

Genetic and morphological insights reveal the hidden and colorful diversity in *Oscarella* sponges

SUPPLEMENTARY INFORMATION

Supplementary Figure 1: Picture of each specimen. Abbreviations: 3PP, 3PP; JAR, Jarre; LEV, Levant; MAI, Maïre; MEJ, Mejean; MUG, Mugel; TIB, Tiboulou du Frioul; and VES, Vesse.

Supplementary Figure 2: Haplotype networks supporting the delimitation of four new *Oscarella* species. Haplotype networks based on the *atp6*, *nad1-atp8*, *tatC*, *cox1* mitochondrial markers and *epic12* nuclear marker. Each haplotype (a, b, c, etc.) is depicted as a circle, with the size of the circle proportional to the number of specimens represented. Numbers between haplotypes indicate the number of mutations separating them. Colors correspond to species delimitation. Note that the *epic12* sequences were not phased. Yet, allelic variation was very limited in most species, with one to two alleles detected per species (except *O. tuberculata*), and a small number of heterozygous individuals were identified in which both allelic variants could be unambiguously recognized by repeated independent PCR amplifications and Sanger sequencing.

Supplementary Figure 3: Maximum likelihood (ML) phylogenetic tree of *Oscarella* species based on eight mitochondrial protein-coding genes (*cox1*, *nad1*, *atp8*, *atp6*, *cox3*, *atp9*, *nad4*, and *nad6*). Only nodes with bootstrap values greater than 85% (1,000 BS replicates) are shown. Dots on the tree indicate the inferred ancestral color state of the common ancestor of *Oscarella* and the last common ancestor of *O. lobularis*, *O. sabinae* sp. nov., *O. tuberculata*, and *O. veronicae* sp. nov. Note that *Pseudocortidium jarrei* is proposed to be renamed *Oscarella jarrei*.

Supplementary Figure 4. Histological semi-thin sections of the four *Oscarella* species newly described in this study. (A) *Oscarella fragilis* sp. nov.; (B) *Oscarella lunettae* sp. nov.; (C) *Oscarella sabinae* sp. nov.; (D) *Oscarella veronicae* sp. nov. Abbreviations: c.c., choanocyte chamber; ex, exopinacocyte; i.c., inhalant canal; v.c., vacuolar cell.

Supplementary Figure 5: Sequence alignment highlighting the unique diagnostic mutation found in the partial mitochondrial genome of *O. veronicae* sp. nov. This sequence, we called SNP, was amplified and sequenced for several specimens belonging to the species complex. The mutation specific to *O. veronicae* sp. nov. is marked by a black arrow.

Supplementary Table 1: List of *Oscarella* spp. specimens. It contains information regarding the sampling, the PCR results (haplotypes), and its assignment to a given species.

Supplementary Table 2: Accession numbers of mitochondrial genomes and haplotypes. Species names with an asterisk correspond to partial mitochondrial genomes. Species names underlined in blue indicate those obtained in the present study.

Supplementary Table 3: Nanopore sequencing coverage and consensus quality statistics for mitochondrial amplicon assemblies. Variants were detected using a minor allele frequency (MAF) threshold of $\geq 30\%$ and a minimum read support of $>30\times$, as defined by the Eurofins amplicon analysis pipeline. Phred quality scores represent the confidence assigned to the consensus base at each position, with higher scores indicating higher base-calling confidence.

Supplementary Table 4: Per-position Nanopore read support for phylogenetically relevant substitutions in mitochondrial assemblies.