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# Supplementary Materials for

## Gene losses in the common vampire bat illuminate molecular adaptations to blood feeding

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**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**

**Table S6: Software used in the present study.**

| Software | Version or *git commit* | URL | Reference | Section |
|---|---|---|---|---|
| ccs | 6.0.0 | https://github.com/PacificBiosciences/ccs | - | genome assembly |
| hifiasm | 0.15.1-r331 | https://github.com/chhylp123/hifiasm | (*111*) | |
| SALSA2 | *1b76bf63efb973583647a1eb95863d33ee6e09ad* | https://github.com/marbl/SALSA | (*112*) | |
| Omni-C mapping pipeline | 0.1 | https://omni-c.readthedocs.io/en/latest/fastq_to_bam.html | - | |
| BWA-MEM | 0.7.17-r1188 | https://github.com/lh3/bwa | (*113*) | |
| pairtools | 0.3.0 | https://github.com/open2c/pairtools | - | |
| cooler | 0.8.11 | https://github.com/open2c/cooler | (*114*) | |
| HiGlass | 2.1.11 | http://higlass.io | (*115*) | |
| SeqKit | 0.13.2 | https://github.com/shenwei356/seqkit | (*116*) | |
| pbmm2 | 1.3.0 | https://github.com/PacificBiosciences/pbmm2 | - | |
| gcpp | 2.0.2-2.0.2 | https://github.com/PacificBiosciences/gcpp | - | |
| freebayes | 1.3.2 | https://github.com/freebayes/freebayes | - | |
| DeepVariant | 1.1.0 | https://github.com/google/deepvariant | (*117*) | |
| Merqury | 1.0 | https://github.com/marbl/merqury | (*118*) | |
| bcftools | 1.12 | https://github.com/samtools/bcftools | (*119*) | |
| RepeatModeler | 2.0.1 | http://www.repeatmasker.org | - | repeat masking |
| RepeatMasker | 4.0.9 | http://www.repeatmasker.org | - | |

| | | | | |
|---|---|---|---|---|
| LASTZ | 1.04.03 | https://github.com/lastz/lastz | (*120*) | genome alignment |
| axtChain | 1.0 | https://github.com/ucscGenomeBrowser/kent | (*121*) | |
| RepeatFiller | 1.0 | https://github.com/hillerlab/GenomeAlignmentTools | (*122*) | |
| chainCleaner | 1.0 | https://github.com/hillerlab/GenomeAlignmentTools | (*123*) | |
| TOGA | 1.0 | https://github.com/hillerlab/TOGA | - | orthology inference and gene loss screen |
| CESAR | 2.0 | https://github.com/hillerlab/CESAR2.0 | (*124*) | |
| BWA-MEM | 0.7.7-r441 | https://github.com/lh3/bwa | (*113*) | |
| Picard | 2.21.4 | http://broadinstitute.github.io/picard | - | |
| UCSC genome browser | - | https://genome.ucsc.edu | (*125*) | |
| MACSE | 2 | https://bioweb.supagro.inra.fr/macse | (*126*) | relaxed selection |
| HmmCleaner | 0.180750 | https://metacpan.org/dist/Bio-MUST-Apps-HmmCleaner | (*127*) | |
| RELAX | 3.1 | https://github.com/veg/hyphy | (*128*) | |
| STAR | 2.7.3 | https://github.com/alexdobin/STAR | (*129*) | gene expression analysis |
| IGV | 2.8.9 | https://software.broadinstitute.org/software/igv | (*130*) | |
| SciPy stats | - | https://scipy.org | (*131*) | whole blood iron measurements |

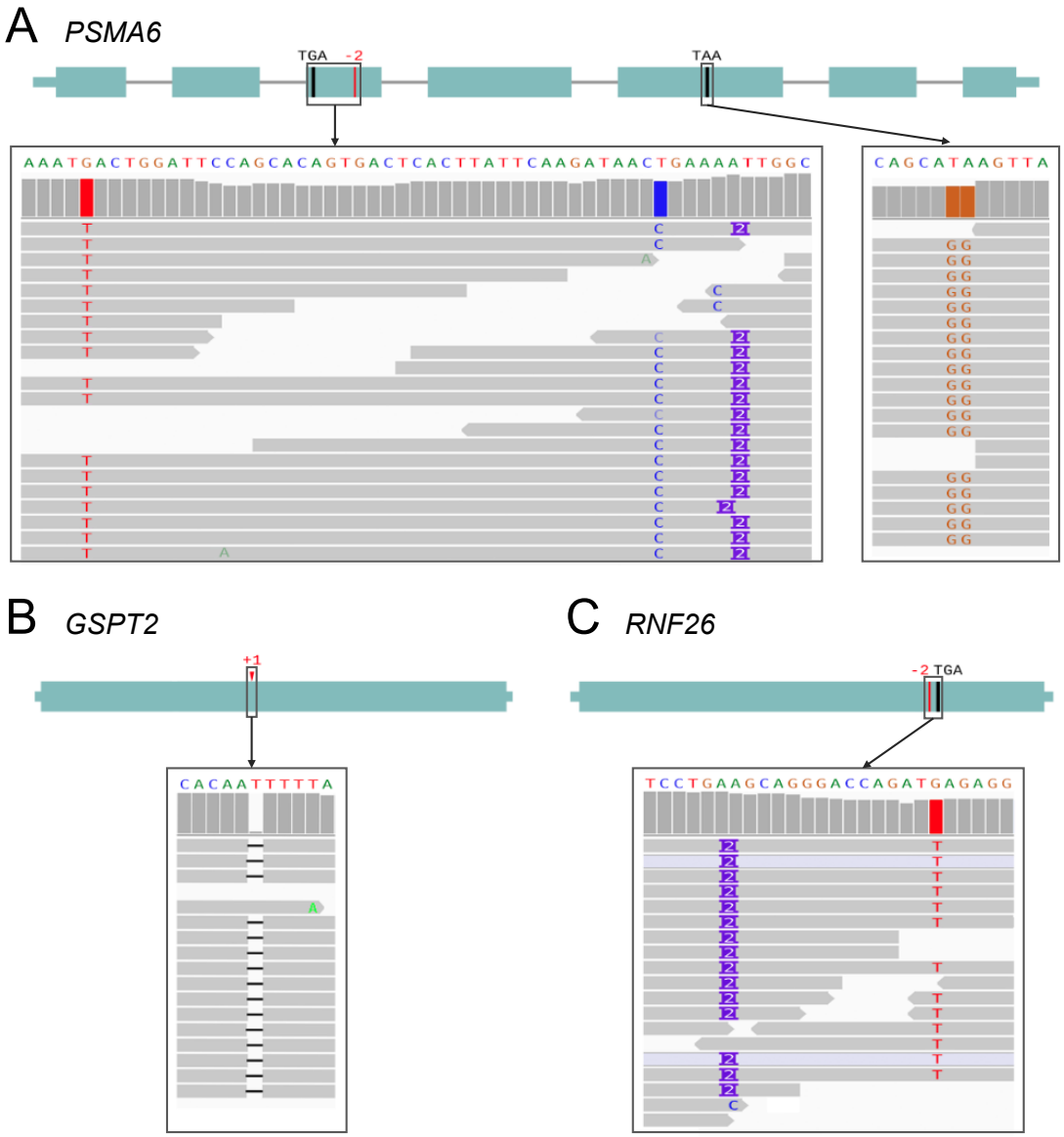


**Figure S1: Base errors in the Illumina-based *Desmodus rotundus* genome assembly mimic gene losses.**

(A) *PSMA6* (Proteasome 20S subunit alpha 6) exhibits three inactivating mutations in two exons in the Illumina-based assembly, indicating loss of this gene. However, aligning the raw Illumina reads that were used to generate this assembly (grey lines in the IGV screenshots (*130*)) provides no support for the inactivating alleles and instead supports the non-inactivating alleles, suggesting that these mutations are base errors in the assembly.

(B) *GSPT2* (G1 to S phase transition 2) exhibits a 1 bp insertion, which is not supported by aligning Illumina reads.

(C) *RNF26* (Ring finger protein 26) exhibits two adjacent mutations, both of which are base errors in the Illumina-based assembly.

Corroborating that the apparent mutations are base errors, all three genes lack any inactivating mutation in our new reference-quality haplotype assemblies.

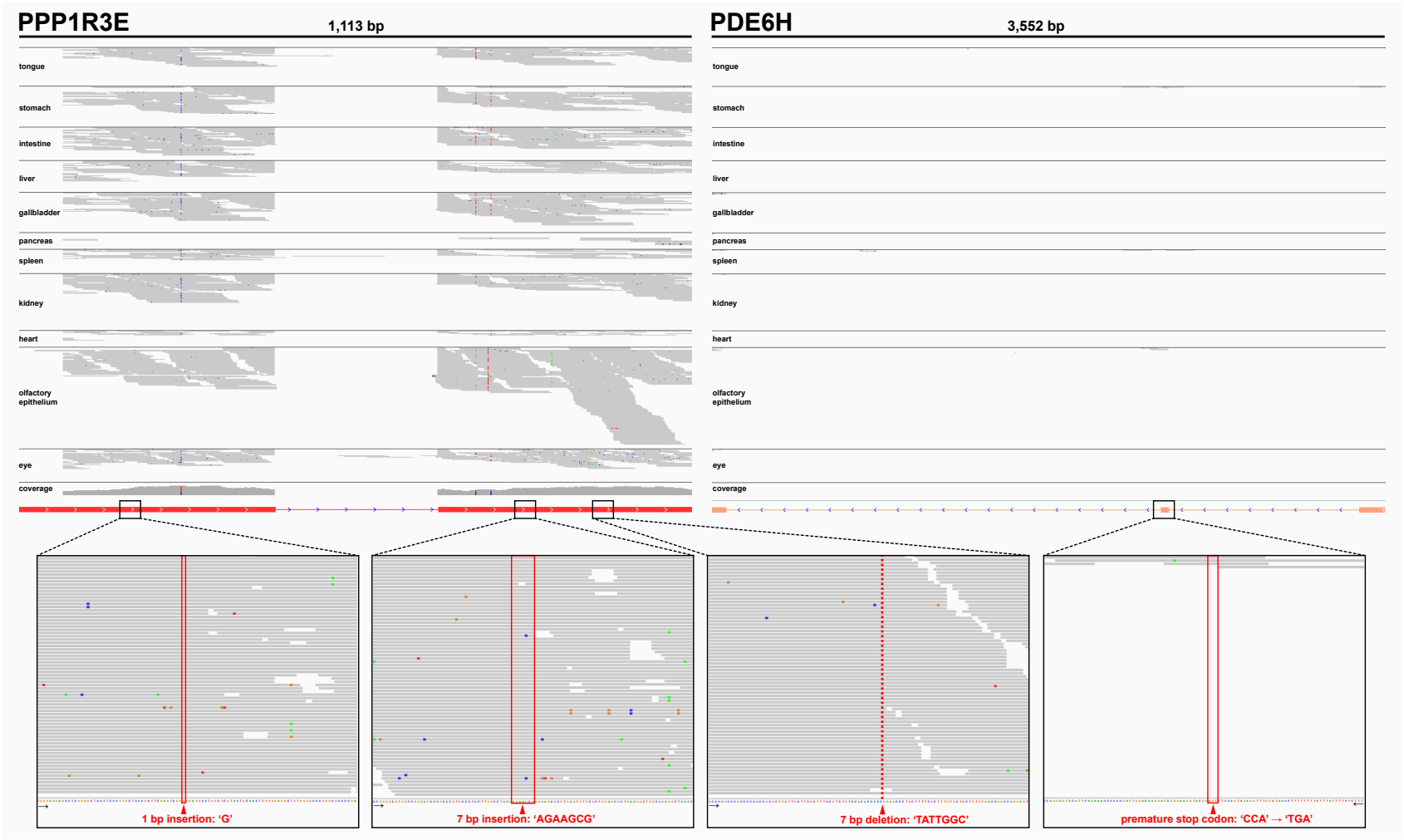


**Figure S2: RNA expression at gene loss loci.**

The upper panels show aligned RNA sequencing reads for 11 tissues (Table S4) and the remnant exons of the lost genes in the *D. rotundus* haplotype 1 assembly. The lower panel displays the merged sequencing read sets zoomed in at the 100 bp regions around inactivating mutations. *PPP1R3E* (left) represents an example of an inactivated gene where expression and splicing is still observed. Importantly, all three inactivating mutations are present in the aligned reads, which confirms that no functional protein is produced. *PDE6H* (right) represents an example of a gene loss where no relevant expression and splicing is detected. Only few reads align, but they support the inactivating mutation.

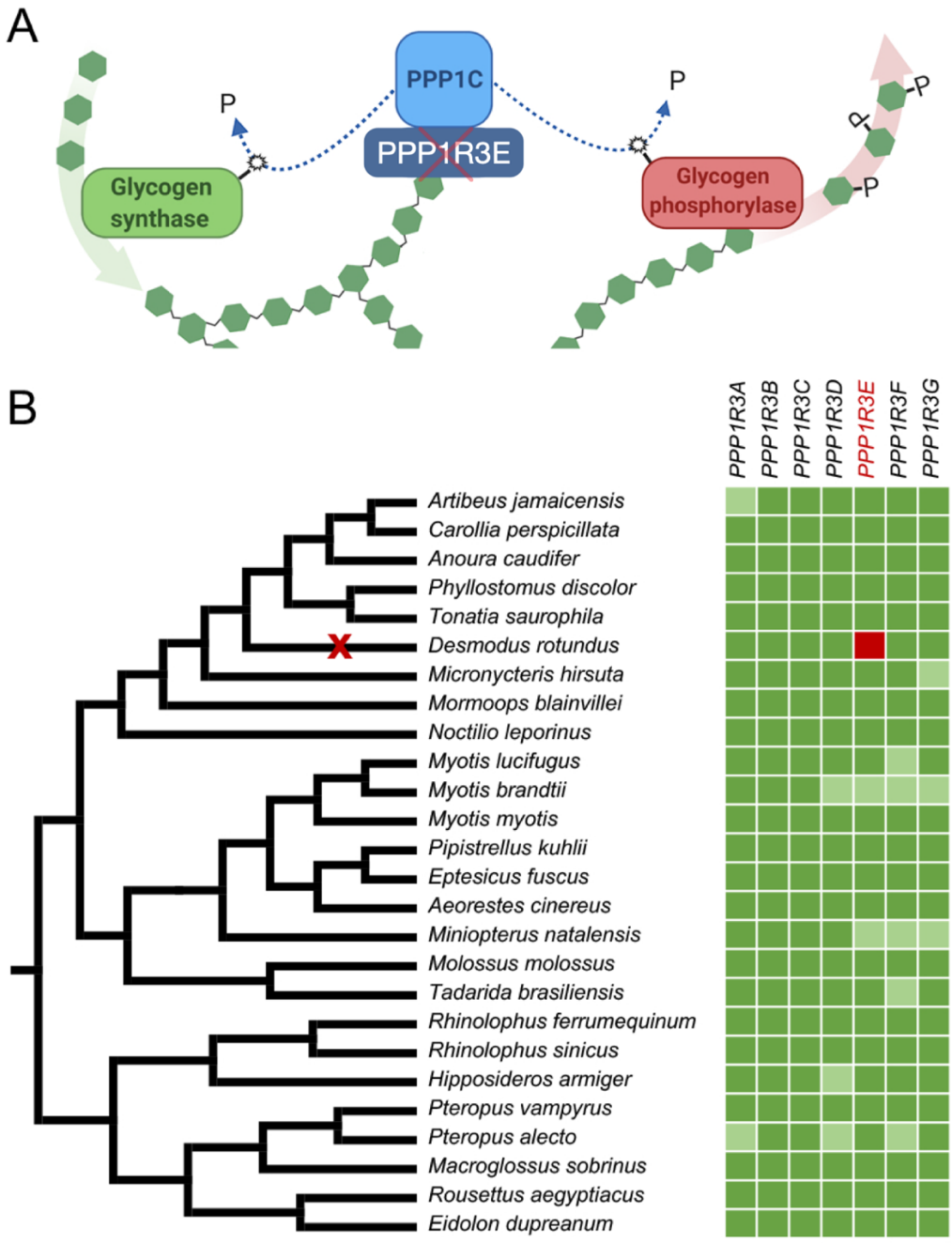


**Figure S3: Presence and loss of regulatory subunits of protein phosphatase 1.**

(A) PPP1R3E directly binds to glycogen and the protein phosphatase 1 catalytic subunit. Protein phosphatase 1 regulates a switch between glycogenesis and glycogenolysis. By dephosphorylating glycogen synthase, it activates glycogenesis. By dephosphorylating glycogen phosphorylase, it inhibits glycogenolysis.

(B) Of all *PPP1R3* genes that encode regulatory subunits, *PPP1R3E* is the only one that is lost in *D. rotundus*. Two green shades indicate genes lacking inactivating mutations, with light green indicating that some parts of the coding region are missing due to assembly gaps or fragmentation.

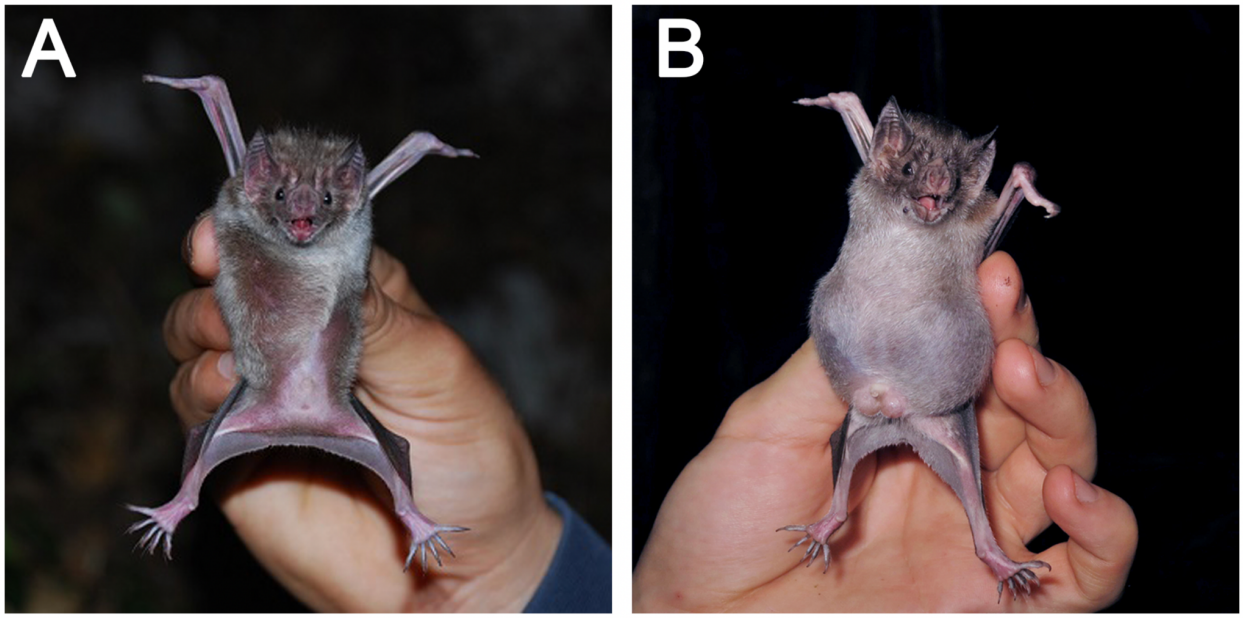


**Figure S4: The stomach of *Desmodus rotundus* evolved into a gastric caecum.**
*D. rotundus* features an unparalleled gastric morphology with a stomach that has been repurposed into a distensible storage structure termed a gastric caecum (*132-134*). Shown are two different *D. rotundus* specimens before (A) and after feeding (B), where the blood-filled gastric caecum causes distention of the entire abdomen. Photographs were kindly provided with permission by Victor Mendoza Sáenz (A, ECOSUR) and Jonathan Flanders (B, Bat Conservation International).
It should be emphasized that while the shown photographs are several years old, the IUCN Bat Specialist Group strongly recommends the use of gloves while handling bats, as bats can harbor pathogens.

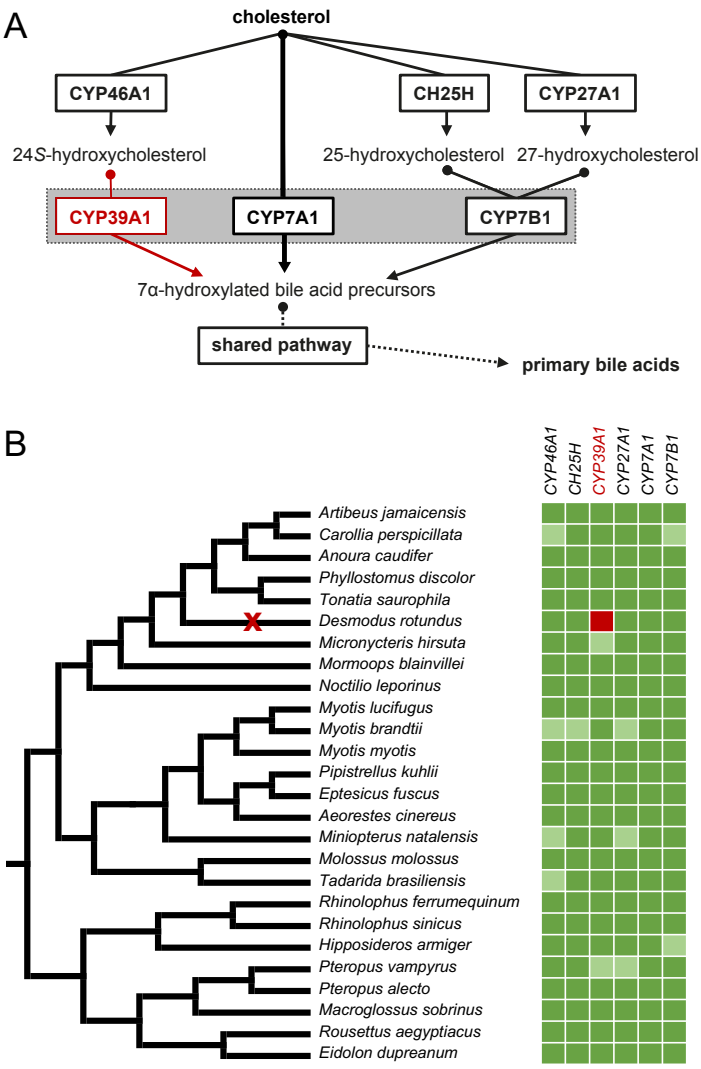


**Figure S5: *Desmodus rotundus* maintains the major and one alternative pathway for bile acid production.**

(A) The role of *CYP39A1* in bile acid synthesis (*135*): In the major pathway of bile acid synthesis (center) cholesterol is directly 7α-hydroxylated by CYP7A1. This enzyme produces the majority of secreted bile acid and takes place in the liver. In two alternative pathways (left and right), cholesterol is in the first step converted to an oxysterol by CYP46A1 (→ 24S-hydroxycholesterol), CH25H (→ 25-hydroxycholesterol) or CYP27A1 (→ 27-hydroxycholesterol). 25- and 27-hydroxycholesterol are then 7α-hydroxylated by CYP7B1, while 24S-hydroxycholesterol is 7α-hydroxylated by CYP39A1. All 7α-hydroxylated bile acid precursors undergo further modifications to form primary bile acids. Of the three respective 7α-hydroxylases (grey box), only the gene encoding CYP39A1 (red) is lost in *D. rotundus*. Since CYP39A1 is highly specific towards 24S-hydroxycholesterol (*76*) and since knockout of the upstream enzyme CYP46A1 in mice does not result in a significantly reduced bile acid pool (*136*), the CYP46A1/CYP39A1 pathway contributes little to the total hepatic bile acid synthesis (*135*).

(B) Presence and loss of genes involved in the initial steps of bile acid synthesis pathways. Two green shades indicate genes lacking inactivating mutations, with light green indicating that parts of the coding region are missing due to assembly gaps or fragmentation.

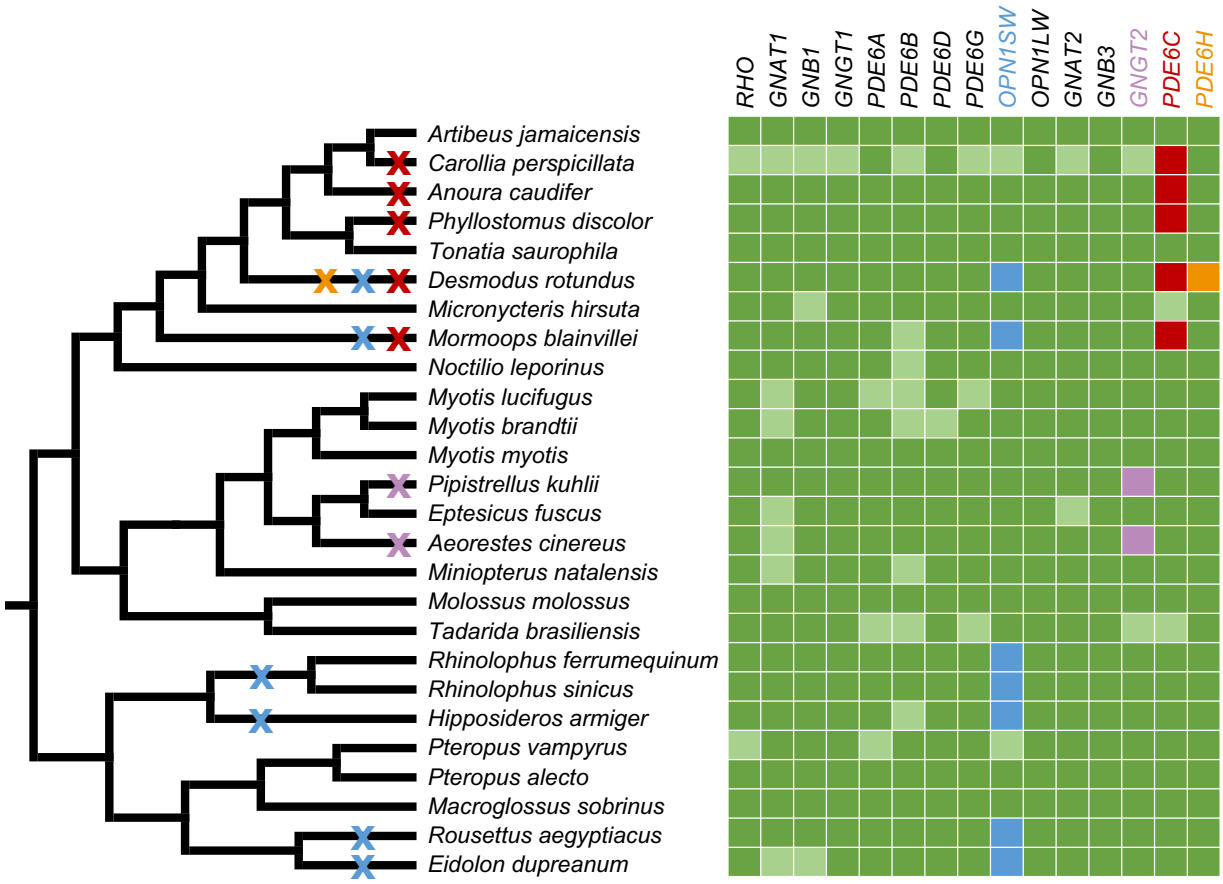


**Figure S6: Presence and loss of genes in the rod and cone phototransduction cascades.**
Two green shades indicate genes lacking inactivating mutations, with light green indicating that some parts of the coding region are missing due to assembly gaps or fragmentation. Blue, purple, red and orange indicate losses of different genes. For *PDE6A*, no orthologous alignments were present at all in the Sanger sequencing-based assembly of *Myotis lucifugus* (grey). Losses of the short-wavelength sensitive opsin (*OPN1SW*) in the vampire bat and other bats have been previously reported (*137-140*).

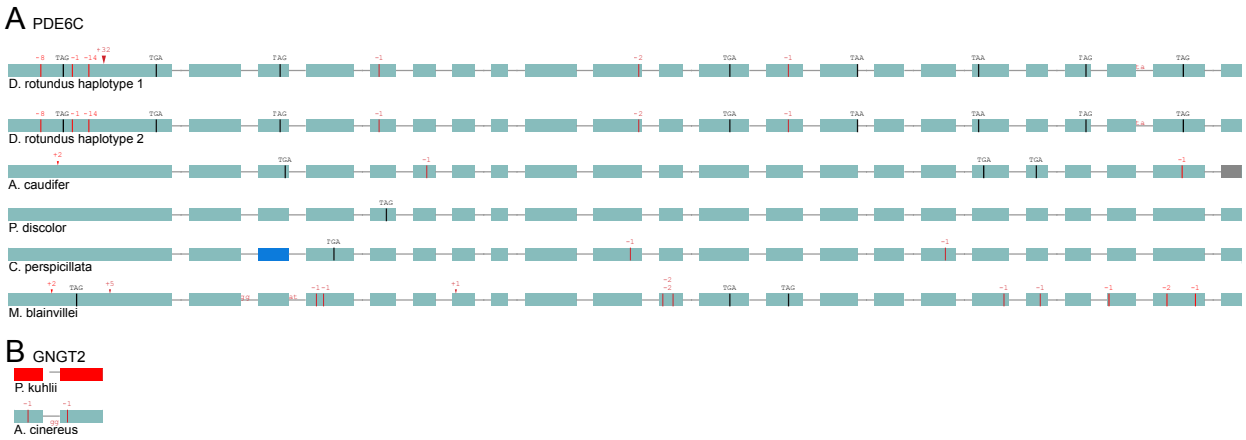


**Figure S7: Inactivating mutations in the cone phototransduction genes *PDE6C* (A) and *GNGT2* (B) in bats.**

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