

## Supplementary Information

### **Parallel and convergent genomic changes underlying independent subterranean colonization across beetles**

### **Genomic exaptation and convergent evolution paved the way to independent subterranean colonization across beetle lineages**

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## Supplementary Tables

**Supplementary Table 1.** Fossil calibration points used for the divergence time estimation with MCMCtree. Codes indicated correspond to those of Figure 1.

| Split codes                     | Fossil                                        | Age (Mya)       | References |
|---------------------------------|-----------------------------------------------|-----------------|------------|
| (1) Root Age - Holometabola     | <i>Srokalarva berthei</i>                     | 358.9           | 1          |
| (2) Crown Coleoptera            | <i>Ponomarenkium belmonthensis</i>            | 307.1 - 251.878 | 2          |
| (3) Coleoptera - Archostemata   | <i>Kirghizocupes proporeius</i>               | 235 - 221.5     | 1          |
| (4) Coleoptera - Adephaga       | <i>Tunguskagyrs planus</i>                    | 254.2 - 252.2   | 1          |
| (5) Coleoptera - Curculionoidea | <i>Archaeorrhynchus</i> and/or <i>Eobelus</i> | 166,1 - 157,3   | 1          |
| (6) Crown Polyphaga             | Undescr. taxon of the series Elateriformia    | 293.69 - 237    | 2          |
| (7) Derodontidae - Clambidae    | <i>Juropeltastica sinica</i>                  | 251.878 - 165   | 2          |

1. McKenna, D. D. *et al.* The evolution and genomic basis of beetle diversity. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 24729–24737 (2019).
2. Cai, C. *et al.* Integrated phylogenomics and fossil data illuminate the evolution of beetles. *R Soc Open Sci* **9**, 211771 (2022).

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|------------------------------------------|-------------------------------------|-----------------------------------------------------------|---------------------------------------------------|-----------------------------------------|------------------------------------------------------|----------------------------------------------------------------|---------------------------------------------|-------------------------------------------|------------------------------------------|------------------------------------------------------------------|------------------------------------------------|------------------------------------------|------------------------------------|-------------------------------------|------------------------------------------------------------|------------------------------------------|
| sensory perception                       | digestive tract morphogenesis       | stem cell differentiation                                 | endocrine system development                      | reproductive system development         | digestive system development                         | chitin-based cuticle development                               | detection of stimulus                       | response to toxic substance               | entrainment of circadian clock           | response to acid chemical                                        | localization of cell                           | calcium ion transport                    | organic acid transport             | regulation of hormone levels        | positive regulation of cytosolic calcium ion concentration |                                          |
|                                          | system process                      | cardiocyte differentiation                                | determination of bilateral symmetry               | cuticle development                     | body morphogenesis                                   | appendage development                                          | urogenital system development               | cellular response to lipid                | response to abiotic stimulus             | response to biotic stimulus                                      | response to stress                             | protein localization to cilium           | acid secretion                     | action potential                    | negative regulation of molecular function                  |                                          |
|                                          |                                     | forebrain neuron differentiation                          | morphogenesis of a branching structure            | motting cycle                           | muscle structure development                         | head development                                               | ectodermal placode formation                | circulatory system development            | detection of abiotic stimulus            | cellular response to environmental stimulus                      | response to xenobiotic stimulus                | retinoic acid receptor signaling pathway | response to endogenous stimulus    | transepithelial transport           | epithelial fluid transport                                 | regulation of molecular function         |
|                                          |                                     |                                                           | inner ear morphogenesis                           | pancreas development                    | developmental growth involved in morphogenesis       | ossification                                                   | motting cycle, chitin-based cuticle         | developmental maturation                  | cranial skeletal system development      | transmembrane receptor protein tyrosine kinase signaling pathway | response to nicotine                           | response to food                         | response to stress                 | secretion                           | xenobiotic transport                                       | negative regulation of protein stability |
| behavior                                 | epithelial cell differentiation     | digestion                                                 | multi-multicellular organism process              | establishment of tissue polarity        | regeneration                                         | blood vessel remodeling                                        | determination of adult lifespan             | response to wounding                      | intracellular receptor signaling pathway | multicellular organismal response to stress                      | mating                                         | mating behavior                          | stem cell fate commitment          | regulation of peptide transport     |                                                            |                                          |
|                                          |                                     |                                                           |                                                   |                                         |                                                      |                                                                |                                             |                                           |                                          |                                                                  |                                                |                                          |                                    |                                     |                                                            |                                          |
| regulation of animal organ morphogenesis | regulation of cell cycle process    | regulation of T cell activation                           | regulation of non-canonical Wnt signaling pathway | regulation of receptor recycling        | regulation of phosphorus metabolic process           | regulation of cell cycle                                       | regulation of cell population proliferation | cellular polysaccharide metabolic process | sulfur compound metabolic process        | transcription, DNA-templated                                     | organic hydrocarbon compound metabolic process | proteolysis                              | male sex differentiation           | multi-organism reproductive process | movement of cell or subcellular component                  |                                          |
|                                          | positive regulation of MAPK cascade | positive regulation of cell population proliferation      | regulation of cytokine production                 | regulation of cell growth               | regulation of locomotion                             | regulation of apoptotic process                                | regulation of immune system process         | carbohydrate metabolic process            | lipid metabolic process                  | wax biosynthetic process                                         | carbohydrate derivative metabolic process      | pyruvate biosynthetic process            | male germ-line sex differentiation | fertilization                       | actin filament-based process                               |                                          |
|                                          | regulation of cell-cell adhesion    | positive regulation of phosphorylation                    | regulation of cell division                       | regulation of protein catabolic process | positive regulation of cellular component biogenesis | negative regulation of small molecule metabolic process        | regulation of lipid metabolic process       | protein hydrolase organization            | lipid catabolic process                  | energy reserve metabolic process                                 | membrane protein proteolysis                   | ATP metabolic process                    | cellular amine metabolic process   | single fertilization                | cell recognition                                           |                                          |
|                                          | regulation of cell adhesion         | negative regulation of transcription by RNA polymerase II | positive regulation of cytokine production        | regulation of circadian rhythm          | regulation of catabolic process                      | positive regulation of hematopoietic stem cell differentiation | regulation of coagulation                   | regulation of growth                      | synapse organization                     | cell junction organization                                       | phosphorus metabolic process                   | secondary amine metabolic process        | cellular amine metabolic process   | reproduction                        | multicellular organismal process                           | cell death                               |
| regulation of cell adhesion              | regulation of cell adhesion         | regulation of cell adhesion                               | regulation of cell adhesion                       | regulation of cell adhesion             | regulation of cell adhesion                          | regulation of cell adhesion                                    | regulation of cell adhesion                 | regulation of cell adhesion               | regulation of cell adhesion              | regulation of cell adhesion                                      | regulation of cell adhesion                    | regulation of cell adhesion              | regulation of cell adhesion        | regulation of cell adhesion         | regulation of cell adhesion                                |                                          |
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|                                          | regulation of cell adhesion         | regulation of cell adhesion                               | regulation of cell adhesion                       | regulation of cell adhesion             | regulation of cell adhesion                          | regulation of cell adhesion                                    | regulation of cell adhesion                 | regulation of cell adhesion               | regulation of cell adhesion              | regulation of cell adhesion                                      | regulation of cell adhesion                    | regulation of cell adhesion              | regulation of cell adhesion        | regulation of cell adhesion         | regulation of cell adhesion                                |                                          |
|                                          | regulation of cell adhesion         | regulation of cell                                        |                                                   |                                         |                                                      |                                                                |                                             |                                           |                                          |                                                                  |                                                |                                          |                                    |                                     |                                                            |                                          |

b

|                                                  |                                                |                                           |                                                           |                                  |                                 |                             |                                  |                                               |                                     |                                                                           |                                           |
|--------------------------------------------------|------------------------------------------------|-------------------------------------------|-----------------------------------------------------------|----------------------------------|---------------------------------|-----------------------------|----------------------------------|-----------------------------------------------|-------------------------------------|---------------------------------------------------------------------------|-------------------------------------------|
| regulation of developmental process              | regulation of multicellular organismal process | regulation of signaling                   | regulation of cell communication                          | regulation of localization       | response to external stimulus   |                             | chemotaxis                       | system process                                | behavior                            | nervous system process                                                    |                                           |
|                                                  | regulation of cellular component organization  | regulation of cellular component movement | positive regulation of signaling                          | regulation of locomotion         |                                 |                             |                                  |                                               |                                     |                                                                           |                                           |
| regulation of anatomical structure morphogenesis | regulation of neuron projection development    | regulation of biological quality          | regulation of cellular macromolecule biosynthetic process | regulation of biological process | response to endogenous stimulus | response to biotic stimulus | defense response                 | developmental process                         |                                     |                                                                           |                                           |
|                                                  |                                                |                                           |                                                           |                                  |                                 |                             |                                  |                                               |                                     |                                                                           |                                           |
| cellular component morphogenesis                 |                                                | supramolecular fiber organization         | post-embryonic animal organ development                   |                                  | actin cytoskeleton organization |                             | multicellular organismal process |                                               | multi-organism reproductive process | reproduction                                                              | movement of cell or subcellular component |
|                                                  |                                                |                                           |                                                           |                                  |                                 |                             |                                  |                                               | biological regulation               | biological process involved in interspecies interaction between organisms | response to stimulus                      |
| instar larval or pupal development               | cell projection organization                   | head development                          | muscle structure development                              | extracellular organization       | actin filament-based process    | locomotion                  | cell communication               | cellular component organization or biogenesis | signaling                           |                                                                           |                                           |

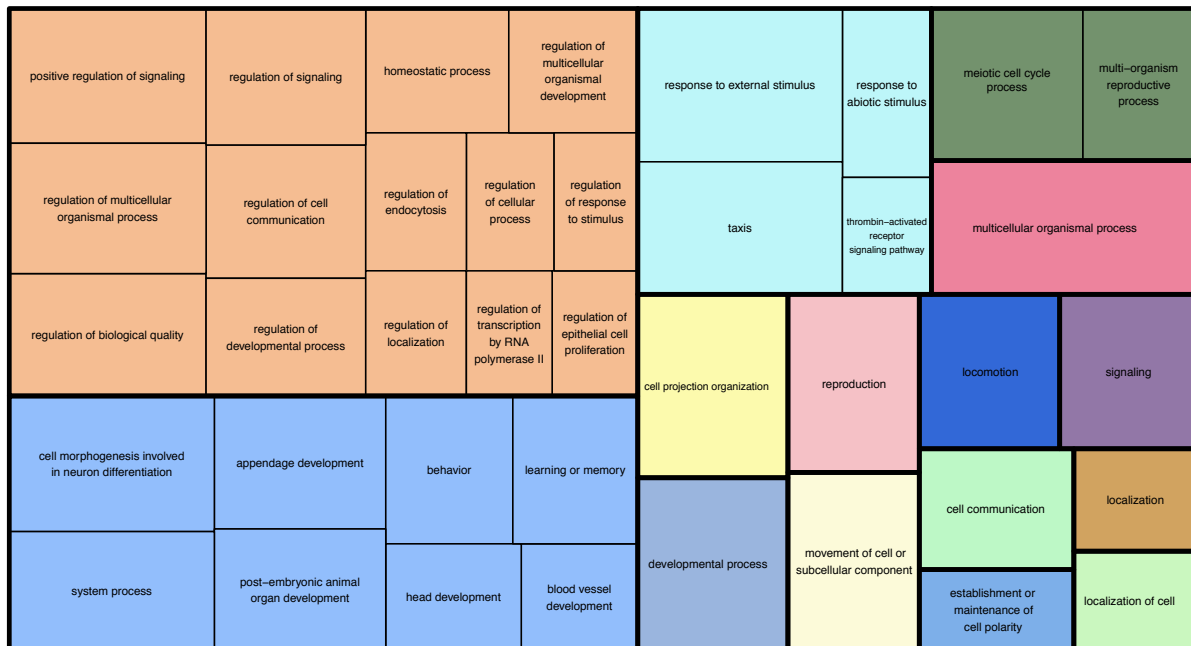
a

## CONTRACTED



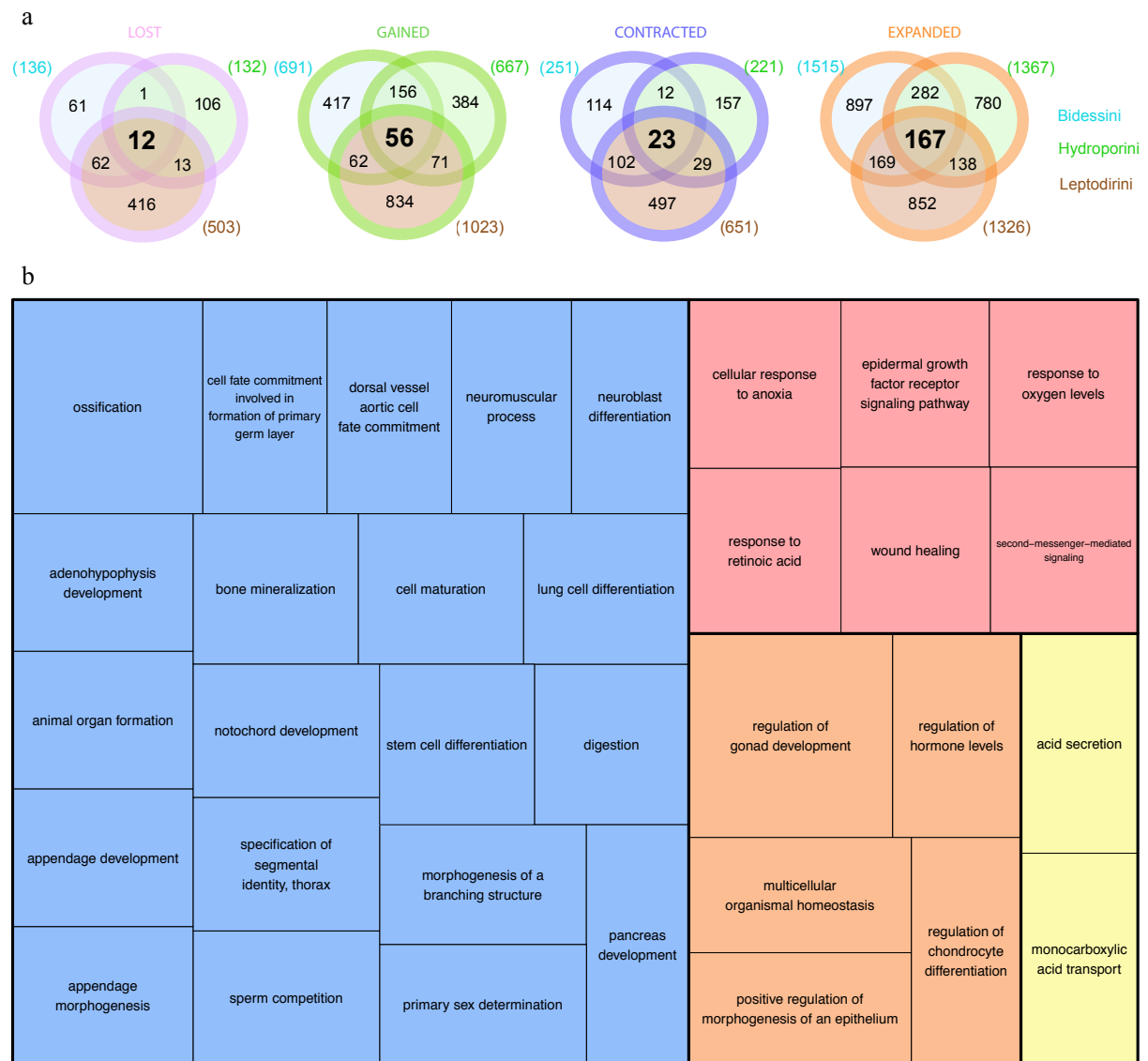
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## EXPANDED

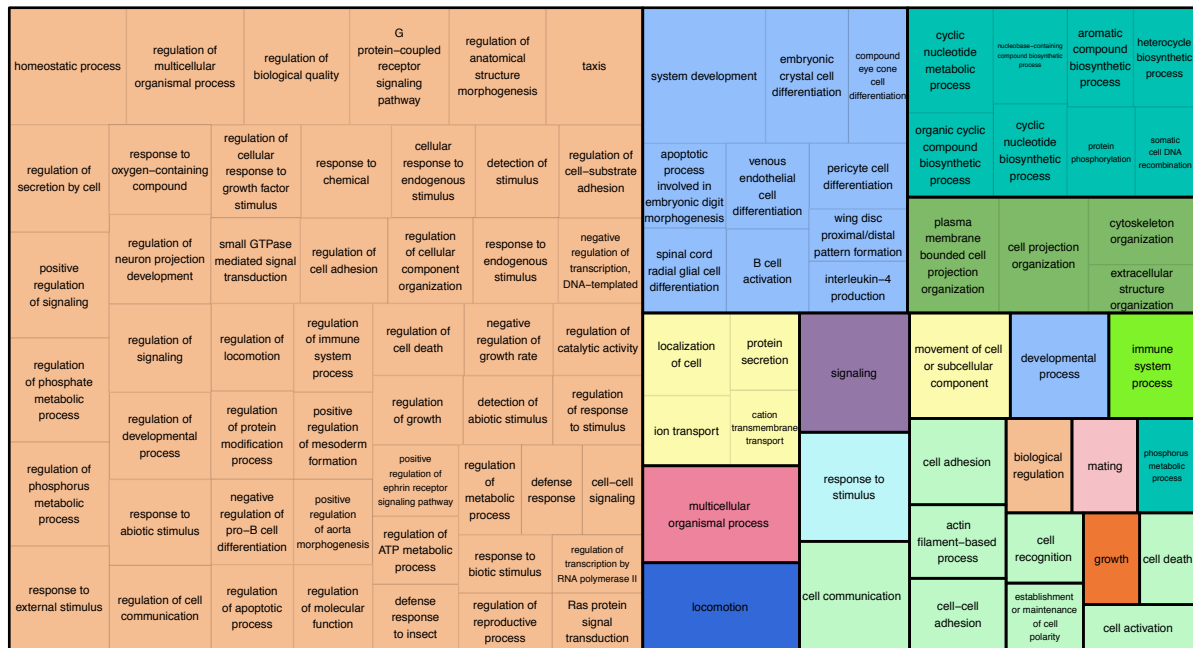


**Supplementary Figure 2.** Treemaps indicating the significantly overrepresented biological processes in the contracted (a), expanded (b) orthogroups in the most recent common ancestor of the tribe Hydroporini obtained with a gene ontology enrichment. The size of the square is scaled to the p-value.



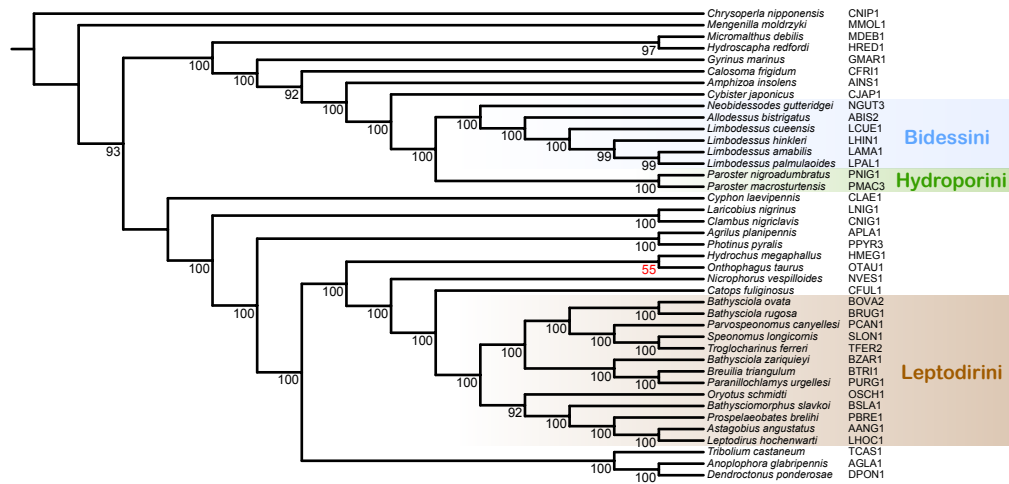


**Supplementary Figure 4.** Parallel evolution among the most recent common ancestors (MCRA) of the tribes Bidessini, Hydroporini and Leptodirini. **a** Venn diagrams indicating the total orthogroups with parallel evolution in the MRCA of the three tribes. **b** Treemap indicating the significantly overrepresented biological processes in the orthogroups parallelly contracted among the three MRCAs.

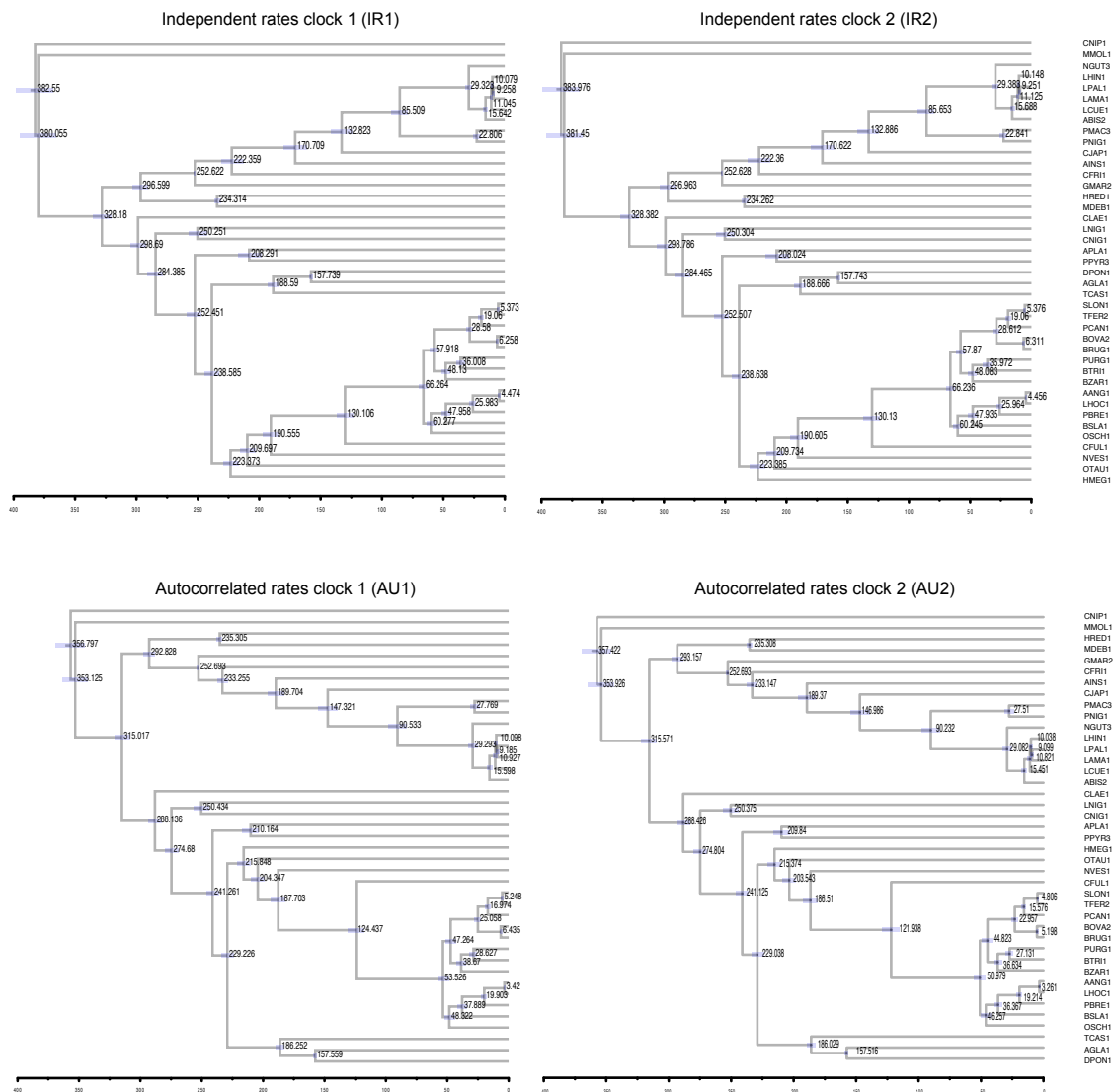


**Supplementary Figure 5.** Treemaps indicating the significantly overrepresented biological processes in the parallelly expanded orthogroups in the most recent common ancestor of the highly modified lineages of the tribe Leptodirini.

a



b



**Supplementary Figure 6. a** Maximum-likelihood phylogenetic tree inferred with 232 orthogroups. **b** Time-calibrated trees indicating the different time divergence estimations obtained with MCMCtree under different molecular clocks and alternative runs.