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DNA analysis of invasive Raccoon (*Procyon lotor*) in France and Belgium

DNA data and DNA genotyping/sequencing

Raccoon (*Procyon lotor*) is a non native species in Europe listed in the EU regulation 1143/2014. This alien mesocarnivore is expanding in Europe. Member States must control its expansion and avoid any further introduction.

The study aims:

- 1) to understand the history of its introduction in France and to trace the putative multiple introductions;
- 2) to study the spatial population structure among the three identified populations and assess gene flow, hence animal movements, between them;
- 3) to investigate the degree of genetic connectivity between the Belgian-German population and the French populations while evidences of contacts have been established.

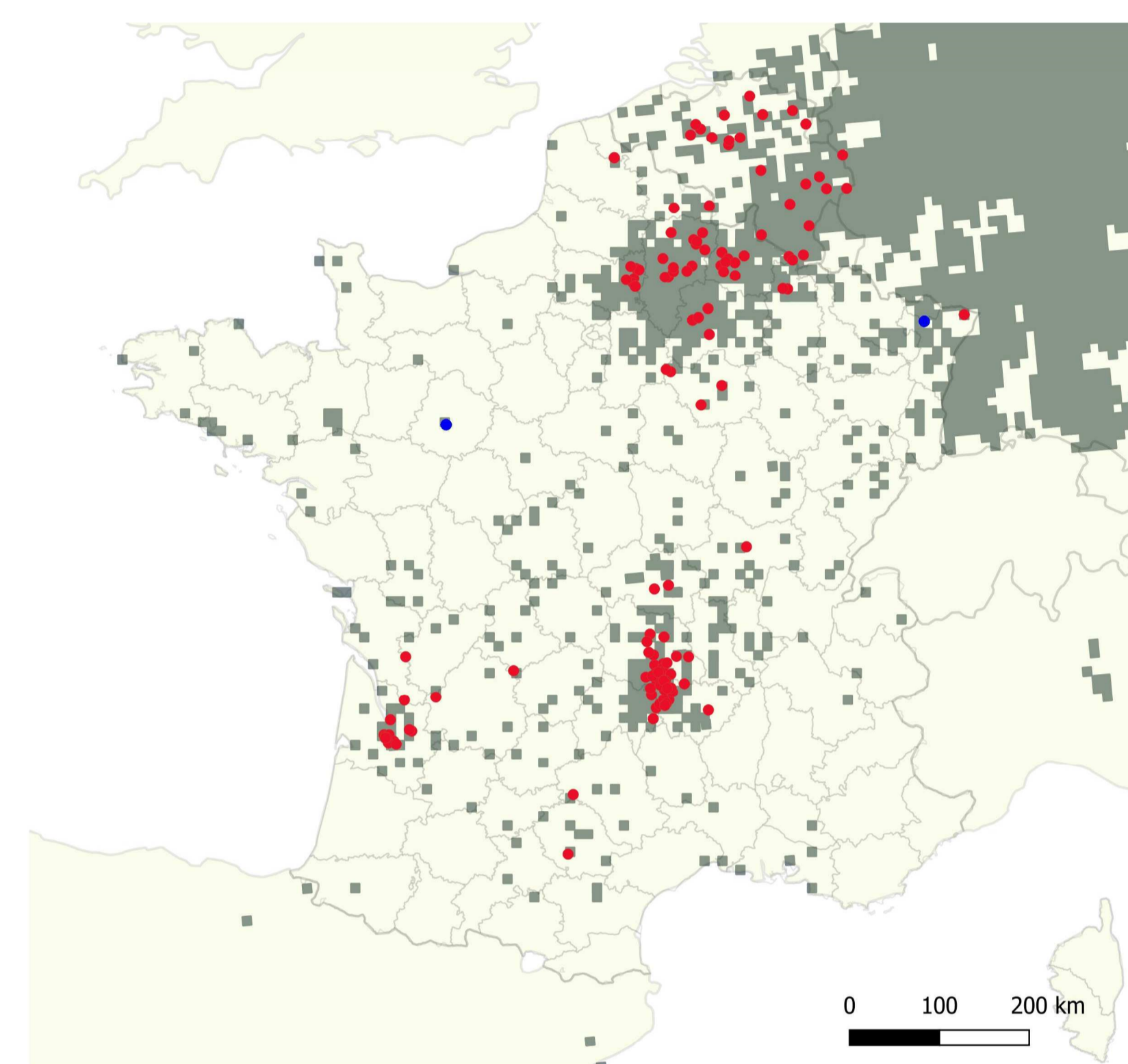
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Maillard, J.-F., Berger, J., Chevret, P., Ruetter, S., Adriaens, T., Schockert, V., Léger, F., Veron, G., Queney, G., Devillard, S., 2020. L'apport de la génétique dans la compréhension de l'évolution des populations de rats laveurs. *Faune Sauvage*. 326, 10–17.

220 tissue samples were collected on raccoons caught by trapping methods or victims of roadkill in 2019 or in previous years in France and Belgium. Tissues were preserved in 95% ethanol. In addition, 15 fur samples were collected on captive raccoons from zoos or illegal pets in France.

Genetic monitoring investigating current population genetic structure is based on microsatellite markers whose rapid evolution highlights that of short-time-scale polymorphism. A panel of 26 specific genetic markers for the raccoon (25 microsatellites and 1 sex marker) was used to achieve this goal.

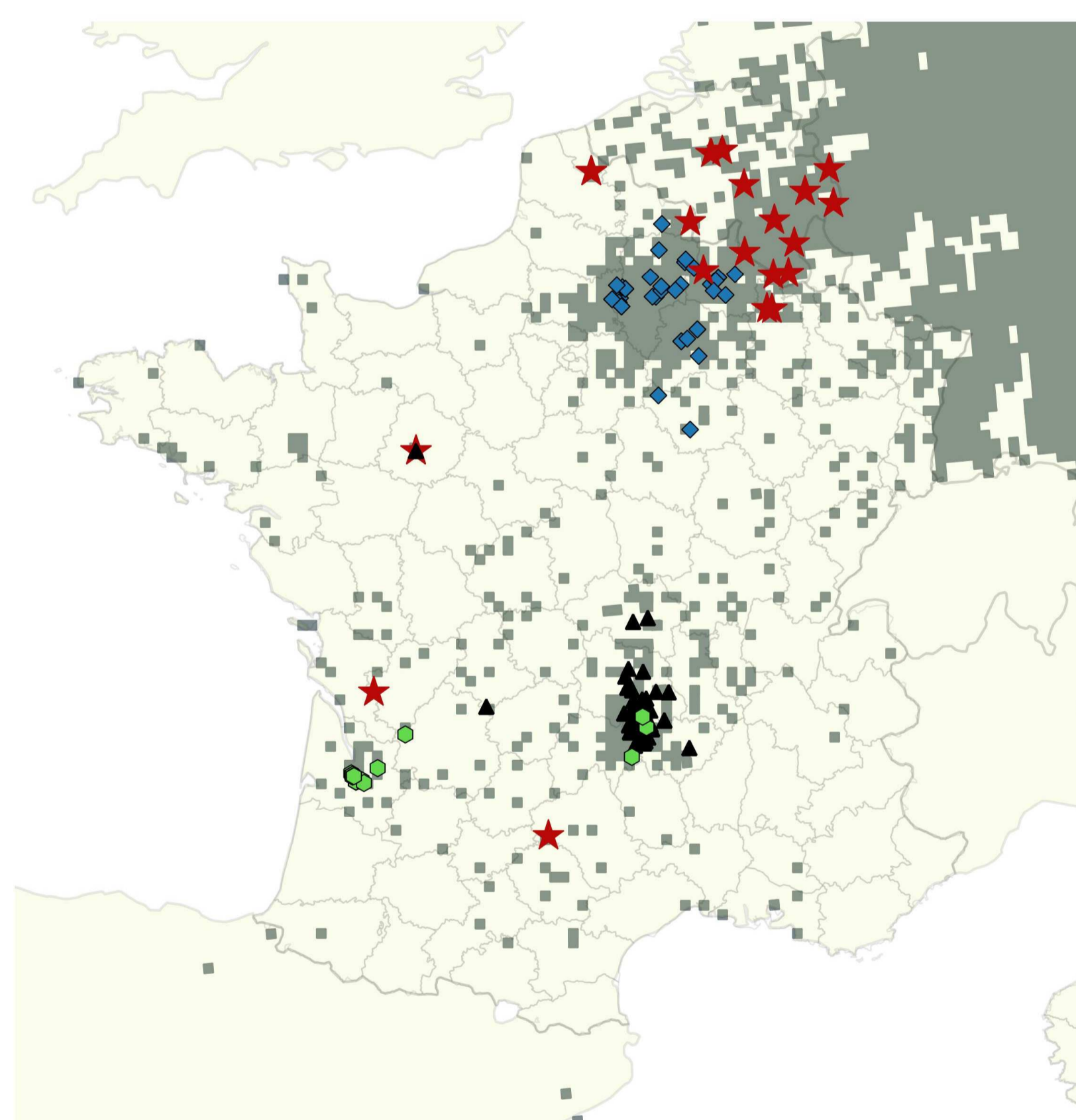
To infer the history of the introduction of raccoons in France, we amplified and sequenced a 566 base pairs from mitochondrial DNA fragment corresponding to a part of the D-loop.



Map 1- Distribution of collected samples (into the wild: red dots; in captivity: blue dots). In grey: raccoon data (10 x 10 km mesh) in the Western part of Europe (Léger & Ruetter, 2014 ; Tsiamis & al., 2017).

Microsatellite DNA results

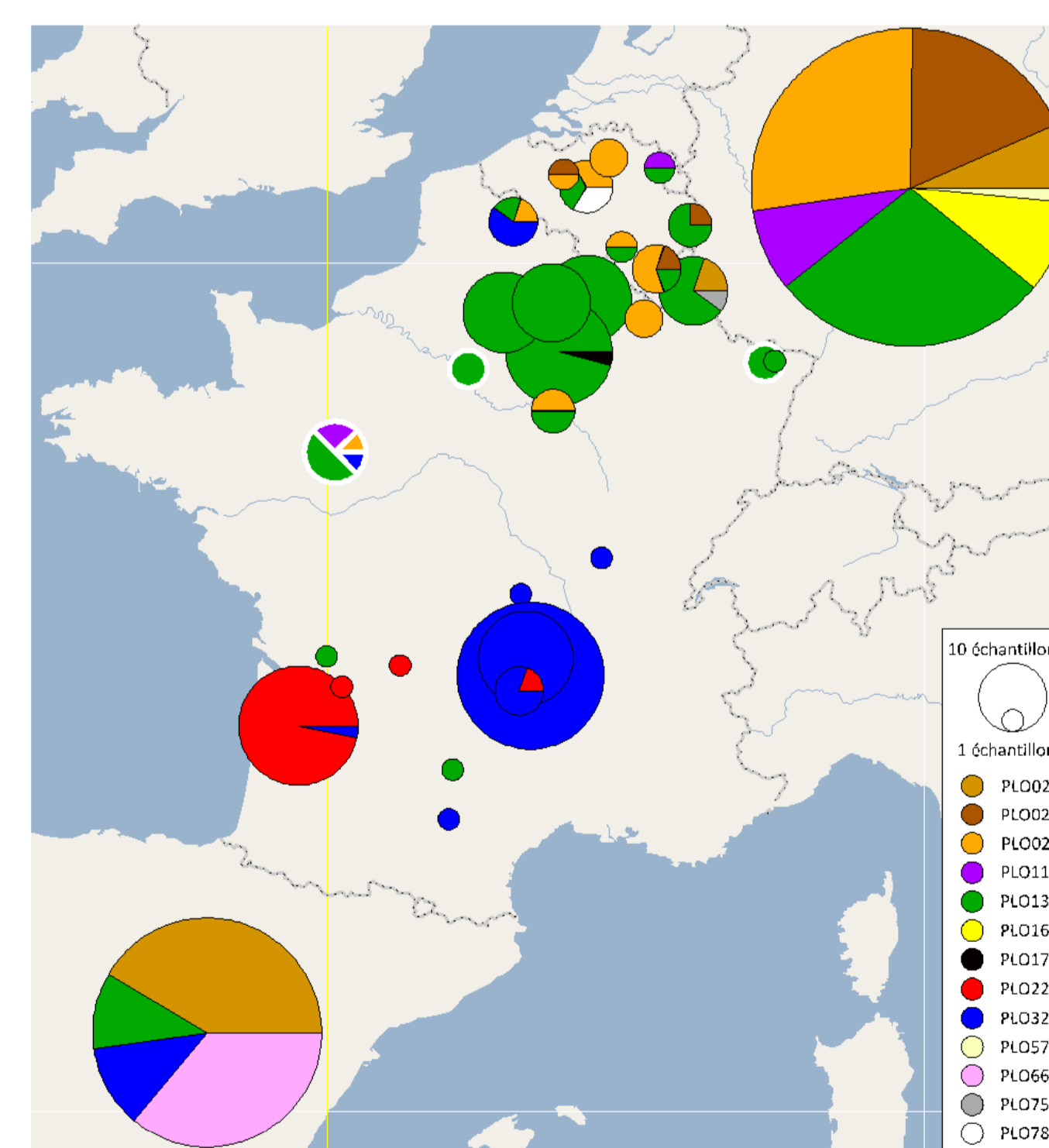
- In France, genetic diversity within each population is low (2.7 alleles per locus) and appears to be limited compared to the Belgian population (5.99);
- As expected for introduced populations, the allelic richness is lower than that found in native ones, with an average of 9.9 alleles per locus and an observed heterozygosity of $H_o = 0.8$ in North America (Cullingham et al., 2008);
- There is a high heterozygosity deficit in each population ($F_{is} = 0.225$; mean heterozygosity observed significantly different from that expected: $H_o = 0.48$ vs $H_e = 0.63$) in agreement with a founder effect due to a recent introduction;
- 4 populations (3 in France and one in Belgium) strongly identified through clustering methods strengthening the pattern of low gene flow ($F_{st} = 0.15$);



Map 2- The four genetic groups of raccoons identified in France and Belgium by clustering methods (DAPC, snapclust, Structure). Only highly assigned individuals (> 80%) are represented.

Mitochondrial DNA results

- France includes 7 haplotypes, while Belgium has 5, which 2 are shared with France. This number is relatively low compared to the 76 haplotypes identified in the native American range of the raccoon (Cullingham & al., 2008);
- French populations are each characterized by one predominant haplotype highlighting the likely multiple introductions in France;
- With common haplotypic diversity patterns, populations from the North of France and Belgium are probably at the start of their genetic mix.



Map 3 - Distribution of French, Belgian, Spanish and German haplotypes (from Fischer & al. 2017 for these last 2 countries) determined from D loop sequences. The captive animals in France are circled in white.

- Cullingham, C.I., Kyle, C.J., Pond, B.A. & White, B.N. 2008. Genetic structure of raccoons in eastern North America based on mtDNA: implications for subspecies designation and rabies disease dynamics. *Canadian Journal of Zoology* 86: 947-958.
- Fischer, M.L., Salgado, I., Beninde, J., Klein, R., Frantz, A.C., Heddergott, M., Cullingham, C.I., Kyle, C.J. & Hochkirch, A. 2017. Multiple founder effects are followed by range expansion and admixture during the invasion process of the raccoon (*Procyon lotor*) in Europe. *Diversity and Distributions* 23: 409-420. <https://doi.org/10.1111/ddi.12538>
- Léger, F. & Ruetter, S. 2014. Raton laveur et chien viverrin : le point sur leur répartition en France. *Faune sauvage* n°302 : 9-16.
- Tsiamis, K., Gervasini, E., Deriu, I., D'amico, F., Nunes, A.L., Addamo, A.M. & Cardoso, A.C. 2017. Baseline Distribution of Invasive Alien Species of Union concern. *Ispra (Italy): Publications Office of the European Union*; 2017, EUR 28596 EN. <https://doi.org/10.2760/772692>



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