Phylogenomics reveal how bacteria adapt to salinity

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Background:

- Different microbial communities in freshwater (F), brackish (B) and marine (M) biomes
- Phylogenomics can reveal previous cross-biome transition events
- Comparing genomes across multiple transitions can reveal convergent evolution related to salinity change

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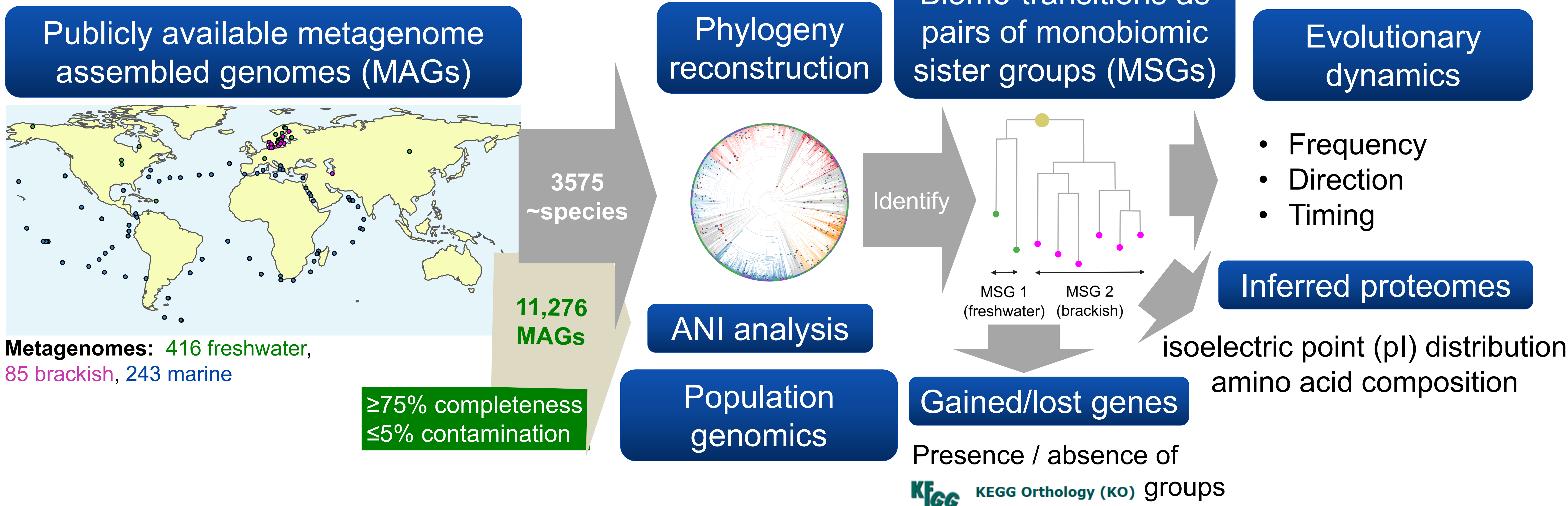10.1101/2022.10.03.5
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Conclusions:

Bacterial adaptation to a different salinity regime:
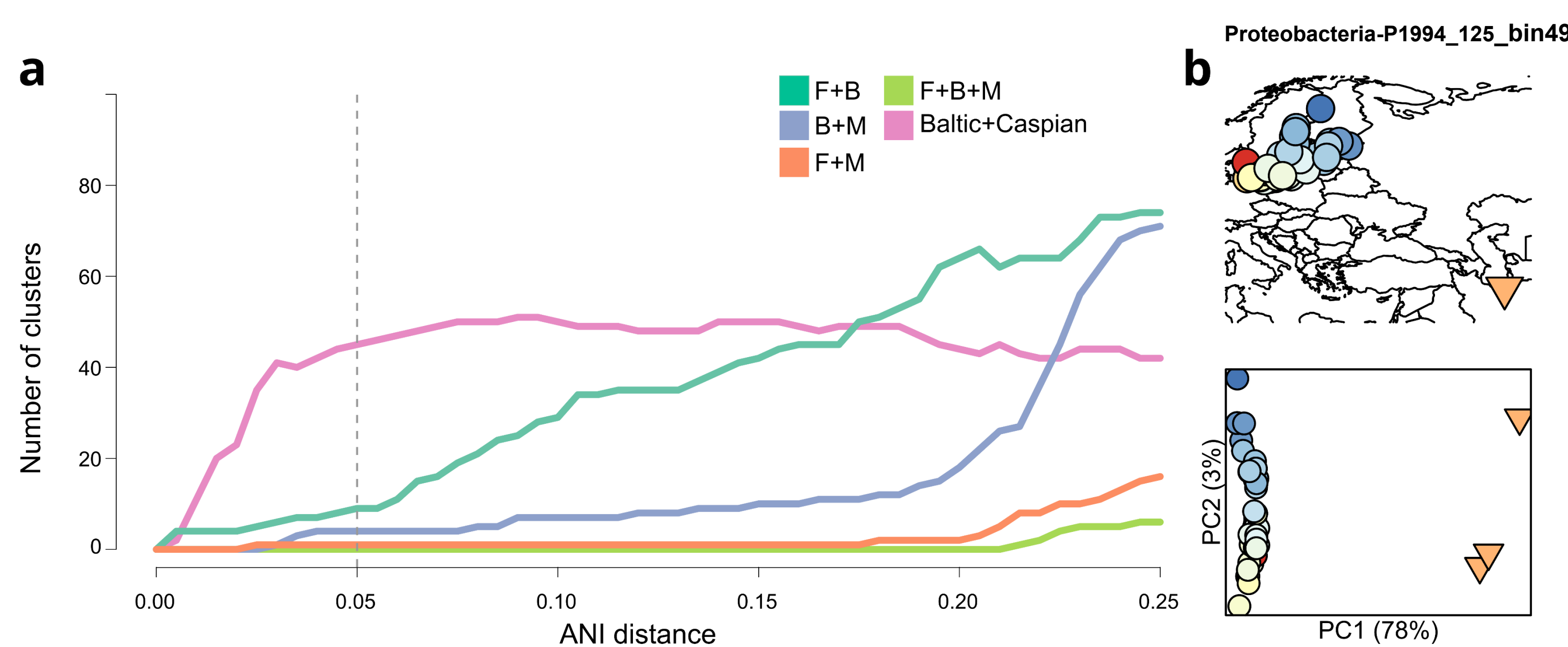- is rare;
- leads to wide-scale changes in proteome properties and composition;
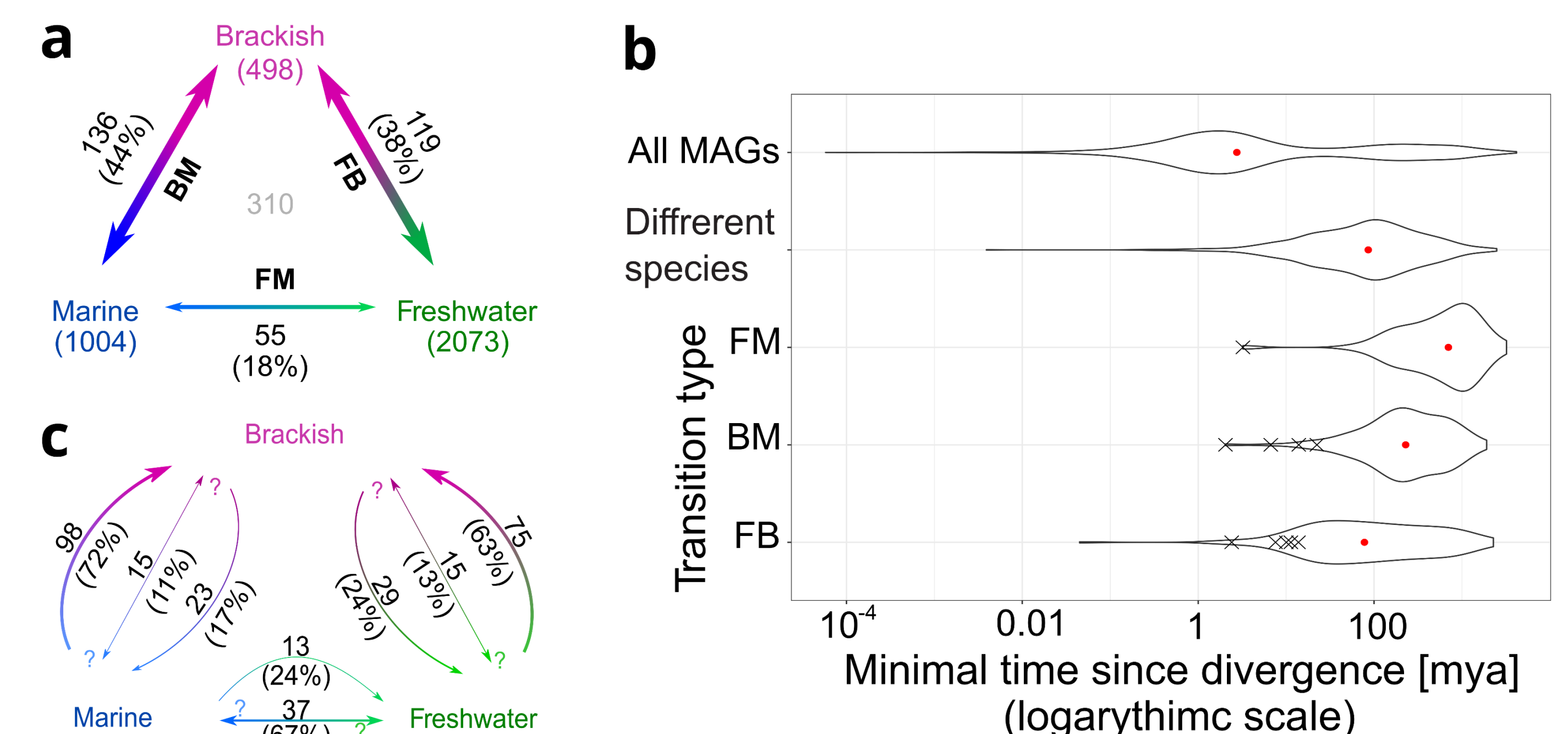- entails gains/loses specific gene functions.

Data analysis

Publicly available metagenome assembled genomes (MAGs)

Metagenomes: 416 freshwater, 85 brackish, 243 marine

3575 ~species

11,276 MAGs

≥75% completeness
≤5% contamination

Phylogeny reconstruction

Biome-transitions as pairs of monobiomic sister groups (MSGs)

Identify

MSG 1 (freshwater) MSG 2 (brackish)

Evolutionary dynamics
- Frequency
- Direction
- Timing

Inferred proteomes
isoelectric point (pI) distribution
amino acid composition

ANI analysis

Population genomics

Gained/lost genes
Presence / absence of KEGG Orthology (KO) groups

Results

Different species inhabit different biomes

a — Legend: F+B, B+M, F+M, F+B+M, Baltic+Caspian
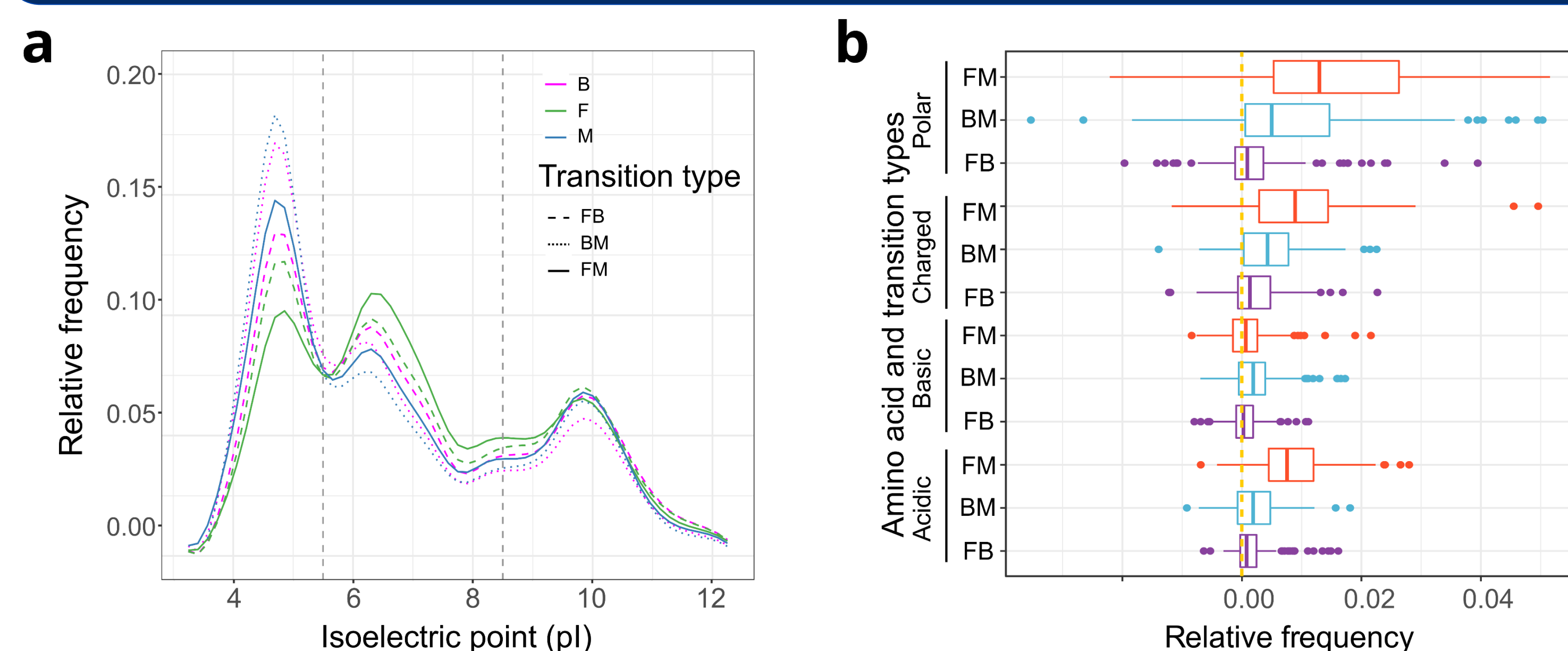
b — Proteobacteria-P1994_125_bin49

Fig. 1 Community separation - ANI analysis and population genomics. a. Number of MAG clusters at different nucleotide distance cut-offs with members of different biome combinations. **b.** Population structure of a brackish genome cluster. The geographic origin of each metagenome sample included for the MAG is shown to the left and a PCoA based on pairwise F_{ST} values.
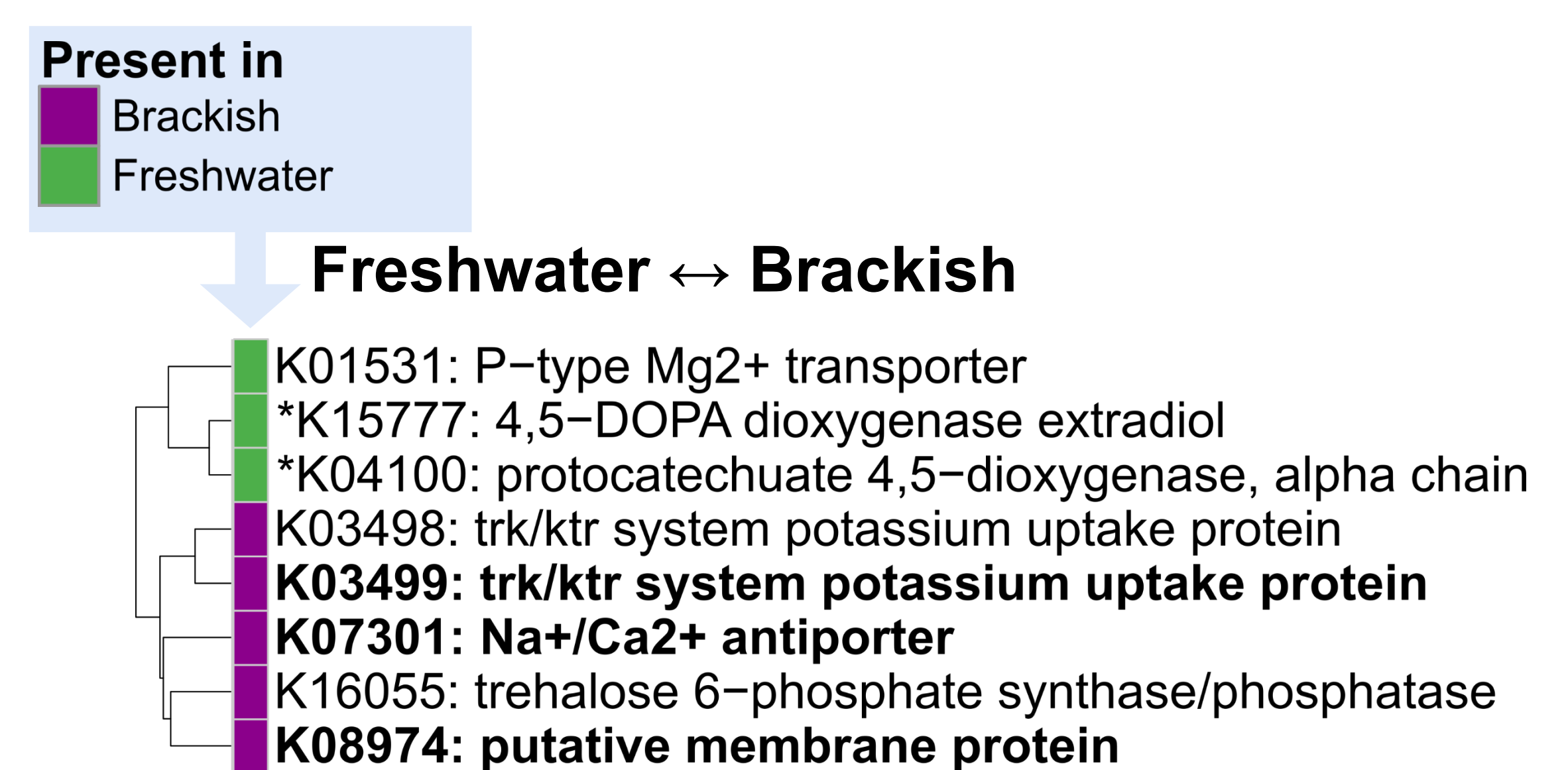
Cross-biome transitions are rare and ancient

a — Brackish (498); Marine (1004); Freshwater (2073)
BM 136 (44%); FB 119 (38%); FM 55 (18%); 310

c — Brackish, Marine, Freshwater transitions: 98 (72%), 15 (11%), 23 (17%), 15 (63%), 29 (24%), 75 (13%), 13 (24%), 37 (67%), 5

b

Fig. 2 Number, timing, and direction of transitions. a. Observed numbers of transitions between the biomes.. **b.** The distributions of estimated minimal times since divergence for all nodes on the unpruned tree ("All MAGs"), nodes corresponding to most recent divergence events ("Different species"), and nodes corresponding to transitions grouped by type. **c.** Numbers (and proportion) of transitions of each type for which the direction of the transition indicated by the arrows was more probable than the two other ancestral biome-states taken together.

### Cross-biome transitions lead to wide-scale changes in proteome composition…

a — Legend: B, F, M; Transition type: -- FB, -- BM, — FM

b — Amino acid and transition types: Polar (FM, BM, FB), Charged (FM, BM, FB), Acidic (FM, BM, FB)

Fig. 3 Changes in proteome properties and composition. a. Averaged distribution of pI values in predicted proteomes of MAGs. **b.** Population structure of a brackish genome cluster. Differences in fractions of the proteomes which the chosen categories of amino acids make up. The lower categories are subsets of the higher ones: polar ⊃ (charged = acidic + basic).

…and gain or loss of specific gene functions

Present in
- Brackish
- Freshwater

Freshwater ↔ Brackish

- K01531: P−type Mg2+ transporter
- *K15777: 4,5−DOPA dioxygenase extradiol
- *K04100: protocatechuate 4,5−dioxygenase, alpha chain
- K03498: trk/ktr system potassium uptake protein
- **K03499: trk/ktr system potassium uptake protein**
- **K07301: Na+/Ca2+ antiporter**
- K16055: trehalose 6−phosphate synthase/phosphatase
- **K08974: putative membrane protein**

Fig. 4 KEGG orthologs (KOs) significantly (FDR < 0.1) differentially present between pairs of freshwater and brackish MSGs. Highlighted KOs were also differentially present across freshwater ↔ marine transitions. KOs were clustered by similarity in presence/absence patterns across all the MSG pairs.
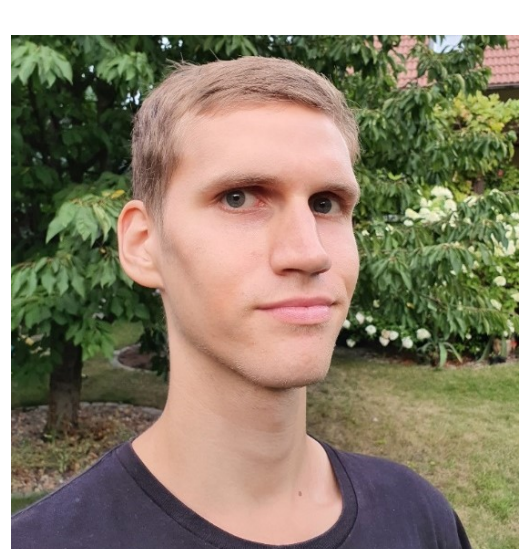
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