

Validated methodology for quantifying infestation levels of dreissenid mussels in environmental DNA (eDNA) samples.

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Supplementary Information

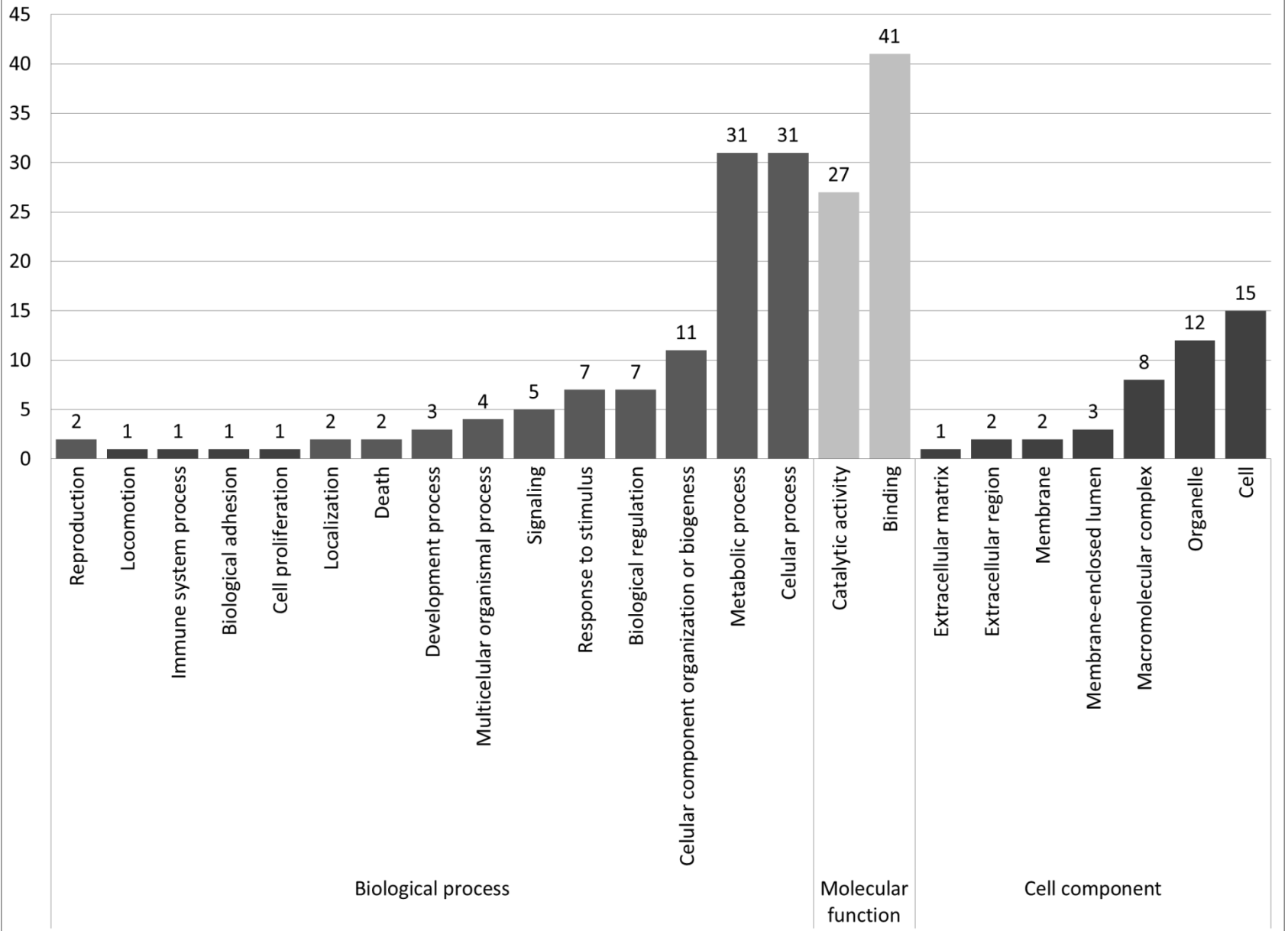
Supplementary Figure S1. Gene Ontology (GO) genes annotation for MPS sequences

Supplementary Figure S2. Agarose gel (1.5%) of representative adult individuals in the species specificity PCR. DP: *D. polymorpha*, DR: *D. rostriformis*, CF: *C. fluminea*, PS: *Pomacea sp.*, MA: *M. auricularia*, Neg: negative PCR control.

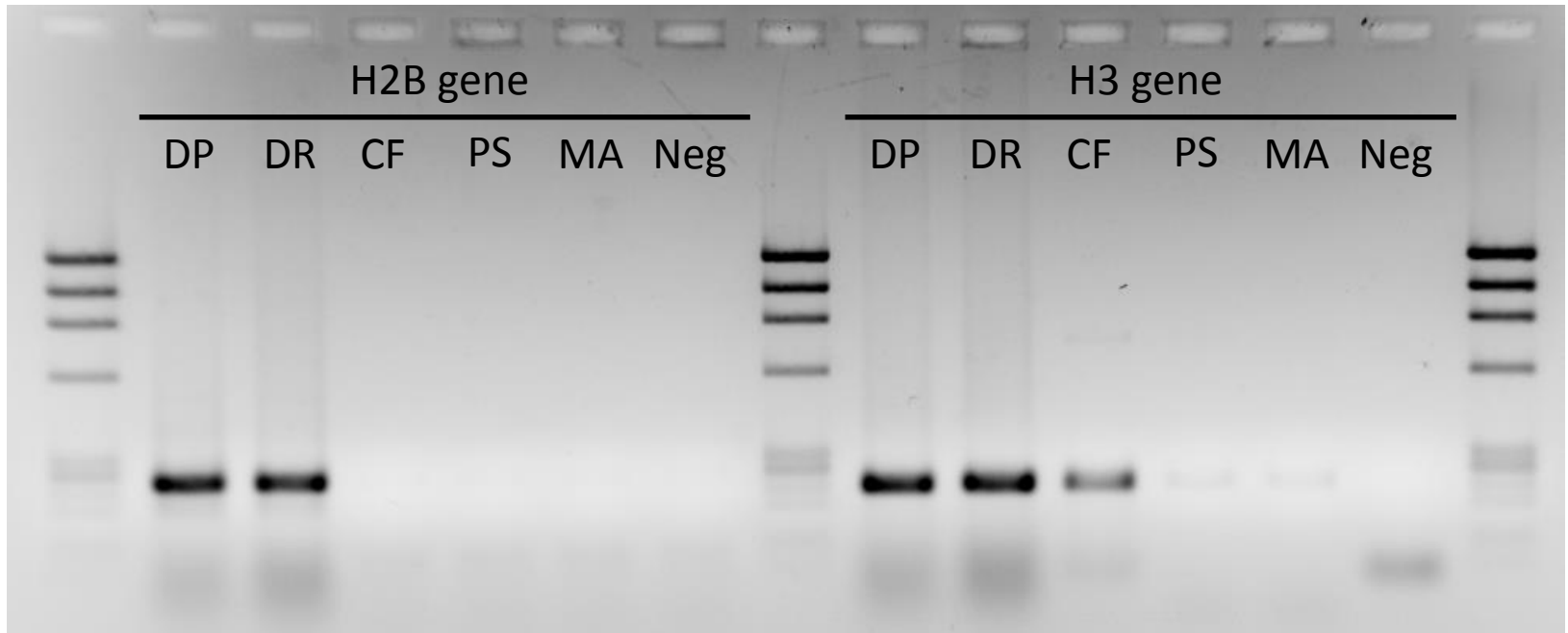
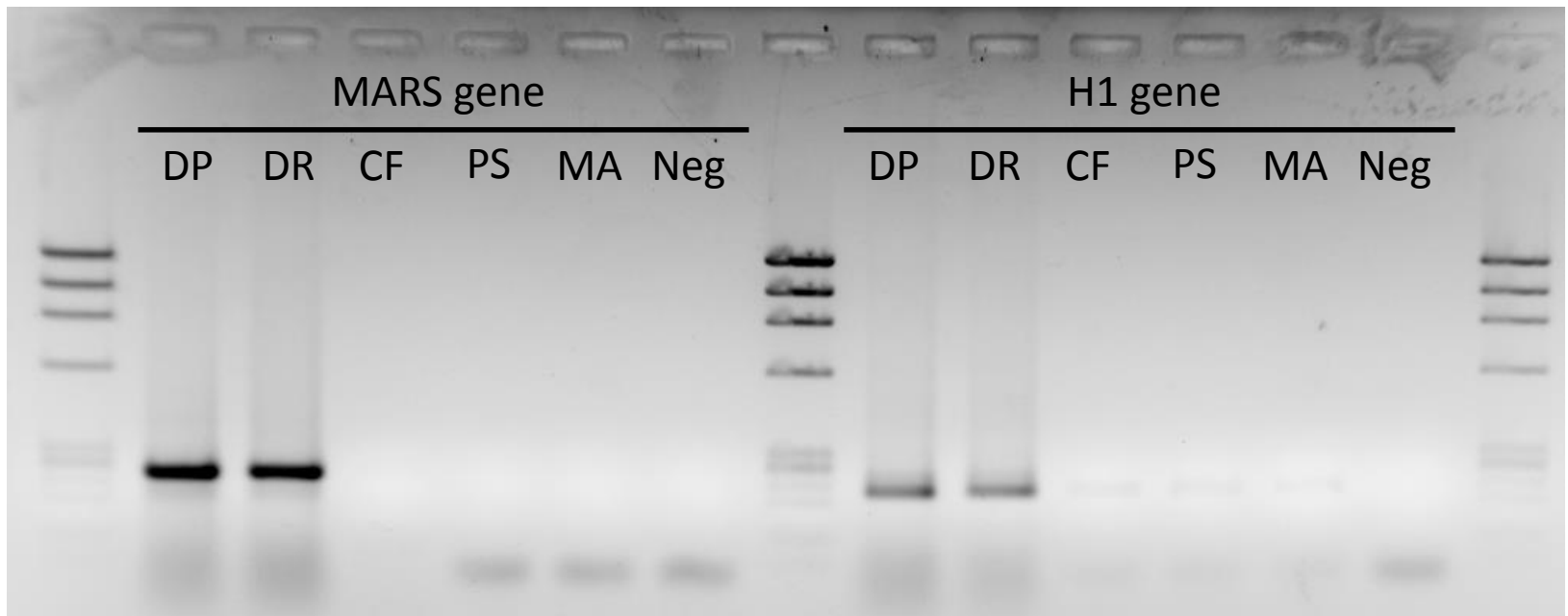
Supplementary Figure S3. Alignment of H2B sequences with the MPS sequence of reference. DP adult tissue represents all H2B sequences for the three *D. polymorpha* adult individuals. DR adult tissue represents H2B sequences for the two *D. rostriformis* adult individuals analyzed. Water samples sequence represents all environmental samples, all of them matching completely with adult *D. polymorpha* H2B sequences.

Supplementary Figure S4. Map of the situation of the water samples analyzed in this study. All locations are situated in the Northwest Iberian Peninsula (grey area). The map was generated by the authors using the vector drawing program *Inkscape version 0.48.1* (<https://inkscape.org/en/>).

GO terms annotation



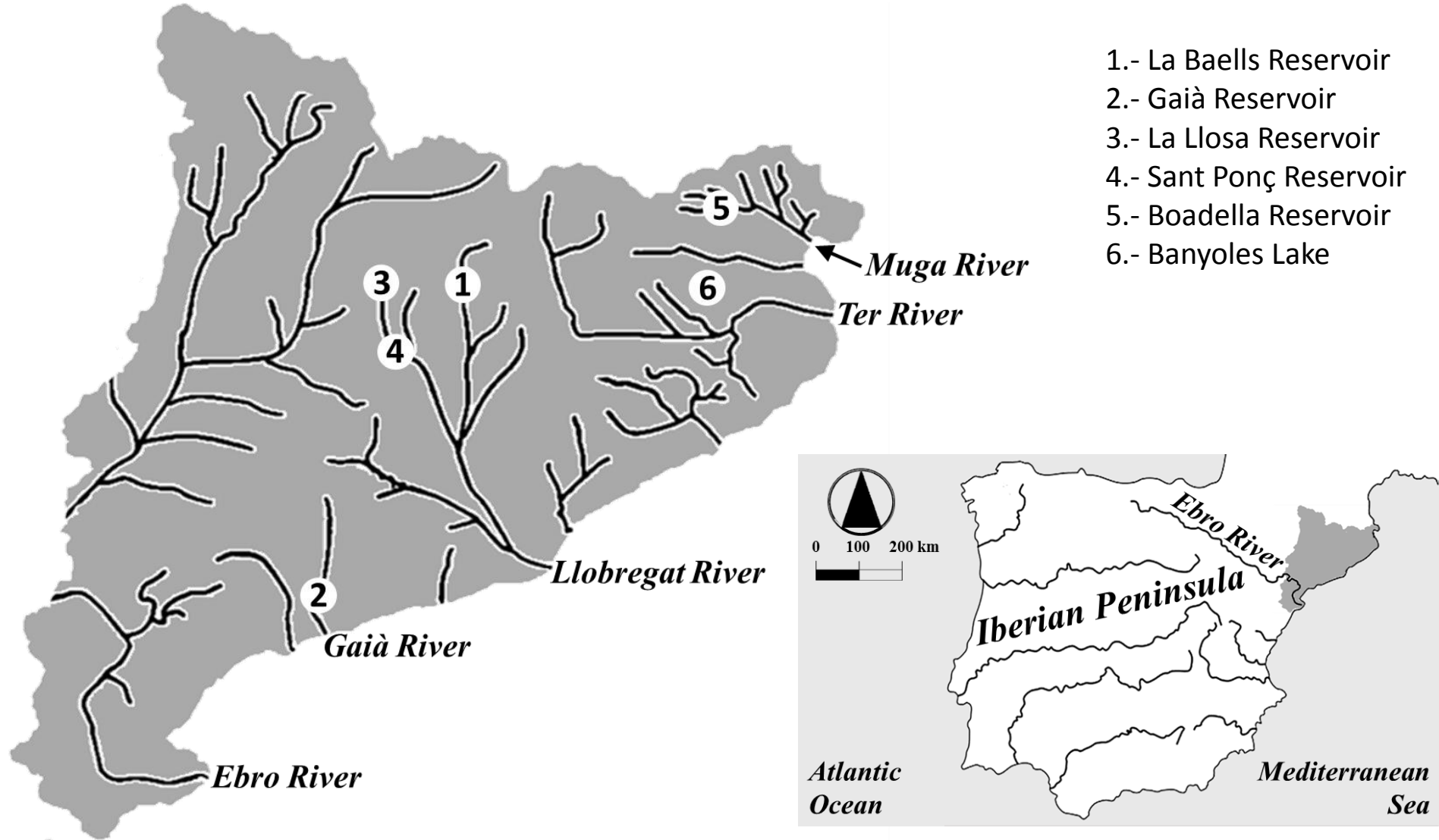
Supplementary Figure S1



Supplementary Figure S2

| | | | | | | | |
|-----------------|------------|------------|------------|-------------|-------------|------------|----------|
| | 1 | 5 | 15 | 25 | 35 | 45 | 55 |
| | | | | | | | |
| Contig000076 | CGCGCGCTCC | ACTGACAAGA | AGAAGCGCAG | GAGGAGGAGG | GAATCCTACG | CCATCTACAT | |
| DP adult tissue | | | | | | | |
| DR adult tissue | | | | |A..... | | |
| Water samples | | | | | | | |
| | | 65 | 75 | 85 | 95 | 105 | 115 |
| | | | | | | | |
| Contig000076 | CTACAAAGTC | TTGAAGCAGG | TGCACCCCGA | CACCGGAGTG | TCCAGCAAGG | CCATGTCGAT | |
| DP adult tissue | | | | | | | |
| DR adult tissue | | C.C..... | | | | |C.. |
| Water samples | | | | | | | |
| | | 125 | 135 | 145 | 155 | 165 | 175 |
| | | | | | | | |
| Contig000076 | CATGAACAGC | TTTGTCAACG | ACATCTTCGA | GCGCATTGCT | GCCGAGGCTT | CCCGCCTTGC | |
| DP adult tissue | | | | | | | |
| DR adult tissue | | | |C..... | | | |
| Water samples | | | | | | | |
| | | 185 | 195 | 205 | 215 | 225 | 235 |
| | | | | | | | |
| Contig000076 | CCACTACAAC | AAGCGATCCA | CCATCACAAG | CAGAGAGATC | CAGACCGCTG | TGCGTCTCCT | |
| DP adult tissue | | | | | | | |
| DR adult tissue | | | | | | | |
| Water samples | | | | | | | |
| | | 245 | 251 | | | | |
| | | | | | | | |
| Contig000076 | GCTGCCTGGT | G | | | | | |
| DP adult tissue | | . | | | | | |
| DR adult tissue | | . | | | | | |
| Water samples | | . | | | | | |

Supplementary Figure S3



Supplementary Figure S4