

Sophie Teullet¹, Rémi Allio^{1,2}, Dave Lutgen^{1,3,4}, Christopher A. Emerling^{1,5}, Marie-Ka Tilak¹, Amandine Magdeleine¹, Benoit de Thoisy^{6,7}, Tristan Lefebure⁸, Guillaume Borrel⁹ & Frédéric Delsuc¹

¹ISEM, Univ Montpellier, CNRS, IRD, Montpellier, France; ²CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ. Montpellier, Montpellier, France; ³Institute of Ecology and Evolution, University of Bern, Bern, Switzerland; ⁴Swiss ornithological Institute, Sempach, Switzerland; ⁵Biology Department, Reedley College, Reedley, CA, USA; ⁶Institut Pasteur de la Guyane, Cayenne, French Guiana, France; ⁷Kwata NGO, Cayenne, French Guiana, France; ⁸Univ. Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR 5023 LEHNA, F-69622, Villeurbanne, France; ⁹Institut Pasteur, Université Paris Cité, UMR CNRS 6047, Evolutionary Biology of the Microbial Cell, Paris, France.

@sophieteullet

Specialized in the consumption of ants and/or termites, myrmecophagous mammals represent a striking example of dietary convergence. They evolved five times independently in armadillos, anteaters, pangolins, and aardwolves (Fig 1). Myrmecophagous species consume large quantities of insects whose chitinous exoskeletons must be digested. Chitinolytic enzymes (Chitinases) can be produced by both the host and its gut microbiota. Five chitinase paralogs (*CHIA1-5*) have been inferred in the last common ancestor of placentals with subsequent losses occurring in non-insectivorous lineages (Emerling *et al*, 2018). Myrmecophagous mammals have distinct *CHIA* gene repertoires (Emerling *et al*, 2018). These genes have been found expressed in the salivary glands and other digestive organs of the Malayan pangolin suggesting a potential role of these genes in the digestion of prey (Cheng *et al*, 2022; Ma *et al*, 2017, 2019). Microbial chitinases have been identified in the gut microbiota of mammals with a chitin-rich diet including myrmecophagous species (Cheng *et al*, 2022; Ma *et al*, 2018; Sanders *et al*, 2015) raising questions about the potential role of the gut microbiome in chitin digestion.

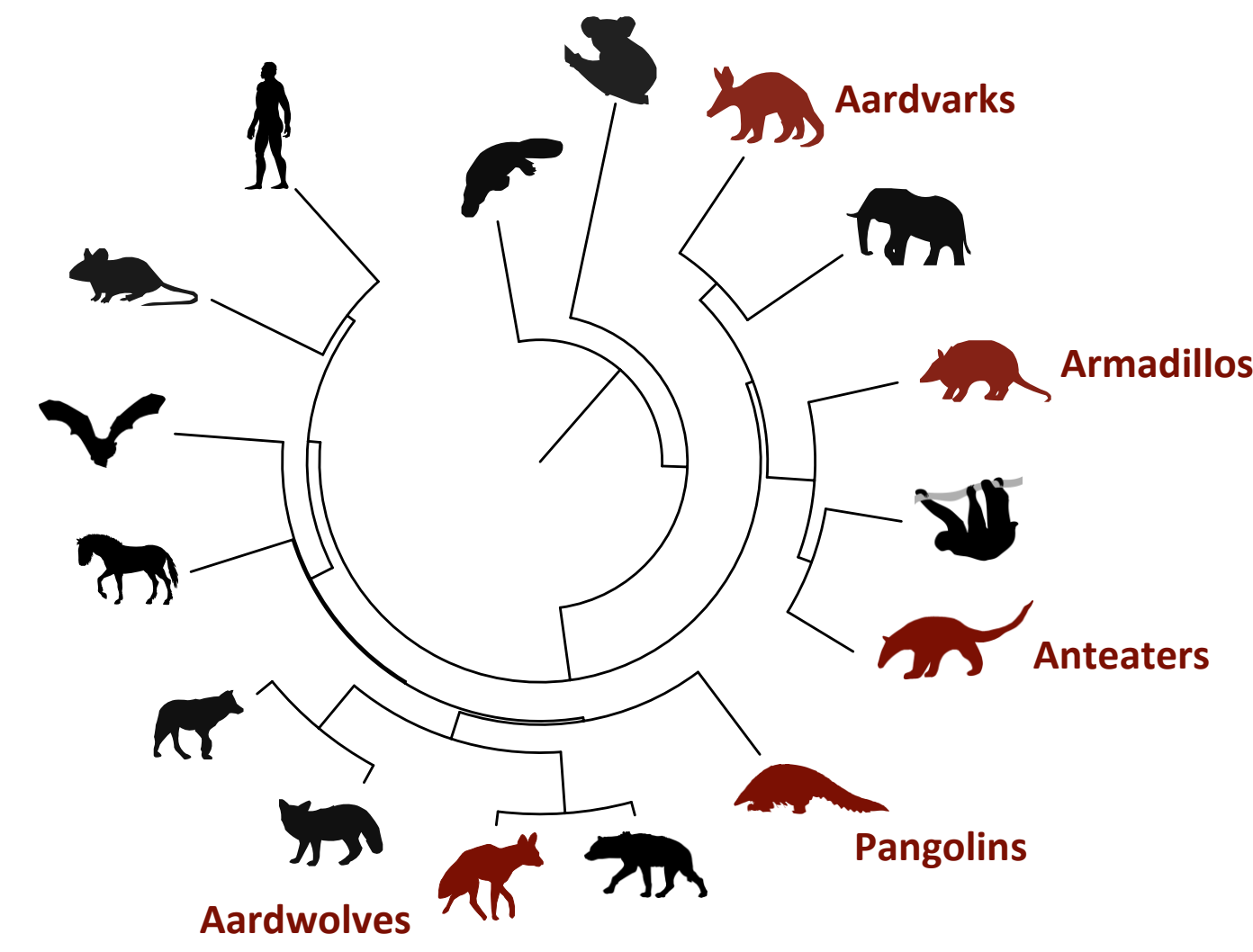


Fig 1. Phylogeny of myrmecophagous mammals (in red).

Do myrmecophagous species rely on endogenous or microbial chitinases (or both) to digest their prey?

Do the same genomic and microbial adaptations are involved in convergent myrmecophagous species?

Molecular tinkering of *CHIA* gene repertoires in ant-eating mammal genomes

Only *CHIA5* is functional in the Malayan pangolin (*Manis javanica*) → phylogenetic constraints: loss probably occurred in the last common ancestor of Pholidota and Carnivora.

Only *CHIA5* is pseudogenized in the southern tamandua (*Tamandua tetradactyla*). (Emerling *et al*, 2018)

Potential role of these genes in chitin digestion?

M&M: Comparative transcriptomics of digestive and non-digestive organs of *M. javanica* (n = 16 organs) and *T. tetradactyla* (n = 10).

Results: *CHIA3* and *CHIA4* found expressed in salivary glands, tongue, liver, stomach of *T. tetradactyla*, *CHIA1* and *CHIA2* in the pancreas (Fig 2) → different uses of the 4 paralogs.

CHIA5 found overexpressed in digestive organs of *M. javanica* (Fig 2) → compensation for having only one functional *CHIA*. (Allio *et al*, 2023, bioRxiv)

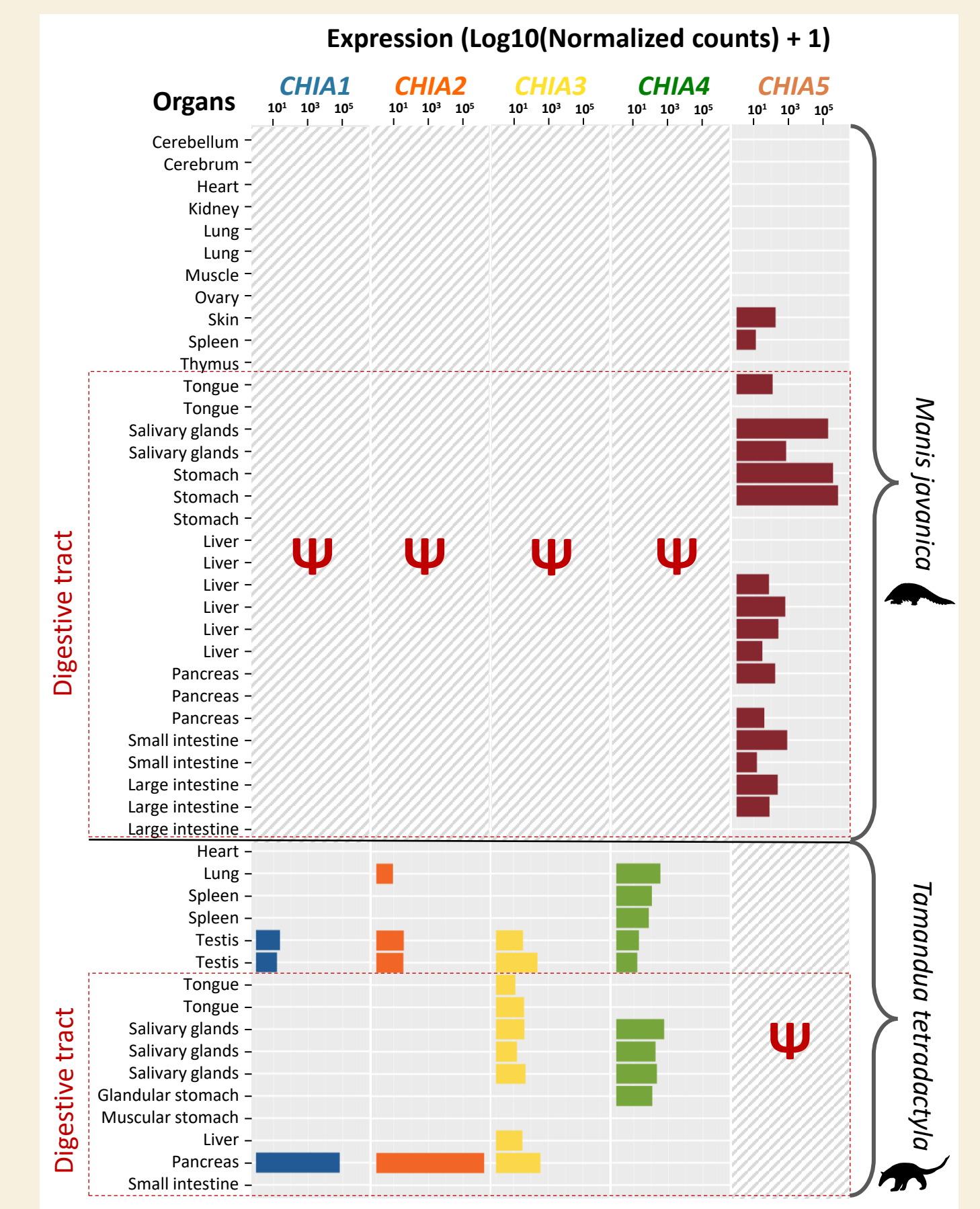
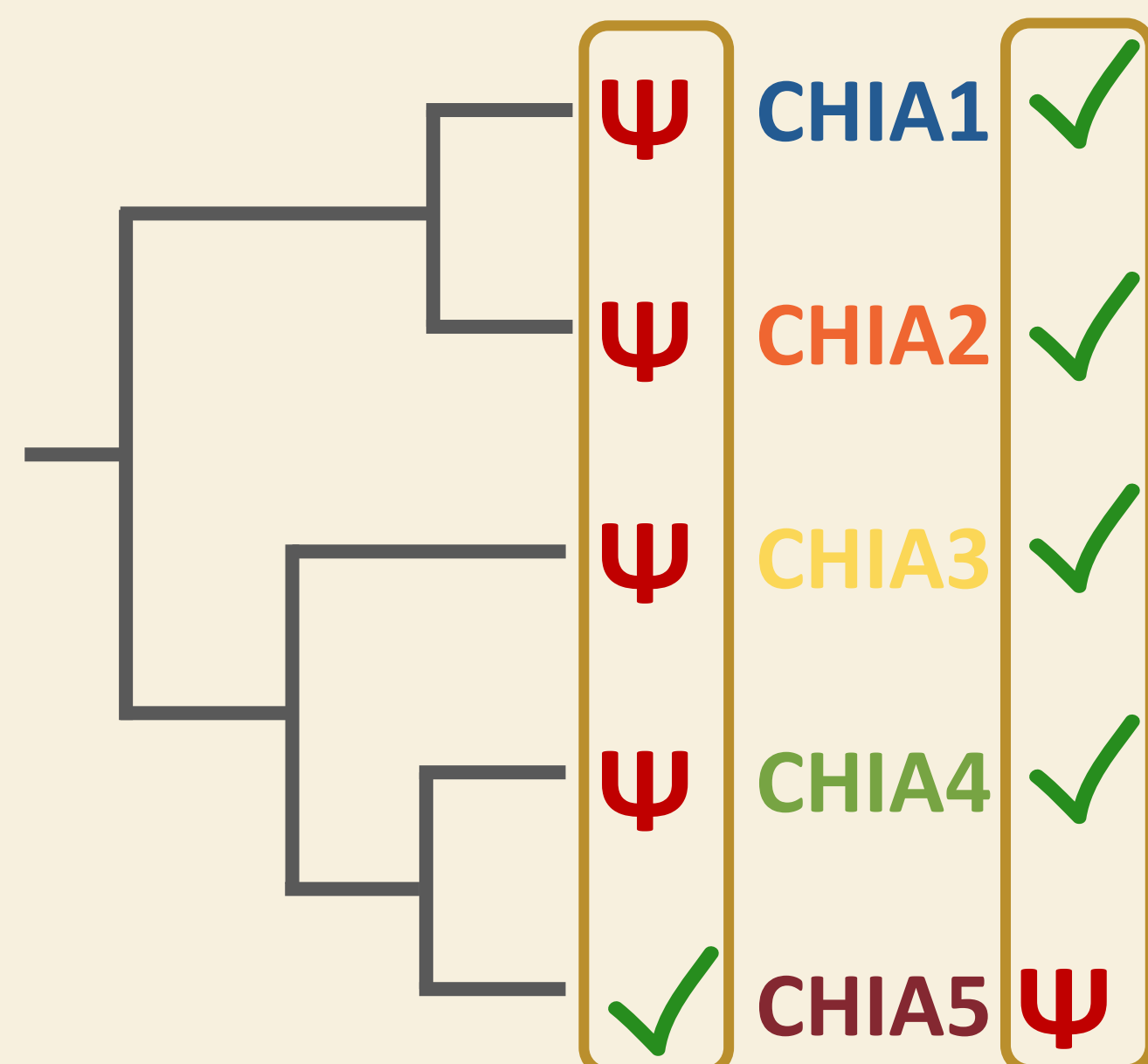


Fig 2. *CHIA* gene expression in digestive and non-digestive organs of the Malayan pangolin and southern tamandua. Ψ indicates pseudogenes.

Role of the gut microbiota in prey digestion

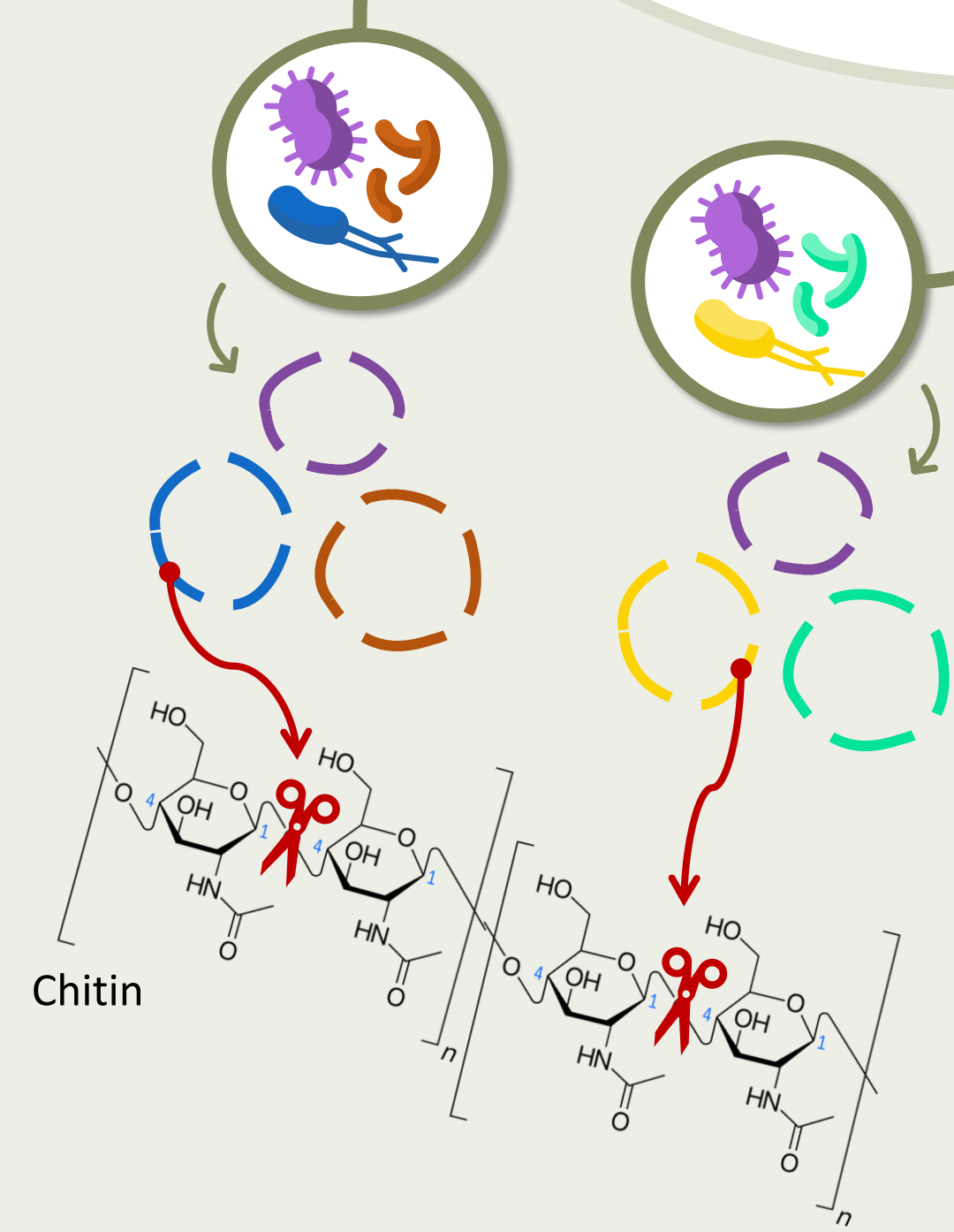
M&M: 29 gut metagenomes of nine myrmecophagous and insectivorous species.

Genome-resolved metagenomics → 314 high-quality bacterial genomes.

Identification of GH18 sequences (GH18 = CAZymes comprising chitinases and chitin-binding enzymes).

Results: Identification of 394 GH18 sequences in 132 bacterial genomes with: 237 sequences having an active chitinolytic site (DXXDXDXE) distributed in 82 bacterial genomes.

Chitinolytic bacterial genomes mainly belong to the Lachnospiraceae, Acutalibacteraceae and Ruminococcaceae bacterial families. (Teullet *et al*, 2023, bioRxiv)



Results: Chitinolytic bacteria shared among closely-related host species (Fig 3) → influence of host phylogeny.

Some shared among distantly-related host species (i.e., *Blautia* sp., *Enterococcus faecalis*) → convergent adaptation to myrmecophagy.

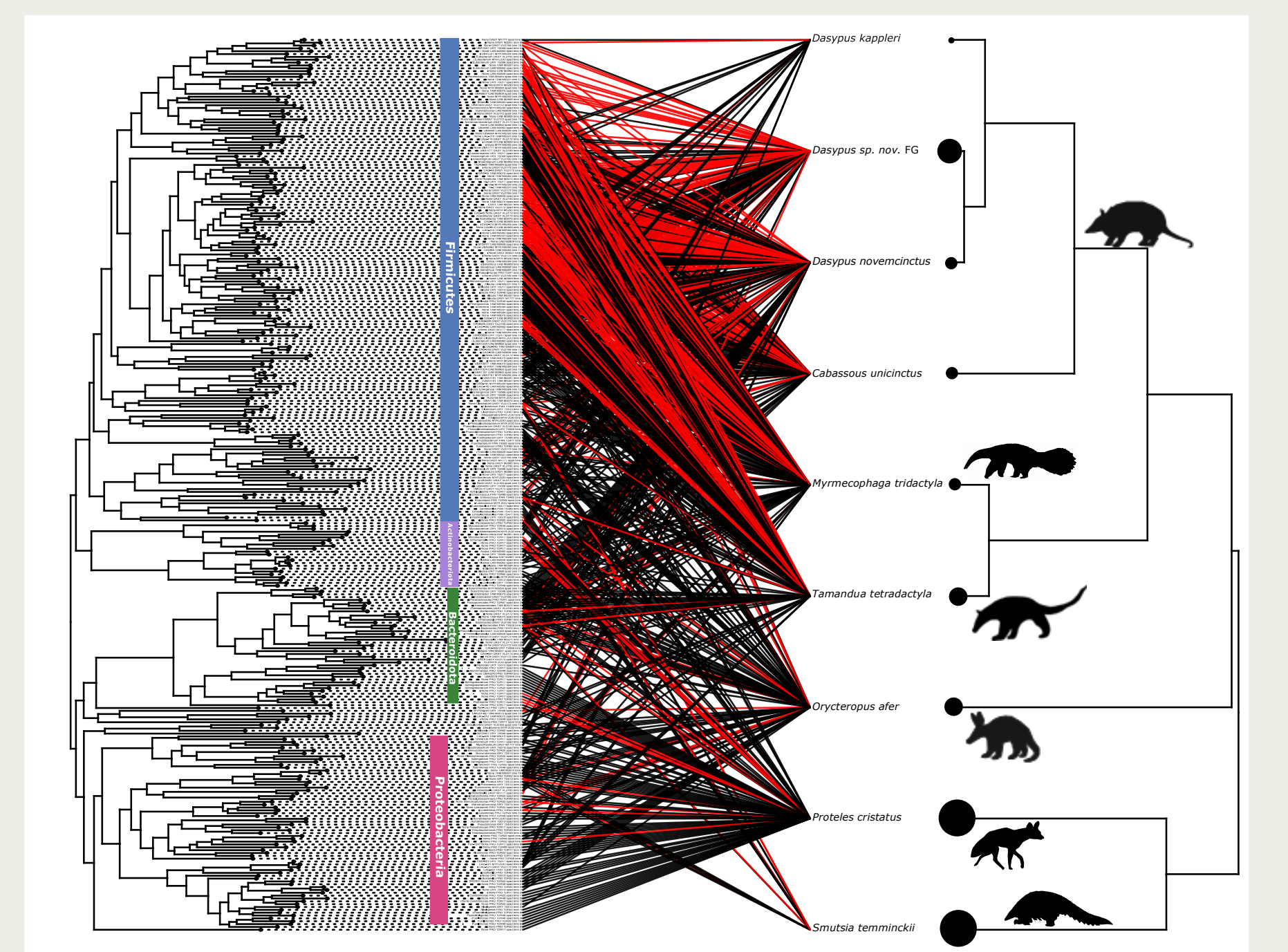


Fig 3. Distribution of chitinolytic bacterial genomes (red links) among nine myrmecophagous and insectivorous species. Phylogenetic trees of the 314 bacterial genomes and host species are respectively represented on the left and right of the graph. Major bacterial phyla are indicated by different colors. Circle sizes at the tips of the host phylogeny are proportional to the number of samples per species (1 to 6).

Both endogenous and microbial chitinases are likely involved in the digestion of prey. Combining (meta)genomics and (meta)transcriptomics would shed further light on their relative contributions to convergent adaptation to myrmecophagy.

References

- Allio R, Teullet S, Lutgen D, Magdeleine A, Koual R, Tilak M-K, Thoisy B de, Emerling CA, Lefebure T, Delsuc F. 2023. Comparative transcriptomics reveals divergent paths of chitinase evolution underlying dietary convergence in ant-eating mammals. bioRxiv. <https://doi.org/10.1101/2022.11.29.518312>.
 Cheng S-C, Liu C-B, Yao X-Q, Hu J-Y, Yin T-T, Lim BK, Chen W, Wang G-D, Zhang C-L, Irwin DM, Zhang Z-G, Zhang Y-P, Yu L. 2022. Hologenomic insights into mammalian adaptations to myrmecophagy. Natl Sci Rev nwa174.
 Emerling, C.A., Delsuc, F., and Nachman, M.W. (2018). Chitinase genes (*CHIA*s) provide genomic footprints of a post-Cretaceous dietary radiation in placental mammals. Science Advances 4, eaar6478.
 Ma, J.-E., Li, L.-M., Jiang, H.-Y., Zhang, X.-J., Li, J., Li, G.-Y., Yuan, L.-H., Wu, J., and Chen, J.-P. (2017). Transcriptomic analysis identifies genes and pathways related to myrmecophagy in the Malayan pangolin (*Manis javanica*). PeerJ 5, e4140.
 Ma, J.-E., Jiang, H.-Y., Li, L.-M., Zhang, X.-J., Li, G.-Y., Li, H.-M., Jin, X.-J., and Chen, J.-P. (2018). The Fecal Metagenomics of Malayan Pangolins Identifies an Extensive Adaptation to Myrmecophagy. Front. Microbiol. 9.
 Ma, J.-E., Jiang, H.-Y., Li, L.-M., Zhang, X.-J., Li, H.-M., Li, G.-Y., Mo, D.-Y., and Chen, J.-P. (2019). SMRT sequencing of the full-length transcriptome of the Sunda pangolin (*Manis javanica*). Gene 692, 208–216.
 Sanders, J.G., Beichman, A.C., Roman, J., Scott, J.J., Emerson, D., McCarthy, J.J., and Girguis, P.R. (2015). Baleen whales host a unique gut microbiome with similarities to both carnivores and herbivores. Nature Communications 6, 8285.
 Teullet S, Tilak M-K, Magdeleine A, Schaub R, Weyer NM, Panatino W, Fuller A, Loughry WJ, Avenant NL, Thoisy B de, Borrel G, Delsuc F. 2023. Metagenomics uncovers dietary adaptations for chitin digestion in the gut microbiota of convergent myrmecophagous mammals. bioRxiv <https://doi.org/10.1101/2023.04.21.537829>.

