

REPLY TO GAUDRY ET AL.:

Cross-validation is necessary for the identification of pseudogenes

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Our article (1) describing a widespread loss of uncoupling protein 1 (UCP1) in cetaceans, sirenians (the manatee), and one pinniped (the Antarctic fur seal, *Arctocephalus gazella*) as a convergent mechanism to minimize heat loss has raised questions from experts in the field of mammalian energy metabolism (2).

Briefly, Gaudry et al. (2) argue, after examining raw high-throughput sequencing data from National Center for Biotechnology Information Sequence Read Archive, that 1) while UCP1 inactivation is likely associated with a higher reliance on insulation in fully aquatic mammals (cetaceans and sirenians), this is likely not the case for pinnipeds; 2) our findings

reflect a misassembly of the *A. gazella* genome (generated by ref. 3); and 3) when UCP1 is lost in pinnipeds, this event is possibly associated with a greater body size (case in point, the northern and southern elephant seals).

Apparent UCP1 loss is observed in *A. gazella* in a genome assembly derived from PacBio sequencing data (v1.2 and subsequent versions) (3), while an assembly derived from Illumina sequencing (v1.1) (4) indicates that the gene is intact (Fig. 1). We are very grateful to Gaudry et al. (2) for pointing out this error. This reminds us that it is not only important to assess the quality of published genomes prior to

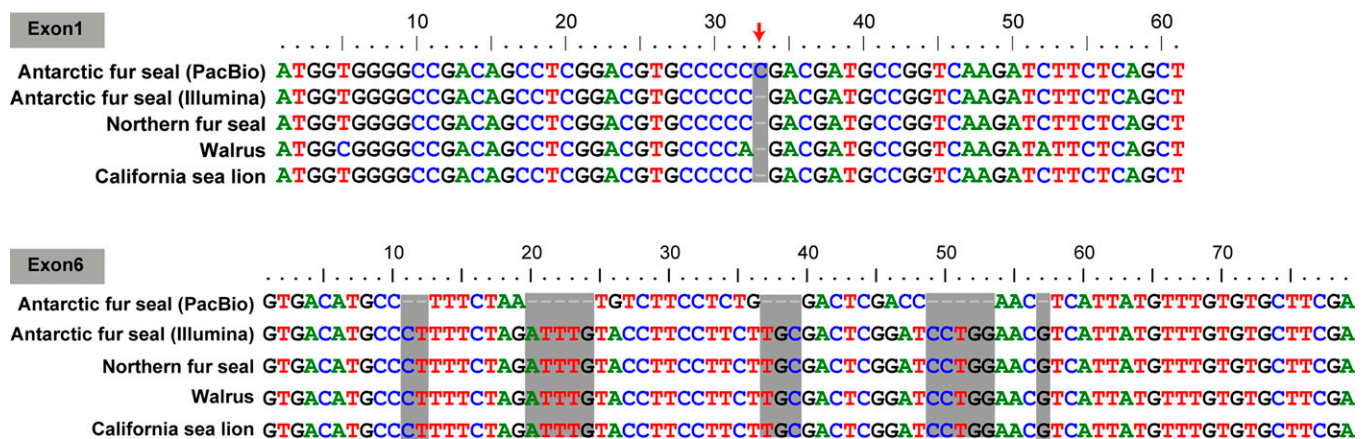


Fig. 1. Differences in exon 1 and exon 6 sequences of UCP1 in Antarctic fur seal PacBio and Illumina genome assemblies. Gray blocks indicate regions unique to the Antarctic fur seal PacBio assembly. For comparison, the sequences of northern fur seal, walrus, and California sea lion are shown.

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The authors declare no competing interest.

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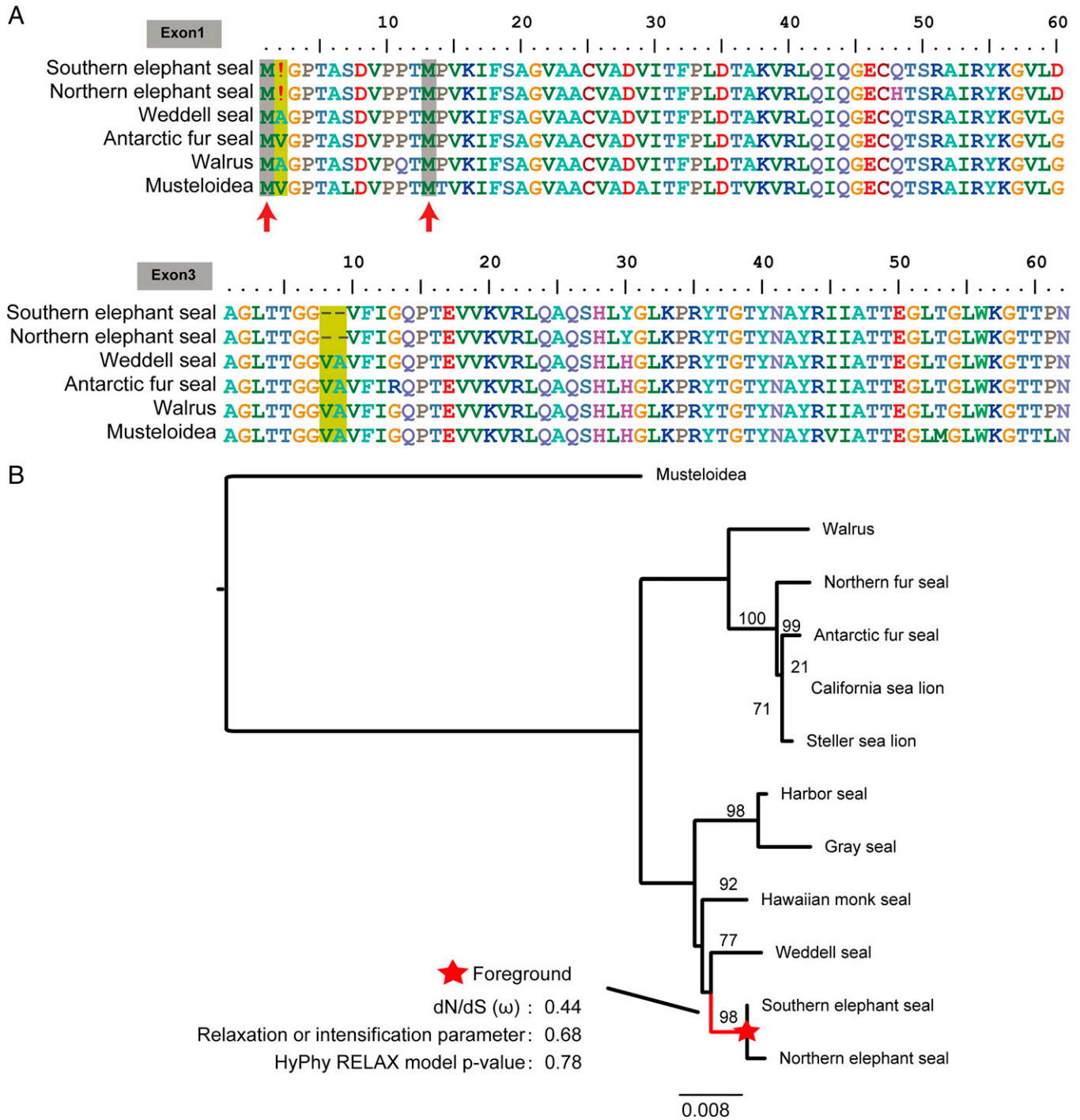


Fig. 2. (A) Alignment of exon 1 and exon 3 of the *UCP1* of southern elephant seal and northern elephant seal. The gray blocks and the red arrows show the position of the start codon, and the yellow blocks indicate amino acids missing in the elephant seals. (B) Summary of relaxed selection test of northern and southern elephant seal *UCP1*. A red star indicates the foreground branch.

data analysis but also necessary to cross-validate using data from multiple sources before conclusions are made.

Gaudry et al. (2) describe pseudogenization of *UCP1* in the northern and southern elephant seals and speculate that this is linked with the large body size of the two species. While this extrapolation is fascinating, we argue that this conclusion is not necessarily valid. Firstly, the frameshift in exon 1 is located in the ostensibly 5' untranslated region; another start codon appears about 10 amino acids later (Fig. 2A). Considering that we did not

detect a signal of relaxed selection in these species (Fig. 2B), we cannot fully confirm, without transcriptomic data, that this gene has been pseudogenized. Secondly, even if this gene has been lost in elephant seals, a link between loss of *UCP1* and body size, in our opinion, is not straightforward. *UCP1* loss could reflect an adaptive thermoregulatory mechanism coincidentally associated with a larger body size. However, we agree with Gaudry et al., in general, that the thermoregulatory strategy is likely to be different between fully aquatic and semiaquatic marine mammals.

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