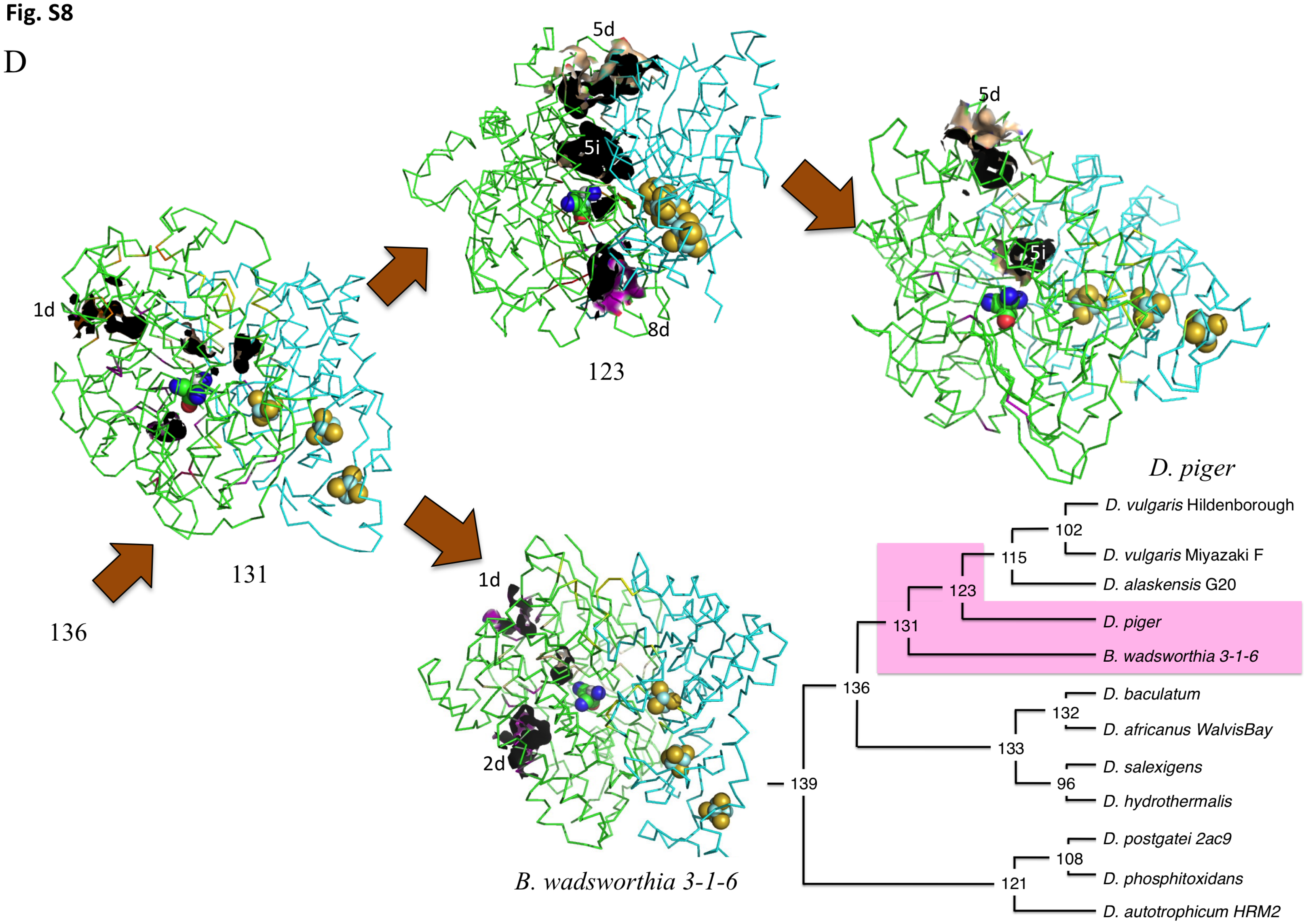

D
5d
5i
8d
123
5d
5i
D. piger
1d
131
136
1d
2d
B. wadsworthia 3-1-6
D. vulgaris Hildenborough
102
115
D. vulgaris Miyazaki F
D. alaskensis G20
123
131
D. piger
B. wadsworthia 3-1-6
136
D. baculatum
132
D. africanus WalvisBay
133
D. salexigens
139
96
D. hydrothermalis
D. postgatei 2ac9
108
D. phosphitoxidans
121
D. autotrophicum HRM2

Fig. S8

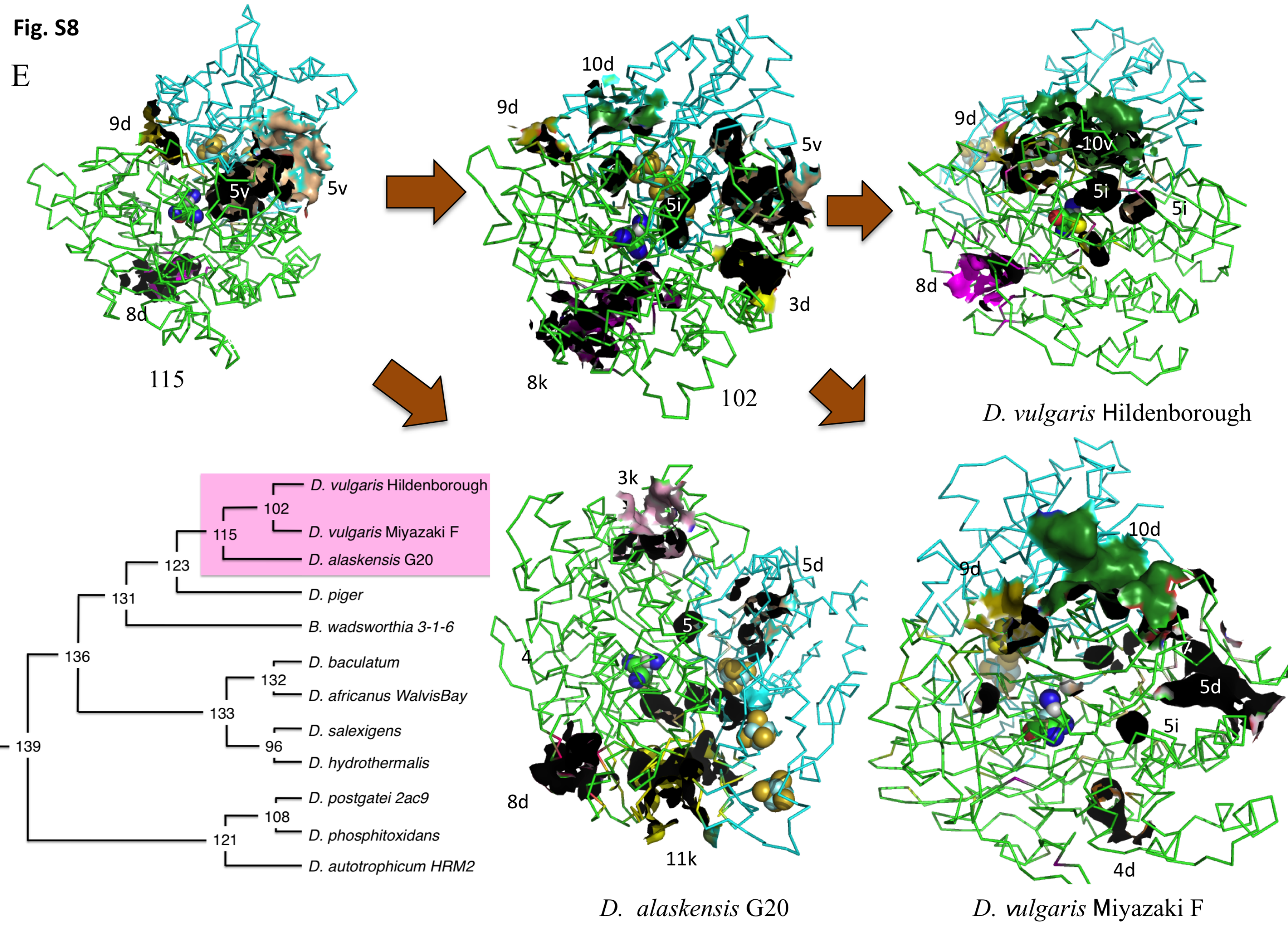
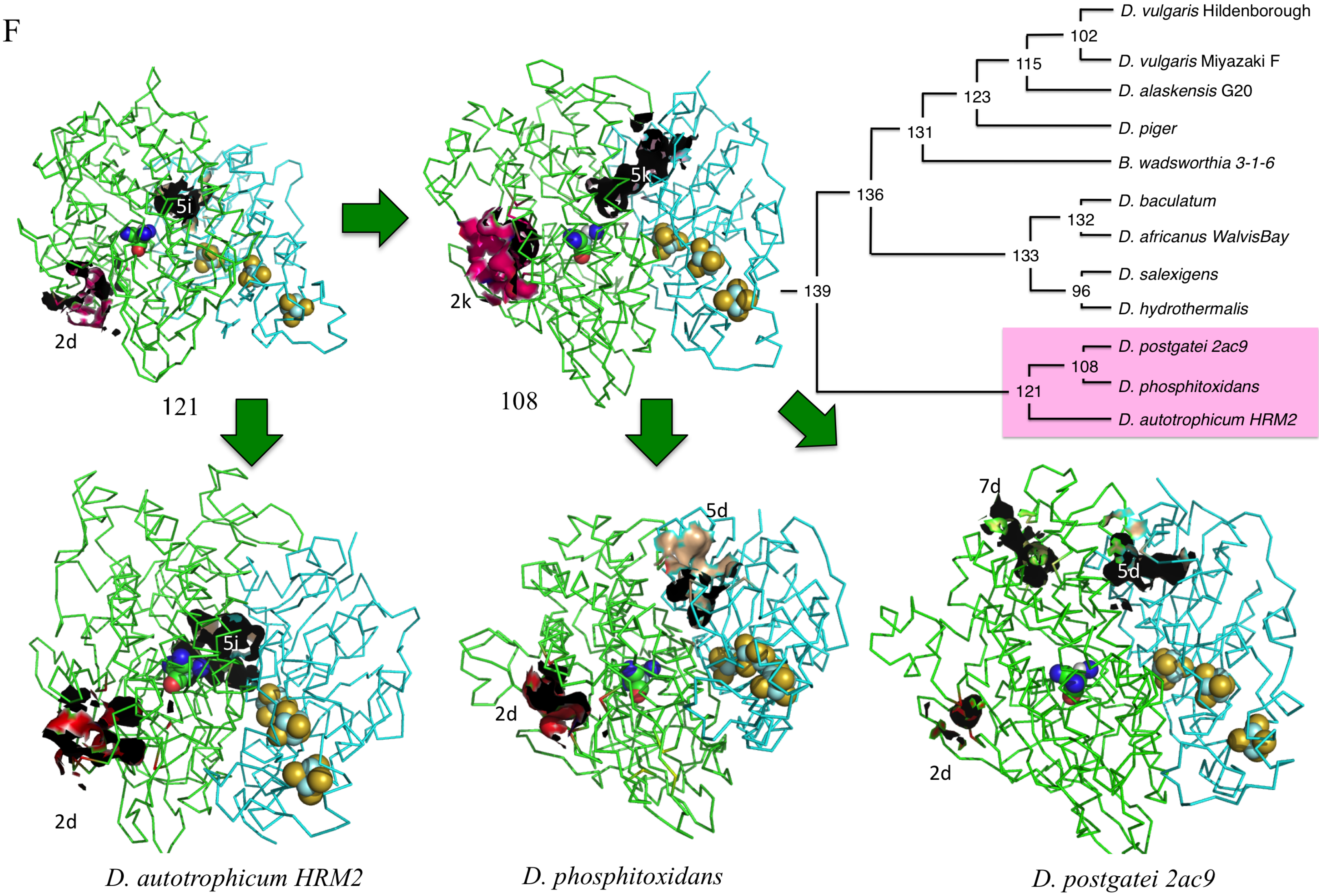

E
9d
5v
5v
8d
115
10d
9d
5v
5i
3d
8k
102
9d
10v
5i
5i
8d
D. vulgaris Hildenborough
D. vulgaris Hildenborough
102
D. vulgaris Miyazaki F
115
D. alaskensis G20
123
D. piger
131
B. wadsworthia 3-1-6
136
D. baculatum
132
D. africanus WalvisBay
133
D. salexigens
96
D. hydrothermalis
139
D. postgatei 2ac9
108
D. phosphitoxidans
121
D. autotrophicum HRM2
3k
5d
5
4
8d
11k
D. alaskensis G20
10d
9d
5d
5i
4d
D. vulgaris Miyazaki F

Fig. S8

F


5i
2d
121
5k
2k
108
D. vulgaris Hildenborough
102
D. vulgaris Miyazaki F
115
D. alaskensis G20
123
D. piger
131
B. wadsworthia 3-1-6
136
D. baculatum
132
D. africanus WalvisBay
133
D. salexigens
96
D. hydrothermalis
139
D. postgatei 2ac9
108
D. phosphitoxidans
121
D. autotrophicum HRM2
5i
2d
5d
2d
7d
5d
2d
D. autotrophicum HRM2
D. phosphitoxidans
D. postgatei 2ac9

Supplementary Figure S8: History of cavity development in [NiFeSe]Hases Comparative morphology of the cavities in [NiFeSe] Hases from the common ancestor 136 to the extant [NiFeSe]Hases *via* the intermediate ancestral forms. Cavities are identified by location and amino acid residues involved, and are designated by numbers and morphological designations, d, k, v, and i. The Ni-Fe and Fe-S clusters are shown in sphere designation, and the large and small subunits are designated in green and blue wire forms, respectively. (A-C) Blue arrows show evolutionary trait under absolutely anaerobic aquatic environments. (D, E) Brown arrows show path to terrestrial divergence including human faeces-derived isolates. (F) Green arrows show the path to marine sediment-derived [NiFeSe] Hases. Phylogenetic tree on each panel A-F highlights the genetic trait where the panel illustrates.

Supplementary Fig. S9

```
                                       10        20        30        40        50        60
                             ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.          ---------------------------------MSLTRRDFVKLCTGTVAGFGISQMFHP
102                          ---------------------------------MSLTRRDFVKLCTGTVAGFGISQMFHP
D. vulgaris Miyazaki F       ---------------------------------MSLNRRDFVKLCTGTVAGFGISQMFHP
115                          ---------------------------------MSLTRRDFVKLCTGTVAGFGVSQMFHP
D. alaskensis G20            ---------------------------------MSLSRRNFVKLCTGTVAGFGVSQMFHP
123                          ---------------------------------MSLTRRDFVKLCTGTVAGFGVSQMFHP
D. piger                     ---------------------------------MSLNRRDFVKLCTGTVAGFGVAQMFHP
131                          ---------------------------------MSLTRRDFVKLCTGTVAGFGVSQMFHP
B. wadsworthia 3-1-6         ---------------------------------MALSRRDFVKLCSGTVAGFGVSQMFHP
136                          ---------------------------------MSLTRRDFVKLCTGTVAGFGVSQMFHP
133                          ---------------------------------MSLTRRDFVKLCTGTVAGFGVSQMFHP
132                          ---------------------------------MSLTRRDFVKLCTGTVAGFGVSQMFHP
D. baculatum                 ---------------------------------MSLSRREFVKLCSAGVAGLGISQIYHP
D. africanus WalvisBay        ---------------------------------MSLDRRDFLKLCTGTVAGLGVSQMVHP
96                           ---------------------------------MSLTRRDFVKMCTGTVAGFGISQMFNP
D. hydrothermalis            ---------------------------------MSLTRRDFVKMCTGTVAGFGISQVFNP
D. salexigens                ---------------------------------MSLTRRDFVKMCTGTVAGFGISQMFNP
121                          -------------------VKDEQTLPDAQQEGTGVSRRDFLKTLTGTAAGIGISQMFNP
D. autotrophicum HRM2         MFYCLSVTRCTSNSIKEVPLKEEQNLSDDQKEGSGVSRRGFLKALTGTAAGIGLSQMINP
D. postgatei 2ac9            -------------------VKDDHMLPETQQEKSGVTRRCFLKTVAGAAAGIGVSQMVNP
108                          -------------------VKDEQTLPDAQQEKAGVSRRNFLKTLTGTAAGIGISQMFNP
D. phosphitoxidans           -------------------VKDKQSLPDAQPKLAGISRRNFLKTLTGTAAGIGISQVFNP
139                          ---------------------------------MSLTRRDFVKLCTGTAAGFGVSQMFHP

                                       70        80        90       100       110       120
                             ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.          AVHEAL-AG--T-LTGERP-PVFWLQGQGCTGCSVTLLNSVHPSIADVLLKVISLEFHPT
102                          AVREAL--G--T-LTGERP-PVFWLQG-GCTGCSVSLLNSVHP-IADVLLKVISLEFHPT
D. vulgaris Miyazaki F       AVCEAI-SG--S-LNGERP-PVLWLQGQGCTGCSVSLLNSVHPTIADVLLKVISLEFHPT
115                          AVREAL--G--T-LTGERP-PVFWLQG-GCTGCSVSLLNSVHP-IADVLLKVISLEFHPT
D. alaskensis G20            AIRDAL-AD--T-LKGDRP-PVLWLQGQGCTGCSVSLLNSVHPSIKDVLLDVISLEFHPT
123                          AIREALG----T-LTGERP-PVFWLQG-GCTGCSVSLLNSVHP-IADVLLKVISLEFHPT
D. piger                     AVREAL---AGT-LTGERP-PVIWLQGQGCTGCSVSLLNNVNPSIADVLLKIISLEYHPT
131                          AIGEAL-----T-LTGERP-PVFWLQG-GCTGCSVSLLNSVHP-IADVLLKVISLEFHPT
B. wadsworthia 3-1-6         AIHEAF---AQT-LTGERP-PVFWVQGQGCTGCSVTLLNSTHPSIADVLLKIISLEFHPT
136                          AIVEAL-----K-LAAEKP-PVFWLQG-GCTGCSVSLLNTVHP-IADVLLEVISLEFHPT
133                          AIVHAL-----KKVAAEKA-PVFWLQG-GCTGCSVSLLNTVHP-IAEVLLEVISLEFHPT
132                          AIVHAL-----KKVAAEKA-PVFWLQG-GCTGCSVSLLNTVHP-IAEVLLEVISLEFHPT
D. baculatum                 GIVHAM-----T-EGAKKA-PVIWVQGQGCTGCSVSLLNAVHPRIKEILLDVISLEFHPT
D. africanus WalvisBay        AIVRAL-----E-KATSGKAPVFWLQGCGCTGCSVSILNSVHPTIADVLLKVISMDFHPT
96                           SVVHAL-----K-KFVPN---VFWLQG-GCTGCSVSILNSVHP-IAEVLLDVINLDYHPT
D. hydrothermalis            SVVHAL-----K-KFVPH---VFWLQGQGCTGCSVSILNSVHPSIAEVLLEVITLDYHPT
D. salexigens                SVVHAL-----K-KFVPN---VFWLQGQGCTGCSVSILNSVHPSIAEVLLDVINLDYHPT
121                          ALVQAL-----K-KGLEHH-PVLWIQGQGCTGCSVSLLNSVDPTIADVLLKVISLQYHPT
D. autotrophicum HRM2         ALVSAM-----K-KGLENH-PVLWIQGQGCTGCSVSLLNSVEPTIAKVLLDIISLQYHPT
D. postgatei 2ac9            TLVKAL-----E-KGLERH-PVLWIQGQACTGCSVSLLNSVDPSIADVLLKVISLQYHPT
108                          ALVQAL-----E-KGLERH-PVLWIQGQGCTGCSVSLLNSVDPTIADVLLKVISLQYHPT
D. phosphitoxidans           ALIQAL-----E-KGLERH-PVLWIQGQGCTGCSVSLLNSVDPSIADVLLKVISLQFHPT
139                          AIVEAL-----K-MAAEKP-PVFWLQG-GCTGCSVSLLNTVHP-IADVLLEVISLQFHPT
```

Fig. S9

```
                                 130       140       150       160       170       180
                          ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.       VMAWEGEHAIEHMRKVAEKFKGKFFLVIEGSVPVEADGKYCIIG-EA-----NHHEISMV
102                       -MAWEGEHAIEHMMKIAEK-KGKFFLVVEGSVPIEAD-KYCIIG-EA-----DHKEISMV
D. vulgaris Miyazaki F    VMAWEGEPAMEHMMKIAEQYKGKYFVVVEGAVPTEADGKYCIIG-EA-----HHKEISMV
115                       -MAWEGEHAIEHMMKIAEK-KGKFFLVVEGSMPIAAD-KYCIIG-EA-----DHKEISMA
D. alaskensis G20         VMAWEGEHAIEHMMKIAEKYEGKFFVVVEGSIPLAADGKYCIIG-DI-----DHKEISIA
123                       -MAGEGEDAFEHMMKIAEK-KGKFFLVVEGSIPI-ADDKYCIVG-EA-----DHKEITMA
D. piger                  VMGGEGEHDAYTHMLNIAKNFKGKFFLAIEGSIPLAKDGRYCVVA-EE-----GHTEITMA
131                       -MAAEGEDAFEHMMKIAEK-KGKFFLVVEGAIPI-ADDKYCIVG-EA-----DHKEITMT
B. wadsworthia 3-1-6      VMAAEGEGAYEHMMRVAEKFKGKFIFAVEGAVPVAHDGKCCVVA-EA-----DHHEVTMT
136                       -MAAEGEKALEHMLEIAEK-KGKFFLVVEGAIPI-ADDNYCIVG-EA-----DHKEITMT
133                       -MAAEGEKALEHMFEIAEK-KGKFFLVVEGAIPT-AEDHYCIVG-EA-----DHKEITMT
132                       -MAAEGEMALEHMFEIAEK-KGKFFLVVEGAIPT-AEDHYCIVG-EA-----DHKEITMT
D. baculatum              VMASEGEMALAHMYEIAEKFNGNFFLLVEGAIPTAKEGRYCVVG-ETLDAKGHHHEITVM
D. africanus WalvisBay    LMGAEGEMAMEHMFEVAKAKKGQYFVVLEGAIPTADE-RYCIIG-DV-----HHKEISIN
96                        -MGSEGDEAWDHMMGVAE-NKGKYILIVEGSVPTAEN-HYCIVGAGA-----DHKEYTMT
D. hydrothermalis         IMGSEGAQAWDHMMGVAGKEQGKYILIVEGSVPTAENGHYCIVGAGA-----DHKEYTMS
D. salexigens             IMGSEGHEAWDYMMSQAEANKGKYIVIVEGSVPTAENGHYCIVGAGA-----DHKEYTMT
121                       VMAGEGETALDHLYEVAEEYKGKFSLVVEGAIPMAADGKYCIVG-EN-----DHKEITMV
D. autotrophicum HRM2     VMASEGETALHNLYRVAEEYKGKFSLVVEGAIPLAANGKYCIVG-ER-----DNKDITMV
D. postgatei 2ac9         IMAGEGKTALENLFGIAREYKGKFSLVVEGAIPTAAEGKYCGVG-EY-----AHKEYTMV
108                       VMAGEGETALDHLYEVAKEYKGKFSLVVEGAIPMAADGKYCIVG-EN-----DHKEITMV
D. phosphitoxidans        VSECEGETAMNHVYEVANEYKGRFSLVIEGAIPVAADGKYCILG-EI-----GHREITMV
139                       -MAAEGEKALEHMFEIAEK-KGKFFLVVEGAIPI-ADDNYCIVG-EE-----DHKEITMT

                                 190       200       210       220       230       240
                          ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.       DALKEFGPNAAAVLAVGTCAAYGGIPAAEGSETGATAVSKFLGDNGIKTPVVNIPGCPPH
102                       DAIKDLAANAAAVLAVGTCAAYGGIPAAEGSETGAMA-SQFFEDNG-KTPVVNIPGCPPH
D. vulgaris Miyazaki F    DAMKSVAANAAAVLAVGTCAAYGGIPAAQGSETGAKSVSQFFKDNGIATPVVNIPGCPPH
115                       DAIKDLAANAAAVLAVGTCAAYGGIPAAEGNVTGAMA-SQFFEENG-KTPVVNIPGCPPH
D. alaskensis G20         DAMRDIAAKSAAVLAVGTCAAYGGIPAAEGNLTEAVSVQQFFEQEGIKTPVVNIPGCPPH
123                       DVIKELAANAAAVLAVGTCAAYGGIPAAEGNVTGAMG-SEFFKENG-KTPVVNIPGCPPH
D. piger                  DLVKKLAPDAAAVLALGTCAAYGGIPAAKGSVTEAMGTGALLKQAGIKTPVVNIPGCPPQ
131                       DVIKELAANAAAVLAVGTCAAYGGIPAAEGNVTGAMG-CEFFKENG-KTPVVNIPGCPPH
B. wadsworthia 3-1-6      EVTKVLAANAAAVLAVGTCAAYGGIPAGKGNETGAMGVSAFLKKEGIPAPVINIPGCPPH
136                       EVIKELATNAAAVLAVGTCAAYGGIPAAEGNLTGAMG-RDFFEENG-KTPVVNIPGCPPH
133                       EVIQELATNAAAVVAVGTCAAYGGIPAAEGNLTGSMG-RDFFEENG-KTPVVNIPGCPPH
132                       EVIQELATNAAAVVAVGTCAAYGGIPAAEGNLTGSMG-RDFFEEKG-KTPVVNIPGCPPH
D. baculatum              ELIRDLAPKSLATVAIGTCAAYGGIPAAAGNVTGSKSVRDFFAEEKIEKLLVNVPGCPPH
D. africanus WalvisBay    QAMLELGKDAAACIALGSCGAYGGIPAAKGNLTGCVGLRDFYQSKNISTPVVNIPGCPPH
96                        EATLEMAKNAAVVVNVGTCAAYGGIPAAEGNLTGSMS-TNFLAENG-KTPVVNIPGCPPH
D. hydrothermalis         EATLEMAKNAAVVVNVGTCAAYGGIPAAEGNLTGSMSVTNFLAENGVKTPVVNIPGCPPH
D. salexigens             EATLEMAKNAAVVVNVGTCAAYGGIPAAEGNLTGSMSVTNFLAENGVKTPVVNIPGCPPH
121                       DMIKELATMAGTVLAVGTCAAYGGIPAAKGNLTGATGVRDFFKENDINTPVVNIPGCPPH
D. autotrophicum HRM2     EMTTELGKMAGSVLAVGTCAAYGGIPAAKGNLTQATGVQNVFKKFDITTPVVNIPGCPPH
D. postgatei 2ac9         DLVKEVAPMAGSCLAVGTCAAYGGIPAAKGNVTGATGCRDFFAANSINTPIVNIPGCPPH
108                       DMVKELAPMAGSVLAVGTCAAYGGIPAAKGNVTGATGVRDFFKANDINTPVVNIPGCPPH
D. phosphitoxidans        DMVQELAPMAGSVLAVGTCAAFGGIPAAKGNVTGATGVRDFFKSKDITTPVVNIPGCPPH
139                       EVIKELATKAAAVLAVGTCAAYGGIPAAEGNLTGAMG-RDFFEENG-KTPVVNIPGCPPH
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Fig. S9

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                               250       260       270       280       290       300
                          ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.       PDWIVGTVVLA--------LDAIKKNGL-------EGGLAE----VVKVLDSDGRPTPFF
102                       PDWIVGTVVVA--------LNAIKENGL-------EAGLAE----VVKLLDADGRPTP-Y
D. vulgaris Miyazaki F    PDWIVGTVVVA--------LNAIKAKGL-------AAGLGD----VVKLLDADGRPTPFY
115                       PDWIVGTVVVA--------LDAIKENGL-------EAGLAE----VVKLLDADGRPTP-Y
D. alaskensis G20         PDWIVGTVVVA--------LDAIKKHGL-------QGGLGE----VVKLLDGDGRPTPFY
123                       PDWIVGTIVVA--------LNAIKENGL-------EAGLAE----VVKLLDADGRPMPY-
D. piger                  PDWIVGTIALA--------LQKIKEKGL-------EAGLAE----VVSLLDSEGRPLPFY
131                       PDWIVGTIVVA--------LNAIKENGL-------EAGLAE----VVKLLDADGRPMPY-
B. wadsworthia 3-1-6      PDWIVGTIGLG--------LQALATNTL---------GLL-----VKQGLDANGRPKAFY
136                       PDWIVGTLVVA--------LNAIKENGL-------EDGLAE----VVKILDDDGRPMLF-
133                       PDWMVGTLVVA--------LNAIKENGL-------EDGLAE----VVKILDDDGRPMLF-
132                       PDWMVGTLVVA--------LNAI----L-------EHGLAE----VVKILDDDGRPMLF-
D. baculatum              PDWMVGTLVAA---------------WSHVLNPTEHPLPE--------LDDDGRPLLFF
D. africanus WalvisBay    PDWIIGSLAFA---------------L-------EHGVEATAKI----LDAEGRPSVFF
96                        PDWMVGTLVVAINAIEEKG--------L-------QGGLAEVVKI----LDDDGRPTP-F
D. hydrothermalis         PDWMVGTLVVAINAIEEKG--------L-------QGGLAEIVTI----LDDEGRPTPFF
D. salexigens             PDWMVGTLVVAINAIEAKG--------L-------QGGLAEVVKI----LDDNGRPTPFF
121                       PDWIVGTIVHL---------------L-------KEGLPE--------LDDDGRPMLFF
D. autotrophicum HRM2     PDWIVGTIAHL---------------L-------TKGLPE--------LDENGRPLLFF
D. postgatei 2ac9         PDWIVLSIVHL---------------L-------EKGIPE--------LDDEGRPMLFF
108                       PDWIVGSIVHL---------------L-------KEGIPE--------LDDDGRPMLFF
D. phosphitoxidans        PDWIVGSIVHL---------------L-------NAGIPE--------LDYDGRPTLFF
139                       PDWIVGTLV---------------HVL-------EDGLPE--------LDDDGRPMLF-
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                               310       320       330       340       350       360
                          ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.       GRNIHENCPYLDKYDEGVMSATFTDKVGCRYDLGCKGPMTMADCFERKWNGGVNWCVQNA
102                       GRNIHENCPYLDKYDEGKMSETFT-KDGCRYD-GCKGPMTMSDCFERKWNGGVNWCVHNA
D. vulgaris Miyazaki F    GRNVHENCPYLEAYDAGKMCETFTKKEGCRYDLGCKGPMSMCDSFERKWNGGVNWCISNA
115                       GRNIHENCPYLDKFDEGKMSETFT-KDGCRYD-GCKGPTTMSDCFERKWNSGVNWCVDNA
D. alaskensis G20         GTNIHDNCPYLPQFEEYVMSETFTQKDGCRYELGCKGPSTMADCYKRKWNNGVNWCISNA
123                       GRNIHENCPYLDKFDEGKLSETFT-KDGCRYD-GCKGPATNSDCFERKWNSGVNWCVDNA
D. piger                  GRNVHENCPYLGKYDEGKFSATFTEKDGCRYDLGCKGPGAYCDSFERKWN-GVNWCVANA
131                       GQNIHENCPYLDKFDEGKLAETFT-KDGCRYD-GCKGPATNSDCFERKWNSGVNWCVDNA
B. wadsworthia 3-1-6      -KNVHMNCPHLSAFEAGHMVKTMSDKDGCRFSMGCKGPRSACDSFERKWNNGVNWCVNNA
136                       GENIHDNCPYLDKFDNDKFAETFT-KDGCRYD-GCKGPATNSDCFKRKWNSGVNWCVDNA
133                       GENIHDNCPYLDKFDNDKFAETFT-KDGCRYD-GCKGPATNSDCFKRKWNSGVNWCVENA
132                       GENIHDNCPYLDKFDNDKFAETFT-KAGCKYD-GCKGPATNSDCFKRKWNNGVNWCVENA
D. baculatum              GDNIHENCPYLDKYDNSEFAETFTKP-GCKAELGCKGPSTYADCAKRRWNNGINWCVENA
D. africanus WalvisBay    GENIHDNCPYLEHFENDNFAATFTQAG-CKYNLGCKGPACNSDCFKRKWNSGMNWCVENA
96                        GENIHDNCPYLEAFDNDEYAEIFT-PDKCRYE-GCKGPSANSDCFKRKWNGGVNWCVENS
D. hydrothermalis         GENIHDNCPYLEAFDNDEYAEVFTDPEKCRYELGCKGPSANSDCFKRKWNGGVNWCVENS
D. salexigens             GENIHDNCPYLEAFDNDEYAEIFTDPVKCRYELGCKGPSANSDCFKRKWNGGVNWCVENS
121                       GENIHDNCPYLDDFDEDIFAETFTDNKGCRMDLGCKGPDTYADCFKRKWNSGLNWCVDNA
D. autotrophicum HRM2     GENIHENCPYLDYFDQDIYSKTFTDKKGCRMELGCKGPDTYADCFRRKWNSGLNWCIENA
D. postgatei 2ac9         GENIHDNCPRLKMYEADQLSQTLSDPKGCRINLGCKGPSTYADCYKRKWNSGLNWCVDNA
108                       GENIHDNCPYLDDYDADLLAETLSDPKGCRMDLGCKGPDTYADCYKRKWNSGLNWCVDNA
D. phosphitoxidans        GENIHDNCPHLDDYDAAFMAATLSDPKGCRMDLGCKGPDTYADCYQRKWNSGLNWCVNNA
139                       GENIHDNCPYLDKFDNDKFAETFT-KDGCRFD-GCKGPATYADCFKRKWNSGVNWCVDNA
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Fig. S9

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                          370       380       390       400       410       420
                   ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden. VCIGCVEPDFPDGKSPFYQA--MSGCTPK--AAPA-GAT-------GRTTIAIDPVTRIE
102                 VCIGCVEPDFPD-KSPFYQ---MSGCTPK--AAPA-GAT-------GKTTIAIDPVTRIE
D. vulgaris Miyazaki F VCIGCVEPDFPDGKSPFYSA--MSGCTPK--AAPA-GAT-------GKATIAIDPVSRIE
115                 VCIGCVEPDFPD-KSPFYE---MSGCTPK--TAPA-GAT-------GKTTIAIDPVTRIE
D. alaskensis G20   VCIGCVEPDFPDGKSPFYES--MSGCTNK--MAAG-GVS-------GKTKIAIDPVTRIE
123                 VCIGCVEPDFPD-KSPFYEM--T---TPK--TAPA-GAN-------GKTTIAIDPVTRIE
D. piger            ICIGCTEPSFPDGQSPFYSN--MA----------------------KATIAIDPVTRIE
131                 VCIGCVEPDFPD-KSPFYEM--T---TSK--TAPA-GAN-------GKTTIAIDPVTRIE
B. wadsworthia 3-1-6 TCIGCTSPTFPDGQSPFYVN--MS----------------------KTVIAIDPVTRLE
136                 VCIGCVEPGFPD-MSPFYEM--T---ASK--TAPA-GAD-------GKIKIAIDPVTRIE
133                 VCIGCVEPGFPD-MSPFYEM--M---ASK--TAPA-GAD-------GKIKIAIDPVTRIE
132                 VCIGCVEPGFPD-MSPFYEM--M---ASK--TAPA-GAD-------GKVKISIDPVTRIE
D. baculatum        VCIGCVEPDFPDGKSPFYVAE-VS----Q--AATP-AAD-------GKVKISIDPLTRVE
D. africanus WalvisBay VCTGCAEPGWPDNFSPFYESM-MS--EKN------------------VKISIDPVTRIE
96                  VCIGCVEPGFPD-MSPFYE-G-MS--SKS--HAPA-GKD-------GKIKIAIDPVTRIE
D. hydrothermalis   VCLGCVEPGFPDEMSPFYEAG-MS--SKS--HAPA-SKD-------GKIKIAIDPVTRIE
D. salexigens       VCIGCVEPGFPDEMSPFYEAG-MS--SKS--HAPA-GKD-------GKIKIAIDPVTRIE
121                 VCIGCVEPGFPDAMSPFYEPA-MAGCKPE--AAPAGATG-------KKIKIAIDPVTRIE
D. autotrophicum HRM2 VCIGCVEPGFPDASSPFYEQS-MAGCKPE--AVPVGAAG-------KKIKVAIDPVTRIE
D. postgatei 2ac9   VCIACVEPGFPDQSSPFYEPA-MSGSKP--------TTGSVGVDSTKKLKISIDPITRIE
108                 VCIGCVEPGFPDAMSPFYEPA-MAGCKPQ--AEPA-ATG-------KKIKIGIDPVTRIE
D. phosphitoxidans  VCIGCVEAGFPDAMSPFYEPAYMAGCQPQ--AEPA-QTG-------KKIKIGIDPVTRIE
139                 VCIGCVEPGFPD-MSPFYEM--T---ACKPATAPA-GAD-------GKIKIAIDPVTRIE

                          430       440       450       460       470       480
                   ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden. GHLKAEVVVENGKVVDARLSGGMYRGFETILRGRDPRDASQIVQRICGVCPTAHSTASVL
102                 GHLKAEVVVENG-VVDARLSGGMYRGFETILRGRDPR-ASQIVQRICGVCPTAHSTASVL
D. vulgaris Miyazaki F GHLKAEVTVENGVVVDARLSGGMYRGFETILRGRDPRDASQIVQRICGVCPTAHSTASCM
115                 GHLKAEVVVENG-VVDARLSGGMFRGFENILRGRDPR-ASQIVQRICGVCPTAHSTASVL
D. alaskensis G20   GHLKAEVVVEGGKVVDAHISGGMFRGFENILRGRDPRDASQIVQRICGVCPTAHSTASVM
123                 GHLKAEVVVEDG-VVDARLSGGMFRGFENILSGRDPR-ASQIVQRICGVCPTAHATASAL
D. piger            GHLKAQVVVENGKVVDAHLTGGMFRGFEQILKGRDPRDSTQIVQRICGVCPTSHAMASAL
131                 GHLKAEVEVEDG-VVDARLSGGMFRGFENILSGRDPR-ASQIVQRICGVCPTAHATASAL
B. wadsworthia 3-1-6 GHLKVEVQVEDGKVADAWITGGMFRGFEAILRGRNPRDASQIVQRICGVCPVAHATASSL
136                 GHLKAEVEVEDG-VVDAWFSGGMFRGFENILIGRDPR-ASQIVQRICGVCPTAHATASAL
133                 GHLKAEVEVEDG-VVDAWFSGGMFRGFENILIGRDPR-ASQIVQRICGVCPTAHSTASAL
132                 GHLKAEVEVEDG-VVDAWFSGGMFRGFENILIGRDPR-ASQIVQRICGVCPTAHSTASAL
D. baculatum        GHLKIEVEVKDGKVVDAKCSGGMFRGFEQILRGRDPRDSSQIVQRICGVCPTAHCTASVM
D. africanus WalvisBay GHLKAEVKVENGVVTDAWMSGGMFRGFENILIGRDPRDATQIVQRICGVCPTAHSTASCL
96                  GHLKAEVVVKDG-VVDAWFSGGMYRGFENILIGRDPR-AAQLTQRLCGVCRTAHSTASTL
D. hydrothermalis   GHLKAEVVVKDGKVVDAWLSGGMYRGFENILVGRDPRDAAQLTQRLCGVCPTAHSTASCL
D. salexigens       GHLKAEVVVKDGKVTDAWLSGGMYRGFENILIGRDPRDAAQLTQRLCGVCPTAHSTASTR
121                 GHLKAEVEVEDGKVVDARFSGGMFRGFENILNGRDPRDATQIVQRICGVCPTAHATASAL
D. autotrophicum HRM2 GHLKVEVEVKGGKVVDARCFGGMFRGFENILTGRDPRDATQIVQRICGVCPTAHATASSL
D. postgatei 2ac9   GHLKAEVEVKNGVVVDARMSGGMYRGFEQILVGRDPRDAVQITQRICGVCPTAHATASSL
108                 GHLKAEVEVEDGKVVDARISGGMYRGFEQILNGSDPRDATQITQRICGVCPTAHATASAL
D. phosphitoxidans  GHLKAEVEVAGGKVVDAHITGGMYRGFEQILYGRDPRDATQITQRICGVCPTAHATASAL
139                 GHLKAEVEVEDG-VVDAWFSGGMFRGFENILIGRDPR-AAQIVQRICGVCPTAHATASAL
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supplementary Fig. S9

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                                490       500       510       520       530       540
                        ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.     ALDEAFGAKVPNNGRITRNLIFGANYLQSHILHFYHLSAQDFVQGPDTAPFVPRFPKSDL
102                     ALDD-FGVKVPTNGRI-RNLIFGANYLQSHILHFYHLAAL-FVQGPDTAPFV-RFAKPDL
D. vulgaris Miyazaki F  ALDNAFKVKVPTNGRLTRNLTFGANYLQSHILHFYHLAALDFVQGPDSAPFVPRFAKPDL
115                     ALDD-FGVKVTTNGRI-RNLILGANYLQSHILHFYHLAAL-FVQGPDTAPFV-RFAKPDL
D. alaskensis G20       ALDKAFGVKVTTNGRLTRNLILGANYMQSHILHFYHLAALDFVQGPETAPFVPRFKNPDL
123                     ALDD-FGVKVTTNGRI-RNLILGANYLQSHILHFYHLAAL-FVQGPDTAPFV-RFAKPDL
D. piger                AQEDAFNIKVTGNGRITRNLILGANYLQSHILHFYHLAALDFVAGPDTAPFVPRFAQPDL
131                     ALDD-FGVKVTTNGRI-RNLILGANYLQSHILHFYHLAAL-YVQGPDTAPFV-RYDNPDL
B. wadsworthia 3-1-6    AIEAVCGVEVPENGRIARNLMLAGNYLQSNILHFYHLGGQDYFHGPDTVPFIPRYRNPDL
136                     ALDD-FGVKVTTNGRI-RNLILGANYLQSHILHFYHLAAL-YVNGPDMAPFV-RYDNPDL
133                     ALDD-FGVKVTTNGRI-RNLIFGANYLQSHILHFYHLAAL-YVNGPDIAPFV-RYDNPDL
132                     ALDD-FGVKVTTNGRI-RNLIFGANYLQSHILHFYHLAAL-YVNGPDIAPFV-RYDNPDL
D. baculatum            AQDDAFGVKVTTNGRITRNLIFGANYLQSHILHFYHLAALDYVKGPDVSPFVPRYANADL
D. africanus WalvisBay  ALDDAFGVKLTTNGRVTRNLIFGANYLQSHILHFYHLAALDYVAGPDVAPFVPRYEKSDL
96                      ALDD-FGVKLTTNGRV-RNLIFGANYLQSHILHFYHLAAL-FVSGPGKAPFV-RFEHPDL
D. hydrothermalis       ALDDAFGAKITTNGRVTRNLIFGANYLQSHILHFYHLTALDFVRGPGKAPFVPRFEQPDL
D. salexigens           ALDDAFGVKLTTNGRVTKNLIFGANYLQSHILHFYHLAALDFVRGPGKAPFVPRFEQPDL
121                     ALDDAFGVKLTNNGRIARNLILGANFLQSHILHFYHLAALDYVNGPDTAPFIPRYDNNDM
D. autotrophicum HRM2   ALDDAFGVKLTNNGRVARNLILGANFLQSHILHFYHLAALDYVNGPEVAPFIPRYKNNDV
D. postgatei 2ac9       ALDDAFGVTLTDNGRIARNLILGANFIQSHILHFYHLAALDYVNGPDTAPFIPRYKHNDI
108                     ALDDAFGVKLTDNGRIARNLILGANFLQSHILHFYHLAALDYVNGPDTAPFIPRYDNNDM
D. phosphitoxidans      ALDDAFGVELTDNGRIARNLILGANFLQSHILHFYHLAALDYCNGPDTAPFIPRYANNDM
139                     ALDD-FGVKVTNNGRI-RNLILGANYLQSHILHFYHLAAL-YVNGPDMAPFV-RYDNPDL

                                550       560       570       580       590       600
                        ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.     RLSKELNKAG---------VDQYIEALEVRRICHEMVALFGGRMPHVQGQVVGGATEIPT
102                     RLPKEMNKA----------VDQYLEALEVRRICHEMVAL-GGRMPHVQGQVVGGATEIPT
D. vulgaris Miyazaki F  RLPKDINAAA---------VDQYLEALEVRRICHEMVAMFGGRMPHVQGQVVGGTTEIPT
115                     RLPKEMNKV----------VDQYLEALEVRRICHEMVAL-GGRMPHVQGQVVGGATEIPT
D. alaskensis G20       RLPSAVNQVA---------VDQYLEALEVRRICHEMVAIFGGRMPHVQGQVVGGTTEIPT
123                     RLPKEMNKV----------VDQYLEALEVRRICHEMVAL-GGRMPHVQGMVVGGATEIPT
D. piger                RLPPEANKVG---------VDQYLEALEVRRIAHEMVALFGGRMPHVQGIVPGGATEMPT
131                     RLPKEMNKV----------VDQYLEALEVRRICHEMVAL-GGRMPHVQGMVVGGATEIPT
B. wadsworthia 3-1-6    RLSEEQNTLA---------MDEYIEALEVRQVCHQLVALFGGRMPHLQGILGGGAAQIPD
136                     RLAKEINKV----------VDQYLEALEIRRICHEMVAL-GGKMPHVQGMVVGGATEIPT
133                     RLAHEINKV----------VDQYLEALEIRRICHEMVAL-GGKMPHVQGMVVGGATEIPT
132                     RLAHEINKV----------VDQYLEALEIRRICHEMVAL-GGKMPHVQGMVVGGATEIPT
D. baculatum            -LTDRIKDGAKADATNTYGLNQYLKALEIRRICHEMVAMFGGRMPHVQGMVVGGATEIPT
D. africanus WalvisBay  RLTPELNKVA---------VDQYLEALNVRLVAHEMVALFGGKMPHVSGQVVGGTTEIPS
96                      RLDEKTNKV----------VDQYVKALEIRRICHEMVAL-GGKMPHVSGQVVGGATEIPT
D. hydrothermalis       RLDEKTNAVA---------VDQYIKALEIRRICHEMVALFGGKMPHISGQVVGGTTEIPT
D. salexigens           RLDEKTNKVA---------VDQYVKALEIRRICHEMVALFGGKMPHVSGQVVGGATEIPT
121                     RLPKEINEVA---------VDQYLEALEIRKICHEMVALFGGKMPHVQGIVVGGTTEIPT
D. autotrophicum HRM2   RLDKATNQVG---------VDQYLEALEIRKICHEMVALLGGKMPHVQGIVVGGTTEIPT
D. postgatei 2ac9       RVSKDINDLC---------VGQYLEALEIRKICHEMVALLGGKMPHVQGIVVGGTTEIPT
108                     RLPKEINDVA---------VGQYLEALEIRKICHEMVALLGGKMPHVQGIVVGGTTEIPT
D. phosphitoxidans      RLPKELNDVA---------VGQYLEALEIRKICHEMVALLGGKMPHVQGIVVGGSTEIPT
139                     RLAKEINKV----------VDQYLEALEIRRICHEMVAL-GGKMPHVQGMVVGGATEIPT
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supplementary Fig. S9

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                                     610       620       630       640       650       660
                             ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.          KEKLVEYAARFKKVRDFVEQKYVPVVYTIGSKYKDMFKVGQGFKAALCVGAFPLDNSGKK
102                          KEA-VEYAARFKKVREFVEEKYVPV-YTVGTVYKDLFKFGQGYKNCISFGAFPLNDDGNE
D. vulgaris Miyazaki F       KEALVEYAARFKKVREFVEKKYVPVAYTVGAAYKDLFKFGQGYKNCISFGAFPLNDAMTE
115                          KEA-VEYAARFKKVRKFVEEKYVPV-YIVGTVYKDLFKFGQGYKNCISFGVFPLNDDGKE
D. alaskensis G20            KEALVEYAARFKKVRKFVEEKYVPVVYLVGSVYKDLFAFGQGYKNCVAFGVFPLNDEGTE
123                          KEA-VEYAARFKKVRKFVEEKYVPV-YIVGSVYKDLFKIGQGYKNCISFGVFPLNDDGKE
D. piger                     KEALLEYAARFKKVRQFIVEKYLPITYIVGSVYKDLFEQGQGVHGCSCFGVFPMTDDGKT
131                          KET-VEYAARFKKVRKFVEEKYVPV-YIMGSVYKDLFKIGQGYKNCISFGVFPLDDDGKE
B. wadsworthia 3-1-6         RETILEYAARMKQVRKFVENRYLPLVYTIASRYMDMFEMAHGYKNALCVGVFPLAKKGE-
136                          KEK-AEYAARFKKVRKFIEEKYVPV-YIMGSVYTDLFKIGEGYKNFIAFGVFPLDDDGKE
133                          KEK-AEYAARFKKVHKFIEEKYVPV-YIMGSVYTDLFKIGDGYKNVIAFGVFPMDDDGKE
132                          KEK-AEYAARFKKVHKFIEEKYVPV-YIMGSVYTDLFKIGDGYKNVIAFGVFPMDDDGKE
D. baculatum                 ADKVAEYAARFKEVQKFVIEEYLPLIYTLGSVYTDLFETGIGWKNVIAFGVFPEDDDYKT
D. africanus WalvisBay       QQKLDEYTKRFKLVRKFIEETYVPTVYVIGKAYADLLKVGDGYKNVISFGVFPMDDSGKE
96                           KEK-AEYASRFKEVQKFIEETYVP-VYLIGSVYKDLFKIGGGYKNAMAYGVFPMDDAGYE
D. hydrothermalis            KEKLAEYASRFKDVQKFIAEVYVPTVYLIGSVYKDLFKIGGGYKNCMAYGVFPMSDGGDD
D. salexigens                KEKLAEYASRFKQVQKFIEETYVPTVYLIGSVYKDLFKIGGGYKNAMAYGVFPMDDAESE
121                          RENLDAYAERFKKVRKFIEEKYVPIVYLLAGPYGDLLKTGVGYKNFIAFGVFPLDDEGN-
D. autotrophicum HRM2        REKLDAYKERFKTVRKFIEERYLPLIYLLAGPYGDLLKTGTGYKNCIAFGVFPLDDAGN-
D. postgatei 2ac9            REALNAYAERFKKIKKFVMEKYIPVIYTLAGPYGDLLKTGVGHKNLVSWGVFPMDSKGN-
108                          REALNAYAERFKKVRKFIVEKYVPIVYLLAGPYGDLLKTGVGHKNLVSWGVFPLDNKGN-
D. phosphitoxidans           REALNAYAERFKKVRQFILEKYVPIVYLLAGPYGDLLKTGVGHKNLVSWGVFPLDNKGN-
139                          KEK-AEYAARFKKVSKFIEEKYVPV-YIMASVYTDLFKIGEGYKNFIAFGVFPLDDDGNE
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                                    670       680       690       700       710       720
                             ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.          HLFMPGVYAKGKDMPFDPSKIKEYVKYSWFAEETTG-LNYKEGKTIPAPDKAGAYSFVKA
102                          LLLKPGVYIDGKDKPFDPKQIKEYVKYSW-DDATTG-LH-KEGKTIPAPDKA-AYSFVKA
D. vulgaris Miyazaki F       LHLKRGVYIDGKDQPFDPKLIKEYVKYSWFDDATTG-LHFSQGKTVPAPNKAGAYSFVKA
115                          LLLKPGVYIDGKDKPFDPKQIKEYVKYSW-DDATTG-LH-NEGKTIPDPDKA-AYSFVKA
D. alaskensis G20            FALKPGVYMDGEDKPFDARLIKEYVKYSWFDDSTTG-LHYTEGQTIPDPEKPGAYSFVKA
123                          LLLKPGVYIDGKDEPFDPKQIKEYVKYSW-DDATTG-LH-NEGKTIPDPDKA-AYSFVKA
D. piger                     HLLKAGVFLNGRDVEFDPKKITEDLKYAWYDDATTG-KGADGAETNPNLDKKDAYSFVKA
131                          LLLKPGVYIDGKDEPFDPKQIKEYVKYSW-DDATTG-LH-NEGKTIPDPDKA-AYSFVKA
B. wadsworthia 3-1-6         QFFNAGAYINGRDEPFDGNRILEDVRYSWFEPAPSG-TPLQKSESNPQVDKEGAYSFIKA
136                          FLLKPGVYIDGKDEAFDPKQIKEYVKYSW-DDATTG-LH-SEGKTIPDPDKA-AYSFIKA
133                          FLLKPGVYIDGKDEAFDPKQIKEYVKYSW-DDASTG-LH-SEGKTIPDPDKA-AYSFIKA
132                          FLLKPGVYIDGKDEAFDPKQIKEYVKYSW-DDASTG-LH-SEGKTIPDPDKP-AYSFIKA
D. baculatum                 FLLKPGVYIDGKDEEFDSKLVKEYVGHSFFDHSAPGGLHYSVGETNPNPDKPGAYSFVKA
D. africanus WalvisBay       TLLKPGVYINGKDVAFDPAKIKEYAKYSWFEDSCSN-LHPSQGKTLPKLGKPGAYSFIKA
96                           FLLKPGVYIDGKDDGFDPKLIKEYTKYSW-TDECSD-LH-SEGKTIPDVHKK-AYSFCKA
D. hydrothermalis            FLIKSGVYVDGKDEKFDPKLIKEFTKYSWYTDECSD-LHPSEGKTIPDVHKKDAYSFCKA
D. salexigens                FLLKPGVYIDGKDAGFDQKLIKEYTKYAWYTDECSD-LHPSKGKTIPDVHKKDAYSFCKA
121                          TLLKPGVYTDGKDKAFDPDKIKEYVKYSWFDDTTTG-LHPSEGKTVPDPDKAGAYSFIKA
D. autotrophicum HRM2        TLLKSGVFTDGKYTDFNSDNIKEYVKHSFFADNTTG-LHPSKGKTVPDPEKATGYSFIKS
D. postgatei 2ac9            TLLKPGVYTDGKDYKVDPTQIKEYVKYSWFEDSTTG-LNPTRGRTRPEPGKAGAYSFIKA
108                          TLLKPGVYTDGKDYAVDPAKIKEYVKYSWFEDTTTG-LNPTEGKTLPDPDKAGAYSFIKA
D. phosphitoxidans           TLLKPGVYTDGKDYAVDPAMIKEYVKYSWFEDSTTG-LNPTEGKTLPNPNKPGAYSFIKA
139                          -LLKPGVYIDGKDEAFDPNQIKEYVKYSW-DDDTTG-LH-SEGKTIPDPDKA-AYSFIKA
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supplementary Fig. S9

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                                    730       740       750       760       770       780
                                ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.         PRYDGLSLEVGPLARMWVNNPELSPVGKKLLKDLFGISAKKFRDLG-EEAAFSLMGRHVA
102                         PRYNGKPVEVGPLA-MWVT-PELSPVGQKLLKDLFGIDAKSFRD-G-DEAAFSLMGRHVA
D. vulgaris Miyazaki F      PRYNGKAVESGPVARMWITNPELSPVGQKLLKDLFKLNAKRFRDLG-DEAAFSVMGRHVA
115                         PRYNGKPVEVGPLA-MWVT-PELSPMGQKLLKDLFGIDAKSFRD-G-DEAAFSLMGRHVA
D. alaskensis G20           PRYNGKPCEVGPLARMWVANPELSPMGQKMLKEHYGIDAKRFRDIG-AEAAFSLMGRHVA
123                         PRYNGKPVEVGPLA-MWVT-PELSPMGQKLLKDLFGIDAKSFRD-G-EDAAFSIMGRHVA
D. piger                    PRYDGEVIEVGPAARMWVANAPLSEVGVKMLKEKFGIEARTIRDLG-WDKVFSIMGRHVA
131                         PRYNGKPVEVGPLA-MWVT-PELSPMGQKLLKDLFGIDAKSFRD-G-EDAAFSIMGRHVA
B. wadsworthia 3-1-6        PLYAGHRVEVGPLARMWINDKPLSPIGQRFFADMFGVRAETFRQIG-EDPAFSIMGRNVA
136                         PRYNGKPVEVGPLA-MWVT-PELSPMGQKLLKDLFGIDAKSFRD-G-EDAAFSIMGRHVA
133                         PRYNGKPVEVGPLA-MWVP-PELSPMGQKLLKDLFGIDAKSFRD-G-EDMAFSIMGRHVA
132                         PRYNGKPVEVGPLA-MWVP-PELSPMGQKLLKDLFGIDAKSFRD-G--DMAFSIMGRHVA
D. baculatum                PRYKDKPCEVGPLARMWVQNPELSPVGQKLLKELYGIEAKNFRDLG--DKAFSIMGRHVA
D. africanus WalvisBay      PRYDGHPVEVGPLARMWVSNPELSPMGKKQLKDLFGINANRFRDLG--DLAFSIMGRHVA
96                          SRYNGKAVEVGPLA-MWVH-PELSPMGKKQLKDLFGIEAKIFRD-GE-DMAFSLMGRHVA
D. hydrothermalis           SRYNGNAVEVGPLARMWIHNPELSPMGKKQLKDLYGITAKNFRDLGE-DMAFSLMGRHVA
D. salexigens               SRYNGKAVEVGPLARMWVHNPELSPMGKKQLKDLFGIEAKMFRDLGE-DMAFSLMGRHVA
121                         PRYNGKPHEVGPLARMWVTNPELSATGQKAL------GVKRLSDIG--DAAFSILGRHVA
D. autotrophicum HRM2       PRYNGKPHEVGPLARMWITNPELSATGQKAL------GVKRMRDIG--DAAFSILGRHIA
D. postgatei 2ac9           PRYNGKPHEGGPLARMWATNPELSKTGQEAL------GVKKLRDIG--DACFSILGRHVA
108                         PRYNGKPHEGGPLARMWATNPELSATGQKAL------GVKRLSDIG--DACFSILGRHVA
D. phosphitoxidans          PRYNGKPHEGGPLARMWATNPELSATGQKEL------GVTRLRDIG--DACFSILGRHVA
139                         PRYNGKPLEVGPLA-MWVT-PELSPMGQKLL------DAKSFRD-G--DAAFSIMGRHVA

                                    790       800       810       820       830       840
                                ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.         RAEETYYMLGAIE--GWLKEIKAGEDTVVMPAVPASAEGTGFTEAPRGSLLHYVKVKDSK
102                         RAEETYYMLNAIE--RWLKEVKAGEET-VASEIP-SAEGIGFTEAPRGSLLHYINIK-SK
D. vulgaris Miyazaki F      RAEETYYMLSAIE--RWLKEVKAGEETFAAAEIPASSEGVGFTEAPRGSLVHYINIKDQK
115                         RAEETYLIPNAIE--RWLKEVKPGEET-VPSEIP-SAEGIGFTEAPRGSLLHYINIK-YK
D. alaskensis G20           RAEETWLTTNYIE--RWLKEVVPGAETYVPSEIPEQAEGTGFTEAPRGSLLHYIDIKDSV
123                         RAEETYLIANAIE--RWLKEVKPGEET-VPSEIP-SAEGIGFTEAPRGSLLHYINIK-YK
D. piger                    RAEEALLIANAVE--GWLKEVKPDGETFTPFEIPQSAEGYGCSEAPRGSLVHYIRVKDQK
131                         RAEETYLIANAIE--RWLKEVKPGEET-VASEIP-TAEGIGFTEAPRGSLLHYINIK-YK
B. wadsworthia 3-1-6        RVEEVYQTLGMIE--YWLHELEPGAQTFALPEVPQAGEGIGFTEAPRGALCHYMRVKNGV
136                         RAEETYLVANAIE--RWLKEVKPGEET-VASEIP-TAEGIGFTEAPRGSLLHYINIK-YK
133                         RAEETYLVANAIED-RWLKEVKPGEET-VTSEIP-TAEGIGFTEAPRGSLLHYINIK-YK
132                         RAEETYLVANAIED-RWLKEVKPGEET-VTSEIP-TAEGIGFTEAPRGSLLHYINIK-YK
D. baculatum                RAEETWLTAVAVE--KWLKQVQPGAETYVKSEIPDAAEGTGFTEAPRGALLHYLKIKDKK
D. africanus WalvisBay      RAEETYLVAKAIE-ERWLKEVKPGKETFVAASIPDSAEGIGFTEAPRGSLLHYVNIKDKK
96                          RAEEAYMVANAIE-DAWLKEVKPGEET-VKTEIP-SAEGLGLTEAPRGSLLHYINIK-SK
D. hydrothermalis           RAEESYLVANAIG-DIWLGEVKEGEETYVKTTMPESGEGVGLTEAPRGSLLHYINIKDSK
D. salexigens               RAEEAYMVANAIQ-DAWLKEVQPGEETYVKTEIPESAEGLGLTEAPRGSLLHYINIKDSK
121                         RAEETLLVAKAME--HWLDEAKPGKETFVAAIPENAEGIGLTEAPRGALLHYIDIKNYK
D. autotrophicum HRM2       RAEETLLVAMQME--RWLEEAKPGLETFVAAPIPENAEGIGLTEAPRGALLHYIDIKNSV
D. postgatei 2ac9           RAEETLLVAKAVE--QWIAQATPGKETFVPAAIPENAEGLGMTEAPRGALLHYVEIKNSV
108                         RAEETLLVAKAME--QWLTQAKPGKETFVPAAIPENAEGLGMTEAPRGALLHYIDIKNYK
D. phosphitoxidans          RAEETALVAAAME--EWLTQAQPGKETFVPAPIPDSAQGLGMTEAPRGALLHYIDIKDQK
139                         RAEETYLVANAIE--HWLKEVKPGEET-VASEIP-NAEGIGFTEAPRGALLHYINIK-YK
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supplementary Fig. S9

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                                   850       860       870       880       890       900
                               ....|....|....|....|....|....|....|....|....|....|....|....|
D. vulgaris Hilden.            IDNYQIVSASLWNCNPRDDMGQRGAVEEALIGIPVDDIQNPVNVARLIRAFDPULGCAVH
102                            IDNYQIVSATLWNCNPRDDMGQRG-VEEALIGIPVPDINNPVNVARLIRAFDPULGCAVH
D. vulgaris Miyazaki F         IDNYQIVSATLWNCNPRDDSGQLGPVERALVGTPVPDISNPVNVARVIRAFDPULGCAVH
115                            IDNYQIVSATLWNCNPRDDMGQRG-VEEALIGIPVPDINNPVNVARLIRAFDPULGCAVH
D. alaskensis G20              ISNYQIVSATLWNCNPRDDMGNRGPVEEALIGTPVPDLENPVNIARLIRAFDPULGCAVH
123                            IDNYQIVSATLWNCNPRDDMGQRG-VEEALIGIPVPDINNPVNVGRIIRAFDPULGCAVH
D. piger                       IDSYQIISATLWNCSPRDDKGRRGPLEEALIGVQVPDINNPVNVGRTIRAFDPULGCAVH
131                            IDNYQIVSATLWNCNPRDDMGQRG-VEEALIGIPVPDINNPVNVGRIIRAFDPULGCAVH
B. wadsworthia 3-1-6           IDDYAVVAASMWNCSPRDDAGKRGAVEEALIGVPVPEVDSPVNVGRVIRAYDPULGCAVH
136                            IDNYQIVSATLWNANPRDDMGQRG-MEEALIGIPVPDIKNPVNVGRIIRSFDPULGCAVH
133                            IDNYQIVSATLWNANPRDDMGQRG-IEEALIGIPVPDIKNPVNVGRIIRSFDPULGCAVH
132                            IDNYQIVSATLWNANPRDDMGQRG-IEEALIGIPVPDIKNPVNVGRIIRSYDPULGCAVH
D. baculatum                   IENYQIVSATLWNANPRDDMGQRGPIEEALIGVPVPDIKNPVNVGRLVRSYDPULGCAVH
D. africanus WalvisBay         IANYQIVSATLWNANPRDDMGKRGPMEQALIGTPVPDAKNPVNVGRVIRAYDPULGCAVH
96                             TANYQMIPATLWNSTPRDDKGQRG-IEEALVGTPVPDPKNPVDISRIIRSFDPULGCAVH
D. hydrothermalis              TANYQMIPATLWNSTPRDDKGMRGTIEEALVGCPVPDPSSPVDISRIIRSFDPULGCAVH
D. salexigens                  TANYQMIPATLWNSTPRDDKGHRGTIEEALVGTPVPDPKNPVDISRIIRSFDPULGCAVH
121                            IANYQITSATIWNANPRDDMGQRGPIEEALIGIPVPDIDNPVNVGRLIRSFDPULGCAVH
D. autotrophicum HRM2          ISNYQITSATIWNANPRDDMEQRGPIEQALIGIPVPDIDSPVNVGRLIRSFDPULGCAVH
D. postgatei 2ac9              ISNYQITSATIWNANPRDDMGQRGPIEEALIGVPVPDVDNPVNVGRLIRAYDPUMGCAVH
108                            IANYQITSATIWNANPRDDMGQRGPIEEALIGVPVPDVDNPVNVGRLIRSFDPULGCAVH
D. phosphitoxidans             IANYQITSATIWNANPRDDMGQRGPIEEALIGVPVPDVNSPVNVGRLIRSFDPULGCAVH
139                            IDNYQIVSATIWNANPRDDMGQRG-MEEALIGIPVPDIDNPVNVGRIIRSFDPULGCAVH

                                   910
                               ....|....|....|....
D. vulgaris Hilden.            VLHAESGKVAVIEVK----
102                            VLHAESGKVSVVEVK----
D. vulgaris Miyazaki F         VLHAESGKVSVVEVK----
115                            VLHAESGKVSVVEVK----
D. alaskensis G20              VLHAESGKVSVVEF-----
123                            VLHAETGKVSVVEVE----
D. piger                       VLHAETG------------
131                            VLHAETGKVSVVEVE----
B. wadsworthia 3-1-6           VLHAKAE------------
136                            VLHAETGKEHVVEVE----
133                            VLHAETGEEHVVNVE----
132                            VLHAETGEEHVVNVE----
D. baculatum                   VLHAETGEEHVVNID----
D. africanus WalvisBay         VLHAETGEKTVVTVE----
96                             VLHAETGEEHVVHVGEGC-
D. hydrothermalis              VLHAETGEEHVYHVGEGC-
D. salexigens                  VLHAETGEEHVVHVGEGC-
121                            VLHAETGKTIVVDVP---L
D. autotrophicum HRM2          VLHAETGKTNVVEIP---L
D. postgatei 2ac9              VLDADTGKQIKVEVP---L
108                            VLDAETGKTIKVDVP---L
D. phosphitoxidans             VLDVETGRTVKVDVP---L
139                            VLHAETGKENVVEVE----
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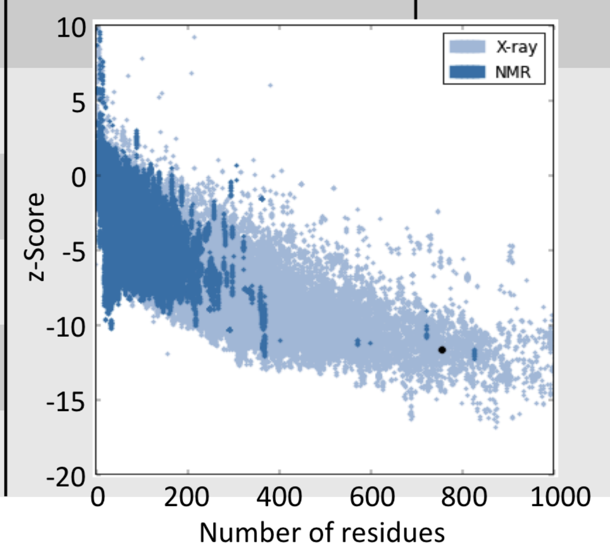
Supplementary Fig. S9: Amino acid sequence alignment and identification of residues involved in the cavity. Amino acid residues that have more non-synonymous substitutions ($\delta N - \delta S > 0$) are marked in yellow. The residues constituting cavities are designated in red. Assemblage names in the left column are grouped as terrestrial origin (highlighted in yellow), clinical isolates (brown letters), deep-sea marine vent origin (highlighted in blue), and sediment origin (highlighted in green). Ternary structural models start with R78 and end at H900.

MD model	z-Score	MD model	z-Score	MD model	z-Score
D. vulgaris Hildenborough	-11.71	*D. baculatum*	-11.22	*D. postgatei* 2ac9	-9.86
2wpn.pdb (*D. vulgaris* H.)	-11.59	1cc1.pdb (*D. baculatum*)	-10.64	*D. phosphit-oxidans*	-9.99
D. vulgaris Miyazaki F	-10.69	*D. africanus* Walvis Bay	-11.20	*D.autotrophicum HRM2*	-11.22
D. alaskensis G20	-11.38	*D. salexigens*	-10.73	108	-11.10
B. wadsworthia 3-1-6	-10.42	*D. hydrothermalis*	-8.96	121	-11.25
D. piger	-11.56	132	-9.69		
102	-10.25	96	-10.30		
115	-10.07	133	-9.92		
123	-9.71	136	-9.75		
131	-9.88	139	-10.42		



Supplementary Table S1: Quality of MD models evaluated by ProSA. ProSA is a knowledge-based program for the evaluation of the model accuracy. The *z*-score indicates overall model quality and measures the deviation of the total energy of the structure, which is displayed in a plot that contains the *z*-scores of all experimentally determined protein chains in current PDB. The range of z-scores between -9 and -14 is acceptable for the [NiFeSe]-Hases with 770 amino acid residue. X-ray crystallographic models 2wpn.pdb and 1cc1.pdb are also evaluated as reference.