Fábio de Andrade Machado

Implicações evolutivas da integração
morfológica do crânio em Caniformia
(Carnivora; Mammalia)

São Paulo
2016

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morfológica do crânio em Caniformia
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Evolutionary consequences of morphological integration in the skull of
Caniformia (Carnivora; Mammalia)

Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Doutor em Ciências, na Área de Genética e Biologia Evolutiva.

Orientador: Gabriel Henrique Marroig Zambonato

São Paulo
2016

Machado, Fabio A.

Implicações evolutivas da integração morfológica
do crânio em Caniformia (Carnivora; Mammalia)

268 páginas

Tese (Doutorado) - Instituto de Biociências da
Universidade de São Paulo. Departamento de
Genética e Biologia Evolutiva.

1. Carnivora
2. Caniformes
3. Morfometria
4. Genética Quantitativa
5. Erro do tipo I
6. Poder estatístico
7. Macroevolução

I. Universidade de São Paulo. Instituto de Biociências.
Departamento de Genética e Biologia Evolutiva.

Comissão Julgadora:

Prof. Dr.

Nome

Prof. Dr.

Nome

Prof. Dr.

Nome

Prof. Dr.

Nome

Prof. Dr.

Nome do Orientador

Aos meus pais, Francisco e Regina, meus companheiros

But the historian (...) has to say
"here is the way it looks at present".
(...) It [the theory] is open to change:
you want to come up with the best
tentative model you can, and if
somebody else shoots it down, great!

All we want is to get closer to
the truth, which we are never quite
going to get to (...). It is kind of
a North Star by which we navigate.
You are never going to get on board your
boat and get to the North Star!

No, it is like a guiding light: you
are trying to get closer and closer to it -
you are trying to get farther and
farther out of your ignorance.

But who knows? It is conceivable that
somebody might come up with some

alternative paradigm to evolution. But if they did, it would have to explain all the evidence as well or better. It is kind of doubtful anybody will, it will be a heck of a boulder to try to push (...) so I don't expect it will happen.

But nobody would rule it out because all results of honest inquiry are based on this skepticism and contain the seed of their own demolition. It may never happen, but you are willing to see any view.

Robert M. Price

Agradecimentos

Em primeiro lugar eu gostaria de agradecer a meu orientador, **Gabriel Marroig** por há 5 anos ter me acolhido em seu laboratório e ter dado a oportunidade de trabalhar com ele e em conjunto com a fantástica equipe que reuniu sobre sua tutela. Foi um período de intenso aprendizado e amadurecimento que com certeza levarei para sempre comigo.

Agradeço aos meus queridos colegas de laboratório, por terem compartilhado comigo nessa jornada, cada um à sua maneira. À **Paula Assis**, obrigado por ter me recebido em Berkeley e ter me introduzido na cultura Norte-Americana. À **Barbara Costa**, pela honestidade e pelo humor ácido. Ao **Guilherme Garcia**, que presenciou a maior parte deste caminho, agradeço pelas conversas francas e abertas. Metade das vezes que tomávamos um café, eu não queria ou sequer precisava. Ao **Alex Hubbe**, pelo interesse mútuo pelos fósseis, pela confiança, pelo respeito e por ter me mostrado o caminho quando cheguei no laboratório. Ao **Diogo Melo**, pelas trocas de ideias, dicas e por ser um colega criativo e desafiador. À **Aninha Pavan**, pelas conversas sobre filogenias, projetos e, acima de tudo, pelo bom humor. À **Anna Penna**, pela amizade que começou no laboratório, mas que espero que não termine por lá. À **Daniela Rossoni**, por estar sempre disposta a aprender em conjunto, a falar e escutar. À **Monique Simon**, por colocar a qualidade, rigor e clareza acima de tudo. Trabalhar com você foi um prazer enorme, e só posso esperar que possamos voltar a fazê-lo. Ao **Thiago Zahn**, pelo companheirismo em terras distantes e nestas, por sempre estar aberto ao diálogo e a ver outros pontos de vista. É bom saber que mesmo na discordância, e talvez principalmente nela, conseguimos achar pontos em comum. Te agradecer pelos dados que ajudaram na realização desta tese soa um tanto menor, porém necessário.

Agradeço à Universidade de São Paulo (e seus funcionários) pela in-

fraestrutura, e o suporte fornecido pelo Programa de Pós Graduação de Genética e Biologia Evolutiva. Também contei com o apoio institucional das seguintes instituições Norte-Americanas: *University of Massachusetts* (Amherst) e *Smithsonian Institute* (Washington). Agradeço principalmente à FAPESP pelas bolsas (auxílio regular e BEPE) que permitiram a realização deste projeto. Agradeço o auxílio da CAPES que me permitiu comparecer a cursos de alta qualidade no exterior. Agradeço ainda ao suporte do Santander (em conjunto com *University of California*) e do auxílio proporcionado pelo *American Museum of Natural History* (New York).

Esse trabalho não poderia ter sido realizado sem o acesso à coleções zoológicas que acomodam os espécimes por mim estudados. Assim, devo a enorme gratidão à todos os curadores e administradores de coleções das coleções por mim visitadas: **Mario de Vivo** e **Juliana Gualda** do Museu de Zoologia da Universidade de São Paulo (São Paulo); **João Oliveira**, **Luiz Flamarion** e **Sérgio Maia Vaz** do Museu Nacional (Rio de Janeiro); **David Flores** e **Sergio Lucero** do *Museu Argentino de Ciencias Naturales Bernardino Rivadavia* (Buenos Aires); **Diego Verzi** e **Itati Olivares** do Museu La Plata (La Plata); **Eileen Lacey** e **Chris Conroy** do *Museum of Vertebrate Zoology* (Berkeley); **Nancy B. Simmons**, **Neil Duncan**, **Eileen Westwig**, **Aja Marcato** e **Eleanor Hoeger** da seção de mamíferos e **John Flynn**, **Jin Meng** e **Judy Galkin** da seção de paleontologia do *American Museum of Natural History* (Nova York); **Kris Helgen**, **Darrin Lunde**, **Esther Langan** e **John Ososky** do *Smithsonian Institute* (Washington); **Bruce Patterson**, **Bill Stanley** (*in memoriam*) da seção de mamíferos e **Bill Simpson** da seção de paleontologia do *Field Museum* (Chicago); **Hopi Hoekstra** e **Judith M. Chupasko** do *Museum of Comparative Zoology* (Cambridge); e **Ted Daeschler** e **Ned Gilmore** da *Academy of Natural Sciences of Drexel University* (Philadelphia). Não posso deixar de agradecer especialmente à **Betsy Dummont** por ter me super-

visionado durante o período de viagem de coleta de dados nos Estados Unidos, sem o qual esta tese não teria sido realizada.

Durante o longo período de coleta de dados a hospitalidade de diversas pessoas foi indispensável. No Rio de Janeiro, fui recepcionado por **Grazielle Giacomo** em um barco construído à imagem do Beagle (que forma de começar as viagens de coleta). Em Buenos Aires, meus tios **Calu Machado** e **Juan Arranz** me ofereceram teto e carinho na minha primeira incursão em terras distantes. Em Nova York, **Silvia Pavan** e **Pedro Peloso** me ofereceram uma casa quando nenhuma mais estava disponível. Em Chicago, **Arthur Porto** e **Emily Robbins** dividiram comigo sua casa, sua comida e seus amigos. Em Cambridge, **Bruno Medeiros** e **Tauana Cunha** me deram um lar. Em Washington, **Daniel Damineli** e **Maitê Portes** me ofereceram um colchão, companhia, amizade, peteca e conversas.

Não posso deixar de agradecer a **Celeste Luna**, a minha maior surpresa dessa jornada. Gracias por estar conmigo en los momentos buenos y difíciles, por la comprensión, la fuerza, el afecto y la amistad. Por lo que eres. Te quiero mucho mi pequenina.

Por ultimo, agradeço minha família e especificamente ao meu pai, **Francisco de Andrade Machado Filho** e minha mãe **Maria Regina Leitão de Andrade Machado**, meus companheiros mais antigos. Passamos por diversas provações e tribulações neste período, mas sempre nos mantendo fortes e unidos. Nunca vou poder colocar em palavras minha gratidão. Sem seu suporte e amor este trabalho teria sido impossível.

Resumo

O fenótipo de caracteres complexos é o produto final da inter-relação entre genes, vias ontogenéticas e efeitos ambientais. A variação desses fatores não apenas influencia o fenótipo final, mas também como caracteres covariam e evoluem de forma integrada. A seleção natural pode influenciar a integração entre caracteres, levando a mudança de padrões de correlação ao longo do tempo. Assim, uma visão integrativa e dinâmica de fenótipos complexos é essencial para a compreensão da história evolutiva destas estruturas. Na tese atual investiguei a integração morfológica de caracteres cranianos em Caniformes sob duas perspectivas. Em uma primeira abordagem investiguei o padrão de integração morfológica das espécies a partir da comparação das variâncias e covariâncias dos caracteres. Os resultados evidenciam dois principais pontos. O primeiro é que houve uma considerável estabilidade na covariância entre caracteres ao longo de toda a história evolutiva de Carnivora, sugerindo a manutenção dos padrões de desenvolvimento no grupo como um todo. O segundo ponto é que, apesar desta estabilidade, espécies da família Canidae apresentam modificações em sua integração morfológica que os tornam mais similares entre si e mais dissimilares com os demais Carnivora. Essas diferenças estão relacionadas principalmente com caracteres da região facial, que apresentaram maior flexibilidade evolutiva, maiores correlações entre caracteres, e contêm uma maior proporção da variância em Canidae que nos demais Carnivora. Em uma segunda abordagem investiguei as propriedades estatísticas de dois testes baseados na teoria de genética quantitativa: o teste de regressão de autovalores e o teste de correlação de componentes principais (PCs). Estes testes avaliam a proporcionalidade entre padrões de covariância genética

e entre-espécies como forma de testar a hipótese nula de deriva genética. Os resultados mostram que o uso de contrastes filogenéticos independentes (PIC) reduz erros do tipo I inflados, principalmente no caso do teste de correlação. Quando PIC são utilizados, o teste de correlação apresenta taxas de erro tipo I nominais para todos os números de espécies. Entretanto, a flutuação do número efetivo populacional (N_e) infla o erro tipo I deste testes. O teste de regressão, apesar de apresentar erro do tipo I inadequado para número de espécies baixo, é robusto a flutuações de N_e . A redução do número de PCs reduz o erro do tipo I a valores nominais à custa de uma redução no poder do teste. O poder de ambos os testes é similar nos diversos cenários avaliados, com uma leve tendência de maior poder para o teste de correlação em números amostrais mais baixos. Adicionalmente, as famílias de Caniformes foram utilizadas como estudo de caso para ambos testes. Os testes foram realizados com métodos paramétricos e não-paramétricos (simulações) e com e sem PIC. Houve rejeição de deriva para quase todas as famílias, com exceção de Mephitidae e Ursidae. Os testes de regressão baseados em simulações se mostraram consistentes com e sem o uso de PIC, apresentando intervalos de confiança menores que os testes paramétricos. Os resultados da presente tese abrem diversas possibilidades de investigação futura, tanto do ponto de vista empírico (em relação a modificações de Canidae e dos processos evolutivos deste grupo e de Ursidae e Mephitidae), assim como metodológicos (aprofundamento das investigações sobre as propriedades dos métodos para investigações macroevolutivas baseados em genética quantitativa).

Palavras-chave: Carnivora, Caniformes, Morfometria, Genética Quantitativa, Erro do tipo I, Poder estatístico, Macroevolução

Abstract

The phenotype of complex characters is the end-product of the interrelations between genes, ontogenetic pathways and environmental effects. The variation in these factors influences not only the final phenotype, but also how characters covary and evolve in an integrated way. Natural selection can influence the interaction among characters, leading to changes in the patterns of integration. Therefore, an integrative and dynamic view of complex phenotypes is essential to the understanding of the evolutionary history of such structures. In the present thesis I investigated the morphological integration of cranial characters in Caniform species in two perspectives. In the first approach I investigated the pattern of morphological integration of the species through the comparison of character variances and covariances. The results of this investigation highlighted two points. The first is that there is considerable stability in the covariance among characters along the evolutionary history of Carnivora, suggesting the maintenance of ontogenetic pathways in the group. The second is that, despite this stability, Canidae species show changes in their morphological integration that make them more similar among each other and more different from the rest of Carnivora. These changes are related mainly to characters from the facial region, which showed a greater evolutionary flexibility, greater correlation among characters, and concentrate a greater proportion of the variance in Canidae than in the rest of Carnivora. In a second approach I evaluated the statistical properties of tests based on quantitative genetics theory: the test of regression of eigenvalues and the test of correlation of principal components (PCs). These tests investigate the proportionality between patterns of genetic and between-species covariance as a way to test the null hypothesis

of genetic drift. The results show that the use of phylogenetic independent contrasts (PIC) reduces the inflated type I error, especially in the case of the correlation test. When PIC are employed, the correlation test shows nominal type I error rates for all species sample sizes. However, the oscillation of the effective population size (N_e) inflates type I error rates of these tests. The regression test, despite showing inadequate type I error rates at small species sample sizes, is robust to the oscillation of N_e . The reduction of the number of PCs reduces type I error rates to nominal values at the expense of statistical power. The power of both tests is similar under different scenarios evaluated, with a slight tendency of the correlation test to perform better at small number of species. Additionally, the Caniform families were used as case studies for both tests. Tests were performed using parametric and non-parametric (simulations) techniques, with and without PIC. The drift hypothesis was rejected for almost all families, with the exception of Mephitidae and Ursidae. The regression tests based on simulations were consistent with and without the use of PIC, showing narrower confidence intervals than the ones for parametric tests. The results of the present thesis open a wide range of future investigation opportunities, both from the empirical (relative to the differences in Canidae patterns of morphological integration or the evolutionary processes underlying Ursidae and Mephitidae diversification) and methodological (further investigations of the properties of the quantitative genetics-based tests for macroevolution) points of view.

Keywords: Carnivora, Caniformes, Morphometrics, Quantitative Genetics, Type I error, Statistical Power, Macroevolution

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Capítulo 1

Introdução

Integração morfológica

Integração morfológica se refere ao fato de que organismos são compostos por diferentes partes (ou caracteres) que apresentam diferentes graus de interconexão entre si. Esse conceito está intrinsecamente ligado à ideia de que organismos são sistemas formados por elementos individualizados (caracteres) que apresentam associações internas e que se influenciam mutuamente. Os elementos e suas interconexões afetam o comportamento do organismo como um todo, que por sua vez regula a estrutura e relações dos elementos que o compõem (Nagel, 1961). Chamamos a um conjunto de caracteres com níveis variáveis de interconexão entre si de caracteres complexos.

O reconhecimento de que os caracteres dos organismos não variam independentemente é um fenômeno há muito tempo reconhecido por filósofos naturais. Aristóteles em seu *Περὶ Ζῴων μορίων* ("As partes dos animais") foi o primeiro autor a estabelecer uma metodologia sistemática para avaliar a relação entre as partes dos animais (órgãos, tecidos, fluidos, etc), apontando para a associação entre os elementos como sendo necessária para a produção de uma certa função orgânica (Mayr, 1961). A visão Aristotélica dos organismos como sistemas integrados foi levada ao extremo com Galeno, que em seu *De Usu Partium Corporis Humani* ("Sobre o uso das partes do corpo humano") publicado no século II, discute não apenas como certas partes contribuem para determinada função, mas também que a composição de um sistema complexo é essenciais para a performance do organismo (Schiefsky, 2007). Em outras palavras, Galeno argumentava, através de uma apresentação abundante de contrafactuais ¹, que mudanças mínimas nas propriedades, organização e configuração das partes dos organismos se-

¹uma afirmação condicional na qual o primeiro enunciado expressa algo contrário aos fatos.

riam prejudiciais para o funcionamento perfeito das funções necessárias ao desenvolvimento, à vida e à reprodução. Galeno era um dos proponentes mais antigos de uma espécie de *Design Inteligente*² metodológico que via a especificidade funcional (isto é, a conexão específica de partes para a realização de uma função) como evidência de uma força criadora por trás da existência de organismos biológicos. Apesar de hoje serem desconsiderados pela ciência, argumentos como o avançado por Galeno não eram considerados anti-científicos na sua época, sendo Galeno na verdade considerado um dos grandes cientistas da antiguidade (Carrier, 2016).

A ideia de que o organismo é constituído por partes que interagem entre si foi de extrema influência no pensamento de Georges Cuvier, o pai da paleontologia moderna. Cuvier era um grande admirador de Aristóteles e, assim como Galeno, acreditava que as diversas partes do organismo deveriam ser avaliadas em relação a um "todo" integrado, cunhando o que ficou conhecido como "O Princípio de Correlação de Partes":

Se os dentes de um animal são como devem ser, de maneira que ele se alimente com carne, podemos ter certeza sem subsequente investigação que todo o sistema de seus órgãos digestivos é apropriado para esse tipo de alimento; e que todo o esqueleto e os órgãos locomotivos, até mesmo seus órgãos sensoriais, são arranjados de tal maneira a torná-lo hábil para a caça e captura de sua presa. Essas relações são condições necessárias para a existência do animal; se fosse de forma contrária, ele não seria capaz de subsistir.

"Extract from a memoir on an animal of which the bones are found in the plaster stone [pierre à plâtre] around Paris, and which appears no longer to exist alive today"(Cuvier, 1798) reproduzido em (Rudwick, 2008, p. 36)

²Design inteligente se refere à versão modernizada dos argumentos clássicos de teologia natural aplicados aos organismos biológicos (Scott, 2009).

Assim, Cuvier reconhece não apenas as necessidades fisiológicas para a associação de partes, mas também a associação imposta por necessidades ecológicas, permitindo a interação de elementos pertencentes a diferentes sistemas (como dentes e sistemas digestivo) que não estão necessariamente ligados aos tipos de funções mecânicas e sistêmicas. Cuvier, em concordância com Galeno, via a especificidade funcional das diversas partes do organismo como impeditivos para a transmutação das espécies, considerando o registro fóssil como evidência de sucessivas criações divinas, e não de transformação gradual ao longo do tempo. Apesar disso, seu discurso era particularmente livre de referências diretas a divindades, e seu princípio de correlação era dado mais como uma lei natural do que como ação direta de uma entidade (Rudwick, 2008).

Talvez o expoente mais influente destes conceitos tenha sido Willian Paley, que consolidou o pensamento da teologia natural até o começo do século XIX. Segundo Paley, a complexidade (e beleza) dos organismos não apenas era evidência *prima facie* da existência de um arquiteto transcendental, ou Deus, mas que o estudo da natureza permitia também examinar as propriedades desta entidade, como sua bondade, unidade e, sobretudo, inteligência (Paley, 1802). Esse pensamento é similar ao exposto por Galeno, de que a especificidade funcional de caracteres complexos era tão grande que só poderia ser algo planejado de antemão e confeccionado com este objetivo em mente: tal planejamento e antevisão, ainda mais quando considerado em escala global, só seria possível se desempenhados por uma divindade com poder para tal. Paley apontava não apenas para como os múltiplos componentes de um sistema se associavam especificamente para a formação dos sistemas biológicos, como também para sua má-formação, como evidência de que alterações poderiam trazer malefícios para o funcionamento dos organismos (Paley, 1802, p.132). Isso reforçaria a ideia de que

espécies estão perfeitamente adaptadas a seus ambientes e que a mutabilidade das espécies é impossível.

Darwin, baseando-se parcialmente nos trabalhos de Saint-Hilaire, interpretou teratologias (ou má-formações) de maneira diferente. Para Darwin, teratologias não eram essencialmente distintas da variação natural das espécies (Darwin, 1859, p. 22), e evidenciavam a existência de leis de variação, que ele chamou de Correlações de Crescimento, que ditavam que diferentes órgãos se desenvolvem em conjunto (1859, p. 24-25). Uma vez que tais variações nem sempre se relacionavam a partes que apresentam funções similares (1859, p. 131-132), a modificação de um caráter por meio de seleção artificial (1859, p. 25) ou natural (1859, p. 84) poderia afetar outros caracteres, o que implica que organismos não necessariamente vão apresentar todas suas partes perfeitamente adaptadas (1859, p. 130, 176) ³. Darwin respondeu aos naturalistas que acreditavam ser a sua teoria a que na verdade implicava que organismos estariam perfeitamente adaptados:

Eles [naturalistas] acreditam que diversas estruturas foram criadas em prol da beleza, para deleite do homem, ou por mera variação. A veracidade de tais doutrinas seria fatal para a minha teoria. Admito plenamente que muitas das estruturas que hoje existem não têm atualmente uma utilidade direta para os seus possuidores. Condições físicas provavelmente têm pouco efeito na estrutura, de certa forma independente de qualquer benefício proporcionado. A correlação de crescimento com certeza teve um papel mais que importante, e a modificação útil de uma parte vai frequentemente ocasionar em outras partes diversas mudanças de nenhum uso direto. Então, novamente caracteres que an-

³Darwin também rejeitava a noção de adaptação perfeita com base em observações empíricas, que demonstravam que populações apresentavam variação em caracteres de importância funcional (Bizzo, 2010). Ademais, sua teoria não demandava que organismos fossem em si perfeitamente adaptados, apenas melhor adaptados do que outros organismos na mesma população (1859, p. 176)

teriormente eram úteis, ou que anteriormente tinham surgido de correlação de crescimento, ou de outra causa desconhecida, podem reaparecer a partir da lei de reversão, ainda que agora de nenhum uso direto. Os efeitos da seleção sexual, quando exibidos para encantar as fêmeas, podem ser chamados úteis apenas em um sentido forçado. Mas, de longe, o mais importante é que a parte principal da organização de cada ser é simplesmente devido à herança; e, conseqüentemente, embora cada ser esteja seguramente bem adaptado para o seu lugar na natureza, muitas estruturas agora não têm relação direta com os hábitos de vida de cada espécie.

Darwin (1859, p. 174)

Podemos então ver que em resposta a diversas críticas à sua teoria, seja sobre a impossibilidade de evolução por especificidade funcional, seja por prever adaptação perfeita dos organismos ao seu ambiente, Darwin recorreu a conceitos de integração morfológica. Assim, Darwin reconhece claramente duas causas para a integração morfológica: como consequência de leis de variação por correlação de crescimento e/ou como resultado da ação de seleção natural, seja atualmente ou no passado (1859, p. 133,175).

Durante o século XX, o conceito de integração morfológica se transformou enormemente. Destacam-se nesse momento as contribuições de Darcy Thompson no seu *On Growth and Form* (1917). Thompson era um crítico da teoria de Darwin, no sentido que esta focava-se em seleção natural, em detrimento de leis de formação, para explicar a forma e estrutura dos organismos:

O morfologista, quando comparando um organismo com outro, despreza a diferença entre eles ponto a ponto, e "caráter" a "caráter". Se ele é forçado de tempos em tempos a admitir a existência de "correlação" entre caracteres (como mostrado por Cuvier há cem anos) e ainda,

apesar de reconhecer este fato de correlação de forma vaga, como um fenômeno que decorre de causas que, exceto em raras circunstâncias, ele não pode investigar; ele então cai rapidamente no hábito de pensar e falar de evolução como se ela tivesse prosseguido nas linhas da sua própria descrição, ponto a ponto, caráter a caráter. Mas se, por outro lado, peixes diversos e dissimilares podem ser referenciados como um todo a funções idênticas de sistemas de coordenadas diferentes, este fato por si só vai constituir uma prova de que há uma "lei de crescimento" abrangente que permeia toda a estrutura em sua integridade, e que algumas forças mais ou menos simples e reconhecíveis estiveram atuando.

Thompson (1917, p. 727)

Assim, Thompson é considerado um dos pioneiros do tratamento matemático dessas leis de associação entre caracteres, e de sua implicação para a variação nos organismos vivos. Julian Huxley expandiu o programa de pesquisa de Thompson em seu *Problems of Relative Growth* (1932), reconhecendo a questão do crescimento diferencial de partes (assim como Thompson) como essencial para a biologia, visto que todos os organismos adquirem sua forma através de crescimento e correlação diferencial de partes.

No livro *Morphological Integration*, os autores Everett Olson e Robert Miller (1958) argumentaram que a integração e interdependência entre caracteres poderia ser mensurada estatisticamente, através da correlação de medidas obtidas de diferenças caracteres. Essa ideia simples, porém poderosa, permitiu testar, como proposto por estudiosos da integração morfológica desde Galeno, se demandas funcionais se refletem em associações de caracteres. Assim, esse livro é considerado um marco no estudo da integração morfológica, delineando a base metodológica do programa de pesquisa da área até os dias de hoje.

Ainda no século XX, diversos autores foram essenciais para o aprofundamento de nossa compreensão da integração morfológica. De maneira geral esses autores argumentaram que os organismos e suas partes deveriam ser compreendidos em termos genéticos, assim trazendo o estudo da integração explicitamente para o âmbito da síntese evolutiva, explicitamente no contexto da genética quantitativa. Abaixo, revejo alguns conceitos básicos desta área e suas interconexões com o conceito de integração morfológica.

Genética quantitativa

Durante a formulação de sua teoria de evolução por seleção natural, Darwin não pôde recorrer a nenhum mecanismo para explicar a herança de caracteres contínuos e teve que se valer de extensivos exemplos, principalmente de variantes domésticos, de que caracteres dessa natureza eram de fato herdados entre progenitores e descendentes. Apesar dessa lacuna teórica, a redescoberta das leis de Mendel na virada do século XX foi recebida com desconfiança por darwinistas. O motivo disso era que, apesar das leis de Mendel⁴ explicarem a mecânica do funcionamento da herança de caracteres, os tipos de caracteres avaliados por Mendel e priorizados por seus seguidores, os Mendelianos, eram de natureza discreta, ou seja, assumiam estados descontínuos. Nesse contexto, mutações seriam a principal força por trás da evolução, permitindo a criação de novos estados discretos de caracteres que, então, podiam ser selecionados ou descartados (De Vries, 1903; Castle, 1905).

⁴(Monaghan e Corcos, 1984)

Lei da Pureza dos Gametas Cada gameta carrega apenas um fator (alelo) referente a cada caráter/gene.

Lei da Segregação Independente Para diversos caracteres, os fatores que determinam cada um se segregam independentemente nos gametas.

Em oposição estavam os Biometristas, seguidores de Darwin que, como ele, viam a variação contínua de caracteres como a força motriz da evolução por seleção natural. Enquanto os Mendelianos apresentavam uma base teórica para explicar a herança de caracteres discretos, aos Biometristas restava lidar com o problema da regressão à média de caracteres contínuos. Colocado sucintamente, o problema de regressão à média se baseia na observação de que, em uma população de indivíduos que variam continuamente em um determinado caráter, a média dos valores fenotípicos da prole vai ser mais próxima da média populacional do que da média dos valores dos pais. Esse era um problema para os biometristas, pois parecia sugerir que caracteres contínuos sempre seriam revertidos para um valor ancestral, demandando constante seleção para sua modificação.

A solução para esse dilema foi formalmente desenvolvida por Fisher (1918), que notou que, à medida que o número de genes aditivos que influencia um caráter cresce, a distribuição de valores fenotípicos dos caracteres se aproxima de uma distribuição contínua (Figura 1.1). Ademais, fenótipos foram modelados como sendo a soma de efeitos genéticos e ambientais, implicando que os valores fenotípicos observados não refletem diretamente os valores genotípicos de cada indivíduo. Isso permitiu modelar a seleção em um caráter contínuo z da seguinte forma:

$$\Delta z = \frac{\sigma_a^2}{\sigma_p^2} s \quad (1.1)$$

(Lush, 1937) onde Δz é a diferença na média do caractere z entre a geração parental e a geração descendente, s é o diferencial de seleção (a diferença entre a média da população total e a população selecionada) e σ_a^2 / σ_p^2 é a razão entre a variância genética aditiva e a variância fenotípica total, também chamada de herdabilidade (h^2). Esse conceito permitiu compreender a regressão à média como um fenômeno emergente da genética dos caracteres

Figura 1.1: Efeitos de número variável de alelos aditivos em um caráter quantitativo em equilíbrio **A-** Dois alelos em um loci. **B-** Quatro alelos em dois loci. **C-** Seis alelos em três loci. **D-** Um número alto de alelos

contínuos, permitindo ainda a estimativa de parâmetros genéticos a partir de observações empíricas. Esse arcabouço teórico possibilitou modelar caracteres contínuos que podiam evoluir de forma gradual e eram herdados mendelianamente, unificando assim as teorias evolutiva e genética.

No contexto de genética quantitativa, a associação entre partes dos organismos é dada pela existência de desequilíbrio de ligação ⁵ entre genes e por pleiotropia ⁶ que se expressam em correlação e covariância (genética) entre caracteres (Falconer et al., 1996). Com base neste conceito, Russel Lande revolucionou o estudo de caracteres associados ao traduzir os conceitos de genética quantitativa em um contexto explicitamente multivariado. Segundo

⁵associação não-aleatória de alelos em diferente loci.

⁶quando um gene afeta múltiplos caracteres.

(Lande, 1979) , a versão multivariada da equação de resposta evolutiva é

$$\Delta \mathbf{z} = \mathbf{G}\mathbf{P}^{-1}\mathbf{s} \quad (1.2)$$

onde $\Delta \mathbf{z} = \{\Delta z_1, \Delta z_2, \dots, \Delta z_n\}$ é um vetor coluna de diferenças entre médias de n caracteres, $\mathbf{G}$ é a matriz $n \times n$ de variância-covariância de efeitos aditivos (*e.g.* elementos $\mathbf{G}_{ii}$ são as variâncias genéticas aditivas do caráter i e elementos $\mathbf{G}_{ij}$ são as covariâncias genéticas aditivas entre os caracteres i e j), $\mathbf{P}$ é a matriz $n \times n$ de variância-covariância fenotípica e $\mathbf{s} = \{s_1, s_2, \dots, s_n\}$ é um vetor de diferenciais de seleção. Pode-se observar que a equação 1.2 é uma tradução direta da equação 1.1 em um contexto multivariado. Lande ainda mostrou que o diferencial de seleção normalizado pela matriz de covariância fenotípica ($\mathbf{P}^{-1}\mathbf{s}$) é equivalente ao gradiente da paisagem adaptativa estimado sobre o fenótipo médio da população (β). Assim a equação 1.2 pode ser expressa como

$$\Delta \mathbf{z} = \mathbf{G}\beta \quad (1.3)$$

Desta forma representa as direções de maior variação de aptidão, e pode ser estimado como sendo um vetor de coeficientes parciais de regressão múltipla do fenótipo sobre a aptidão (Lande e Arnold, 1983). Assim, β pode ser compreendido como a direção e intensidade da seleção sobre a média em uma população.

A equação de Lande também modela a dinâmica de caracteres que não estão sendo selecionados, mas evoluem em decorrência de covariâncias genéticas e seleção indireta em outros caracteres. Para exemplificar isso, considere um sistema de dois caracteres. Para $\beta = \{\beta_1, \beta_2\}^t$ a resposta evolutiva

em cada caráter será

$$\Delta z_1 = \mathbf{G}_{11}\beta_1 + \mathbf{G}_{12}\beta_2 \quad (1.4a)$$

$$\Delta z_2 = \mathbf{G}_{21}\beta_1 + \mathbf{G}_{22}\beta_2 \quad (1.4b)$$

A presença de qualquer covariância genética não-nula entre caracteres ($\mathbf{G}_{12} = \mathbf{G}_{21} \neq 0$) implica que a resposta evolutiva em um caráter, mesmo na completa ausência de seleção direta, vai ser influenciada pela seleção presente em outro caráter. De certa forma, essa é uma formalização das ideias de Darwin (1859) para a seleção indireta de caracteres que acata as críticas de Thompson (1917), por explicitamente quantificar a interconexão de partes, e a forma como ela interage com a seleção natural. Assim, o estudo das covariâncias genéticas e fenotípicas codificadas nas matrizes $\mathbf{G}$ e $\mathbf{P}$, de sua variação e de suas consequências para a evolução fenotípica dos diversos grupos é uma escolha natural para o estudo de integração morfológica em sistemas complexos (Cheverud, 1984; Roff, 2002; Stepan et al., 2002; Arnold et al., 2008).

O Crânio

A morfologia do crânio de mamíferos é o resultado da complexa interação de um número grande de processos ontogenéticos (Hallgrímsson et al., 2007). O crânio pode ser subdividido em três principais regiões: o neurocrânio, derivado do dermatocrânio, o basicrânio, derivado do condrocânio e a região da face, derivada inicialmente do splancnocrânio, recebendo adições subsequentes do dermatocrânio. O basicrânio é uma região ontogeneticamente estável, crescendo principalmente por ossificação endocon-

dral, atingindo a morfologia adulta muito cedo durante o desenvolvimento (Farkas et al., 1992). A face e o neurocrânio apresentam desenvolvimentos mais tardios, crescendo principalmente por ossificação intramembranosa nas suturas estimuladas pelo crescimento dos órgãos acomodados nestas regiões (Opperman, 2000). Apesar destes elementos morfológicos apresentarem distintos padrões de desenvolvimento, interações epigenéticas entre regiões do crânio são bastante consistentes entre diferentes linhagens, podendo ser importantes fontes de variação morfológica (Cornette et al., 2014). Assim, visto o grande número de vias ontogenéticas envolvidas na sua determinação, esperamos que o crânio seja influenciado por um grande número de genes, sendo um modelo ideal para o emprego dos modelos de genética quantitativa. Além disso, visto que tais vias influenciam diversos caracteres ao mesmo tempo, esperamos que existam diferentes graus de associação entre as múltiplas partes, impostas por correlações de crescimento (Darwin, 1859; Thompson, 1917; Huxley, 1932).

A variação nos hábitos de vida das espécies normalmente está associada a diferenças na morfologia craniana. Isso pode se dar através de diferenças na ecomorfologia alimentar, visto que diferentes tipos de itens alimentares impõem diferentes demandas biomecânicas no crânio (e.g. Wroe e Milne, 2007; Christiansen, 2008; Figueirido et al., 2009; Jones e Goswami, 2009; Figueirido et al., 2010; Slater et al., 2009, 2010; Tseng e Wang, 2011; Figueirido et al., 2011, 2013; Meloro et al., 2015; Tseng e Flynn, 2015; Jones et al., 2015). O crânio também reúne parte do sistema nervoso central e a maioria dos órgãos sensoriais, sendo o tamanho relativo destes órgãos importante na determinação da forma final da estrutura (e.g. Radinsky, 1981a,b, 1982; Finarelli, 2008; Finarelli e Flynn, 2009; Van Valkenburgh et al., 2014; Bird et al., 2014; Ranslow et al., 2014). Esses fatores interagem entre si de forma complexa, potencialmente impactando a relação entre seus elementos, se tradu-

zindo em diferenças na integração morfológica entre as espécies (Cheverud, 1982; Cheverud et al., 1983). Assim, a análise de um grupo com grandes diferenças ecológicas pode nos fornecer importantes informações a respeito da evolução da integração morfológica. Na presente tese, eu investiguei a variação na integração morfológica de um grupo que se encaixa perfeitamente nesta descrição: os Caniformes.

Caniformes

A ordem Carnivora apresenta uma divisão basal em duas principais subordens: Caniformes e Feliformes. Feliformes contém as famílias viventes Felidae, Viverridae, Hyaenidae, Herpetidae, Eupleridae, Nandiniidae e Prionodontidae. Com exceção de Felidae, Feliformes é considerado um grupo principalmente do velho mundo. Apesar de apresentar formas extremamente especializadas como, por exemplo, os tigres-dentes-de-sabre ou o termitívoro *Proteles cristata*, são usualmente considerados menos diversos que sua subordem irmã, tanto em termos de número de espécies, quando em ecologia e forma (Goswami, 2010). De fato, classicamente apenas quatro famílias eram reconhecidas (Hyaenidae, Viverridae, Herpestidae e Felidae), evidenciando a grande similaridade fenotípica entre representantes dos diferentes taxons reconhecidos atualmente (Gaubert et al., 2005).

Caniformes (Figura 1.2), por outro lado, é um grupo extremamente diverso em quase todos os sentidos. Podem ser considerados o único grupo de animais (com exceção do homem) que conseguiu dominar todos os continentes, em parte por causa dos Pinipedia. Ecologicamente, é o único táxon de mamíferos que apresenta exemplares extremamente adaptados tanto para o terrestre quanto para o ambiente aquáticos (Goswami, 2010). Caniformes não são exclusivamente carnívoros, como é evidenciado pelos pan-

Figura 1.2: Exemplos das Famílias de Caniformes. Linha de cima da esquerda para a direita: Canidae, Ursidae, Ailuridae. Linha do meio da esquerda para a direita (Pinipedia): Odobenidae, Otariidae, Phocidae. Linha de baixo da esquerda para a direita (Musteloidea): Procyonidae, Mephitidae, Mustelidae.

das gigante (*Ailuropoda melanoleuca*) e vermelho (*Ailurus fulgens*), que são animais especializados na ingestão de bambu. Contudo, grande parte das espécies pode ingerir carne ou pelo menos matéria animal como parte da dieta (Kissling et al., 2014). Apesar disso, a dieta dos diferentes grupos varia muito em termos de composição (Van Valkenburgh, 1988; Pineda-Munoz e Alroy, 2014), fator este que parece influenciar o fenótipo dos grupos (Asahara et al., 2016). Adicionalmente, mesmo dentro de grupos especializados em um item alimentar, a estratégia de captura de presas varia consideravelmente, influenciando a interação entre morfologia e ecologia alimentar das espécies (e.g. Van Valkenburgh e Wayne, 1994; Sacco e Van Valkenburgh, 2004; Slater et al., 2009, 2010; Tseng e Wang, 2011; Tseng, 2013; Figueirido et al., 2013; Kienle e Berta, 2015; Tseng et al., 2016).

O táxon Caniformes é composto por 9 famílias atuais:

Canidae é a segunda família mais diversa de Caniformes, com cerca de 37 espécies viventes atualmente descritas, e todas elas pertencem a subfamília Caninae (Tedford et al., 2009). Os Caninae são a única subfamília de canídeos que abandonou a América do Norte (centro de origem de Canidae) povoando a Ásia, Europa, África, América do Sul e, em tempos recentes, Austrália. Canídeos atuais são, em sua maioria, animais de médio a grande porte apresentando variação considerável em socialidade (Bekoff et al., 1981; Biben, 1983). Ecologicamente podem ser considerados em sua maioria generalistas, com algumas espécies podendo ser classificadas como hipercarnívoras (*Canis lupus*, *Lycaon pictus*, *Cuon alpinus* e *Speothos venaticus*, Slater et al., 2009) e ao menos uma espécie termitívora (*Otocyon megalotis*, Clark Jr, 2005). Apresentam um pós-crânio especializado para corrida e cursorialidade (Van Valkenburgh, 1985; Andersson, 2005), recaindo sob o crânio a demanda da aquisição e processamento de alimento (Slater et al., 2009).

Canidae é grupo irmão de todas as demais famílias de Caniformes que, em conjunto, formam o clado Arctoidea (Flynn et al., 2005; Nyakatura e Bininda-Emonds, 2012; Eizirik et al., 2010; Doronina et al., 2015).

Ursidae é a primeira família que se separa das demais de Arctoidea e, de maneira similar a Canidae, todas as espécies viventes de Ursidae pertencem a uma única subfamília (Ursinae). Apesar de apresentarem um número extremamente reduzido de espécies –apenas oito no total– ursídeos apresentam uma enorme diversidade ecológica e morfológica, como pode ser observado pelo contraste entre seus membros especialistas: o panda *Ailuropoda melanoleuca*, um animal especialista em matéria vegetal dura, o urso-polar *Ursus maritimus*, um hipercarnívoro exclusivo e o insetívoro *Melursus ursinus* (Sacco e Van Valkenburgh, 2004). As demais espécies desta família também podem ser consideradas curiosas de um ponto de vista mais amplo por serem onívoros de grande porte (Mattson, 1998; Sacco e Van Valkenburgh, 2004; van Heteren et al., 2015), algo particularmente raro para Carnivora (Carbone et al., 2007; Tucker et al., 2016).

Odobenidae é a primeira das 3 famílias de Pinipedia, a superfamília de Caniformes semiaquáticos. Atualmente Odobenidae é representada por uma única espécie vivente, a morsa *Odobenus rosmarus*. A morsa é facilmente reconhecida pelo seu grande tamanho, hábito marinho, comportamento gregário e, obviamente, pela presença de enormes caninos em forma de presa. Curiosamente, os demais dentes da morsa são extremamente reduzidos e pouco funcionais, provavelmente em decorrência da especialização para o consumo de moluscos bivalves através de um mecanismo de sucção (Fay, 1985). Apesar das modificações ob-

servadas na espécie vivente de morsa, os primeiros membros desta família no registro fóssil apresentavam uma morfologia generalizada piscívora, similar à de leões-marinhos (Berta et al., 2007).

Otariidae ou leões-marinhos, são considerados os mais terrestres dos pinipédios, principalmente por apresentarem alguma funcionalidade de seus apêndices posteriores em terra (Berta et al., 2007). As espécies viventes fazem parte de uma irradiação recente que foi extremamente bem sucedida na colonização do Oceano Pacífico e do hemisfério sul, incluindo América do Sul, África e Austrália (Yonezawa et al., 2009; Churchill et al., 2014). Os leões-marinhos são considerados conservativos, apresentando uma morfologia piscívora generalista. Dimorfismo sexual é comum no grupo e fortemente associado à presença de haréns, chegando ao extremo no leão-marinho *Otaria byronia* (Cullen et al., 2014).

Phocidae é a família de pinipédios mais diversificada, apresentando 19 espécies viventes subdivididas em duas subfamílias principais- Phocinae e Monachinae (Fulton e Strobeck, 2010). Phocinae é composta de espécies principalmente piscívoras que habitam os mares do hemisfério norte, incluindo uma espécie dulcícola (*Pusa caspica*). Monachinae, por outro lado, apresenta uma maior diversidade de formas e hábitos alimentares (Adam e Berta, 2002; Jones e Goswami, 2009; Kienle e Berta, 2015), como evidenciado, por exemplo, pelas espécies especialistas em vertebrados (a foca-leopardo *Hydrurga leptonyx*) e filtradores de krill (foca-caranguejeira *Lobodon carconophaga*). Podem ser encontradas nos oceanos Atlântico, Pacífico e Antártico (Fulton e Strobeck, 2010). Diferente de Otariidae, apresentam maior diversidade de estratégias reprodutivas, com espécies monomórficas que se reprodu-

zem isoladamente e de forma dispersa, à espécies que formam harens e apresentam dimorfismo sexual extremo, como no gênero de elefantes-marinhos *Mirounga* (Cullen et al., 2014).

Ailuridae, em conjunto com as próximas três famílias, constitui a superfamília Musteloidea. Ailuridae é a segunda família monotípica de Caniformes, com apenas uma única espécie vivente, o panda vermelho (*Ailurus fulgens*). Devido a sua convergência morfológica com o panda gigante (*Ailuropoda melanoleuca*), e seu pequeno tamanho e morfologia generalizada, o posicionamento de *Ailurus* foi incerto desde o seu descobrimento, sendo reconhecido ora como sendo associado a Ursidae, ora associado à Musteloidea. Filogenias moleculares encerraram o debate, demonstrando que *Ailurus* é de fato um Musteloidea (Flynn et al., 2005; Eizirik et al., 2010; Morlo e Peigné, 2010; Nyakatura e Bininda-Emonds, 2012; Doronina et al., 2015), e que todas as similaridades com o panda gigante são fruto de convergência adaptativa devido ao consumo de matéria vegetal dura (Figueirido et al., 2010, 2013).

Mephitidae foi durante muito tempo considerada uma subfamília de Mustelidae, entretanto evidências moleculares demonstraram que se tratava de uma família à parte, possivelmente irmã do clado formado por Procyonidae e Mustelidae (Flynn et al., 2005; Eizirik et al., 2010; Doronina et al., 2015). São caracterizados pela presença de glândulas de secreção nocivas que podem ser usadas em um "spray" contra predadores (Stankowich et al., 2014). Apesar de pouco diversos (12 espécies) o status taxonômico de muitas espécies ainda é disputado (e.g. *Conepatus humboldtii*, Schiaffini et al., 2013), evidenciando a falta de revisões no grupo. Em sua maioria, habitam as Américas, com a exceção de *Mydaus*, originário do Sudeste Asiático. São onívoros oportunistas

que usam suas fortes garras para cavar atrás de tubérculos, insetos e invertebrados (Nowak, 1999).

Procyonidae é um grupo de 18 espécies de animais onívoros parcialmente ou completamente arborícolas presentes nas Américas do Norte e Sul. Apresentam variação de ecologia e forma, com espécies frugívoras de rosto curto como o kinkajou (*Potos flavus*) e os olingos (gênero *Olingo*) e espécies insetívoras de rosto alongado, como os quati (gênero *Nasua*) (Myers, 2000; Nowak, 1999). Ambos os gêneros de animais de maior porte (*Procyon* e *Nasua*) apresentam espécies miniaturizadas relacionadas à invasão de ambientes insulares (*Procyon pygmaeus*, de Villa-Meza et al., 2011) e de altitude (espécies do subgênero *Nasuella*, Helgen et al., 2009).

Mustelidae é a família mais diversa de Carnívora, contendo 56 espécies viventes, estando presentes em todos os continentes do mundo, com a exceção da Austrália, Antártica e ilhas oceânicas (Wilson e Reeder, 2005). São principalmente carnívoros, explorando uma enorme diversidade de itens alimentares. Apresentam diferentes graus de especialização, como o hipercarnívoro Carcaju (*Gulo gulo*), capaz de abater presas até 5 vezes o seu tamanho (Pasitschniak-Arts e Larivière, 1995), ou a lontra marinha (*Enhydra lutris*), que se utiliza de ferramentas para quebrar invertebrados marinhos mais resistentes (Estes, 2001; Wilson e Reeder, 2005). Talvez o Carnívora mais especializado seja o *Mustela nigripes*, que se alimenta quase que exclusivamente de uma única espécie de roedor, o cão-da-pradaria (*Cynomys ludovicianus*; Hillman e Clark, 1980). Mustelidae é caracterizada pela presença de um corpo mais alongado, pernas posteriores mais curtas, tamanho reduzido e rosto pouco alongado. Esse padrão corpóreo permitiu a invasão dos

mais diversos habitats, com formas escaladoras (*e.g.* *Martes*), semi-fossoriais (*e.g.* *Mustela*) e semi aquáticas (as lontras, Lutrinae).

Objetivos e Estrutura da tese

O objetivo da presente tese é avaliar as consequências evolutivas da variação do padrão de integração morfológica em Caniformes. Especificamente, esse objetivo pode ser decomposto em duas perguntas específicas que são abordadas em capítulos distintos.

No Capítulo 3 intitulado "Morphological Integration in the skull of Carnivora (Mammalia): Interplay between modularity, flexibility, patterns and magnitude" apresento uma análise da diversidade de padrões de integração morfológica em Carnivora. Estudos prévios de integração morfológica em mamíferos tem demonstrado que o padrão de covariância é surpreendentemente conservado para o grupo como um todo (Marroig e Cheverud, 2001; Oliveira et al., 2009; Shirai e Marroig, 2010; Marroig e Cheverud, 2010; Hubbe et al., 2016; Haber, 2016), provavelmente em decorrência da estabilidade de rotas de desenvolvimento (Cheverud, 1982, 1984). Carnivora, especificamente, foi avaliada no contexto de genética quantitativa (Porto et al., 2009, 2013), mas tais análises foram restritas a no máximo quatro espécies, evidenciando a similaridade no padrão de covariância destas espécies com o de grupos pertencentes a outras ordens. Análises específicas de clados menos inclusivos tem revelado a presença de diferenças pontuais que podem ser informativas (*e.g.* Oliveira et al., 2009; Shirai e Marroig, 2010; Hubbe et al., 2016; Haber, 2016), o que justificaria uma investigação mais aprofundada do grupo. Inicialmente esse capítulo trataria apenas da comparação entre espécies de Caniformes, porém resultados preliminares apontaram que existia uma distinção em Canidae (Apêndices B e C). Visto que Canidae é grupo

Figura 1.3: Exemplos de crânios de cada Família de Caniformes. A- Canidae (*Canis lupus* -MNRJ32366). B- Ursidae (*Ursus arctos*- MVZ437). C- Ailuridae (*Ailurus fulgens*- USNM259379). D- Otariidae (*Otaria flavescens*-AMNH73120). E- Phocidae (*Phoca hispida*- MVZ123999). F- Odobenidae (*Odobenus rosmarus*- MVZ119066). G- Procyonidae (*Procyon cancrivorus*-MZUSP19794). H- Mephitidae (*Mephitis macroura*- FMNH5432). I- Mustelidae (*Mustela putorius*- AMNH2546).

irmão das demais famílias de Caniformes, restava a dúvida de se o padrão observado nessa família era plesiomórfico, e logo similar a Feliformes, ou derivado, sendo exclusivo de Canidae. Sendo assim, o escopo deste capítulo foi expandido para avaliar Carnivora como um todo com a utilização de dados de Feliformes coletados por um colega de laboratório (Zahn, 2016).

No Capítulo 4 intitulado "Error rates of quantitative genetics tests applied to macroevolutionary data" apresento uma investigação dos aspectos estatísticos de testes de genética quantitativa em contextos macroevolutivos. Esse métodos confrontam a variação intra e interespecífica de forma a avaliar a hipótese nula de deriva genética como sendo suficiente para explicar o padrão de variação observado entre espécies (Lande, 1979; Ackermann e Cheverud, 2004). O objetivo deste capítulo foi investigar as propriedades estatísticas (erro do tipo I e poder) de dois testes específicos, um baseado na proporcionalidade de variâncias nas escalas intra e interespecífica (teste de regressão) e outro baseado no surgimento de correlações entre espécies em combinações de caracteres que são independentes intraespecificamente (teste de correlação; Ackermann e Cheverud, 2004; Marroig e Cheverud, 2004). O erro do tipo I para o teste de regressão foi avaliado previamente em duas publicações. Ackermann e Cheverud (2004) passageiramente citam que o erro do tipo I foi verificado por simulações, mas não oferece nenhuma descrição detalhada dos parâmetros empregados. Prôa et al. (2013) realizam um estudo inteiramente voltado para avaliar a adequação da utilização de matrizes fenotípicas **P** como substituto de **G** para testes de regressão e mostram que, quando ambas matrizes são similares, o teste apresenta erros do tipo I adequados. Aqui eu expando essas investigações, investigando as propriedades não apenas do teste de regressão, mas também o de correlação em um contexto comparativo, avaliando e mensurando o impacto de dependência filogenética, erro amostral e variação demográfica no erro do

tipo I e poder.

Ambos capítulos citados acima foram produzidos em inglês, em formato de artigo. A tese ainda conta com um capítulo metodológico (Capítulo 2) que delinea os métodos empregados, amostra total e análises preliminares, um capítulo de conclusões (Capítulo de 5) e cinco apêndices. O Apêndice A traz a lista completa de espécimes analisados. O apêndice B traz uma comparação de diferentes métodos para a análise da similaridade de matrizes de covariância e correlação. O apêndice C é o pôster apresentado no congresso Evolution 2015, que contém alguns resultados preliminares que ajudaram na tomada de decisões subsequentes no desenvolvimento da tese. Os Apêndices D e E são os apêndices que contêm o material suplementar dos Capítulos 3 e 4, respectivamente. Por fim, o Apêndice F contém as publicações aceitas durante o desenvolvimento da tese.

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Capítulo 2

Materiais e Metodos

Amostragem

Coleções e esforço amostral

O presente projeto se baseou inteiramente na disponibilidade de materiais osteológicos em coleções de museus de história natural e zoologia. O grupo Caniformes é um dos poucos táxons de mamíferos que está presente em todos os continentes do mundo, incluindo Antártica. Devido a sua ampla distribuição, coleções brasileiras e regionais não são o suficiente para amostrar o grupo de forma satisfatória. Sendo assim, o projeto necessitou da visita a diversas coleções da América do Norte e do Sul. Coleções da América do Sul foram escolhidas principalmente pela conveniência e baixo custo, enquanto coleções Norte-Americana foram visitadas pois apresentam acervos extensos representativos de diversas regiões do mundo.

No total foram visitadas 11 coleções, sendo elas: Museu de Zoologia da Universidade de São Paulo (MZUSP, São Paulo), Museu Nacional (MN, Rio de Janeiro), Museu Paraense Emilio Goeldi (MPEG, Belém), Museo Argentino de Ciencias Naturales Bernardino Rivadavia (MACN, Buenos Aires), Museo de La plata (FCNyM, La plata), American Museum of Natural History (AMNH, New York), National Museum of Natural History of the Smithsonian Institution (USNM, Washington), Museum of Comparative Zoology (MCZ, Harvard), Field Museum (FMNH, Chicago) e Academy of Natural Sciences of Drexel University (ANSP, Philadelphia). No total, foram gastos 2000 horas de esforço amostral, resultando em um total de 5626 espécimes amostrados de 166 espécies (Tabela 2.1). A lista completa de espécimes se encontra no Apêndice A. As amostras e a taxonomia utilizadas nos capítulos 3 e 4 podem ser encontrados nas tabelas D.1 e E.1, respectivamente.

Tabela 2.1: Esforço amostral por instituição visitada. n- total de exemplares medidos. dias- total de dias úteis gastos na instituição. Esforço- total de horas gastas com amostragem. Horas/dia- Horas por dia gastas com a amostragem. n/hora- Espécimes medidos por hora. n/dia- espécimes medidos por dia. Valores "totais" são a soma para n, dias e esforço, e valores médios para horas/dia, n/hora e n/dia.

Instituição	País	n	dias	esforço (h)	horas/dia	n/hora	n/dia
MNRJ	BRA	132	10.5	63	6	2.10	12.57
MPEG	BRA	112	10	60	6	1.87	11.20
MZUSP	BRA	327	43	172	4	1.90	7.60
FCNyM	AR	105	7	56	8	1.88	15.00
MACN	AR	236	13	104	8	2.27	18.15
AMNH	EUA	1123	61	366	6	3.07	18.41
ANSP	EUA	76	4	28	7	2.71	19.00
FMNH	EUA	684	22	176	8	3.89	31.09
MCZ	EUA	106	4	32	8	3.31	26.50
MVZ	EUA	1488	68	612	9	2.43	21.88
USNM	EUA	1230	41	328	8	3.75	30.00
Outros*	BRA	7	1	3	3	2.33	7.00
total		5626	284.5	2000	6.75	2.63	18.20

*- espécimes não-tombados de *Canis familiaris* de coleções veterinárias.

Marcos anatômicos e medidas

Definição e identificação dos Marcos Anatômicos

A escolha de marcos anatômicos seguiu trabalhos prévios do nosso grupo (e.g. Cheverud, 1982, 1996; Ackermann e Cheverud, 2000; Cheverud et al., 1983; Marroig e Cheverud, 2001; Oliveira et al., 2009; Hubbe et al., 2016), principalmente as propostas de Porto et al. (2009). A configuração de marcos anatômicos do crânio de Caniformes pode ser vista na Figura 2.1. Todos os marcos anatômicos utilizados estão localizados nas suturas entre 3 ossos ou na extremidades de suturas (marcos anatômicos do tipo I e II, respectivamente *sensu* Bookstein, 1997).

Apesar dos marcos utilizados serem homólogos e presentes em todas as espécies analisadas, sua identificação não foi trivial em todos os casos.

Isso se deve a dois fatores principais. Primeiro, Caniformes apresentam uma grande variabilidade no tempo de fechamento de suturas cranianas (Goswami et al., 2013), com famílias, como Mustelidae e Procyonidae, nas quais os ossos do crânio se fusionam completamente muito cedo na vida do animal e outros grupos, como Phocidae, no qual grande parte das suturas nunca se fecha. Segundo, em alguns grupos a anatomia e formas cranianas são extremamente variáveis, com mudanças radicais da posição de marcos anatômicos (ver descrição abaixo). Esse segundo fator por si só não seria problemático, mas em conjunto com o primeiro torna a identificação de marcos anatômicos com base em análises comparativas complicada.

Para contornar essas questões, inicialmente se avaliou um método que consiste na utilização de uma luz emitida através do côndilo occipital dos espécimes de forma a tornar as suturas mais visíveis. Entretanto em muitas espécies de maior porte, como no caso de Pinipedia, os ossos eram espessos demais para a luz atravessar. Adicionalmente, e mais preocupantemente, nos grupos mais fusionados - Musteloidea principalmente - esse método se mostrou impreciso. Isso foi verificado com a utilização de séries ontogenéticas disponíveis, nas quais foi observado que o padrão evidenciado pela iluminação representava mais a impressão das circunvoluções do cérebro do que o padrão de suturas ainda visíveis em indivíduos juvenis. Isso também foi observado em grupos nos quais as suturas estavam visíveis ao longo de quase toda a ontogenia, como Canidae. Sendo assim, a solução encontrada foi a utilização de séries ontogenéticas para diagnosticar a localização dos marcos anatômicos. Isso foi feito principalmente através de: **(1)** a observação da posição dos marcos nos juvenis; **(2)** a relação destes marcos com outras estruturas cranianas e; **(3)** sua modificação ao longo da série ontogenética, especialmente a manutenção de sua posição relativa nos diversos estágios de desenvolvimento. Foram utilizadas séries para a espécie sendo

Figura 2.1: Marcos anatômicos em ambas as vistas digitalizadas sobre o crânio de um espécime de *Canis lupus*. Pontos negros e brancos representam marcos restritos às vistas A e Z, respectivamente. Estrelas são pontos comuns a ambas as vistas.

medida ou, alternativamente, para espécies do mesmo gênero, quando as primeiras não estavam disponíveis. Essa metodologia possibilitou inferir a posição dos marcos anatômicos para todas as espécies de Caniformes.

Abaixo descrevo a anatomia dos marcos utilizados e comento brevemente sobre mudanças anatômicas que influenciaram no trabalho.

NSL - Sutura mais anterior entre ambos nasais. Em muitos indivíduos um dos ossos estava ausente ou deslocado. Foi comum observar, principalmente em canídeos e procionídeos, uma leve abertura de ambos os nasais, o que deslocaria o marco posteriormente. Em tais casos foi pos-

sível observar a presença de ranhuras que indicavam o ponto de encontro entre os ossos, auxiliando o posicionamento preciso do marco.

NA - Sutura entre os ossos nasais e o frontal. Esse ponto é geralmente não-ambíguo e facilmente localizado em todos os grupos. Muitas vezes os nasais diferem em tamanho, principalmente em grupos com face mais alongada, como Canidae. Nesses casos escolhia-se o ponto mais posterior para demarcar o ponto.

PM - Ponto mais lateral da sutura premaxilar-maxilar em vista ventral. Em algumas espécies, a sutura entre a pré-maxila e a maxila estende-se a partir do plano médio do palato até a região interna (ou lingual) do dente canino. Quando isto ocorreu, **PM** foi registrado no encontro da sutura com o canino.

MT - Ponto mais posterior da sutura entre o palatino e maxila no palato ósseo. Trabalhos prévios definiram este ponto como sendo a ponta da tuberosidade da maxila (Porto et al., 2009). Entretanto, tal estrutura não está presente na maior parte dos táxons aquáticos e alguns terrestres, que apresentam um contínuo entre a asa do pterigoide e a série dentária. A sutura acima descrita está em associação próxima com a tuberosidade da maxila sempre que esta está presente. Assim, a sutura foi usada como uma aproximação do ponto para os taxons investigados.

IS - Ponto médio da maxila na porção inferior do septo entre os incisivos.

PNS - Ponto mais posterior da sutura entre os palatinos. Na maior parte das espécies isso também correspondeu ao ponto mais posterior do palato ósseo, exceto em *Arctonyx* (Mustelidae), na qual as asas do pterigoide se fusionam medialmente, formando uma extensão do palato ósseo.

ZI - Ponto mais inferior e posterior da sutura zigomático-maxilar. Na maioria dos caniformes, a região anterior do zigomático faz uma curvatura característica, na qual uma porção pequena do maxilar contribui para a formação do arco. Isso descreve um desenho em "C" (que pode ser visto na Figura 2.1), que é comum a quase todos os Caniformes, exceto Phocidae. Nesse grupo, o maxilar avança muito mais posteriormente na região ventral do arco. Assim, a sutura inferior entre o maxilar e o zigomático se encontra no meio do arco, e não no encontro deste com a região lateral do rosto. De qualquer forma, o ponto foi marcado nessa mesma sutura, na posição mais ventral, o que nem sempre correspondia à ponta da projeção da maxila (que podia se encontrar na lateral interna do arco, muitas vezes inacessível).

ZS - Ponto mais superior da sutura zigomático-maxilar. A posição anatômica desse marco variou bastante entre grupos. Enquanto em Ursidae e Canidae, por exemplo, o zigomático encontrava com a maxila na região lateral do rosto, em Phocidae e alguns Mustelidae a maxila contribui para a formação do arco, fazendo com que o marco fosse deslocado posteriormente.

ZYGO - Ponto mais posterior da sutura temporal-zigomático.

EAM - Ponto anterior do meato auditivo externo. No caso de alguns animais maiores (*e.g.* Ursidae, Otariidae), o canal auditivo se expande, formando um tubo alongado. Nesses casos o ponto foi marcado próximo à borda do canal auditivo, na região externa.

TS - Sutura temporal-esfenoidal-basioccipital. Em Phocidae, essa região encontrava-se extremamente modificada. O encontro entre os ossos ocorria no interior de uma fenestra, inacessível pelo equipamento. Nestes casos,

marquei o ponto na sutura entre o temporal e o basioccipital na altura da bula timpânica.

JP - Borda mais lateral e posterior do forame jugular, na sutura entre o basioccipital e o temporal. Porto et al. (2009) definiram este ponto como sendo na base do processo jugular, na sutura entre o basioccipital e o temporal. Porém em algumas espécies o processo juglar encontra-se distante desta sutura, e muitas vezes extremamente modificado, sendo de difícil detecção. Visto que a sutura entre o basioccipital e o temporal sempre se encontrava associada ao forame jugular, este ponto foi escolhido.

APET - Ponto anterior da crista petrosal. Essa crista pode ser frágil em muitos animais menores. No caso dessa estrutura estar quebrada em ambos os lados do crânio, o marco foi estimado como sendo o ponto mais anterior da depressão formada por essa crista no basicrânio.

BA e OPI - Lábio ventral e dorsal do forame magno, respectivamente. Nas espécies maiores o forame pode ser muito robusto, dificultando a identificação de um ponto específico para ambos marcos. A forma dos lábios podia variar também, desde achatado, com uma superfície variando desde reta até arredondada. Por essas peculiaridades, esses marcos foram sempre marcados ou na região mais extrema dos lábios, ou no ponto médio (quando os lábios apresentavam um achatamento).

AS - Sutura occipital-parietal-temporal. Apesar de ser precisamente definido, o ponto varia consideravelmente de posição entre diferentes grupos. O motivo disso é que o temporal apresenta dois componentes referentes à região esquamosal e ao petromastóide. Em grupos que apresentam a caixa craniana expandida (e.g. Mustelidae, Phocidae, Odobenidae), ambas partes são indiferenciáveis, enquanto em grupos com

caixa craniana menos alongada (*e.g.* Ursidae, Canidae) a região petromastóide se encontra bastante reduzida no sentido antero-posterior. Neste último caso o petromastóide varia muito de tamanho, principalmente no sentido dorso-ventral, algo particularmente evidente em Canidae. Adicionalmente, na maioria das espécies de lontras (*Lutrolineae*), o parietal sofre uma modificação extrema, dominando a caixa craniana, se estendendo até a região ventral do crânio. Essa extensão do parietal era acompanhada pela redução da região petromastóide no sentido dorso-ventral fazendo com que o ponto AS fosse registrado na região ventral do crânio, próximo ao ponto JP. Sendo assim, apesar da definição do marco se manter a mesma em todo grupo, a posição relativa do marco variou bastante entre grupos.

LD - Sutura entre os parietais e interparietais. Visto que o interparietal assume diversas formas ao longo da evolução dos mamíferos (Koyabu et al., 2012), esse ponto pode se encontrar em regiões muito diferentes do crânio. Em Canidae e Ursidae, por exemplo, o interparietal avança por entre os parietais pela região dorsal do crânio. Em Otariidae e em Mustelidae, o interparietal forma um contínuo com o supra-occipital (Koyabu et al., 2012), sendo que o **LD** fica definido como o ponto mais posterior e dorsal do crânio. Isso não traz problemas para a definição do marco, mas pode ter implicações para a interpretação das distâncias baseadas neste ponto.

BR - Sutura entre os frontais e parietais. Algumas espécies podem apresentar uma crista sagital pronunciada que pode influenciar na medição do ponto. Via de regra, quando a crista é pouco pronunciada o ponto foi marcado no topo da crista. Porém em espécies de crista muito pronunciada, como *Otaria flavescens*, o ponto foi marcado nas laterais da

crista, próximo à base, sobre a sutura entre o frontal e o parietal.

PT e TSP - Sutura frontal-parietal-esfenóide e temporal-esfenóide-parietal, respectivamente. Esses pontos se mostraram consideravelmente variáveis ao longo de todos os grupos, com muitos casos onde não era possível distingui-los por haver um único ponto de encontro entre frontal-temporal-parietal-esfenóide. Adicionalmente, em alguns casos, existem projeções ventrais e posteriores do frontal, formando uma nova sutura estendida entre o frontal e o temporal, dando a impressão de que ambos os pontos se "inverteram" no sentido antero-posterior. Nesses casos o TSP foi marcado como sendo o encontro entre temporal-frontal-esfenóide e o PT como o encontro do frontal-parietal-temporal. Em Phocidae, esses pontos podiam encontrar-se deslocado ventralmente, principalmente em espécies com o encéfalo mais expandido, como *Mirounga*, *Pusa* e *Phoca*.

Digitalização dos espécimes

As medidas morfométricas foram obtidas através da digitalização de marcos anatômicos no crânio dos espécimes. A digitalização dos marcos se deu com a ajuda de um sistema Microscribe MLX (Immersion Corporation - San Jose, California) que permite o registro de coordenadas tri-dimensionais (Figura 2.2A). Os espécimes foram fixados em massa de modelar não-orgânica em duas posições distintas, chamadas de vista A e Z (Figura 2.2B-C e 2.1). A vista A apresenta 25 marcos, dos quais 7 se encontram na linha média do crânio e 18 são bilaterais (9 pares). A vista Z apresenta 11 marcos, dos quais 7 são da linha média e 4 são bilaterais (2 pares). A massa utilizada teve que ser resistente o suficiente para manter o espécime na sua posição, mas não tão resistente que impedisse ser modelada com facilidade. Dada a grande

variação de tamanho e de forma em Caniformes, diferentes materiais tiveram que ser utilizados, dependendo sempre do tamanho e da resistência dos espécimes medidos e as condições ambientais da área de trabalho. Isso se deve ao fato de que massas de modelar respondem à temperatura, se tornando mais resistentes em ambientes mais frios e mais macias em ambientes mais quentes. Se a massa era macia demais, ela podia não proporcionar sustentação adequada, além de soltar partes, principalmente dentro de suturas, o que pode eventualmente danificar espécimes de coleções. Nos casos em que isso ocorreu, instrumentos cirúrgicos e de dentista foram utilizados para limpar o espécime. Adicionalmente, no caso de animais de grande porte, como *Mironga*, *Odobenus* e *Hydrurga*, sacos de areia (ou outros objetos pesados) foram utilizados como apoio adicional, uma vez que a quantidade de massa era insuficiente para mantê-los imóveis.

As três coordenadas espaciais de cada marco foram então registradas (digitalizadas) em uma planilha de Microsoft Excel com a utilização de um software próprio do equipamento (Figura 2.3). Antes da digitalização de cada espécime, suas informações – como sexo, localidade, subespécie, identificador, coletor e etc – foram registradas (Figura 2.3A). A calibração do equipamento foi verificada antes de cada seção de digitalização pela medição de uma distância conhecida em uma régua (Figura 2.3D). Se a diferença estava acima da especificada pelo fabricante, o equipamento era recalibrado segundo o manual do fabricante.

Cada espécime foi medido duas vezes, o que ajudou a minimizar possíveis erros de medição (Figura 2.3B). Durante o processo de digitalização dos espécimes, distâncias fixas foram escolhidas para averiguar a precisão de cada ponto (Figura 2.3C). Se as distâncias ultrapassavam em muito o erro do equipamento, o indivíduo era medido novamente. Se diferenças persistissem, o equipamento era recalibrado. Aqui eu utilizei distâncias arbitrárias

que não correspondiam necessariamente às distâncias que de fato foram utilizadas nas análises. Isso foi feito para não inflar artificialmente a precisão das medidas que foram de fato incorporadas nas análises.

Após a medição todos os espécimes foram fotografados com uma câmera Cannon EOS Rebel T3i nas vistas dorsal, lateral e ventral, com auxílio de um tripé ou estativa. A mandíbula dos espécimes, quando disponível, foi fotografada em vista lateral de ambos os lados, alinhando os dois maiores planos da estrutura com o plano da imagem. Todas as fotos foram tiradas com uma escala. Para minimizar os efeitos de paralaxe, a câmera foi colocada o mais distante possível do espécime e este foi colocado ao centro da imagem.

Unificação das vistas

A princípio, todas as medidas podem ser obtidas nas configurações de marcos referentes a vista A e Z isoladamente. Entretanto, devido à diferenças na anatomia dos grupos, alguns marcos não poderão ser acessado pelas vistas convencionais para alguns grupos, como foi o caso dos marcos PT para Phocidae e AS para Lutreolinae (ver acima). Nesses casos, visto que existiam marcos anatômicos comuns a ambas as vistas, fez-se um procedimento de unificação das vistas para a obtenção de uma configuração completa na qual todas as medidas podiam ser tomadas.

Para cada réplica de cada espécime a unificação das vistas foi feita através da obtenção da matriz de rotação $\mathbf{R}$ que melhor satisfizesse

$$\mathbf{A}_c = \mathbf{Z}_c \mathbf{R} \quad (2.1)$$

onde $\mathbf{A}_c$ e $\mathbf{Z}_c$ são as matrizes de marcos comuns em ambas as vistas. Estas matrizes estão centralizadas, isto é, suas coordenadas estão centralizadas no

Figura 2.2: A- estação de trabalho para a digitalização dos crânios. B- Posição para a digitalização dos marcos anatômicos da vista A. C- Posição para a digitalização dos marcos anatômicos da vista Z. Ver a Figura 2.1 para marcos referentes às diferentes vistas.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	MUSEUM	MZUSP	A										
2	SPECIES	Gallctis cuja											
3	SUBSPECIES												
4	ID MUSEU	19821											
5	SEX	m											
6	COL	ABC-Finep											
7	DATE	1976											
8	DET	Mwillig&Mmares											
9	DATE DET	1988											
10	LOCATION	Exu (Fazenda Batente), Pe											
11	MANDIBLE	x											
12	OBS												
13			B										
14													
15	FIRST MEASUREMENT												
16	A												
17	BR	653.10	9.81	33.13	BR	653.05	9.79	33.20	0.095	0.000	0.165	0.023	0.018
18	NON	646.70	6.83	64.69	NON	646.77	6.77	64.61	0.117	0.163	0.191	0.181	0.303
19	NLE	649.57	6.81	75.65	NLE	649.44	6.79	75.60	0.143	0.116	0.154	0.138	0.180
20	IS	657.59	8.90	83.15	IS	657.34	8.60	83.07	0.397	0.165	0.000	0.103	0.281
21	MP	662.22	9.92	70.01	MP	661.93	9.73	69.90	0.369	0.230	0.016	0.169	0.333
22	PNS	668.72	12.24	53.37	PNS	668.80	12.19	53.76	0.407	0.338	0.295	0.167	0.002
23	BA	680.69	17.74	23.34	BA	680.69	17.56	23.20	0.222	0.072	0.153	0.224	0.097
24	OPI	674.30	16.46	16.53	OPI	674.25	16.18	16.65	0.309	0.086	0.145	0.032	0.136
25	EAM	666.86	29.51	38.98	EAM	667.07	29.49	38.97	0.216	0.129	0.139	0.245	0.076
26	JP2	675.26	28.71	27.01	JP2	674.75	29.32	26.38	1.019	0.219	0.717	0.472	0.708
27	JP	673.99	24.02	27.60	JP	674.21	24.41	27.37	0.503	0.512	0.441	0.465	0.517
28	APET	674.09	19.42	36.86	APET	673.33	19.43	37.62	1.076	0.498	0.877	0.965	0.635
29	TS	671.99	25.54	38.86	TS	671.26	25.99	39.36	0.996	0.098	0.398	0.653	0.029
30	ZYGI	660.34	30.57	49.45	ZYGI	660.60	30.72	49.43	0.307	0.158	0.235	0.218	0.039
31	TSP	662.41	25.04	44.51	TSP	662.43	25.06	44.54	0.046	0.044	0.054	0.135	0.097
32	PT	659.24	22.25	47.61	PT	659.21	22.19	47.74	0.146	0.023	0.103	0.000	0.198
33	PZIG	648.94	18.08	57.74	PZIG	648.81	17.92	58.12	0.436	0.259	0.405	0.273	0.068
34	ZI	658.88	25.99	63.58	ZI	658.86	26.02	63.70	0.125	0.069	0.081	0.012	0.110
35	PM	658.75	12.38	80.07	PM	658.72	12.36	80.17	0.102	0.027	0.155	0.043	0.113
36	DATA	655.35	12.05	80.61	DATA	655.36	12.20	80.42	0.384	0.224	0.451	0.402	0.257

Figura 2.3: Planilha utilizada para a coleta de dados. A- Informações gerais sobre o espécimes sendo medido. B- Duas réplicas das coordenadas tri-dimensionais dos marcos anatômicos. C- Diferenças entre algumas medidas selecionadas para averiguar diferenças entre as duas digitalizações. D- Local para a verificação da calibração do equipamento.

centróide das configurações. Segundo (Schönemann, 1966), a matriz de rotação $\mathbf{R}$ que minimiza a soma dos quadrados das distâncias entre os marcos é dada por

$$\mathbf{R} = \mathbf{V}\mathbf{D}^*\mathbf{U} \quad (2.2)$$

na qual $\mathbf{V}$, $\mathbf{U}$ e $\mathbf{D}$ (sendo essa última utilizada para produzir $\mathbf{D}^*$) são obtidas através da decomposição de valor-singular da matriz $\mathbf{A}_c^t\mathbf{Z}_c$

$$\mathbf{A}_c^t\mathbf{Z}_c = \mathbf{U}\mathbf{D}\mathbf{V} \quad (2.3)$$

Os elementos d_{ij}^* da matriz $\mathbf{D}^*$ são obtidos diretamente a partir dos ele-

mentos d_{ij} da matriz $\mathbf{D}$ da seguinte forma:

$$d_{ij}^* = \begin{cases} d_{ij}/|d_{ij}|, & \text{se } i = j \\ 0, & \text{se } i \neq j \end{cases} \quad (2.4)$$

As configurações de marcos finais foram obtidas como

$$\mathbf{X} = \begin{bmatrix} \mathbf{A}_{-c} \\ \mathbf{Z}^* \end{bmatrix} \quad (2.5)$$

onde $\mathbf{A}_{-c}$ é a matriz de marcos de $\mathbf{A}$ sem os pontos comuns e $\mathbf{Z}^* = \mathbf{Z}\mathbf{R}$. $\mathbf{A}_{-c}$ e $\mathbf{Z}$ encontram-se centralizadas nos marcos comuns.

Medidas

O conjunto de medidas utilizadas no presente trabalho (tabela 2.2) foi escolhido principalmente por três motivos. Primeiramente essas medidas buscam quantificar variações morfológicas restritas a um ou poucos ossos ou estruturas cranianas, permitindo assim a compreensão de processos ontogênicos, genéticos ou funcionais localizados (Pearson e Davin, 1924; Cheverud, 1982). Secundariamente, essas medidas já foram extensivamente analisadas por nosso grupo (e.g. Cheverud, 1982, 1996; Ackermann e Cheverud, 2000; Cheverud et al., 1983; Marroig e Cheverud, 2001; Porto et al., 2009; Oliveira et al., 2009; Hubbe et al., 2016) o que permite a comparação direta dos resultados. Por último, análises de genética quantitativa desses caracteres tem apontado para a similaridade no padrão de covariâncias fenotípicas e genotípicas destes caracteres em grupos tão dispares quanto marsupiais (Porto et al., 2015), roedores (Garcia et al., 2014) e primatas (Cheverud, 1982; Marroig e Cheverud, 2010) tornando-os ideais para o tipo de análise realizado aqui. Os caracteres foram alocados nos módulos e regiões cranianas estabelecidas por Cheverud (1982), seguindo as sugestões de Porto et al.

(2009).

As medidas foram obtidas calculando-se a distância linear entre um par de marcos anatômicos com a utilização do teorema de Pitágoras. Quando possível, medidas presentes em ambos os lados do crânio foram obtidas e a média de ambos valores foram usadas para representar a respectiva distância nas análises subsequentes. As medições foram tomadas para cada réplica medida, possibilitando assim obter dois conjuntos de medidas para cada espécime.

Erro de medição

Para estimar a confiabilidade das medições calculei a repetibilidade das medidas para espécies com 25 ou mais indivíduos (ver Capítulo 3). A repetibilidade pode ser definida como a porcentagem da variação total das medidas que pode ser atribuída às diferenças entre espécimes e que não está associada ao erro de medição intra-individual. A repetibilidade foi estimada como a proporção da variância total que pode ser explicada pelas réplicas segundo um modelo linear (Lessells e Boag, 1987).

As médias de repetibilidades foram sempre superiores a 0.91 quando avaliadas por espécies (Tabela 2.3) e superiores a 0.93 quando avaliadas por caráter (Tabela 2.4), com exceção de APET-TS (média=0.86) e BA-OPI (média=0.89), que também apresentaram os menores valores mínimos (BA-OPI para *Vulpes velox*=0.64; APET-TS para *Martes foina*=0.59, *Martes americana*=0.60, *Mustela altaica*=0.59). Baixos valores de BA-OPI podem ser devido ao fato de que ambos pontos BA e OPI são marcos anatômicos do tipo II, o que dificulta identificação precisa do mesmo ponto em ambas as réplicas. Adicionalmente, a região do forame magno pode ser muito robusta, principalmente em espécimes maiores, o que dificulta mais ainda a medição

Tabela 2.2: Medidas cranianas empregadas no presente estudo. As medidas são definidas por dois marcos anatômicos e são obtidas como a distância euclidiana entre eles. Ver figura 2.1 para localização dos marcos anatômicos.

Região	Modulo	Medida
Face	Nasal	IS-NSL
Face	Nasal	NSL-NA
Face	Nasal	NSL-ZS
Face	Nasal	NA-PNS
Face	Oral/Nasal	IS-PNS
Face	Oral/Nasal	NSL-ZI
Face	Oral	IS-PM
Face	Oral	PM-ZS
Face	Oral	PM-ZI
Face	Oral	PM-MT
Face	Oral	ZI-MT
Face	Oral	MT-PNS
Face	Oral/Zigomático	ZS-ZI
Face	Zigomático	PT-ZYGO
Face	Zigomático	ZI-ZYGO
Face	Zigomático	ZI-TSP
Face	Zigomático	EAM-ZYGO
Face	Zigomático	ZYGO-TSP
Neurocrânio	Abóboda/Zigomático	PT-TSP
Neurocrânio	Abóboda	NA-BR
Neurocrânio	Abóboda	BR-PT
Neurocrânio	Abóboda	BR-APET
Neurocrânio	Abóboda	PT-APET
Neurocrânio	Abóboda	PT-BA
Neurocrânio	Abóboda	PT-EAM
Neurocrânio	Abóboda	LD-AS
Neurocrânio	Abóboda	BR-LD
Neurocrânio	Abóboda	OPI-LD
Neurocrânio	Abóboda	PT-AS
Neurocrânio	Abóboda	JP-AS
Neurocrânio	Base	PNS-APET
Neurocrânio	Base	APET-BA
Neurocrânio	Base	APET-TS
Neurocrânio	Base	BA-EAM
Neurocrânio	Base	BA-OPI

Tabela 2.3: Repetibilidade por espécie (n >24)

	Min.	Mean	Max.		Min.	Mean	Max.
<i>Ailuropoda melanoleuca</i>	0.893	0.981	0.999	<i>Mellivora capensis</i>	0.861	0.984	1.000
<i>Ailurus fulgens</i>	0.930	0.979	0.999	<i>Melogale moschata</i>	0.796	0.966	0.997
<i>Aonyx cinerea</i>	0.856	0.973	0.998	<i>Melogale personata</i>	0.809	0.963	0.997
<i>Arctocephalus australis</i>	0.944	0.994	1.000	<i>Melursus ursinus</i>	0.933	0.989	1.000
<i>Arctocephalus gazella</i>	0.683	0.914	0.998	<i>Mephitis macroura</i>	0.870	0.963	0.998
<i>Arctonyx collaris</i>	0.954	0.992	1.000	<i>Mephitis mephitis</i>	0.831	0.965	0.998
<i>Atelocynus microtis</i>	0.893	0.981	0.998	<i>Mirounga angustirostris</i>	0.932	0.996	1.000
<i>Bassaricyon alleni</i>	0.741	0.950	0.996	<i>Mirounga leonina</i>	0.988	0.998	1.000
<i>Bassaricyon medius</i>	0.747	0.941	0.996	<i>Monachus tropicalis</i>	0.829	0.972	0.999
<i>Bassariscus astutus</i>	0.612	0.920	0.994	<i>Mustela altaica</i>	0.590	0.956	0.999
<i>Bassariscus sumichrasti</i>	0.724	0.962	0.998	<i>Mustela erminea</i>	0.712	0.951	0.997
<i>Callorhinus ursinus</i>	0.891	0.988	1.000	<i>Mustela frenata</i>	0.802	0.969	0.999
<i>Canis adustus</i>	0.814	0.980	0.999	<i>Mustela itatsi</i>	0.764	0.949	0.998
<i>Canis aureus</i>	0.864	0.981	0.999	<i>Mustela kathiah</i>	0.842	0.967	0.998
<i>Canis dingo</i>	0.896	0.988	1.000	<i>Mustela nigripes</i>	0.731	0.954	0.998
<i>Canis familiaris</i>	0.884	0.983	1.000	<i>Mustela nivalis</i>	0.745	0.958	0.999
<i>Canis latrans</i>	0.896	0.985	0.999	<i>Mustela putorius</i>	0.817	0.972	0.998
<i>Canis lupus</i>	0.939	0.995	1.000	<i>Mustela sibirica</i>	0.810	0.965	0.997
<i>Canis lycaon</i>	0.901	0.984	0.999	<i>Mustela vision</i>	0.851	0.969	0.999
<i>Canis mesomelas</i>	0.807	0.976	0.997	<i>Nasua narica</i>	0.855	0.978	0.999
<i>Canis rufus</i>	0.843	0.980	0.998	<i>Nasua nasua</i>	0.879	0.983	0.999
<i>Cerdocyon thous</i>	0.891	0.978	0.999	<i>Nyctereutes procyonoides</i>	0.773	0.969	0.998
<i>Chrysocyon brachyurus</i>	0.930	0.988	0.999	<i>Odobenus rosamarus</i>	0.907	0.983	1.000
<i>Conepatus chinga</i>	0.925	0.983	0.999	<i>Otaria byronia</i>	0.972	0.996	1.000
<i>Conepatus leuconotus</i>	0.868	0.956	0.996	<i>Otocyon megalotis</i>	0.868	0.976	0.999
<i>Conepatus semistriatus</i>	0.877	0.979	0.998	<i>Pagophilus groenlandicus</i>	0.895	0.986	0.998
<i>Cuon alpinus</i>	0.746	0.974	0.997	<i>Phoca hispida</i>	0.953	0.989	1.000
<i>Cystophora cristata</i>	0.921	0.992	1.000	<i>Phoca vitulina</i>	0.837	0.990	1.000
<i>Eira barbara</i>	0.829	0.977	0.999	<i>Poecilogale albinucha</i>	0.809	0.965	0.998
<i>Enhydra lutris</i>	0.931	0.979	0.999	<i>Potos flavus</i>	0.878	0.971	0.998
<i>Erignathus barbatus</i>	0.651	0.915	0.998	<i>Procyon cancrivorus</i>	0.873	0.979	0.999
<i>Eumetopias jubatus</i>	0.953	0.995	1.000	<i>Procyon lotor</i>	0.873	0.979	0.999
<i>Galictis cuja</i>	0.868	0.977	0.998	<i>Pteronura brasiliensis</i>	0.930	0.985	0.999
<i>Galictis vittata</i>	0.916	0.976	0.999	<i>Speothos venaticus</i>	0.881	0.980	0.998
<i>Gulo gulo</i>	0.890	0.980	0.999	<i>Spilogale angustifrons</i>	0.593	0.926	0.996
<i>Helarctos malayanus</i>	0.720	0.952	1.000	<i>Spilogale gracilis</i>	0.672	0.959	0.998
<i>Ictonyx libyca</i>	0.789	0.946	0.998	<i>Spilogale putorius</i>	0.727	0.952	0.998
<i>Ictonyx striatus</i>	0.863	0.961	0.998	<i>Taxidea taxus</i>	0.892	0.982	0.999
<i>Leptonychotes weddelli</i>	0.775	0.948	0.995	<i>Tremarctos ornatus</i>	0.918	0.990	1.000
<i>Lobodon carcinophaga</i>	0.933	0.990	0.999	<i>Urocyon cinereoargenteus</i>	0.833	0.975	0.998
<i>Lontra canadensis</i>	0.691	0.951	0.997	<i>Urocyon litoralis</i>	0.698	0.958	0.996
<i>Lontra longicaudis</i>	0.775	0.976	0.999	<i>Ursus americanus</i>	0.973	0.996	1.000
<i>Lutra lutra</i>	0.861	0.980	0.999	<i>Ursus arctos</i>	0.964	0.996	1.000
<i>Lycalopex culpaeus</i>	0.851	0.982	0.998	<i>Ursus maritimus</i>	0.948	0.988	0.999
<i>Lycalopex griseus</i>	0.855	0.980	0.998	<i>Ursus thibetanus</i>	0.915	0.985	0.999
<i>Lycalopex gymnocercus</i>	0.883	0.980	0.998	<i>Vulpes chama</i>	0.804	0.974	0.998
<i>Lycalopex sechurae</i>	0.844	0.969	0.997	<i>Vulpes lagopus</i>	0.801	0.972	0.997
<i>Lycalopex vetulus</i>	0.778	0.964	0.997	<i>Vulpes macrotis</i>	0.826	0.975	0.998
<i>Lycaon pictus</i>	0.865	0.979	0.998	<i>Vulpes ruppelli</i>	0.785	0.965	0.996
<i>Martes americana</i>	0.598	0.958	0.997	<i>Vulpes velox</i>	0.635	0.936	0.995
<i>Martes flavigula</i>	0.735	0.971	0.999	<i>Vulpes vulpes</i>	0.800	0.978	0.998
<i>Martes foina</i>	0.592	0.938	0.997	<i>Vulpes zerda</i>	0.668	0.921	0.990
<i>Martes pennanti</i>	0.727	0.980	0.999	<i>Zalophus californicus</i>	0.651	0.978	1.000
<i>Martes zibellina</i>	0.732	0.944	0.996	<i>Zalophus wolfebaeki</i>	0.924	0.990	0.999
<i>Meles meles</i>	0.892	0.976	0.999				

Tabela 2.4: Repetibilidade por caráter

	Min.	Mean	Max.		Min.	Mean	Max.
APET-BA	0.886	0.975	1.000	OPI-LD	0.832	0.963	0.999
APET-TS	0.590	0.861	0.988	PM-MT	0.824	0.976	1.000
BA-EAM	0.882	0.975	0.999	PM-ZI	0.841	0.979	1.000
BA-OPI	0.635	0.899	0.990	PM-ZS	0.856	0.976	1.000
BR-APET	0.720	0.985	1.000	PNS-APET	0.921	0.979	1.000
BR-LD	0.815	0.981	0.999	PT-APET	0.913	0.977	0.999
BR-PT	0.910	0.991	1.000	PT-AS	0.919	0.986	1.000
EAM-ZYGO	0.760	0.956	0.997	PT-BA	0.862	0.994	1.000
IS-NSL	0.844	0.974	1.000	PT-EAM	0.651	0.975	0.999
IS-PM	0.712	0.936	0.999	PT-TSP	0.892	0.984	0.999
IS-PNS	0.852	0.993	1.000	PT-ZYGO	0.683	0.987	1.000
JP-AS	0.651	0.923	0.995	ZI-MT	0.839	0.967	0.999
LD-AS	0.721	0.970	0.999	ZI-TSP	0.837	0.990	1.000
MT-PNS	0.727	0.961	0.999	ZI-ZYGO	0.747	0.977	1.000
NSL-NA	0.844	0.991	0.999	ZS-ZI	0.888	0.973	0.996
NSL-ZI	0.946	0.992	1.000	ZYGO-TSP	0.873	0.987	1.000
NSL-ZS	0.897	0.988	1.000	ZI-PT	1.000	1.000	1.000
NA-BR	0.919	0.995	1.000	ZYGO-PT	1.000	1.000	1.000
NA-PNS	0.798	0.992	1.000				

precisa do mesmo ponto repetidas vezes. No caso da distância APET-TS, as repetibilidades mais baixas provavelmente se devem ao fato de que em muitos casos a crista petrosal estava quebrada, sendo sua posição estimada pela presença de sulcos deixados pela estrutura. Curiosamente, nenhuma outra medida com o marco APET apresentou repetibilidades consideradas baixas, o que leva a crer que o tamanho diminuto desta medida também pode contribuir para esse padrão. A inclusão ou exclusão de ambas as medidas não alteraram os padrões gerais observados no Capítulo 3, e por isso foram mantidas em todas as análises.

Integração morfológica

Cálculo das matrizes

Para espécies com 25 ou mais indivíduos amostrados obtive as matrizes de correlação e covariância entre os caracteres. Ao calcular tais matrizes, é importante identificar e controlar efeitos indesejados nas medidas, como variação geográfica e dimorfismo sexual. Para isso, testei o efeito destes fatores (sexo e subespécie e suas interações) através de Análises de Variância Univariada (ANOVAs) e Multivariada (MANOVA). Efeitos considerados relevantes foram removidos através de um modelo linear multivariado, e os resíduos desse modelo foram usados como dados para a estimativa das matrizes de covariância V através da seguinte fórmula

$$\mathbf{V} = \mathbf{DD}^t \frac{1}{df} \quad (2.6)$$

sendo $\mathbf{DD}^t$ a matriz dos produtos cruzados dos resíduos do modelo linear e df os graus de liberdade dos dados. No caso de espécies com o efeito de fatores controlados, df é igual aos graus de liberdade residuais do modelo linear. Isso é equivalente a multiplicar a matriz de covariância estimada por $(n - 1)/df$, corrigindo para a perda de graus de liberdade através do modelo linear. No caso de não haverem fatores para serem corrigidos, $\mathbf{DD}^t$ é apenas a matriz de produtos cruzados dos dados centralizados, e $df = n - 1$, que é equivalente à estimativa de matriz de covariância amostral. Ver tabela D.1 para os efeitos controlados e o número amostral por espécie.

A partir da matriz $\mathbf{V}$ estimada, a matriz de correlação $\mathbf{C}$ pode ser calcu-

lada como

$$\mathbf{C} = \mathbf{S}^{-1}\mathbf{V}\mathbf{S}^{-1} \quad (2.7)$$

com $\mathbf{S}$ sendo uma matriz diagonal preenchida com os desvios padrões das variáveis.

Comparação de matrizes

As matrizes de correlação e de covariância foram comparadas entre os diferentes táxons pelo método de *random skewers* ou RS (Cheverud e Marroig, 2007). Esse método consiste na criação de vetores aleatórios de mesmo tamanho que o número de caracteres sob investigação (35, no presente caso) e a multiplicação desses vetores pelas matrizes $\mathbf{V}$ ou $\mathbf{C}$ de duas espécies distintas, produzindo pares de vetores resposta (um para cada matriz comparada). A correlação entre esses vetores resposta é igual ao cosseno entre eles, e a média das correlações entre todos os vetores aleatoriamente gerados é tida como uma medida de similaridade entre essas matrizes. Valores mais próximos de 1 indicam maior similaridade estrutural entre as matrizes, enquanto valores mais próximos de 0 indicam que as matrizes são mais dissimilares.

O método RS pode ser interpretado diretamente dentro do contexto da teoria de genética quantitativa, onde os vetores gerados podem ser vistos como gradientes de seleção (β) aleatórios aplicados nas matrizes de covariância genética $\mathbf{G}$, produzindo respostas evolutivas Δz , ou

$$\Delta z = \mathbf{G}\beta \quad (2.8)$$

(Lande, 1979). Visto que para o crânio de mamíferos a matriz $\mathbf{G}$ é suficientemente similar ao seu análogo fenotípico $\mathbf{P}$ (Porto et al., 2009), a comparação

Figura 2.4: Representação esquemática do método de *Random Skewers* para um único gradiente de seleção (β). No lado esquerdo- elipses correspondentes a matrizes de covariância para dois caracteres. Do lado direito- comparação direta das respostas (Δz s) provenientes de cada matriz. Cor indica a resposta (Δz) produzida por cada matriz. Ver texto para descrição.

das matrizes fenotípicas através desse método pode ser interpretada como um teste da hipótese de que os táxons comparados respondem de forma similar à seleção natural (ver Capítulo 3).

A figura 2.4 exemplifica o RS para um único gradiente de seleção e diferentes matrizes G com dois caracteres. As matrizes **A**, **B** e **C** apresentam os mesmos componentes principais, porém com variância distribuídas de forma distintas: **A** apresenta variação distribuída mais homogeneamente entre seus dois PCs (*i.e.* menor correlação/integração entre caracteres); **B** apresenta o primeiro PC com mais variância que o segundo (*i.e.* maior correlação/integração entre caracteres); **C** é um caso intermediário entre **A** e **B**. **D** é equivalente a **C** porém levemente rotacionada, implicando que seus PCs são diferentes das outras matrizes. Δz s de cores distintas são as respostas evolutivas de cada matriz ao mesmo gradiente de seleção β . Nesse exemplo é fácil de observar que, apesar da seleção ser a mesma em todos os casos, a resposta evolutiva varia bastante em função da geometria de G .

A mudança na magnitude da integração/correlação entre os caracteres afeta diretamente Δz_s , sendo que o ângulo entre Δz_s reflete a diferença na esfericidade da matriz, com **C** sendo intermediária à **A** e **B**. A mudança de padrão, como exemplificado na diferença entre **C** e **D** também modifica consideravelmente a resposta. É interessante notar que a resposta de **A** e **D** nesse exemplo em particular são muito similares, o que poderia nos fazer concluir que ambas matrizes são estruturalmente similares quando elas de fato não são. É por esse motivo que, para quantificar a similaridade das matrizes, é importante medir a correlação de vetores ao longo do máximo possível de dimensões. Isso é feito através da geração de um número grande de vetores aleatórios de forma a obter uma estimativa não enviesada de similaridade.

No próximo capítulo eu foco na comparação de matrizes de covariância empregando o RS. Apesar de outras medidas de similaridade e comparação de matrizes existirem, eu decidi restringir as análises a apenas RS por três principais motivos adicionais. Primeiro porque o RS em matrizes de covariância apresenta uma interpretação trivial dentro do contexto de genética quantitativa, como exposto em Cheverud e Marroig (2007). Segundo porque o RS apresenta uma extensão que permite investigar quais caracteres estão ligados às diferenças entre os grupos (Marroig et al., 2011). Em terceiro, uma comparação entre diferentes estatísticas empregadas em matrizes de covariância e correlação revelou que, mesmo quando diferentes métodos apresentam valores absolutos distintos (figura B.1) e correlações baixas entre si (tabela B.1), o principal padrão observado foi consistente entre as diferentes medidas (figuras B.2-B.5). Esta comparação se encontra no Apêndice B. Assim, a utilização de RS para análises de similaridade de matrizes é consistente com a avaliação de outras medidas de similaridade/dissimilaridade e permite um melhor entendimento dos padrões observados e clareza na exposição dos resultados.

Repetibilidade de matrizes

Visto que covariâncias são estimativas, espera-se que elas não reflitam a estrutura de covariância populacionais, principalmente quando números amostrais são baixos (Cheverud, 1996; Marroig et al., 2012). A similaridade entre matrizes, então, deve levar em conta a capacidade daquele número amostral em recuperar estrutura de covariância populacional intraespecífica. Se uma amostra específica possui correlação abaixo de um (0.8, por exemplo) com outras matrizes obtidas de uma mesma população, então valores próximos à autocorrelação devem ser interpretados como indistintos empiricamente da matriz original, mesmo que tal valor seja diferente de 1. Segundo Cheverud (1996) uma estatística de similaridade r_{obs} entre matrizes pode ser ajustada da seguinte maneira

$$r_{adj} = \frac{r_{obs}}{\sqrt{t_1 t_2}} \quad (2.9)$$

onde t_1 e t_2 são valores de repetibilidade (autocorrelação) de cada uma das duas matrizes comparadas. Logo, ao invés de comparar o valor bruto de similaridade contra um valor de 1.0 (total correlação/similaridade estrutural), a comparação é feita entre o valor observado e a média geométrica das repetibilidades das matrizes. A repetibilidade foi estimada usando um método de Monte Carlo (Marroig e Cheverud, 2001) onde a matriz de covariância é utilizada para gerar múltiplas amostras aleatórias. A partir destas amostras uma nova matriz de covariância ou correlação é obtida e comparada com a matriz original utilizando a estatística sendo avaliada (RS no presente caso). A média de similaridade entre todas as amostras e a matriz original é tida como a repetibilidade da matriz.

A figura 2.5 mostra as repetibilidades para todas as espécies com $n > 5$ e evidencia a presença de um padrão bimodal, com um decréscimo constante

Figura 2.5: Repetibilidades empíricas estimadas para todas as espécies com $n > 5$. Linha escura é um ajuste do tipo *loess*. Polígonos cinza são os intervalos de confiança.

de repetibilidades para amostras entre 100-25 e uma redução mais abrupta para valores abaixo de 25. A maioria das amostras superiores a 25 apresentaram repetibilidades maiores que 0.9, sugerindo que esse é um limite amostral confiável.

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Chapter 3

Morphological Integration in the skull of Carnivora (Mammalia): Interplay between modularity, flexibility, patterns and magnitude

1 Introduction

2 *"Every organized individual forms an entire system of its own, all*
3 *the parts of which naturally correspond, and concur to produce a cer-*
4 *tain definite purpose, by reciprocal reaction, or by combining towards*
5 *the same end. Hence none of these separate parts can change their forms*
6 *without a corresponding change in the other parts of the same animal,*
7 *and consequently each of these parts, taken separately, indicates all the*
8 *other parts to which it has belonged"*

9 *-Georges Cuvier 1827, p. 90*

10 Morphological integration refers to the fact that different phenotypic
11 traits of organism are not fully independent from each other, and tend to
12 covary in different degrees (Olson and Miller, 1958). This pattern has long
13 been noticed by students of anatomy such as Georges Cuvier, the father
14 of paleontology, who recognized not only that parts were contingently as-
15 sociated with each other but that they had to be, in order for organisms
16 to perform their specific functions (Cuvier and Jameson, 1827). Although
17 Cuvier concluded that integration (and its association with functional de-
18 mands) was an impediment to the transmutation of species, Darwin argued
19 that correlations among traits should be understood as "laws of variation"
20 arising from correlations of ontogenetic growth (Darwin, 1859). In his view,
21 integration is a constraint on the generation of variants, but not an abso-
22 lute one, given that natural selection could ultimately overcome it in order
23 to fine tune an species to its environment. Thus, the understanding of how
24 populational variation is generated and how it interacts with natural selec-
25 tion in order to produce adaptations is a key component of evolutionary
26 investigations (Lande, 1979; Lande and Arnold, 1983; Hansen and Martins,
27 1996).

Genetically speaking, covariation among traits is the product of pleiotropy and linkage disequilibrium (Falconer et al., 1996; Lynch et al., 1998; Hansen, 2006). The covariation that arises from these factors influence not only how tightly related traits are in a population, but mainly how they will be inherited jointly (Hallgrímsson et al., 2009). This is especially important in the context of the study of natural selection because, in the presence of genetic covariances, selection on a character can indirectly influence the evolutionary response on another character.

According to the multivariate breeder's equation (Lande, 1979), the change in mean multivariate phenotype from one generation to the other will be

$$\Delta \mathbf{z} = \mathbf{G}\boldsymbol{\beta} \quad (3.1)$$

where $\Delta \mathbf{z} = \{\Delta z_1, \Delta z_2, \dots, \Delta z_n\}$ is a column vector of mean changes on n traits; $\mathbf{G}$ is the $n \times n$ matrix of variance-covariance of additive genetic effects (e.g. elements G_{ii} are the additive genetic variances of trait i and elements G_{ij} are the additive genetic covariances between traits i and j), and $\boldsymbol{\beta} = \{\beta_1, \beta_2, \dots, \beta_n\}$ is the column vector of selection gradients, or the selection on each character that is independent of the selection on others (Lande, 1979; Lande and Arnold, 1983). To exemplify the importance of this concept, consider a system of two traits. For $\boldsymbol{\beta} = \{\beta_1, \beta_2\}$ the evolutionary response for each trait will be

$$\Delta z_1 = G_{11}\beta_1 + G_{12}\beta_2 \quad (3.2a)$$

$$\Delta z_2 = G_{21}\beta_1 + G_{22}\beta_2 \quad (3.2b)$$

Its easy to see that, in the presence of non-zero genetic covariances ($G_{12} = G_{21} \neq 0$), the evolutionary response of a trait will not only be a function of

genetic variance and direct selection, but also indirect selection and genetic covariance (Lande and Arnold, 1983; Agrawal and Stinchcombe, 2009; Marroig et al., 2011). This suggests that the investigation of species divergence (*i.e.* $\Delta\mathbf{z}$) alone can, at best, paint an incomplete picture of the evolutionary processes at work (see Grabowski, 2016; Savell et al., 2016 for some empirical examples).

In comparative analysis, one of the main goals is to investigate past selective regimes in order to explain the observed diversity of forms. In a quantitative genetic framework, this is done by retrospectively estimating β s from inferred $\Delta\mathbf{z}$ s (*e.g.* Ackermann and Cheverud, 2004; Marroig et al., 2004; Marroig and Cheverud, 2005, 2010; Martínez-Abadías et al., 2012; Marroig et al., 2012; Schroeder et al., 2014; Grabowski, 2016; Savell et al., 2016), which can be done by simply rearranging Equation (3.1) as

$$\beta = \mathbf{G}^{-1}\Delta\mathbf{z} \quad (3.3)$$

If $\mathbf{G}$ differs greatly between species, estimated β s can be imprecise, leading to wrong conclusions about the evolutionary history of a group (Turelli, 1988). So, to evaluate how well quantitative genetic theory can be applied in a macroevolutionary context, a preliminary investigation of the similarity of the patterns of morphological integration expressed in $\mathbf{G}$ for different species is a necessary first step.

There are two main ways in which differences in morphological integration are usually investigated: the first is related to the patterns – how characters are interrelated – and the second is related to the magnitude – how strong are the interactions among characters. Differences in pattern and magnitudes of integration can lead to modularity, *i.e.* the fact that characteres from one set (module) tend to have stronger correlations with other elements from the same set than with characteres from other sets (Olson and

75 Miller, 1958; Berg, 1960; Wagner et al., 2007; Klingenberg, 2008). Genetically
76 speaking, modularity pertains to the fact that characters vary in their inter-
77 connections due to pleiotropic effects (Wagner et al., 2007). Changes in the
78 underlying pleiotropic effects can then alter both the magnitude of correla-
79 tions (via increase pleiotropy between traits, Pavlicev et al., 2008), and pat-
80 terns of integration and modularity (via changes in patterns of pleiotropic
81 effects, Melo and Marroig, 2015).

82 The mammalian skull is an ideal model for the study of morphologi-
83 cal integration in a comparative context. It is a complex structure made by
84 the sum of simpler discrete units, or bones, which combine to form a larger
85 integrated whole. The overall number and position of bones in the skull
86 is remarkably conserved within mammals (Schoch, 2006), allowing for a
87 phylogenetically broader comparison of trait measurements (Pearson and
88 Davin, 1924). Ontogenetically speaking, even though each bone can be de-
89 velopmentally individualized, general ontogenetic factors affect more than
90 one structure, producing different degrees of morphological integration and
91 modularity (Cheverud, 1982; Hall, 2005; Hallgrímsson et al., 2007, 2009).
92 Lastly, the skull is an important morpho-functional complex, combining di-
93 verse organs and functions that play important roles in the species ecology
94 and evolution (Cheverud, 1982).

95 Previous large scale investigations of the morphological integration of
96 the skull in mammals have painted a coherent picture. While matrices from
97 different species respond similarly to natural selection (Marroig and Cheverud,
98 2001; Oliveira et al., 2009; Shirai and Marroig, 2010a; Marroig and Cheverud,
99 2010; Hubbe et al., 2016; Haber, 2016), overall magnitudes of overall inte-
100 gration tends to vary widely between even closely related species (Mar-
101 roig and Cheverud, 2001; Oliveira et al., 2009). Furthermore, modularity
102 patterns seem conserved among the major mammalian clades (Goswami,

2006a; Porto et al., 2009; Shirai and Marroig, 2010a; Porto et al., 2013), reinforcing the idea that ontogenetic pathways and genetic architecture have been relatively stable throughout mammalian evolution. However, despite this stability at large scales (Goswami, 2006a; Porto et al., 2009, 2013), analyses within mammalian orders sometimes highlights differences at some species or genus (Oliveira et al., 2009; Shirai and Marroig, 2010b; Hubbe et al., 2016) or even between families (Haber, 2016). Thus, even though we expect that morphological integration will generally be conserved at broader scales, the investigation of less inclusive clades can still lead to significant insight into the evolution of morphological integration.

The objective of the present contribution is to evaluate the stability (or not) of the patterns of morphological integration of the skull in Carnivoran mammals. Carnivores are one of the most species-rich clades of large Mammals, containing almost 300 extant species, being the only order that can be consider to have populated all continents on Earth, including Antarctica. Carnivora displays an impressive diversity of skull shape disparity (Radinsky, 1981a,b, 1982; Drake and Klingenberg, 2010) which is accompanied by an equally impressive diversity of feeding strategies, ecological specializations and morphological variation. For these reasons, the group is considered to be an ideal natural model for the study of adaptation in the macroevolutionary scale (Goswami, 2010), especially regarding skull shape evolution (e.g. Wroe and Milne, 2007; Christiansen, 2008; Figueirido et al., 2009; Jones and Goswami, 2009; Figueirido et al., 2010; Slater et al., 2009, 2010; Tseng and Wang, 2011; Figueirido et al., 2011b, 2013; Meloro et al., 2015; Tseng and Flynn, 2015; Jones et al., 2015).

The morphological integration the Carnivoran skull has been investigated previously in two main works which have shown that that patterns of integration can change as a function of ecological demands (Meiri et al.,

2005; Goswami, 2006b). While Meiri et al. (2005) evaluated cranial measurements directly (*e.g.* length, height), their main findings were related to variation and covariation of teeth morphology (see also Dayan et al., 2002). Goswami (2006b) on the other hand explicitly evaluated morphological integration of the skull using geometric morphometrics, and found that, despite being very similar among groups, patterns of integration correlated with both phylogeny (suggesting a phylogenetically structured variation) and with diet at less inclusive taxa. These last findings were, however, based on reduced species (30 spp) and intraspecific (15-22 specimens) samples, and did not explicitly investigate what are the actual modifications on the patterns of integration and their possible evolutionary consequences.

Here we aim to further our knowledge of the evolution of morphological integration within Carnivora by using one of the most comprehensive datasets in an explicit quantitative genetics context. We combine matrix comparison and pattern recognition techniques in order to identify the presence of any differences in the morphological integration among groups. To describe the identified patterns we integrate modularity and quantitative genetics methods to dissect the differences and similarities among groups.

Materials and Methods

Sample

More than 8300 specimens were evaluated and measured from the following institutions: Museu de Zoologia da Universidade de São Paulo (MZUSP, São Paulo), Museu Nacional (MNRJ, Rio de Janeiro), Museu Paraense Emílio Goeldi (MPEG, Belém), Museo Argentino de Ciencias Naturales Bernardino Rivadavia (MACN, Buenos Aires), Museo de La Plata (FCNyM, La Plata), American Museum of Natural History (AMNH, New York), National Mu-

seum of Natural History of the Smithsonian Institution (USNM, Washington), Museum of Comparative Zoology (MCZ, Harvard), Field Museum (FMNH, Chicago), Academy of Natural Sciences of Drexel University (ANSP, Philadelphia), Museum für Naturkunde (ZMB_Mam, Berlin), Naturhistorisches Museum Wien (NMW, Vienna), Muséum National d'Histoire Naturelle (MNHN, Paris), Zoologisches Forschungsmuseum Alexander Koenig (ZFMK, Bonn), Přírodovědecké muzeum - Národní muzeum (NMP, Prague), University Museum of Zoology, Cambridge (UMZC, Cambridge - UK), Senckenberg Naturmuseum Frankfurt (SMF, Frankfurt am Main) and Naturalis Biodiversity Centre (Naturalis, Leiden). Caniforms were measured by FAM and Feliforms were measured by TMZ. Taxonomy followed recent phylogenetic studies (Arnold et al., 2010; Fulton and Strobeck, 2010; Slater et al., 2012; Agnarsson et al., 2010; Nyakatura and Bininda-Emonds, 2012).

We included only adult individuals in the analysis. Given the high taxonomic heterogeneity of the sample, different criteria to infer specimen adulthood were established. For most species, age was inferred mostly by the eruption of the teeth and the level of closure of the basioccipital synchondrosis. Given the high diversity of suture closure timing in carnivorans (Goswami et al., 2013), some taxa (e.g. Phocids) never show high (if any) levels of suture-closure, while others (e.g. Mustelids and Procyonids) show high degree of bone fusing very early in live. So, in addition to the aforementioned criteria, adulthood was also established comparatively by observation of the largest individuals and characteristics that they might possess. For example, all adult fissipeds show a smoother bone surface than its sub-adults counterparts. For pinnipeds, adulthood was mainly established by size and shape comparisons and evaluation of tooth-wear. Young adult specimens were sampled in some taxa, especially within Feliforms and particularly for species with lower sample sizes. Given that the covariation pattern of the

skull is expected to be similar among ontogenetic stages (Hubbe et al in prep), for these taxa age (adult/young adult) was included as a factor to be controlled for (see below). Also, due to the rarity of some taxa in the visited institutions, we included captive animals in the dataset. Those individuals were carefully evaluated before measurement, and any evident deformation was basis for exclusion. We prioritized animals with sufficient information in order to identify if: 1-they were wild-caught, and 2-how much time they spent in captivity. Those two criteria were used to minimize deformations in the skull that could arise from prolonged captivity (Hartstone-Rose et al., 2014). The effect of captivity was controlled in matrix estimation (see below).

The removal of juveniles, individuals with uncertain taxonomic affinities, outliers and species with insufficient samples produced a total of 6,793 specimens (4,442 Caniformes/2,351 Feliformes) from 144 species (94 Caniformes/50 Feliformes) included in 81 genera (45 Caniformes/ 31 Feliformes), representing 16 carnivoran families (with Prionodontidae being the only absent family). This is one of the largest and most comprehensive datasets for exploring intraspecific morphological integration in the skull of Carnivorans (Table D.1).

Morphometrics and data processing

The morphometric measurements were obtained through digitized anatomical landmarks of the skull (Figure C.1) with the aid of a Microscribe MLX system (Immersion Corporation - San Jose, California). Landmarking followed previous works from the research group (e.g.Cheverud, 1982, 1996b; Ackermann and Cheverud, 2000; Cheverud et al., 1983; Marroig and Cheverud, 2001; Porto et al., 2009; Oliveira et al., 2009; Hubbe et al., 2016, Figure C.1). Skulls were digitized in two views to allow for the sampling of the whole

212 skull. To unify these views, both configurations were mean-centered on the
213 common landmarks and rotated in order to minimize their squared dis-
214 tances (Schönemann, 1966). From the final configurations, we obtained a
215 set of 35 interlandmark distances to describe the skull morphology, follow-
216 ing Porto et al. (2009). The list of measurements can be found on Table 2.2.
217 When measurements for both sides of the skull were available, bilaterally
218 symmetrical measurements were averaged between sides, producing a set
219 of 35 measurements. Each individual was measured twice and repeatability
220 was estimated as the proportion of the total variance that can be explained
221 by replicates on a linear model (Lessells and Boag, 1987). All measurements
222 were averaged between replicates for further analysis.

223 Outliers identification and normality inspection were done using graph-
224 ical methods (Zuur et al., 2010) with both univariate and multivariate tech-
225 niques. For the univariate case, we produced Quantile-Quantile plots (QQ-
226 plots) for individual measurements for each species. QQ-Plots confront em-
227 pirical quantiles and theoretical ones (from a normal distribution in this
228 case) and help evaluate departures from normality and identify possible
229 outliers in the sample. For the multivariate case, the squared Mahalanobis
230 distance between each individual and the sample mean was estimated and
231 plotted against the theoretical quantile from a Chi-squared distribution (Filz-
232 moser et al., 2005). These methods were used along with other diagnostic
233 methods (e.g. histograms and dotplots) to help identify and remove mea-
234 surements or individuals from the dataset. Complementarily we performed
235 multiple Lilliefors test for normality on each variable from each species.
236 These procedures were performed on raw data and on the residuals of the
237 linear model, after controlling for unwanted factors (see below). Together
238 with the graphical results this suggests that there are no notable deviations
239 from normality in this dataset.

Figure 3.1: Landmarks shown on a *Canis lupus* skull.

240 In the present work we chose to focus on the use of the classically mea-
241 surements set of measurements used by our group instead of focusing on
242 geometric morphometrics techniques. There are some known issues with
243 the estimation of covariances of Procrustes-superimposed landmarks (Zelditch
244 et al., 2012; Linde and Houle, 2009; Walker, 2000; Torcida et al., 2013) and
245 of measures of repeatability (von Cramon-Taubadel et al., 2007) that could
246 potentially affect our interpretations (see Garcia et al., 2015 for more de-
247 tails). Furthermore, these measurements are originally chosen in order to
248 reflect specific dimensions of skull bones and structures in order to allow
249 for a localized understanding of ontogenetic, genetic and functional pro-
250 cesses (Cheverud, 1982; Pearson and Davin, 1924). While in classical mor-
251 phometrics it is possible to alternate between a collection of traits and a sin-
252 gle trait without losing equivalency -every column of a multivariate dataset
253 is a meaningful trait on it own- the same cannot be said for geometric mor-
254 phometrics, where each column (a coordinate of a landmarks) of the mul-
255 tivariate dataset only has meaning in relationship to other columns (other
256 coordinates of the same landmarks and other landmarks through Procrustes
257 superposition). Given that some of the techniques used here depends heav-
258 ily on the capacity to bisect the phenotype (Marroig et al., 2011), the use
259 of measurements, and not superimposed landmark configurations, is con-
260 sidered more adequate. Lastly, the quantitative genetics of these measure-
261 ments has been extensively studied and the similarity between genotypic
262 and phenotypic covariation patterns have been well established for multi-
263 ple taxa (Porto et al., 2009; Garcia et al., 2014; Porto et al., 2015; Marroig
264 and Cheverud, 2010). Because of these reasons, this set of measurements
265 and choice of methodology are best suited to the objectives of the present
266 investigation.

The Cheverud Conjecture

Obtaining accurate estimates of $\mathbf{G}$ can be problematic for most non-model organisms, as is the case for most mammalian species. This is because genetic variance and covariance have to be estimated through experimental breeding of large colonies (Steppan et al., 2002; McGuigan, 2006). While this can be achieved for some species in a laboratory setting (*e.g.* Cheverud, 1982; Cheverud et al., 1983; Lofsvold, 1986, 1988; Atchley and Zhu, 1997; Klingenberg and Leamy, 2001; Klingenberg, 2003; Wagner et al., 2008; Hlusko and Mahaney, 2009, and for rare exceptions in the wild (*e.g.* Wilson et al., 2007; Poissant et al., 2008; Martínez-Abadías et al., 2009), for most mammals reasonable direct estimates of $\mathbf{G}$ are highly unlikely. Phenotypic estimates of covariance (or $\mathbf{P}$ matrices) are, on the other hand, far easier to obtain. They can be estimated without information regarding genealogy and are (usually) better estimated, because sample sizes of $\mathbf{G}$ are based on the number of families, rather than individuals (Cheverud, 1988). Additionally, there is a wealth of evidence suggesting that phenotypic estimates of covariance largely reflects the underlying geometry of $\mathbf{G}$, particularly for morphological characters (Cheverud, 1988, 1996b; Roff, 1995; House and Simmons, 2005; Porto et al., 2009; Marroig and Cheverud, 2010; Garcia et al., 2014; Porto et al., 2015), thus allowing for the use of $\mathbf{P}$ as a surrogate of $\mathbf{G}$. This idea, sometimes called "The Cheverud Conjecture" (Cheverud, 1988), has been widely used on macroevolutionary studies (*e.g.* Ackermann and Cheverud, 2004; Marroig et al., 2004; Revell, 2007; Revell et al., 2010; Marroig and Cheverud, 2010; Schroeder et al., 2014; Grabowski, 2016; Savell et al., 2016).

The direct comparison of $\mathbf{P}$ s on a broad phylogenetic context can also be used itself as evidence in support of Cheverud's conjecture. This is because $\mathbf{P} = \mathbf{G} + \mathbf{E}$, where $\mathbf{E}$ is a matrix of environmental (or residual) covariances. If

we observe high similarities between **Ps**, than that either means that the underlying **Gs** are different and **Es** somehow compensate for these differences, or that both **Gs** and **Es** are similar among groups. Cheverud (1988) argued that, based on the prevalence of phenocopies in morphological characteres (enviromental perturbations that mimic mutations or genetic variantion), the last interpretation is more plausible, a fact that would also explains the similarity found for the **Ps** and **Gs** in different datasets. So, if we observe that **P** matrices are similar among species, we can conclude that the underlying **Gs** are stable, and that **P** and **G** for each species are structurally similar (Marroig and Cheverud, 2001). Therefore, the observation of a high similarity among **Ps** can give us not only information about the genetic architecture of individual species or groups, but also the validity of the extension of quantitative genetics theory into a macroevolutionary context.

Covariance Matrix estimation

Carnivorans are known to show intraspecific variation in skull morphology due to a myriad of factors. For example *Cerdocyon thous*, a middle-sized South-American canid, shows considerable variation in size (Martinez et al., 2013) and shape (Machado and Hingst-Zaher, 2009) along its geographic range, a variation that coincides with classically described subspecies (Machado and Hingst-Zaher, 2009), a pattern common in other species (e.g. Pocock, 1935; Jolicoeur, 1959; Timm-Davis et al., 2015; Helgen et al., 2013; Nascimento, 2014; Milenković et al., 2010; Howell et al., 2016; Hernández-Romero et al., 2015). In the study of integration patterns, this kind of variation can be a nuisance because differences of this sort usually affect the mean values of populations, as is usually evidenced by the results of Analyses of Variance (ANOVAs). If large, these differences can distort matrix estimates because the covariance pattern will reflect inter-group instead of

322 intra-group covariation (see Figure 3.2). Because of this, sources of variation
323 that are not of interest, such as geographical/subspecies variation, sexual
324 dimorphism, age and captivity status have to be removed prior to the esti-
325 mation of the phenotypic covariance matrix $\mathbf{P}$.

326 The influence of these factors was evaluated on a case-by-case basis.
327 When information about subspecies or sex were lacking, these factors were
328 first inferred through other means (*e.g.* geographical information, cranial
329 morphology, skins, etc). Secondly, to validate these estimates, we performed
330 Linear Discriminant Analyses (LDAs) using the individuals with full infor-
331 mation as training sets. The discriminant functions obtained were then ap-
332 plied to the individuals with unknown information and the resulting classi-
333 fication was confronted with the inferred one. In cases where both classifica-
334 tions matched, the classification was applied to that specimen. Also, in cases
335 where no previous estimate was possible (*e.g.* lack of geographical infor-
336 mation or clear sexual dimorphism), information was also assigned if LDA
337 posterior probabilities of classification were high (*e.g.* > 0.7 for sexual di-
338 morphism, lower for factors with more classes). All effects were then tested
339 for through univariate and multivariate ANOVAs (MANOVAs) and evalu-
340 ated with principal component analysis (PCA). Those factors that showed
341 large effect sizes were controlled for using a multivariate linear model ap-
342 proach. The covariance matrix of the residuals of the linear model can be
343 interpreted as the pooled within-groups covariance matrix. Table D.1 shows
344 the sources of variation controlled for in each species. Species with no fac-
345 tors to be controlled were processed through a linear model with just the
346 intercept.

Figure 3.2: Example of the impact of differences in group averages in the estimate of the covariance matrix. **A-** 3 groups with little differentiation between their means. **B-** The group averages were multiplied by 2 in relation to A. **C-** The group averages were multiplied by 5 in relation to A. Dashed ellipsis represents the covariance matrix of the full sample without controlling for fixed (group) effects. raw and res refers to the Random Skewers similarity between the sample covariance matrix (empirical) and the populational covariance matrix used in the simulation for matrices estimated on raw and residual data.

Matrix similarity, repeatability and rarefaction analysis

Matrices were compared using the Random Skewers, or RS (Cheverud, 1996b; Cheverud and Marroig, 2007) method. In RS, each matrix under comparison is multiplied by a set of randomly generated vectors ("skewers"), and each pair of response vectors is compared through vector correlation. The mean correlation between all pairs of vectors is taken as a similarity statistic between both matrices. The RS method can be fully interpreted within the theoretical framework of quantitative genetics, in which the skewers can be viewed as random selection gradients (β) that are applied on a population with a genetic covariance matrix $\mathbf{G}$, producing an evolutionary responses $\Delta\mathbf{z}$, as for Equation (3.1). Given that we are using $\mathbf{P}$ as a proxy for $\mathbf{G}$, the use of RS can be interpreted as a measure of the capacity of different species to respond similarly to natural selection.

Since covariances are sample estimates, their precision can be negatively influenced by low sample sizes, therefore misrepresenting the true matrix similarity among groups. To circumvent that problem we corrected observed similarities as $r_{adj} = r_{obs} / (\sqrt{t_1 t_2})$, where r_{adj} is the adjusted similarity, r_{obs} is the observed similarity and $\sqrt{t_1 t_2}$ is the harmonic mean between the repeatabilities t_1 and t_2 of the matrices being compared, or the maximum correlation expected for that comparison (Cheverud, 1996b). The r_{adj} obtained this way represents a conservative value of similarity, taking into account the prior expectation that the matrices are equivalent among Mammalian taxa (Marroig and Cheverud, 2001; Oliveira et al., 2009; Porto et al., 2009; Hubbe et al., 2016).

To calculate matrix repeatabilities previous works have utilized two different approaches: Monte Carlo (Marroig and Cheverud, 2001; Porto et al., 2009; Oliveira et al., 2009) and Bootstrap (Goswami et al., 2015; Hubbe et al., 2016). These methods are used to generate multiple replicated datasets in

order to calculate new covariance matrices. On the Monte Carlo approach, the estimated covariance matrix is used to generate multiple random multivariate data sets, while in the Bootstrap approach the original data is resampled with replacement multiple times. In both cases, the resulting dataset is used to calculate a new covariance matrix to be compared to the original one (through RS in the present case). The average similarity is taken as the repeatability measure of that matrix. To evaluate the impact of different (re)sampling methods, we performed a rarefaction analysis of repeatability using both methods on the *Canis lupus* sample, with sample sizes ranging from 5 to 105. For each sample size 100 subsamples were drawn from the original data (without replacement) and for each sample 1000 Bootstrap and Monte Carlo replicates were produced, totaling 10100000 operations.

In order to evaluate the appropriate sample size for matrix comparison we used two methods in addition to the rarefaction analysis of repeatabilities. First, we obtained repeatabilities for all species with more than 5 specimens, in order to investigate if there is any clear drop in matrices with small sample sizes. Secondly, we performed a similar rarefaction analysis on the *Canis lupus* sample, comparing subsample estimates with the full sample matrix, in order to evaluate the precision of estimates from smaller sample sizes. This rarefaction was performed with sample sizes ranging from 5 to 105 and with 100 subsamples for each sample size (10100 operations). For each subsample the matrix was estimated and compared to the full sample covariance matrix through RS. Following Goswami (2006b), we confronted rarefied matrix similarity with empirical similarities among species in order to evaluate what is an adequate sample size. For the species with sufficient samples, we calculated the pairwise raw and adjusted similarity (RS) values.

Cluster Analysis

To inspect the presence of any clustering in the patterns of morphological integration as revealed by the RS analysis, we used a data-mining technique called k-medoids (Kaufman and Rousseeuw, 1987). This technique is analogous to the k-means method, but differs by being applicable directly on a distance or dissimilarity matrix without loss of information. The k-medoids algorithm consists in the selection of k elements of the sample, the so called "medoids", and their associated clusters in order to minimize the criteria L defined as

$$L = \sum_{i=1}^k \sum_{j \in C_i} d_{ij} \quad (3.4)$$

where j are the elements of the i^{th} cluster and d_{ij} is the distance or dissimilarity between element j and the medoid of the cluster i. For a fixed value of k, the exact solution was given by Gordon and Vichi (1998) and is implemented on package *clue* of R Core Team (2016). To implement this procedure on matrix similarity, we transformed the RS matrix into a distance matrix by simply obtaining $1 - RS$. The application of the k-medoids algorithm in this case could be viewed as a way of finding clusters of species with matrices that are more similar among themselves than to other groups of species.

In order to find the best number of k partitions, the above procedure was repeated for $k = 1, \dots, n$. Given that the increase of k leads necessarily to a decrease in L (e.g. if $k = n$ then $L = 0$), this criteria cannot be used to compare among different values for k. Instead, we utilized the Calinski-Harabasz (CH) criterion (Caliński and Harabasz, 1974) which is defined as

$$CH_k = \frac{SS_B}{SS_W} * \frac{(n - k)}{(k - 1)} \quad (3.5)$$

in which SS_B and SS_W are the between and within-cluster sum of dissimilar-

ities respectively. According to this criterion, as k approaches n , the second hand of the equation approaches 0, consequently penalizing more complex arrangements. The CH criterion is convenient because it has a direct relation with F – ratios used on Analyzes of Variance (ANOVAs). For dissimilarity matrices, the SS_B e SS_W portions of the equation can be calculated as the sum of distances between groups and the sum of distances within groups respectively (McArdle and Anderson, 2001; Anderson, 2001). Given that the CH criterion is not defined for one group ($SS_B = 0$) we employed a non-parametric multivariate analysis of variance (NP-MANOVA, Anderson, 2001) on the cluster obtained for $k = 2$ in order to evaluate the validity of that grouping. The significance of the NP-MANOVA was obtained by 10000 permutations of the distance matrix (Anderson, 2001). All analyses were done on the uncorrected and corrected values of RS. Additionally we performed the analysis using taxonomic groupings that corresponded to the clusters found adding the coefficient of morphological integration r^2 as a covariate to investigate if the observed patterns could be due only to variation in morphological integration. This was done using a type II NP-MANOVA implemented in the package RVAideMemoire (Hervé, 2016).

Modularity, Evolutionary flexibility, magnitude of integration and percentage of variation

To investigate the nature of differences between covariance structures we obtained modularity indexes, evolutionary flexibility, magnitude of integration and percentage of variation explained for each species (Porto et al., 2009; Marroig et al., 2009).

Firstly, we tested modularity hypotheses based on functional matrix theory (Moss ML, 1960) adapted primarily for primates (Cheverud, 1982) and further verified on all major mammalian clades, including Carnivora (Porto

et al., 2009, 2013). This was done by first building "hypothetical matrices":
i.e. trait*trait matrices that contains 1s for all entries within modules and 0s
for all other entries (Cheverud, 1995, 1996b). Each covariance matrix was
then transformed into their correlation counterpart and the correlation be-
tween those matrices and the hypothetical ones were calculated by taking
the Pearson product-moment correlation between the lower triangle of both
matrices without the diagonal. Since the entries on a correlation matrix are
not independent data points, the significance of those correlations were as-
sessed through 1000 permutations (Cheverud, 1982).

Following Porto et al. (2009) we tested the Oral, Nasal, Zygomatic, Vault
and Base cranial module hypotheses as well as the two main cranial regions-
Face and Neurocranium- containing characters of somatic/late and neu-
ral/early growth, respectively. Additionally, we also tested composite hy-
potheses that include more than one module/region. This is done by sum-
ming different hypothetical matrices and setting all values larger than 1 to
1. Specifically we used two composite hypotheses: "Total" that includes all
modules and "Neuroface" that evaluates both cranial regions jointly.

As a measure of modularity we calculated the AVGratio for all modular-
ity hypotheses. The AVGratio is calculated as the ratio between the average
correlation of traits within a given hypothetical module (AVG+; 1s in the
hypothetical matrix) and the average correlation among other traits and be-
tween modules (AVG-; 0s in the hypothetical matrix, Cheverud, 1995, 1996a;
Marroig et al., 2004). If the ratio is higher than 1, this means that the char-
acters inside a module are more integrated than those outside or between
modules. If the value is lower than 1, then the within-module correlations
are lower than those outside the module.

Flexibility expresses the capacity of a species to respond evolutionarily
in the same direction of natural selection and is measured as the vector cor-

480 relation between the selection gradient β and the evolutionary response $\Delta\mathbf{z}$.
 481 Since this measure depends on the direction of selection we need to mea-
 482 sures along all directions of the phenotypic space to obtain a single value for
 483 each species. To do that, we utilize a method that is analog to RS, in which
 484 a number of random normalized selection gradients are generated and ap-
 485 plied to each matrix using the multivariate breeders equation (Lande, 1979).
 486 Flexibility is calculated for each pair of β and $\Delta\mathbf{z}$ and the average flexibility
 487 $\bar{f}$ is used as an overall measure of evolutionary flexibility (Marroig et al.,
 488 2009). This method is usually employed on the full phenotypic space, but it
 489 can be easily adapted to explore the flexibility of a subset of characters or
 490 modules representing different directions (Garcia et al., 2014). The value of
 491 flexibility for that subset can be calculated as $\bar{f}$ based on randomly gener-
 492 ated normalized vectors that have positive values for the entries associated
 493 with that subset and 0 for everything else. This was done for each individ-
 494 ual modular hypothesis and for all individual traits. All $\bar{f}$ were calculated
 495 on 1000 random vectors with the exception of the ones for individual traits
 496 because only one normalized vector is possible in those cases.

497 To measure the overall morphological integration of the skull we calcu-
 498 lated the average coefficient of determination of each matrix $\bar{r}^2$. This coeffi-
 499 cient is simply the average of the squared correlation coefficients among all
 500 traits. Since $\bar{r}^2$ measures global integration of the skull, we also calculated
 501 the average r^2 for each character as the average square correlation between
 502 one character and all others (each line/column of a correlation matrix). We
 503 could also calculate average r^2 values for modular hypothesis, but that in-
 504 formation is already contained in the AVGratio, specifically AVG+.

505 Finally, we also calculated the proportion of variance contained in each
 506 character and module in order to inspect shifts in the distribution of vari-
 507 ation of the skull among different groups. We also calculated the relative

amount of variance associated with size as it is correlated with modularity, flexibility and integration measures (Porto et al., 2009; Marroig et al., 2009). Here size is considered to be the main factor influencing all characters (Wright, 1968) and its variance can be calculated as the eigenvalue associated with the first eigenvector of the covariance matrix of linear measurement data (Bookstein, 1989).

To investigate differences in the pattern of morphological integration between the clusters found, we tested the differences between groups using t-tests on modularity indexes (AVGratio, AVG+, AVG-), flexibility ($\bar{f}$ for all skull, for modules/regions and characters), morphological integration indexes ($\bar{r}^2$ for all skull and for characters) and relative variances (for each character, cranial modules/regions and size). Given that measures of modularity, relative variation and flexibility are thought to be influenced by (or correlated to) $\bar{r}^2$ (Porto et al., 2009; Marroig et al., 2009), we also performed Analyses of Covariances (ANCOVAs) on these measures using $\sqrt{\bar{r}^2}$ as a covariate. The square root was obtained in order to linearize the data and to put the variable on the same scale as AVG+ and AVG-. Multiple tests were corrected for using Bonferroni.

Selection Response Decomposition

In order to diagnose which characters are responsible for the observed disparity in morphological integration patterns we employed the Selection Response Decomposition (Marroig et al., 2011). This method is based on the same principle as RS in which selection gradients are applied to two matrices and their responses are compared. On SRD, instead of comparing $\Delta \mathbf{z}$ as a whole, the evolutionary response is decomposed into vectors which contains the elements of the matrix multiplication $\mathbf{G}\beta$. For a given character i

the vector of evolutionary response is arranged as follows

$$[G_{i1}\beta_1, G_{i2}\beta_2, \dots, G_{in}\beta_n] \quad (3.6)$$

The sum of the elements of this vectors is equal to the evolutionary response of that individual character (Lande and Arnold, 1983; Marroig et al., 2011; Savell et al., 2016). These character vectors are then compared between species through vector correlation, producing a distribution of SRD similarity values between two species for each character. For a large number of group comparisons, one can construct a pairwise matrix of similarity between species analogous to RS for each character. This method is implemented in the *evolqg* R package (Melo et al., 2015).

In order to measure the influence of each character on the variation of morphological integration, each SRD matrix was correlated with the RS matrix through matrix correlation, using 1000 permutations to assess the significance. Each matrix was also subjected to the k-medoids method in order to inspect if the same clusters seen in RS can be found for each individual character SRD matrix. Finally, we performed NP-MANOVAs on each SRD matrix using the groupings suggested by the analysis of the RS in order to diagnose which characters are more associated with the differences found.

Results

Repeatability, rarefaction and minimum sample size

The rarefaction analysis of repeatability estimates on the *Canis lupus* sample indicates that both Monte Carlo and Bootstrap methods give on average the same result when sample sizes are greater than 8 (Figure 3.3A), Pearson's $r=0.998$). When sample sizes are small ($n=5$), the Monte Carlo approach

Figure 3.3: Rarefaction analysis of repeatabilities estimated through Monte Carlo and Bootstrap approaches on the *Canis lupus* sample. The black line has slope=1 and intercept=0 representing when both repeatability estimates would be equal. Numbers represent sample sizes.

shows a bias towards higher values. Additionally, the standard deviation of the estimates is higher for the Bootstrap method when sample sizes are smaller than 18 (Figure 3.3B). Given that both methods provided the same result for the sample sizes chosen here (see below) we focus only on the repeatabilities estimated through Monte Carlo. Figure 2.5 shows the empirical Monte Carlo repeatabilities for all species with $n > 5$. The curve shows a bimodal pattern, with a monotonic decrease in repeatabilities from sample size 100 to 25, and an abrupt reduction of repeatabilities from 25 to 5. The majority of values for $n > 25$ are above 0.9 and no value is lower than 0.7, even for $n = 5$.

The rarefaction analysis of the similarity between subsamples and the full sample of the *Canis lupus* matrix shows that sample sizes > 35 have a similarity to the original matrix > 0.9 (Figure 3.4A), and higher on average than all empirical similarities (Figure 3.4B). Values between 35 and 25 have $RS = 0.8 - 0.9$ (Figure 3.4A) and are on average superior to 95% of the empirical similarities between families values and 90% of the between genera values.

Figure 3.4: Analysis of rarefaction on the *Canis lupus* sample. **A.** Matrix similarity (RS) between subsamples of the matrix and the full sample matrix compared to empirical similarities. **B.** Average percentile of the rarefied value on the empirical distributions. BF- Between Families. F- Within families and between genera. G- Within genera. All- Full sample. Dots represents distribution of sample sizes >24 showing that the majority of the sample is larger than 35.

573 Given these results we chose to include all species with $n > 25$. By doing this,
 574 we minimize the effects of low repeatabilities for samples $n < 25$ and allow
 575 for broad scale comparisons while including many rare taxa (Figure 3.4B,
 576 Table D.1).

577 Matrix similarity and Cluster Analysis

578 Figure C.2 shows the 10296 pairwise Random Skewers comparisons for all
 579 144 species. All observed and corrected values were highly correlated (Pear-
 580 son's $r = 0.993$). Henceforth, all corrected values will be displayed between
 581 parenthesis, unless otherwise stated. Values ranged from 0.442 – 0.902 (0.470 –
 582 0.956) with an average of 0.705 ± 0.070 (0.749 ± 0.072). The highest val-
 583 ues were observed for within-genera comparisons, ranging from 0.66 – 0.90
 584 (0.72 – 0.95), with an average value of 0.80 ± 0.05 (0.85 ± 0.05). Values for
 585 comparisons within families but between different genera (henceforth "be-

586 tween genera") had an intermediate value, ranging from 0.61 – 0.90 (0.66 –
 587 0.94) with average values of 0.77 ± 0.06 (0.82 ± 0.06). The lowest values were
 588 observed for comparisons between families, that ranged from 0.44 – 0.88
 589 (0.47 – 0.88) with an average of 0.70 ± 0.07 (0.73 ± 0.07). Only three com-
 590 parisons had a corrected value < 0.5 and they all involved the comparison
 591 between a Piniped (*Odobenus rosmarus* and *Mirounga leonina*) and a Fissiped
 592 (*Mephitis macroura* and *Hemigalus derbyanus*).

593 There seems to be a clear demarcation between Canidae species and
 594 the rest of Carnivora (Figure C.2). RS values within Canidae ranged from
 595 0.699 – 0.903 (0.742 – 0.956), with an average of 0.809 ± 0.041 (0.856 ± 0.041),
 596 while the values without Canidae ranged from 0.444 – 0.901 (0.471 – 0.945),
 597 with an average of 0.711 ± 0.067 (0.756 ± 0.069). Values between Canidae and
 598 non-canid Carnivores ranged from 0.481 – 0.844 (0.506 – 0.887) with an av-
 599 erage of 0.679 ± 0.066 (0.726 ± 0.069). This suggests that canid species are
 600 more similar among each other and slightly more dissimilar to the rest of rest
 601 of the Carnivora.

602 According to the CH-index criteria for the k-medoids clustering method,
 603 the optimal partition of the data is $k=2$ for both observed and corrected val-
 604 ues (Figure 3.6A). The NP-MANOVA analysis on the two resulting clusters
 605 supports the separation for both observed and corrected values (Table 3.1).
 606 For the observed RS values, the clusters largely differentiates Canidae (with
 607 5 exceptions: *Speothos venaticus*, *Lycaon pictus*, *Canis adustus*, *Nyctereutes pro-*
 608 *cyonoides* and *Lycalopex culpaeus*) from non-canid Carnivora (Figure 3.6B).
 609 For the corrected values, the results are not so clear cut, with all species of
 610 Canidae forming one cluster, along with some species from other families.

611 The result of the NP-MANOVA using the distinction between Canidae
 612 and non-canid Carnivora as a factor ("Canidae" factor) shows that the dif-
 613 ference between groups is also significant, with Canidae explaining slightly

Figure 3.5: Heat map representing pairwise Random Skewers comparisons. Values below and above the diagonal are the observed and corrected similarities, respectively. Values at the diagonal are repeatabilities. 1-28-Canidae; 29-33- Ursidae; 34- Odobenidae; 35-40- Otariidae; 41-49-Phocidae; 50-58-Mephitidae; 59-Ailuridae; 60-68-Procyonidae; 70-95-Mustelidae; 96-Nandiniidae; 97-118-Felidae; 119-129- Viverridae; 130-133- Hyaenidae, 134-143-Herpestidae, 144-145- Eupleridae

Table 3.1: Non-Parametric Manova analysis of k-medoid Clusters and Taxonomy classification. *Sum Sq*- Sum of Squares.*Mean Sq*- Mean Squares.*Df*- Degrees of Freedom.*R*²- Coefficient of determination (values between parenthesis is the coefficient of determination adjusted for multiple factors *R*_{adj}).*F*- Pseudo-F statistic. *p*- probability given the null-hypothesis.

Observed	<i>Sum Sq</i>	<i>Mean Sq</i>	<i>Df</i>	<i>R</i> ²	<i>F</i>	<i>p</i>
Cluster	0.254	0.254	1	0.185(0.179)	32.231	0.001
Residuals	1.119	0.008	142	0.815		
Total	1.373		143			
Canidae	0.288	0.288	1	0.209(0.203)	37.633	0.001
Residuals	1.085	0.008	142	0.791		
Total	1.373		143			
Family	0.653	0.047	14	0.476(0.414)	8.361	0.001
Residuals	0.720	0.006	129	0.524		
Total	1.373		143			
Corrected	<i>Sum Sq</i>	<i>Mean Sq</i>	<i>Df</i>	<i>R</i> ²	<i>F</i>	<i>Pr(>F)</i>
Cluster	0.298	0.298	1	0.217(0.189)	34.289	0.001
Residuals	1.235	0.009	142	0.899		
Total	1.533		143			
Canidae	0.318	0.318	1	0.231(0.226)	37.095	0.001
Residuals	1.215	0.009	142	0.885		
Total	1.533		143			
Family	0.809	0.058	14	0.589(0.541)	10.295	0.001
Residuals	0.724	0.006	129	0.527		
Total	1.533		143

Figure 3.6: K-medoids clustering analysis on similarity matrix through Ramdon Skewers (RS). **A.** Calinski-Harabasz index by number of cluster for observed and corrected RS values. **B.** Species cluster assigment for the optimal partition of the similarity matrix ($k=2$) for the K-medoids clustering analysis. Each dot represent one species. Black and gray dots are cluster assigned for observed and corrected similarities respectively. Rectangular frames highlight the groupings found for the observed RS similarity.

more variation than the detected cluster (Table 3.1). Additionally, Canidae alone explains almost half of the variation that is explained by using all families as factors (Canidae: $R_{adj} \approx 0.20$); All Families: ($R_{adj} \approx 0.40$, Table 3.1). When we test the differences of Canidae using r^2 as a covariate we find that the difference between groups is still significant even if we account for the variation in the overall magnitude of morphological integration (Table 3.2). Also, the addition of r^2 to the NP-MANOVA barely changed the amount of variation explained by Canidae/non-canid Carnivora (observed- $R^2 = 0.210$; corrected- $R^2 = 0.220$), while the variation of morphological integration explained a significant (albeit smaller) amount of variation in RS (observed- $R^2 = 0.140$; corrected- $R^2 = 0.113$). Together these results suggests that Canidae have the most distinct pattern of morphological integration within Order Carnivora. The next tests were conducted in order to explicitly diagnose the differences between this family and the others.

Modularity, Evolutionary flexibility, magnitude of integration and percentage of variation

The results for the modularity tests can be found in Table 3.3. Modularity was statistically significant ($p < 0.05$) mainly for the Nasal and Oral hypotheses, with rejection of the null hypothesis in 67.4 and 77.1% of the cases, respectively. The Zygomatic module was largely undetected, with the exception of a few canids, otariids, procyonids and hyenids, being detected only in 4.2% of the cases. Both the Base and Vault modules were not detected in any case. The Face was detected in almost all instances (91.7%), while the Neurocranium was detected in none. For composite hypotheses, NeuroFace was detected in almost all cases (97.9%) while the Full hypothesis was detected on a few (34.7%). When we look at the family level, the results seems to be consistent with the overall pattern, with the Oral mod-

Figure 3.7: Overall morphological integration (r^2) of different families of Carnivora. Each dot represents one species.

ule being detected in the majority of cases, followed by the Nasal and then the Zygomatic modules (when detected). The only clear exception seems to be Canidae, that had a perfect rate of detection for the Nasal module (100%), followed by the Oral module (88.9%). A χ^2 -squared test for similarity of proportions showed, however, that after correction for multiple tests no family deviated from what was expected for the full sample ($p > 0.1$ for all tests), including the Nasal and Oral modules for Canidae ($p = 0.103$ and 1.000 respectively).

Overall morphological integration (r^2) varied widely among families (Figure 3.7). Both the t-tests and the ANCOVAs with $\sqrt{r^2}$ as a covariate indicate that Canidae have a Nasal module that has a higher AVGratio, evolutionary flexibility and proportion of variation than non-canid carnivorans (Table 3.4, Figures 3.8 to 3.10). Both tests also indicate that Canids have a more variable Oral module, a less flexible and variable Base, as well as less variable Vault and Zygomatic modules (Figures 3.9 and 3.10). Additionally, the AN-

Table 3.2: Type II Non-Parametric Manova analysis on RS for the distinction between Canidae and non-canid Carnivora with r^2 as a covariate. *Sum Sq*- Sum of Squares. *Mean Sq*- Mean Squares. *Df*- Degrees of Freedom. R^2 - Coefficient of determination. *F*- Pseudo-F statistic. *p*- probability given the null-hypothesis.

Observed	<i>Sum Sq</i>	<i>Mean Sq</i>	<i>Df</i>	R^2	<i>F</i>	<i>p</i>
r^2	0.193	0.193	1	0.140	30.244	0.001
Canidae	0.289	0.289	1	0.210	45.362	0.001
r^2 :Canidae	0.001	0.001	1	0.001	0.168	0.992
Residuals	0.892	0.006	140	0.649		
Total	1.373		143	1.000		
Corrected						
r^2	0.155	0.155	1	0.113	20.501	0.001
Canidae	0.303	0.303	1	0.220	40.061	0.001
r^2 :Canidae	0.003	0.003	1	0.002	0.403	0.821
Residuals	1.058	0.008	140	0.770		
Total	1.533		143	1.117		

Table 3.3: Modularity tests performed on functional matrix hypotheses. Values are percentages of times that a given hypothesis was rejected through matrix permutation tests. Module Hypotheses- Oral, Nasal, Zygomatic, Vault, Base. Region Hypotheses- Face, Neurocranium. Composite hypotheses- NeuroFace, Full

family	n	Oral	Nasal	Zygomatic	Vault	Base	Face	Neurocranium	NeuroFace	Full
Canidae	27	0.889	1.000	0.037	0.000	0.000	0.963	0.000	1.000	0.407
Ursidae	5	0.600	0.600	0.000	0.000	0.000	1.000	0.000	1.000	0.000
Odobenidae	1	1.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000
Otariidae	6	0.167	0.500	0.333	0.000	0.000	1.000	0.000	0.833	0.000
Phocidae	9	0.889	0.556	0.000	0.000	0.000	1.000	0.000	1.000	0.111
Mephitidae	9	0.556	0.222	0.000	0.000	0.000	0.667	0.000	1.000	0.111
Ailuridae	1	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	1.000
Procyonidae	9	0.667	0.778	0.222	0.000	0.000	1.000	0.000	1.000	0.667
Mustelidae	27	0.815	0.556	0.000	0.000	0.000	0.889	0.000	0.926	0.333
Nandiniidae	1	1.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000
Felidae	22	0.818	0.682	0.000	0.000	0.000	0.955	0.000	1.000	0.182
Viverridae	11	0.818	0.636	0.000	0.000	0.000	0.818	0.000	1.000	0.636
Hyaenidae	4	0.500	0.500	0.250	0.000	0.000	0.750	0.000	1.000	1.000
Herpestidae	10	0.900	0.700	0.000	0.000	0.000	0.900	0.000	1.000	0.600
Eupleridae	2	1.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000
Total	144	0.771	0.674	0.042	0.000	0.000	0.917	0.000	0.979	0.347

Table 3.4: T-tests and ANCOVA analyses (with $\sqrt{r^2}$ as a covariate) for the differences between Canidae and non-canid Carnivora for modularity-based statistics. *AVGrat*- Ratio between average correlarions within module (AVG+) to average correlation outside module (AVG-). *Flex.*- Flexibility. *Var(%)*- percentage of variance. Positive and negative values indicate increased or decreased values for Canidae respectively. The Full* entry refers to different concepts for AVGrat (a composite hypothesis) and Flex. (flexibility for the entire structure). *-values significant at $\alpha < 0.05$ withouth correction for multiple tests. **-values significant at $\alpha < 0.05$ with correction for multiple tests.

<i>T-Test</i>	<i>AVGrat.</i>	<i>Flex.</i>	<i>Var(%)</i>
Oral	0.001	0.030**	0.065**
Nasal	0.328**	0.054**	0.061**
Zygomatic	-0.013	-0.014	-0.027**
Vault	-0.035	0.000	-0.046**
Base	0.062	-0.040**	-0.018**
Face	0.108	0.022**	0.064**
Neurocranium	-0.048*	-0.032**	-0.064**
NeuroFace	0.034		
Full*	0.026	0.002	
Size			-0.002
<i>ANCOVA</i>			
Oral	-0.066	0.020*	0.072**
Nasal	0.259**	0.043**	0.067**
Zygomatic	-0.017	-0.028**	-0.025**
Vault	-0.039	-0.021*	-0.060**
Base	0.071	-0.054**	-0.016**
Face	0.050	0.019*	0.076**
Neurocranium	-0.044	-0.047**	-0.076**
NeuroFace	0.000		
Full*	-0.004	-0.019**	
Size			0.044**

COVA detected a decrease in flexibility of the Zygomatic module as well as a decrease in the overall flexibility and increase in size variance (Figures 3.9 and 3.10). For the cranial regions both tests detects an increase in flexibility and variance in the Face and a decrease of both variables in the Neurocranium (Figures 3.9 and 3.10).

Selection Response Decomposition and Individual Characters

The k-means analyses for SRD matrices consistently returned $k=2$ as the preferred partition scheme according to the CH index. Only a few characters showed some degree of taxonomic arrangement, mostly those concentrated on the Nasal and Oral modules. These arrangements however were not as clear cut as the ones observed for RS (Figure 3.11). Given that we want to investigate the distinction between Canidae and non-canid Carnivora, we also used this taxonomic distinction as a factor in NP-MANOVAS for each SRD matrix. The results show that 17 out of 18 characters in the Face showed significant difference between groups, as well as 12 out of 17 characters of the Neurocranium (Table 3.5 and Figure 3.12A). From those characters, the ones that have the largest coefficients of determination ($R^2 = 0.161 - 0.245$) are found in the Nasal and Oral modules, while the lowest values are found in the Vault (with the exception of LD-AS; $R^2 = 0.167$).

With the exception of two characters (BA-OPI and JP-AS) all SRD matrices showed a positive significant correlation with the RS matrix according to the the matrix permutation tests Table 3.5. All SRDxRS correlations using corrected values for RS were on average 0.03 lower than the ones for observed RS values. The degree of correlation varied greatly between cranial modules and regions. The Face showed the highest correlations (Figure 3.12B), with 14 (out of 18) correlations being higher than 0.5, while the

Figure 3.8: Relationship between $AVG+$ and $AVG-$ for different modularity hypotheses. Black- Canidae. Gray- Non-canid Carnivora. Dashed line- $AVG+ = AVG-$.

Figure 3.9: Relationship between the morphological integration index $\sqrt{r^2}$ and flexibility measured for each module. Black- Canidae. Gray- Non-canid Carnivora.

Figure 3.10: Relationship between the morphological integration index $\sqrt{r^2}$ and percentage of total variation explained by each module, region and size. Black- Canidae. Gray- Non-canid Carnivora.

Figure 3.11: K-medoids clustering analysis on similarity matrix through the SRD matrices for $k=2$. Different colors represents different group identity. Character are sorted in order of lower (up) to higher (down) SRD-RS correlation.

Neurocranium showed only 6 correlations higher than 0.5 (out of 17). The highest values (0.69 and 0.77 for observed values) were found for the characters shared by both the Nasal and Oral modules, while the lowest significant values were distributed among the Base and Vault modules (Table 3.5).

Both t-tests and ANCOVAs on individual character flexibilities agrees that there is a significant increase of flexibility in characters from the Nasal, Oral and Vault modules, as well as a decrease of flexibility in characters from the Zygomatic, Vault and Base modules for canid species (Tables 3.6 and 3.7 and fig. 3.12C). Overall, the ANCOVA detects more decreases in flexibility on the Zygomatic, Vault and Base. For the character-specific $\bar{r}^2$, t-tests detect only one increase in integration on the Nasal region and decreases throughout the skull modules (Table 3.6). The ANCOVAs of character-specific $\bar{r}^2$ on the other hand showed that integration increased throughout the skull, mainly in the Nasal and Oral modules (Table 3.7 and fig. 3.12D). Finally, both tests agrees that there is an increase in relative variance associated with the Facial region, while the Neurocranium shows a decrease in relative variance (Tables 3.6 and 3.7 and fig. 3.12E).

Discussion

Similarity of G among Carnivora

Here we conducted the most comprehensive analysis of the patterns of morphological integration of the skull in Carnivora so far. We included 144 species of almost all families currently recognized, producing the largest between species comparison of morphological integration in Mammals. The analysis of matrix similarity through Random Skewers (RS) showed that, despite not being identical, phenotypic matrices of covariance **P** showed high values of similarity (Figure C.2). These values were as high as the ones

Figure 3.12: Graphical representation of the results from the character-based analysis represented on the skull of *Canis lupus*. **A-** Coefficient of determination R^2 of NP-MANOVAS between Canidae and the rest of Carnivora; **B-** Matrix correlation between SRD matrices and the RS matrix; **C-** Significant differences in character-specific flexibility; **D-** Significant differences in character-specific integration; **E-** Significant differences in character-specific relative variance. **B-D** refers to the significant differences found on the ANCOVA analysis using $\sqrt{r^2}$ as a covariate (see for full results Table 3.6).

Table 3.5: Summary of RSxSRD matrix correlations and coefficient of determination (R^2) for the NP-MANOVA between taxonomic groups (Canidae/Non-canid Carnivora). * $p < 0.05$. ** - $p < 0.01$.

Region	Module	Character	RSxSRD	R^2
Face	Nasal	IS-NSL	0.604-0.570**	0.143**
Face	Nasal	NSL-NA	0.430-0.401	0.011
Face	Nasal	NSL-ZS	0.618-0.591*	0.161**
Face	Nasal	NA-PNS	0.590-0.553*	0.202**
Face	Oral/Nasal	IS-PNS	0.774-0.753**	0.245**
Face	Oral/Nasal	NSL-ZI	0.696-0.666**	0.180**
Face	Oral	IS-PM	0.463-0.429	0.077**
Face	Oral	PM-ZS	0.697-0.672**	0.177**
Face	Oral	PM-ZI	0.633-0.596*	0.107**
Face	Oral	PM-MT	0.713-0.678**	0.162**
Face	Oral	ZI-MT	0.300-0.273	0.025*
Face	Oral	MT-PNS	0.504-0.472	0.143**
Face	Oral/Zygomatic	ZS-ZI	0.350-0.320	0.051**
Face	Zygomatic	PT-ZYGO	0.606-0.573*	0.060**
Face	Zygomatic	ZI-ZYGO	0.522-0.489	0.105**
Face	Zygomatic	ZI-TSP	0.599-0.556	0.096**
Face	Zygomatic	EAM-ZYGO	0.338-0.298	0.057**
Face	Zygomatic	ZYGO-TSP	0.637-0.603**	0.090**
Neurocranium	Vault/Zygomatic	PT-TSP	0.244-0.226	0.030*
Neurocranium	Vault	NA-BR	0.518-0.502	0.028*
Neurocranium	Vault	BR-PT	0.269-0.238	0.023*
Neurocranium	Vault	BR-APET	0.471-0.424	0.042**
Neurocranium	Vault	PT-APET	0.354-0.334	0.012
Neurocranium	Vault	PT-BA	0.546-0.518*	0.063**
Neurocranium	Vault	PT-EAM	0.495-0.471	0.018
Neurocranium	Vault	LD-AS	0.346-0.333	0.167**
Neurocranium	Vault	BR-LD	0.278-0.291	0.057**
Neurocranium	Vault	OPI-LD	0.266-0.238	0.022
Neurocranium	Vault	PT-AS	0.439-0.415	0.025*
Neurocranium	Vault	JP-AS	0.137-0.123	0.025*
Neurocranium	Base	PNS-APET	0.470-0.438	0.086**
Neurocranium	Base	APET-BA	0.494-0.458*	0.136**
Neurocranium	Base	APET-TS	0.257-0.247	0.001
Neurocranium	Base	BA-EAM	0.607-0.577*	0.157**
Neurocranium	Base	BA-OPI	0.183-0.166	0.034*

Table 3.6: T-tests for the differences between Canidae and non-canid Carnivora for individual characters analyses. Flex.- Difference in character-specific flexibility. $\sqrt{r^2}$ - Difference in mean character-specific integration. Var(%)- Difference in the percentage of variation explained by character. Positive and negative values indicate increased or decreased values for Canidae respectively. *- $p < 0.05$ without correction for multiple tests. **- $p < 0.05$ after correction for multiple tests (Bonferroni)

Region	Module	Character	Flex.	$\sqrt{r^2}$	Var(%)
Face	Nasal	IS-NSL	-0.009	-0.045*	-0.007**
Face	Nasal	NSL-NA	0.006	0.086**	0.039**
Face	Nasal	NSL-ZS	0.036**	0.023*	0.019**
Face	Nasal	NA-PNS	-0.049**	-0.044**	-0.017**
Face	Oral/Nasal	IS-PNS	0.019*	-0.010	0.010**
Face	Oral/Nasal	NSL-ZI	0.041**	-0.017	0.016**
Face	Oral	IS-PM	0.019*	-0.019	0.001**
Face	Oral	PM-ZS	0.085**	-0.007	0.023**
Face	Oral	PM-ZI	0.029**	0.003	0.016**
Face	Oral	PM-MT	0.049**	0.003	0.019**
Face	Oral	ZI-MT	0.002	-0.090**	-0.007**
Face	Oral	MT-PNS	-0.064**	-0.064**	-0.008**
Face	Oral/Zygomatic	ZS-ZI	-0.054*	-0.013	-0.006**
Face	Zygomatic	PT-ZYGO	-0.017	-0.042*	-0.010**
Face	Zygomatic	ZI-ZYGO	-0.016	-0.015	-0.006**
Face	Zygomatic	ZI-TSP	0.013	-0.042*	-0.005
Face	Zygomatic	EAM-ZYGO	-0.025	-0.031	-0.005**
Face	Zygomatic	ZYGO-TSP	-0.001	-0.056**	-0.009**
Neurocranium	Vault/Zygomatic	PT-TSP	0.157**	0.001	0.015**
Neurocranium	Vault	NA-BR	0.001	-0.073*	-0.035**
Neurocranium	Vault	BR-PT	0.003	-0.040*	-0.006**
Neurocranium	Vault	BR-APET	-0.008	-0.035*	-0.006**
Neurocranium	Vault	PT-APET	0.005	-0.017	-0.002*
Neurocranium	Vault	PT-BA	-0.012	-0.027*	-0.007**
Neurocranium	Vault	PT-EAM	0.006	-0.005	-0.001
Neurocranium	Vault	LD-AS	0.132**	-0.101*	0.003
Neurocranium	Vault	BR-LD	0.01	-0.015	-0.006
Neurocranium	Vault	OPI-LD	0.084**	-0.050*	0.006*
Neurocranium	Vault	PT-AS	-0.024	-0.017	-0.008**
Neurocranium	Vault	JP-AS	-0.028	0.025	0.001
Neurocranium	Base	PNS-APET	-0.007	0.013	0.005*
Neurocranium	Base	APET-BA	-0.023	-0.032*	-0.006**
Neurocranium	Base	APET-TS	0.014	-0.066**	-0.002**
Neurocranium	Base	BA-EAM	-0.054**	-0.041*	-0.011**
Neurocranium	Base	BA-OPI	-0.098**	0.010	-0.003**

Table 3.7: ANCOVAs (with overall $\sqrt{r^2}$ as a covariate) for the differences between Canidae and non-canid Carnivora for individual characters. Flex- Difference in character-specific flexibility. r^2 - Difference in mean character-specific integration. Var(%)- Difference in the percentage of variation explained by character. Positive and negative values indicate increased or decreased values for Canidae respectively. *- $p < 0.05$ without correction for multiple tests. **- $p < 0.05$ after correction for multiple tests (Bonferroni).

Region	Module	Character	Flex	$\sqrt{r^2}$	Var(%)
Face	Nasal	IS-NSL	-0.022	-0.022*	-0.005
Face	Nasal	NSL-NA	-0.036*	0.135**	0.038**
Face	Nasal	NSL-ZS	0.019	0.059**	0.019**
Face	Nasal	NA-PNS	-0.067**	-0.027*	-0.016**
Face	Oral/Nasal	IS-PNS	0.006	0.017*	0.014**
Face	Oral/Nasal	NSL-ZI	0.024**	0.009	0.017**
Face	Oral	IS-PM	0.006	0.010	0.002**
Face	Oral	PM-ZS	0.074**	0.023*	0.025**
Face	Oral	PM-ZI	0.008	0.038**	0.016**
Face	Oral	PM-MT	0.034**	0.038**	0.020**
Face	Oral	ZI-MT	-0.024	-0.071**	-0.007**
Face	Oral	MT-PNS	-0.086**	-0.042*	-0.008**
Face	Oral/Zygomatic	ZS-ZI	-0.085**	0.017	-0.007**
Face	Zygomatic	PT-ZYGO	-0.033**	-0.023*	-0.009**
Face	Zygomatic	ZI-ZYGO	-0.043**	0.017	-0.005
Face	Zygomatic	ZI-TSP	-0.015	-0.018	-0.005
Face	Zygomatic	EAM-ZYGO	-0.065**	0.001	-0.006**
Face	Zygomatic	ZYGO-TSP	-0.018	-0.040**	-0.007**
Neurocranium	Vault/Zygomatic	PT-TSP	0.137**	0.009	0.014**
Neurocranium	Vault	NA-BR	-0.041**	-0.052**	-0.039**
Neurocranium	Vault	BR-PT	-0.030	-0.018	-0.006**
Neurocranium	Vault	BR-APET	-0.031*	-0.009	-0.005**
Neurocranium	Vault	PT-APET	-0.017	0.000	-0.003**
Neurocranium	Vault	PT-BA	-0.033**	-0.005	-0.007**
Neurocranium	Vault	PT-EAM	-0.021	0.018	-0.002
Neurocranium	Vault	LD-AS	0.099**	-0.086**	0.002
Neurocranium	Vault	BR-LD	-0.016	-0.007	-0.010*
Neurocranium	Vault	OPI-LD	0.049*	-0.028*	0.006*
Neurocranium	Vault	PT-AS	-0.058**	0.007	-0.01**
Neurocranium	Vault	JP-AS	-0.052	0.043*	0.001
Neurocranium	Base	PNS-APET	-0.039*	0.053**	0.005*
Neurocranium	Base	APET-BA	-0.044**	-0.009	-0.005**
Neurocranium	Base	APET-TS	-0.007	-0.050*	-0.003**
Neurocranium	Base	BA-EAM	-0.067**	-0.021*	-0.01**
Neurocranium	Base	BA-OPI	-0.124**	0.026	-0.004**

709 observed among Old World Monkeys (Oliveira et al., 2009), Xenarthrans
710 (Hubbe et al., 2016) and of Ruminants (Haber, 2014), but were lower on
711 average than what was observed for New World Monkeys (Marroig and
712 Cheverud, 2001). However, when we look at less inclusive taxa such as fam-
713 ilies, the average similarity goes up, being compared to that of New World
714 Monkeys and within Ruminant families. In fact, the similarity among differ-
715 ent families is compatible to that seen between both sub-orders of primates
716 (Porto et al., 2009) and between Ruminant families (Haber, 2016), suggesting
717 that patterns of morphological integration are as conserved within Carni-
718 vore as they are within other orders of mammals published so far. Modularity
719 analysis reinforces this idea, showing that the modular patten of the skull is
720 shared by all Carnivora (Table 3.3), reflecting the conclusion that a similar
721 ontogenetic program is shared by all therian mammals (Goswami, 2006a;
722 Porto et al., 2009).

723 Goswami (2006b) was the only previous study that formally evaluated
724 the similarity in morphological integration of the skull between Carnivora
725 species. That study found much higher similarities on average between species
726 then those presented here, with values superior to 0.9 of analysis including
727 size. While both studies shared many anatomical landmarks and had a sim-
728 ilar number of traits (this study - 35 interlandmark distances; Goswami - 31
729 landmarks), they diverge mainly on morphometric techniques employed.
730 While here we focused on the use of classically established measurements
731 of cranial dimensions, Goswami (2006b) work was based on the a geomet-
732 ric morphometric representation of the data. The correlation matrix was ob-
733 tained as the congruence coefficient between pairwise coordinates (Goswami
734 and Polly, 2010), producing landmark-by-landmark matrices. While this can
735 help explain the difference in similarity values (Klingenberg, 2008), the use
736 of this landmark representation of morphometric data could be questioned.

It is not entirely clear what kind of morphospace is being represented, nor how it can be analyzed through quantitative genetics means. For example, given that the selection gradient β is a vector of partial regression coefficients of fitness onto traits (Lande and Arnold, 1983), how would a β of individual landmarks be estimated, and what would it represent? Even though we can easily do that using other morphometric methods, including Procrustes data (Martínez-Abadías et al., 2012; Mitteroecker and Bookstein, 2011), the representation of data dependency through congruence coefficient and related methods still lacks clear connections with evolutionary theory. Therefore, the values found for (Goswami, 2006b) does not clearly translate into a quantitative genetics context. This does not mean that matrices of congruence coefficient between landmarks has no place in the investigation of morphological integration, though. A recent study showed that this type of matrix representation can be successfully used to investigate modularity (Goswami and Finarelli, 2016) reaching similar conclusions to analysis of both Procrustes coordinates and classical linear measurements (Cheverud, 1982). Additionally, preliminary analyses of the correlation between covariance matrix similarity and ecological factors has corroborated some of the conclusions presented by Goswami (2006b) for Feliformes (Zahn, 2016), showing that both approaches can reveal similar signals in the data. These analyses are currently being expanded to encompass all Carnivora.

In quantitative genetic terms, high levels of $\mathbf{P}$ similarity give us confidence that the underlying genetic covariance is most likely stable across species (Marroig and Cheverud, 2001). This is because, as previously explained, $\mathbf{P}$ is the sum of genetic ($\mathbf{G}$) and environmental ($\mathbf{E}$) covariances (Falconer et al., 1996) and, if $\mathbf{P}$ s are similar, than that can be due to similarity in either $\mathbf{G}$, $\mathbf{E}$ or in both. Given that $\mathbf{G}$ matrix for the characters analyses

here seems to be conserved across mammalian Orders (Porto et al., 2009; Hubbe et al., 2016), it is more probable that similarity values found for **P**s of Carnivora are due to a stable **G**. In fact, the similarity between *Akodon* (Cricetidae; Rodentia) **G** matrix and Canidae **P** matrix is as high as the average similarity observed within families, suggesting that we don't need to postulate any additional phenomena, other than **G** stability, to explain observed similarities. This does not exclude the possibility of the similarity being influenced by similar **E**s between species. However, because of morphological integration is an expression of ontogeny, **G** and **E** probably share similar structure, because both genetic and environmental factors act on the same developmental pathways (Cheverud et al., 1983; Cheverud, 1988; Klingenberg, 2008). In other words, even if **E** is affecting **P** similarities, the fundamental cause is the conservation of ontogenetic pathways and, thus, of **G**.

According to quantitative genetic theory (Lande, 1980; Cheverud, 1984, 1996a), stability of **G** is possible when the amount of variation introduced by mutation is equivalent to the amount removed by selection. In that case

$$\mathbf{G} = \mathbf{W}^{1/2}(\mathbf{W}^{-1/2}\mathbf{U}\mathbf{W}^{-1/2})^{1/2}\mathbf{W}^{1/2} \quad (3.7)$$

where **U** is the matrix of mutational effects and **W** is the matrix of the pattern of stabilizing selection on additive values. Because stabilizing selection is independent of directional selection (Lande, 1980; Cheverud, 1984), the mean phenotype can evolve while **W** remains the same, thus maintaining **G** stable.

Cheverud (1984) argued that **W** is a sum of both "external" (given by environmental and ecological factors) and "internal" stabilizing selection (given by internal coherence of ontogenetic pathways). If we consider only the diversity of feeding habits in Carnivora, the existence of a similar ex-

791 ternal stabilizing selection on the skull across Carnivora would be rather re-
792 markable. Carnivora includes durophagous bone-crackers (*Hyena*) and bamboo-
793 eaters (*Ailurus* and *Ailuropoda*), pack-hunting hypercarnivores (*e.g.* *Panthera*
794 *leo*, *Lycaon pictus*, *Cuon alpinus*, *Speothos venaticus*, *Canis lupus*), termitivores
795 (*Proteles cristata*, *Otocyon megalotis*), large omnivores (*Ursus*), piscivores (*e.g.*
796 *Phoca*, *Pusa*), filter feeders (*Lobodon carcinophaga*), suction feeders (*e.g.* *Odoben-*
797 *nus rosmarus*) to name a few. Biomechanical studies have shown that even
798 slight changes in feeding strategy can impose great stress on skulls (Slater
799 et al., 2009; Slater and Van Valkenburgh, 2009), playing important roles in
800 the evolution of carnivoran morphology (Slater et al., 2010; Tseng, 2013;
801 Figueirido et al., 2013; Tseng and Flynn, 2015). Since biomechanical propri-
802 eties are functions of the relationship between variables, we would expect
803 that those combinations of characters that optimize performance would be
804 privileged by stabilizing natural selection (Rayner, 1985). Given that a broad
805 range of feeding habits all converge to a similar covariance structure (im-
806 plying similarity in stabilizing selection), one would have to conclude that
807 biomechanical performance is not the main component of the stabilizing se-
808 lection on the skull morphology of Carnivora. Then, if similar **Ws** exists,
809 they would have to be influencing general aspects of the functionality of
810 skull, such as the fit between lower mandible and cranium (Figueirido et al.,
811 2013), or mainly be a product of internal stabilizing selection.

812 This is not to say that biomechanical factors do not affect the evolution of
813 skull morphology in Carnivora, as they clearly do (*e.g.* Mattson, 1998; Sacco
814 and Van Valkenburgh, 2004; Figueirido et al., 2009; Sicuro and Oliveira,
815 2010; Damasceno et al., 2013; Figueirido et al., 2011a; Werdelin and Wesley-
816 Hunt, 2014; Kienle and Berta, 2015), but that ontogenetic constraints might
817 then be playing a greater role in the structuring of within species variation
818 (Cheverud, 1996a), influencing the evolution of complex morphologies.

Difference in Canidae

The k-medoids cluster analysis performed on RS similarity values indicated that species of Canidae, despite being similar to other families, clustered separately, not only by responding differently to selection, but also by responding more similarly among each other (Figures C.2 and 3.6). Results obtained from observed and corrected values of RS differed in some aspects. While observed values produced a cluster with only Canidae, with the exception of a few species, the corrected values clustered all canid species together, along with a few representatives of other families. The only consistent result for observed and corrected values is that almost all Canidae clustered separately from almost all other Carnivora, with the exceptions being inconsistent among analysis. The NP-MANOVAs analysis pointed out that the differentiation of Canidae explains more variation in RS than the k-medoids cluster, a fact that was even more obvious for the corrected values (Table 3.1). The NP-MANOVAs also highlighted that corrected values of RS presents more variation than the observed ones (total Sum of Squares in Table 3.1), which might be explained by the fact that repeatabilities are estimated with error, even for high sample sizes (Figure 3.3). The introduction of an extra source of error increases variance, potentially making the discrimination of subtle differences in covariance structure more difficult (*i.e.* decrease in power).

The separation of Canidae from the rest of Carnivora could not be explained by differences in overall morphological integration (r^2), with the differentiation between Canidae and the rest of Carnivora explaining always more variation in matrix similarity than r^2 (Table 3.2). The identification of taxa with different patterns of covariation is not in itself surprising. For an analysis within Catharrhini, (Oliveira et al., 2009) identified differences in *Homo* and *Gorilla* related to the increased modularity of

the neurocranium (Porto et al., 2009). Shirai and Marroig (2010b) showed that *Metachirus* diverged from the rest of Didelphimorphia by having a low $\bar{r}^2$. For living Xenarthra, Hubbe et al. (2016) showed that *Myrmecophaga* responded differently to selection in relation to other species because some traits had larger variances and covariances. However, despite differences in single species or genus, studies showing differences between more inclusive taxa are rare. Haber (2014) was the only work to identify a distinction between two families (bovids and cervids), pointing to alterations in the correlation structure in the nasal and oral modules, mirroring findings presented here. However, variations in overall integration can influence individual correlations and modularity patterns (Porto et al., 2009 and Figures D.1 and D.2 here). Since both ruminant families also diverge in overall integration (Haber, 2016), it's not clear if observed differences can be attributed to changes in overall integration alone. The use of ANCOVAs using $\bar{r}^2$ as a covariate allowed us to circumvent these issues and to dissect more clearly the nature of such differences. For this reason we focus on the discussion of ANCOVAs results below. Furthermore, by employing a taxonomically rich sample with 14 families and identifying changes in only one of them we reinforced both the ideas of conservation of covariance structure among Carnivora and differentiation only in Canidae.

It is unlikely that the results presented here are due to sampling differences. Our rarefaction analysis shows that the select sample is adequate to diagnose differences between families (Figure 3.4), and a correlation analysis between a matrix of pairwise geometric means of sample sizes and RS matrix revealed low value of correlation ($r = 0.150$), despite being significant. However, when we separate both sub-orders, Caniformes species showed no significant correlation ($r = 0.097$) while Feliformes show a higher significant correlation ($r = 0.380$). Despite this, the k-medoids analysis showed

no tendency to cluster species with similar samples, instead producing a taxonomically structured cluster around Canids.

Changes in Canidae covariance structure were not associated to shifts in overall morphological integration (as shown by the fact that $\bar{r}^2$ values for Canidae always falls within the range observed for the rest of Carnivora, Figures 3.8, 3.9, D.1 and D.2), but were associated with a decrease in overall flexibility after controlling for differences in $\bar{r}^2$ (Figure 3.9 and table 3.4). Even though $\bar{f}$ and integration are tightly negatively associated (Marroig et al., 2009; Goswami et al., 2014), the example of Canidae clearly shows that both can be decoupled by changes in patterns of integration (*i.e.* modularity, distribution of in variances and correlations, etc). This makes sense if we understand overall integration measures as they are: statistical summaries representing ensemble proprieties of matrices, such as averages of squared correlations (as presented here) or variances of eigenvalues (Wagner, 1984), for example. In both cases, different patterns of correlations among characters or of distribution of eigenvalues can map into the same statistic value ("many-to-one mapping"), possibly obfuscating subtle differences in covariance structure. While flexibility might also present similar many-to-one mapping pattern, our empirical result suggests that the cases were that happens might differ, even if slightly, for $\bar{r}^2$ and $\bar{f}$. This conclusion is in contrast to the one advanced by Haber (2016), who used simulations of randomly generated matrices and concluded that "the commonly observed tight inverse relationship between $rSDE(\mathbf{P})$ [relative Standard Deviation of Eigenvalues, a measure highly correlated with $\bar{r}^2$, Pavlicev et al., 2009; Haber, 2011] and $\bar{f}$ can be explained by their technical relationship, and does not in itself imply anything regarding the evolutionary implications of either" (Haber, 2016, Supplementary Online Material). Contrary to that however, the author also concluded that both $rSDE(\mathbf{P})$ and $\bar{f}$ differed in their sensitivity to modular-

ity, but dismissed this possibility as relevant, probably because parameters necessary for that effect to take place fell outside of the empirical parameter values under evaluation. Despite that, Haber (2016) results shows that more modular matrices will be slightly less flexible than less modular matrices with the same overall integration, as is the case for Canidae. Therefore we argue that these simulations and our empirical results actually shows the role of differences in pattern of morphological integration in shaping the evolutionary potential.

The K-medoids cluster analysis (Figure 3.11), NP-MANOVAS and matrix correlation with RS (Table 3.5) of individual selection response decomposition (SRD) matrices all point to the conclusion that Canidae differences are mainly concentrated in the Facial region (Figure 3.12A-B). ANCOVAs using $\bar{r}^2$ as covariate shows the Canidae is different by having more relative variance (as % of total variance) on the Oral and Nasal modules while having less variation on the Zygomatic, Vault and Base (Table 3.3). These changes in relative variance are associated with significant increases in flexibility at the Nasal and decreases of flexibility at the Zygomatic and Base modules (Figure 3.9 and table 3.4). Additionally, analysis of modularity shows that the Nasal is more modular, having a higher AVG-ratio associated to an increase in the intra-module correlations (Figures 3.8, D.1 and D.2 and table 3.4). Individual character analysis largely reflects these results (Table 3.7), showing that increases in character variance are usually associated to increases in character flexibility (Figure 3.12C,D). Analysis of character-based integration also shows an increase in Nasal, Oral and Base characters and a decrease in the Vault and Zygomatic, despite affecting different traits than those highlighted by the analysis of the variance and flexibility (Figure 3.12D). The only characters that showed an increase in all three measures in Canidae were related to some general dimensions of the skull, namely palatal length

(PM-MT), neurocranium height (PNS-NON), size of the parietal (BR-NON; Figure 3.12C-D). Jointly, these results lead us to the conclusion that Canidae has a more modular and flexible rostrum associated with an increase in variance and in morphological integration in traits related to the facial region, specifically to the Nasal and Oral modules.

The evolution of morphological integration on a quantitative genetic framework is a topic of intense debate and consensus on the causes of changes is still lacking (see Melo et al., 2016 for a recent review). Theoretical models have argued that both stabilizing (Lande, 1980) and directional selection (Pavlicev et al., 2011) can play an important part in changing the covariance pattern in **G**. Simulation analysis broadly confirm these expectations even under different models (Melo and Marroig, 2015; Jones et al., 2014). Specifically, Melo and Marroig (2015) model shows that, for a system with many traits, directional correlational selection on the same direction ("corridor selection") on a module and stabilizing selection on the rest of the traits increases not only intra-module correlation, but also increases the variation associated with directions that contrasts the selected and non-selected modules. This description matches what happens on the facial region of canids, specifically on the Nasal module. Additionally, the fact that RS similarities between Canidae and the other families is still high, despite these differences, reinforces the idea that the same stabilizing selection pattern is at play, at least for non facial modules. Thus, the combination of the same general stabilizing selection pattern of Carnivora plus a family-specific past history of direction selection on facial modules could explain differences observed in Canidae.

The causes for this selection on facial dimensions (mostly length) could be many. The relative length of the skull is tightly associated with changes in diet, mainly with carnivory. Canids with relative shorter snouts have an

959 increased bite force (Damasceno et al., 2013) and mechanical performance
960 for large prey capture (Slater et al., 2009), consistent with the fact that larger
961 body size leads to increased levels of carnivory in Carnivora (Van Valken-
962 burgh et al., 2004; Van Valkenburgh, 2007), via increase energetic demands
963 (Carbone et al., 2007; Tucker et al., 2016). Canids also shows a positive al-
964 lometric relationship between respiratory turbinal surface area and skull
965 length, contrary to the isometric relationship observed for arctoid Carni-
966 vores (Green et al., 2012) and Feliformes (Van Valkenburgh et al., 2014),
967 probably linked to the foraging necessities of hypercarnivores. Therefore,
968 forces associated with dietary shifts in Canidae seems to be antagonistic
969 in nature, probably imposing a more complex adaptive landscape on facial
970 traits, leading to increase integration, variance and flexibility.

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Chapter 4

Error rates of quantitative genetics tests applied to macroevolutionary data

1 *"We may often falsely attribute to correlation of growth, structures*
2 *which are common to whole groups of species, and which in truth are*
3 *simply due to inheritance; for an ardent progenitor may have acquired*
4 *through natural selection some one modification in structure, and, af-*
5 *ter thousands of generations, some other and independent modification;*
6 *and these two modifications, having been transmitted to a whole group*
7 *of descendants with diverse habits, would naturally be thought to be*
8 *correlated in some necessary manner"*

9 *-Charles Darwin 1859, p. 133*

10 **Introduction**

11 The study of the causes of phenotypic diversification is one of the main
12 goals of macroevolutionary studies. Ever since Darwin first proposed it, bi-
13 ologists have sought to use the theory of natural selection to explain biolog-
14 ical diversity on the planet (*e.g.* Fisher, 1930). Despite much interest, how-
15 ever, formal applications of theoretical evolutionary models to macroevolu-
16 tion were scarce for a long time. In a seminal paper, Gould and Lewontin
17 (1979) strongly criticized the practices of biologists when it came to adap-
18 tive explanations. According to them, contemporary researchers were spe-
19 cially inclined to explain away any biological feature as the product of se-
20 lection without addressing alternative hypothesis, such as drift and biolog-
21 ical constraints (*i.e.* Maynard-Smith et al., 1985). The next decades were fol-
22 lowed by an intense development of what is now referred as "Phylogenetic
23 Comparative Methods", allowing for the test of a wide range of hypotheses
24 about adaptive evolution in a macroevolutionary context, partly overcom-
25 ing Gould and Lewontin's critique.

26 Even though some phylogenetic comparative methods do not have any

necessary association to explicit biological models (*e.g.* Giannini and Goloboff, 2010), the most influential ones are those based on quantitative genetics theories of continuous traits (Felsenstein, 1985; Hansen and Martins, 1996). Under these theories, traits are modeled as being influenced by a large number of alleles with infinitesimal genetic additive effects, allowing for the statistical modeling of evolutionary processes such as drift, stabilizing and directional selection. Despite the abundance of comparative methods, most of the implementations focus on univariate cases, and even though multivariate methods are slowly being developed (*e.g.* Felsenstein, 2008; King and Butler, 2009; Bartoszek et al., 2012; Goolsby et al., 2016), the majority of these methods ignore the effect of genetic correlations on evolutionary outcomes, another aspect of Gould and Lewontin's critique (Cheverud, 1984).

Russell Lande was the first to formally extend the quantitative genetic theory into a multivariate framework (Lande, 1979). In this context, phenotypic evolution is influenced not only by the available additive genetic variance, but also by the genetic covariance between characters, which are jointly expressed as a covariance matrix of additive effects called $\mathbf{G}$. $\mathbf{G}$ is central for the evolution of complex phenotypic traits because it dictates how populations will behave under genetic drift and other evolutionary forces. According to Lande (1979) multivariate genetic drift is analogous to a random sampling of genetic additive effects and can be modeled as

$$\mathbf{B} = \frac{t}{N_e} \mathbf{G} \quad (4.1)$$

where $\mathbf{B}$ is the covariance matrix of evolutionary changes ($\Delta\mathbf{z}$ s), t is the elapsed time since divergence from the ancestral population in generations and N_e is the effective population size (Table 4.1). Given that $\frac{t}{N_e}$ is an unknown quantity in the vast majority of macroevolutionary studies, this expectation is translated into the investigation of the proportionality between

53 **B** and **G** (Lofsvold, 1988; Ackermann and Cheverud, 2002, 2004; Marroig
 54 et al., 2004; Marroig and Cheverud, 2004). This concept is very powerful
 55 because, by modeling drift in a multivariate context, we can provide a null-
 56 expectation of phenotypic evolution while incorporating genetic covaria-
 57 tion between characters. We can then test if a given macroevolutionary pat-
 58 tern is sufficiently explained by drift+genetic constraints or if natural selec-
 59 tion has to be invoked.

60 In the simplest case where all populations derive from the same ances-
 61 tral group and there is no gene-flow among them, **B** will be identical to the
 62 covariance matrix among population means, or between-groups covariance
 63 matrix. This is because, since drift is modeled as a Gaussian random walk,
 64 the expectation is equal to the initial phenotype, making the average of the
 65 population means a good estimate of the ancestral trait value. The standard
 66 calculation of the covariance among population means will be based on the
 67 cross-product of the differences between species means and the ancestral
 68 phenotype (*i.e.* $\Delta \mathbf{z}s$).

69 For phylogenetically structured populations, Felsenstein (1985) showed
 70 that **B** will not be equal to the between-group covariance matrix because of
 71 the presence of correlation introduced due to phylogenetic non-independence
 72 (see also Hansen and Martins, 1996). In this case, Felsenstein argued that, if
 73 we have a tree with branch lengths proportional to the expected amount
 74 of evolution (Garland Jr et al., 1992; Garland, 1992), then **B** will be better
 75 estimated as the covariance matrix of Phylogenetic Independent Contrasts
 76 (PIC) of the original data (Felsenstein, 1988), or $\mathbf{B}_{PIC}$ (Table 4.1). If we as-
 77 sume that generation time, N_e and **G** are constant through the phylogeny,
 78 then the magnitude of evolution will only depend on t (in years), allowing
 79 for the use of a phylogeny with divergence times to estimate $\mathbf{B}_{PIC}$.

80 The objective of the present work is twofold. Firstly we call attention

to this underappreciated theoretical framework for the study of multivariate phenotypic evolution. Secondly, we evaluate the statistical proprieties of two tests employed in the macroevolutionary studies under a quantitative genetics framework in order to provide guidance to researchers potentially interested in their application.

Principal Component-Based Tests of Drift

Ackermann and Cheverud (2002) first introduced two distinct methods to evaluate proportionality between $\mathbf{G}$ and $\mathbf{B}$ based on Principal Component Analysis (PCA). PCA is a common exploratory statistical tool and is based on the eigen-decomposition of a covariance matrix $\mathbf{A}$ as follows

$$\mathbf{A} = \mathbf{V}\mathbf{\Lambda}\mathbf{V}^{-1} \quad (4.2)$$

where $\mathbf{V}$ contains the standardized eigenvectors and $\mathbf{\Lambda}$ is a diagonal matrix containing the eigenvalues of $\mathbf{A}$. For a square symmetrical matrix the eigenvectors, or Principal Components (PCs), are orthogonal to each other and can be interpreted as a new set of independent variables. The covariance matrix of these PCs is equal to $\mathbf{\Lambda}$, and each eigenvalue is equivalent to the variance of each corresponding eigenvector. One can project the between-species data $\mathbf{X}$ onto this new space as

$$\mathbf{Y} = \mathbf{XV} \quad (4.3)$$

The covariance matrix of $\mathbf{Y}$ is equivalent to $\mathbf{B}$ projected onto the PCs, or $\mathbf{B}^*$. Alternatively $\mathbf{B}^*$ can be calculated by directly projecting $\mathbf{B}$ onto the PC space using Equation (4.2). If we premultiply by $\mathbf{V}^{-1}$ and postmultiply by $\mathbf{V}$, we can project a covariance matrix onto the space defined by $\mathbf{V}$. Given that the proportionality of $\mathbf{G}$ and $\mathbf{B}$ under neutral evolution is invariant to arbitrary

Table 4.1: Glossary of symbols used and their corresponding description.

Symbol	Description
$\mathbf{G}$	Genetic variance-covariance matrix.
$\mathbf{B}$	Between-groups variance-covariance matrix. Covariance matrix of evolutionary changes ($\Delta\mathbf{z}$ s) for independent populations.
$\mathbf{B}_{PIC}$	Between-groups variance-covariance matrix estimated on phylogenetic independent contrasts. Covariance matrix of evolutionary changes ($\Delta\mathbf{z}$ s) for phylogenetically structured populations.
$\mathbf{W}$	Pooled within-groups covariance matrix used as an approximation of the ancestral $\mathbf{G}$.
$\mathbf{C}$	Variance-covariance matrix of directional selection gradients (β s).
$\mathbf{A}^*$	* denotes projection of a variance-covariance matrix $\mathbf{A}$ on the principal components of $\mathbf{G}$.
$\mathbf{A}_{cor}$	"cor" denotes a correlation matrix counterpart of a variance-covariance matrix $\mathbf{A}$.
t	Time in generations.
N_e	Effective population size.
e	Effect size; the size of the directional selection vector $\mathbf{s}$ simulated.

rotations of the phenotypic space (as long as both matrices are rotated by the same set of $\mathbf{V}$), the comparison of $\mathbf{\Lambda}$ (or $\mathbf{G}^*$) and $\mathbf{B}^*$ is equivalent to the comparison between $\mathbf{G}$ and $\mathbf{B}$.

In order to develop a formal test of proportionality between matrices using PCA, Ackermann and Cheverud (2002) noticed that, by log-transforming Equation (4.1), we can write the comparison between $\mathbf{G}^*$ and $\mathbf{B}^*$ as a linear regression equation

$$\log(\text{var}(PC_i)) = a + b \log(\lambda_i) + \epsilon \quad (4.4)$$

were $a = \log(\frac{t}{N_e})$ is the intercept, b is the slope, $\text{var}(PC_i)$ are the variances of $\mathbf{B}^*$, λ_i are the eigenvalues (variances) of $\mathbf{G}^*$ and ϵ is a gaussian error term. By using ordinary least-squares (OLS) regression analysis, one can adjust the values for the slope and compare it to the expected value under proportionality $b = 1$. This can be achieved by either observing the parametric confidence interval of b (Ackermann and Cheverud, 2002) or by applying a t-test to directly calculate p -values for the null hypothesis (Prôa et al., 2013).

Ackermann and Cheverud (2002) also argued that, since PCs are orthogonal to each other on $\mathbf{G}$, under drift the same set of PCs should also be orthogonal on $\mathbf{B}$. This can be done by obtaining the correlation matrix of $\mathbf{Y}$ (Equation (4.3)) or, alternatively, by transforming $\mathbf{B}^*$ on its correlation counterpart $\mathbf{B}_{cor}^*$. Because under the expectation of drift $\mathbf{B}^*$ is a diagonal matrix, its corresponding $\mathbf{B}_{cor}^*$ is by definition an identity matrix $\mathbf{I}$ (*i.e.* no correlation between the species scores on the PCs of $\mathbf{G}$). Therefore, any deviation of $\mathbf{B}_{cor}$ from $\mathbf{I}$ can be seen as evidence to reject proportionality. This can be done by two main ways: firstly is the application of Bartlett χ^2 which tests if a square matrix deviates from $\mathbf{I}$ and, secondly, by the presence of significant correlations between the projections of species means on the PCs of $\mathbf{G}$. This second test has the advantage of also providing information about the direction of

129 selection (Marroig and Cheverud, 2004).

130 The use of these two methods –the regression approach and the corre-
131 lation approach– in conjunction is convenient because they allow for the
132 evaluation of different aspects of the covariance structure. While the regres-
133 sion focuses on the diagonal of $\mathbf{G}^*$, the correlation test investigates the off-
134 diagonal elements of this matrix, constituting two lines of evidence to test
135 the hypothesis of neutral evolution.

136 Type I error (false positives) rates for the regression test were previously
137 explored in two papers (Ackermann and Cheverud, 2002; Prôa et al., 2013),
138 but type II error rates (false negatives) or power (rate of rejection of a false
139 null-hypothesis, or 1-(type II error rate)) were previously discussed (Ack-
140 ermann and Cheverud, 2002), but not systematically investigated. In the
141 present contribution we calculate type I error rate and power of both regres-
142 sion and correlation tests of neutral evolution of multivariate quantitative
143 characters on phylogenetically structured populations. We employed quan-
144 titative genetic theory in order to simulate phenotypic evolution by drift
145 and natural selection on a phylogeny. Because species are phylogenetically
146 related, we expect that analysis using phylogenetic comparative methods
147 will outperform analysis on raw data, at least for the correlation test. Be-
148 cause variances are not affected by phylogenetic relationships, we expect
149 that the regression test will be more consistent between phylogenetic and
150 non-phylogenetic approaches.

151 As a case study we use Caniformes (Carnivora) skull morphological
152 evolution. Caniforms are a diverse group of mammals, containing 9 fam-
153 ilies (2 of which are monospecific) and more than 160 species, allowing for
154 the examination of a range of species sample sizes. Additionally, caniforms
155 phylogenetic relationships between species are fairly well known (Arnold
156 et al., 2010; Agnarsson et al., 2010; Nyakatura and Bininda-Emonds, 2012;

Slater et al., 2012), providing a robust framework for simulation analysis. Finally, cranial morphology and evolution has been intensively studied for this group and is generally agreed that morphological diversity is influenced by ecological factors, mainly feeding ecology (e.g. Wroe and Milne, 2007; Jones and Goswami, 2009; Figueirido et al., 2010; Slater et al., 2009, 2010; Tseng and Wang, 2011; Figueirido et al., 2011b, 2013). Most studies, however, do not take into account the role of genetic constraints nor explicitly test the null hypothesis of drift. Here we test the null hypothesis of drift for these groups.

Materials and Methods

Sample, measurements and matrices

The caniform phylogeny was obtained from Slater et al. (2012). High-level phylogenetic relationship seems robust and concordant with most published phylogenies (Arnold et al., 2010; Fulton and Strobeck, 2010; Slater et al., 2012; Agnarsson et al., 2010; Nyakatura and Bininda-Emonds, 2012). We manually added newly described species to the tree topology in order to maximize species sample (Figure 4.1, see Appendix E for details about modifications).

We obtained 35 cranial measurements from 5088 individuals as distances between anatomical landmarks. The effects of confounding factors such as sex or subspecies were removed from the sample using a multivariate linear model. Pooled within-group covariance matrices $\mathbf{W}$ for cranial traits were estimated for each node of the phylogeny by pooling the residuals of all daughter species and adjusting for lost of degrees of freedom due to the removal of within and between species effects. The taxonomy used and sample sizes can be seen on Table E.1. Details about sample, data processing,

Figure 4.1: Caniform Phylogeny modified from Slater et al. (2012). A- Canidae; B- Ursidae; C- Pinipedia; D- Mephitidae; E- Procyonidae; F- Mustelidae.

measurements and estimation of **W**s can be found in Chapters 2 and 3.

Given that covariance matrices were considered highly similar among Carnivora species (Chapter 3), **W** matrices can be confidently used as substitutes for **G** in order to model phenotypic evolution and to investigate the mode of evolution on our empirical example (Cheverud, 1982, 1996; Ackermann and Cheverud, 2000; Cheverud et al., 1983; Marroig and Cheverud, 2001; Oliveira et al., 2009; Hubbe et al., 2016). Because **W** are estimates with sampling error, matrices were controlled for noise according to Marroig et al. (2012), by first investigating the Second-derivative variance of the eigenvalues of each **W** matrix. When this values stabilizes near zero, the eigenvalue of the following eigenvectors is raised (extended) to the value of the stable eigenvector. This is done in order to minimize the effect of small eigenvalues on the inverted matrix. Extended versions of the **W** matrices were only used when matrix inversion was necessary.

Model of phenotypic evolution

Phenotypic evolution was modeled by incorporating both natural selection and drift as

$$\mathbf{B} = t(e\mathbf{GCG} + \frac{\mathbf{G}}{N_e}) \quad (4.5)$$

were t and N_e are the time in generations and the effective population size, as in Equation (4.1), **G** is the genetic covariance matrix, **C** is the covariance matrix of selection gradients and **B** here is the matrix of covariance of evolutionary responses $\Delta\mathbf{z}$ s (Lande, 1979; Felsenstein, 1988; Zeng, 1988; Hansen and Martins, 1996; Arnold et al., 2001). e is the effect size controlling the relative importance (intensity) of natural selection (**GCG**) in relation to drift ($\mathbf{G}/N_e$; see Table 4.1 for a glossary of the terms of the model).

$\mathbf{C}$ was modeled as $\mathbf{s}^t \mathbf{s} + \mathbf{I}/1000$, where $\mathbf{s}$ is a random vector of norm=1 (t denotes transpose) and $\mathbf{I}$ is an identity matrix. This last step was added to ensure that $\mathbf{C}$ was positive-definite. Since for $e = 1$ we want selection and drift to have the same relative importance, both $\mathbf{GCG}$ and $\mathbf{G}/N_e$ were scaled to have the same trace. However, by altering e , $\mathbf{CGC}$ can be arbitrarily big or small in relation to $\mathbf{G}/N_e$. If e tends to zero, then it is easy to see that Equation (4.5) collapses into Equation (4.1). Thus, we are able to alter not only the intensity of directional selection, but the mode of evolution, by adjusting only the parameter e . In addition to simulating species means we also performed a Monte Carlo sampling of within-species variation to evaluate the impact of sampling on both mean species phenotype and on the estimation of $\mathbf{W}$ (following Prôa et al., 2013). A detailed description of the model and the R code used to implement it can be found at Appendix E. All analysis were done on the R programming environment (R Core Team, 2016).

To observe the impact of variation in species sample size on type I error and power, the simulations were conducted on each clade of the phylogeny. For each node we estimated $\mathbf{W}$ (see Chapter 4) and extracted the phylogeny of that particular clade. We restricted the analysis to nodes that had a pooled sample larger than 35 and that had more than the minimum proposed sample of 4 species (Ackermann and Cheverud, 2002), resulting in a total of 91 clades from various taxonomic ranks. The Table E.2 shows the summary of the number of simulations performed for each analysis described below.

We also performed all analysis on sets of randomly selected species (and trees) following Cressler et al. (2015), but since preliminary results for type I error and power were basically the same, we only report the clade-based analysis for clarity. Because there is a strong correlation between pooled within-group sample size (*i.e.* sample used to estimate $\mathbf{W}$) and number of

species ($r = 0.93$) we only display results in relation to species sample size.

Type I error rates and Power

Simulations were performed using eight values for $e=[0, 0.001, 0.01, 0.1, 1, 10, 100, 1000]$. As stated above, when $e = 0$ the simulation is equivalent to neutral evolution, while for all other values of e the relative contribution of **GCG** varies exponentially. For each run both the regression and the correlation tests were conducted. For the regression test, the deviation of the estimated slope from 1 was accessed through a t-test. For the correlation tests each pairwise correlation of $\mathbf{B}_{cor}^*$ was subjected to a significance test and the resulting p-values were corrected for multiple comparisons using the Bonferroni correction. The null-hypothesis was rejected if the matrix showed any significant correlation. In cases where species n was smaller than the number of variables, we performed the analysis by evaluating the correlation among only the first n PCs (Marroig and Cheverud, 2004).

For each run, the tests were done in four different ways. Firstly, all tests were conducted using raw untransformed data and on PIC estimated for the clade under analysis. For PIC, all the operations were performed by simply substituting $\mathbf{B}$ by $\mathbf{B}_{PIC}$ where needed (Revell, 2007). Secondly, for both raw and PIC data, tests were performed by using the original $\mathbf{W}$ or by using the resampled $\mathbf{W}$ for each run of the simulation. This was done in order to inspect the impact of sampling of $\mathbf{W}$ on the test results.

For all values of e , the rate of rejection of the null hypothesis was calculated. For $e = 0$, the rate is equal to the type I error rate, while for non-zero values of e the rate equals to the Power of the test. The simulations were run for each clade- e combination.

Since the PCs are sorted according to their eigenvalues, it is easy to remove axis that explain smaller proportion of the total variation. For multi-

262 variate morphometrics this can be specially useful because the reduction of
 263 the number of variables can lead to a better estimation of statistical parame-
 264 ters, specially in the case of covariance and correlation matrices (Friedman,
 265 1989). Additionally, the last eigenvalues of a covariance matrix are thought
 266 to be associated with noise (Marroig et al., 2012), and their removal is some-
 267 times proposed as a way to improve statistical inferences (Strauss, 2010; Mit-
 268 teroecker and Bookstein, 2011). To assess if the reduction of the dimension-
 269 ality of the data affects statistical proprieties of the neutral evolution tests,
 270 we remove the last 5, 10, 15, 20, 25 and 30 PCs, and calculated error I rate
 271 and power for clades with small sample size (5–6 species).

272 **Variation in N_e**

273 One of the underling assumptions of the use of the quantitative genetic
 274 model for drift (Equation (4.1)) is that effective population size N_e have to
 275 be constant throughout the evolutionary history of the group. However, its
 276 highly unlikely that this will hold for large evolutionary scales, with some
 277 groups showing abrupt differences in N_e between sister taxa or even along
 278 the history of a species. Calculations of N_e are generally not available for all
 279 species within a large groups and even if that was the case, estimation of
 280 ancestral N_e along all phylogeny are not trivial, even if possible.

281 To evaluate the potential effects of variation in N_e on the statistical per-
 282 formance of the tests we artificially altered the branch lengths of the phy-
 283 logeny, as to simulate increase or decrease of rates of neutral evolution along
 284 that tree (Venditti et al., 2011; Baker et al., 2016). In practice, since the neutral
 285 simulations are based on multiplying draws from $\mathbf{G}$ by the branch length
 286 as a representation of t/N_e (see Appendix E), artificially increasing (or de-
 287 creasing) branch lengths can be interpreted as a proportional decrease (or
 288 increase) in N_e .

For each run of the simulation a number of scalars equals to the number of branches were drawn from a normal distribution with mean 0 and five different values for standard deviation ($\sigma=[0.1, 0.7, 1.3, 1.9, 2.5]$). The exponential of each scalar was obtained and then multiplied by the branch length to simulate an alteration in the rate of drift following Equation (4.1). This produced a range of scenarios where the N_e was rather stable on one side ($\sigma = 0.1$, 95% range of scalars: 0.822 – 1.217) and variable on the other ($\sigma = 2.5$, 95% range of scalars: 0.007 – 134.390). If we assume baseline N_e of 20000, then the simulations can be seen as varying N_e from 16440-24331 on one extreme ($\sigma = 0.1$) and 149-2685796 on the other ($\sigma = 2.5$). Type I error rates were calculated as described above. The PIC version of the regression and correlation tests used to access type I error rates were performed on the original unscaled phylogeny.

Empirical example: Caniform Families

To evaluate the performance of the tests on a empirical datasets we performed both regression and correlation tests for neutral evolution on the 7 non-monospecific Caniform Families: Canidae, Ursidae, Otariidae, Phocidae, Mephitidae, Procyonidae and Mustelidae. Polled sample size was on average 173, full sample size ranged from 463-2350 and species sample size ranged from 8-57 among families (Table E.1).

Drift tests were performed on raw and PIC data and significance was accessed by both parametric tests and simulations. For parametric correlation tests we used the full dataset and also a reduced number of PCs (always less PCs than the number of species, as for Ackermann and Cheverud, 2004; Marroig and Cheverud, 2004), always controlling for multiple correlations using Bonferroni. Regression analysis was performed using OLS regression and differences of the estimated slope from 1 (the null-expectation) were

316 tested through t-tests.

317 For non-parametric tests, the simulations previously described for neu-
318 tral evolution (with sampling error on W) were used to construct null distri-
319 butions based on the family-level phylogeny and W matrix. For each family
320 10000 simulations were conducted and all the statistics were calculated at
321 each run. Empirical values were considered significant if they fell outside
322 95% intervals calculated from simulations.

323 For the correlation analysis a multiple test correction should be employed.
324 However, if we apply Bonferroni on empirical p-values obtained through
325 10000 simulations, the lowest corrected value would be insufficient to re-
326 ject the null hypothesis at $\alpha = 0.05$ for all cases (minimum corrected p -
327 *value* = 0.0595). For that reason, we also recorded the correlation values
328 that fell completely outside the simulated empirical distribution (*i.e.* when
329 $p > 0.0001$).

330 Results

331 Type I error and Power

332 For the analysis of raw data, the type I error rate (false positives) for the
333 regression test of neutral evolution was greatly impacted by the resampling
334 of W through Monte-Carlo (Figure 4.2, upper left panel). When we assumed
335 that G was estimated without error (no resampling), type I error rates were
336 nominal ($\alpha = 0.05$) independent of sample size. However if we account
337 for sampling error through Monte-Carlo resampling of W , error rates were
338 greatly inflated, mainly for samples lower than 10, where the median error
339 rate was of 0.21, with values as high as 0.98. Correlation tests, on the other
340 hand, showed inappropriate type I error rates independent of sampling er-
341 ror on W , showing small error rates at lower species samples (< 6) that

342 quickly escalates to 1.00 error rates at $n \approx 15$ (Figure 4.2, lower left panel).

343 For the analysis of phylogenetic independent contrasts (PIC) data type I
344 error rates for the regression tests also showed nominal values for all sam-
345 ples sizes when within-group sampling was not accounted for. However,
346 when within-group error was introduced, values were slightly inflated, with
347 a median error of 0.9 for small sample sizes ($n < 10$), reaching values as high
348 as 0.48 (Figure 4.2, upper right panel). For correlation tests on PIC data, both
349 with and without sampling error on **W** showed nominal error rates through-
350 out all sample sizes (Figure 4.2, lower right panel).

351 Sampling error on **W** seems to affect mainly the power of regression
352 analysis, being higher when the resampling of **W** was not performed (Fig-
353 ure 4.3, first and second columns). Bellow I describe mainly the results for
354 the simulations with sampling error.

355 For the lowest values of the effect size e (0.001-0.1) the rates of rejection
356 of the null hypothesis (power) were virtually indistinguishable from the
357 simulations without directional selection (Figure 4.3, first row). For $e = 1$,
358 both regression and correlation tests on PICs behave similarly, with an al-
359 most linear increase in power with the increase of n (Figure 4.3, middle
360 row). The regression test on raw data shows a similar but more moderate
361 increase in power with sample size for the same effect size. For $e = 10$,
362 regression tests on PIC data shows power=1.00 at $n=20$, while analysis on
363 raw data fails to reach that value for $n < 40$. Correlation analysis on raw
364 data reaches power=1.00 at $n=15$, while this analysis on PIC also fails to
365 reach power=1.00 for $n < 40$ (Figure 4.3, forth row). For higher values of e
366 (100-1000) regression tests shows a rapid increase in power, with raw data
367 reaching power=1.00 at sample sizes closer to 20, while PIC data reaches the
368 same asymptote at samples closer to 15. For correlation tests, power=1.00 is
369 reached at $n = 5$ (Figure 4.3, last two rows), with values for $n = 4$. being

Figure 4.2: Type I error rates for regression and correlation tests of drift on raw and PIC data. B- Analysis without resampling of W. WB-Analysis with resampling of W.

370 higher for raw data than for PIC data.

371 Because power was close to 0.05 in all tests (with exception of correlation
372 analysis on raw data) for $e \leq 1$, the effect of variation of the number of
373 PCs on power was only evaluated for effect sizes larger than $e = 1$. For
374 those cases, differences in PC numbers impacted power similarly, the only
375 difference being the baseline power for the full set of vectors. Thus, here
376 we focus on the results for $e = 1000$, where the effects of the reduction of
377 dimensionality are clearly illustrated (Figure 4.4).

378 Both type I error rates and power for all analysis were effected by the
379 reduction of the number of PCs, with the exception of the type I error of
380 the correlation analysis on PIC data, that showed nominal values ($\alpha = 0.05$)
381 in all simulations (Figure 4.4). Analogously, correlation analysis of raw data
382 also showed no difference in type I error rates, but all values were superior
383 to the nominal value. For regression analysis, the decrease in the number of
384 PCs allowed for the reduction of type I errors for both raw and PIC data. For
385 PIC analysis, nominal levels were reached with PCs=20, while for raw data,
386 nominal levels were reached only with PC=10. Regarding power, both re-
387 gression analysis (PIC and Raw data) showed an increase in power as more
388 PCs were retained, with the exception that analysis on PIC tended to show
389 superior values for simulations accounting for sampling error. Correlation
390 analysis showed high power for low number of PCs retained, and showed
391 a decrease in power as more PCs were retained. This pattern was more pro-
392 nounced for PIC than for raw data.

393 **Variation in N_e**

394 Variation in N_e had no discernible effect on the regression analysis per-
395 formed on either raw data or PIC data for all levels of σ Figure 4.5. For
396 correlation analysis the increase in the variation of N_e along the phylogeny

Figure 4.3: Statistical power for regression and correlation tests of drift on raw and PIC data. Rows are different values of effect size e . B- Analysis without resampling of W. WB-Analysis with resampling of W. Lines are Lowess fits to the data.

Figure 4.4: Effect of varying varying numbers of principal components (PCs) on Type I error and Power for analysis of clades with sample sizes of 5-6. B-Analysis without resampling of W. WB-Analysis with resampling of W.

397 results in an increase of type I error rates for both PIC and raw data. Even
398 though correlation analysis of PIC and raw data have different error rates
399 when σ is low, both show a similar error rate when values of σ were greater,
400 with a type I error rate equal to 1 for values larger than 10 for raw data and
401 20 for PIC.

402 **Caniformes**

403 For the regression test, the parametric approach on Raw data rejected the
404 null hypothesis for Canidae, Ursidae, Procyonidae and Mustelidae (Table 4.2
405 and fig. 4.6). The non-parametric simulations-based tests rejected drift for
406 those families plus Otariidae and Phocidae, failing to reject the null hypoth-
407 esis only for Mephitidae (Figure 4.6). The analysis on PIC data rejected drift
408 for Canidae, Procyonidae and Mustelidae, while the simulation approach
409 rejected drift for Canidae, Otariidae, Phocidae, Procyonidae and Mustelidae
410 (Table 4.2 and fig. 4.7). Overall, the non-parametric test on PIC data rejected
411 on more occasions (4 tests), while the parametric regression on PIC rejected
412 the least times (3 tests).

413 For correlation tests on raw data, all tests, both parametric and non-
414 parametric, managed to reject the null hypothesis of drift, with the excep-
415 tion of the parametric test for Ursidae (Table 4.2). For the analysis of PIC
416 data, both parametric and non-parametric tests agreed in which cases drift
417 was rejected and also in the approximate number of significant correlations
418 (either if they were corrected or not for multiple tests, Table 4.2). Which
419 PCs were correlated varied among different methods (Table 4.3). Almost all
420 correlations found on the non-parametric PIC tests were also found on the
421 parametric PIC tests. Parametric tests on raw data found the most correla-
422 tions, while non-parametric tests found significant correlation in just a few
423 cases.

Figure 4.5: Type I error rates for different simulations varying the N_e along the phylogeny. Different values on the right of each row represents 95% of the simulated N_e assuming a baseline of $N_e = 20000$. Lines, from top to bottom, represent $\sigma=[0.1, 0.7, 1.3, 1.9, 2.5]$, respectively. See text for further description. B- Analysis without resampling of W. WB-Analysis with resampling of W.

Figure 4.6: Regression analysis of drift on raw data. $\log(\lambda_i)$ - Log-eigenvalues of the $\mathbf{W}$ matrix. $\log(\text{var}(PC_i))$ - Log-variances of the species means or PICs projected onto $\mathbf{W}$. Gray lines- Regression lines obtained from 100 simulations of drift. Black lines- Ordinary Least Squares regression estimate.

Figure 4.7: Regression analysis of drift on PIC data. $\log(\lambda_i)$ - Log-eigenvalues of the $\mathbf{W}$ matrix. $\log(\text{var}(PC_i))$ - Log-variances of the species means or PICs projected onto $\mathbf{W}$. Gray lines- Regression lines obtained from 100 simulations of drift. Black lines- Ordinary Least Squares regression estimate.

Table 4.2: Drift tests for the Caniform families. n- species or PIC sample sizes. Clade age- Age of the clade in million years. Raw-analysis performed on raw data. PIC- analysis performed on PIC data. Regression: b - slope estimate; CI_{slope} - Parametric slope interval; CI_{null} - Confidence interval of the null hypothesis built by simulation. Correlation: Parametric- Number of significant correlations using the parametric method. Non-Parametric- Number of significant correlations using the parametric method. For correlation tests, values before and after "/" are numbers of significant correlations for analysis without and with multiple test correction, respectively. See text for details for non-parametric multiple test correction.

<i>Data=Raw</i>	n	Clade age	<i>b</i>	Regression		Correlation		Comparable with drift?
				CI_{slope}	CI_{null}	Parametric	Non-Parametric	
Canidae	36	13.73	1.209	1.063<>1.354	0.889<>1.084	258/66	89/5	No
Ursidae	8	19.09	0.825	0.655<>0.994	0.829<>1.085	54/0	59/1	No
Otariidae	13	3.94	1.129	0.980<>1.278	0.838<>1.041	61/1	64/1	No
Phocidae	19	14.68	1.093	0.981<>1.206	0.840<>1.086	102/7	30/1	No
Mephitidae	11	9.98	1.040	0.859<>1.222	0.812<>1.104	219/19	127/6	No
Procyonidae	14	18.88	1.293	1.115<>1.470	0.828<>1.093	145/5	61/1	No
Mustelidae	57	21.08	1.274	1.142<>1.406	0.916<>1.060	319/133	203/26	No
<i>Data=PIC</i>								
Canidae	35	13.73	1.161	1.032<>1.291	0.939<>1.036	177/24	176/23	No
Ursidae	7	19.09	0.848	0.680<>1.016	0.843<>1.063	61/0	59/0	Yes
Otariidae	12	3.94	1.090	0.950<>1.230	0.854<>1.028	52/0	50/0	No
Phocidae	18	14.68	1.035	0.916<>1.154	0.889<>1.032	93/2	93/2	No
Mephitidae	10	9.98	1.004	0.850<>1.158	0.862<>1.057	61/0	61/0	Yes
Procyonidae	14	18.88	1.185	1.036<>1.335	0.874<>1.046	90/2	85/2	No
Mustelidae	56	21.08	1.129	1.034<>1.224	0.949<>1.028	236/41	229/37	No

Table 4.3: Significant correlation between the scores of species (raw) or independent contrasts (PIC) on the Principal Components of W

	raw		PIC	
	Parametric	Non-Parametric	Parametric	Non-Parametric
Canidae	1-2, 6, 9, 18, 20, 23, 26, 29, 34, 35; 2-3, 6, 9, 18, 23, 25, 26, 28, 34; 3-6, 8, 23, 28; 4-17, 20; 6-8, 9, 16, 18, 23, 25, 28, 29, 30, 34; 7-10; 8-16, 23, 25, 28, 9-10, 18, 20, 23, 26, 28, 29, 34, 35; 12-20; 16-23, 25; 18-28; 20-29, 34, 35; 23-25, 28, 29; 25-28, 29, 34; 26-35; 28-29, 34; 29-34	-	1-18, 20, 35; 2-3, 6, 8, 16, 23; 3-4, 8; 6-8, 23, 28; 7-10; 8-16, 25; 9-10; 16-25; 20-35; 23-28; 25-29, 34; 29-34; 32-33	1-9, 20, 35; 2-3, 6, 8, 16, 23; 3-8, 16; 6-8, 23; 8-16, 25; 9-10; 16-25; 20-35; 23-28; 25-29, 34; 29-34; 32-33
Ursidae	-	-	-	-
Otariidae	12-35	-	-	-
Phocidae	4-25, 30; 5-30; 12-15; 13-30; 24-31; 28-29	-	4-25; 28-29	4-25; 28-29
Mephitidae	5-7, 11, 13, 33; 7-11, 13, 17, 30; 11-13, 22, 33; 13-15, 17, 28, 33; 15-33; 18-24; 24-34; 28-33	-	-	-
Procyonidae	1-2, 3; 2-21; 3-13; 9-29	-	1-2; 3-23	1-2; 3-23
Mustelidae	1-2, 4, 5, 6, 7, 15, 16, 19, 20, 27, 29, 32, 33; 2-4, 5, 6, 9, 13, 16, 19, 20, 22, 23, 25, 26, 27, 29, 32, 33; 3-7, 8, 10, 12, 17, 21, 28, 31; 4-6, 7, 8, 15, 16; 5-6, 12, 13, 20, 21, 23, 25, 26, 29, 32; 6-9, 16, 19, 20, 22; 7-8, 10, 15, 21, 28; 8-10, 12, 13, 15, 17, 21, 23, 25, 26, 28, 31; 9-13, 14, 19, 20, 22, 33; 10-12, 17, 19, 28, 30; 12-13, 21, 23, 25, 26, 31; 13-14, 20, 21, 25, 29, 32; 14-21; 15-21, 28; 16-19, 22, 27, 30; 17-21, 22, 23, 25, 26, 28, 30; 19-20, 22, 30; 20-22, 25, 29, 32; 21-23, 25, 26, 28, 31; 22-24, 30; 23-25, 26; 25-26, 28; 26-31; 27-32; 28-30; 29-32, 33	1-2, 4, 6; 2-5, 6; 5-26; 7-15; 8-21; 25-26	1-2, 4, 5, 6, 15; 2-5, 6, 16, 22, 29, 33; 4-6, 8; 5-19; 7-8, 31; 8-21, 31; 9-33; 10-17; 11-31; 12-13, 31; 13-20; 14-21, 33; 16-19, 22, 30; 17-24, 26, 28, 35; 21-31; 22-24, 30; 25-26; 26-28, 35; 28-30; 29-33	1-2, 4, 5, 6, 15; 2-5, 16, 29, 33; 3-7; 4-8; 5-19; 7-8; 8-21, 31; 9-33; 11-31; 12-13, 31; 13-20; 14-21, 33; 16-19, 22, 30; 17-24, 26, 28; 22-30; 25-26; 26-28, 35; 27-32; 28-30; 29-33

Discussion

Here we investigated the statistical properties of two quantitative genetics tests of neutral evolution on a macroevolutionary framework. Both methods rely on the theoretical prediction that interspecific and intraspecific patterns of covariation will match under drift (Lande, 1979). The rejection of this expectation can be seen as evidence that the observed pattern does not match the expectation under drift, opening the door for natural selection as an explanatory hypothesis (Ackermann and Cheverud, 2002, 2004; Marroig and Cheverud, 2004). However, when comparing species in a comparative context, phylogenetic dependency might influence the results, mainly when we are exploring correlations among characters (Díaz-Uriarte and Garland Jr, 1996). Because phylogenetic relationships affects correlations and covariances among traits, we expected that correlation tests would be more sensitive to phylogenetic dependence than regression tests.

The use of a Monte-Carlo resampling procedure to simulate within-species sampling error influenced greatly the results for the regression analysis, inflating type I error rates for low sample sizes and generally reducing power, except when strength of selection was high (large effect sizes e) and samples were large (Figures 4.2 and 4.3). This is in clear contrast to what was reported by Ackermann and Cheverud (2002), in which type I error rates were deemed adequate even for low samples. Our simulations shows that when we do not resample the $\mathbf{W}$ matrix we obtain adequate type I error rates. So, if Ackermann and Cheverud (2002) performed simulations without accounting for sampling error on $\mathbf{W}$, than that would explain the results reported by them.

The fact that sampling error effect type I error rates and power might be related to the estimation of the eigenvalues of $\mathbf{W}$. Because Ordinary Least-Square regressions only models error on the dependent variable (the vari-

ances $var(PC_i)$ of $\mathbf{B}$ in this case, Equation (4.4)), the introduction of error on the independent variable (eigenvalues λ_i of $\mathbf{W}$, Equation (4.4)) can be seen as violating OLS model assumptions, making estimates of confidence interval and significance inaccurate (Sokal and Rohlf, 1995).

Analysis performed on PIC data outperforms non-PIC analysis by having adequate type I error rates and by having higher power, a fact more evident for regression analysis (Figures 4.2 and 4.3). Even though Revell (2007) reached a similar result, in that case the author was testing the matrix correlation between the full $\mathbf{G}$ and $\mathbf{B}$, which includes both variances and covariances among traits. For the regression test as present here, however, covariances between PCs (traits) are not included in the analysis, and the use of PIC should not influence the result of the test. This is because phylogenetic relationships are thought to introduce covariance among traits, but should not influence the trait variances under drift (Hansen and Martins, 1996). However, Rohlf (2006) have shown that the task of comparative methods, specifically of General Least Squares and PIC, is to improve estimates of statistical parameters on phylogenetically structure data. In other words, statistical dependency imposed by the phylogenetic structure increases the standard error of the estimated variances of $\mathbf{B}$, a fact that, when combined with sampling error on $\mathbf{W}$, helps to disrupt the expected proportionality between λ_i and $var(PC_i)$. Using comparative methods might help quantitative genetic analysis by providing a better estimate of not only covariances, but also of variances of $\mathbf{B}$ (Revell, 2007).

Correlation analysis showed the most drastic difference between analysis of raw and PIC data. While analysis of raw data showed inadequate type I error rates for almost all sample sizes, PIC analysis showed nominal 5% levels for all samples (Figure 4.2), a well known result for the analysis of correlation on phylogenetically structured data (Díaz-Uriarte and Garland Jr,

1996). This is further corroborated by the fact that if we apply transformations on the tree in order to make it more "star-like" (*i.e.* make tips more independent of each other), type I error rates for raw data analysis falls as a function of the transformation parameter (Figure E.1). This is consistent for what was found by Revell (2007), where linearly decreasing speciation rates (a "star-like" tree) resulted in similar values for correlations between **B** and **G** both with PIC or raw data.

For regression analysis the reduction of the number of PCs can help to bring type I errors down to nominal levels for regression analysis (Figure 4.4). Since PCs associated with lower eigenvalues are thought to contain potential source of error and noise (Strauss, 2010; Mitteroecker and Bookstein, 2011; Marroig et al., 2012), particularly for small sample sizes, the reduction of the number of PCs might remove potential sources of noise and within-group error. Additionally this could help in the analysis of groups lacking a phylogeny: by adjusting the number of PCs, one could obtain adequate type I error rates for the analysis even for analysis of raw data. Thus, regression analysis can be a very important tool in the preliminary investigation of natural selection for under-studied groups.

The removal of PCs, however, can lead to lower power for the regression analysis, imposing a trade-off between type I error and power for low sample sizes. In this context, the use of PIC is particularly advantageous because, despite presenting the same trade-off, it allows the retention of more PCs in order to have adequate type I error. For example, to obtain nominal levels of type I error in raw data we would have to retain approximately 10 PCs, what would lead to a maximum power of 0.6, while with PIC we could retain 20 PCs to have the same type I error, leading to a maximum power of 0.8. So even though regression analysis can be performed in the absence of phylogenetic information, the use of comparative methods can greatly

508 improves the results.

509 Curiously, the removal of more PCs effects correlation analysis by in-
510 creasing average power, not lowering it (Figure 4.4). This might be the be-
511 cause, as the number of variables increases in relation to sample size, power
512 goes down. Thus, the reduction of PCs for correlation analysis suggested
513 by Marroig and Cheverud (2004) might not be justified in order to reduced
514 type I errors (which are unchanged for different number of retained axis,
515 Figure 4.4) but to increase power. Also, differently from the regression anal-
516 ysis, correlation analysis should not be performed in the absence of phylo-
517 genetic information, independently of how many PCs are retained.

518 Correlation analysis were particularly sensitive to fluctuations in N_e (Fig-
519 ure 4.5). The higher the fluctuation in N_e , the larger the inflation of type
520 I error rates, specially for PIC data. One premise of the use of PIC is that
521 branch lengths will be proportional to the expected divergence between
522 species (Felsenstein, 1985). Even though it is usually assumed that expected
523 divergence is directly proportional to time under drift, this is evidently not
524 true when N_e fluctuates (Equation (4.1)). In those cases, PIC estimates will
525 be imprecise because the standardization by time will not translate into a
526 standardization by expected divergence.

527 In our simulations, as N_e fluctuation increases, the mismatch between
528 branch lengths expressed in years and the expected amount of evolution
529 will go up, reflecting in a inflated type I error for correlation tests on PIC.
530 Correlation analysis on raw data seems to be less affected by fluctuations
531 in N_e because of inflated type I errors to begin with. When N_e fluctuation is
532 high, error I rates resemble the rate of rejection of the null hypothesis when
533 we introduce selection for both raw and PIC data (Figure 4.3), probably be-
534 cause we are altering rates of evolution, mimicking the effects of selection
535 (Garland, 1992; Martins et al., 2002), rendering correlation tests inadequate

in this case.

Even though it has been long shown that violation of the underlying model leads to a poor performance of comparative methods (Díaz-Uriarte and Garland Jr, 1996; Garland, 1992; Martins et al., 2002), the use of single-rate models can be justified by assuming a mutation-drift-equilibrium model where the variance added by mutation and the amount of variance lost by drift are equal. In that case we can rewrite Equation (4.1) as

$$\mathbf{B} = \mathbf{M}2t \quad (4.6)$$

where $\mathbf{M}$ is a covariance matrix of mutational effects. This has the convenient propriety of making the rate of evolution independent of population size (Lande, 1979, 1980; Felsenstein, 1988; Lynch, 1989; Hansen and Martins, 1996), thus making the use of quantitative genetics tests of proportionality adequate even in the presence of demographic variation.

In the absence of selection, equilibrium can be reached after $4N_e$ generations irrespective of initial conditions (Turelli et al., 1988). For Caniformes, if we take female sexual maturity as a minimum benchmark for generation time, we would have a maximum (conservative) values of 6.6 years (obtained from the AnAge database; Tacutu et al., 2012). Furthermore, if we assume an average N_e of 200000, than Caniformes clades would have reached equilibrium at a maximum age of 1.3 million years. Even though this is not a problem for the analysis of the Caniform families evaluated here (Table 4.2), it can pose an issue for less inclusive and younger taxa, such as tribes and genus, or even larger clades if N_e or generation time are high. In cases where the populations have not reached equilibrium, the regression test might be an alternative because, differently from the correlation test, regression tests were surprisingly robust even for large fluctuations of N_e , showing similar type I error rates for all values of σ (Figure 4.5).

The investigation of the Caniform families confirms the expectations from the simulations. For both tests, analyses performed on PIC data reject drift in less cases than analysis on raw data, as would be expected by the lower type I error of the former Table 4.2. For correlation data of PIC, both parametric and non-parametric methods produced very similar results Table 4.3, probably because the non-parametric procedure for correcting for multiple tests was adequate for PIC data. Parametric and non-parametric correlation tests of raw data, on the other hand, differed greatly, both in number and in what correlations were found Table 4.3. This suggests that the use of simulations produce confidence intervals of the null hypothesis that are too wide to be reliable for raw data. For regression analysis, on the other hand, simulations presented narrower confidence intervals, rejecting drift in more cases than the parametric tests Table 4.2. This further reinforces the idea that OLS regressions might not be adequate to test proportionality between **B** and **W** because it fails to construct adequate confidence intervals for the null hypothesis. Since models for phenotypic evolution required for the test of quantitative genetics are simple and readily implementable (*i.e.* brownian motion Lande, 1976), simulations for regression analysis should always be preferred when a phylogeny is available.

Carnivora in general is considered a test case for adaptive evolution due to its diversity of ecology and associated morphology (Goswami, 2010). Thus, the fact that we failed to identify deviation of drift for two families, Mephitidae (skunks) and Ursidae (bears), is worth further attention.

Mephitidae skull adaptations has received little attention. Despite the fact that the family has been included in ecomorphological analysis of more inclusive taxa (Frischia et al., 2007; Van Valkenburgh, 2009; Figueirido et al., 2010; Schiaffini and Prevosti, 2013; Tseng and Flynn, 2015; Dumont et al., 2016), skunks are usually described as having a similar omnivore feeding

ecology. Furthermore, no previous work on the group have included the most morphologically divergent genus, the *Mydaus*, probably because its placement within Mephitidae was controversial till recently (Sato, 2016). For these reasons it is unclear how we can interpret the results presented here for this family. It could be the case that, because skunks are ecologically and morphologically conserved, the signal of selection might be difficult to detect. Further studies of the ecomorphology of this family will surely help to elucidate these issues.

Ursidae is a species poor family that has a considerable diversity in feeding ecology, from omnivorous diet (*U. arctos*, *U. thibetanus*, *U. americanus*, *H. malayanus*), insectivores (*M. ursinus*), hypercarnivores (*U. maritimus*) and herbivores (*A. melanoleuca*, *T. ornatus*). The fact that our PIC analysis suggests that the evolution of the skull in bears is consistent with drift is at odds with other interpretations of the association between morphology of the skull and feeding ecology (e.g. Figueirido and Soibelzon, 2009; Figueirido et al., 2009, 2010, 2011b, 2013). However, it has been shown that mandibular and dental morphology have stronger ecomorphological signal than cranial traits specifically for bears (Sacco and Van Valkenburgh, 2004; Christiansen, 2008; van Heteren et al., 2015) and for Carnivores in general (Figueirido et al., 2011a). Additionally, some aspects of bear skull morphology can be present in species that uses very different food sources (e.g. large diastema in the carnivorous *U. maritimus*, a trait associated with increased selection and manipulation of vegetal matter, Mattson, 1998), indicating a generally well adapted skull shape for a variety of habits. Alternatively, adaptations to specific food sources can be rare and restricted to a few cases (e.g. hypercarnivores and herbivores, Figueirido et al., 2009; Slater et al., 2010; Figueirido et al., 2010, 2011a), thus making selection difficult to detect.

Both Mephitidae and Ursidae are the least diverse families analyzed

618 here. Our simulations shows that, for small sample sizes, the regression
619 analysis have an inflated type I error (Figure 4.2), making the lack of rejec-
620 tion of the null hypothesis for both families even more surprising. However,
621 small samples are associated with low power, something that is particularly
622 problematic when strength of selection is small (Figure 4.3). Therefore, the
623 combination of low samples and weak (or at least rare) selection could make
624 the detection of natural selection through these methods difficult. The ad-
625 dition of fossil species and the analysis of other traits (*e.g.* mandibular and
626 dental morphology) might help to clarify this issue.

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Capítulo 5

Conclusões

Padrão de integração em Canidae e relações com domesticação de *C. familiaris*

Na presente tese eu demonstrei que Carnivora como um todo apresenta um padrão conservado de integração morfológica (Capítulo 3). Visto que o grupo apresentam uma enorme diversidade de hábitos e estratégias alimentares, tal fenômeno provavelmente se deve à ação de seleção estabilizadora interna, resultando na manutenção das vias ontogenéticas que atuam na formação do crânio (Cheverud, 1996). Apesar desta estabilidade, foi diagnosticada uma distinção da família Canidae das demais, que são mais similares entre si e mais dissimilares em relação a espécies de outras famílias. Essa mudança está associada ao aumento da flexibilidade, integração e variância relativa na região da face das espécies de canídeos.

Tendo este tipo de resultado em mente, é difícil não pensar nas possíveis conexões do fenômeno aqui detectado e a diversidade morfológica no cachorro doméstico *Canis familiaris*. Excetuando-se a variação extrema de tamanho e aspectos da morfologia "mole", como pelagem, forma de orelhas e cauda, a variação do tamanho relativo do rosto de cachorros é extrema, rivalizando a variação vista não apenas em Canidae selvagem (Wayne, 1986), mas em Carnivora como um todo (Drake e Klingenberg, 2008). O fato das espécies de Canidae apresentarem uma face mais modular, podendo responder mais diretamente à seleção natural, sugere uma possível explicação para a grande diversidade de forma craniana observada nos cachorros.

A proposta de que Canidae como um todo é estruturalmente distinto dos demais Carnivora em sua capacidade de resposta à seleção não é nova. Laidlaw et al. (2007) demonstraram que, assim como cachorros domésticos, Canidae apresentava uma taxa elevada de sequências repetitivas puras (não interrompidas por mutações pontuais) no genoma. A presença de sequên-

cias mais puras, quando encontradas em genes regulatórios, potencializa a capacidade da espécie de criar nova variação por duplicação de material genômico, variação esta que pode subsequentemente ser selecionada (Fondon e Garner, 2004; Laidlaw et al., 2007). Assim, diferenças na arquitetura genética de Canidae podem ter sido essenciais para o sucesso da domesticação de cachorros.

Evidências adicionais para a "domesticabilidade" de Canidae vem do famoso experimento com raposas de Belyaev, na antiga União Soviética e atual Rússia (Belyaev et al., 1985; Trut, 1999; Trut et al., 2009; Kukekova et al., 2008; Trut et al., 2004). Nesse experimento de 40 anos, indivíduos de *Vulpes vulpes* selvagens foram selecionados para menor agressividade, o que resultou em uma miríade de modificações fenotípicas em poucas gerações, inclusive em caracteres cranianos ligados à face (Figura 5.1). A diminuição de agressividade, medo e estresse em animais domesticados parecem estar associados à redução do tamanho e da função da glândula adrenal (Trut et al., 2009). Wilkins et al. (2014) notaram que, assim como a adrenal, todos os caracteres ligados à domesticação (compondo a chamada "Síndrome de domesticação") estão ligados ontogeneticamente ao desenvolvimento de células da crista neural. Assim, a redução do efeito destas células no desenvolvimento (seja por redução de migração das células, ou de efeitos de expressão local) poderia explicar as diversas modificações observadas em animais domesticados. Então, a modificação de caracteres da face em animais domesticados pode se dar por resposta indireta à seleção para maior docilidade, através da diminuição da glândula adrenal, mediada pela redução da influencia de células da crista neural (Wilkins et al., 2014). Apesar de evolução correlacionada de caracteres ser comum na maioria das espécies domesticadas ("Síndrome de domesticação"), Canidae (tanto cachorros quanto raposas) é o grupo que apresenta um padrão de modificações

Figura 5.1: Crânio de indivíduos selvagens (esquerda) e domesticados (direita) de *Vulpes vulpes*. Reproduzido de Trut et al. (2004).

que podem ser consistentemente correlacionadas com o desenvolvimento da crista neural (Sanchez-Villagra e Geiger, 2016). Uma maior variabilidade genética associada à genes regulatórios ligados à crista neural pode ajudar a explicar porque canídeos são mais variáveis em sua morfologia craniana.

Apesar dos canídeos selvagens viventes apresentarem certa conservação morfológica, táxons extintos apresentavam morfologias e hábitos de vida extremamente variados (Slater, 2015). A diversidade fenotípica em táxons fosseis pode ser tão extrema a ponto de produzir morfologias similares às encontradas em cachorros domésticos atuais (*e.g.* Figura 5.2), o que é particularmente impressionante, visto que cachorros domésticos são um exemplo de diversidade morfológica extrema (*e.g.* Drake e Klingenberg, 2010). Isso sugere que, de acordo com os resultados encontrados aqui, a maleabilidade morfológica de *C. familiaris* provavelmente esteve presente em toda a

diversificação de Canidae, apenas sendo coaptada recentemente para a produção de variantes domésticos. Assim, a investigação da domesticação de cachorros, e do registro fóssil podem ser fontes valiosas de informação para a compreensão da evolução de Canidae (Fondon e Garner, 2004; Laidlaw et al., 2007; Sanchez-Villagra e Geiger, 2016).

Testes de Deriva no Contexto de Métodos Filogenéticos Comparativos

Os anos recentes tem visto um número crescente de Métodos Filogenéticos Comparativos para o estudo de padrões macroevolutivos. Essa tendência tem em parte sido estimulada pelo crescimento do poder de computadores pessoais e pelo acesso a plataformas de programação versáteis, como o R (R Core Team, 2016). Isso tem encurtado o caminho entre desenvolvimentos teóricos e implementação de software, com muitos artigos sobre novos métodos sendo publicados em conjunto com implementações prontas para o uso. Entretanto, uma base teórica sólida nem sempre é o suficiente para contornar problemas de implementação e a interação dos métodos com os dados. A avaliação dos méritos e limitações de diferentes métodos e implementações, até para os classicamente estabelecidos, é essencial para o progresso do ramo de análises comparativas (Cooper et al., 2016).

Aqui eu apresentei uma investigação das propriedades estatísticas de dois métodos baseados em teoria de genética quantitativa, o de regressão e o de correlação, para o teste da hipótese de evolução neutra (Capítulo 4). Ambos os métodos se baseiam na predição teórica de que padrões de covariação intraespecíficos e interespecíficos vão ser similares sob deriva (Lande, 1979). A rejeição dessa expectativa pode ser vista como evidência de que o padrão observado não se adequa a expectativa sob deriva, abrindo

Figura 5.2: Comparação dos crânios de *C. familiaris* e *Borophagus secundus* (F:AM31004). Escala= 5cm.

a porta para seleção natural como uma hipótese explicativa (Ackermann e Cheverud, 2002, 2004; Marroig e Cheverud, 2004)

A utilização de contrastes filogenéticos independentes (PIC) claramente melhoram a performance de ambos os testes, sendo mandatório no caso do teste de correlação. Isso pode ter um impacto significativo para conclusões de trabalhos baseados neste ultimo método (*e.g.* Ackermann e Cheverud, 2002; Marroig e Cheverud, 2004), uma vez que a dependência filogenética entre as linhagens introduz correlação entre os fenótipos dos descendentes mesmo sob deriva. Por outro lado, o teste de regressão pode ser adequado sem o uso de informação filogenética através do PIC. Esse teste entretanto só apresenta erro do tipo I adequado para um número de espécies elevado, fato este que pode influenciar a conclusão de trabalhos baseados em amostras reduzidas (*e.g.* Ackermann e Cheverud, 2004; Schroeder et al., 2014).

Os resultados das simulações indicam ainda que não parece existir uma sugestão única para os interessados no estudo de evolução multivariada. Enquanto o teste de correlação apresenta erros do tipo I adequados para quase todos os números amostrais de espécie, ele é bastante impactado por potenciais flutuações no numero efetivo populacional (N_e) das espécies. Esse problema é provavelmente contornável em clados mais inclusivos, por terem tido tempo o suficiente de atingir equilíbrio entre mutação e deriva (Lande, 1979, 1980; Felsenstein, 1988; Lynch, 1989; Hansen e Martins, 1996). Porém, a medida que clados se tornam menores e mais jovens, este problema pode afetar substancialmente a conclusão deste teste. O teste de regressão, por outro lado, é mais robusto a variações de N_e , o que poderia torna-lo indicado para populações recentes. Esse teste, entretanto, apresenta erro do tipo I inflado quando poucas espécies são analisadas, podendo impedir o uso de ambos os testes em clados pequenos e jovens. O erro do tipo I inflado para o teste de regressão pode de certa forma ser contornado

pela exclusão de componentes principais de menor importância. Essa prática porém leva a uma queda do poder, o que pode impedir a detecção do sinal de seleção natural, o que também é potencialmente problemático em clados menores. Visto isso, os testes de regressão e deriva, quando empregados com cautela, podem ser ferramentas valiosas no estudo da evolução fenotípica multivariadas.

As técnicas aqui apresentadas diferem dos demais métodos comparativos pois não requerem nenhuma hipótese explícita a respeito de que tipo de fatores podem estar influenciando a evolução adaptativa do grupo. Assim, não é necessária a proposição de nenhum tipo de "nicho" ou "zona adaptativa" (*e.g.* Hansen, 1997; Butler e King, 2004), ou de variáveis independentes (*e.g.* Felsenstein, 1985; Grafen, 1989; Martins e Hansen, 1997; Hansen et al., 2008), permitindo a investigação da evolução fenotípica até mesmo quando o conhecimento a respeito do grupo é limitado. Ainda, estas técnicas não são inconsistentes com os tipos de métodos supracitados, podendo ser utilizados em conjunto para uma melhor exploração de hipóteses evolutivas (*e.g.* Simon et al., 2016).

Cada teste individual ainda apresenta peculiaridades vantajosas. O teste de regressão pode ser aplicado na ausência de informação filogenética (desde que seja ajustado o número de componentes principais), o que pode ser particularmente vantajoso em grupos sem informação filogenética precisa, como grupos fósseis. O teste de correlação, por outro lado, apresenta erro do tipo I adequados até mesmo para número de espécies muito baixos, permitindo a análise de dados com alta dimensionalidade até com clados pequenos. Assim, estas técnicas constituem um recurso pouco apreciado e com um grande potencial para a análise de dados multivariados.

Perspectivas

Os resultados da presente tese abrem dois tipos de possíveis linhas de investigação sendo um de natureza metodológica e outro de natureza empírica. Do ponto de vista metodológico as possibilidades ainda são diversas. O método de simulação filogenética empregado aqui é plástico o suficiente para expandir o arcabouço de hipóteses que podem ser explorados pelos testes de deriva. De fato, a simulação permite a construção da distribuição de estatísticas referentes à hipótese nula, mas nada nos impede de construir distribuições referentes a outras hipóteses. Isso pode ser feito não apenas alterando o padrão de seleção diretamente como um parâmetro nas equações de genética quantitativa (Equação 4.5), mas também simulando outros padrões de seleção através da modificação dos tamanhos de ramos nas árvores (Venditti et al., 2011; Baker et al., 2016). Outras direções são ainda possíveis, como a avaliação do impacto de extinções, da inclusão de espécies fósseis e de erro na estimativa da filogenia.

Em termos de questões empíricas, as considerações colocadas acima sobre o Capítulo 3 delineiam uma possível linha de pesquisa relacionada a origem da diferença em padrão de integração morfológica em Canidae, e suas possíveis interconexões com o processo de domesticação e com o registro fóssil. Ainda do ponto de vista empírico, o Capítulo 3 levanta duas possíveis lacunas de conhecimento ligadas às famílias dos cangambás (Mephitidae) e dos ursos (Ursidae). Os testes sob contrastes para ambos os grupos falharam em rejeitar a hipótese de deriva, o que levanta possibilidades sobre como esses animais se adaptaram para suas diferentes dietas atuais. Visto que ambos grupos apresentam amostras reduzidas, fato que também pode ter afetado estes resultados, a avaliação de espécies fósseis pode ser essencial para aumentar o poder dos testes e esclarecer essas questões.

Por último, análises mais aprofundadas sobre a evolução dos Carnivora

ainda são necessários. A inclusão, na presente tese, dos dados de Feliformes provenientes da dissertação de mestrado de Zahn (2016) foram essenciais para diagnosticar com clareza a distinção de Canidae dos demais Carnivora, porém muitas relações ainda ficam em aberto. Inclusive, análises preliminares de Zahn (2016) demonstraram diversas associações entre integração morfológica e fatores ecológicos que merecem uma melhor investigação em um contexto mais amplo.

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Apêndices

Apêndice A

Espécimes

Sexo

♂ Machos

♀ Fêmeas

○ Sexo indefinido

Museu

AMNH American Museum of Natural History (Nova York, Estados Unidos)

ANSP Academy of Natural Sciences of Drexel University (Philadelphia, Estados Unidos)

FCNyM Facultad de Ciencias Naturales y Museo de La plata (La Plata, Argentina)

FMNH Field Museum of Natural History (Chicago, Estados Unidos)

MACN Museo Argentino de Ciencias Naturales Bernardino Rivadavia (Buenos Aires, Argentina)

MCZ Museum of Comparative Zoology (Cambridge, Estados Unidos)

MN Museu Nacional (Rio de Janeiro, Brasil)

MPEG Museu Paraense Emilio Goeldi (Belém, Brasil)

MZUSP Museu de Zoologia da Universidade de São Paulo (São Paulo, Brasil)

USNM National Museum of Natural History of the Smithsonian Institution (Washington, Estados Unidos)

Canidae

Atelocynus microtis - FMNH 98080♂, FMNH 93955♂, FMNH 52491○, FMNH 98081♂, FMNH 60676♀, FMNH 110949♂, FMNH 60674♂, FMNH 57836○, FMNH 60675♀, MN 650○, MPEG 8866○, MPEG 4260♂, MPEG 854○, MPEG 6541♀, MPEG 4252♀, MPEG 6540♂, MVZ 136657♂, MVZ 157978♂, MZUSP 19751♂, MZUSP 19753♂, MZUSP 4320♀, MZUSP 19750○, MZUSP 19754♂.

Canis adustus - AMNH 216344♂, AMNH 116335♀, AMNH 52049♂, AMNH 81039♀, AMNH 160990♀, AMNH 116333♂, AMNH 160989♀, AMNH 160997♀, AMNH 116334♀, AMNH 114255♂, AMNH 114259♀, AMNH 54212♀, AMNH 27725♀, AMNH 179136♂, AMNH 52057♂, FMNH 27248♀, FMNH 32936♂, FMNH 83641♀, FMNH 18939♂, FMNH 18948♂, FMNH 42121♂, FMNH 38184♂, FMNH 38185♀, FMNH 95999♀, FMNH 73036♀, FMNH 95933♂, FMNH 73037♀, MCZ 44289♀, MCZ 44290♂, MCZ 37939♀, MCZ 45890♀, MVZ 149525♂, MVZ

155564♂, MVZ 118415♂, MVZ 149524♂, USNM 61759♂, USNM 470131♀, USNM 399437♂, USNM 182343♂, USNM 181488♀, USNM 182348♂, USNM 162137♂, USNM 350071♂, USNM 318095♀, USNM 299841♀, USNM 350072♀, USNM 342087♀, USNM 342085♂, USNM 342088♀.

Canis anthus - AMNH 81041♂, AMNH 81040♀, AMNH 27726♀, AMNH 27732♀, AMNH 27735♀, AMNH 27733♀, AMNH 27739♀, AMNH 187714♂, AMNH 114175♂, FMNH 27155♂, FMNH 27147♂, FMNH 27152♀, FMNH 27156♂, FMNH 27150♂, MVZ 101014♀, MVZ 173749♀, MVZ 173765♀, FMNH 140116♂, FMNH 107340♂, FMNH 107231♀, FMNH 107227♀, FMNH 107234♀, FMNH 107232♂, FMNH 107337♀, FMNH 107230♂, FMNH 107338♀, FMNH 107336♂, FMNH 140115♀, FMNH 121349♀, FMNH 107228♂, FMNH 107229♀, FMNH 140119♂, FMNH 107236♂, FMNH 140118♀, FMNH 105807♂, FMNH 140117♂, FMNH 105742♀, FMNH 106723♂, FMNH 106722♂, FMNH 105740♀, FMNH 105744♂, FMNH 106724♂, FMNH 105741♂, FMNH 105743♀, FMNH 108364♂, FMNH 140124♂, FMNH 107223♂, FMNH 75647♂, FMNH 98921♀, FMNH 89967♂, FMNH 89966♀, FMNH 107226♀, FMNH 140122♂, FMNH 96233♂.

Canis aureus - AMNH 88708♀, AMNH 88709♂, AMNH 88712♂, AMNH 54516♂, AMNH 54515♀, FMNH 103912♀, FMNH 103913♀, FMNH 103914♀, FMNH 103920♀, FMNH 103910♀, FMNH 103916♀, FMNH 29757♀, FMNH 29790♂, FMNH 29756♀, FMNH 103909♂, FMNH 112371♂, FMNH 112364♂, FMNH 57264♂, FMNH 97773♀, FMNH 112372♂, FMNH 35672♂, FMNH 83076♂, FMNH 83078♀, FMNH 91242♀, MZUSP 2568♀, AMNH 169447♀, FMNH 27158♀.

Canis dingo - AMNH 197747♂, AMNH 197746♀, AMNH 197748♂, AMNH 65848♂, AMNH 173525♂, AMNH 65847♂, AMNH 18077♂, AMNH 65845♂, AMNH 65846♂, AMNH 183434♂, AMNH 183433♀, AMNH 153629♀, AMNH 154465♂, AMNH 153664♂, AMNH 153663♂, FMNH 129799♂, FMNH 33598♀, FMNH 119851♂, FMNH 54203♂, MCZ 329♂, MCZ 8025♂, MCZ 21590♀, MCZ 21391♂, MCZ 21593♂, MCZ 28026♂, MCZ 47062♀, MCZ 21591♂, MVZ 119676♀, MVZ 119675♂, MVZ 119812♀, MVZ 152803♀, MVZ 175994♂, MVZ 133283♀, MVZ 124399♀, MVZ 129306♂, MVZ 129305♀, MVZ 119677♂, MVZ 152804♂, USNM 284435♀, USNM 270381♂, USNM 238468♀, USNM 197076♂, USNM 284437♀, USNM 254718♀, USNM 277393♀, USNM 49705♂, AMNH 151842♀, AMNH 104074♀, AMNH 151839♂, AMNH 151803♀, AMNH 151841♂.

Canis familiaris - USNM 175867♀, USNM 289972♂, USNM 38989♂, USNM 506608♀, USNM 581987♂, USNM 785♂, USNM 21995♀, USNM 21077♂, USNM 21989♂, USNM 289968♂, USNM 582945♀, USNM 200888♂, USNM 785♂, USNM 289967♀, USNM 22044♂, USNM 289973♂, USNM 241137♂, USNM 349500♀, USNM 22194♂, USNM 22141♂, USNM 122748♀, USNM 124645♀, USNM 273290♂, USNM 187987♀, USNM 272209♀, USNM 36967♂, USNM 6610♂, USNM 22308♂, USNM 6611♂, USNM 22690♂, USNM 275155♂, USNM 274104♂, USNM 347608♂, USNM 8222♂, USNM 6612♂, USNM 261035♂, USNM 34805♂, USNM 20805♂, USNM 174670♂, USNM 22252♂, USNM 22558♂, USNM 122751♀, USNM 122759♀, USNM 23224♂, USNM 36073♂, USNM 21992♂, USNM 21998♂, USNM 22563♂, USNM 16532♀, USNM 289971♂, USNM 23005♂, USNM 122749♂, USNM

187984♂, USNM 21630, USNM 35140♀, USNM 219940, USNM 270172♂, USNM 122750♂, USNM 187988♂, USNM 269321♂, USNM 1879820, USNM 251443♂, USNM 86370♀, USNM 21135♂, USNM 187976♂, USNM 187977♀, USNM 187980♂, USNM 21993♂, USNM 219970, USNM 21988♂, USNM 154310, USNM 518126♀, USNM 5145130, USNM 534281♂, USNM 505225♀, USNM 399050♀, USNM 289976♂, USNM 398215♀, USNM 3989080, USNM 289977♀, USNM 238168♂, USNM 347480♀, USNM 396991♀, USNM 3990490, USNM 347609♀, USNM 347441♀, USNM 398536♂, USNM 3500000.

Canis himalayensis - ANSP 17498♂, ANSP 17497♂, ANSP 174960, MCZ 248700, MCZ 24873♂, USNM 172654♀, USNM 198458♀, USNM 198460, USNM 172655♀, USNM 198457♂.

Canis indica - FMNH 44469♂, FMNH 44467♀, FMNH 44471♀, FMNH 44470♀, FMNH 460790, FMNH 994170.

Canis latrans - MN 32369♀, MVZ 36167♀, MVZ 35445♀, MVZ 35446♂, MVZ 37959♂, MVZ 35447♂, MVZ 35443♀, MVZ 35444♀, MVZ 112798♀, MVZ 112799♀, MVZ 35442♂, MVZ 37962♀, MVZ 3245♀, MVZ 37961♂, MVZ 3247♂, MVZ 3246♂, MVZ 37960♀, MVZ 40173♀, MVZ 40174♀, MVZ 36469♀, MVZ 44168♂, MVZ 106928♀, MVZ 70469♂, MVZ 97594♂, MVZ 97599♀, MVZ 97598♀, MVZ 97597♂, MVZ 184044♂, MVZ 97595♀, MVZ 97596♂, MVZ 21414♂, MVZ 70500♀, MVZ 21545♂, MVZ 107350♂, MVZ 16260♂, MVZ 21410♀, MVZ 70501♀, MVZ 21547♂, MVZ 25899♂, MVZ 70499♂, MVZ 21546♀, MVZ 107247♂, MVZ 34307♂, MVZ 70498♂, MVZ 70470♂, MVZ 70472♂, MVZ 70471♂, MVZ 16256♀, MVZ 51978♂, MVZ 12876♂, MVZ 16258♀, MVZ 34755♀, MVZ 37284♂, MVZ 51660♀, MVZ 16257♀, MVZ 8260♂, MVZ 84961♂, MVZ 37292♂, MVZ 51623♀, MVZ 88064♂, MVZ 37294♂, MZUSP 2570♀, MZUSP 28846♀.

Canis lupus - FMNH 7657♀, FMNH 7619♀, FMNH 21750♀, FMNH 21751♂, FMNH 128794♂, FMNH 138790♂, FMNH 138795♀, FMNH 138773♂, FMNH 138783♀, FMNH 138774♀, FMNH 138772♂, FMNH 138779♀, FMNH 138782♀, FMNH 138781♀, FMNH 13778♂, FMNH 138784♀, FMNH 128780♂, FMNH 138785♀, FMNH 138792♂, FMNH 138787♂, FMNH 138793♂, FMNH 138788♀, FMNH 138789♀, FMNH 138791♀, FMNH 138786♂, FMNH 138797♂, FMNH 150997♀, FMNH 72961♂, FMNH 72962♀, MCZ 39658♀, MCZ 50508♂, MN 32366♂, MVZ 8321♂, MVZ 59682♀, MVZ 109615♂, MVZ 5681♂, MVZ 32422♂, MVZ 57343♂, MVZ 35380♀, MVZ 76253♀, MVZ 129254♂, MVZ 47276♀, MVZ 47277♀, MVZ 74827♀, MVZ 138927♂, MVZ 74828♂, MVZ 31973 - B♂, MVZ 109614♂, MVZ 74829♀, MVZ 109617♂, MVZ 35387♂, MVZ 35381♂, MVZ 31042♀, MVZ 31043♂, MVZ 12454♂, MVZ 12455♂, MVZ 12457♂, MVZ 34228♂, MVZ 28003♀, MVZ 28004♀, MVZ 28001♂, MVZ 28002♂, MVZ 29772♀, MVZ 30515♂, MVZ 31234♂, MVZ 184046♀, MVZ 159321♀, MVZ 76254♂, MVZ 95177♂, MVZ 99746♀, MVZ 4777♂, MVZ 44166♂, MVZ 4776♂, MVZ 184047♀, MVZ 984♂, MVZ 77345♂, MVZ 123980♀, MVZ 77346♂, MVZ 77742♂, MVZ 119835♀, MVZ 88226♀, MVZ 88225♂, MVZ 123978♂, MVZ 119833♂, MVZ 84200♀, MVZ 119834♀, MVZ 119836♂, MVZ 119832♀, MVZ 125603♀, MVZ 123999♂, MVZ 122232♀, MVZ 123981♂, MVZ 123983♀, MVZ 125604♂, MVZ 123982♂, MVZ 125607♂, MVZ 122234♂, MVZ 125606♀, MVZ 122233♀, MVZ 122230♂, MVZ 122231♂, MVZ 128114♂, MVZ 224392♂, MVZ 171944♀, MVZ

33389♂, MVZ 184048♂, MZUSP 2569♂.

Canis lycaon - ANSP 2262♂, ANSP 2261♂, ANSP 2259♂, FMNH 129295♂, FMNH 129294♂, FMNH 21207♀, FMNH 153798♀, FMNH 147636♂, FMNH 147637♀, FMNH 147638♂, FMNH 147639♀, FMNH 160108♂, FMNH 147640♂, FMNH 153802♀, FMNH 140894♂, FMNH 165352♂, FMNH 160106♂, FMNH 153799♂, FMNH 160110♂, FMNH 160107♀, FMNH 51773♀, MCZ 8045♂, MCZ 326♂, MCZ 51378♀, MCZ 40936♂, MCZ 56610♀, MCZ 56611♂, MCZ 56612♀, MCZ 62506♀, USNM 140562♂, USNM 140561♂, USNM 268194♂, USNM 170566♂, USNM 168820♂, USNM 223171♀, USNM 242289♂, USNM 242290♀, USNM 180798♂, USNM 170692♂, USNM 170621♂, USNM A45561♂, USNM A45560♂, USNM 289995♀, USNM 265071♂, USNM 243395♂, USNM 347917♀, USNM 265072♂, USNM 178452♀, MVZ 77344♂, MVZ 123562♂, MVZ 77342♀, MVZ 77343♂.

Canis mesomelas - AMNH 3770♂, AMNH 233009♂, AMNH 80652♂, AMNH 233010♂, AMNH 27736♀, AMNH 27731♂, AMNH 34731♂, AMNH 34734♂, AMNH 34732♂, AMNH 187716♀, AMNH 114267♂, AMNH 187727♀, AMNH 54208♀, AMNH 54211♂, AMNH 54213♂, AMNH 54206♂, AMNH 179139♂, AMNH 179137♂, AMNH 179140♀, AMNH 54209♂, AMNH 169095♀, AMNH 114177♂, AMNH 82398♀, AMNH 114179♀, AMNH 114180♀, AMNH 205145♂, AMNH 205146♀, AMNH 187715♂, AMNH 187711♀, AMNH 114228♂, AMNH 187713♀, AMNH 187712♂, MVZ 117811♀, MVZ 117810♀, MVZ 117808♂, MVZ 118420♀, MVZ 118419♀, MVZ 117809♂, MVZ 165153♀, MVZ 165156♂, MVZ 165142♂, MVZ 165141♂, MVZ 117807♂, MVZ 117802♂, MVZ 118416♂, MVZ 165152♀, MVZ 1651157♀, MVZ 165154♀, MVZ 165158♂, MVZ 165150♀, MVZ 165140♀, MVZ 165151♀, MVZ 165145♂, MVZ 165143♂, MVZ 165147♂, MVZ 165146♂, MVZ 99398♂, MZUSP 2571♂.

Canis rufus - AMNH 5450/4609♂, AMNH 100225♀, MVZ 95809♀, MNHN 235608♀, MNHN 235607♀, MNHN 235594♂, MNHN 234438♂, MNHN 235612♂, MNHN 235595♀, MNHN 235596♀, MNHN 234417♂, MNHN 235611♂, MNHN 235613♀, MNHN 235609♂, MNHN 235599♀, MNHN 235598♀, MNHN 235597♂, MNHN 235610♂, MNHN 236536♀, MNHN 235616♂, MNHN 235614♀, MNHN 236542♀, MNHN 236541♀, MNHN 236539♂, MNHN 236538♀, MNHN 236547♀, MNHN 236546♀, MNHN 236545♀, MNHN 236544♂, MNHN 236543♀, MNHN 236552♀, MNHN 236551♀, MNHN 236550♂, MNHN 236549♀, MNHN 236548♀, MNHN 242557♀, MNHN 242556♀, MNHN 242555♂, MNHN 242554♂, MNHN 236554♀, MNHN 242563♂, MNHN 242562♂, MNHN 242560♀, MNHN 242559♀, MNHN 242568♂, MNHN 242567♀, MNHN 242566♀, MNHN 242565♀, MNHN 242564♂.

Canis simensis - AMNH NA(214799), AMNH 214798♀, AMNH 81001♂, FMNH 8170♂, FMNH 27010♀, FMNH 28492♂, FMNH 27012♀, FMNH 32940♂, FMNH 27011♂, MCZ 38289♂.

Cerdocyon thous - MZUSP 2480♂, MZUSP 2470♀, MZUSP 3340♂, MZUSP 3103♂, MZUSP 6636♂, MZUSP 2481♂, MZUSP 2478♀, MZUSP 3183♂, MZUSP 4152♂, MZUSP 2476♀, MZUSP 19741♂, MZUSP 2472♂, MZUSP 2917♂, MZUSP 486♀, MZUSP 487♂, MZUSP 1165♂, MZUSP 2831♀, MZUSP 8903♂, MZUSP 856♂, MZUSP 1164♂, MZUSP 4135♂, MZUSP 1166♂, MZUSP 29019♂, MZUSP

2919♀, MZUSP 31060♂, MZUSP 2833♀, MZUSP 2835♀, MZUSP 2831♀, MZUSP 3763♀, MZUSP 3762♀, MZUSP 453♂, MZUSP 2834♂, MZUSP 13797♀, MZUSP 9011♀, MZUSP 32617♀, MZUSP 3097♀, MZUSP 3037♂, MZUSP 3038♂, MZUSP 3041♂, MZUSP 4220♂, MZUSP 3030♀, MZUSP 3040♀, MZUSP 3102♂, MZUSP 3045♀, MZUSP 2918♂, MZUSP 2922♂, MZUSP 2921♂, MZUSP 3101♂, MZUSP 3031♂, MZUSP 3486♂, MZUSP 19739♂, MZUSP 3100♂, MZUSP 3033♂, MZUSP 3034♀, MZUSP 3098♀, MZUSP 3032♂, MZUSP 3039♀, MZUSP 3104♀, MZUSP 3042♀, MZUSP 3035♂, MZUSP 6640♂, MZUSP 4216♂, MZUSP 4217♂, MZUSP 19742♂, MZUSP 19738♂, MZUSP 3777♂, MZUSP 4215♂, MZUSP 4221♀, MZUSP 7028♂, MZUSP 3372♂, MZUSP 3373♂, MZUSP 6315♂, MZUSP 19757♂, MZUSP 2599♀, MZUSP 2601♂, MZUSP 2600♂, MZUSP 2595♀, MZUSP 2602♀, MZUSP 3504♂, MZUSP 2598♀, MZUSP 2596♂, MZUSP 6314♂, MZUSP 1933♀, MZUSP 19793♀, MZUSP 9686♂, MZUSP 8732♂, MZUSP 2757♂, MZUSP 19755♀, MZUSP 518♂, MZUSP 514♂.

Chrysocyon brachyurus - FCNyM 2.IV.02.4♂, FCNyM 1686♂, FCNyM 5.X.99.1♂, FCNyM 1684♀, FCNyM 564♂, FCNyM 695♂, FCNyM 6♂, FCNyM 2024♀, MACN 24201♂, MACN 24043♂, MACN 23983♂, MACN 19146♂, MACN 371♂, MACN 5349♂, MACN 30198♂, MACN 373♂, MACN 23984♂, MACN 20646♂, MACN 3029♂, MACN 23456♂, MACN 13466♂, MN 3041♀, MN 70999♂, MN 69817♀, MN 71086♂, MN 68175♂, MN 4815♂, MN 36666♂, MN 4899♀, MN 69817♀, MN 70999♂, MN 71086♂, MN 640♂, MN 664♂, MN 17501♂, MN 1049♂, MN 17502♂, MPEG 1419♀, MVZ 120924♀, MVZ 119566♂, MZUSP 32629♀, MZUSP 32505♂, MZUSP 34774♂, MZUSP 3090♀, MZUSP 32041♀, MZUSP 32044♀, MZUSP 32503♂, MZUSP 32042♀, MZUSP 32043♂, MZUSP 19733♀, MZUSP 525♂, MZUSP 32199♂, MZUSP 32039♂, MZUSP 32056♂, MZUSP 31981♂, MZUSP 28870♀, MZUSP 9420♂, MZUSP 19732♂, MZUSP 2479♂, MZUSP 3025♀, MZUSP 3700♂, MZUSP 3338♂, MZUSP 19736♂.

Cuon alpinus - AMNH 60775♂, AMNH 54544♀, AMNH 54842♂, AMNH 54984♂, AMNH 101882♂, AMNH 101773♀, AMNH 102083♀, ANSP 15276♂, ANSP 17499♂, FMNH 36001♂, FMNH 33500♂, FMNH 91246♂, FMNH 39182♂, FMNH 35814♂, FMNH 35813♀, MCZ 19566♂, MCZ 30382♂, MCZ 35929♂, MCZ 35919♀, MNHN 83522♂, MNHN 196976♂.

Lycalopex culpaeus - FCNyM 5.III.36.9♀, FCNyM 1620♀, FCNyM 1749♂, MACN 15096♂, MACN 15090♀, MACN 15092♂, MACN 15112♀, MACN 15107♂, MACN 15097♀, MACN 15102♂, MACN 15105♀, MACN 15110♂, MACN 15115♀, MACN 15086♂, MACN 15114♀, MACN 15099♂, MACN 15093♀, MACN 15085♂, MACN 15095♀, MACN 15104♀, MACN 15108♂, MACN 15088♀, MACN 15098♀, MACN 15101♂, MACN 15106♀, MACN 15056♀, MACN 15053♂, MACN 15068♀, MACN 15059♂, MACN 15081♂, MACN 15084♂, MACN 15070♂, MACN 15076♀, MACN 15069♂, MACN 15073♀, MACN 15082♂, MACN 15083♀, MACN 15054♂, MACN 15067♂, MACN 15077♀, MACN 15235♀, MACN 2229♂, MACN 15238♂, MACN 15221♀.

Lycalopex fulvipes - FMNH 23814♀, FMNH 23815♂.

Lycalopex griseus - FCNyM 1892♂, FCNyM 1889♂, FCNyM 1903♂, FCNyM 1890♂, FCNyM 1893♂, FCNyM 712♂, FCNyM 451♂, FCNyM 21.XII.01.27♂, FCNyM 1899♂, FCNyM 1901♂, FCNyM 240♂, FCNyM 559♂, FCNyM 441♂,

FCNyM 701♂, FCNyM 696♂, MACN 20208♀, MACN 50419♀, MACN 50420♀, MACN 50490♀, MACN 15263♂, MACN 15262♂, MACN 15269♀, MACN 15265♂, MACN 14540♂, MACN 15264♀, MACN 15186♂, MACN 15189♂, MACN 20814♂, MACN 13781♀, MACN 20206♀, MACN 20205♀, MACN 20207♂, MACN 36480♀, MACN 2320♂, MACN 20278♀, MACN 23728♀, MACN 741♀, MACN 23663♀, MACN 23662♂, MACN 23729♀.

Lycalopex gymnocercus - MACN 2753♂, MACN 54133♀, MACN 14409♀, MACN 15390♀, MACN 15389♂, MACN 15388♂, MACN 15364♂, MACN 532♀, MACN 50443♀, MACN 24472♂, MACN 13299♀, MACN 2935♂, MACN 13313♂, MACN 39194♀, MACN 49160♀, MACN 49148♂, MACN 49139♂, MACN 49134♀, MACN 49149♀, MACN 35241♀, MACN 50504♀, MACN 50494♂, MACN 50495♂, MACN 50501♂, MACN 49159♀, MACN 49167♂, MACN 50502♂, MACN 50500♀, MACN 50491♀, MACN 50503♀, MACN 15601♂, MACN 13327♂, MACN 13331♂, MACN 14386♀, MACN 15758♂, MACN 15759♂, MACN 15769♀, MACN 15768♂, MACN 15765♂, MACN 15770♀, MACN 15767♀, MACN 15772♂, MACN 15766♂, MACN 15771♀, MN 2355♀.

Lycalopex sechurae - AMNH 46528♂, AMNH 46527♀, AMNH 46529♂, AMNH 46531♀, AMNH 46533♂, AMNH 63709♂, AMNH 46532♀, AMNH 46530♂, AMNH 46525♂, FMNH 80953♂, FMNH 80955♂, FMNH 21129♂, FMNH 80954♀, FMNH 19971♀, FMNH 19541♂, FMNH 19972♀, FMNH 80962♀, FMNH 80963♀, FMNH 80956♀, FMNH 80958♂, FMNH 80961♀, FMNH 80960♂, FMNH 80959♂, FMNH 80964♀, FMNH 80957♀, FMNH 80968♀, FMNH 80965♂, FMNH 80969♂, FMNH 80966♂, FMNH 53913♂, FMNH 80967♂, FMNH 53911♂, USNM 121172♀, USNM 181150♂, USNM 121171♀, USNM 545110♂, USNM 545109♂.

Lycalopex vetulus - AMNH 133928♂, AMNH 133926♂, AMNH 133927♂, AMNH 133937♂, AMNH 133929♂, AMNH 70091♀, AMNH 245699♂, AMNH 349♂, AMNH 391♂, AMNH 100100♀, AMNH 100091♂, FMNH 20747♂, MCZ NA♂, MN 651♂, MN 71047♂, MN 71093♂, MN 4909♂, MN 5151♀, MN 71112♀, MN 71047♂, MN 71160♂, MN 71122♂, MZUSP 30588♂, MZUSP 30589♀, MZUSP 30597♂, MZUSP 30598♀, MZUSP 825♀, MZUSP 3047♂, MZUSP 3048♂, MZUSP 1084♂, MZUSP 3050♀, MZUSP 1016♂, MZUSP 1018♀, MZUSP 1075♂, MZUSP 3046♂, MZUSP 1012♂, MZUSP 1011♀, MZUSP 3049♀, MZUSP 1014♂, MZUSP 1015♀.

Lycaon pictus - AMNH 83128♂, AMNH 83129♂, AMNH 27672♂, AMNH 114243♂, AMNH 114248♂, AMNH 114253♀, AMNH 114250♀, AMNH 1142247♂, AMNH 114249♂, AMNH 114252♀, AMNH 114251♀, AMNH 114246♂, AMNH 9038♀, AMNH 82080♂, AMNH 82082♂, AMNH 82081♀, AMNH 82085♂, AMNH 82083♂, AMNH 82084♂, AMNH 82086♂, AMNH 82087♀, AMNH 82088♀, AMNH 85254♂, AMNH 81814♂, AMNH 81813♂, AMNH 81847♂, AMNH 81853♂, AMNH 81852♂, AMNH 101691♀, AMNH 150153♂, AMNH 164162♂, FMNH 35123♂, FMNH 33478♂, FMNH 20770♂, FMNH 35121♂, FMNH 35122♂, FMNH 127812♂, FMNH 20769♂, FMNH 127813♀, FMNH 24326♀, FMNH 32931♀, FMNH 32930♀, FMNH 32932♀, FMNH 34588♂, FMNH 34587♂, MCZ 13233♂, MVZ 184049♂, MVZ 124258♂, MVZ 4842♂, MVZ 117806♂.

Nyctereutes procyonoides - AMNH 22792♀, AMNH 84342♀, AMNH 43142♂,

AMNH 43140♀, AMNH 59323♂, AMNH 60193♂, AMNH 59322♀, AMNH 38326♂, AMNH 45333♀, AMNH 58370♀, AMNH 84438♂, AMNH 57340♀, AMNH 60123♀, AMNH 57113♂, AMNH 184572♂, AMNH 249766♀, AMNH 248767♀, FMNH 59013♂, FMNH 33614♂, FMNH 57169♀, FMNH 36002♂, FMNH 33616♂, MCZ 222♂, MCZ 3143♀, MCZ 24853♂, MCZ 24860♂, MVZ 119062♂, MNHN 218153♂, MNHN 240170♀, MNHN 254641♀, MNHN 255532♀, MNHN 256104♂, MNHN 256103♂, MNHN 255536♂, MNHN 290623♀, MNHN 20944♂, MNHN 260687♂, MNHN 503941♀, MNHN 270347♂, MNHN 54875♂, MNHN 522061♀, MNHN 290658♀.

Otocyon megalotis - AMNH 422960♀, AMNH 70030♂, AMNH 233012♀, AMNH 63993♂, AMNH 114♂, AMNH 42960♀, AMNH 83647♀, AMNH 169092♂, AMNH 42969♂, AMNH 114273♂, AMNH 114284♂, AMNH 114262♂, AMNH 233013♂, AMNH 114269♀, AMNH 114266♂, AMNH 114265♂, AMNH 169093♀, AMNH 114264♂, AMNH 114282♂, AMNH 179142♂, AMNH 114283♂, AMNH 114271♂, AMNH 179143♂, AMNH 114263♂, AMNH 82397♂, AMNH 161153♂, AMNH 161154♂, AMNH 161151♂, AMNH 34265♂, AMNH 161152♂, AMNH 161155♂, AMNH 85155♂, AMNH 85156♀, FMNH 1469♂, FMNH 38189♀, FMNH 85882♂, FMNH 38190♀, FMNH 38419♂, FMNH 73043♀, FMNH 73041♂, FMNH 127816♂, FMNH 127814♂, FMNH 127820♂, FMNH 127815♀, FMNH 73040♂, FMNH 73042♀, FMNH 43334♂, MVZ 118425♂, MVZ 184051♂, MVZ 99399♂, MVZ 173742♀.

Speothos venaticus - AMNH 98640♂, AMNH 184688♂, AMNH 136285♂, AMNH 37472♂, AMNH 175306♂, AMNH 76805♀, AMNH 76806♀, AMNH 76035♂, AMNH 98558♂, AMNH 98560♀, AMNH 167846♂, AMNH 98559♀, FMNH 87861♂, FMNH 121544♂, FMNH 60290♀, FMNH 125402♂, MACN 33154♂, MACN 16510♂, MACN 5067♂, MCZ 41096♂, MCZ 42086♀, MCZ 28056♀, MN 25670♂, MN 3033♂, MN 25668♂, MN 3035♂, MN 68179♂, MPEG 5614♂, MPEG 1780♂, MPEG 1042♂, MPEG 6535♂, MPEG 6536♂, MPEG 8794♀, MPEG 1394♀, MPEG 1778♂, MPEG 111♀, MPEG 5615♀, MPEG 8793♂, MPEG 26180♂, MVZ 126276♂, MVZ 184054♂, MZUSP 19743♂, MZUSP 19744♂, MZUSP 2685♂, MZUSP 2684♀, MNHN 270370♀, MNHN 270368♂, MNHN 544414♀, MNHN 179047♀, MNHN 521045♀, MNHN 307650♀, MNHN 270165♀, MNHN 538307♀, MNHN 314048♂, MNHN 253504♂, MNHN 582465♂, MNHN 398030♀, MNHN 395841♂.

Urocyon cinereoargenteus - MVZ 115415♂, MVZ 2316♀, MVZ 3681♂, MVZ 49520♀, MVZ 2304♂, MVZ 110884♀, MVZ 2327♀, MVZ 49516♂, MVZ 7096♂, MVZ 121587♂, MVZ 2325♀, MVZ 7095♂, MVZ 8828♀, MVZ 19080♂, MVZ 2330♀, MVZ 2328♂, MVZ 90628♀, MVZ 90627♂, MVZ 94841♂, MVZ 27868♀, MVZ 94840♂, MVZ 121588♀, MVZ 3250♂, MVZ 16249♀, MVZ 3959♀, MVZ 81326♀, MVZ 81325♂, MVZ 94843♂, MVZ 37955♀, MVZ 37958♂, MVZ 2331♀, MVZ 37957♀, MVZ 4536♀, MVZ 37953♂, MVZ 42783♀, MVZ 37954♂, MVZ 37628♀, MVZ 4540♂, MVZ 27869♂, MVZ 7093♂, MVZ 94842♂, MVZ 4539♂, MVZ 4538♂, MVZ 3249♂.

Urocyon littoralis - MVZ 52148♀, MVZ 47295♀, MVZ 113602♀, MVZ 113601♂, MVZ 38929♂, MVZ 52147♂, MVZ 47296♂, MVZ 31200♂, MVZ 31199♂, MVZ 31198♀, MVZ 31197♀, MVZ 7098♀, MVZ 7097♂, MVZ 109863♀, MVZ 39925♂,

MVZ 133162♂, MVZ 133161♀, MVZ 109865♀, MVZ 169864♀, MVZ 109866♀, MVZ 38360♀, MVZ 38259♂, MVZ 38358♀, MVZ 38357♀, MVZ 38356♀, MVZ 38355♀, MVZ 38352♀, MVZ 38351♂, MVZ 38353♂, MNHN 15663♂, MNHN 188081♀, MNHN 188080♀, MNHN 45229♀, MNHN 188086♂, MNHN 188083♀, MNHN 188082♀, MNHN 188085♂, MNHN 188084♂, MNHN 188087♂, MNHN 47118♂, MNHN 12548♂, MNHN 307394♂, MNHN 307393♀, MNHN 47111♀, MNHN 3073396♀, MNHN 47115♂, MNHN 47114♂, MNHN 47113♂, MNHN 47112♂, MNHN 92033♂, MNHN 92029♂, MNHN 60674♀, MNHN 92030♀, MNHN 20784♀, MNHN 60673♀.

Vulpes bengalensis - AMNH 54517♂, AMNH 54517♂, AMNH 54526♀, FMNH 83083♀, FMNH 94095♂, FMNH 105550♀, MNHN 38577♂, MNHN 327159♂, MNHN 8552♂, MNHN 257970♀.

Vulpes chama - AMNH 148759♂, AMNH 81760♂, FMNH 85881♀, FMNH 38421♂, FMNH 38422♀, MCZ 20077♂, MCZ 37021♀, MNHN 296103♂, MNHN 470140♂, MNHN 384117♀, MNHN 368439♀, MNHN 296101♀, MNHN 296102♀, MNHN 368438♀, MNHN 429120♀, MNHN 368437♂, MNHN 253245♂, MNHN 253403♂, MNHN 469835♀, MNHN 470141♂, MNHN 469834♂, MNHN 469836♀, MNHN 469833♀, MNHN 469831♂, MNHN 469832♀.

Vulpes corsac - ANSP 12094♂, MCZ 41148♂.

Vulpes ferrilata - ANSP 17502♂, ANSP 17506♂, ANSP 17503♀, ANSP '7504♀, FMNH 142276♂, MCZ 38088♂.

Vulpes lagopus - AMNH 34518♂, AMNH 34517♀, AMNH 34519♂, AMNH 34520♂, AMNH 34521♂, MVZ 123987♂, MVZ 116914♀, MVZ 40072♂, MVZ 40073♀, MVZ 40076♂, MVZ 40077♂, MVZ 40078♂, MVZ 40079♂, MVZ 40080♀, MVZ 40081♂, MVZ 40082♂, MVZ 40083♂, MVZ 40084♀, MVZ 40085♀, MVZ 40087♀, MVZ 40074♀, MVZ 40086♂, MVZ 40088♂, MVZ 40089♂, MVZ 40090♂, MVZ 40091♀, MVZ 40092♂, MVZ 40094♂, MVZ 40095♂, MVZ 40096♀, MVZ 40097♂, MVZ 43267♂, MVZ 134450♂, MVZ 43269♀, MVZ 43268♂, MVZ 43270♂, MVZ 43271♀, MVZ 43272♀, MVZ 43273♀, MVZ 43274♀, MVZ 43275♂, MVZ 43276♀, MVZ 43277♂, MVZ 43278♂, MVZ 43279♂, MVZ 43280♂, MVZ 43281♀, MVZ 43288♂, MVZ 43283♂, MVZ 46713♂, MVZ 46715♀, MVZ 46714♂.

Vulpes macrotis - MVZ 10582♀, MVZ 61937♀, MVZ 61938♀, MVZ 61939♀, MVZ 61940♂, MVZ 61941♂, MVZ 51726♂, MVZ 61942♂, MVZ 84585♂, MVZ 10584♀, MVZ 10583♂, MVZ 84586♀, MVZ 10585♀, MVZ 7091♂, MVZ 25903♂, MVZ 25902♂, MVZ 114366♂, MVZ 114367♀, MVZ 2339♂, MVZ 7092♂, MVZ 92559♀, MVZ 79866♂, MVZ 57330♀, MVZ 74811♀, MVZ 74810♂, MVZ 7090♂, MVZ 25904♀, MVZ 51646♀, MVZ 51644♂, MVZ 31890♂, MVZ 31891♀, MVZ 31892♀, MVZ 31895♂, MVZ 31888♂, MVZ 31894♂, MVZ 31893♂, MVZ 28063♂, MVZ 28062♂, MVZ 28061♀, MVZ 28060♀, MVZ 31886♀, MVZ 122322♂, MVZ 31889♀, MVZ 31887♂, MVZ 28064♂, MVZ 28059♀, MVZ 28058♀, MVZ 119047♂, AMNH 19019♀, AMNH 589♂.

Vulpes pallida - AMNH 82198♂, AMNH 82295♀, AMNH 82296♀, FMNH 104722♂, FMNH 91325♀, MNHN 410817♂, MNHN 378689♀, MNHN 268464♀, MNHN 401318♂, MNHN 378690♂, MNHN 401317♂, MNHN 410918♀, MNHN 410536♂, MNHN 410533♂, MNHN 410526♂, MNHN 410532♂, MNHN 410524♂.

Vulpes rueppellii - FMNH 107333♂, FMNH 107334♂, FMNH 121350♂,

FMNH 107264♂, FMNH 140125♀, FMNH 140134♀, FMNH 140139♀, FMNH 140126♂, FMNH 121351♂, FMNH 107262♂, FMNH 106761♂, FMNH 107335♂, FMNH 140128♂, FMNH 140132♂, FMNH 140135♀, FMNH 140129♂, FMNH 140138♂, FMNH 140131♂, FMNH 20386♂, FMNH 107263♀, FMNH 140137♂, FMNH 140133♂, FMNH 140130♀, FMNH 140127♀, FMNH 107251♀, FMNH 107238♂, FMNH 107249♂, FMNH 107246♂, FMNH 106147♂, FMNH 107237♀, FMNH 107250♀, FMNH 107248♀, FMNH 107239♀, FMNH 107247♀, FMNH 140144♀, FMNH 140140♀, FMNH 140145♂, FMNH 140141♂, FMNH 140142♀, FMNH 140143♀, FMNH 140150♂, FMNH 107244♀, FMNH 107245♀, FMNH 140149♂, MNHN 321959♀, MNHN 321963♂, MNHN 321961♀, MNHN 328549♀, MNHN 154248♀, MNHN 321964♀, MNHN 321965♂, MNHN 321960♂, MNHN 401316♀, MNHN 476037♂, MNHN 476035♀, MNHN 476036♂, MNHN 401314♂, MNHN 401312♂, MNHN 401315♀.

Vulpes velox - AMNH 80127♂, AMNH 14811♂, AMNH 14810♀, AMNH 15871♂, AMNH 15870♂, AMNH 24419♂, AMNH 16609♀, MVZ 88140♂, MNHN 130059♀, MNHN 108319♀, MNHN 108318♂, MNHN 67599♀, MNHN 108260♂, MNHN 136566♀, MNHN 135129♂, MNHN 145876♂, MNHN 145875♀, MNHN 145877♂, MNHN 145874♀, MNHN 146311♂, MNHN 146312♂, MNHN 146317♀, MNHN 146313♂, MNHN 146227♂, MNHN 146226♂, MNHN 146315♂, MNHN 146318♀, MNHN 146316♂, MNHN 146321♀, MNHN 146322♀, MNHN 146323♀, MNHN 146325♀, MNHN 146324♀, MNHN 1347♀, MNHN 1343♀, MNHN 1345♂, MNHN 514948♂, MNHN 289140♀.

Vulpes vulpes - MVZ 222287♂, MVZ 222275♀, MVZ 222274♂, MVZ 222270♀, MVZ 222272♂, MVZ 222294♂, MVZ 222293♀, MVZ 222295♂, MVZ 222288♀, MVZ 222292♂, MVZ 222276♀, MVZ 222291♂, MVZ 222296♀, MVZ 222297♂, MVZ 222298♀, MVZ 222308♂, MVZ 222301♀, MVZ 222302♀, MVZ 222309♂, MVZ 222303♀, MVZ 222304♂, MVZ 222307♀, MVZ 222312♀, MVZ 222313♂, MVZ 222315♂, MVZ 222310♀, MVZ 222321♀, MVZ 222322♀, MVZ 222324♀, MVZ 222329♀, MVZ 222330♀, MVZ 222331♀, MVZ 222332♂, MVZ 222334♂, MVZ 222306♂, MVZ 222338♀, MVZ 222342♂, MVZ 222343♂, MVZ 222344♂, MVZ 222345♂, MVZ 222348♀, MVZ 222349♀, MVZ 222351♀, MVZ 222352♂, MVZ 222353♀, MVZ 222354♀, MVZ 222355♀, MVZ 222356♂, MVZ 222358♂, MVZ 222359♂, MVZ 222360♀, MVZ 222362♀, MVZ 222364♀, MVZ 222367♀.

Vulpes zerda - FMNH 140200♂, FMNH 74991♂, FMNH 140198♂, FMNH 79807♂, FMNH 140199♀, FMNH 174990♂, FMNH 140196♂, FMNH 92266♀, FMNH 93864♀, FMNH 140197♂, FMNH 93863♂, FMNH 93634♂, FMNH 93635♀, FMNH 92726♂, FMNH 92723♀, FMNH 92724♂, FMNH 84570♀, FMNH 89868♀, FMNH 91746♀, FMNH 91743♂, FMNH 89927♂, FMNH 84569♀, FMNH 89716♂, FMNH 927225♂, FMNH 107278♂, FMNH 91748♀, FMNH 106605♀, FMNH 107274♂, FMNH 107273♂, FMNH 107279♂, FMNH 140202♂, FMNH 140204♀, FMNH 100109♀, FMNH 106645♂, FMNH 140203♀, FMNH 106601♀, FMNH 140207♂, FMNH 140207♂, FMNH 140208♀, FMNH 106644♂, FMNH 107275♀, FMNH 107280♂, FMNH 89940♀, FMNH 89934♂, MVZ 184064♂.

Ursidae

Ailuropoda melanoleuca - AMNH 87242o, AMNH 147746♀, AMNH 110454o, AMNH 110452o, AMNH 110451♀, AMNH 89029o, AMNH 89030♀, ANSP 21719o, ANSP 15050o, FMNH 31128♀, FMNH 36758♀, FMNH 39514o, FMNH 31128♀, USNM 258425o, USNM 258423o, USNM 259027♂, USNM 258836♂, USNM 259028o, USNM 259074♂, USNM 259029o, USNM 259400♀, USNM 259075♂, USNM 259401♂, USNM 259402♂, USNM 258834♂, USNM 258835♂, USNM 399447o, USNM 259076♀.

Helarctos malayanus - AMNH 35484♀, AMNH 116♀, AMNH 296♀, AMNH 35364♀, AMNH 28254♀, AMNH 103987♀, AMNH 60772♂, ANSP 20235♀, ANSP 19844♀, ANSP 3151♂, ANSP 12933♀, ANSP 13403♂, MVZ 4816♀, MVZ 4162♀, USNM 197254♀, USNM 153839♂, USNM 123138♂, USNM 153835♀, USNM 145580♂, USNM 145580♂, USNM 153836♀, USNM 153837♂, USNM 198715♀, USNM 142344♂, USNM 198713♂, USNM 151866♂, USNM 198714♀, USNM 197255♀, USNM 153838♂, USNM 358645♀, USNM 291756♂.

Melursus ursinus - AMNH 22720♀, AMNH 22896♂, AMNH 35602♀, AMNH 35898♀, AMNH 90388♀, AMNH 54464♀, AMNH 54467♂, AMNH 54466♀, AMNH 54465♂, ANSP 2934♀, ANSP 21577♂, ANSP 4231♂, ANSP 11827♀, ANSP 12946♀, ANSP 11973♀, ANSP 12797♀, FMNH 99524♂, FMNH 99523♂, FMNH 30253♂, FMNH 32750♀, FMNH 27442♂, FMNH 27441♀, USNM 199518♀, USNM 253300♀, USNM 253301♀.

Tremarctos ornatus - AMNH 217682♂, AMNH 70164♀, AMNH 256812♂, AMNH 149303♀, AMNH 42473♀, AMNH 67732♂, AMNH 149302♀, ANSP 19163♂, FMNH 47052♂, FMNH 20311♀, FMNH 85499♂, FMNH 85498♂, FMNH 41294♂, FMNH 78678♀, FMNH 88488o, FMNH 123369♀, FMNH 134484♀, FMNH 142010♀, MACN 17831♂, MVZ 124111♂, USNM 210323♂, USNM 210322♀, USNM 271418♂, USNM 271421♂, USNM 271419♂, USNM 271420♂, USNM 210324♂, USNM 282620♀, USNM 582002♀, USNM 194309♀, USNM 443519♀, USNM 155575o, USNM 170656o, USNM 170657♀.

Ursus americanus - MVZ 120840♂, MVZ 18980♀, MVZ 19705♀, MVZ 20746♂, MVZ 10504♂, MVZ 133134o, MVZ 4723♂, MVZ 13764♀, MVZ 19233♂, MVZ 38338♂, MVZ 81580♂, MVZ 30117♂, MVZ 81581♂, MVZ 27993♂, MVZ 24331♂, MVZ 162979♀, MVZ 114216♂, MVZ 162978♀, MVZ 162984♀, MVZ 162982♀, MVZ 16269♂, MVZ 162985♂, MVZ 99741♂, MVZ 128921♂, MVZ 103898♂, MVZ 184150♂, MVZ 4763♂, MVZ 31017♂, MVZ 71805♂, MVZ 81376o, MVZ 162996♀, MVZ 163008♂, MVZ 163006♀, MVZ 163009♀, MVZ 163001♂, MVZ 163004♀, MVZ 163005♂, MVZ 163002♂, MVZ 163003♀, MVZ 68843♂, MVZ 16375♂, MVZ 30116♂, MVZ 14712♀, MVZ 68854♀, MVZ 162998♀, MVZ 162997♀, MVZ 21399♀, MVZ 162994♀, MVZ 162990♀, MVZ 162988♀.

Ursus arctos - MVZ 213♂, MVZ 4728♂, MVZ 4704♂, MVZ 437♀, MVZ 216♂, MVZ 4725♂, MVZ 217♂, MVZ 214♂, MVZ 215♂, MVZ 4774♂, MVZ 221♀, MVZ 218♂, MVZ 9522♂, MVZ 9521♀, MVZ 8942♀, MVZ 8945♂, MVZ 4385♂, MVZ 184151♂, MVZ 4699♂, MVZ 4364♀, MVZ 324♀, MVZ 323♂, MVZ 9523♀, MVZ 4363♂, MVZ 123990♂, MVZ 93882♂, MVZ 281♀, MVZ 251♂, MVZ 4354♂, MVZ 4386♂, MVZ 4356♀, MVZ 4355♂, MVZ 4357♂, MVZ 4692♀, MVZ 970♂, MVZ 971♀, MVZ 129392♂, MVZ 220♀, MVZ 4333♀, MVZ

4753♀, MVZ 125610♂, MVZ 222♂, MVZ 4724♂, MVZ 46628♂, MVZ 43895♂, MVZ 100057♂, MVZ 46638♂, MVZ 100056♂, MVZ 100058♀, MVZ 46637♂, MVZ 100060♂, MVZ 46633♀, MVZ 46642♀, MVZ 100049♀, MVZ 46641♀, MVZ 100050♀, MVZ 100045♀, MVZ 100051♂, MVZ 100048♂, MVZ 100046♂, MVZ 100047♀, MVZ 46631♀, MVZ 46636♀, MVZ 100052♂, MVZ 46625♀, MVZ 8857♂.

Ursus maritimus - AMNH 15685♂, AMNH 19260♀, AMNH 215283♀, AMNH 19508♂, AMNH 75245♂, AMNH 75244♂, AMNH 14053♂, AMNH 292♂, AMNH 34421♀, AMNH 293♂, AMNH 23792♂, AMNH 238789♂, AMNH 238793♀, AMNH 238794♂, AMNH 238799♀, AMNH 238798♂, AMNH 238802♀, AMNH 238814♀, AMNH 238807♂, AMNH 238811♀, AMNH 238810♀, AMNH 238805♂, AMNH 238809♂, AMNH 238939♂, AMNH 238940♂, AMNH 238941♂, AMNH 238946♂, AMNH 238943♂, AMNH 238944♂, AMNH 238945♂, AMNH 23895♂, AMNH 238948♂, AMNH 238947♂, AMNH 238950♀, AMNH 238953♀, AMNH 238954♀, AMNH 238673♂, AMNH 238676♂, AMNH 238679♂, AMNH 238677♂, AMNH 238883♀, AMNH 238681♀, MVZ 126857♂, MVZ 125564♀, MVZ 119839♀, MVZ 12487♂, MVZ 119065♂, MVZ 46712♂, MVZ 123991♀, MVZ 128061♂, MVZ 4680♂, USNM 215714♂, USNM 223176♂, USNM 212590♀, USNM 225604♂, USNM 227100♂, USNM 227105♂, USNM 227099♂, USNM 227104♂, USNM 258621♂, USNM 228307♂, USNM 228308♂, USNM 229293♂, USNM 228310♀, USNM 262477♂, USNM 228309♀, USNM 258623♂, USNM 258622♂, USNM 336269♂, USNM 265099♀, USNM 336270♂, USNM 107501♂, USNM 107500♀, USNM 107499♀, USNM 275124♀, USNM 275117♂, USNM 512112♀, USNM 291019♂, USNM 282825♂, USNM 512113♂, USNM 512105♀, USNM 512103♀, USNM 512113♂, USNM 512115♀, USNM 512116♂, USNM 512126♂, USNM 512120♂, USNM 512123♂, USNM 512124♀, USNM 512157♀, USNM 512149♀, USNM 512136♀, USNM 512163♀.

Ursus thibetanus - AMNH 35496♂, AMNH 35489♂, AMNH 35016♀, AMNH 23086♀, AMNH 119476♂, AMNH 100396♂, AMNH 80248♀, AMNH 70320♂, AMNH 84389♂, AMNH 110457♂, AMNH 11089♂, AMNH 114544♂, AMNH 57075♂, AMNH 45293♂, AMNH 57076♂, ANSP 12596♂, ANSP 12658♂, ANSP 13492♂, ANSP 17355♂, ANSP 15036♀, ANSP 14924♂, ANSP 11947♂, ANSP 12148♂, FMNH 25712♀, FMNH 31785♂, FMNH 31794♂, FMNH 99349♂, FMNH 26924♂, FMNH 26923♂, FMNH 89896♂, MVZ 4821♂, MVZ 4817♂, USNM 84092♀, USNM 36565♂, USNM 38195♂, USNM 22998♂, USNM 187866♂, USNM 22004♀, USNM 83453♂, USNM 258646♂, USNM 199684♀, USNM 258430♂, USNM 218152♂, USNM 259099♀, USNM 258647♀, USNM 240669♀, USNM 358644♂, USNM 240669♀, USNM 221064♀, USNM 258593♂, USNM 271090♂.

Odobenidae

Odobenus rosmarus - AMNH 149381♂, AMNH 232559♂, AMNH 213190♀, AMNH 24407♀, AMNH 121♂, AMNH 41216♂, AMNH 1562♀, AMNH 77935♂, AMNH 77986♂, AMNH 90779♀, AMNH 90877♂, AMNH 79901♀, AMNH 179982♂, AMNH 14230♀, AMNH 19268♂, AMNH 19267♀, AMNH 19271♀,

AMNH 19272♂, AMNH 19273♂, AMNH 19276♂, AMNH 19277♀, AMNH 19278♀, AMNH 19281♂, AMNH 19282♂, AMNH 19284♀, AMNH 19283♀, AMNH 19285♀, AMNH 19288♂, AMNH 19589♀, AMNH 19298♀, AMNH 70099♂, AMNH 15085♀, AMNH 15086♀, AMNH 15087♀, AMNH 15088♀, AMNH 15089♀, AMNH 15091♀, AMNH 15092♂, AMNH 15602♂, AMNH 15706♂, AMNH 15708♂, AMNH 15707♂, AMNH 14068♂, AMNH 14069♂, AMNH 14071♀, AMNH 11047♀, AMNH 14046♂, AMNH 11048♂, AMNH 11049♂, AMNH 31646♀, AMNH 73301♀, AMNH 73303♂, AMNH 73302♂, AMNH 29903♂, AMNH 29902♀, AMNH 29906♂, AMNH 29907♀, AMNH 29908♂, FMNH 5508♀, FMNH 5505♀, FMNH 26889♀, FMNH 140831♂, FCNyM 1501♂, FCNyM 1502♂, MVZ 119066♂, MVZ 125559♂.

Otariidae

Arctocephalus australis - AMNH 205918♂, AMNH 254569♂, AMNH 254564♂, AMNH 254563♂, AMNH 254562♂, AMNH 254568♀, AMNH 254565♂, AMNH 25461♀, AMNH 254567♀, AMNH 205917♂, AMNH 205916♂, FCNyM 14.IV.48.14♂, FCNyM 1061♂, MACN 24732♂, MACN 21863♂, MACN 29769♂, MACN 21862♂, MCZ 1125♂, MCZ 1108♂, MCZ 1126♂, MVZ 139504♂, USNH 504900♀, USNH 239140♂, USNH 36664♂, USNH 484934♀, USNH 504899♀, USNH 504896♀, USNH 501117♀, USNH 504897♀, USNH 484938♀, USNH 50119♂, USNH 501120♀, USNH 504895♂, USNH 504898♀, USNH 484936♂, USNH 501118♀, USNH 501067♀, USNH 484935♀, USNH 23331♂, USNH 396062♂, USNH 396921♂, USNH 504891♀, USNH 550479♂, USNH 550480♂.

Arctocephalus galapagoensis - AMNH 100319♀, AMNH 100341♂, AMNH 100342♀, USNH 259790♂, USNH 259832♀.

Arctocephalus gazella - FCNyM 25.VI.02.8♂, FCNyM 25.VII.02.12♂, FCNyM 25.VI.02.10♂, FCNyM 25.VI.02.15♂, FCNyM 25.VI.02.11♂, FCNyM 25.VI.02.14♂, FCNyM 25.VII.02.9♂, FCNyM 25.VI.02.22♂, FCNyM 25.VI.02.20♂, FCNyM 25.VI.02.17♂, FCNyM 25.VI.02.18♂, FCNyM 25.VI.02.19♂, FCNyM 26.VI.02.16♂, FCNyM 25.VI.02.24♂, MACN 23666♂, MACN 21062♂, MACN 21351♂, MACN 21756♂, MACN 21996♂, MACN 21353♂, MACN 21754♂, MACN 21060♂, MACN 21760♂, MACN 21350♂, MACN 21759♂, MACN 21349♂, MACN 21755♂, MACN 21859♂, MACN 21761♂, MACN 20436♂, MACN 21857♂, MACN 21352♂, USNH 392266♂, USNH 550091♂, USNH 392266♂, USNH 550090♀.

Arctocephalus pusillus - AMNH 80083♂, AMNH 100045♀, AMNH 81704♂, AMNH 81707♂, AMNH 81705♂, AMNH 81706♂, AMNH 81709♂, AMNH 81708♂, AMNH 81711♂, AMNH 81710♂, AMNH 81714♀, AMNH 81713♂, MCZ 56582♂, USNH 484928♂, USNH 396063♀.

Arctocephalus townsendi - MVZ 191003♂, USNH 83618♀.

Arctocephalus tropicalis - MN 50791♂, MN 50790♂, MN 29680♂.

Callorhinus ursinus - AMNH 77796♂, AMNH 245299♂, AMNH 77898♂, AMNH 71170♀, AMNH 100335♀, AMNH 100334♂, FMNH 47266♂, FMNH 1546♂, FMNH 1546♂, FMNH 81505♀, MCZ 128♂, MCZ 1088♂, MVZ 35090♀, MVZ 35355♂, MVZ 35086♂, MVZ 175109♀, MVZ 153256♀, MVZ 140846♀.

MVZ 220255♂, MVZ 88910♀, MVZ 138677♀, MVZ 115223♂, MVZ 115219♀, MVZ 115217♀, MVZ 115228♀, MVZ 115222♀, MVZ 115218♀, MVZ 115221♂, MVZ 115220♀, MVZ 115225♀, MVZ 115224♂, MVZ 115227♀, MVZ 115226♂, MVZ 184135♀, MVZ 184136♂, MVZ 122102♀, USNH 286130♀, USNH 286135♂, USNH 286132♀, USNH 286144♂, USNH 283699♂, USNH 286219♂, USNH 286204♀, USNH 286214♂, USNH 286234♂, USNH 286230♀, USNH 12740♂, USNH 12740'♀, USNH 16322♂, USNH 16321♂, USNH 38155♀, USNH 38225♂, USNH 47087♂, USNH 47084♂, USNH 38226♀, USNH 47086♂, USNH 47088♂, USNH 47083♂.

Eumetopias jubatus - AMNH 185111♂, AMNH 38400♂, AMNH 77801♂, AMNH 77749♀, AMNH 77883♂, AMNH 143664♂, AMNH 164286♂, AMNH 62261♂, AMNH 60797♂, AMNH 62262♂, AMNH 62264♂, AMNH 181383♂, AMNH 38401♀, FMNH 29939♂, FMNH 33770♂, FMNH 29941♂, FMNH 29942♀, FMNH 29934♂, FMNH 29932♂, FMNH 29933♂, MVZ 101430♂, MVZ 91069♂, MVZ 191004♀, MVZ 208598♂, MVZ 138679♂, MVZ 118620♀, MVZ 4897♂, MVZ 4117♂, MVZ 88876♀, MVZ 4967♀, MVZ 4964♂, MVZ 8827♂, MVZ 138680♂, MVZ 186325♂, MVZ 186326♀, MVZ 172086♀, USNH 47104♀, USNH 3631♂, USNH 4701♂, USNH 7140♂, USNH 6906♂, USNH 4702♂, USNH 8162♀, USNH 15861♀, USNH 8163♀, USNH 13217♂, USNH 21108♂, USNH 21297♂, USNH 21302♀, USNH 21307♂, USNH 21305♂, USNH 21299♀, USNH 21309♀, USNH 21303♂, USNH 21523♀, USNH 21304♂, USNH 23457♀, USNH 21537♀, USNH 22077♂, USNH 22072♂, USNH 15277♂.

Neophoca cinerea - USNH 504729♂, USNH 571463♂.

Otaria byronia - AMNH 73120♂, AMNH 73121♂, AMNH 73119♂, AMNH 73122♀, AMNH 77800♀, AMNH 77799♀, AMNH 77910♂, AMNH 77918♂, AMNH 77917♂, AMNH 205919♂, AMNH 77920♂, FMNH 22247♂, FMNH 54243♂, FMNH 20852♂, FMNH 50789♂, FMNH 50790♀, FMNH 50791♀, MACN 41226♂, MACN 21994♂, MACN 21984♂, MACN 1313♂, MACN 25168♂, MACN 2727♂, MACN 24851♂, MACN 24733♂, MACN 2326♂, MACN 22851♂, MACN 21995♂, MACN 22371♂, MACN 1314♂, MACN 21738♀, MACN 20578♀, MACN 20579♀, MACN 1311♀, MACN 21742♀, MACN 22853♀, MACN 20596♀, MN 50787♂, MN 50788♂.

Phocarcos hookeri - USNH 344986♀, USNH 344983♀, USNH 344981♂, USNH 344980♂, USNH 484531♂, USNH 344982♂, USNH 344985♂, USNH 484526♂.

Zalophus californianus - AMNH 180502(m), AMNH 180459♀, AMNH 180458♂, AMNH 179998♂, AMNH 180515♂, AMNH 19395♂, AMNH 13907♀, AMNH 180461♀, AMNH 180667♂, AMNH 180455♂, AMNH 180457♀, AMNH 180453♀, AMNH 18452♀, FMNH 104796♀, MVZ 175110♂, MVZ 186327♂, MVZ 186328♂, MVZ 18632♂, MVZ 102818♂, MVZ 172065♂, MVZ 172068♂, MVZ 172067♂, MVZ 172069♂, MVZ 172070♂, MVZ 172071♂, MVZ 172073♂, MVZ 172076♂, MVZ 172077♂, MVZ 1369♂, MVZ 1368♂, MVZ 172066♂, MVZ 113720♂, MVZ 172085♂, MVZ 172083♂, MVZ 186330♂, MVZ 139211♂, MVZ 139212♂, MVZ 4895♀, MVZ 4103♀, MVZ 4108♂, MVZ 113721♀, MVZ 172097♀, MVZ 172099♀, MVZ 135008♀, MVZ 4110♀.

Zalophus wolfebaeki - AMNH 63946♂, AMNH 63947♀, AMNH 214781♂, AMNH 99465♀, AMNH 214780♂, AMNH 214782♂, AMNH 99463♂, AMNH

99464♂, AMNH 99461♂, AMNH 99462♂, AMNH 73664♂, FMNH 51760♂, FMNH 51759♀, FMNH 51748♀, NA 125503♀, USNH 23332♂, USNH 23280♂, USNH 23278♀, USNH 23277♂, USNH 23281♂, USNH 23279♂, USNH 23276♂.

Phocidae

Cystophora cristata - AMNH 184660♀, AMNH 95♂, AMNH 150417♂, AMNH 97♂, AMNH 10199♂, AMNH 108♀, AMNH 96♀, AMNH 100135♀, AMNH 184659♂, AMNH 232565♂, AMNH 212185♂, USNM 21043♂, USNM 188845♂, USNM 6576♀, USNM 38233♂, USNH 188852♀, USNH 188850♀, USNH 188848♀, USNH 188846♂, USNH 6577♀, USNH 188865♂, USNH 188861♂, USNH 188862♂, USNH 188853♀, USNH 188854♂, USNH 188866♂, USNH 188855♀, USNH 188867♀, USNH 188868♀, USNH 188864♂, USNH 188863♂, USNH 188882♀, USNH 188877♀, USNH 188889♀, USNH 188869♀, USNH 188881♀, USNH 188871♀, USNH 188890♀, USNH 188891♀, USNH 188895♀, USNH 188894♀, USNH 188898♀, USNH 188899♀, USNH 188900♂, USNH 188896♀, USNH 188903♂, USNH 188904♂, USNH 188897♀, USNH 188893♀, USNH 188905♂.

Erignathus barbatus - AMNH 22943♂, AMNH 75254♂, AMNH 75254♂, AMNH 75249♂, AMNH 22942♂, AMNH 19347♂, AMNH 98♂, AMNH 28097♂, AMNH 99♂, AMNH 19313♀, AMNH 73331♂, AMNH 28♂, AMNH 10136♂, AMNH 18166♂, AMNH 143901♂, AMNH 18165♀, AMNH 10202♀, AMNH 10135♂, AMNH 18167♂, AMNH 15774♂, FMNH 81503♂, FCNyM 1055♂, MVZ 124013♀, MVZ 124019♂, MVZ 125584♀, MVZ 128914♀, MVZ 128913♂, MVZ 125582♂, MVZ 124021♀, MVZ 125581♂, MVZ 124015♀, MVZ 125577♂, MVZ 125576♀, MVZ 125575♀, MVZ 125569♀, MVZ 125578♀, MVZ 124016♂, MVZ 125573♀, MVZ 128124♂, MVZ 128916♂, MVZ 125570♂, MVZ 124020♀.

Halichoerus grypus - AMNH 103♂, AMNH 125591♂, AMNH 125593♂, AMNH 100191♀, AMNH 173535♀, AMNH 69489♂, AMNH 69487♂, AMNH 69488♂, AMNH 69490♂, FMNH 81506♂, MVZ 140005♂, MVZ 186579♀, USNH 19837♀, USNH 38237♀, USNH 35291♂, USNH 446405♀, USNH 446406♀, USNH 4747♀, USNH 504481♂.

Histiophoca fasciata - AMNH 130246♂, AMNH 130245♂, MCZ 52239♂, MCZ 52240♂, MVZ 51387♂, MVZ 51385♂, USNH 16484♂, USNH 504959♂, USNH 504960♂, USNH 311771♂, USNH 399449♂, USNH 571367♂.

Hydrurga leptonyx - AMNH 36200♂, AMNH 48157♂, AMNH 77914♂, AMNH 173537♂, ANSP 2488♂, FCNyM 1059♂, FCNyM 691♂, FCNyM 14.IV.48.11♂, FCNyM 26.IV.00.14♂, MN 53662♂, MVZ 127753♂, USNH 270315♀, USNH 269533♀, USNH 15758♂, USNH 275205♀, USNH 270326♂, USNH 275298♀, USNH 550358♂, USNH 550360♂, USNH 277255♀, USNH 396931♂, USNH 550359♂, USNH 571676♂.

Leptonychotes weddellii - AMNH 85517♂, AMNH 85512♀, AMNH 88446♂, AMNH 212192♂, AMNH 88447♀, AMNH 88450♀, AMNH 88453♀, AMNH 88454♀, AMNH 88454♀, AMNH 88452♂, AMNH 88469♂, AMNH 88473♀, AMNH 88489♂, AMNH 88482♂, AMNH 88485♂, AMNH 88492♂, AMNH 88488♂, AMNH 88590♀, AMNH 88499♂, AMNH 88501♂, AMNH 88507♀, AMNH 88508♀, AMNH 88506♀, AMNH 88505♀, AMNH 88504♀, AMNH

88510♀, AMNH 88513♀, AMNH 88511♀, AMNH 88514♂, AMNH 88515♂, AMNH 88520♂, AMNH 88521♂, AMNH 88528♂, AMNH 88541♂, AMNH 88547♂, AMNH 88551♂, AMNH 88548♂, AMNH 88557♂, AMNH 88556♂, AMNH 212171♀, AMNH 30574♂, FCNyM 31.xII.02.24♂, FCNyM 14.IV.48.12♂, MACN 49252♂, MACN 22610♂, MACN 20628♂, MACN 22854♂, MACN 1327♂, MACN 1919♂, MACN 20628♂, MCZ 51874♀, MCZ 51710♀, MCZ 51731♂, MCZ 51712♀, MCZ 51711♀.

Lobodon carcinophaga - AMNH 88546♂, AMNH 88445♂, AMNH 88449♂, AMNH 88484♂, AMNH 88487♂, AMNH 88474♀, AMNH 88490♂, AMNH 88493♂, AMNH 88494♂, AMNH 88496♂, AMNH 88498♂, AMNH 85510♂, AMNH 85513♂, AMNH 88529♂, AMNH 88530♂, AMNH 88534♂, AMNH 88536♂, AMNH 88533♂, AMNH 88539♂, AMNH 88538♂, AMNH 88540♂, AMNH 88537♂, AMNH 88549♂, AMNH 88552♂, AMNH 88543♂, FMNH 43885♀, FCNyM 30.XII.02.11♂, FCNyM 22.III.99.10♂, FCNyM 474♂, MACN 1920♂, MCZ 52287♀, MCZ 51851♂, MVZ 127754♂, MVZ 127752♀, MVZ 127757♂, MVZ 127751♀, USNH 269722♀, USNH 550077♀, USNH 270324♀, USNH 275204♂, USNH 550078♀, USNH 270385♀, USNH 270325♂, USNH 550080♀, USNH 550083♂.

Mirounga angustirostris - AMNH 77930♀, AMNH 32676♂, AMNH 32678♂, AMNH 32677♀, AMNH 32679♂, FMNH 41251♂, FMNH 51440♂, FMNH 41253♀, MVZ 162301♀, MVZ 162298♂, MVZ 162299♂, MVZ 162302♂, MVZ 162300♂, USNH 20927♀, USNH 21887♀, USNH 38234♀, USNH 21890♀, USNH 219058♂, USNH 260867♂, USNH 255975♀, USNH 265353♂, FMNH 41249♀.

Mirounga leonina - AMNH 77923♂, AMNH 77928♂, AMNH 48151♂, AMNH 48152♂, AMNH 48156♀, AMNH 48155♀, AMNH 48154♀, AMNH 48160♀, AMNH 48159♀, AMNH 48161♂, AMNH 48162♂, AMNH 48163♂, AMNH 48153♂, AMNH 48166♀, AMNH 77911♀, AMNH 77912♀, AMNH 77916♂, AMNH 77919♂, AMNH 70240♂, AMNH 34904♂, FMNH 10886♂, FCNyM 947♂, FCNyM 26.IV.00.13♂, FCNyM 1504♂, MACN 22614♀, MACN 22612♀, MACN 22613♀, MACN 2491♂, MACN 20608♂, MACN 1326♂, MN 50786♀, USNH 15336♀, USNH 239141♀, USNH 504927♀, USNH 550379♀, USNH 484893♂.

Monachus monachus - AMNH 73608♀, USNH 219059♂.

Neomonachus schauinslandi - USNH 181250♂, USNH 243840♂, USNH 243838♂, USNH 243842♀, USNH 243839♀, USNH 395786♂, USNH 334573♂, USNH 243849♀, USNH 243844♂, USNH 395787♂, USNH 334577♂, USNH 395997♂, USNH 395761♂, USNH 20468♂, USNH 504892♂, USNH 395995♂, USNH 396000♂, USNH 504893♂, USNH 504926♂.

Neomonachus tropicalis - AMNH 19600♀, AMNH 15666♂, AMNH 77741♂, AMNH 15896♀, AMNH 10421♂, ANSP 4963♂, USNH 100354♂, USNH 100355♀, USNH 225443♂, USNH 100368♂, USNH 100364♀, USNH 100360♀, USNH 100356♀, USNH 100357♀, USNH 100361♂, USNH 100365♀, USNH 100369♂, USNH 100363♂, USNH 100358♂, USNH 100358♂, USNH 100370♀, USNH 100366♀, USNH 100359♀, USNH 100362♀, USNH 100367♀, USNH 102524♀, USNH 102525♀, USNH 102528♀, USNH 100372♀, USNH 100371♀, USNH 100373♀, USNH 102532(*Monachus tropicalis*), USNH 102530♀, USNH 102523♂, USNH 102531♀, USNH 102527♀, USNH 102526♀, USNH 102529♂, USNH

A. ESPÉCIMES

102533♀, USNH 102534♀, USNH 102535♀, USNH 102536♀, USNH 102537♀.

Ommatophoca rossii - MACN 48259♀, MCZ 51852♂, MCZ 52305♂, USNH 270316♀, USNH 275206♂, USNH 270321♀, USNH 339989♂, USNH 302975♂.

Pagophilus groenlandicus - AMNH 150419(x), AMNH 186435♂, AMNH 180016♂, AMNH 10138♂, AMNH 73327♂, AMNH 10140♂, AMNH 10139♂, AMNH 10141♂, AMNH 10142♂, AMNH 10148♂, AMNH 10144♂, AMNH 10180♀, AMNH 10146♂, AMNH 19307♂, AMNH 10150♂, AMNH 10149♂, AMNH 10143♂, AMNH 10145♂, AMNH 10152♀, AMNH 10147♂, AMNH 10151♀, AMNH 10155♂, AMNH 10178♂, AMNH 10157♀, AMNH 10158♀, AMNH 10156♀, AMNH 10153♂, AMNH 10154♀, AMNH 10179♂, FMNH 9866♂, FMNH 30369♀, MCZ 8985♀, MCZ 8983♂, MCZ 8984♂, MCZ 8959♀, MCZ B8988♀, MCZ B8990♂, MCZ B8987♀, MCZ 8991♂, MCZ 8986♂, MCZ 9374♂, MCZ 9006♂, MCZ 8992♂, MCZ 8989♂, MCZ 7734♂, MCZ 7736♂, MCZ 7724♂, MCZ 60604♀, MCZ 60716♀, MCZ 64093♂, MCZ 64092♂.

Pusa caspica - USNH 34165♂, USNH 341616♂, USNH 341617♂.

Pusa hispida - AMNH 75256♂, AMNH 28099♀, AMNH 28098♀, AMNH 90801♀, AMNH 75253♂, AMNH 22941♂, AMNH 90800♂, AMNH 73330♂, AMNH 187♂, AMNH 10214♂, AMNH 104♂, AMNH 105♂, AMNH 107♂, AMNH 10212♂, AMNH 10217♂, AMNH 10210♂, AMNH 10215♂, AMNH 10211♂, AMNH 10213♂, AMNH 19305♂, AMNH 19308♂, FMNH 30367♀, FMNH 67404♂, FMNH 30366♂, FMNH 30361♀, MCZ 7747♀, MCZ 6297♀, MCZ 6296♂, MCZ 6295♀, MCZ 7742♂, MCZ 7743♂, MCZ 7741♂, MCZ 6969♂, MCZ 37621♂, MCZ 7745♂, MCZ 7746♂, MCZ 21809♂, MCZ 47066♂, MCZ 29804♂, MCZ 47447♂, MCZ 8512♂, MCZ 60957♀, MVZ 123996♀, MVZ 125568♀, MVZ 124000♀, MVZ 123999♀, MVZ 123998♂, MVZ 124003♂, MVZ 124002♀, MVZ 124004♂, MVZ 124001♀, MVZ 125612♀, MVZ 123997♀, MVZ 125611♀, MVZ 134146♀.

Phoca largha - AMNH 18169♀, AMNH 15817♂, AMNH 19843♂, AMNH 18169♀, AMNH 212250♂, MCZ 6970♂.

Pusa sibirica - AMNH 206594♂, AMNH 185195♀, AMNH 185595♀, MCZ 12654♂, MVZ 140629♀, USNH 175690♀, USNH 550028♂, USNH 550034♂, USNH 550029♂.

Phoca vitulina - MVZ 172131♀, MVZ 172132♂, MVZ 172129♂, MVZ 172130♀, MVZ 172119♀, MVZ 191005♀, MVZ 172121♀, MVZ 175105♂, MVZ 172120♂, MVZ 196047♂, MVZ 172125♀, MVZ 172109♂, MVZ 172107♀, MVZ 172117♀, MVZ 172106♂, MVZ 172113♂, MVZ 172105♂, MVZ 196035♀, MVZ 196048♀, MVZ 196043♂, MVZ 196046♀, MVZ 196045♂, MVZ 172104♂, MVZ 208580♀, MVZ 140849♂, MVZ 4757♀, MVZ 208593♀, MVZ 172115♀, MVZ 208588♂, MVZ 208579♂, MVZ 208578♂, MVZ 172082♂, MVZ 172110♂, MVZ 175103♀, MVZ 172112♂, MVZ 208572♂, MVZ 175107♀, MVZ 175102♀, MVZ 153262♂, MVZ 153260♂, MVZ 175106♀, MVZ 175101♂, MVZ 175108♀, MVZ 175104♀, MVZ 208569♀, MVZ 208575♀, MVZ 114778♂, MVZ 208584♀, MVZ 208585♀, MVZ 208559♂, MVZ 208587♂, MVZ 172111♂, MCZ 11455♀.

Mephitidae

Conepatus chinga - FMNH 29301♂, FMNH 29293♀, FMNH 29294♂, FMNH 29291♀, FMNH 29292♀, FMNH 29280♂, FMNH 29282♂, FMNH 29300♂, FMNH 29286♀, FMNH 29298♀, FMNH 29289♂, FMNH 29288♂, FMNH 106002♂, FMNH 29287♀, FMNH 29297♀, FMNH 27673♀, FMNH 29285♀, FMNH 29299♂, FCNyM 20.XII.00.6♂, FCNyM 2007♂, FCNyM 5.V.99.5♂, FCNyM 2.Vi.60.13♂, FCNyM 5.V.99.7♂, FCNyM 11.IX.98.8♂, FCNyM 8.IX.98.3♂, FCNyM 5.V.99.4♂, MN 1501♀, MN 3130♂, MN 3128♂, MN 1500♂, MN 13385♂, MN 59335♂, MN 4873♂, MPEG 22231♂, MPEG 22189♂, MPEG 22232♂, MPEG 22233♂, MPEG 22190♂, MVZ 39875♂, MZUSP 1009♂, MZUSP 3538♂, MZUSP 229♂, MZUSP 2310♂, MZUSP 1010♀, MZUSP 2309♂, MZUSP 228♂, MZUSP 3068♂, MZUSP 3067♂, FMNH 54330♂, FMNH 54329♂, MACN 4910♀, MACN 499♂, MACN 36322♂, MACN 30393♂, MACN 32251♀, MACN 13714♀, MACN 4915♀, MACN 50421♂, MACN 36324♂, MACN 30102♀, MACN 36728♂, MACN 34330♂, USNM 172792♂, USNM 172794♂, USNM 172793♂, FCNyM 8.V.59.4♀, FCNyM 8.V.59.6♀, FCNyM 7.X.46.1♂, FCNyM 1582♂, FCNyM 658♂, FCNyM 1727♂, FCNyM 3.X.94.1♂, FCNyM 330♂, FCNyM 1277♂, FCNyM 10.II.99.1♂, FMNH 49720♂, FMNH 49732♀, FMNH 49733♀, FMNH 49734♂, FMNH 49723♂, FMNH 106007♂, FMNH 52486♀, FMNH 49724♀, FMNH 49725♂, FMNH 49726♂, FMNH 49727♀, FMNH 49728♀, FMNH 49729♀, FMNH 49731♀, MVZ 114775♂, MVZ 117776♀, MVZ 114940♀, MVZ 135823♂, MVZ 114941♀, FMNH 24355♂, FMNH 21356♀.

Conepatus humboldtii - FCNyM 1.xii.00.5♂, FCNyM 1.XII.00.4♂, MACN 16475♂, MACN 4942♂, MACN 2049♀, MACN 2874♀, MACN 2872♂, MACN 13297♂, MACN 15549♂, MACN 1988♀, MACN 36332♂, MACN 1051♂, MACN 14084♂, MACN 15573♀, MVZ 162288♂, MVZ 159444♂, MVZ 172241♂, MZUSP 2313♂, MZUSP 258♂, MZUSP 1088♂, MZUSP 257♂, MZUSP 2315♂, FCNyM 533♂, FCNyM 702♂, FCNyM 325♂, FCNyM 2026♂, FCNyM 2027♂, FCNyM 2025♂, FCNyM 2028♂.

Conepatus leuconotus - AMNH 128470♂, AMNH 129709♀, AMNH 128471♂, AMNH 129710♂, AMNH 128472♀, AMNH 128997♀, AMNH 128473♂, AMNH 29281♂, MVZ 98295♂, MVZ 132165♂, MVZ 132164♀, MVZ 132161♂, MVZ 98298♀, MVZ 132170♂, MVZ 132169♀, MVZ 132166♂, MVZ 132160♂, MVZ 132159♀, MVZ 132168♂, MVZ 132161♂, MVZ 31331♀, MVZ 117020♀, MVZ 106116♂, MVZ 80336♂, MVZ 52002♀, MVZ 85320♀, MVZ 85318♀, MVZ 109609♂, MVZ 109608♀, MVZ 109606♂, MVZ 62707♂, MVZ 121106♂, MVZ 109607♀, MVZ 76291♀, MVZ 74335♂, MVZ 121824♂, MVZ 32491♂, MVZ 32493♂, MVZ 32494♂, MVZ 32495♂, MVZ 32497♂, MVZ 32496♂.

Conepatus semistriatus - AMNH 172190♀, FMNH 13852♀, FMNH 44336♀, FMNH 125113♂, FMNH 22202♀, FMNH 18770♂, FMNH 38061♂, FMNH 58560♀, FMNH 19680♀, FMNH 19976♂, MACN 3162♀, MVZ 135824♀, MVZ 84332♀, MZUSP 8453♀, MZUSP 2649♂, USNM 65763♂, USNM 281452♂, USNM 65762♀, USNM 281464♂, USNM 281457♂, USNM 281461♀, USNM 281462♀, USNM 281459♀, USNM 281455♀, USNM 281460♂, USNM 281465♀, USNM 281463♀, USNM 281458♀, USNM 281456♂, USNM 443291♂, USNM 442414♀, USNM 372745♀, USNM 443295♀, USNM 443294♀, USNM 443293♀,

USNM 388244♂, USNM 443289♀, USNM 443285♀, USNM 443576♂.

Mephitis macroura - FMNH 8683♂, FMNH 8682♀, FMNH 5432♂, FMNH 13996♂, FMNH 15043♀, FMNH 13997♀, MVZ 100116♀, MVZ 100115♂, MVZ 100114♂, MVZ 91167♂, MVZ 100122♂, MVZ 100121♂, MVZ 100119♂, MVZ 100118♂, MVZ 100117♂, MVZ 100125♂, MVZ 100124♀, MVZ 100123♂, MVZ 85323♀, MVZ 85322♀, MVZ 85321♀, MVZ 128496♂, MVZ 9502♂, MVZ 50256♀, MVZ 85326♂, MVZ 85325♂, MVZ 85324♂, MVZ 132138♀, MVZ 132139♀, MVZ 132136♂, MVZ 132137♂, MVZ 132135♂, MVZ 132134♂, MVZ 98294♂, MVZ 98292♀, MVZ 98293♀, MVZ 132143♀, MVZ 132142♂, MVZ 132140♀, MVZ 132144♂, MVZ 132145♂, MVZ 132151♂, MVZ 132146♂, MVZ 132148♂, MVZ 132150♀.

Mephitis mephitis - MVZ 59815♀, MVZ 59811♂, MVZ 102294♀, MVZ 227171♀, MVZ 184110♂, MVZ 149008♂, MVZ 125184♂, MVZ 34995♂, MVZ 34994♀, MVZ 59817♀, MVZ 59816♂, MVZ 59814♀, MVZ 32329♂, MVZ 62850♀, MVZ 90336♀, MVZ 59818♀, MVZ 34996♀, MVZ 14640♂, MVZ 28066♀, MVZ 93613♂, MVZ 936112♀, MVZ 23693♀, MVZ 163329♂, MVZ 11742♀, MVZ 21135♂, MVZ 21134♂, MVZ 23669♂, MVZ 21568♀, MVZ 101327♀, MVZ 24417♀, MVZ 23694♂, MVZ 19732♀, MVZ 19742♀, MVZ 19741♂, MVZ 19740♂, MVZ 19739♀, MVZ 19738♂, MVZ 19736♀, MVZ 108073♀, MVZ 21570♂, MVZ 21569♀, MVZ 3763♀, MVZ 38854♀, MVZ 88170♂, MVZ 88169♀, MVZ 149772♀, MVZ 95469♀, MVZ 90449♀.

Mydaus javanensis - AMNH 106636♂, AMNH 31811♀, AMNH 102701♂, AMNH 102702♀, ANSP 14084♀, ANSP 14083♂, FMNH 68730♂, FMNH 68729♀, MNHN 156287♂, MNHN 34894♂, MNHN 121498♂, MNHN 121497♀.

Mydaus marchei - FMNH 62882♀, FMNH 62877♂, FMNH 1158♂, FMNH 62878♂, MNHN 144958♀, MNHN 478276♀, MNHN 478278♂, MNHN 478277♂.

Spilogale angustifrons - FMNH 74120♂, FMNH 74119♂, FMNH 74118♂, FMNH 74117♀, FMNH 73338♂, FMNH 48512♂, USNM 51152♂, USNM 88154♂, USNM 20437♂, USNM 50822♂, USNM 334672♂, USNM 79017♂, USNM 55585♂, USNM 120101♂, USNM 50824♀, USNM 34912♂, USNM 50823♂, USNM 81716♀, USNM 50821♂, USNM 81717♂, USNM 334674♀, USNM 337938♀, USNM 337826♂, USNM 334671♂, USNM 337937♂, USNM 339972♂, USNM 337640♂, USNM 334673♂, USNM celeris♀, USNM 334675♂, USNM 337641♀, USNM 361358♀, USNM 565456♂, USNM 565454♂, USNM 565455♂, USNM 77916♂, USNM 73520♂, USNM 73519♂, USNM 73521♂, USNM 73524♂, USNM 73527♀, USNM 73528♂, USNM 75321♀.

Spilogale gracilis - MVZ 34993♀, MVZ 47169♂, MVZ 4076♂, MVZ 4077♀, MVZ 21916♂, MVZ 21915♀, MVZ 16579♂, MVZ 149009♀, MVZ 28731♀, MVZ 7390♂, MVZ 16264♀, MVZ 77324♂, MVZ 58873♀, MVZ 58872♂, MVZ 24826♀, MVZ 24825♂, MVZ 38298♀, MVZ 124139♀, MVZ 14641♂, MVZ 59966♂, MVZ 59965♂, MVZ 107392♂, MVZ 107391♂, MVZ 44279♀, MVZ 107395♀, MVZ 107394♀, MVZ 107393♂, MVZ 21564♀, MVZ 23623♂, MVZ 23622♀, MVZ 4279♀, MVZ 4278♂, MVZ 23695♂, MVZ 23670♀, MVZ 8956♂, MVZ 122743♂, MVZ 19727♂, MVZ 115413♂, MVZ 3612♂, MVZ 101507♂, MVZ 90447♂, MVZ 21567♀, MVZ 21566♂, MVZ 38878♀, MVZ 19728♀, MVZ 21565♀, MVZ 22119♀, MVZ 201322♂.

Spilogale putorius - FMNH 7735♂, FMNH 7734♂, FMNH 21920♀, FMNH 6875♀, FMNH 6876♂, FMNH 6877♂, FMNH 6878♂, FMNH 10960♀, FMNH 10959♂, FMNH 51373♂, FMNH 49145♀, FMNH 199852♂, FMNH 199842♀, FMNH 199837♂, FMNH 199838♀, FMNH 199839♂, FMNH 199840♂, FMNH 10961♀, FMNH 199846♀, FMNH 199847♀, FMNH 199845♀, FMNH 199843♀, FMNH 199844♀, FMNH 199848♂, FMNH 199850♂, FMNH 199849♂, FMNH 199841♂, FMNH 5438♀, USNM 70308♂, USNM 17184♂, USNM 70305♂, USNM 70306♂, USNM 70307♂, USNM 64019♀, USNM 70309♂, USNM 231609♂, USNM 243510♀, USNM 70304♂, USNM 64021♂, USNM 263620♀, USNM 17185♂.

Spilogale pygmaea - AMNH 175183♂, MVZ 155581♂, MVZ 155581♂, MVZ 155580♀, MVZ 155579♂, USNM 523998♀.

Ailuridae

Ailurus fulgens - AMNH 146682♂, AMNH 35387♂, AMNH 119474♀, AMNH 35433♀, AMNH 146778♀, AMNH 169496♀, AMNH 119676♀, AMNH 35495♀, AMNH 110455♀, AMNH 117408♀, ANSP 19185♂, ANSP 21583♀, ANSP 20128♀, ANSP 13493♀, ANSP 12160♀, FMNH 36749♂, FMNH 36753♂, FMNH 36750♂, FMNH 36752♀, FMNH 36751♂, FMNH 36754♀, FMNH 36755♀, FMNH 36756♀, FMNH 36757♀, FMNH 114384♀, FMNH 60762♀, FMNH 57324♀, FMNH 57336♀, FMNH 60452♀, FMNH 60497♂, FMNH 60046♀, FMNH 44875♂, FMNH 65803♀, FMNH 104950♂, MCZ 3579♀, MCZ 52237♀, MVZ 136208♀, MVZ 13215♀, MVZ 123701♂, MVZ 119483♂, USNM 259379♀, USNM 256098♀, USNM 258350♀, USNM 252146♂, USNM 252091♀, USNM 258051♀, USNM 305771♂, USNM 396950♀, USNM 252087♀.

Procyonidae

Bassaricyon alleni - AMNH 98709♀, AMNH 70532♀, AMNH 142223♀, AMNH 67706♀, FMNH 104935♀, FMNH 86908♀, FMNH 86909♀, FMNH 62079♀, FMNH 41501♀, FMNH 65787♀, FMNH 65988♂, FMNH 65805♂, FMNH 65789♂, FMNH 42502♂, MACN 5081♂, MACN 5082♂, MACN 3168♀, MCZ 37923♂, MCZ 37922♀, MCZ 37920♀, MVZ 155220♀, MVZ 153646♂, MVZ 155219♂, USNM 281484♂, USNM 281483♀, USNM 281482♀, USNM 194315♀, USNM 544415♀, USNM 443279♀, USNM 443718♀, USNM 281485♂, USNM 443717♀.

Bassaricyon gabbii - AMNH 140334♀, AMNH 30748♀, USNM 516945♂, USNM 516946♀, USNM 316230♀, USNM 338859♀, USNM 337632♂, USNM 324294♀, ANSP 1116♂, MCZ 38506♀.

Bassaricyon medius - AMNH 37797♀, FMNH 86852♀, FMNH 69578♀, FMNH 90049♀, FMNH 90051♀, FMNH 29180♂, USNM 397350♂, USNM 598997♀, USNM 363342♀, USNM 324296♀, USNM 179158♀, USNM 171138♂, USNM 307036♂, USNM 363344♂, USNM 179053♂, USNM 363343♂, USNM 324295♂, USNM 305751♂, USNM 335768♂, USNM 305753♂, USNM 338894♂, USNM 305750♂, USNM 335771♂, USNM 335770♂, USNM 338348♀, USNM 307037♀, USNM 310666♀, USNM 284903♀, USNM 284934♀, USNM 284933♀, USNM

284935♀, USNM 284773♀, USNM 305752♀, USNM 335767♀, USNM 307035♀, USNM 305748♀, USNM 335769♀, USNM 305749♀, MVZ 124112♂.

Bassaricyon neblina - FMNH 70723♂, FMNH 70725♂, FMNH 90052♀, FMNH 70724♂, FMNH 70721♀, FMNH 89220♀, FMNH 70726♂, FMNH 85818♂.

Bassariscus astutus - MVZ 38870♀, MVZ 21561♂, MVZ 38871♂, MVZ 99969♂, MVZ 9531♀, MVZ 22108♂, MVZ 21560♀, MVZ 21559♀, MVZ 21558♂, MVZ 21557♀, MVZ 59695♂, MVZ 59695♂, MVZ 192085♀, MVZ 38877♀, MVZ 106180♀, MVZ 34306♂, MVZ 34305♂, MVZ 34304♀, MVZ 59698♀, MVZ 59697♀, MVZ 184143♀, MVZ 229631♀, MVZ 12889♂, MVZ 12888♀, MVZ 12887♂, MVZ 12886♀, MVZ 91165♂, MVZ 117019♀, MVZ 117018♂, MVZ 51995♂, MVZ 51994♀, MVZ 51993♂, MVZ 51991♂, MVZ 51990♀, MVZ 94907♀, MVZ 52000♂, MVZ 51999♂, MVZ 51998♀, MVZ 51997♀, MVZ 51996♂, NA 80329♂, NA 80328♂, NA 74334♂, NA 74333♀, NA 80331♂, NA 80330♂.

Bassariscus sumichrasti - AMNH 10089♂, AMNH 135927♀, AMNH 137987♀, AMNH 135333♂, AMNH 137990♂, AMNH 135584♂, AMNH 177074♂, AMNH 148976♂, AMNH 148804♂, AMNH 148803♂, AMNH 145160♀, AMNH 232477♀, AMNH 254529♀, AMNH 214894♂, AMNH 178706♂, AMNH 175090♀, AMNH 68524♀, AMNH 172199♀, MVZ 98275♀, MVZ 98278♂, MVZ 98277♂, MVZ 132073♂, MVZ 98276♀, MVZ 98282♂, MVZ 184144♂, MVZ 98281♂, MVZ 98280♀, MNHN 181256♀, MNHN 180213♀, MNHN 181254♀, MNHN 108290♂, MNHN 181257♂, MNHN 15973♂, MNHN 133185♀, MNHN 292254♀, MNHN 340740♀, MNHN 340738♀, MNHN 340737♀, MNHN 70820, MNHN 340735♀, MNHN 8077♂, MNHN 8978♀, MNHN 13850♂, MNHN 181255♂, MNHN 13849♂, MNHN 340736♂, MNHN 340739♂.

Nasua narica - AMNH 141977♂, AMNH 14062♂, AMNH 135970♀, AMNH 141855♂, AMNH 141854♂, AMNH 139317♂, AMNH 126182♀, AMNH 126183♀, AMNH 173530♂, AMNH 145938♂, AMNH 123278♂, AMNH 123276♀, AMNH 685250, AMNH 165973♀, AMNH 145936♀, AMNH 145151♀, AMNH 149144♀, AMNH 145153♀, AMNH 145157♀, AMNH 145946♂, AMNH 145650♂, AMNH 145947♀, AMNH 143968♂, AMNH 145932♂, AMNH 145933♂, AMNH 145651♀, AMNH 145652♂, AMNH 145942♀, AMNH 145960♀, AMNH 145152♀, AMNH 145943♀, AMNH 172192♂, AMNH 17261♀, AMNH 17256♂, AMNH 28434♂, AMNH 28958♀, AMNH 29439♀, AMNH 28433♂, MVZ 132107♀, MVZ 23840♂, MVZ 98260♀, MVZ 98259♀, MVZ 132112♀, MVZ 122109♀, MVZ 132114♀, MVZ 132116♀, MVZ 132115♀, MVZ 98265♂.

Nasua nasua - MPEG 1207♂, MPEG 1531♀, MPEG 87♂, MPEG 4268♀, MPEG 5516♂, MPEG 5526♂, MPEG 5522♂, MPEG 5512♂, MPEG 5514♂, MPEG 5555♂, MPEG 5536♀, MPEG 5535♂, MPEG 5534♂, MPEG 5533♂, MPEG 5554♂, MPEG 5530♀, MPEG 5545♂, MPEG 5552♀, MPEG 5553♀, MPEG 5549♀, MZUSP 7153♂, MZUSP 7154♀, MZUSP 7124♂, MZUSP 19776♀, MZUSP 5537♂, MZUSP 5555♀, MZUSP 7121♂, MZUSP 5544♀, MZUSP 5245♀, MZUSP 19791♂, MZUSP 7156♂, MZUSP 5556♀, MZUSP 5243♀, MZUSP 19779♀, MZUSP 19783♂, MZUSP 19781♀, MZUSP 25456♂, MZUSP 19780♂, MZUSP 19784♀, MZUSP 19675♂, MZUSP 5538♂, MZUSP 802♂, MZUSP 4983♂, MZUSP 5250♀, MZUSP 5543♀, MZUSP 19775♂, MZUSP 19769♂, MZUSP 5547♂, MZUSP 7157♀, MZUSP 5540♀, MZUSP 5230♀, MZUSP 3150♂, MZUSP 8637♀, MZUSP

5228♀, MZUSP 21333♀, MZUSP 3148♂, MZUSP 2576♂, MZUSP 3149♂, MZUSP 2891♂, MZUSP 2809♂, MZUSP 7977♂, MZUSP 7379♂, MZUSP 7522♂, MZUSP 7800♀, MZUSP 2650♀, MZUSP 8301♀, MZUSP 8302♂, MZUSP 6311♂, MZUSP 6312♀, MZUSP 6310♂, MZUSP 28543♀, MZUSP 28542♀, MZUSP 28541♂, MZUSP 6309♂.

Nasuella meridensis - MZUSP 2528♂, USNM 143658♀.

Nasuella olivacea - AMNH 181493♀, AMNH 18489♀, AMNH 32358♂, AMNH 33049♂, AMNH 67084♂, FMNH 86850♂, FMNH 89236♂, FMNH 70747♀, FMNH 70746♂, FMNH 70748♂, FMNH 70744♂, FMNH 70749♀, FMNH 70745♂, FMNH 70742♂, FMNH 70741♂, FMNH 70743♂, USNM 240034♀.

Potos flavus - MACN 5085♂, MACN 5084♂, MACN 5083♂, MACN 32146♀, MN 4866♀, MN 4867♀, MN 4874♀, MN 4876♂, MN 4877♀, MN 4881♂, MN 4880♀, MN 5162♀, MN 5689♀, MN 5714♀, MN 5722♀, MN 5716♀, MN 5721♂, MN 32409♂, MN 3098♂, MN 32406♀, MN 10972♀, MN 10965♀, MN 10968♂, MN 10969♂, MN 10966♂, MN 10970♀, MN 10971♂, MN 32419♂, MN 32321♀, MN 11396♂, MN 10992♀, MN 32423♀, MN 5639♂, MN 32415♀, MN 555000♂, MN 4903♂, MN 4902♀, MN 4851♀, MPEG 4342♀, MPEG 7083♂, MPEG 4347♀, MPEG 7084♂, MPEG 4348♀, MPEG 6529♂, MPEG 4334♀, MPEG 4339♀, MPEG 4345♂, MPEG 4333♂, MPEG 1974♂, MPEG 4341♀, MPEG 4340♂, MPEG 4336♀, MPEG 4335♀, MPEG 4337♂, MPEG 22891♂, MPEG 38540♂, MPEG 22854♂, MPEG 12264♂, MPEG 19715♂, MPEG 22605♂, MZUSP 5563♀, MZUSP 7381♀, MZUSP 8951♀, MZUSP 4055♂, MZUSP 7382♀, MZUSP 21330♀, MZUSP 7519♂, MZUSP 7520♀, MZUSP 5534♂, MZUSP 8645♂.

Procyon cancrivorus - AMNH 62869♂, AMNH 61601♀, AMNH 214734♀, AMNH 94247♀, AMNH 215129♂, MACN 13816♀, MACN 5069♂, MACN 32254♀, MACN 16190♀, MACN 5070♀, MN 3087♀, MN 4897♀, MN 11203♀, MN 23887♀, MN 7256♀, MN 28802♀, MN 25657♀, MN 7612♀, MN 23885♂, MN 7625♀, MPEG 4210♀, MPEG 6821♀, MPEG 721♀, MPEG 4208♂, MZUSP 19794♂, MZUSP 1180♂, MZUSP 2982♀, MZUSP 2809♂, MZUSP 2825♂, MZUSP 2337♂, MZUSP 2306♂, MZUSP 1437♂, MZUSP 22363♂, MZUSP 22364♂, MZUSP 21627♂, MZUSP 22418♂, MZUSP 22362♂, MZUSP 22365♂, MZUSP 22366♂, MZUSP 21628♀, MZUSP 1673♂, MZUSP 2824♂, MZUSP 19795♀, MZUSP 19852♀.

Procyon lotor - MVZ 24602♀, MVZ 16545♂, MVZ 102281♀, MVZ 18468♀, MVZ 90703♂, MVZ 109255♂, MVZ 107708♀, MVZ 102286♀, MVZ 18472♀, MVZ 121228♂, MVZ 11739♂, MVZ 21125♀, MVZ 11737♂, MVZ 138990♂, MVZ 16266♀, MVZ 16267♀, MVZ 7082♀, MVZ 7084♀, MVZ 16666♂, MVZ 34159♀, MVZ 34160♀, MVZ 28000♂, MVZ 101810♂, MVZ 101813♂, MVZ 101808♀, MVZ 101807♂, MVZ 101809♀, MVZ 128839♂, MVZ 102287♀, MVZ 12983♂, MVZ 101325♀, MVZ 101326♂, MVZ 101626♀, MVZ 101812♀, MVZ 101811♂, MVZ 33592♂, MVZ 33593♀, MVZ 33594♂, MVZ 97902♀, MVZ 97903♂, MVZ 97905♂, MVZ 99294♂, MVZ 97904♀, MVZ 108056♀, MVZ 138991♂, MVZ 32450♀, MVZ 32451♂, MVZ 141821♂, MVZ 34753♂, MVZ 7080♀, MVZ 3240♀, MVZ 33617♂, MVZ 3611♀, MVZ 8990♂.

Procyon pygmaeus - MCZ 55723♂, MNHN 108512♀, MNHN 108509♂, MNHN 108510♀, MNHN 108508♀.

Mustelidae

Aonyx cinerea - FMNH 38009♀, FMNH 88612♀, FMNH 121229♀, FMNH 88611♀, FMNH 74281♀, FMNH 125384♂, FMNH 62868♀, FMNH 135739♂, FMNH 62869♂, FMNH 129494♀, MVZ 170987♀, MVZ 184090♀, USNM 151879♀, USNM 114465♀, USNM 155324♂, USNM 198054♀, USNM 19183♀, USNM 122840♂, USNM 144434♀, USNM 481273♀, USNM 478283♂, USNM 535212♀, USNM 314979♂.

Aonyx congicus - MVZ 19210♂, USNM 175520♀.

Arctonyx albogularis - AMNH 32258♀, AMNH 57373♂, AMNH 84391♂, AMNH 41475♂, AMNH 113710♂, AMNH 85025♂, AMNH 57118♂, AMNH 117412♂, AMNH 110460♀, AMNH 110464♂, AMNH 45294♂, AMNH 110461♀, ANSP 15037♀, FMNH 39506♀, FMNH 39373♀, USNM 259015♀.

Arctonyx collaris - AMNH 87394♂, AMNH 37221♂, ANSP 15178♂, ANSP 15279♀, FMNH 31779♂, FMNH 38027♂, FMNH 38025♀, MVZ 186563♂, USNM 277852♂, USNM 83517♂, USNM 83516♂, USNM 258860♂.

Arctonyx hoevenii - ANSP 20230♀, ANSP 20232♀.

Eira barbara - MN 68625♀, MN 29976♂, MN 6089♀, MN 6085♂, MN 6088♂, MN 5619♂, MN 63452♀, MN 5649♂, MN 10990♂, MN 30003♂, MN 74385♂, MN 5512♀, MN 7611♀, MN 29977♀, MN 68357♂, MN 68358♀, MN 3102♀, MN 3107♀, MN 75096♂, MN 4904♂, MN 4906♀, MN 4907♀, MN 5163♂, MPEG 331♂, MPEG 134♂, MPEG 1523♂, MPEG 5625♂, MPEG 5629♂, MPEG 5627♀, MPEG 5632♀, MPEG 10014♂, MPEG 10013♀, MZUSP 594♀, MZUSP 5188♀, MZUSP 5189♂, MZUSP 926♀, MZUSP 5192♀, MZUSP 5140♀, MZUSP 5191♂, MZUSP 3672♀, MZUSP 10145♀, MZUSP 10143♀, MZUSP 5186♀, MZUSP 5187♂, MZUSP 5275♂, MZUSP 5195♀, MZUSP 19806♀, MZUSP 19845♀, MZUSP 19803♀, MZUSP 19804♂, MZUSP 19799♀, MZUSP 19809♀, MZUSP 19802♀, MZUSP 22901♂, MZUSP 19810♂, MZUSP 19807♂, MZUSP 19800♀, MZUSP 7029♂, MZUSP 3375♂, MZUSP 4244♀, MZUSP 3917♀, MZUSP 4243♀, MZUSP 4242♀, MZUSP 2726♀, MZUSP 2237♂, MZUSP 2727♂, MZUSP 5933♀, MZUSP 2728♂, MZUSP 6584♂, MZUSP 6811♀, MZUSP 2972♂, MZUSP 2975♂, MZUSP 1162♀, MZUSP 2976♂, MZUSP 19817♀, MZUSP 1161♀, MZUSP 1807♂, MZUSP 2848♂, MZUSP 2975♂, MZUSP 2974♂, MZUSP 2924♀, MZUSP 488♂, MZUSP 7699♀, MZUSP 3760♂, MZUSP 3184♂, MZUSP 2468♂, MZUSP 2469♀.

Enhydra lutris - MVZ 6956♀, MVZ 163330♂, MVZ 116213♀, MVZ 114550♀, MVZ 116611♀, MVZ 140630♂, MVZ 184092♂, MVZ 154700♀, MVZ 135831♂, MVZ 184093♂, MVZ 34236♂, MVZ 113396♂, MVZ 119824♂, MVZ 84812♂, MVZ 184096♂, MVZ 186320♂, MVZ 123192♂, MVZ 123194♀, MVZ 123196♂, MVZ 123198♂, MVZ 123197♂, MVZ 123199♀, MNHN 146438♂, MNHN 146439♂, MNHN 146440♀, MNHN 146441♀, MNHN 36266♀, MNHN 285413♂, MNHN 263315♂, MNHN 285425♂, MNHN 271748♂, MNHN 286368♀, MNHN 286382♂, MNHN 286374♂, MNHN 286379♂, MNHN 285481♂, MNHN 285433♂, MNHN 288813♂, MNHN 288799♀, MNHN 288815♀, MNHN 288818♀, MNHN 286398♀, MNHN 288798♀, MNHN 288810♂, MNHN 288811♀, MNHN 288812♂.

Galictis cuja - AMNH 235992♂, AMNH 33281♂, AMNH 205832♀, AMNH 212544♂, AMNH 38983♀, FCNyM 2020♂, FCNyM 15.V.97.42♀, FCNyM 8.V.59.6♂, FCNyM 23.X.98.3♀, FCNyM 6.III.36.25♂, FCNyM 2034♂, MACN 23291♂,

MACN 13939♀, MACN 23604♂, MACN 16520♂, MACN 16254♀, MACN 13963♂, MACN 13075♂, MACN 3096♂, MACN 3095♂, MPEG 22230♀, MPEG 22188♂, MPEG 22229♀, MVZ 114774♂, MVZ 85164♂, MZUSP 19821♂, MZUSP 6650♂, MZUSP 6467♂, MZUSP 8454♂, MZUSP 978♀, MZUSP 9633♂, MZUSP 3066♂, MZUSP 231♀, MZUSP 232♂, MZUSP 1247♂, USNM m♂, USNM 271573♂, USNM 172796♂, FCNyM 1014♂, FCNyM 704♂, FCNyM 1261♂, FCNyM 388♂, FCNyM 3. V.99.4♂, FCNyM 671♂, FCNyM 674♂, FCNyM 1262♂, FCNyM 1028♀, FCNyM 1706♂, FCNyM 1705♂.

Galictis vittata - AMNH 6287♂, AMNH 35257♀, AMNH 98568♂, AMNH 98571♂, AMNH 98572♀, AMNH 98569♂, AMNH 77695♂, AMNH 76630♀, AMNH 173910♂, AMNH 76856♀, MACN 5091♀, MPEG 8522♂, MPEG 4221♀, MPEG 4220♀, MPEG 5651♂, MPEG 4222♂, MPEG 4223♂, MPEG 22603♂, MPEG 1139♂, MPEG 6520♀, MPEG 6524♂, MPEG 22604♂, MPEG 8861♂, MVZ 184098♂, MVZ 157992♀, MVZ 155227♀, MVZ 155226♀, USNM 292156♀, USNM 443283♂, USNM 443720♀, USNM 395050♂, USNM 281481♀, USNM 460074♀, USNM 361041♀, USNM 362246♀.

Gulo gulo - AMNH 34506♀, AMNH 34500♀, AMNH 37432♂, AMNH 37432♂, AMNH 19842♀, AMNH 22933♂, AMNH 19485♂, AMNH 37433♀, AMNH 22932♂, AMNH 137270♀, AMNH 37431♀, AMNH 182935♀, AMNH 169501♂, AMNH 165766♀, AMNH 34448♀, AMNH 182934♀, AMNH 109♀, AMNH 18263♂, MVZ 32807♀, MVZ 16373♀, MVZ 33475♀, MVZ 4346♂, MVZ 22121♂, MVZ 22120♀, MVZ 119843♂, MVZ 4682♀, MVZ 4683♀, MVZ 119842♂, MVZ 4218♀, MVZ 4217♀, MVZ 128119♂, MVZ 44167♀, MVZ 21981♂, MVZ 123822♂, MVZ 123994♀, MVZ 101129♀, MVZ 184100♀, MVZ 184099♂, MVZ 122236♂, MVZ 122238♀, MVZ 122239♂, MVZ 4799♀, MVZ 33267♀, MVZ 43633♀, MVZ 43632♂, MVZ 122237♂, MVZ 43625♀, MVZ 34396♀, MVZ 43631♂, MVZ 119841♂, MVZ 30049♂.

Hydrichtis maculicollis - AMNH 51829♀, AMNH 51825♀, AMNH 51828♂, AMNH 51827♂, AMNH 239613♂, AMNH 89807♂, AMNH 89808♂, AMNH 119955♂, AMNH 82402♂, USNM 220396♂, USNM 429136♀.

Ictonyx libyca - MVZ 34190♂, USNM 379079♀, USNM 410550♀, USNM 454406♂, USNM 454405♂, USNM 402154♂, USNM 454407♂, USNM 402156♂, USNM 402155♀, USNM 476028♂, USNM 410542♂, USNM 410553♂, USNM 410554♀, USNM 410541♀, USNM 410549♀, USNM 410547♀, USNM 410546♂, USNM 410544♀, USNM 410552♂, USNM 350097♀, USNM 410543♂, USNM 350096♂, USNM 317111♂, USNM 317113♀, USNM 402158♂, USNM 410548♀, USNM 410540♂, USNM 476028♂, USNM 402157♂, USNM 342062♀, USNM 342065♂, USNM 342064♂, USNM 342063♂, USNM 342061♀, USNM 317110♂, USNM 317109♀, USNM 350095♀, USNM 317108♀.

Ictonyx striatus - AMNH 80666♂, AMNH 80664♂, AMNH 116288♀, AMNH 116284♂, AMNH 116285♂, AMNH 116287♂, AMNH 116286♂, AMNH 116282♀, AMNH 116283♂, AMNH 116280♀, AMNH 116295♀, AMNH 116298♂, AMNH 116293♀, AMNH 116290♂, AMNH 116297♀, AMNH 116296♂, AMNH 116291♂, AMNH 116301♀, AMNH 116304♂, AMNH 116300♂, AMNH 116308♀, AMNH 116311♀, AMNH 116307♂, AMNH 116318♂, AMNH 116309♀, AMNH 116320♂, AMNH 168959♂, AMNH 168961♂, AMNH 168958♀, AMNH 168962♂, AMNH

168965♂, AMNH 168964♂, AMNH 168966♂, AMNH 168968♂, AMNH 168967♂, AMNH 168975♀, AMNH 168974♂, AMNH 168972♀, AMNH 168969♂, AMNH 168973♀, MVZ 118428♂, MVZ 117814♂, MVZ 118436♂, MVZ 118438♂, MVZ 118429♂, MVZ 117815♀, MVZ 118432♀.

Lontra canadensis - FMNH 196096♀, FMNH 171158♀, FMNH 171149♀, FMNH 171155♂, FMNH 167045♀, FMNH 176621♂, FMNH 176621♂, MVZ 46661♀, MVZ 51577♂, MVZ 19098♂, MVZ 19152♂, MVZ 33621♀, MVZ 38872♀, MVZ 36530♀, MVZ 23665♂, MVZ 24768♀, MVZ 25335♂, MVZ 24779♂, MVZ 25336♂, MVZ 27853♂, MVZ 29243♀, MVZ 208♂, MVZ 208♂, MVZ 285♂, MVZ 4415♂, MVZ 21242♂, MVZ 21243♂, MVZ 19087♀, MVZ 12475♀, MVZ 4400♂, MVZ 4401♀, MVZ 986♀, MVZ 4338♂, MVZ 19088♂, MVZ 25554♀, MVZ 61451♂, MVZ 19153♂, MVZ 12653♂, MVZ 38894♂, MVZ 19154♂, MVZ 35356♂, MVZ 33318♂, MVZ 44571♂, MVZ 38989♂, MVZ 38990♂, MVZ 38988♀.

Lontra felina - ANSP 2446♂, FMNH 24226(f), FMNH 24223♂, FMNH 49884♂, FMNH 41242♂, MVZ 141632♂, MVZ 141631♂, MVZ 141868♂, MVZ 119556♂, USNM 25768♂, USNM A24785♂, USNM 512791♀.

Lontra longicaudis - AMNH 78512♀, AMNH 4765♀, AMNH 67099♂, AMNH 98589♂, AMNH 188309♂, AMNH 205176♂, AMNH 133949♀, AMNH 205902♀, AMNH 133950♀, AMNH 207729♀, AMNH 142247♀, AMNH 14633♂, AMNH 142248♂, AMNH 23507♂, AMNH 23494♀, AMNH 29841♂, AMNH 38121♀, AMNH 33191♀, AMNH 29840♂, AMNH 6678♂, AMNH 63958♂, AMNH 96286♀, AMNH 80092♂, AMNH 41940♂, AMNH 74458♀, AMNH 64114♀, AMNH 214498♂, AMNH 71861♂, AMNH 230840♂, AMNH 71860♀, FMNH 69582♂, FMNH 62265♀, FMNH 16554♂, FMNH 93536♂, FMNH 68320♀, FMNH 93535♀, FMNH 68321♀, FMNH 98079♂, FMNH 78461♂, MN 22255♂, MN 25628♂, MN 50800♂, MN 3031♂, MN 3020♂, MN 30002♂, MN 25680♂, MVZ 153652♀, MVZ 170966♂, MVZ 116823♂, MZUSP 5171♂, MZUSP 5172♀, MZUSP 1080♀, MZUSP 10366♂.

Lontra provocax - FMNH 24222♀, FMNH 24224♂.

Lutra lutra - AMNH 206592♂, AMNH 32257♀, AMNH 44788♀, AMNH 1294♂, AMNH 36598♀, AMNH 240918♀, AMNH 89♂, ANSP 20234♀, ANSP 2001♂, ANSP 11395♂, ANSP 5264♂, ANSP 6844♀, FMNH 81491♂, FMNH 85860♂, FMNH 39181♀, FMNH 104393♂, FMNH 75863♂, FMNH 75858♀, FMNH 75859♀, FMNH 75861♂, FMNH 75860♀, FMNH 995225♀, FMNH 99385♂, FMNH 99383♂, FMNH 99384♂, FMNH 97843♀, MVZ 34264♀, USNM 259466♀, USNM 199613♀, USNM 358573♂, USNM 188617♀, USNM A01292♂, USNM 188616♀, USNM 154157♂, USNM A21120♀, USNM 188615♂, USNM 874♀, USNM 327735♂.

Lutra sumatrana - FMNH 66014♂, USNM 142337♀, USNM 309015♂, USNM 309014♀, USNM 142336♀.

Lutrogale perspicillata - AMNH 99610♂, AMNH 163186♂, AMNH 212978♂, FMNH 63799(f).

Lyncodon patagonicus - FCNyM 1255♂, FCNyM 27.III.96.1♂, FCNyM 1256♂, FCNyM 6.III.36.32♂, MACN 31214♂, MVZ 163429♂.

Martes americana - MVZ 33627♀, MVZ 115553♀, MVZ 24822♂, MVZ 33626♂, MVZ 24823♂, MVZ 32808♂, MVZ 38294♂, MVZ 38293♂, MVZ 38292♂, MVZ

23041♂, MVZ 23040♂, MVZ 13770♀, MVZ 13769♂, MVZ 13768♂, MVZ 13767♂, MVZ 34163♂, MVZ 34162♀, MVZ 22113♂, MVZ 22110♀, MVZ 22109♂, MVZ 36574♂, MVZ 13773♂, MVZ 13772♂, MVZ 13771♀, MVZ 69634♂, MVZ 216770♂, MVZ 23043♂, MVZ 23042♀, MVZ 46665♀, MVZ 46664♀, MVZ 62776♂, MVZ 62775♂, MVZ 62774♂, MVZ 37495♀, MVZ 37494♀, MVZ 37493♀, MVZ 95042♂, MVZ 2086653♀, MVZ 97313♀, MVZ 97310♀, MVZ 62777♂, MVZ 32801♂, MVZ 97312♀.

Martes flavigula - AMNH 101633♀, AMNH 80155♀, AMNH 106620♂, AMNH 80110♀, AMNH 102881♂, AMNH 106621♂, AMNH 106622♀, AMNH 171164♀, AMNH 27615♀, AMNH 43148♂, AMNH 43147♀, AMNH 110474♀, AMNH 110472♀, AMNH 57046♂, AMNH 110473♀, AMNH 38331♂, AMNH 55904♂, AMNH 59317♀, AMNH 85044♀, AMNH 84445♀, AMNH 113029♀, AMNH 206591♀, AMNH 87391♂, AMNH 171167♂, AMNH 171165♂, AMNH 163581♂, AMNH 163580♀, AMNH 163585♂, AMNH 163587♀, AMNH 163582♂, AMNH 163584♂, AMNH 163586♂, AMNH 163583♀, AMNH 107135♀, AMNH 103999♀, AMNH 106062♀, AMNH 106061♀, AMNH 117629♀, AMNH 117619♀, AMNH 117621♂, AMNH 84894♀, ANSP 15279♂, ANSP 19266♂, ANSP 15278♂.

Martes foina - AMNH 163433♀, AMNH 163434♂, AMNH 163435♀, AMNH 163436♀, AMNH 69516♀, AMNH 69517♂, AMNH 6289♀, AMNH 70182♂, AMNH 70236♀, FMNH 35827♂, MVZ 77293♂, MVZ 77294♂, USNM 173300♂, USNM 173299♂, USNM 173298♂, USNM 173297♀, USNM 173296♂, USNM 173295♂, USNM 173306♂, USNM 173305♂, USNM 173304♀, USNM 173303♀, USNM 173302♀, USNM 173301♀, USNM 173309♂, USNM 173308♂, USNM 173307♀, USNM 173310♂, USNM 173313♀, USNM 173315♀, USNM 174075♂, USNM 174078♀, USNM 198481♂, USNM 198485♀, USNM 173311♂, USNM 174076♂, USNM 174077♂, USNM 198483♀, USNM 198482♀, USNM 198484♂, USNM 173314♂.

Martes martes - AMNH 69527♀, AMNH 36633♀, AMNH 183359♀, FMNH 84721♂, USNM 1291♂, USNM 1291♂, USNM 188101♂, USNM 1964♀, USNM 188100♀.

Martes melampus - AMNH 184575♂, AMNH 184574♀, MVZ 114424♂, USNM 13832♂, USNM 13833♂, USNM 115684♂.

Martes pennanti - AMNH 121557♂, AMNH 12558♂, MVZ 16596♂, MVZ 21396♂, MVZ 24740♂, MVZ 23686♀, MVZ 23668♂, MVZ 196716♂, MVZ 43621♀, MVZ 54935♂, MVZ 54934♂, MVZ 44543♀, MVZ 44532♀, MVZ 43624♂, MVZ 43623♀, MVZ 43615♀, MVZ 43619♀, MVZ 43618♂, MVZ 43617♀, MVZ 44534♀, MVZ 44533♂, MVZ 44531♂, MVZ 31129♀, MVZ 31093♀, MVZ 29809♂, MVZ 29791♂, MVZ 29811♂, MVZ 186282♂, MVZ 23884♂, MVZ 23883♂, MVZ 132532♂, MVZ 97899♀, MVZ 20955♀, MVZ 185230♀, MVZ 31133♀, MVZ 31132♂, MVZ 29813♀, MVZ 29812♀, MVZ 186281♂, MVZ 186283♀.

Martes zibellina - AMNH 97799♂, AMNH 97800♂, MVZ 4943♂, USNM 113948♀, USNM 113991♀, USNM 113990♀, USNM 21986♂, USNM 113980♂, USNM 113969♀, USNM 113960♂, USNM 20884♂, USNM 113993♀, USNM 113970♀, USNM 113962♀, USNM 49483♂, USNM 113949♀, USNM 49484♂, USNM 113951♀, USNM 113950♀, USNM 113971♂, USNM 113981♂, USNM 113992♂, USNM 113972♀, USNM 113985♂, USNM 1173974♂, USNM 113982♂,

USNM 113963♂, USNM 83449♂, USNM 113952♂, USNM 113954♀, USNM 83451♂, USNM 113965♀, USNM 83450♀, USNM 113953♀, USNM 113975♀, USNM 113983♀, USNM 113956♂, USNM 113944♂, USNM 113966♂, USNM 113977♀, USNM 113976♀, USNM 113986♀, USNM 113984♀.

Meles anakuma - AMNH 18698♀, USNM 23934♂.

Meles leucurus - AMNH 85048♀, AMNH 57117♀, AMNH 84356♂, ANSP 16691♀, ANSP 15032♂, FMNH 36735(m), FMNH 57164♀, FMNH 57195♀, MVZ 398774♂, USNM 199041(m), USNM 242566♀.

Meles meles - AMNH 69513♂, AMNH 69512♂, AMNH 69503♂, AMNH 173524♂, AMNH 70602♂, AMNH 1295♂, AMNH 99346♂, AMNH 70603♂, AMNH 97794♂, ANSP 2449♂, MVZ 184106♂, MVZ 34265♂, MVZ 21980♂, MVZ 129663♀, MVZ 129662♂, USNM 188600♂, USNM 38434♂, USNM 534227♀, USNM 319225♀, USNM 257677♂, USNM 154154♂, USNM 257676♂, USNM 188601♂, ANSP 4047♂, ANSP 525♂, ANSP 2450♂, ANSP 3310♂.

Meles canescens - AMNH 69511♂, AMNH 88700♀, AMNH 88698♂, FMNH 97837♂, FMNH 98435♂, FMNH 98434♂, FMNH 47418♂, USNM 171964♂, USNM 327733♀, USNM 327732♂, ANSP 17516♀.

Mellivora capensis - AMNH 34262♂, AMNH 34263♂, AMNH 69499♂, AMNH 81831♂, AMNH 81232♀, AMNH 51951♀, AMNH 51952♂, AMNH 169085♂, AMNH 119622♂, AMNH 83450♂, ANSP 12158♂, ANSP 3814♂, FMNH 35694(FMNH), FMNH 85510♂, FMNH 29794♂, FMNH 43298♂, MVZ 184107♂, MNHN 537650♂, MNHN 175751♂, MNHN 296107♂, MNHN 164700♀, MNHN 283341♂.

Melogale everetti - FMNH 108979♀, FMNH 108980♀, FMNH 49254♀, USNM 301103♀, USNM 293171♂, USNM 293172♀, USNM 293170♂, USNM 83969♂, USNM 292893♂, USNM 292891♂, USNM 292887♀, USNM 292888♀, USNM 292890♂, USNM 292892♀, USNM 292894♂, USNM 292889♂.

Melogale moschata - AMNH 43168♂, AMNH 44709♀, AMNH 85028♀, AMNH 43167♀, AMNH 60191♀, AMNH 45336♂, AMNH 85026♂, AMNH 57033♀, AMNH 58271♀, AMNH 5693♂, AMNH 58272♂, AMNH 59980♀, AMNH 60031♀, AMNH 59978♂, AMNH 60087♂, AMNH 60059♂, AMNH 60034♀, AMNH 60058♀, AMNH 60032♀, AMNH 60035♀, AMNH 38329♀, AMNH 85031♀, AMNH 185123♂, AMNH 185122♂, AMNH 185121♂, AMNH 184976♀, AMNH 185389♂, AMNH 185390♀, AMNH 184544♂, AMNH 184975♀, AMNH 184977♂, AMNH 184974♂, MVZ 186566♂, MVZ 186565♀, MVZ 174840♂, MVZ 174839♀, MVZ 184108♀, MNHN 330696♂, MNHN 330698♀, MNHN 294294♀, MNHN 330706♂, MNHN 330702♂, MNHN 330699♀, MNHN 330700♀, MNHN 294295♂, MNHN 330701♂, MNHN 330695♂, MNHN 358572♂.

Melogale orientalis - AMNH 102086♀, AMNH 102074♀, AMNH 102076♂, AMNH 102695♀, AMNH 102075♀, AMNH 102089♂, AMNH 102088♀, MVZ 186567♀, MNHN 121501♂, MNHN 522010♂, MNHN 522009♂, MNHN 522011♀, MNHN 522012♂, AMNH 31806♀.

Melogale personata - FMNH 30888♂, FMNH 37996♀, FMNH 37994♀, FMNH 37997♀, FMNH 37995♀, FMNH 39129♀, FMNH 30887♂, USNM 257831♂, USNM 297171♂, USNM 253410♀, USNM 505242♂, USNM 258015♂, USNM 355329♂, USNM 258014♂, USNM 356605♂, USNM 356828♂, USNM 357294♂, USNM 358076♀, USNM 356929♀, USNM 261614♀, USNM 356604♂, USNM

356597♀, USNM 356598♂, USNM 356599♀, USNM 356600♂, USNM 356601♂, USNM 543102♂, USNM 357293♂, USNM 357743♂, USNM 356931♂, USNM 357625♂, USNM 357739♂, USNM 357626♂, USNM 357741♂, USNM 357740♂, USNM 358077♀, USNM 356930♂, USNM 357742♂, USNM 358071♂, USNM 358078♀, USNM 358105♂, USNM 358073♀, USNM 358074♀, USNM 358075♀, USNM 358072♀.

Mustela africana - AMNH 61813♀, MPEG 6515♂, MPEG 6528♀, MPEG 293♀, MPEG 6527♂, MPEG 289♂, MPEG 292♀, MPEG 22448♂, MPEG 22449♀, MPEG 22443♂, MPEG 22446♀, MPEG 6526♂, MPEG 22450♂, USNM 255119♂.

Mustela altaica - AMNH 117605♂, AMNH 117604♀, FMNH 49923♀, FMNH 44770♂, FMNH 44348♀, FMNH 44769♂, FMNH 46039♀, USNM 62110♂, USNM 198476♂, USNM 198475♂, USNM 198474♀, USNM 84059♀, USNM 198473♀, USNM 84058♀, USNM 176038♀, USNM 176037♂, USNM 176036♂, USNM 176035♀, USNM 176034♀, USNM 176033♂, USNM 354422♂, USNM 354421♂, USNM 270534♂, USNM 270608♀, USNM 270609♀.

Mustela erminea - MVZ 43811♂, MVZ 43810♂, MVZ 41064♂, MVZ 41063♀, MVZ 41061♂, MVZ 40177♂, MVZ 40175♀, MVZ 53438♂, MVZ 53783♀, MVZ 86689♂, MVZ 8668♂, MVZ 86687♂, MVZ 32577♀, MVZ 32576♂, MVZ 32575♂, MVZ 32573♂, MVZ 43816♀, MVZ 43815♀, MVZ 43813♂, MVZ 43812♂, MVZ 53419♂, MVZ 53413♂, MVZ 53411♂, MVZ 53410♂, MVZ 53416♂, MVZ 53417♂, MVZ 53414♂, MVZ 10686♂, MVZ 10683♂, MVZ 43403♂, MVZ 43402♂, MVZ 43401♂, MVZ 43400♂, MVZ 43399♂, MVZ 43398♂, MVZ 43397♂, MVZ 43394♂, MVZ 43393♂, MVZ 43392♂, MVZ 43391♂, MVZ 43384♂, MVZ 43385♂, MVZ 43386♂, MVZ 43387♂, MVZ 43388♂, MVZ 43389♂, MVZ 43390♂, MVZ 43395♂, MVZ 43396♂.

Mustela eversmanni - AMNH 60102♂, AMNH 45605♀, AMNH 84312♀, AMNH 85382♀, AMNH 45606♀, AMNH 57338♂, FMNH 134578♀, USNM 172631♂, USNM 155161♂, USNM 155162♂, USNM 188449♂, USNM 175441♂, USNM 175439♀, USNM 22191♀, USNM 38365♂, USNM 188448♀, USNM 269134♀, USNM 259792♂, USNM 22192♂, MVZ 41206♂.

Mustela felipei - FMNH 89745♂, FMNH 70999♂.

Mustela frenata - MVZ 32421♂, MVZ 41038♂, MVZ 51973♂, MVZ 97346♂, MVZ 14700♀, MVZ 114508♂, MVZ 51666♂, MVZ 120868♀, MVZ 85158♀, MVZ 79821♂, MVZ 66010♂, MVZ 100040♀, MVZ 107357♂, MVZ 108067♀, MVZ 3761♀, MVZ 91059♀, MVZ 119049♀, MVZ 95821♂, MVZ 95822♀, MVZ 95823♀, MVZ 95824♂, MVZ 95825♂, MVZ 46723♂, MVZ 119050♀, MVZ 119051♂, MVZ 119052♂, MVZ 32820♂, MVZ 114560♂, MVZ 129256♂, MVZ 112020♂, MVZ 23909♀, MVZ 54957♂, MVZ 119807♀, MVZ 40302♂, MVZ 90474♀, MVZ 19723♀, MVZ 19722♂, MVZ 19721♂, MVZ 19720♂, MVZ 19719♂, MVZ 19718♂, MVZ 69999♂, MVZ 69995♂.

Mustela itatsi - AMNH 37850♂, USNM 140895♂, USNM 140894♀, USNM 140893♂, USNM 140892♂, USNM 140890♂, USNM 20942♂, USNM 140896♂, USNM 140897♂, USNM 140898♂, USNM 140899♂, USNM 140900♂, USNM 140901♂, USNM 140902♀, USNM 140903♂, USNM 140904♂, USNM 140905♂, USNM 140906♂, USNM 140908♂, USNM 140909♂, USNM 140911♂, USNM 140912♂, USNM 140913♂, USNM 140914♂, MVZ 113425♂.

Mustela kathiah - AMNH 43172♀, AMNH 114883♂, AMNH 163579♂, AMNH 56940♀, AMNH 47879♂, AMNH 44575♂, AMNH 60195♂, AMNH 87393♂, AMNH 45502♀, AMNH 150090♂, AMNH 47880♂, FMNH 39121♀, FMNH 39363♂, FMNH 75808♀, FMNH 84808♂, FMNH 84809♂, FMNH 75809♂, FMNH 32504♀, FMNH 32503♂, FMNH 32502♂, FMNH 39122♂, USNM 254587♀, USNM 254411♀, USNM 258180♂.

Mustela lutreola - AMNH 206590♀, ANSP 504♂, ANSP 495♂, USNM 38466♂.

Mustela nigripes - AMNH 2546♂, AMNH 22894♂, AMNH 22820♂, AMNH 41994♂, AMNH 1203♂, AMNH 140397♂, AMNH 42567♂, AMNH 121610♂, AMNH 70590♂, AMNH 35041♂, AMNH 40078♂, FMNH 25623♀, FMNH 167200♂, FMNH 25621♂, FMNH 25622♂, MVZ 77840♂, MVZ 55212♀, MVZ 220147♂, MVZ 66019♂, MVZ 70209♂, MVZ 184119♀, MVZ 78134♂, USNM 234118♂, USNM 228233♂, USNM 247073♀, USNM 32771♀, USNM 22311♂, USNM 22427♂, USNM 30066♂, USNM 30065♂, USNM 30064♀, USNM 110772♂, USNM 188450♂, USNM 188453♂, USNM 83994♂, USNM 83993♀, USNM 83992♂, USNM 188454♂, USNM 188452♀, USNM 188451♂, USNM 188456♀, USNM 188455♂, USNM 188457♀, USNM 188458♂, USNM 224450♂, USNM 234971♀, USNM 234973♂, USNM 232400♂, USNM 234970♀.

Mustela nivalis - MVZ 118875♂, MVZ 118713♂, MVZ 118715♂, MVZ 140472♀, MVZ 14471♀, MVZ 134457♂, MVZ 134452♂, MVZ 134456♀, MVZ 129405♀, MVZ 128907♂, MVZ 121202♂, MVZ 43288♂, MVZ 121201♂, MVZ 40059♀, MVZ 43289♂, MVZ 125600♂, MVZ 128911♀, MVZ 134458♂, MVZ 106863♂, MVZ 121141♀, MVZ 150827♀, MVZ 184120♀, MVZ 129406♀, MVZ 118720♂, MVZ 118717♂, MVZ 128708♀, MVZ 118719♀, MVZ 118721♀, MVZ 118873♂, MVZ 118718♀, MVZ 118874♂, MVZ 171952♂, MVZ 123587♂, MVZ 129383♂, MVZ 171951♀, MVZ 41213♀, MVZ 41212♂, MVZ 78308♂, MVZ 171954♀, MVZ 171953♀, MVZ 41031♂, MVZ 53801♂, MVZ 41030♀.

Mustela nudipes - ANSP 6674♂, ANSP 20233♂, FMNH 88607♂, FMNH 88290♂, USNM 151878♂, USNM 301102♂, USNM 267386♂, USNM 489385♂, USNM 489386♂.

Mustela putorius - AMNH 77730♂, AMNH 69520♂, AMNH 69519♂, AMNH 36631♂, AMNH 163437♂, AMNH 69521♂, AMNH 77727♀, AMNH 69518♂, AMNH 35093♂, AMNH 18019♂, AMNH 243106♀, AMNH 200850♂, AMNH 3528♀, AMNH 181985♀, AMNH 235238♀, AMNH 235224♂, AMNH 235239♀, AMNH 235240♀, AMNH 235241♀, AMNH 235233♀, AMNH 235242♀, AMNH 235237♀, AMNH 235244♀, AMNH 235243♀, AMNH 235245♀, AMNH 235246♀, AMNH 235250♀, AMNH 235248♀, FMNH 22530♀, FMNH 123765♀, FMNH 199823♀, FMNH 60690♂, FMNH 123658♂, FMNH 150736♂, FMNH 23447♂, MVZ 34262♂, MVZ 122052♂, MVZ 77296♂, MVZ 77295♂.

Mustela sibirica - AMNH 85414♂, AMNH 85430♂, AMNH 85440♂, AMNH 85495♂, AMNH 85409♂, AMNH 85419♂, AMNH 85439♀, AMNH 85410♂, AMNH 85413♀, AMNH 85411♂, AMNH 85412♂, AMNH 45354♀, AMNH 32265♀, AMNH 45350♂, AMNH 32266♂, AMNH 32267♂, AMNH 114878♂, AMNH 114880♂, AMNH 110469♀, AMNH 110467♂, AMNH 110471♂, AMNH 110468♀, FMNH 35995♂, FMNH 35994♂, FMNH 46040♀, FMNH 46041♂, FMNH 18922♂, FMNH 18921♂, FMNH 45976♂, FMNH 41016♂, FMNH 37805♂,

FMNH 37806♂, FMNH 36695♂, FMNH 37807♂, FMNH 37804♂, FMNH 36694♂, FMNH 35465♂, FMNH 104392♂, MVZ 41208♂, MVZ 30484♂.

Mustela subpalmata - USNM 283268♂, USNM 283267♀, USNM 283266♀, USNM 277284♀, USNM 277282♀, USNM 277283♂, USNM 317101♂, USNM 317102♂, USNM 317104♂, USNM 317103♂, MVZ 107719♀.

Mustela vison - MVZ 19298♂, MVZ 12012♂, MVZ 12016♀, MVZ 12014♂, MVZ 12015♀, MVZ 12013♂, MVZ 12011♀, MVZ 90445♂, MVZ 44140♂, MVZ 24731♀, MVZ 24730♀, MVZ 24640♂, MVZ 23690♀, MVZ 23659♂, MVZ 69090♂, MVZ 13774♀, MVZ 38924♀, MVZ 44141♂, MVZ 34164♀, MVZ 23051♂, MVZ 23050♀, MVZ 23049♀, MVZ 36464♀, MVZ 34237♂, MVZ 33620♀, MVZ 31112♀, MVZ 31111♂, MVZ 34956♂, MVZ 34955♂, MVZ 10851♀, MVZ 10850♂, MVZ 24771♂, MVZ 24767♂, MVZ 24766♂, MVZ 23666♂, MVZ 27852♂, MVZ 39882♀, MVZ 13775♀, MVZ 12905♂, MVZ 12904♂, MVZ 12903♂.

Poecilogale albinucha - AMNH 118614♂, AMNH 119660♂, AMNH 86491♂, AMNH 116322♂, AMNH 180890♂, AMNH 86490♀, FMNH 26050♂, FMNH 26047♀, FMNH 26053♀, FMNH 26056♂, FMNH 26054♀, FMNH 26340♀, FMNH 26338♀, FMNH 26341♀, FMNH 26342♂, FMNH 26344♀, FMNH 26345♀, FMNH 26349♂, FMNH 26347♂, FMNH 26348♀, FMNH 27580♀, FMNH 27582♂, FMNH 27585♂, FMNH 27587♂, FMNH 27588♂, FMNH 38145♂, FMNH 26546♂, FMNH 36039♂, FMNH 149355♀, FMNH 177236♂, FMNH 177235♂, FMNH 214727♂, USNM 377040♂.

Pteronura brasiliensis - AMNH 77735♂, AMNH 214394♀, AMNH 73587♂, AMNH 91711♂, AMNH 91712♂, AMNH 93732♂, AMNH 74431♀, AMNH 71858♀, AMNH 74430♂, AMNH 74432♀, AMNH 30190♂, AMNH 30191♀, AMNH 98594♂, AMNH 92834♂, FMNH 20015♀, FMNH 41233♂, FMNH 70768♂, FMNH 98078♂, MN 77231♂, MN 50803♂, MN 67470♀, MN 42695♂, MN 73635♂, MN 32700♂, MN 50802♂, MPEG 5602♂, MPEG 5605♀, MPEG 5603♂, MPEG 5604♀, MPEG 685♂, MPEG 5652♂, MZUSP 1658♂, MZUSP 7018♀, MZUSP 7025♂, MZUSP 7019♀, MZUSP 7024♀, MZUSP 2323♂, MZUSP 7020♂, MZUSP 7022♂, MZUSP 7021♀, MZUSP 32375♂, MZUSP 5173♀, MZUSP 5890♂.

Taxidea taxus - MVZ 1017769♀, MVZ 24733♀, MVZ 121834♀, MVZ 114582♂, MVZ 46656♀, MVZ 7076♀, MVZ 124431♂, MVZ 154699♂, MVZ 124431♂, MVZ 11336♂, MVZ 165913♂, MVZ 90625♀, MVZ 87958♂, MVZ 59755♂, MVZ 225023♂, MVZ 32964♀, MVZ 19746♂, MVZ 34166♀, MVZ 19747♀, MVZ 7078♂, MVZ 184130♂, MVZ 40629♂, MVZ 40628♂, MVZ 40627♂, MVZ 40626♂, MVZ 67775♂, MVZ 84959♀, MVZ 46527♀, MVZ 45904♂, MVZ 37280♂, MVZ 8277♂, MVZ 8276♂, MVZ 90855♂, MVZ 76251♂, MVZ 41463♂, MVZ 41465♀, MVZ 41466♂, MVZ 41467♂, MVZ 97200♀, MVZ 41500♂.

Vormela peregusna - AMNH 183358♂, FMNH 112447♀, FMNH 103964♂, FMNH 112448♀, FMNH 48482♂, USNM 200322♂, USNM 200321♂, USNM 15996♀, USNM 154995♀, USNM 200792♂.

Apêndice B

Comparação entre medidas de similaridade de matrizes

Comparação de matrizes é uma tópico de intenso debate, principalmente no contexto de genética quantitativa, na qual a estabilidade das matrizes de covariância fenotípica/genotípica tem importantes consequências para o estudo de evolução em escala micro (*e.g.* Brodie III, 1993; Whitlock et al., 2002; Wood e Brodie, 2015; Careau et al., 2015; Roff, 2000; Puentes et al., 2016) e macroevolutiva (*e.g.* Roff, 2000; Arnold et al., 2008; Roff, 2003; Prôa et al., 2013,3). Ao longo dos anos, diversas estatísticas de comparação de matrizes foram desenvolvidas e empregadas neste contexto (*e.g.* Flury, 1987, 1984; Cheverud et al., 1983; Roff, 2002; Cheverud e Marroig, 2007; Hansen e Houle, 2008; Calsbeek e Goodnight, 2009; Mitteroecker e Bookstein, 2009; Roff et al., 2012; Garcia, 2012). Abaixo eu comparo quatro medidas atualmente implementados no pacote produzido pelo laboratório *EVOLQG* (Melo et al., 2015): RS, correlação de matrizes, Krz_{Cor} , PCA_{sim} .

O método RS foi explicado em detalhes no Capítulo 2. Correlação de matrizes, nada mais é do que o coeficiente de correlação de Pearson dos elementos não-diagonais das matrizes sendo comparadas. A correlação de Krzanowski (1979) mede a similaridade dos subespaços definidos pelos principais eixos de variação (componentes principais, PCs) das matrizes sendo comparadas. A correlação de Krzanowski entre duas matrizes A e B é calculada como

$$Krz_{Cor}(A,B) = \frac{1}{k} \sum_{i=1}^k \sum_{j=1}^k \cos^2 \theta_{ij}$$

onde k é o numero de componentes principais selecionados, θ_{ij} é o ângulo entre o PC i da primeira matriz e o PC j da segunda matriz. Se usarmos todos os PCs de ambas matrizes, $Krz_{Cor}(A,B)$ é igual a 1. Sendo assim, sugere-se avaliar apenas um subconjunto de PCs para produzir uma estatística informativa, normalmente a metade menos um (17 PCs no presente caso).

Visto que essa estatística não leva em conta a variância explicada por

cada PC, Singhal e Seborg (2006) propôs o uso de uma derivação da correlação de Krzanowski chamada *PCA similarity*, e é calculada como

$$PCA_{sim}(A,B) = \frac{\sum_{i=1}^k \sum_{j=1}^k (\lambda_i^A \lambda_j^B) \cos^2 \theta_{ij}}{\sum_{i=1}^k \lambda_i^A \lambda_i^B}$$

onde λ_i^A e λ_j^B são os autovalores i e j das matrizes A e B respectivamente. Note que essa estatística, diferente da correlação original de Krzanowski por avaliar todos os PCs. Assim o PCA_{sim} avalia não apenas o alinhamento relativo dos autovetores de ambas as matrizes, como também a quantidade de variação contida em cada um deles.

Métodos

As similaridades de matrizes, tanto de covariância quanto de correlação, foram estimadas par-a-par produzindo, para cada estatística, uma matriz de similaridade entre espécies. Isso resultou em oito matrizes par-a-par (quatro estatísticas, dois tipos de matrizes), que foram comparadas entre si por correlação de matrizes. Para ilustrar o padrão evidenciado em cada matriz de similaridade eu empreguei dois métodos gráficos. O primeiro consiste em produzir um "mapa de calor", onde a intensidade de similaridade de matrizes é expressa através de uma escala de cor. O segundo método emprega Análises de Coordenadas Principais (PCOA) para resumir a variação entre matrizes a poucos eixos de variação.

PCOA é uma análise de decomposição espectral aplicada em uma matriz de distâncias, ao invés de uma matriz de variância-covariância. Assim, os autovetores obtidos através de uma PCOA representam os valores das observações nos eixos de máxima variação daquele espaço. Para um espaço euclidiano, a dispersão das observações em um PCOA é equivalente às projeções das variáveis originais nos autovetores de uma PCA regular. Visto

que o método requer a utilização de uma matriz de distância, eu obtive $1 - S$, onde S é a matriz de similaridades obtidas pelas diversas estatísticas. A matriz resultante é uma matriz de dissimilaridade e pode ser analisada regularmente por PCOA.

A seguir apresento a comparação entre os diferentes métodos quando aplicados nas matrizes de covariância e correlação. Foram obtidas as estatísticas de similaridade para todas as espécies com mais de 24 espécimes e as repetibilidades foram calculadas através de 1000 réplicas por Monte Carlo.

Resultados

Para matrizes de covariância os valores médios de similaridades corrigidos em ordem decrescente foram: PCA_{sim} (0.82), seguidos por RS (0.80), Krz_{Cor} (0.78) e $MatrixCor$ (0.60). Em termos de variação, Krz_{Cor} apresentou o menor desvio padrão (0.03), seguidos por RS (0.08), PCA_{sim} (0.08) e $MatrixCor$ (0.14). Para matrizes de correlação os padrões foram similares ($PCA_{sim} = 0.82 \pm 0.05$; $RS = 0.80 \pm 0.05$, $Krz_{Cor} = 0.77 \pm 0.03$ e $MatrixCor = 0.57 \pm 0.10$). Tanto RS quanto PCA_{sim} apresentaram similaridades de matrizes de correlação mais elevadas e menos variáveis do que as similaridades de covariâncias, enquanto similaridades por Krz_{Cor} e $MatrixCor$ apresentaram valores mais similares entre os dois tipos de matriz, com uma leve tendência para valores mais baixos em matrizes de correlação (figura B.1).

Os valores de correlação entre as diferentes estatísticas foram similares para as similaridades corrigidas e observadas, e por isso foco na descrição dos padrões observados. Para matrizes de covariância, RS, PCA_{sim} e correlação de matrizes apresentaram padrões de similaridade equivalentes (Tabela B.1, figura B.2), com correlações entre as diferentes estatísticas superiores a 0.89, enquanto todas as correlações com Krz_{Cor} foram inferiores a 0.43. Para matrizes de correlação o padrão foi similar, com exceção do fato de as

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Figura B.1: Comparação das estatísticas de similaridade de matrizes. **A-** Valores observados. **B-** Valores corrigidos para repetibilidade. *RS-* *Random Skewers*, *KrzCor.-* Correlação de Krzanowski, *PCASim.-* *PCA similarity*, *MatrixCor.-* Correlação de matrizes. *cov-* Matrizes de covariância, *cor-* Matrizes de correlação.

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Tabela B.1: Correlação entre medidas de similaridade de matrizes. Caselas em cinza representam comparações entre matrizes de covariância e correlação para a mesma estatística. Valores em negrito se referem a comparações para matrizes de covariância, e valores sublinhados se referem a comparações para matrizes de correlação. RS- *Random Skewers*, KrzCor- Correlação de Krzanowski, PCA_{sim} - *PCA similarity*, Matrix Cor.- Correlação de matrizes.

		RS		KrzCor		PCA_{sim}		Matrix Cor.	
Observados		cov	cor	cov	cor	cov	cor	cov	cor
RS	cov	1.0000	0.7032	0.4256	0.5114	0.8989	0.5886	0.9085	0.6369
	cor	0.7032	<u>1.0000</u>	0.3258	<u>0.3988</u>	0.6563	<u>0.8902</u>	0.6295	<u>0.7455</u>
KrzCor	cov	0.4256	0.3258	1.0000	0.7296	0.2896	0.1815	0.2987	0.3575
	cor	0.5114	<u>0.3988</u>	0.7296	<u>1.0000</u>	0.4368	<u>0.2912</u>	0.4568	<u>0.4388</u>
PCA_{sim}	cov	0.8989	0.6563	0.2896	0.4368	1.0000	0.7075	0.9241	0.6182
	cor	0.5886	<u>0.8902</u>	0.1815	<u>0.2912</u>	0.7075	<u>1.0000</u>	0.5988	<u>0.6912</u>
Matrix Cor.	cov	0.9085	0.6295	0.2987	0.4568	0.9241	0.5988	1.0000	0.6836
	cor	0.6369	<u>0.7455</u>	0.3575	<u>0.4388</u>	0.6182	<u>0.6912</u>	0.6836	<u>1.0000</u>
Corrigidos									
RS	cov	1.0000	0.6900	0.4249	0.5043	0.8949	0.5685	0.9126	0.6331
	cor	0.6900	<u>1.0000</u>	0.3070	<u>0.3689</u>	0.6108	<u>0.8665</u>	0.6186	<u>0.7538</u>
KrzCor	cov	0.4249	0.3070	1.0000	0.7348	0.2965	0.1710	0.3213	0.3567
	cor	0.5043	<u>0.3689</u>	0.7348	<u>1.0000</u>	0.4184	<u>0.2459</u>	0.4576	<u>0.4263</u>
PCA_{sim}	cov	0.8949	0.6108	0.2965	0.4184	1.0000	0.6751	0.9268	0.6031
	cor	0.5685	<u>0.8665</u>	0.1710	<u>0.2459</u>	0.6751	<u>1.0000</u>	0.5926	<u>0.7141</u>
Matrix Cor.	cov	0.9126	0.6186	0.3213	0.4576	0.9268	0.5926	1.0000	0.6753
	cor	0.6331	<u>0.7538</u>	0.3567	<u>0.4263</u>	0.6031	<u>0.7141</u>	0.6753	<u>1.0000</u>

correlações entre PCA_{sim} e RS com *MatrixCor* serem mais reduzidas (0.69 e 0.75 respectivamente). Adicionalmente, para matrizes de covariância há uma padronização evidente nas similaridade para todas as estatísticas, com a família Canidae apresentando valores mais elevados de similaridade entre si do que com outros grupos (ver capítulo 3), com a exceção aparente de *KrzCor* (figura B.2). Apesar disso, esse padrão não foi evidente quando as mesmas estatísticas foram calculadas sob as matrizes de correlação (figura B.3).

De forma resumida, podemos dizer que as matrizes de correlação se mostraram mais similares (ao menos segundo RS e PCA_{sim}) do que as matrizes de covariância. A estatística *KrzCor* se demonstrou a mais estável, com pouca variação tanto para matrizes de covariância quanto de correlação. RS, PCA_{sim} e *MatrixCor* apresentam padrões de similaridade altamente corre-

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Figura B.2: Mapa de calor de similaridade de matrizes de covariância segundo diferentes estatísticas. Valores mais claros são menores e valores mais escuros são maiores. RS- *Random Skewers*, KrzCor- *Correlação de Krzanowski*, PCAsim- *PCA similarity*, Matrix Cor.- *Correlação de matrizes*.

Figura B.3: Mapa de calor de similaridade de matrizes de correlação segundo diferentes estatísticas. Valores mais claros são menores e valores mais escuros são maiores. RS- *Random Skewers*, KrzCor- *Correlação de Krzanowski*, PCAsim- *PCA similarity*, Matrix Cor.- *Correlação de matrizes*.

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lacionado, apesar de diferirem muito em valor absoluto, com PCA_{sim} apresentando os maiores valores de similaridade e $MatrixCor$ os menores.

A inspeção dos gráficos de PCOA para as matrizes de covariância (Figura B.4) e correlação (Figura B.5) demonstram que, apesar de haver diferenças entre os gráficos como seria esperado através das análises de correlação, Canidae sempre emerge como um grupo particularmente distinto dos demais Caniformes. A investigação da relação das distâncias Riemmanianas entre matrizes (Mitteroecker e Bookstein, 2009; Bookstein e Mitteroecker, 2013) produziu um resultado muito similar ao observado para outras estatísticas (Figura C.3), principalmente para RS (Figura B.4), com a exceção da inversão do eixo que discrimina Canidae dos demais Caniformes. O resultado desta investigação preliminar foi exposto em formato de poster durante a reunião Evolution 2015 e pode ser visto no Apêndice C.

Essas comparações sugerem que estatísticas diferentes podem apresentar valores médios e variâncias distintas e mesmo assim evidenciar o mesmo padrão de similaridades entre os grupos. Isso se deve provavelmente ao fato de que todas as estatísticas avaliam aspectos comuns da matriz, mesmo que a similaridade entre duas matrizes seja calculadas de forma diferente. Isso parece ser particularmente válido para RS, PCA_{sim} e $MatrixCor$, os quais avaliam a matriz como um todo e apresentaram correlações altas entre si. Krz_{Cor} distoou desse padrão, apresentando correlações baixas com demais medidas sugerindo que, ao não quantificar a distribuição de variação nos diversos autovetores das matrizes, essa medida não reflete bem a capacidade de responder similarmente à seleção, como medido por RS.

Figura B.4: Análise de Coordenadas Principais das similaridades de matrizes por RS , Krz_{Cor} (krz), PCA_{sim} (pcs) e M_{cor} (mcor) para matrizes de covariância.

Figura B.5: Análise de Coordenadas Principais das similaridades de matrizes por RS , Krz_{Cor} (krz), PCA_{sim} (pcs) e M_{cor} (mcor) para matrizes de correlação.

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Appendix C

Poster Evolution 2015

Introduction

Evolutionary constraints are properties of populations that hinder the generation of variation of a given kind or bias the production of variants on a given direction Maynard-Smith et al. (1985). The interaction between constraints and the adaptive regime of a population can lead to different evolutionary outcomes Porto et al. (2015), thus being important in the understanding of macroevolutionary patterns of morphological variation.

The objective of the ongoing project is to investigate variation in morphological integration of the skull in caniform carnivorans, and the potential constraints that these might entail. Here, we investigate the similarities and differences in patterns of morphological integration within the group Caniformes. This is done by the investigation of the phenotypic covariance matrix $\mathbf{P}$, which contains information about the variation and correlation of phenotypic characters. $\mathbf{P}$ (and its genetic counterpart $\mathbf{G}$) matrices are never expected to be identical between populations due to its finite size (sampling effects) not to mention differences in local selection Marroig and Cheverud (2001). For that reason, it is important not to evaluate if species diverge in pattern of covariation, but if the observed differences are large enough to produce different evolutionary outcomes. Thus, we use quantitative genetic theory to evaluate how similarly species would respond to natural selection.

Materials

We analyzed a total of 4,708 specimens from 104 species. Morphometric measurements were obtained through digitized anatomical landmarks of the skull (Figure C.1) with the aid of a Microscribe MLX system. We estimated 33 interlandmark distances and calculated covariance matrices for these traits.

Methods

Prior to **P** estimation, sources of variation that were not of interest (*e.g.* geographical/subspecies variation, sexual dimorphism, etc) were controlled for using a linear model approach.

To evaluate if species would respond similarly to natural selection, matrices were compared using the Random Skewers (RS) method and corrected for repeatability using non-parametric bootstrap. To illustrate the pattern of matrix similarity, we performed a principal coordinate analysis (PCO) over the Riemannian distance between the correlation matrices for 97 taxa with larger samples. The species on the extremes of the main axis of variation were investigated using the Selection Decomposition Vector (SRD) method.

To evaluate the variation in morphological integration in caniforms, we used the coefficient of variation of eigenvalues (ICV). The ICV is positively correlated with evolutionary constraints and negatively correlated with evolutionary flexibility Marroig et al. (2009).

Results

The results of the Random Skewers analyses were high, with a mean value of 0.736, ranging from 0.464-0.962 (Figure C.2). For the full sample, 95% of all observations scored higher than 0.6, but this value raises to 0.7 for within-family comparisons. When we look at individual families, Canidae and Phocidae show the 95% of the within family comparisons higher than 0.76, and for Ursidae the value is 0.73. For Procyonids and Otariidae the value is closer to 0.7 and for Mephitidae and Mustelidae the values are 0.67 and 0.63, respectively.

It is worth noting that species with small sample size tended to show lower values of RS on average. Nevertheless, these species showed high

Figure C.1: Anatomical landmarks for this study exemplified in a *Canis lupus* skull in lateral and ventral views.

Figure C.2: Pairwise Random Skewers similarity for all 104 taxa. Cells on the diagonal are matrix repeatabilities. Values under diagonal are original values and values above it are corrected for repeatabilities.

values when compared to closely related taxa.

The result of the principal coordinate analysis (Figure C.3) places Canidae into a distinct region of the PCO when compared to other families. Additionally, Mustelidae shows more variation in the first two PCO axes, occupying regions of the "matrix-space" not sampled by the other taxa. SRD analysis between species on extremes of the first PCO axis (not shown) suggests that differences associated with the posterior nasal, anterior zygomatic and base regions of the skull are responsible for the observed pattern.

The magnitude of integration as measured through ICV shows a broad variation within each family, with substantial overlap between families.

Discussion

Our results show that caniform carnivorans respond similarly to natural selection, with RS scores for comparisons within the same family showing higher values than comparisons between families, thus suggesting the presence of a phylogenetic patterning in the variation of **P** structure. In the PCO analysis, all families tended to cluster together, with the exception of Canidae, which shows no overlap with other families in the first axis, and Mustelidae, which overlaps with the other families but shows a wider distribution. ICV variation within families largely overlaps, suggesting that overall integration is not playing a major role in the observed differences of covariation patterns between families.

The results from SRD analyses of the extreme species on the first PCO axis indicate that the differences between Mustelids and Canidae are concentrated in regions that differ in structure between taxa. This might suggest that the divergence in **P** mirrors some aspects of the morphological variation between both groups and could be an indicative of the interaction of morphological diversification and patterns of morphological integration

Figure C.3: Principal Coordinate Analysis of the pair-wise Riemannian distances between correlation matrices.

Figure C.4: Density distribution of morphological integration index (ICV) estimates. Dots represent individual species estimates.

(e.g. Porto et al. (2015); Melo and Marroig (2015); Marroig and Cheverud (2005)). Future macroevolutionary studies will further elucidate the possible interplay between morphological diversification and variation in covariation patterns for this group.

Acknowledgements

We acknowledge the help of the curatorial staff from the following institutions: AMNH, ANSP, FMNH, MACN, MCZ, MLP, MNRJ, MPEG, MVZ, MZUSP, NMNH. We also thank FAPESP for financial support (grants No.2011/21674-4 and 2013/22042-7)

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Appendix D

Apêndice do Capítulo 3

Table D.1: Sample sizes and factors controled for each species analysed. SSP- Subspecies or Geographical variation. SEX- sexual dimorphism. CAP- Captivity. AGE- Age cathegories. Ø - no effects controled. + additive effects. * interaction effects.

Family	Genus	Species	Control	n		
Canidae	Canis	Canis adustus	SSP+SEX	38		
		Canis anthus	SSP+SEX	54		
		Canis familiaris dingo	SSP+SEX	46		
		Canis latrans	SSP	61		
		Canis lupus	SSP+SEX	107		
		Canis lycaon	Ø	48		
		Canis mesomelas	Ø	57		
		Canis rufus	SEX	46		
		Lycaon pictus	SSP	50		
		Cerdocyon	Cerdocyon thous	Ø	77	
		Chrysocyon	Chrysocyon brachyurus	Ø	57	
		Lycalopex	Lycalopex culpaeus	SEX	44	
			Lycalopex griseus	Ø	39	
			Lycalopex gymnocercus	SEX	45	
			Lycalopex sechurae	Ø	37	
			Lycalopex vetulus	Ø	39	
			Nyctereutes	Nyctereutes procyonoides	Ø	41
			Otocyon	Otocyon megalotis	SSP	51
		Speothos	Speothos venaticus	Ø	56	
	Urocyon	Urocyon cinereoargenteus	SEX	44		
		Urocyon littoralis	SEX+SSP	54		
	Vulpes	Vulpes chama	Ø	26		
		Vulpes lagopus	SEX	52		
		Vulpes macrotis	SEX	48		
		Vulpes rueppellii	SEX	58		
		Vulpes velox	Ø	37		
		Vulpes vulpes	SEX	54		
		Vulpes zerda	Ø	45		
	Ursidae	Tremarctos	Tremarctos ornatus	Ø	28	
		Ursus	Ursus americanus	SEX	48	
Ursus arctos			SEX	67		
Ursus maritimus			SEX	84		
Ursus thibetanus			SSP+CAP	46		
Odobenidae	Odobenus	Odobenus rosmarus	Ø	60		
Otariidae	Arctocephalus	Arctocephalus australis	SEX+SSP	36		
		Arctocephalus gazella	Ø	35		
	Callorhinus	Callorhinus ursinus	SEX	55		
	Eumetopias	Eumetopias jubatus	SEX	53		
	Otaria	Otaria byronia	SEX	37		
	Zalophus	Zalophus californianus	Ø	38		

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Table D.1 – continued from previous page

Family	Genus	Species	Control	n
Phocidae	<i>Cystophora</i>	<i>Cystophora cristata</i>	SEX	41
	<i>Erignathus</i>	<i>Erignathus barbatus</i>	Ø	39
	<i>Leptonychotes</i>	<i>Leptonychotes weddellii</i>	Ø	51
	<i>Lobodon</i>	<i>Lobodon carcinophaga</i>	Ø	44
	<i>Mirounga</i>	<i>Mirounga leonina</i>	SEX	33
	<i>Neomonachus</i>	<i>Neomonachus tropicalis</i>	Ø	37
	<i>Phoca</i>	<i>Phoca vitulina</i>	SEX	53
	<i>Pusa</i>	<i>Pagophilus groenlandicus</i>	Ø	47
		<i>Pusa hispida</i>	Ø	51
Mephitidae	<i>Conepatus</i>	<i>Conepatus chinga</i>	Ø	92
		<i>Conepatus humboldtii</i>	Ø	29
		<i>Conepatus leuconotus</i>	SEX+SSP	41
		<i>Conepatus semistriatus</i>	Ø	39
	<i>Mephitis</i>	<i>Mephitis macroura</i>	SEX+SSP	45
		<i>Mephitis mephitis</i>	SEX+SSP	47
	<i>Spilogale</i>	<i>Spilogale angustifrons</i>	Ø	43
		<i>Spilogale gracilis</i>	SEX	48
		<i>Spilogale putorius</i>	SSP	40
Ailuridae	<i>Ailurus</i>	<i>Ailurus fulgens</i>	Ø	48
Procyonidae	<i>Bassaricyon</i>	<i>Bassaricyon alleni</i>	SEX	30
		<i>Bassaricyon medius</i>	SEX	40
	<i>Bassariscus</i>	<i>Bassariscus astutus</i>	SEX+SSP	46
		<i>Bassariscus sumichrasti</i>	SEX+SSP	45
	<i>Nasua</i>	<i>Nasua narica</i>	SEX	44
		<i>Nasua nasua</i>	SEX+SSP	60
	<i>Potos</i>	<i>Potos flavus</i>	SSP	63
	<i>Procyon</i>	<i>Procyon cancrivorus</i>	SEX	41
		<i>Procyon lotor</i>	SEX	52
Mustelidae	<i>Eira</i>	<i>Eira barbara</i>	SEX	77
	<i>Enhydra</i>	<i>Enhydra lutris</i>	SEX	43
	<i>Galictis</i>	<i>Galictis cuja</i>	SEX+SSP	45
		<i>Galictis vittata</i>	SEX+SSP	34
	<i>Gulo</i>	<i>Gulo gulo</i>	SEX	52
Mustelidae	<i>Ictonyx</i>	<i>Ictonyx libyca</i>	SEX+SSP	36
		<i>Ictonyx striatus</i>	SEX+SSP	47
		<i>Poecilogale albinucha</i>	Ø	33
	<i>Lontra</i>	<i>Lontra canadensis</i>	SSP	46
		<i>Lontra longicaudis</i>	SSP	46
	<i>Lutra</i>	<i>Lutra lutra</i>	SEX+SSP	37
	<i>Martes</i>	<i>Martes americana</i>	Ø	43
		<i>Martes flavigula</i>	SEX	44
		<i>Martes foina</i>	SEX+SSP	39
		<i>Martes pennanti</i>	SEX+SSP	34
		<i>Martes zibellina</i>	Ø	40

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Table D.1 – continued from previous page

Family	Genus	Species	Control	n
Nandiniidae Felidae	<i>Melogale</i>	<i>Melogale moschata</i>	∅	46
		<i>Melogale personata</i>	∅	45
	<i>Mustela</i>	<i>Mustela erminea</i>	SEX	49
		<i>Mustela frenata</i>	SEX	42
		<i>Mustela nigripes</i>	SEX	48
		<i>Mustela nivalis</i>	SEX+SSP	43
		<i>Mustela putorius</i>	SEX+SSP	39
		<i>Mustela sibirica</i>	SSP+SEX	39
		<i>Mustela vison</i>	∅	37
	<i>Pteronura</i>	<i>Pteronura brasiliensis</i>	∅	35
	<i>Taxidea</i>	<i>Taxidea taxus</i>	SEX	36
	<i>Nandinia</i>	<i>Nandinia binotata</i>	SEX+AGE+SSP	49
	<i>Acinonyx</i>	<i>Acinonyx jubatus</i>	SEX+SSP+AGE	50
	<i>Caracal</i>	<i>Caracal caracal</i>	SEX+AGE	50
	<i>Catopuma</i>	<i>Catopuma temminckii</i>	SEX+AGE	40
	<i>Felis</i>	<i>Felis catus</i>	AGE	29
		<i>Felis margarita</i>	AGE+SEX*CAT	49
		<i>Felis silvestris</i>	AGE+SEX*CAT	49
	<i>Leopardus</i>	<i>Leopardus geoffroyi</i>	SEX+SSP+CAT	47
		<i>Leopardus guttulus</i>	SEX	43
		<i>Leopardus mitis</i>	SEX+SSP+AGE	73
		<i>Leopardus wiedii</i>	SEX+SSP	50
	<i>Leptailurus</i>	<i>Leptailurus serval</i>	SEX*SSP+AGE+CAP	51
	<i>Lynx</i>	<i>Lynx lynx</i>	SSP+AGE	50
		<i>Lynx rufus</i>	SSP+AGE+SEX	46
	<i>Neofelis</i>	<i>Neofelis diardi</i>	SEX+AGE+CAT	40
	<i>Panthera</i>	<i>Panthera leo</i>	SEX+SSP+CAT	50
		<i>Panthera onca</i>	AGE+SSP+SEX	54
		<i>Panthera pardus</i>	SEX+SSP+AGE	52
		<i>Panthera tigris</i>	SEX+AGE+SSP+CAT	48
		<i>Panthera uncia</i>	SEX+SSP+AGE	46
	<i>Prionailurus</i>	<i>Prionailurus bengalensis</i>	SSP	50
	<i>Puma</i>	<i>Puma concolor</i>	SSP+AGE+SEX	50
		<i>Puma yagouaroundi</i>	SSP+SEX	50
Viverridae	<i>Arctictis</i>	<i>Arctictis binturong</i>	SSP+AGE	50
	<i>Arctogalidia</i>	<i>Arctogalidia stigmatica</i>	SEX	31
	<i>Civettictis</i>	<i>Civettictis civetta</i>	SEX*SSP	51
	<i>Genetta</i>	<i>Genetta genetta</i>	SSP*SEX	57
		<i>Genetta maculata</i>	SSP+SEX+CAT+AGE	59
		<i>Genetta servalina</i>	SSP	44
	<i>Hemigalus</i>	<i>Hemigalus derbyanus</i>	SSP+SEX+AGE	34
	<i>Paguma</i>	<i>Paguma larvata</i>	SSP+CAT	50
	<i>Paradoxurus</i>	<i>Paradoxurus musanga</i>	SSP+AGE	50
	<i>Viverra</i>	<i>Viverra zibetha</i>	SSP+AGE	48

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Table D.1 – continued from previous page

Family	Genus	Species	Control	n
Hyaenidae	<i>Viverricula</i>	<i>Viverricula indica</i>	SSP+AGE+SEX	56
	<i>Crocuta</i>	<i>Crocuta crocuta</i>	SEX*SSP+AGE	50
	<i>Hyaena</i>	<i>Hyaena hyaena</i>	AGE+CAT+SSP	51
	<i>Parahyaena</i>	<i>Parahyaena brunnea</i>	AGE+SSP+SEX	49
	<i>Proteles</i>	<i>Proteles cristata</i>	SSP+AGE	44
Herpestidae	<i>Atilax</i>	<i>Atilax paludinosus</i>	SEX+SSP	50
	<i>Crossarchus</i>	<i>Crossarchus obscurus</i>	Ø	31
		<i>Crossarchus platycephalus</i>	AGE+CAP	39
	<i>Cynictis</i>	<i>Cynictis penicillata</i>	SSP*CAP	53
	<i>Galerella</i>	<i>Galerella sanguinea</i>	SSP	36
	<i>Helogale</i>	<i>Helogale parvula</i>	SSP+AGE+CAP	50
	<i>Mungos</i>	<i>Mungos mungo</i>	SSP+AGE	62
	<i>Suricata</i>	<i>Suricata suricatta</i>	AGE+CAT	51
	<i>Urva</i>	<i>Urva javanica</i>	SEX+SSP	50
		<i>Urva urva</i>	AGE+SEX+SSP	44
	<i>Cryptoprocta</i>	<i>Cryptoprocta ferox</i>	AGE+SEX*CAP	34
Eupleridae	<i>Galidia</i>	<i>Galidia elegans</i>	SEX+AGE+SSP	47

Figure D.1: Relationship between morphological integration index $\sqrt{r^2}$ and $AVG-$ for different modularity hypotheses. Black- Canidae. Gray- Non-canid Carnivora. Dashed line- $AVG+=AVG-$.

Figure D.2: Relationship between morphological integration index $\sqrt{r^2}$ and $AVG+$ for different modularity hypotheses. Black- Canidae. Gray- Non-canid Carnivora. Dashed line- $AVG+=AVG-$.

Appendix E

Apêndice do Capítulo 4

Phylogeny, Taxonomy and Sample Sizes

Since there is no updated phylogeny including all species of Caniformes, we focused on the most complete dated phylogeny of the whole group (Slater et al., 2012). This phylogeny was modified according to new information, or information that was not included in that particular study. Bellow we provide a detailed description of the modifications done and the references that support them (when necessary).

- Canidae
 - The position of *P. vetulus* was set as sister to *P. culpaeus* (Perini et al., 2010) to ensure monophiletism of the genus.
 - *V. chama* and the clade formed by all other *Vulpes* with the exception of *V. cana* and *V. zerda* (Nyakatura and Bininda-Emonds, 2012).
 - *V. velox* as set as sister to *V. macrotis* (Mercure et al., 1993).
 - *V. pallida* as set as sister to *V. corsac* (Bininda-Emonds et al., 1999; Clark Jr et al., 2008).
 - *V. ferrilata* as set as sister to the clade formed by *V. corsac* and *V. pallida* (Bininda-Emonds et al., 1999; Clark Jr et al., 2008).
 - *V. bengalensis* as fixed in a polytomy along with *V. chama* and the clade formed by all other *Vulpes* with the exception of *V. cana* and *V. zerda* (Nyakatura and Bininda-Emonds, 2012).
 - *C. aureus* label was substituted for *C. anthus* (Koepfli et al., 2015).
 - *C. aureus* was set as sister to all other *Canis* with the exception of *C. mesomelas*, *C. adustus*, *L. pictus* and *C. alpinus* (Koepfli et al., 2015).

- *C. indica* was set as sister to *C. lupus* and *C. himalayensis* as set as sister to both (Koepfli et al., 2015).
- *C. rufus* was set as sister to *C. latrans* and *C. lycaon* as set as sister to both (Rutledge et al., 2012).
- Procyonidae
 - *P. pygmaeus* was placed in a polytomy along with other *Procyon* (de Villa-Meza et al., 2011).
 - *Bassaricyon* phylogeny was set according to Helgen et al. (2013).
 - *N. olivacea* and *N. meridensis* were set as sister species associated with *N. narica* (Helgen et al., 2009).
- Mephitidae
 - *C. humboldtii* was added as sister *C. chinga* and *C. semistriatus* was added as sister to both (Schiaffini et al., 2013).
 - All *Spilogale* were set in a polytomy because lack of studies for these species.
- Phocidae
 - *Neomonachus* was set as sister to both *Monachus* species (Scheel et al., 2014).
- Mustelidae
 - *M. canescens* was set as sister of *M. meles* and both were set as sister to clade formed by *M. anakuma* and *M. leucurus* (Abramov and Puzachenko, 2013; İbiş et al., 2015).
 - *L. patagonicus* was set as sister to *Galictis* (Sato et al., 2012).

- All *Melogale* were set in a polytomy because lack of studies for these species.
- *A. congicus* was set as sister to the clade formed by other *Aonyx* and *Lutrogale* because there was no study detailing its placement.
- *M. subpalmata* was set as sister to *M. nivalis* (Nyakatura and Bininda-Emonds, 2012).
- *M. kathiah* was set as sister to the clade including sister to *M. nivalis* (Sato et al., 2012).
- *M. itatsi* was set as sister to the clade including *M. sibirica* and *M. eversmanni* (Sato et al., 2012).
- *M. felipei* and *M. africana* were set as a clade sister to *M. frenata* (Arnold et al., 2010; Nyakatura and Bininda-Emonds, 2012).
- *L. provocax* was set as sister to *L. felina* (Arnold et al., 2010; Nyakatura and Bininda-Emonds, 2012).
- *Arctonyx* species were set on a polytomy (Helgen et al., 2008).

Taxonomic affinities within Carnivora are far from resolved, and nomenclature reassessments and new species descriptions are not infrequent, mainly due to the use of molecular techniques (e.g. Zrzavy and Ricankova, 2004; Bardeleben et al., 2005; Helgen et al., 2008, 2013; Bornholdt et al., 2013; Prevosti et al., 2013; Schiaffini et al., 2013). Explicit subspecies validations are rare for fissipeds (but see Berta and Churchill, 2012), interfering with the swift allocation of specimens based on geographic data (essential for controlling for effects on **W** matrix estimation, see Chapter D). Consequently, taxonomic guides such as Nowak (1999) and Wilson and Reeder (2005) were found to be lacking for particularly problematic taxa, given that they pre-date newer taxonomic works for those groups. For these reasons, taxonomic decisions were drawn from numerous sources, mainly Mammalian Species

reports (see References for full list) and the previously mentioned works. The only exception was the taxonomy of pinnipeds, which followed Berta and Churchill (2012). The resulting taxonomy can be found on Table E.1 along with the sample-size for each species.

Table E.1: Taxonomy and sample sizes

Family	Genus	Species	n
Canidae	<i>Atelocynus</i>	<i>Atelocynus microtis</i>	23
	<i>Canis</i>	<i>Canis adustus</i>	38
		<i>Canis anthus</i>	54
		<i>Canis aureus</i>	27
		<i>Canis himalayensis</i>	10
		<i>Canis indica</i>	6
		<i>Canis latrans</i>	61
		<i>Canis lupus</i>	107
		<i>Canis lycaon</i>	48
		<i>Canis mesomelas</i>	57
		<i>Canis rufus</i>	46
		<i>Canis simensis</i>	10
		<i>Cuon alpinus</i>	21
		<i>Lycaon pictus</i>	45
	<i>Cerdocyon</i>	<i>Cerdocyon thous</i>	77
	<i>Chrysocyon</i>	<i>Chrysocyon brachyurus</i>	57
	<i>Lycalopex</i>	<i>Lycalopex culpaeus</i>	44
		<i>Lycalopex fulvipes</i>	2
		<i>Lycalopex griseus</i>	39
		<i>Lycalopex gymnocercus</i>	45
		<i>Lycalopex sechurae</i>	37
		<i>Lycalopex vetulus</i>	39
	<i>Nyctereutes</i>	<i>Nyctereutes procyonoides</i>	41
	<i>Otocyon</i>	<i>Otocyon megalotis</i>	51
	<i>Speothos</i>	<i>Speothos venaticus</i>	56
	<i>Urocyon</i>	<i>Urocyon cinereoargenteus</i>	44
		<i>Urocyon littoralis</i>	54
	<i>Vulpes</i>	<i>Vulpes bengalensis</i>	10
		<i>Vulpes chama</i>	26
		<i>Vulpes corsac</i>	3
		<i>Vulpes ferrilata</i>	4
		<i>Vulpes lagopus</i>	52
		<i>Vulpes macrotis</i>	48
		<i>Vulpes pallida</i>	17
		<i>Vulpes rueppellii</i>	58

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Table E.1 – continued from previous page

Family	Genus	Species	n
Mephitidae	<i>Conepatus</i>	<i>Vulpes velox</i>	37
		<i>Vulpes vulpes</i>	54
		<i>Vulpes zerda</i>	45
		<i>Conepatus chinga</i>	92
		<i>Conepatus humboldtii</i>	29
		<i>Conepatus leuconotus</i>	41
		<i>Conepatus semistriatus</i>	39
		<i>Mephitis macroura</i>	45
		<i>Mephitis mephitis</i>	47
	<i>Mydaus</i>	<i>Mydaus javanensis</i>	11
	<i>Mydaus</i>	<i>Mydaus marchei</i>	8
	<i>Spilogale</i>	<i>Spilogale angustifrons</i>	43
		<i>Spilogale gracilis</i>	48
		<i>Spilogale putorius</i>	40
		<i>Spilogale pygmaea</i>	6
Ursidae	<i>Ailuropoda</i>	<i>Ailuropoda melanoleuca</i>	20
	<i>Helarctos</i>	<i>Helarctos malayanus</i>	25
	<i>Melursus</i>	<i>Melursus ursinus</i>	25
	<i>Tremarctos</i>	<i>Tremarctos ornatus</i>	28
	<i>Ursus</i>	<i>Ursus americanus</i>	48
		<i>Ursus arctos</i>	67
		<i>Ursus maritimus</i>	84
		<i>Ursus thibetanus</i>	46
Otariidae	<i>Arctocephalus</i>	<i>Arctocephalus australis</i>	36
		<i>Arctocephalus galapagoensis</i>	4
		<i>Arctocephalus gazella</i>	35
		<i>Arctocephalus pusillus</i>	14
		<i>Arctocephalus townsendi</i>	2
		<i>Arctocephalus tropicalis</i>	3
	<i>Callorhinus</i>	<i>Callorhinus ursinus</i>	55
	<i>Eumetopias</i>	<i>Eumetopias jubatus</i>	53
	<i>Neophoca</i>	<i>Neophoca cinerea</i>	2
	<i>Otaria</i>	<i>Otaria byronia</i>	37
	<i>Phocarctos</i>	<i>Phocarctos hookeri</i>	6
	<i>Zalophus</i>	<i>Zalophus californianus</i>	38
		<i>Zalophus wolfebaeki</i>	15
Phocidae	<i>Cystophora</i>	<i>Cystophora cristata</i>	41
	<i>Erignathus</i>	<i>Erignathus barbatus</i>	39
	<i>Halichoerus</i>	<i>Halichoerus grypus</i>	9
	<i>Histiophoca</i>	<i>Histiophoca fasciata</i>	10
	<i>Hydrurga</i>	<i>Hydrurga leptonyx</i>	13
	<i>Leptonychotes</i>	<i>Leptonychotes weddellii</i>	51
	<i>Lobodon</i>	<i>Lobodon carcinophaga</i>	44
	<i>Mirounga</i>	<i>Mirounga angustirostris</i>	22

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Table E.1 – continued from previous page

Family	Genus	Species	n
Procyonidae	<i>Monachus</i>	<i>Mirounga leonina</i>	33
		<i>Monachus monachus</i>	2
	<i>Neomonachus</i>	<i>Neomonachus schauinslandi</i>	19
		<i>Neomonachus tropicalis</i>	37
	<i>Ommatophoca</i>	<i>Ommatophoca rossii</i>	7
	<i>Pagophilus</i>	<i>Pagophilus groenlandicus</i>	47
	<i>Pusa</i>	<i>Pusa caspica</i>	2
		<i>Pusa hispida</i>	51
		<i>Pusa sibirica</i>	9
	<i>Phoca</i>	<i>Phoca largha</i>	6
		<i>Phoca vitulina</i>	53
		<i>Phoca vitulina</i>	53
	<i>Bassaricyon</i>	<i>Bassaricyon alleni</i>	30
		<i>Bassaricyon gabbii</i>	9
		<i>Bassaricyon medius</i>	40
		<i>Bassaricyon neblina</i>	8
	<i>Bassariscus</i>	<i>Bassariscus astutus</i>	46
		<i>Bassariscus sumichrasti</i>	45
	<i>Nasua</i>	<i>Nasua narica</i>	44
		<i>Nasua nasua</i>	60
		<i>Nasuella meridensis</i>	2
		<i>Nasuella olivacea</i>	17
	<i>Potos</i>	<i>Potos flavus</i>	63
	<i>Procyon</i>	<i>Procyon cancrivorus</i>	41
		<i>Procyon lotor</i>	52
		<i>Procyon pygmaeus</i>	3
Mustelidae	<i>Aonyx</i>	<i>Aonyx capensis</i>	2
		<i>Aonyx cinerea</i>	24
		<i>Aonyx congicus</i>	2
		<i>Arctonyx albogularis</i>	16
	<i>Arctonyx</i>	<i>Arctonyx collaris</i>	12
		<i>Arctonyx hoevenii</i>	2
	<i>Eira</i>	<i>Eira barbara</i>	77
	<i>Enhydra</i>	<i>Enhydra lutris</i>	43
	<i>Galictis</i>	<i>Galictis cuja</i>	45
		<i>Galictis vittata</i>	34
	<i>Gulo</i>	<i>Gulo gulo</i>	52
	<i>Hydrictis</i>	<i>Hydrictis maculicollis</i>	11
	<i>Ictonyx</i>	<i>Ictonyx libyca</i>	36
		<i>Ictonyx striatus</i>	47
	<i>Lontra</i>	<i>Lontra canadensis</i>	46
		<i>Lontra felina</i>	11
		<i>Lontra longicaudis</i>	46
		<i>Lontra provocax</i>	2

Continues on next page

Table E.1 – continued from previous page

Family	Genus	Species	n
	<i>Lutra</i>	<i>Lutra lutra</i>	37
		<i>Lutra sumatrana</i>	5
	<i>Lutrogale</i>	<i>Lutrogale perspicillata</i>	4
	<i>Lyncodon</i>	<i>Lyncodon patagonicus</i>	6
	<i>Martes</i>	<i>Martes americana</i>	43
		<i>Martes flavigula</i>	44
		<i>Martes foina</i>	39
		<i>Martes martes</i>	9
		<i>Martes melampus</i>	5
		<i>Martes pennanti</i>	34
		<i>Martes zibellina</i>	40
	<i>Meles</i>	<i>Meles anakuma</i>	2
		<i>Meles leucurus</i>	10
		<i>Meles meles</i>	25
		<i>Meles canescens</i>	11
	<i>Mellivora</i>	<i>Mellivora capensis</i>	22
		<i>Melogale everetti</i>	15
		<i>Melogale moschata</i>	46
		<i>Melogale orientalis</i>	14
		<i>Melogale personata</i>	45
	<i>Mustela</i>	<i>Mustela africana</i>	13
		<i>Mustela altaica</i>	25
		<i>Mustela erminea</i>	49
		<i>Mustela eversmanni</i>	14
		<i>Mustela felipei</i>	2
		<i>Mustela frenata</i>	42
		<i>Mustela itatsi</i>	25
		<i>Mustela kathiah</i>	22
		<i>Mustela lutreola</i>	2
		<i>Mustela nigripes</i>	48
		<i>Mustela nivalis</i>	43
		<i>Mustela nudipes</i>	9
		<i>Mustela putorius</i>	39
		<i>Mustela sibirica</i>	39
		<i>Mustela subpalmata</i>	10
		<i>Mustela vison</i>	37
	<i>Poecilogale</i>	<i>Poecilogale albinucha</i>	33
	<i>Pteronura</i>	<i>Pteronura brasiliensis</i>	35
	<i>Taxidea</i>	<i>Taxidea taxus</i>	36
	<i>Vormela</i>	<i>Vormela peregusna</i>	8
		Total	5088

Tree transformation

Differences in tree shape might influence the performance of tests because of two main reasons: firstly, if phylogenetic dependence introduces correlation among traits, then transforming a tree into a "star-like" phylogeny (no dependence between taxa) would improve type I error rates and power for the analysis of the raw data. Secondly, since different tree shapes are expected under different diversification scenarios, we can investigate the performance of the tests under different situations.

Here we chose to transform the tree according to an "Early-Burst" (EB) model

$$r(t) = r(0)\exp(at) \quad (\text{E.1})$$

where a is the rate change parameter and $r(0)$ and $r(t)$ are rates of diversification at time 0 and t , respectively. a was modeled as varying from -1 to 2 (31 values), with lower values producing a more "tip-heavy" tree (more diversification at the tips) and higher values producing a more "star-like" phylogeny (more diversification at the base).

We used the EB model because it manipulates the rates of speciation events through time and is not necessarily associated to the mode of phenotypic evolution, such as Ornstein–Uhlenbeck or Pagel- λ transformations. This transformation was applied to the full tree and investigations of different values of a were conducted on the full sample only. Power was investigated only for $e = 1$.

Figure E.1 shows the effects of applying an "Early Burst" transformation on both type I error rates and power. Type I error rates for the full sample (all Caniformes) are largely unaffected by the transformation of the tree according to an "Early Burst" model, with the exception that, as the values of

parameter a increases (producing a more "star-like" phylogeny Figure E.1B), than the error rates for the correlation analysis on raw data approaches the nominal value ($\alpha = 0.05$). The only test that had its power affected due to tree transformation was the regression analysis, that showed a decrease in power for lower values of e (producing a more "tip-heavy" phylogeny Figure E.1A).

Figure E.1: A- Type I and power for different values for a (Early Burst parameter). Power was accessed for $e = 1000$. B- Transformations applied to Caniform phylogeny to investigate effect of tree shape on the quantitative genetics tests of neutral evolution.

Simulations

The code used for the simulations can be seen bellow. Number between brackets in the bellow description refers to specific lines on the R code.

Simulations were carried out using the `sim.corrs` function from the R package (Revell, 2012) [27 – 28]. This function models the evolution along a tree as a phylogenetically structured random walk on the space defined by a given covariance matrix $\mathbf{D}$. This is done by sampling a normal distribution np times, where n is the number of transitions (ancestral-descendent pairs) on a tree and p is the number of traits. Each draw is assigned to a transition and is multiplied by its branch length. The resulting data is then multiplied by the Cholesky factor of $\mathbf{D}$, and the transitions are then summed up from the base to the tips of the phylogeny in order to produce average values for the species.

$\mathbf{D}$ was modeled through Equation (4.5) using the pooled within-covariance matrix $\mathbf{P}$ as a surrogate for $\mathbf{G}$ and a randomly produced $\mathbf{C}$ [10 – 13]. Given that we initially want selection and drift to have the same relative importance when the effect size $e = 1$, all branch lengths of the phylogeny were set to be proportional to $\frac{t}{N_e}$ [5,24 – 25]. This gives us branch lengths proportional to the amount of divergence expected due to drift (Felsenstein, 1988). Thus, for $e = 1$, then we can rewrite Equation (4.5) as

$$\mathbf{B} = b_l(\mathbf{A} + \mathbf{G}) \quad (\text{E.2})$$

where b_l is the scaled branch lengths. So according to the above equation, for each generation both selection and drift will have an equal trace of $1/N_e$. This do not mean that selection will depend on N_e , but it establishes a baseline where both selection and drift contribute equally (*i.e* they have to be scaled by the same factor).

Given that phenotypic evolution is modeled by the sum of the selection and drift [27 – 30], we can simulate both components independently as

$$\mathbf{B} = \mathbf{B}_{drift} + \mathbf{B}_{selection} \quad (\text{E.3a})$$

$$\mathbf{B}_{drift} = b_l \mathbf{G} \quad (\text{E.3b})$$

$$\mathbf{B}_{selection} = b_l e \mathbf{A} \quad (\text{E.3c})$$

To ensure that the simulated data had the same rate of evolution as the observed data, we obtained the observed and the simulated $tr(\mathbf{B}_{PIC})$. The square-root of the ratio between both is a scaling factor that, if applied to the simulated data, ensures that $tr(\mathbf{B}_{PIC})$ of both will be equal [31-33]. Since the final data will be re-scaled, then the actual scale of the simulated data can be arbitrary. For this reason, both the traces of $\mathbf{G}$, and $\mathbf{GCG}$ were set to 1 to facilitate the calculations [9,13-14].

After simulating the evolution of species means we then drew from $\mathbf{P}$ a sample equal to the sample size of each species, with means equal to a vector of 0s [35]. The resulting data was then average and added to each species means in order to simulate sampling error on species averages [36-39]. Additionally, to account for sampling error on this matrix, the simulated intraspecific data was retained and used to re-estimate $\mathbf{W}$ [35]. Since both original $\mathbf{W}$ and simulated within-group data were stored, both original $\mathbf{W}$ and re-sampled $\mathbf{W}$ can be used on the calculations to evaluate impact of sampling error. Table E.2 summarizes the total number of simulations per analysis.

Table E.2: Summary of the number of simulations per analysis. N_e - number of different effective population sizes; e - number of different effect size of directional selection; a - number of different Early-Burst parameter used; Clades- number of clades used; PCs- number of different amounts of PCs retained, Runs- number of runs performed for each case. See text for the description of each analysis.

tests	N_e	e	a	Clades	PCs	runs	Total
Error rates	1	8	1	91	1	1000	7.28E+05
Tree transformation	1	2	10	1	1	1000	2.00E+04
Oscillations in N_e	5	1	1	91	1	1000	4.55E+05
Dimension reduction	1	8	1	18	6	1000	8.64E+05
						total=	2.07E+06

R code

Simulation of multivariate phenotypic evolution with drift and selection

Description

Simulates multivariate phenotypic evolution along a phylogenetic tree. Simulations can be performed with both drift or drift plus directional selection with varying intensity.

Usage

```
1 sim_phylo(G, means, phy, n.s, inter=1000, parallel=FALSE,
  ↪ selection=1, efsiz=0, gen_time=1e-06, Nef=1000, Nef_
  ↪ osc="no", Nef_par=NULL)
```

Arguments

G Genetic covariance matrix

means Dataframe of species average phenotypic values. Data should be arranged as speciesXcharacters with lines named after species.

phy Phylogenetic tree used on the simulation. Tip labels should correspond to rownames on *means*.

<i>n.s</i>	Vector containing sample size for each species on the same order as in <i>means</i> .
<i>iter</i>	Number of iterations to be performed.
<i>parallel</i>	Logical. If simulations are going to be performed in parallel. Uses package <i>doParallel</i> .
<i>selection</i>	Numeric. Number of random vectors to be used as vectors of directional selection.
<i>efsize</i>	Numeric. Effect size. If=1, then both selection and drift have similar magnitudes.
<i>gen_time</i>	Numeric. Generation time in the scale of the tree. For million years $1e06 = 1year$.
<i>Nef</i>	Numeric or vector. Effective sample size (N_e) for the population. If numeric, than the same N_e is fixed throughout the tree. If a vector, should be the estimate N_e for each node of the tree.
<i>Nef_osc</i>	Character. If N_e should be variable. Default "no". If "norm", than oscillations of N_e are exponentials of values drawn from a normal distribution. If "unif", than values of N_e are drawn from a uniform distribution.
<i>Nef_par</i>	Numeric of vector. Default NULL. If <i>Nef_osc</i> ="norm" than a numeric value for the sd of the distribution should be provided. If <i>Nef_osc</i> ="unif" than a vector of length 2 should be provided giving lower and upper bounds of N_e . Unused if <i>Nef_osc</i> ="no".

Values

<i>sims</i>	List of outputs of each simulation of length <i>inter</i> . Each entry is a list containing the simulated averages (data.s), the resampled within-group distribution (ws), the simulated random vectors of selection (svec), the ratio between the empirical rate of evolution and the simulated one (rate.ratio) and the GCG matrix (A).
<i>vcv</i>	The input covariance matrix <i>G</i> .
<i>phy</i>	The input phylogeny <i>phy</i> .
<i>n</i>	The number of species.
<i>n.s</i>	The input sample size for each species <i>n.s</i> .
<i>pics</i>	The phylogenetic independent contrasts for the observed data.
<i>means</i>	The input matrix of species averages <i>means</i> .

```

1  sim_phylo<-function(G,means,phy,n.s,inter=1000, parallel=
    ↪ FALSE, selection=1,efsize=0, gen_time=1e-06, Nef=1000,
    ↪ Nef_osc="no",Nef_par=NULL){
2    vcv<-G
3    pics<-apply(means, 2, function(x) pic(x, phy))
4    rate<-tr(var(pics))
5    phy$edge.length <- phy$edge.length / gen_time
6    n<-length(phy$tip.label)
7
8    bm.sim <- function(z) {
9      G <- G / tr(G)
10     svec <- NULL
11     svec <- matrix(Normalize(rnorm(dim(G)[1]*selection)),
        ↪ dim(G)[1], selection)

```



```

12   C <- svec %*% t(svec) + diag(35)*0.0001
13   C <- C/tr(C)
14   A <- (G %*% C %*% G) / tr(G %*% C %*% G)
15   A <- efsize * A
16
17   if(Nef_osc=="no")   Nef_osc <- Nef else{
18     if(Nef_osc=="norm") Nef_osc <- Nef * exp(rnorm(length(
      ↪ phy$edge.length), Nef_par)) else{
19     if(Nef_osc=="unif") Nef_osc <- runif(length(phy$edge
      ↪ .length), min = Nef_par[1], max = Nef_par[2])
20   }
21 }
22
23 phy1 <- phy
24 phy1$edge.length <- phy$edge.length / Nef_osc
25 phy$edge.length <- phy$edge.length / Nef
26
27 drift<- sim.corrs(phy1, G)
28 if(efsize!=0) sel <- sim.corrs(phy, A) else sel <- 0
29
30 data.s <- (drift + sel)
31 rate.s <- tr(var(apply(data.s, 2, function(x) pic(x, phy
      ↪ )))))
32 s <- sqrt(rate/rate.s)
33 data.s <- data.s * s
34
35 ws<-rmvnorm(sum(n.s),sigma=vcv)
36 sps<-rep(rownames(data.s),times=n.s)
37 de<-data.frame(sps=factor(sps,unique(sps)),ws) %>%
38   group_by(.,sps) %>% summarize_each(funs(mean))

```

```
39     data.s<-data.s+de[,-1]
40     list(data.s=data.s, ws=ws, svec=svec, rate.ratio=s^2, A=
        ↪ A, svec=svec)
41 }
42 data.s<-alply(1:inter, 1, bm.sim, .parallel = parallel)
43 list(sims=data.s, vcv=vcv, phy=phy, n=n, n.s=n.s, pics=pics,
        ↪ means=means)
44 }
```

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Apêndice F

Publicações

1. Simon, M. Machado, F. A. e Marroig, G. (in press). High evolutionary constraints limited adaptive responses to past climate changes in toad skulls. *Proceedings of the Royal Society B*.
2. Santos, M. M. D., Silva, F. M. D., Hingst-Zaher, E., Machado, F. A., Zaher, H., e Prudente, A. L. D. C. (in press). Cranial adaptations for feeding on snails in species of *Sibynomorphus* (Dipsadidae: Dipsadinae). *Zoology*.
3. Carvalho, A. L. G., Sena, M. A., Peloso, P. L. V., Machado, F. A., Montesinos, R. e Silva, H. R., et al. (2016). A new *Tropidurus* (Tropiduridae) from the Semiarid Brazilian Caatinga: Evidence for conflicting signal between mitochondrial and nuclear loci affecting the phylogenetic reconstruction of South American collared lizards. *American Museum Novitates*, 3852, 1–68.
4. Bernardo, P. H., Machado, F. A., Murphy, R. W. e Zaher, H. (2012). Redescription and morphological variation of *Oxyrhopus clathratus* Duméril, Bibron and Duméril, 1854 (Serpentes: Dipsadidae: Xenodontinae). *South American Journal of Herpetology*, 7(2), 134–148.
5. Fausto, S., Machado, F. A., Bento, L. F. J., Iamarino, A., Nahas, T. R. e Munger, D. S. (2012). Research Blogging: Indexing and registering the change in Science 2.0. *Plos One*, 7(12), e50109.
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