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# Bridging the gap between micro and macroevolution in island systems using biomechanics and dynamic modelling

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► **To cite this version:**

Maxime Taverne. Bridging the gap between micro and macroevolution in island systems using biomechanics and dynamic modelling. Animal biology. Museum national d'histoire naturelle - MNHN PARIS, 2021. English. NNT : 2021MNHN0005 . tel-03352708

**HAL Id: tel-03352708**

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Submitted on 23 Sep 2021

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***Dani gigantum humeris insidentes***



## RESUME DE LA THESE

L'émergence de la diversité phénotypique peut résulter de processus évolutifs tels que la sélection naturelle ou sexuelle, les effets fondateurs, ou des processus stochastiques comme la dérive génétique. On considère aujourd'hui qu'une part non-négligeable de la diversité actuelle a pour origine des radiations adaptatives pendant lesquels la colonisation de nouvelles niches écologiques est accompagnée d'une variation phénotypique. Les systèmes insulaires peuvent être comparés à des laboratoires à ciel ouvert parce qu'ils offrent des répliques de populations naturelles, essentiels pour répondre à des questions centrales en biologies des organismes et de l'évolution. Les radiations insulaires offrent des opportunités intéressantes d'étudier comment la variation morphologique évolue en relation avec les contraintes environnementales, comme le suggère la théorie écologique. Cependant, il reste encore à comprendre dans quelle mesure les variations morphologiques peuvent donner lieu à des changements significatifs dans l'écologie et l'accomplissement des organismes. Nous proposons de répondre à cette question en utilisant un système insulaire, l'archipel croate de la mer Adriatique, où sont établies des populations de deux espèces de lézards du genre *Podarcis* (*P. melisellensis* et *P. sicula* : Sauria, Lacertidae) qui sont restées isolées les unes des autres depuis la fin de la dernière glaciation. Pour ce faire, nous avons combiné des observations de terrain, des dissections quantitatives et des analyses en morphométrie géométrique 3D pour comprendre les relations entre les variations à l'échelle macro-évolutive de la force de morsure, du comportement, de la forme du crâne et de sa musculature avec les contraintes environnementales, comme l'exploitation des ressources ou la compétition intraspécifique. Nous avons ensuite eu recours à des méthodes d'ingénierie (modélisation dynamique multi-corps, et modélisation en éléments finis) pour tester les conséquences biomécaniques des variations morphologiques subtiles observées au sein des populations.

Plus spécifiquement, nous avons dans un premier temps caractérisé la variabilité du régime alimentaire au sein des populations de l'archipel par l'analyse des contenus stomacaux. Le but de cette première étude était de comprendre quelles caractéristiques du milieu étaient susceptibles d'influencer l'accès aux ressources. Nous avons mis en évidence que les deux espèces d'intérêt occupaient des niches écologiques différentes, et que *P. sicula* était capable d'élargir le champ de ressources utilisées dans les environnements les plus pauvres. Ceci a révélé que la variation de régime alimentaire entre les populations était permise par une spécialisation au niveau de l'individu, donnant

lieu à des différences inter-individuelles plus marquées. Contrairement à nos prédictions basées sur la théorie de la division de ressources, le dimorphisme sexuel dans les dimensions de la tête n'était pas associé à un dimorphisme de régime alimentaire. Enfin, le relâche écologique semble promouvoir la diversification des régimes alimentaires. Ainsi, nous observons chez certaines populations des changements de régimes typiquement insectivores vers des régimes omnivores composés en grande partie de végétaux.

Dans un second temps, nous avons exploré si des paramètres proximaux (dimensions du corps et de la tête) ou ultimes (écologiques, relatifs à la structure de l'habitat) étaient susceptibles d'influer sur la force de morsure, grâce notamment à des acquisitions sur le terrain de forces de morsure *in vivo*. Cette seconde étude a révélé que la variation dans le régime alimentaire décrite dans la partie précédente dépendait de l'isolation de l'île (et en particulier de son éloignement par rapport à la grande île la plus proche), probablement en modulant la diversité et l'abondance des ressources disponibles. La consommation d'items durs tels que les plantes et les proies dures sont associées à des forces de morsure plus élevées, nécessaires pour augmenter l'efficacité de la digestion et pour réduire le temps de transport intra-oral. L'augmentation de la force de morsure était associée à une variation de dimensions clés du crâne. La phylogénie n'impactait que peu les résultats, ce qui suggère que les contraintes écologiques locales sont fortes et favorisent l'émergence de patrons évolutifs convergents des dimensions de la tête et de la force de morsure dans des contextes environnementaux similaires.

Ensuite, bien que les niveaux maximums de 'performance' soient très informatifs, le lien logique qui doit être fait entre fonction et valeur sélective n'a de sens qu'à travers le prisme du comportement, puisque ce dernier module le contexte dans lequel la performance est considérée. Nous avons donc exploré comment un changement de régime alimentaire pouvait influencer la cinématique des mâchoires. Pour cela nous avons filmé des individus issus de deux populations récemment séparées grâce à des caméras à haute vitesse, tout en leur proposant des proies de taille, mobilité et dureté différentes. Ceci a révélé que les propriétés de la proie influaient sur la façon dont les lézards bougent leur mâchoire, mais également qu'une augmentation de la largeur de niche écologique en conditions naturelles était associée à une plus grande flexibilité et une plus grande stéréotypie des mouvements. Cela suggère un raffinement du contrôle moteur chez la population au régime le plus généraliste.

Ensuite, nous avons mené une étude sur les relations entre forme du crâne, architecture musculaire, force de morsure et régime alimentaire. Ceci a montré que la façon la plus parcimonieuse d'augmenter la force de morsure était d'augmenter la force des adducteurs externes, et que l'investissement relatifs

dans les différents groupes musculaires reflétaient la spécialisation du régime alimentaire (vers un régime composé de plantes, ou de proies dures, par exemple). Cela suggère que les changements dans la morphologie et la musculature sont spécifiques à chaque usage du système masticateur dans les conditions imposées par la consommation de ces items. Ainsi, il existe une forte covariation entre forme du crâne et force de morsure, surtout liée à des effets allométriques, et aussi entre force des muscles et forme du crâne, liée à des effets mécaniques plus localisés.

Enfin, nous avons mené une étude combinant deux méthodes de modélisation biomécanique. Celle-ci avait pour objectif de tester comment le système musculosquelettique de l'appareil masticateur répondait aux contraintes imposées par le régime alimentaire et par la compétition intraspécifique. Pour cela nous avons simulé des morsures à des angles d'ouverture de mâchoire variables représentant la position à l'équilibre du système lors de la morsure d'éléments clés : 0° (élément fin comme une plante), 20° (élément de taille intermédiaire comme un arthropode), 45° (élément de grande taille comme un congénère). Nous avons donc réussi à déterminer la fonction de chaque groupe musculaire en fonction des situations, et mis en évidence que la morphologie du crâne et de la mandibule était responsable d'une part non-négligeable de la génération de force de morsure, en impactant directement l'efficacité du système à convertir la force musculaire en force de morsure. De plus, nous avons démontré qu'une augmentation de la force de morsure, nécessaire dans le cas d'un changement de régime vers des items plus exigeants, génère des contraintes mécaniques plus fortes, et que l'évolution de la morphologie crânienne permettait la dissipation et la meilleure distribution du stress généré. De plus, les patrons observés à l'échelle micro-évolutive par le biais des modèles biomécaniques sont congruents avec les patrons macro-évolutifs observés au niveau de l'archipel.

En résumé, la présente étude suggère que la variation de la forme du crâne influence l'efficacité mécanique du système musculosquelettique de l'appareil masticateur. De plus, les variations de morphologie, musculature, comportement et performance reflètent des spécialisations de régime alimentaire. Nos résultats apportent une démonstration de la façon dont les changements micro-évolutifs de la morphologie peuvent finalement aboutir à des patrons macro-évolutifs de variation écologique. Ils suggèrent également que le système insulaire de l'Adriatique se trouve dans une phase initiale de radiation adaptative.

## ACKNOWLEDGEMENTS

On ne vit pas, on ne grandit pas, et on n'accomplit rien seul. Ce qui est vrai dans la vie l'est au moins autant dans la recherche. Le petit monde de la science est pour ainsi dire « a life in a nutshell ». Le travail d'équipe, en communauté, nous apprend que rien ne peut être entrepris si ce n'est dans le partage et l'entraide. A ce jour j'ai surtout beaucoup reçu. En dressant ci-après la liste (non-exhaustive) des personnes qui ont participé, de près ou de loin, à la réalisation de mes rêves et de mes projets, je prends conscience de la chance immense que vous tous m'avez offerte. Cette thèse vous est dédiée.

Anthony, impossible de commencer ces remerciements par quelqu'un d'autre. Que de chemin parcouru depuis notre rencontre et mon entrée progressive dans le monde de la science et de la recherche. Je n'ai aucun mal à dire que cette année 2014, qui a vu la naissance de notre première collaboration, aura marqué pour moi le début d'une ère d'enrichissement professionnel et personnel incroyable. Depuis, tu as toujours été un pilier, cumulant les fonctions de mentor, pédagogue, guide spirituel, guide de voyage, conseiller en dégustation de cigares cubains et de liqueurs, et ami. Si j'en suis là aujourd'hui c'est indubitablement grâce à toi, alors pour toute ta patience, ta confiance, et ton dévouement, MERCI.

Many grateful thoughts to my co-director Michael Fagan. Thank you so much for being part of this multidisciplinary project. I have been so lucky to work with someone so enthusiastic in tackling pure biological questions, and to interact with biomechanics although it is not my original field. Our collaboration has been really easy going and enriching, and despite some unexpected adventures during the last year, I think it turned out to be quite productive! Thank you again for your patience and for your commitment, and wish you some pleasant last few years mentoring students (I secretly cherish that I was one of your very lasts!).

A big thank you to Pete Watson for his tremendous work and most valuable help during the last (but not least) part of the project. Schedule was tight, circumstances were exceptional, the amount of work was massive, but you kept me in line and in time! Many thanks for everything you did, for your advice, for your time, and thank you for sending me some assistance, sometimes long after dinner time!

Merci Anne-Claire Fabre pour ton soutien et ton aide, non seulement durant cette thèse mais également depuis le stage que tu m'as permis de faire en M2.

I would like to properly thank the members of the jury, Paul O'Higgins, Raoul Van Damme, Mehran Moazen and Panayiotis Pafilis, for agreeing to review my work. I am aware of how time-consuming it can be to give a constructive feedback on a 3-years research project. For that, I am flattered to share it with you and, hopefully, to have the opportunity to further discuss about it with you.

Many thanks to the members of my thesis advisory committee: to Antigoni Kaliontzopoulou and Dominique Adriaens, for your time, your journeys to Paris, for all your good advice and for the interesting discussions that we had; à Éric Guilbert, pour ta gentillesse et ton engagement avec l'ED pour un bon déroulement de ces réunions et des thèses en général.

A la direction de l'ED 227, Nathalie Machon, Jérôme Sueur et Julien Gasparini, un grand merci pour le fantastique travail que vous faites au quotidien et toute l'année pour les doctorants. J'ai sincèrement apprécié de pouvoir participer un temps à la vie de cette ED à vos côtés. Un grand merci également aux coordinatrices Anaïs Ranera et Tiphonie Chica-Lefort.

I would like to address my sincere gratitude to Sorbonne Universités and the Idex Super for funding this whole project, to the doctoral school of the Museum, the Société des Amis du Muséum, the Company of Biologists, and the Carl Gans Collections and Charitable Fund for awarding me with some precious funding, and for supporting my work.

Big up to the Team Croatia, including Duje Lisičić, Marko Glogoski, Anamaria Štambuk, Oscar Mira, Iva Sabolić, and Zoran Tadić. Special thanks to Duje, not only for his precious help on the field, but for all the great time we spent in Split and on the islands, and for his hospitality. No Duje, I won't dare to start writing even a slight part of the cursed sentence. Let's not pronounce it EVER AGAIN. Many thanks to the crew of the Lastovo Otočje Nature Park: Bruna Đuković, Alexandra Horvat, Ivan Ivelja, Ivan Jurica, Marijo Bačko, and Viktor Šantić for generously enabling us to go safely to the islands.

Merci infiniment à Hugo Dutel pour sa participation à ce projet, autant que pour ses conseils, son enthousiasme et sa passion de la science qui ont été inspirants. Many thanks to you, as well as to Flora Gröning for co-organizing an awesome workshop on skull biomechanics. So helpful and such a right timing for my PhD project!

Je tiens tout particulièrement à remercier Thierry Decamps pour son immense gentillesse. Depuis mon arrivée dans le labo début 2014, tu as toujours fait tout ce que tu pouvais pour les étudiants. Merci aussi pour ton aide technique, notamment pour filmer mes bêtes. D'ailleurs, ces bêtes ne pourraient pas aussi bien se porter sans les soins et l'attention de Khalid Etefia! Merci à toi. Merci aussi à Hugues

Clamouze, bricoleur hors-pair, qui travaille le bois et le plexi comme personne, et qui a confectionné l'installation nécessaire à la prise de vidéos en 3D.

Une grosse pensée également aux stagiaires que j'ai eu la chance d'encadrer durant cette thèse : Leslie Michal, Julie Lemoine, Louise Martin, Christelle Faivre, Camille Gautier et Joao Duarte Da Silva. Travailler avec vous a été un réel plaisir et surtout un apprentissage précieux.

Merci aux gestionnaires Yamso Sekpon, Manuela Dafonseca, et Nadine Comte, pour votre travail nécessaire au bon fonctionnement de l'unité. Grâce à vous j'ai pu partir en congrès et en mission en toute tranquillité !

Une très grosse pensée à mes anciens encadrants de stage (je ne citerai pas les personnes que j'ai déjà remerciées, même s'ils le mériteraient quand même), sans qui je n'aurais pas pu me forger l'expérience et la maturité dont j'avais besoin pour me lancer dans cette chose un peu folle qu'est la thèse. Dans l'ordre chronologique donc : Jean-Philippe Hugnot et Daria Mamaeva, avec qui j'ai fait mon premier saut dans la recherche à Montpellier ; Raphaël Cornette qui m'a encadré lors de mon premier stage au Muséum en co-direction avec Anthony, et qui depuis lors a toujours été disponible pour des conseils ; Alexandra Houssaye (et encore toi Raph !) pour l'encadrement de mon M1, mais aussi et surtout pour ta franchise, ton sens de l'engagement avec toute l'équipe et avec tes étudiants, tes conseils avisés ; Christine Böhmer et Marc Herbin pour l'encadrement de mon M2, pour votre confiance et l'accès aux collections. Quel plaisir immense, toutes ces dissections fantastiques !

A Emmanuelle Pouydebat, Anick Abourachid, Rémi Hackert, Pauline Provini et Sam Van Wassenbergh, bien que nous n'ayons pas eu l'occasion de collaborer sur un projet, vous avez fait partie intégrante de mon aventure au Muséum, et fait vivre notre équipe. A Fabienne Aujard, un immense merci pour votre engagement sans faille, et pour toute l'énergie que vous avez insufflée dans notre unité. Funevol et Mecadev ont été un environnement de travail très spécial grâce à vous tous, et je suis très heureux d'en avoir fait partie.

A tous les (anciens, futurs anciens, actuels, et futurs) doctorants du labo : Ana, Camille, Christophe, Colline, Cyril, Fanny, Julie, Laurie, Marjorie, Pablo, Priscila, Rémi, Rohan et Romain, un immense merci pour ces grosses barres, ces gros moments de râlage à la cantine, de procrastination assumée dans le bureau, de soutien dans les moments de pétage de câble, pour les apéros et les petits dej' sur la terrasse. La vie de labo, elle se passait là !

Spéciale dédicace à tous mes petits lézards croates ; rien n'aurait été possible sans vous. J'espère que la contribution de ce travail à la construction du savoir sera à la hauteur de votre (occasionnellement funeste) collaboration.

A ce stade des remerciements, paradoxalement, la tâche se corse. J'ai donc décidé d'adresser aux personnes suivantes mes amitiés et affectueuses pensées de la manière la plus simple qui soit. Car je n'ai pas le talent narratif pour résumer en quelques lignes ce qu'elles représentent pour moi. Pendant le temps passé au Muséum (il faut dire que je fais déjà un peu partie des meubles), j'ai fait de belles rencontres en les personnes d'Alexis, Séverine et Artémis, qui se sont avérées être des amitiés irremplaçables. Je pense évidemment à Jacked et Laurent, et notre amitié qui remonte à tant d'années. Et même si nous sommes tous les trois séparés de centaines, voire de milliers de kilomètres, vous savoir à portée de train ou de téléphone est très important depuis mon départ du sud. Du sud d'ailleurs, m'a en quelque sorte accompagné Louis, que je remercie sincèrement pour tous les moments partagés, où il m'incrustait aux soirées SKEMA, à toutes nos journées jeu, ou notre week-end au Puy de Fou avec Caroline. A Florian, devenu ma Dream Team dès notre première rencontre, et toujours resté à ce jour un pilier, un sémaphore dans ma vie. A Elliott, aka Fi, grâce à qui j'ai vécu un master EPIQUE ! Enfin à Nathanaël, et tous les moments géniaux (que je ne compte plus !) qu'on a partagés depuis la licence.

A mes parents, Jean-François et Annie, une infinie gratitude pour m'avoir enseigné à toujours persévérer. A mon père particulièrement, pour n'avoir pas cessé de croire en moi.

Quand je regarde en arrière, sur ces trois années de travail, mais aussi sur les (au hasard) 28 dernières années, il y a une personne qui a tout particulièrement toujours cru en moi. Quelqu'un qui m'a sans cesse défendu, contre les gens, ou les contre les épreuves de la vie ; qui m'a porté, qui m'a relevé, qui m'a secoué quand il le fallait, mais qui me poussait à chaque fois en avant, parce qu'il m'a toujours donné le meilleur exemple. Merci grand frère.

Enfin, à la personne la plus importante, la plus lumineuse, qui partage ma vie et supporte mes airs grognons. A toi Jessica, car chaque moment est plus beau quand on le vit ensemble. Un jour promis, on ira sur l'île des lapins.



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# **General introduction**

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## GENERAL INTRODUCTION

### *Islands: natural laboratories for evolutionary biologists*

Archipelagos represent only 3.5% of the total land area on earth, yet host 15 to 20% of the known terrestrial species (Whittaker et al. 2017). These hotspots of diversity are unfortunately also the scene of high extinction rates, since 60% of the documented extinctions over the last five centuries involve island endemics (Whittaker & Fernández-Palacios, 2007; Tershy et al. 2015; Johnson et al. 2017). Darwin (1845), referring to the Galapagos, declared that he “felt near, both in space and time, to that great fact, that mystery of mysteries, the first appearance of new beings upon the Earth” illustrating the importance of islands in our understanding of evolutionary biology. He and other naturalists, like Wallace in his *Island Life* (1880), quickly realized the crucial role that island systems (but also their continental counterparts, e.g. lakes and river streams, see Fryer, 1996) might play in the understanding of evolution and speciation.

Darwin had the intuition that an organism’s isolation was key to witness evolution in action. Long before the discovery of DNA and the description of the allele-based Mendelian genetics, he provided evidence, with the support of artificial selection, that interrupting the interbreeding of lineages allowed the selection of distinct phenotypes (Darwin, 1859). Later, it was confirmed that gene flow barriers were key events underlying the emergence of new lineages (as elegantly presented by Dawkins, 2009). In this sense, islands and archipelagos are particularly insightful study systems and represent a type of system in which colonization events are often accompanied with the interruption of gene flow, hence providing opportunities for allopatric speciation to occur, especially on smaller islands (Mayr, 1963).

In addition to being isolated environments, islands have strict boundaries, and are often relatively small, which makes them easier to study than similar environments on the mainland. That is to say, the sub-sample of individuals studied are likely representative of the whole population. Abiotic factors, such as climate and geological characteristics are also likely to be more homogeneous among the islands of an archipelago, or at least within each island. Archipelagos often also provide a number of natural replicates, which allow evolutionary biologists to statistically test their hypotheses. Finally, islands generally host simpler ecosystems with less complex interaction chains (Kueffer, Drake & Fernández-Palacios, 2014). Because islands are isolated environments which provide simpler ecosystems and replicates, they are considered model systems by evolutionary biologists, and serve to address key

questions in evolutionary biology involving both the descriptions of patterns and the understanding of processes (McArthur, 1967; Losos & Ricklefs, 2009). To illustrate, the following questions have been addressed using island ecosystems.

- “How does the presence of competitors drive ecological and anatomical divergence in natural populations?”

This question can be tackled either by studying model systems including several communities differing in their interactions, from being purely sympatric to including one or several competitors, and to explore the relationship between community structure and the anatomical and ecological traits of interest (Rainville et al., in press); or by observing the course of a natural invasion of one species into the habitat of a closely-related one (Stuart et al. 2014); or by experimentally introducing a competitor in a competitor-free limited environment and follow how both introduced and endemic species evolve over time (Nevo, 1972; Losos et al. 2006).

- “How does predation pressure drive evolutionary responses?”

The island syndrome is a well-documented set of physiological and anatomical characteristics that are usually observed in island populations and that differ from those observed in their mainland counterparts. Among other traits, those typically include nanism in large species, gigantism in small species, as well as smaller clutches (Novosolov et al. 2013; Baeckens & Van Damme, 2020). Moreover, the lack of predation pressure in archipelagos is often associated with a loss of anti-predator behavior in species which usually encounter predators in mainland ecosystems (Blumstein & Daniel, 2005; Brock et al. 2014; Cooper, Pyron & Garland, 2014; Jolly, Webb & Phillips, 2018). The use of islands has permitted to highlight the relevance of predation pressure as a driver of phenotypic and behavioral evolution. In some studies, predators were excluded (Calsbeek & Cox, 2010). In others, predators were introduced onto some islands (Losos, Schoener & Spiller, 2004). These experiments revealed that lizards modulate their behavioral responses and their habitat use relative to the novel predation pressure, ultimately resulting in phenotypic changes.

- “How does population density influence behavior and natural selection?”

This question has been addressed in an interesting field experiment where population density on six islands of the Bahamas and the presence or absence of predators of lizards were manipulated (Calsbeek & Cox, 2010). Once more, island systems played an important role in allowing to replicate experiments

in natural habitats, and to illustrate the influence of density, hence intraspecific competition, on the evolution of the phenotype (here body size and limb length) and behavior (Calsbeek & Smith, 2007, 2008).

### ***Drivers of evolution in island systems***

Island systems typically impose strong selective constraints on organisms. Moreover, ecological release often occurs on islands (Buckley & Jetz, 2007) because top predators, i.e. carnivorous mammals, are generally absent (MacArthur & Wilson, 1967; Grant, 1972; Losos & Queiroz, 1997). This usually favors mesopredators like lizards, which are freed from predation pressure and can increase their population density (Novosolov et al. 2016). However, the diversity and biomass of arthropods, which represent a major part of the diet of lizards, often collapses on smaller islands (Olesen & Jordano, 2002; Olesen & Valido, 2003). Therefore, intraspecific competition for food access increases due to a relative low food abundance and high lizard density. Additionally, because a lower ratio between speciation and extinction is usually observed in archipelagos, interspecific competition decreases whereas intraspecific competition increases (Pafilis et al. 2009; Itescu et al. 2017). Because islands are environments with clear boundaries, immigration and emigration are typically low or inexistent. Insular populations are thus subject to genetic drift and to the effects of climatic instabilities and seasonal changes.

Many lizards are territorial animals which display several key behaviors. Whereas females freely explore the habitat in search of food, males often defend territories (Husak et al. 2006). This way, they secure access to females for mating, and to protect territories where food is available and where safe basking spots are present. The evolution of insular populations of lizards is partly driven by food availability which can lead to food partitioning to reduce niche overlap and thus competition (Schoener, 1974; Herrel et al. 1999). However, on islands intraspecific levels of competition are typically high, leading to frequent male-male combat and greater levels of intraspecific aggression (Donihue et al. 2016). Compared to their mainland counterparts, insular lizards thus face low levels of interspecific competition and predation but more intraspecific aggression. The evolution of these populations is consequently partly predictable and is expected to be mostly driven by local constraints, including resource availability, and social interactions including aggression and mating. This aspect is corroborated by the frequent observation of phenotypic convergence among lizards on different islands offering habitats of similar characteristics (Losos, 1992; Mahler et al. 2013).

***Interspecific analyses of macroevolutionary patterns in the Caribbean – Anolis lizards as a model***

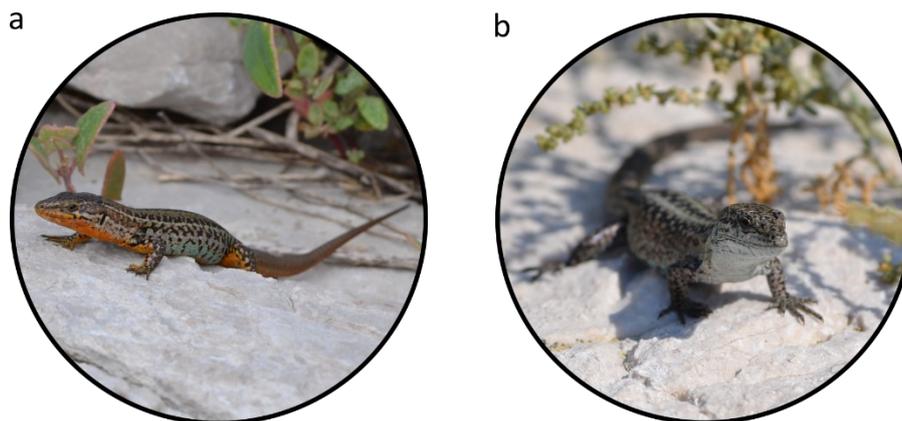
Despite the fact that islands are isolated, invasion events still occur. The species that accidentally arrive on an island often find a largely competitor-free environment that favors ecological release. They also face a unique set of biotic and abiotic factors which drive the populations on each island to take distinct evolutionary trajectories. One of the most famous cases of radiation and diversification in archipelagos is the case of the *Anolis* lizards in the Caribbean. The anole radiation in the Greater Antilles was demonstrated to be the result of the ecological differentiation of originally similar and closely related species which have occupied distinct niches for the last 15 or 20 Mya (Iltis & Vincent, 2006). The utilization of different niches was accompanied by morphological divergence, including for example variation in toepad area, tail length and limb length. By quantifying the importance of this morphological variation in generating variation in ecologically relevant performance traits (i.e. sprint speed, jump distance, clinging ability) in the context of microhabitat use, previous studies have demonstrated the adaptive nature of the observed morphological variation (Losos, 2009). The radiation of anoles is thus thought to be the result of directional selection, ultimately resulting in phenotypic diversification and speciation. Interestingly, macroevolutionary patterns observed in this system are suggested to originate primarily from microevolutionary changes in anatomy, behavior and physiology (as illustrated by Schoener & Schoener, 1983). However, the propensity of subtle variation in phenotypes to generate variation in performance, at magnitudes likely to be under selection, remains unclear. Fortunately, other insular radiations might provide insights to tackle this question.

***Intraspecific macroevolutionary variation across islands***

Island systems oftentimes also provide examples of radiation events that are more recent, and which consequently can inform us on evolutionary processes on shorter time scales. Small land-bridge islands in the Cyclades, Greece, have been separated for about 10.000 years. A recent study (Donihue et al. 2016) has highlighted the importance of intraspecific interactions, in particular of the level of aggression, in shaping the evolution of bite force and head dimensions within a single species (*Podarcis erhardii*). This study also showed that levels of aggression rather than diet were the likely driver of phenotypic variation in these animals, and that levels of aggression increased on smaller islands where population densities are higher. This study thus suggested that the evolution of distinct phenotypes consecutive to the invasion of small depauperate islands is possibly driven by social interactions. To test this idea an intentional introduction of specimens of *P. erhardii* onto small lizard-free islets (Pafilis et al. 2019;

Donihue et al. 2020) was conducted. This experiment aims to understand the evolutionary drivers of the observed inter-island variation in phenotype. To do so the year-to-year changes in ecology, population density, bite force, diet, and morphology from the time of the colonization of these novel environments is quantified. This will allow to better understand the factors driving the evolution of organisms in harsh environmental conditions. This experiment will provide interesting data on the early response of organisms to an introduction into a novel habitat, and to understand whether these rapid changes are plastic or adaptive. Moreover, it can inform on the nature of the evolutionary trajectory of populations living in small and resource-limited environments, whether it be continuous or episodic, and to maybe identify early stages of a radiation.

Another interesting case concerns an archipelago of land-bridge islands in the Adriatic, near the Croatian shore. Three *Podarcis* species differentiated in this part of Europe 1.5 Mya ago: *P. muralis*, *P. melisellensis* and *P. sicula* (Figure 1).



**Figure 1:** Pictures of the two wall-lizards species studied in the present work. a: *Podarcis melisellensis*, b: *Podarcis sicula*. The adults of both species are of similar size (snout-vent length: from 55 to 65mm in females, from 60 to 70mm in males; intact tail length: 100 to 120mm in females, 110 to 130mm in males). They look very much alike, but *P. melisellensis* is recognizable by the three color-morphs displaying yellow, orange or white throat and belly.

The latter two species of wall lizards took advantage of the low sea-level at the end of the last ice age (approximately 18.000 years ago) to invade what would become an archipelago once the sea-levels rose again (Sikora, Mihanović & Vilibić, 2014). The formation of these land-bridge islands created many populations that have remained separated (Podnar, Mayer & Tvrčković, 2004, 2005), although some unintentional introductions by maritime traffic in ancient times also took place. Interestingly, whereas both species can be found in sympatry, they are never in syntopy on the same island. The islands of the Adriatic archipelago are very diverse in size, vegetation cover, habitat structure and isolation

(Figure 2), and thus represent an interesting opportunity to study how more subtle variation in ecology may be associated with phenotypic changes. Subsequent to an introduction experiment on two small islets by Nevo et al. (1972), a research team went back to the two islands 36 years later (Herrel et al. 2008). They found that populations had rapidly diverged in their ecological dynamics, digestive anatomy, head morphology, and bite force. They further showed that these changes were associated with a dietary specialization suggesting that unlike in the Aegean system, diet is likely an important selective agent driving the evolution of phenotypic variation. The present thesis focuses on the Adriatic because the divergence-time between populations is recent enough to address microevolutionary questions, yet old enough to detect patterns of covariation between phenotype and ecology.



**Figure 2:** Pictures of four islands included in the present study illustrating the diversity in size, structure and vegetation cover (a: Mali Barjak, b: Pod Kopište, c: Pod Mrčaru, d: Sušac).

### ***Evolution of phenotypes***

Any biological structure is the result of the interplay between the phylogenetic heritage of the organism, its function, and its development (D’Arcy Thompson, 1942; Gould & Lewontin, 1979; Goodwin & Trainor, 1980; Pigliucci & Kaplan, 2000). The morphology of an organism thus reflects the constraints imposed by the physical characteristics of the environment (Sagnes et al. 1997; Fish, 1998; Fish et al. 2008; Segall et al. 2019; Hedenström, 2002; Altshuler et al. 2015; Hedenström & Johansson, 2015) within the limits imposed by its genetic and developmental repertoire. Moreover, functional and constructional trade-offs may limit the expression of a given phenotype (Cheverud, 1982; Barel et al. 1989; Herrel et al. 2009). Consequently, complex integrated systems such as the vertebrate feeding or locomotor systems may not represent mechanically optimized structures (Zweers, 1979; Wake & Roth, 1989).

Because of the ecological peculiarities of island ecosystems (especially resource use, intraspecific competition, and sexual selection, as suggested previously), strong selective constraints are imposed

on organisms. Therefore, in lizards, diet and the anatomy of the musculoskeletal system are strongly correlated, especially in relation to feeding and locomotion. In particular, the functional characteristics of food items consumed are often correlated with bite force and underlying head anatomy (Aguirre et al. 2003; Herrel & O'Reilly, 2006; Herrel & Holanova, 2008). This is likely because the physical and functional properties of food items impose mechanical constraints on the feeding system. They in turn impact head morphology in line with the demands on force, mobility, or speed needed to cope with the type of food consumed. Unfortunately, the nature of the mechanical constraints imposed onto the musculoskeletal elements during a behavior, as well as their magnitude and direction are not always easily quantifiable. Whether and how these constraints drive the relationships between morphological variation and performance variation remains thus unclear. However, modeling tools provide powerful means to address some of these questions.

The first tool is multibody dynamics analysis (MDA). MDA is a computational method that allows the dynamic analysis of motion and force in articulated, connected systems composed of rigid or elastic bodies. The advantage of MDA is that it allows the resolution of multiple complex equations of motion. Widely used since the 90's in the automotive industry and aeronautics, it has found numerous applications in biomechanics, especially in the simulation of musculoskeletal function and the investigation of the relationships between bone geometry, muscular architecture and properties, forces and motion (in fish: Westneat, 2003; in pigs: Langenbach et al. 2002, 2006; in primates: Curtis et al. 2008; in lizards: Curtis et al. 2010). These models allow the quantification of motion generated by known forces and muscle activation patterns (forward dynamics), or inversely, what forces and muscle activation patterns are necessary to make the model move in a specified manner (inverse dynamics).

The second computational tool is finite-element analysis (FEA) which estimates the stress and strain or deformation of a structure when loaded, given its geometry and its intrinsic material properties (see Rayfield, 2007; Panagiotopoulou, 2009; Bright, 2014 for a historical and comprehensive review of the method). It relies on the discretization of the structure into a mesh of interconnected simple geometric elements. Originally developed for engineering analyses, it has been applied to orthopaedic medicine for many years and more recently to the study of organismal morphology (see O'Higgins et al. 2011 for a review). It can be used to compute the effects of loading on the structural performance of a musculoskeletal system, that is to say, its ability to distribute and to dissipate loading. The quantitative and qualitative description of the strain distribution patterns allows the investigation of the functional consequence of variation in bone morphology thus bridging the link between form and function. The loading data generated by MDA simulations are essential to build realistic FEA models (Rayfield, 2019).

***Goals of this thesis***

The objective of the present work is to understand whether subtle phenotypic variation observed within a species can give rise to changes in performance and subsequently ecology. Therefore, we aim to investigate whether the physical and mechanical characteristics associated with the selective pressures encountered by organisms on islands can explain the evolution of morphological diversity.

The present study uses the Adriatic system described earlier to address these questions. First, we characterized the ecological and dietary variability among populations in this system (Chapter 1). Second, we investigated how ecological differences among populations are associated with variation in bite force, an ecologically relevant performance trait (Chapter 2). Next, we investigated the behavioral responses of the lizards in relation to the type of food consumed through the study of their jaw kinematics (Chapter 3). Chapter 4 investigates patterns of covariation between diet, bite force, muscle architecture, and skull and mandible shape. Finally, the functional relevance of morphological variation was assessed using MDA and FEA modelling tools in Chapter 5.

We hypothesized that: 1) populations differ in their diet, and that competition for food access is greater on smaller islands, 2) variation in diet is associated with variation in bite force, with more forceful bites being associated with the consumption of difficult-to-reduce food items like hard arthropods or plants, 3) dietary shifts are accompanied with the ability of lizards to modulate their behavioral repertoire to cope with the particular functional properties of the prey item consumed, 4) bite force covaries with skull morphology, which reflects dietary specialization and is constrained by the space and mechanical demands associated with the differential investment in jaw muscles, 5) subtle morphological variation reflects the mechanics of biting and force transmission. Ultimately, we hypothesize that microevolutionary changes in morphology through their mechanical impact on performance can be under selection, and ultimately result in the emergence of the phenotypic and ecological diversity observed across the islands included in our study.



# **Chapter 1**

## **Ecology**

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## Diet variability among insular populations of *Podarcis* lizards reveals diverse strategies to face resource-limited environments

- 2019. *Ecology and Evolution* 9: 12408-12420 -

### ABSTRACT

Access to resources is a dynamic and multi-causal process that determines the success and survival of a population. It is therefore often challenging to disentangle the factors affecting ecological traits like diet. Insular habitats provide a good opportunity to study how variation in diet originates, in particular in populations of mesopredators such as lizards. Indeed, high levels of population density associated with low food abundance and low predation are selection pressures typically observed on islands. In the present study, the diet of eighteen insular populations of two closely related species of lacertid lizards (*Podarcis sicula* and *Podarcis melisellensis*) was assessed. Our results reveal that despite dietary variability among populations, diet taxonomic diversity is not impacted by island area. In contrast, however, diet disparity metrics, based on the variability in the physical (hardness) and behavioral (evasiveness) properties of ingested food items, are correlated with island size. These findings suggest that an increase in intraspecific competition for access to resources may induce shifts in functional components of the diet. Additionally, the two species differed in the relation between diet disparity and island area suggesting that different strategies exist to deal with low food abundance in these two species. Finally, sexual dimorphism in diet and head dimensions is not greater on smaller islands, in contrast to our predictions.

**Keywords:** Croatia, diet, insularity, lizard, *Podarcis*, sexual dimorphism

## INTRODUCTION

Access to resources in ecosystems and communities is a dynamic and multi-causal process that determines the success and survival of a population. The mechanisms underlying the flux of resources between species sharing the same environment have been investigated in some detail (Lindeman, 1942; Petchey et al. 2008; Sousa et al. 2008; Yvon-Durocher & Allen, 2012) and many factors have been shown to influence dietary variation in vertebrate communities. On one hand, biotic factors including intra and inter-specific interactions such as competition between closely related species (Pacala & Roughgarden, 1982), predation (Szarski, 1962; Hawlena & Pérez-Mellado, 2009), or male-male combat and territory defense (Edsman, 1986) may impact the resources available to an animal. On the other hand, abiotic constraints such as climate or season will influence food availability and thus constrain what is available for an animal to eat (Renton, 2001; Korslund & Steen, 2006). Because of the complexity and the multiplicity of the factors affecting diet, it is often difficult to disentangle these effects.

Whereas mainland ecosystems are highly complex, islands may provide simpler ecosystems where the factors that drive variation in diet can be more easily understood (Losos & Ricklefs, 2009; Kueffer, Drake & Fernandez-Palacios, 2014). First, immigration and emigration are limited on islands. Second, islands often host species-poor communities compared to similar ecosystems on the mainland as a result of a lower ratio between speciation and extinction (Losos & Schluter, 2000; Losos & Ricklefs, 2009). Smaller islands also often lack top-predators (Grant, 1972; Losos & Queiroz, 1997), especially carnivorous mammals (MacArthur & Wilson, 1967). As a consequence, predation pressure and inter-specific competition are often reduced on islands (Thomas, Meiri & Phillimore, 2009). This may induce ecological release (Buckley & Jetz, 2007), also called niche expansion, a phenomenon where a population takes advantage of the low intensity of predation pressure to thrive and to broaden their niche width. Meso-predators in particular have been demonstrated to show this phenomenon (Litvaitis & Villfuerte, 1996; Ritchie & Johnson, 2009). Consequences of the ecological release, also known as the island syndrome, impact many aspects of the organisms' ecology, such as aggressiveness, body size, life expectancy, and dietary habits (Adler & Levins, 1994; Meiri, Dayan & Simberloff, 2006).

Ecological release often drives an increase in population density in insular communities (Case, 1975; Hasegawa, 1994; Buckley & Jetz, 2010) through density compensation. Although not all organisms show this, most vertebrate taxa do, especially lizards (Case, 1975; Schoener, 1989; Pérez-Mellado et al. 2008) which have been shown to drastically increase their population density when the diversity in species number

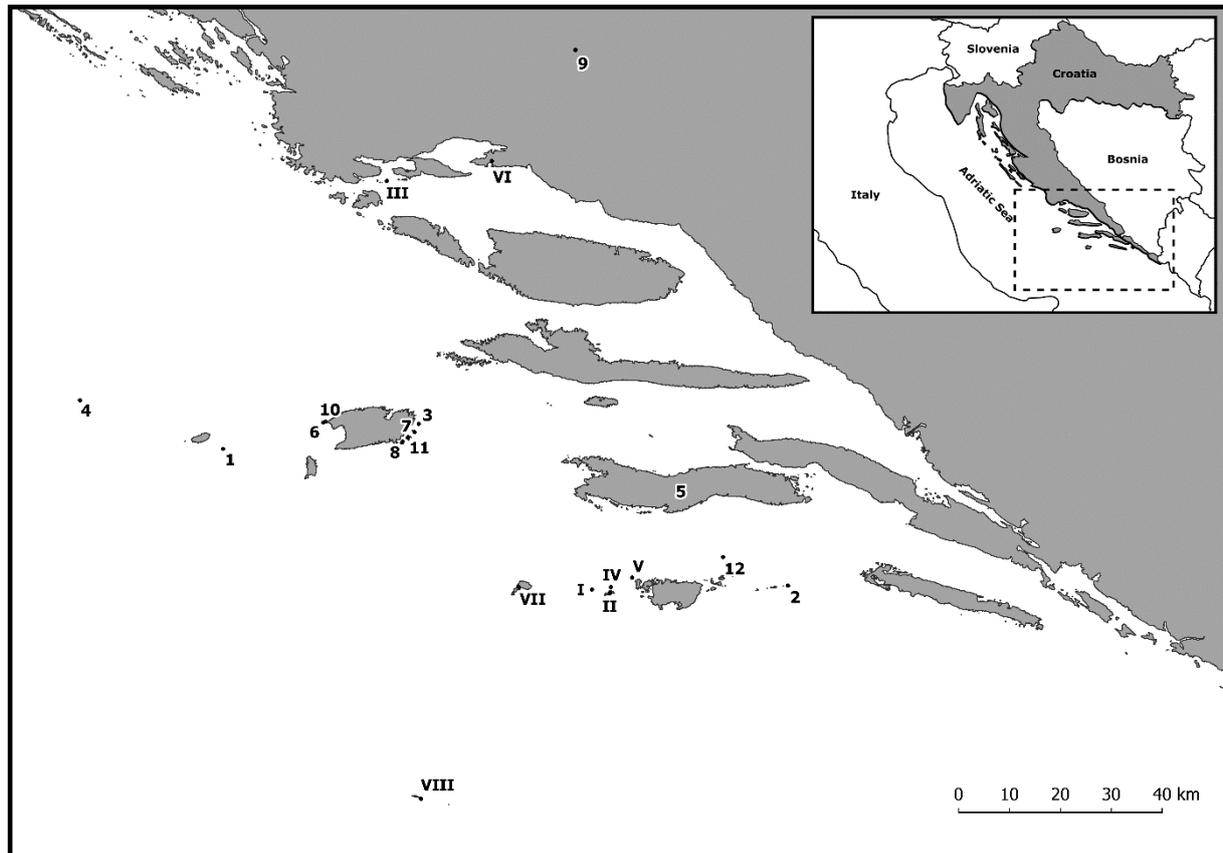
within the community decreases. In this sense, insular populations diverge from their mainland relatives by displaying a higher ratio between intra and interspecific competition (Pafilis et al. 2009; Itescu et al. 2017).

However, prey diversity also drops dramatically on islands. Arthropods, an important food resource for mesopredators such as lizards, do not compensate low diversity by increasing population density, which leads to a collapse of prey biomass on islands (Olesen & Jordano, 2002; Olesen & Valido, 2003). Consequently, the typical food resources may become too scarce to provide the needs of an increased population of mesopredators, resulting in dietary changes and the inclusion of new food sources (e.g., less nutritional items such as plants) (Van Damme, 1999; Bolnick 2001). Therefore, high population densities combined with low food abundance will result in intense competition for access to food which may subsequently drive dietary variation in insular populations.

Previous studies have indeed suggested that intraspecific competition for resource access can increase variation in diet within insular populations (Svanbäck & Bolnick, 2007). This is likely the case because ecological release promotes morphological variation enabling animals to exploit novel food resources (Thomas, Meiri & Phillimore, 2009). The intrapopulation diversification of diet subsequent to the invasion of new habitats with low food abundance was suggested to be advantageous by reducing diet overlap and increase food partitioning. Therefore, intense intraspecific competition associated with food scarcity may drive dietary adaptations. Examples of diet diversification in such populations are common; for example, in birds (MacArthur, Diamond & Karr, 1972) and lizards (at the interspecific level, see Schoener, Slade & Stinson, 1982; Herrel, Vanhooydonck & Van Damme, 2004; at the intraspecific level, see Schoener, 1967; Brown & Pérez-Mellado, 1994; Sagonas et al. 2014; Donihue et al. 2016).

The present study aims to characterize the diversity of diet across insular populations of two closely related species of lacertid lizards: seven populations of *Podarcis sicula* (Rafinesque-Schmaltz, 1810) and eleven of *Podarcis melisellensis* (Braun, 1877). The populations of interest (Figure 1) provide a unique opportunity to unravel the factors affecting dietary diversity as they live in ecologically relatively simple insular systems. They often share their habitat with few other lizard species, but most frequently with the rock specialist *Dalmatolacerta oxycephala* (Duméril & Bibron, 1839) and the nocturnal gecko *Hemidactylus turcicus* (Linnaeus, 1758). Consequently, resource availability and intraspecific competition are likely the main drivers of variation in diet. These lizards were studied on several islands in the middle Adriatic, off the coast of Croatia. These islands further differ in size, yet are within a relatively short distance from one another.

*Podarcis sicula* is known to be more aggressive than its close relative *P. melisellensis*. It is also invasive in many areas world-wide suggesting it is a more generalist species. The way both species deal with the variable intensity of intraspecific competition may thus differ and might have an impact on dietary variation. Moreover, sexual dimorphism, especially in body size, is important in lizards living on small



**Figure 2:** Location of the populations of *Podarcis sicula* (I: Bijelac; II: Kopshte; III: Pijavica; IV: Pod Kopshte; V: Pod Mrčaru; VI: Split; VII: Sušac; VIII: Mala Palagruža) and *Podarcis melisellensis* (1: Brusnik; 2: Glavat; 3: Grebeni; 4: Jabuka; 5: Korčula; 6: Mali Barjak; 7: Mali Parzanj; 8: Ravnik; 9: Sinj; 10: Veli Barjak; 11: Veli Budikovac; 12: Veli Tajana) included in the present study.

and depauperate islands (Schoener, 1977), and has been suggested as determinant factor in food partitioning between males and females (Perry, 1996). Finally, the populations included in the present study live on islands of very diverse size, structure, and vegetation cover that all may impact population density and food availability (Polis & Hurd, 1996).

We formulate four hypotheses that will be tested in this study: (1) we expect to find significant differences between species, sexes, and islands; (2) we expect that the diversity in diet is correlated with island size since food diversity and abundance are dependent on island size; (3) we expect the populations from the smallest islands to present greater levels of sexual dimorphism as a response to increased intraspecific competition for food resources; and (4) we expect to find a lower disparity in the diet of the populations of *P. melisellensis* compared to populations of the more generalist *P. sicula*.

## MATERIAL AND METHODS

### *Specimens*

Following the issuance of the permit from the Ministry of the Environmental Protection of the Republic of Croatia, Directorate for Nature Protection, specimens of *P. sicula* were captured across eight sites (including one continental site), and *P. melisellensis* across twelve sites (including one continental site) (Figure 1). The two species were never found to coexist on the islands. In total, 535 adult animals were captured by noose or by hand at the end of the summer 2016 (Table 1). The snout-vent length (SVL), and linear head dimensions such as head length (HL), head width (HW), head height (HH), lower jaw length (LJL), quadrate-to-tip length (QT) and coronoid-to-tip length (CT) of all individuals were measured using digital calipers (Mitutoyo absolute digimatic;  $\pm 0.01\text{mm}$ ).

**Table 1:** List of the specimens and their origin (\* continental sites)

Species	Site	Symbol	Area (m <sup>2</sup> )	Date	Females	Males
<i>Podarcis melisellensis</i>	Brusnik	BR	49,455	08.27.2016	22	20
	Glavat	GL	18,430	09.09.2016	9	12
	Greben	GR	51,690	08.31.2016	12	18
	Jabuka	JA	22585	08.28.2016	21	17
	Korčula	KR	$2.79 \times 10^8$	10.08.2016	7	11
	Mali Barjak	BM	6,232	08.26.2016	14	18
	Mali Paržanj	PZ	13,403	09.01.2016	13	12
	Ravnik	RV	226,605	09.03.2016	6	11
	Sinj #	SI	-	09.12.2016	5	11
	Veli Barjak	BU	18,116	08.30.2016	19	21
	Veli Budikovac	BD	316,748	09.02.2016	17	17
	Veli Tajan	VT	20,127	09.08.2016	7	8
<i>Podarcis sicula</i>	Bijelac	BI	5,530	09.08.2016	7	13
	Kopište	KO	738,726	09.06.2016	14	19
	Mala Palagruža	PG	26,510	09.10.2016	8	12
	Pijavica	PI	11,037	09.14.2016	13	12
	Pod Kopište	PK	35,835	09.06.2016	15	22
	Pod Mrčaru	PM	13,514	09.05.2016	12	24
	Split #	ST	-	09.12.2016	9	7
	Sušac	SU	4,025,460	09.04.2016	15	5

***Island area***

The area of each island was determined using Google Earth.

***Stomach contents analysis***

All individuals were stomach flushed immediately after capture using a syringe with water and a ball-tipped steel needle (Herrel et al. 2006; Measey et al. 2011). The animals were tapped gently on the sides of the jaw, resulting in a typical gaping response. A small plastic-ring was then inserted between the jaws allowing the introduction of the needle into the pharynx. Palpation helped to detect the position of the needle and to further insert it through the digestive tract into the stomach. The water was then slowly squeezed out of the syringe and food was pushed out with the water. We maintained the flow of water until only water flowed out of the stomach. The stomach contents were preserved in individual vials containing a 70% aqueous ethanol solution and labelled. Animals were measured and marked using a non-toxic marker to distinguish them and to make sure that they were manipulated only once. A recovering time of 12 hours was then observed before releasing the animals to their exact site of capture.

***Characterization of the diet***

The prey items of each stomach content were identified down to the lowest possible taxonomic order following Chinery (1986), and counted. Each food item (including plant matter) was weighted using a Mettler electronic balance ( $\pm 0.1$  mg), and a dial caliper (Mitutoyo  $\pm 0.2$  mm) was used to determine their length and width. The volume of each prey item was calculated following a spheroid equation:

$$Volume = \frac{4}{3} \times \pi \times \frac{L}{2} \times \left(\frac{W}{2}\right)^2$$

where L is the length of the item, and W its width. However, prey items are not always intact preventing the estimation of their volume. To account for this, we measured the length of several key-body parts (i.e. abdomen, head, or wing) and the total length and the width of approximately 15 intact prey items per taxa. For each taxon, these measurements were used to establish a reference equation that further

allowed us to estimate the length and the width, and subsequently the total volume, of the damaged prey when a key-body part was found in the stomach contents.

**Table 2:** Functional categories of prey taxa identified in the stomach contents.

Hardness	Evasiveness	Prey taxa
Soft	Sedentary	Acari
		Aphidoidea
		Araneae
		(Insecta) Larvae
		Pseudoscorpionida
	Intermediate	Thysanura
		Chilopoda
		Embioptera
		Heteroptera
Medium	Sedentary	Diptera
		Lepidoptera
	Evasive	Diplopoda
		Isoptera
Hard	Sedentary	Orthoptera
		Gastropoda
	Intermediate	Homoptera
		Coleoptera
	Evasive	Formicidae
		Flying Hymenoptera

Prey were classified according to their functional properties in terms of hardness and evasiveness (Table 2), following Vanhooydonck, Herrel & Van Damme (2007). Hardness included three categories (soft, medium and hard) as has been previously established by quantifying the forces needed to crush a prey item (Herrel, Van Damme & De Vree, 1996; Andrews & Bertram, 1997; Herrel, Verstappen & De Vree, 1999; Herrel et al. 1999, 2001; Aguirre et al. 2003). The criterion of evasiveness referred to the ability of the prey to escape a predator before capture, and also includes three categories (sedentary, intermediate, and evasive). Plant matter was not included in these calculations and was considered separately. Thus, total of seven food categories were considered in the analyses.

The relative numerical (N %) and mass (M %) abundance were calculated for each functional food category for each individual, along with the relative contribution of each food category to the whole stomach content. An index of relative importance (IRI) was then calculated following the equation:

$$IRI = (N\% + M\%) \times O\%$$

where  $O\%$  is the frequency of occurrence of each prey category (in other words, the proportion of stomachs containing at least one item of the designated food category; Pinkas, 1971; Martin, Twigg & Robinson, 1996; Twigg et al. 1996).

Since it is not possible to calculate means for the frequency data, the IRI was determined for each prey category, for each sex, and each site. Based on all measured and calculated variables, four diet sub-datasets are retained and considered separately for the statistical analyses: the first three describe the numerical and mass abundance, and IRI for each of the seven food categories, and the last sub-dataset includes five prey dimensions. These are Lmax (the maximum length of any prey item ingested by an individual), Lmin (the minimum length), Wmax (the maximum width), Wmin (the minimum width), and Vmax (the maximum volume of any prey item ingested by an individual).

### ***Statistical analyses***

All statistical analyses were carried out using R (v3.5.1, R Core Team 2018). The relative numerical and mass abundance data, which values ranged between 0 and 1, were arcsin-transformed to normality. Values of prey dimensions and of head dimensions were  $\log_{10}$ -transformed. Prior to any analysis, the normality and the homogeneity of variances in each sub-dataset were tested by a Shapiro test and a Bartlett's test.

### ***Variability in diet***

A three-way MANOVA was performed on the whole diet dataset with sex, species, and site as factors as well as for a possible interaction between these factors to test for their effect on diet. Next, MANOVAs were computed for each sex and species separately, with the site as factor (function "manova" in the package "stats"). Tukey's post-hoc tests (function "TukeyHSD" in the package "stats") were used to explore which islands differed from one another.

***Diversity and disparity***

The taxonomical diversity of prey was determined through the calculation of the Shannon-Wiener's diversity index ( $H'$ ). In the following equation,

$$H' = - \sum_{i=1}^S p_i \log(p_i)$$

' $S$ ' is the total number of prey taxa found in an individual stomach, and ' $p_i$ ' is the relative abundance of the prey taxon, calculated as follows:

$$p_i = \frac{n_i}{N}$$

where  $N$  is the total number of prey items found in a stomach and  $n_i$  the total number of prey items of the taxon  $i$ . As it is commonly used in ecological studies, we used the logarithm base 2 of this metric for analysis. Because the distribution of  $H'$  was found to be bi-modal, the difference in sex, species and island was investigated with Mann-Whitney-Wilcoxon tests ("`wilcox.test`" function in "`stats`" package). A possible relationship between diversity in diet and island area or the proportion of plants in the diet was then tested by means of Spearman rank correlation tests (function "`cor.test`" from the "`stats`" package). For the latter analysis the index was averaged by population or sub-population to avoid pseudo replication.

The disparity index calculates the hyper-volume occupied by a subset in ecological space. Based on the two numerical and mass abundance datasets, it was computed using the function "`disparity.per.group`" from the "`dispRity`" package (Guillerme, 2018) in R. The comparison of the disparity of subsets allowed us to test for differences between species, sexes, and islands. This was done using the "`test.disparity`" function which uses a Mann-Whitney-Wilcoxon test of comparison of the medians, coupled with a Bonferroni correction multiple comparisons when required. We also tested for a relationship between disparity and island size. To do so, the disparity metric "`sum`" was extracted for each site, sex, and species,  $\log_{10}$ -transformed and regressed against island area. All linear regressions involving island area exclude mainland sites and the island Korčula. Whereas continental sites have no area associated, Korčula is a very large island close to the mainland and thus its community is expected to behave similar to a mainland one.

***Sexual dimorphism in diet, prey size, head dimensions and SVL***

For both species separately, principal component analyses (PCA) were performed on the data sets describing numeric and volumetric prey consumption, prey dimensions, and head dimensions including all individuals using the function “prcomp” from the “stats” package. This allowed for a reduction in dimensionality. The contribution of each specimen along the three first principal components (PC) was extracted and used to calculate the mean contribution of each sex of each population on these axes.

The sexual dimorphism (SD) for each site was determined as follows:

$$SD = \sqrt{(m_1 - f_1)^2 + (m_2 - f_2)^2 + (m_3 - f_3)^2}$$

where  $m_i$  and  $f_i$  refer respectively to the mean contribution of the males and the females of the population of interest along with the  $PC_i$ .

This method allowed us to calculate three measures of sexual dimorphism:  $SD_d$  (diet),  $SD_p$  (prey size) and  $SD_h$  (head dimensions). Two other sexual dimorphisms,  $SD_{svl}$  (body size) and  $SD_{disp}$  (disparity) were obtained as the difference in these variables between males and females. We tested for differences between species in each SD, then for a possible relationship between each SD, using Mann-Whitney-Wilcoxon tests, and between SD and island area with a non-parametric Spearman rank correlation test.

## RESULTS

### ***Variability in diet***

The calculation of the IRI scores provides a qualitative overview of the trends in diet variability across islands, species, and sexes (Table 3). In the majority of the populations of both species the two most representative prey categories are hard and intermediate evasive prey. However, plant matter is less common in the diet of *P. melisellensis* compared to *P. sicula*. For example, on Sušac plant matter represents 76% of the food eaten by *P. sicula* (Table 3).

### ***Quantification of the diet variability***

The three-way MANOVA performed on the whole dataset reveals a strong species ( $F_{14,482}=17.69$ ,  $P<0.001$ ), sex ( $F_{14,482}=2.32$ ,  $P=0.004$ ), and site ( $F_{252,6930}=2.57$ ,  $P<0.001$ ) effect on diet composition. However, no interaction is detected between these three factors ( $F_{266,6930}=1.01$ ,  $P=0.411$ ).

When considering the dataset describing the numerical abundance of the seven food categories, the diet of both species is significantly different ( $F_{7,527}=17.44$ ,  $P<0.001$ ). Although males and females did not differ regarding this dataset (*P. melisellensis*:  $F_{1,320}=1.47$ ,  $P=0.176$ ; *P. sicula*:  $F_{1,205}=0.84$ ,  $P=0.551$ ), the results of the three-way MANOVA justify to carrying out further analyses by sex. For both species, strong differences are detected between islands (*P. melisellensis*: females:  $F_{77,980}=1.61$ ,  $P<0.001$ , males:  $F_{77,1148}=2.29$ ,  $P<0.001$ ; *P. sicula*: females:  $F_{49,585}=1.98$ ,  $P<0.001$ , males:  $F_{49,742}=2.81$ ,  $P<0.001$ ). If we consider the variation in diet by sex and species separately, variables that globally appear to drive the differences among islands are the proportion of plants, the proportion of soft and medium hard prey and the proportion of evasive prey. The dietary proportions of hard prey do not discriminate islands, except in males of *P. melisellensis* ( $F_{11,164}=2.02$ ,  $P=0.029$ ). The analyses on the mass proportion data show similar results, differences between islands being strongly driven by the volumetric proportion of plants, soft, medium, and evasive prey, and poorly influenced by hard prey, except again in males of *P. melisellensis* ( $F_{11,164}=2.11$ ,  $P=0.016$ ).

**Table 3:** IRI scores of the seven food categories (*Sedent*: sedentary preys, *Inter*: prey of intermediate evasiveness, *f*: females, *m*: males). The highest IRI for each prey functional criteria in each population is marked with a \*. Scores of plant matter that overcomes at least one of the two previously marked scores of the same population are also indicated by \*.

Species	Island	Sex	Hardness			Evasiveness			Plant
			Soft	Medium	Hard	Sedent.	Inter.	Evasive	
<i>P. melisellensis</i>	BR	m	597	38	1796*	103	2889*	149	55
		f	931	10	2984*	649	2881*	125	12
	GL	m	1059	56	4101*	1636*	785	951	153
		f	1260	5	3438*	1727*	1207	365	60
	GR	m	999*	84	704	205	797	971*	19
		f	1098	0	1172*	334	689	872*	22
	JA	m	1863*	111	717	733	937*	935	156
		f	1548*	183	515	1049*	569	585	357
	KR	m	789	1237	3727*	1743	828	1886*	0
		f	1128	252	4323*	2795*	720	769	0
	BM	m	1145*	624	243	628	1925*	80	9
		f	592	207	885*	729	1177*	29	109
	PZ	m	823*	155	265	307	2000*	0	62
		f	2685*	17	1461	870	2711*	591	4
	RV	m	797	5	1886*	221	1554*	596	28
		f	351	0	1428*	211	520*	413	458
	SI	m	268	495	2294*	241	773	1432*	63
		f	471	331	2264*	351	690	1694*	11
	BU	m	827	0	3405*	418	3929*	43	31
		f	969	2	2921*	953	3108*	0	67
BD	m	361	450	1068*	235	1025*	546	222	
	f	633	41	1341*	119	1823*	356	6	
VT	m	296	1055	1930*	1800*	981	39	275	
	f	936*	572	628	1098	1167*	107	352	
<i>P. sicula</i>	BI	m	2043*	445	1098	1587	394	1950*	298
		f	1410*	50	1076	727	219	1992*	86
	KO	m	301	573	1037*	565	1001*	154	1296*
		f	456	78	1671*	432	1211*	343	1217*
	PI	m	741	131	1162*	485	892*	627	1411*
		f	642*	97	610	544*	387	374	1678*
	PK	m	876	24	1150*	297	2178*	150	916
		f	703*	59	595	331	943*	234	2248*
	PM	m	126	216	368*	178	248*	210	1398*
		f	121	82	530*	192	260*	134	2565*
	ST	m	549	2452*	1186	1294*	1010	952	508
		f	516	165	2661*	870	1113*	532	352
	SU	m	7	0	703*	8	703*	0	4403*
		f	133	29	503*	68	786*	21	4804*
	PG	m	865*	586	130	312	130	1767*	58
		f	981	1826*	82	1246*	82	1138	0

**Diet diversity and disparity**

The Mann-Whitney-Wilcoxon test performed on the diversity index ( $H'$ ) shows a significant signal of species ( $P < 0.001$ ) and island ( $P < 0.001$ ), but not sex ( $P = 0.141$ ). *P. melisellensis* consumes, on average, a greater diversity of prey than *P. sicula* ( $H'_m = -0.12$ ,  $H'_s = -0.31$ ). The taxonomic diversity of prey ingested does not correlate with the island area, neither when both species are considered together ( $P = 0.065$ ,  $\rho = -0.461$ ), nor when species are considered separately (*P. melisellensis*:  $P = 0.132$ ,  $\rho = -0.515$ ; *P. sicula*:  $P = 0.139$ ,  $\rho = -0.642$ ). Interestingly, a strong negative correlation exists between  $H'$  and the proportion of plants included in the diet ( $P < 0.001$ ,  $\rho = -0.181$ ). This, however, only holds for *P. sicula* ( $P < 0.001$ ,  $\rho = -0.369$ ; *P. melisellensis*:  $P = 0.357$ ,  $\rho = 0.053$ ).

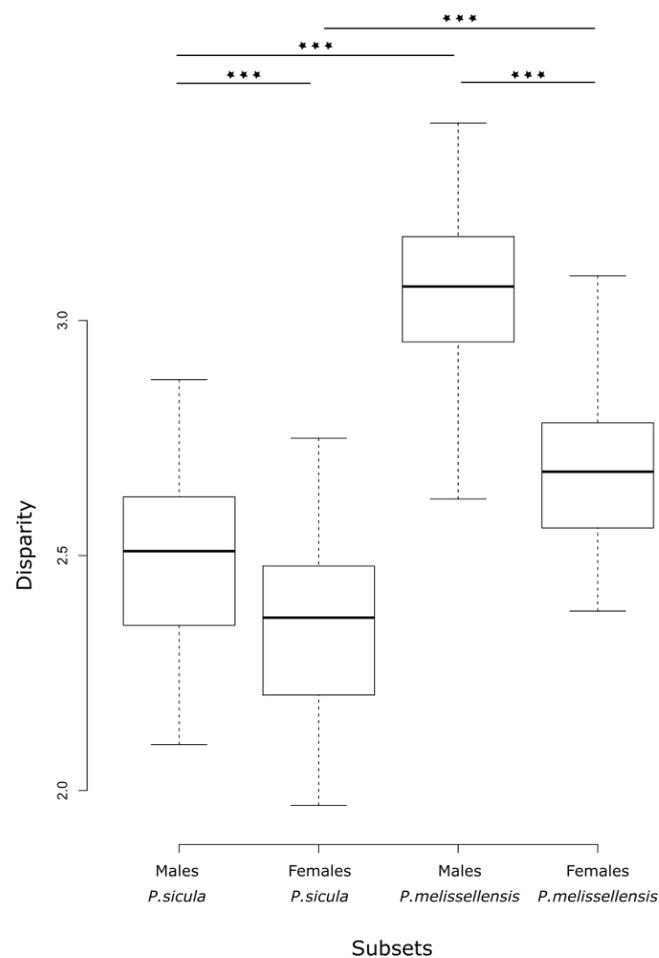
**Table 4:** Results of disparity analyses are given here: *n*, the number of specimens of each subset; *obs*, the observed disparity; *median*, the median value of disparity; and four different percentiles. Then results of Wilcoxon tests between each subset, the associated *W*-values and *P*-values (*s*: *sicula*, *m*: *melisellensis*). \* represents significant *P*-values.

Subsets	n	obs	median	2.5%	25%	75%	97.5%
<i>sicula</i>	207	2.430	2.395	2.101	2.301	2.472	2.687
<i>melisellensis</i>	328	2.917	2.883	2.659	2.819	2.968	3.125
males	290	3.004	2.968	2.759	2.897	3.052	3.179
females	245	2.727	2.705	2.435	2.623	2.778	2.937
males <i>sicula</i>	114	2.506	2.509	2.161	2.353	2.625	2.815
females <i>sicula</i>	93	2.348	2.368	1.984	2.204	2.475	2.703
males <i>melisellensis</i>	176	3.093	3.072	2.705	2.955	3.175	3.363
females <i>melisellensis</i>	152	2.704	2.678	2.447	2.560	2.782	3.042

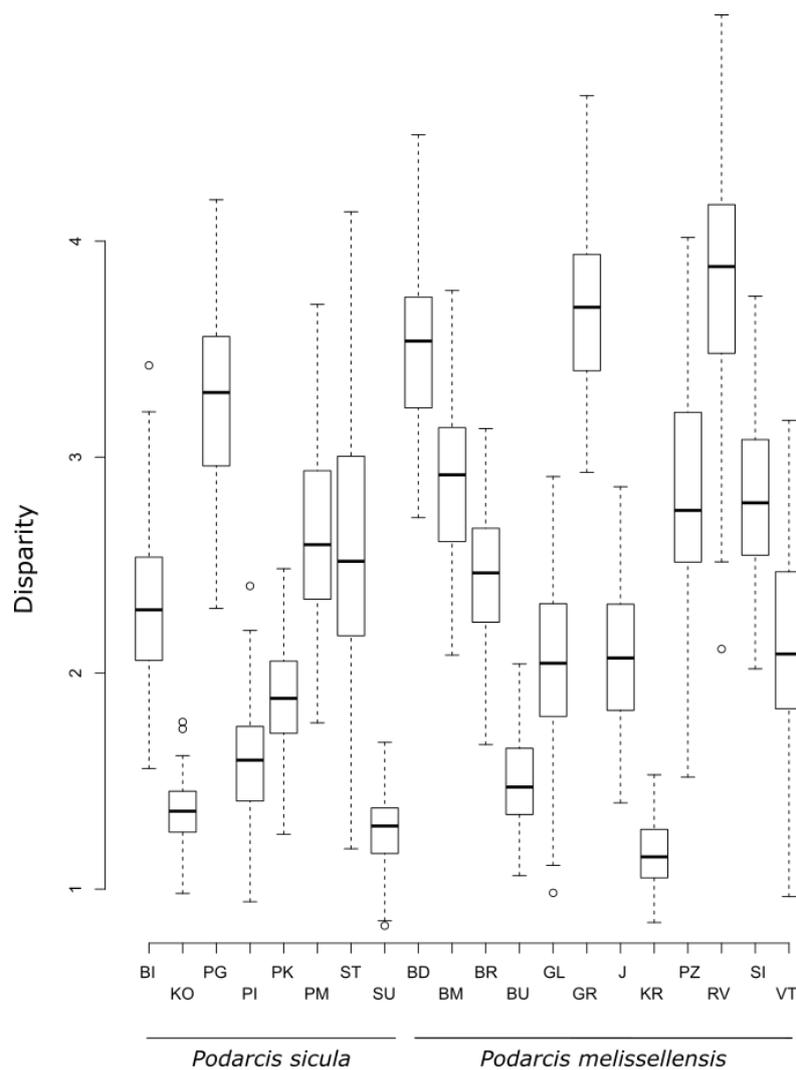
Test	W	p-value
<i>sicula</i> / <i>melisellensis</i>	54	<0.001*
males / females	541	<0.001*
males <i>s</i> / females <i>s</i>	6986	<0.001*
males <i>s</i> / males <i>m</i>	126	<0.001*
males <i>s</i> / females <i>m</i>	2338	<0.001*
females <i>s</i> / males <i>m</i>	51	<0.001*
females <i>s</i> / females <i>m</i>	882	<0.001*
males <i>m</i> / females <i>m</i>	9377	<0.001*

Analyses performed on the numerical and the mass proportion datasets reveal that the disparity in diet differs between species and sexes (Figure 2). Indeed, the disparity is greater in *P. melisellensis* than in *P. sicula* ( $P < 0.001$ ), and greater in males than in females ( $P < 0.001$ ). This difference remains statistically significant, even after a Bonferroni correction, and when each sex of each species is considered separately ( $P < 0.001$ ) with males of *P. sicula* having lower disparity than females of *P. melisellensis* (Table 4). Moreover, the difference in diet disparity between sexes ( $SD_{disp}$ ) in *P. melisellensis* is greater than in *P. sicula* ( $P < 0.001$ ).



**Figure 3:** Results of the comparison of the disparity magnitude between species and sexes (middle bars represent median, boxes values represent the standard deviation, and the whiskers the minimum and maximum values).

Our results also show a great variability among islands (Figure 3). The lizards with the lowest disparity in diet are those from Kopište, Sušac, Veli Barjak and Korčula whereas Mala Palagruža, Veli Budikovac, Grebeni and Ravnik have the highest disparity. The disparity magnitude, estimated by the metric “sum” from the “dispRity” package, correlates positively with the island area for females of *P. melissellensis* ( $P=0.011$ ,  $R^2=0.516$ , intercept=-0.546, slope=0.246) and negatively for females of *P. sicula* ( $P=0.039$ ,  $R^2=0.527$ , intercept=0.755, slope=-0.110).



**Figure 4:** Results of the comparison of the disparity magnitude between populations (middle bars represent median, boxes values represent the standard deviation, and the whiskers the minimum and maximum values).

**Sexual dimorphism**

Spearman rank tests did not reveal any significant relationships between island area, the degree of sexual dimorphism in morphology, or diet (Table 5). Mann-Whitney-Wilcoxon tests did not show any difference in the sexual dimorphism between the two species except in head dimensions, for which sexual dimorphism is greater in *P. melisellensis* than in *P. sicula* ( $P=0.006$ ,  $mean_s = -0.746$ ,  $mean_m = -0.604$ ).

**Table 5:** Results of the Spearman tests for rank correlation between the different sexual dimorphisms ( $SD_d$ : in diet,  $SD_p$ : in prey dimensions,  $SD_{svl}$ : in body size,  $SD_h$ : in head dimensions,  $SD_{disp}$ : in diet disparity) and island area. The table gives the rho-value and the P-value of each test. Pairs marked with an "x" were not tested.

		<i>P. melisellensis</i>						
Variable	area	$SD_d$	$SD_p$	$SD_{svl}$	$SD_h$	$SD_{disp}$		
<i>P. sicula</i>	area	0.236	0.321	0.321	0.393	-0.624	$\rho$	
		0.514	0.367	0.367	0.263	0.060	P	
	$SD_d$	-0.071	x	0.200	0.357	x	$\rho$	
		0.906	x	0.583	0.313	x	P	
	$SD_p$	-0.035	x	x	0.345	0.478	x	$\rho$
		0.963	x	x	0.331	0.166	x	P
	$SD_{svl}$	-0.107	-0.428	0.536	x	x	-0.163	$\rho$
		0.839	0.356	0.236	x	x	0.657	P
	$SD_h$	0.178	-0.071	0.392	x	x	-0.163	$\rho$
		0.713	0.906	0.396	x	x	0.657	P
	$SD_{disp}$	0.428	x	x	0.178	0.250	x	$\rho$
		0.353	x	x	0.713	0.594	x	P

## DISCUSSION

The present study highlights the inter- and intra-specific variability in dietary resources consumed by two closely related species of *Podarcis* lizards. The lack of an interaction between the factors of the general three-way MANOVA (species, sex, and island) suggests that variation in diet differs by island irrespective of the sex and species considered. Similarly, differences between sexes are independent of variation between species and islands, and finally variation between species is not dependent on the island nor the sex of the individual.

### ***Among island variability in dietary diversity***

Insularity profoundly affects the dynamics of a population. Intra-specific competition, enhanced in insular habitats compared to similar ecosystems on mainland, is known to promote dietary diversification (Svanbäck & Bolnick, 2007). We hypothesized that intraspecific competition would increase in parallel to population density when island size decreases (Donihue et al. 2016). We then predicted that taxonomic diversity in diet would be inversely proportional to island size. Interestingly, our results do not show any relationship between island size and dietary diversity. Two hypotheses can be formulated to explain this result. First, the diversity of prey available can be so high that its variation, impacted by island area, does not affect diversity in diet. Second, below a certain diversity threshold of available prey, lizards do not change their dietary width but a shift in the diet resources exploited does occur, resulting in an apparent stasis in the diversity in diet. These hypotheses are supported by the fact that most lacertid lizards are known to be mostly food generalists (Arnold, 1987, Díaz, 1995) whose diet globally corresponds to the available resources (Pollo & Perez-Mellado, 1988; Mou & Barbault, 1986). It has been previously suggested that food generalists would rather feed on a constant prey diversity while specialists would take a greater variety of prey when available resource diversity drops (Olsen et al. 2008), which is congruent with our results. Even if food availability, and consequently diet, fluctuate over the year, diet diversity in lacertid lizards tends to remain constant (Pérez-Mellado et al. 1991; Diaz & Carrascal, 1993). In this case it seems that the opportunistic feeding habits of lizards are a possible explanation for the lack of relationships between taxonomic prey diversity in the diet and island size. However, an investigation on food availability on these islands is required to test for food electivity in these populations and could shed further light on the observed patterns.

Insular populations of *P. sicula* might also shift to a more omnivorous diet, as is seen on the islands Sušac, Pijavica and Pod Mrčaru, where populations include an important proportion of plant items into their diets. Eating plants requires more foraging time, thus exposing lizards to potential predators (Szarski, 1962; Adamopoulou & Legakis, 2002; Hawlena & Pérez-Mellado, 2009). On islands this shift is often thought to be facilitated by a reduction in predation pressure and is enhanced on the smallest islands (Schoener, Slade & Stinson, 1982). Plant consumption is highly variable among islands, ranging from strictly insectivorous populations (e.g. Brusnik or Mala Palagruža) to almost completely herbivorous populations (e.g. Sušac or Pod Mrčaru). The large amount of plant material found in stomachs of *P. sicula* lizards (see also Herrel et al. 2008; Vervust et al. 2010) seems to be enabled by their generalist habits.

### ***Dimorphism and within-population resource partitioning***

The ability to deal with different prey items is often accompanied with changes in head dimensions (Arnold 1987) providing a mechanical advantage (e.g. bite force, gape) in the processing of items of different size and hardness. Territorial males typically bite harder than females thus enabling them to obtain access to a broader range of food resources (Herrel, Verstappen & De Vree, 1999; Herrel et al. 2001). The results of the present study confirm our prediction that disparity in diet, which represents the total diversity of items ingested at the population level, is higher in males than in females (although this dietary dimorphism does not hold when considering taxonomic diversity). Nevertheless, an amplification of sexual dimorphism concomitant with a decrease in island size was not confirmed, neither in diet data nor in head dimensions. The feeding apparatus in lizards is influenced by more than just feeding constraints. Sexual selection driving male-based sexual dimorphism in head size, and consequently bite force, is also considered important in male-male interactions (Lailvaux et al. 2004; Huyghe et al. 2005). In *Podarcis* lizards particularly, the intensity of sexual dimorphism in head size is often, yet not always, correlated with the intensity of dimorphism in prey size ingested (see Vincent & Herrel, 2007 for a review), suggesting that natural selection may not always be the main factor responsible for the emergence of the head dimorphism. Furthermore, despite a clear difference in head dimensions and diet composition between males and females, the lack of any relationship between sexual dimorphism in head dimensions and dimorphism in diet suggests a central role of sexual selection in the emergence of head size dimorphism in the two species examined here. In summary, our results show that 1) a male-based sexual dimorphism in head dimensions and diet (disparity, prey dimensions, and prey proportions) exists, 2) that the intensity of dimorphism in head dimensions does not correlate with

that in diet, suggesting that it is primarily driven by sexual selection, and 3) that the intensity of dimorphism is not amplified on the smallest islands, suggesting that if increased intraspecific competition is occurring on small islets, this likely results in both sexes occupying different microhabitats.

### ***Inter-specific variation in diet***

Disparity in the diet of *P. melisellensis* is higher than that in *P. sicula*. This result is counter-intuitive since it is generally assumed that *P. sicula* is an invasive and more generalist species likely consuming a wide array of prey resources (Zuffi & Giannelli, 2013). Moreover, although diet disparity is positively correlated with the island area in *P. melisellensis*, disparity increases with decreasing area in *P. sicula*. This underlines the ability of the latter to forage on a wider variety of food items when food availability is reduced. Bolnick and collaborators (2010) described several patterns of ecological release following island invasion. The total niche width of the population (TNW) has two additive components that contribute to its variation: the within-individual and the between-individual variance (respectively WIC and BIC). An increase in the total niche width can be due to an increase in within-individual variance (parallel release) or to an increase in between-individual variance (niche variation release) (Van Valen, 1965; Grant et al. 1976), or both. Despite the fact that our data does not allow us to address these issues precisely we suggest that dietary disparity, being an estimate of the total dietary variance of the population, can be likened to the total niche width. Moreover, Shannon's diversity index may be a relevant substitute for variance (Bolnick et al. 2002) and is calculated at the individual level. An index of diversity that remains constant with island size variation would suggest that the within-individual component remains constant, while the total population niche width and subsequently the between-individual component, represented by the disparity, would follow the pattern previously described. Following this scheme, it is probable that the populations of *P. sicula* increase their niche width when islands are smaller, and that populations of *P. melisellensis* do the opposite. In both species, this variation in population niche width appears to be driven by the between-individual component, suggesting the occurrence of individual specialization towards different types of food items (Bolnick et al. 2010). As this only holds for females of both species, it also implies that males and their diet are under the strong influence of sexual selection.

### ***Limits and robustness of the present study***

Studying diet presents many difficulties (Carretero, 2004). Diet in lizards is, for example, highly variable between seasons (Gadsen & Palacios-Orona, 1997; Mamou et al. 2016). To prevent seasonal biases, we sampled the stomach contents of all our populations within a 45 day-long period (19 days when excluding Korčula which was sampled slightly later). This enables a reliable comparison of the diet between sites, yet does not account for possible island-specific seasonal variation in diet. Intraspecific competition and resource availability are also highly season-dependent. We sampled at the end of the summer when food availability is lowest in these Mediterranean habitats (Karamaouna, 1987), thus increasing the likelihood of detecting differences between islands if specialization occurs. Moreover, nectarivory has been shown to be quite common across lacertid species, especially among *Podarcis* (Pérez-Mellado & Traveset, 1999). Although nectar can provide alternative resource opportunities, it cannot be detected in the stomachs and possibly impacts dietary patterns. Isotope analyses of the stomach contents could help determining in what amount the lizards include nectar into their diet. Finally, even if the regressions associated with the patterns described here are statistically significant, they explain only a small part of the overall variance. Yet, it is important to note that despite the huge variability and contingency of diet sampling (individuals, location of sampling, time of the day, sunshine, temperature, state of digestion, etc.) we were able to detect biologically meaningful trends. Diet therefore appears to be a central ecological variable impacted by habitat structure and biotic interactions with conspecifics in these two species of *Podarcis* lizards.

### ***Conclusion***

The present study illustrates the diversity in diet across insular populations of two *Podarcis* species within the same geographic area. Significant differences in dietary composition were found between sexes, among populations, and between species. The lack of a relationship between taxonomic diversity in diet and island area may be explained by the opportunistic habits described for these species. In the case of low food abundance conditions, animals might shift their diet towards more omnivorous diet, as is observed in *P. sicula* that includes a significant amount of plant matter in its diet on some islands. The investigation of disparity suggests that both species have different strategies when facing food scarcity on the smallest islands. Indeed, in contrast to females of *P. melisellensis*, females of *P. sicula* seem to be able to widen their food niche to counteract the low prey abundance. Although diet disparity was found to be greater in males than in females, neither this dimorphism nor

the dimorphism in diet or head dimensions was amplified on the smaller islands. These two last results suggest that dietary specialization in males is also driven by other important factors such as sexual selection. In this sense, our observations on the dimorphism of head dimensions and diet suggest that the differences between sexes might be explained by the occupation of different microhabitats rather than different food niches.



# **Chapter 2**

## **Performance**

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## Proximate and ultimate drivers of variation in bite force in the insular lizards

### *Podarcis melisellensis* and *Podarcis sicula*

- 2020. *Biological Journal of the Linnean Society* **131**: 88-108 -

#### ABSTRACT

Bite force is a key performance trait in lizards since biting is involved in many ecologically relevant tasks, including foraging, fighting, and mating. Several factors have been previously suggested to impact bite force in lizards, such as head morphology (proximate factors), or diet, intraspecific competition, and habitat characteristics (ultimate factors). However, these have been generally investigated separately and mostly at the interspecific level. We tested which factors drive variation in bite force at the population level and to what extent. Our study includes 20 populations of two closely-related lacertid species, *Podarcis melisellensis* and *Podarcis sicula*, which inhabit islands in the Adriatic. We found that lizards with more forceful bites have relatively wider and taller heads, and consume more hard prey and plant material. Island isolation correlates with bite force, likely by driving the resource availability. Bite force is only poorly explained by proxies of intraspecific competition. The linear distance from a large island and the proportion of difficult-to-reduce food items consumed are the ultimate factors that explain most of the variation in bite force. Our findings suggest that the way in which morphological variation affects bite force is species-specific, likely reflecting the different selective pressures operating on the two species.

**Keywords:** bite force, head dimensions, intraspecific competition, island area, island isolation, lizard, *Podarcis melisellensis*, *Podarcis sicula*, resource use.

## INTRODUCTION

Whole-organism performance has been defined as a measure of the ability of an animal to execute an ecologically relevant task (Huey & Stevenson, 1979; Arnold, 1983; Lailvaux & Irschick, 2006). Because whole-organism performance traits may increase survival or fitness (Wainright, 1991; Le Galliard et al. 2004; Miles, 2004; Lailvaux & Irschick, 2006; Calsbeek & Meyers, 2007), it is generally considered that natural selection acts upon performance rather than on the underlying morphological or physiological traits (Irschick et al. 2008). This view is supported by the concept of many-to-one mapping of form to function, which suggests that morphology and performance can sometimes be decoupled, with many morphologies giving rise to the same functional output (Wainwright et al. 2005). Studying the performance of an animal in an ecological context thus contributes to a better understanding of evolutionary processes and helps to explain how variation in morphology arises in different ecological contexts (Arnold, 1983; Kingsolver & Schemske, 1991; Brodie et al. 1995; Kingsolver & Pfennig, 2007).

Numerous studies have considered bite force to be a key performance trait since biting is involved in many essential tasks in vertebrates including feeding, mating (Leboeuf, 1972; Herrel et al. 1996), inter- (Snekser et al. 2008), intraspecific competition (Blanchard et al. 2001), food manipulation (Jones & Kamil, 1973; Reghem et al. 2011), communication (Derryberry et al. 2012), parental care (Balshine-Earn & Earn, 1998), predator defense, and even locomotion (Williams & Bunkley-Williams, 2006). Variation in bite force has been extensively studied as the acquisition of bite force data is relatively straightforward in small vertebrates and the measures highly repeatable (Herrel et al. 2005; Anderson et al. 2008; Lappin & Jones, 2014). Biting is essential in resource acquisition and food processing and it has been previously shown that a high bite force is advantageous, rendering digestion more efficient by increasing the surface area available to digestive juices or microorganisms (e.g. in turtles: Bjorndal et al. 1990; Bjorndal & Bolten, 1992). A direct association between the ability to deal with tougher food items and an increase in bite force has also been suggested for lizards (Verwajen et al. 2002; Herrel et al. 2004a,b; Herrel & Holanova, 2008; Jones & Lappin, 2009), turtles (Herrel et al. 2002), and mammals (Aguirre et al. 2003; Fabre et al. 2017). Moreover, studies have established that the inclusion of plant material in the diet is often associated with higher bite forces, especially in lizards (Herrel, Aerts & De Vree, 1998; Herrel et al. 2004a; Herrel, 2007; Herrel et al. 2008; Sagonas et al. 2014).

In lizards, biting is also important in the context of several other fitness-relevant tasks. First, male lizards often display territorial behaviors when defending resources and sexual partners (Irschick, et al. 2007), which may lead to male-male combat (Stamps, 1977). Bite force is a good predictor of the outcome of these interactions with males that bite more forcefully typically winning fights (Lailvaux et al. 2004; Huyghe et al. 2005; Husak et al. 2006; Lailvaux & Irschick, 2007; Husak et al. 2009). Second, biting may allow males to immobilize females during mating by firmly gripping their necks or abdomens (Noble & Bradley, 1933; Herrel et al. 1996). Higher bite forces may thus increase the reproductive success of males (Lappin & Husak, 2005; Herrel et al. 2010). However, in some species females avoid males with the greatest bite forces, possibly to avoid injury (Huyghe et al. 2012).

The drivers of variation in bite force in lizards are relatively well understood, at least at the proximate level. Indeed, numerous studies have established the morphological and anatomical basis for the variation in bite force. Lizards with taller and wider heads typically bite harder (Herrel et al. 2001a; Verwaijen et al. 2002; Lappin et al. 2006; Huyghe et al. 2009; Herrel et al. 2010; Wittorski et al. 2015). The morphological variation associated with variation in bite force has been often linked to an increase in space available for jaw muscles (Herrel et al. 2007), the size and architecture of the jaw muscles (Huyghe et al. 2008), or the optimization of lever arms (Herrel et al. 2001a). Whereas these previous studies have contributed to unraveling the drivers of variation in bite force, they typically focus on interspecific studies (Herrel et al. 2001b; Vanhooydonck et al. 2010; Kaliontzopoulou et al. 2012), ontogenetic variation (Herrel et al. 2006), or variation between males and females within a species (Brecko et al. 2008; Sagonas et al. 2014; Lopez-Darias et al. 2015). However, analyses dealing with variation across populations of the same species are relatively rare (but see Bonneaud et al. 2016; Donihue et al. 2016). Yet, population-level studies are especially insightful in helping understand the drivers of variation in performance given that they are not confounded by phylogenetic effects.

The present study aims to explore and quantify the drivers of variation in bite force across 18 insular and two continental populations of two *Podarcis* species (eight populations of *Podarcis sicula* – Rafinesque Schmaltz, 1810; and 12 of *Podarcis melisellensis* – Braun, 1877). A recent study (Taverne et al. 2019) has demonstrated that these populations differ in diet, ranging from an almost strictly insectivorous to a largely omnivorous diet. Moreover, the islands that these lizards inhabit are located in the southern Adriatic, off the coast of Croatia, and differ in size, habitat structure and substrate. Indeed, the islands range from very small rocky islets to bigger islands with greater and more complex vegetation cover and topography. The island structure may affect the type and the intensity of social

interactions and intraspecific competition within each population (Svanbäck & Bolnick, 2007; Thomas et al. 2009; Donihue et al. 2016). The formation of these land bridge islands (Marjanac & Marjanac, 2004; Sikora et al. 2014) as well as the time of isolation and genetic divergence have been partially resolved in the literature (Gorman et al. 1975; Podnar et al. 2004; Podnar et al. 2005) allowing us to explore the historical factors that drive variation in morphology and performance in lizards across these islands.

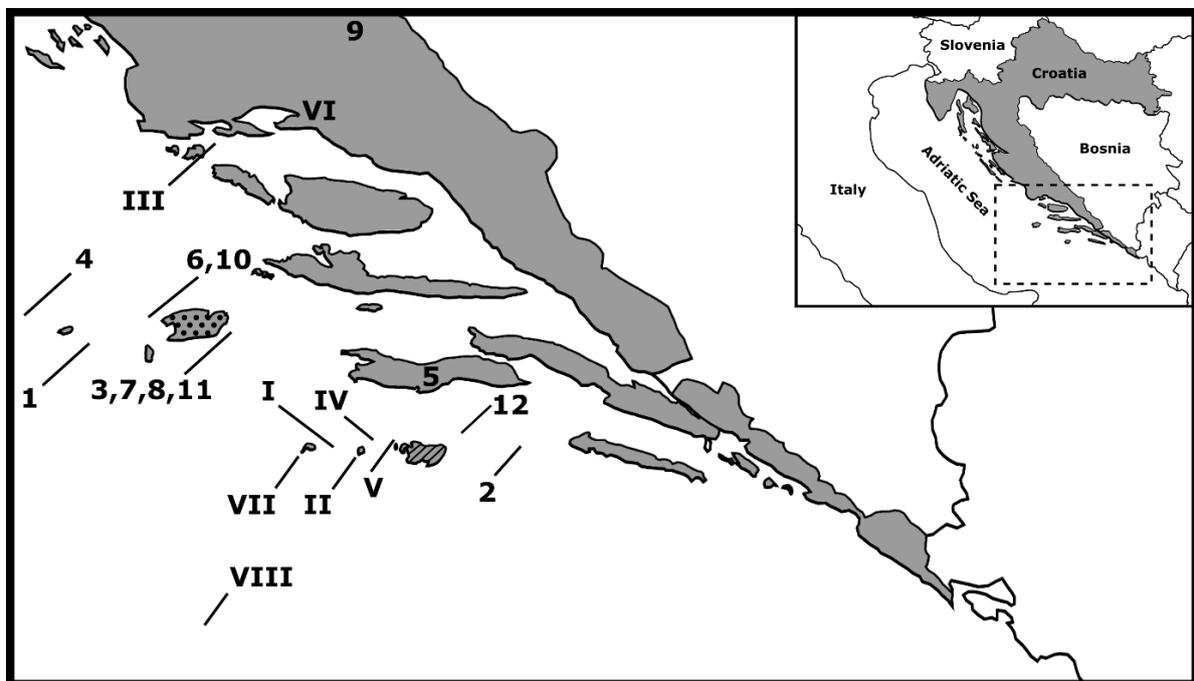
First, we explored the impact of proximate factors such as head shape and size on variation in bite force. Second, we explore the relationships between the functional properties of the food items ingested by the lizards (data from Taverne et al. 2019), food niche breadth and bite force. Intraspecific competition between males is common. Because of this, males often display higher performance levels associated with a sexual dimorphism in head size and shape (Herrel et al. 1996; Verwaijen et al. 2002). Here, we calculated the degree of sexual dimorphism in head dimensions and use it as a proxy for the intensity of sexual selection (Vanhooydonck et al. 2009; Vanhooydonck et al. 2010) and tested its role as a driver of variation in bite force. Additionally, we tested whether two proxies for intraspecific competition (i.e. the number of regenerated tails and the number of missing toes; see Vervust et al. 2009) impacted bite force. As insular populations typically experience an increase in the level of intraspecific competition compared to their mainland counterparts (Pafilis et al. 2009) this may be an important driver of variation in bite force (Donihue et al. 2016). Finally, island size and isolation have been shown to significantly impact the diversity and abundance of prey and vegetation (Itescu et al. 2019), thus impacting the resources available and population densities (Polis & Hurd, 1996). As such, island size and isolation may also be drivers of variation in bite force. By comparing two closely related species living on geographically close islands of similar size and structure, we further aim to explore whether the drivers of variation in bite force are similar in different species under similar contexts, or whether species-specific patterns are observed.

We predict 1) that snout-vent length as well as head size and shape should be good predictors of bite force, 2) that the degree of sexual dimorphism should be correlated with increased bite force in males, 3) that higher bite force should be correlated to food niche breadth, 4) that island size and remoteness should be respectively negatively and positively correlated with bite force, 5) that variation in bite force between populations should not be related to the phylogenetic relationships between populations.

## MATERIAL & METHODS

### *Specimens*

Twenty populations of *Podarcis* lizards were sampled: eight populations of the Italian wall lizard *Podarcis sicula* and twelve populations of the Dalmatian wall lizard *Podarcis melisellensis*, including one mainland site for each (Figure 1). These populations are located in the Adriatic, off the coast of Croatia. In total, 535 adult animals (between 15 and 42 individuals per population) were captured by noose or by hand during the late summer of 2016 (Table 1), with permission from the Croatian Ministry of the Environment (permit n° 517-07-1-1-1-16-6). We measured all individuals using digital calipers (Mitutoyo absolute digimatic;  $\pm 0.01\text{mm}$ ; see Figure S1) and recorded snout-vent length (SVL) and linear head dimensions including head length (HL), head width (HW), head height (HH), lower jaw length (LJL), quadrate to jaw tip length (QT), and coronoid to jaw tip length (CT).



**Figure 5:** Location of the populations of *Podarcis sicula* (I: Bijelac, II: Kopište, III: Pijavica, IV: Pod Kopište, V: Pod Mrčaru, VI: Split, VII: Sušac, VIII: Mala Palagruža) and *Podarcis melisellensis* (1: Brusnik, 2: Glavat, 3: Greben, 4: Jabuka, 5: Korčula, 6: Mali Barjak, 7: Mali Paržanj, 8: Ravnik, 9: Sinj, 10: Veli Barjak, 11: Veli Budikovac, 12: Veli Tajan) included in the present study. Lines point to the locations of the islands that are too small to be represented on the map. The large islands Lastovo and Vis are respectively hatched and dotted.

Two other functionally relevant variables were calculated: the in-lever for jaw opening (open = LJJ – QT) and the in-lever for jaw closing (close= QT – CT).

### ***In-vivo bite force measurements***

*In-vivo* bite force was measured using an isometric Kistler force transducer (type 9203) connected to a Kistler charge amplifier (type 5995, Kistler Inc., Winterthur, Switzerland; see Herrel et al. 1999a for a detailed description of the set-up). Lizards were made to bite the plates of the transducer, left bare, at least five times, and the greatest bite force across the five trials was retained as an estimate of an individual's maximum bite force. Gape angle was standardized across trials by adjusting the distance between the bite plates for each individual to maintain gape angle constant at around 30°. Bite position was standardized by assuring that the tips of the jaws of each lizard were up against the metal stop mounted on the device. Bite force was log<sub>10</sub>-transformed before statistical analyses.

Table 3: origin of specimens and island characteristics (# mainland populations)

Species	Site	Symbol	Date	Females	Males	Area (m <sup>2</sup> )	Distance from big island (m)	Distance from mainland (m)
<i>P. melisellensis</i>	Brusnik	BR	08.27.2016	22	20	13791	20949	55695
	Glavat	GL	09.09.2016	9	12	3379	16491	23071
	Grebeni	GR	08.31.2016	12	18	9187	1004	48768
	Jabuka	J	08.28.2016	21	17	22585	48002	62875
	Korčula	KR	10.08.2016	7	11	2.79 × 10 <sup>8</sup>		1367
	Mali Barjak	BM	08.26.2016	14	18	3632	725	46643
	Mali Paržanj	PZ	09.01.2016	13	12	2671	838	49969
	Ravnik	RV	09.03.2016	6	11	55140	489	51456
	Sinj *	SI	09.12.2016	5	11	-	-	-
	Veli Barjak	BU	08.30.2016	19	21	1246	255	46679
	Veli Budikovac	BD	09.02.2016	17	17	63852	748	50844
	Veli Tajan	T	09.08.2016	7	8	2702	6862	19649
<i>P. sicula</i>	Bijelac	BI	09.08.2016	7	13	734	10233	38550
	Kopište	KO	09.06.2016	14	19	155049	6544	36747
	Mala Palagruža	PG	09.10.2016	8	12	5036	60280	91549
	Pijavica	PI	09.14.2016	13	12	2059	1799	1799
	Pod Kopište	K	09.06.2016	15	22	7915	7340	36152
	Pod Mrčaru	M	09.05.2016	12	24	2931	2630	31933
	Split *	ST	09.12.2016	9	7	-	-	-
	Sušac	SU	09.04.2016	15	5	853927	23013	48958

### ***Island characteristics***

The values of the island area were taken from Duplančić et al. (2004). The shortest linear distance from the continent and the shortest linear distance from the nearest large island (here, either Lastovo or Vis) were calculated using Google Maps and ImageJ (Figure 1, Table 1).

### ***Stomach content analysis***

All individuals were stomach flushed right after capture using a syringe with ball-tipped steel needle (Herrel et al. 2006; Measey et al. 2011) and stomach contents were analyzed as described in Taverne et al. (2019). The diet consisted mostly of arthropods, arachnids, myriapods, gastropods and plant material (leaves, seeds, fruits and flowers) in various proportions. Diet across populations ranged from strictly insectivorous (e.g. Mali Barjak) to omnivorous, as some populations included a significant part of plant matter in their diet (e.g. Sušac, Pod Mrčaru). The maximum and the minimum length, the maximum and minimum width, and the maximum volume of the food items in each stomach was recorded. The volume of a prey item was calculated following a spheroid equation (ellipsoid volume =  $(4/3) \pi (L/2) (W/2)^2$ , with L the length of the item and W its width). Every prey taxon was then attributed to functional groups based on prey hardness and evasiveness as proposed by Vanhooydonck et al. (2007). The relative contribution of each of the six functional groups (soft prey, prey of medium hardness, hard prey, sedentary prey, prey of intermediate evasiveness, and evasive prey) and of plant material to the total mass of the stomach contents was then calculated. The hardness of each food item was further estimated using the following regression equations proposed by Herrel & O'Reilly (2006) that relate prey length and hardness, according to the item hardness category:

$$\text{(soft)} \quad \log_{10}[\text{prey hardness } (N)] = 0.997 \times \log_{10}[\text{prey length } (mm)] - 1.379$$

$$\text{(medium)} \quad \log_{10}[\text{prey hardness } (N)] = 1.780 \times \log_{10}[\text{prey length } (mm)] - 1.942$$

$$\text{(hard)} \quad \log_{10}[\text{prey hardness } (N)] = 1.582 \times \log_{10}[\text{prey length } (mm)] - 1.365$$

The maximal and mean hardness of the prey in each stomach were retained for further analyses.

A diet disparity metric was calculated for each sex of each population (see Taverne et al. 2019). To do so the hyper-volume occupied by each one of these subsets in the ecospace was estimated using the

“disparity.per.group” function from the “dispRity” package (Guillerme, 2018). Finally, an estimate of the taxonomic diversity of prey found in the stomachs, the Shannon diversity index, was calculated for each individual following the equation:

$$H' = - \sum_{i=1}^S \frac{n_i}{N} \log\left(\frac{n_i}{N}\right)$$

where S is the total number of prey taxa found in a stomach,  $n_i$  the total number of prey items of the taxon i and N the total number of items found in a stomach.

### **Phylogenetic analysis**

For the purpose of phylogenetic analysis, mitochondrial cytochrome-*b* sequences were obtained from GenBank (accession numbers in Table S1) for 14 out of the 20 populations used in this study (Table 1). DNA samples for three additional *P. sicula* populations (Bijelac, Kapište and Pod Mrčaru) and three *P. melisellensis* populations (Sinj, Veli Barjak and Veli Tajan) were collected during sampling in the summer of 2016. A piece of lizard tail tissue was taken from two individuals per population and preserved in 96% ethanol. DNA was extracted from approximately 15 mg of lizard tail tissue using commercial DNA extraction kit and the provided protocol (Sigma-Aldrich GenElute Mammalian Genomic DNA Miniprep Kit). Quality and quantity of the extracted DNA were examined by agarose gel electrophoresis and spectrophotometric measurement on nanodrop (Thermo Scientific 2000c).

Optimized protocols from Podnar et al. (2004, 2005) were used to obtain mitochondrial cytochrome-*b* sequences. For both *P. sicula* and *P. melisellensis* populations, 40-50 ng of extracted DNA was amplified in a 25 µL reaction containing 5X Iproof buffer, nH<sub>2</sub>O, 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.4 uM of each primer (Table S2) and 0.5 U iProof Polymerase (Bio-Rad). PCR conditions included initial denaturation for 2 min at 98 °C, followed by 35 cycles of 10 s at 98 °C, 20 s at 50 °C, 90 s at 72 °C, and a final extension of 7 min at 72 °C.

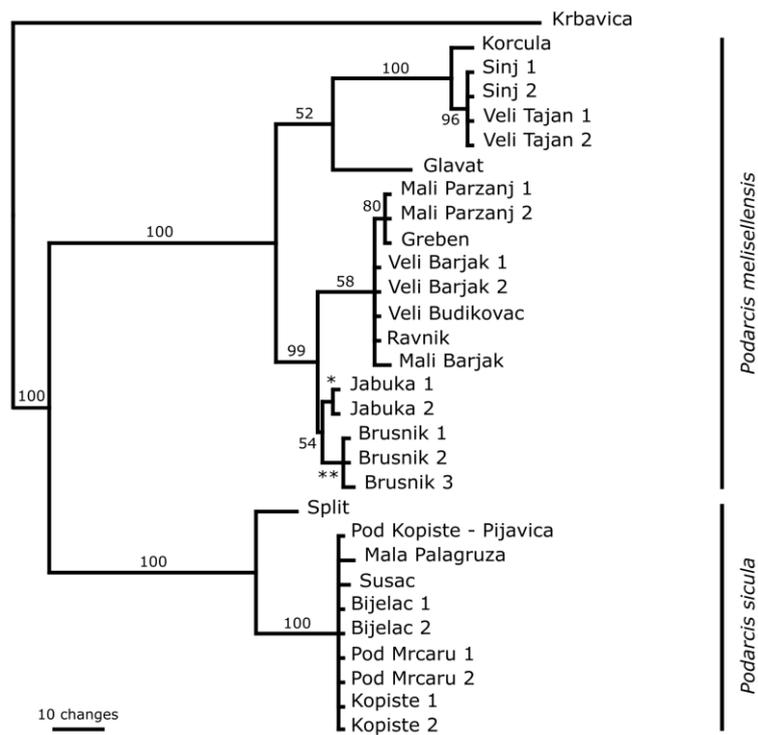
Samples from Bijelac, Kapište and Pod Mrčaru were further reamplified to account for the presence of cytochrome-*b* nuclear pseudogene sequences (numts) in the *P. sicula* populations (Podnar et al. 2005). Reamplification was performed using 1 µL of the amplification mix and included the same conditions as the amplification, apart from the used primers (Table S2), reaction volume which was set to 50 µL

and the PCR annealing temperature that was adjusted to 55 °C. All PCR reactions were performed in Bio-Rad Gradient Thermal Cycler. Macrogen sequencing company (Amsterdam, Netherlands) provided services of PCR product purification and bidirectional sequencing using primers listed in Table S2.

The chromatograms from the 12 processed samples were loaded in Geneious 4.8.5 (Biomatters Ltd.), corrected manually, aligned and trimmed to the same length together with the sequences from the other previously published populations (Table S1). The optimal nucleotide substitution model was determined with jModelTest 2.1.10 (Guindon & Gascuel, 2003; Darriba et al. 2012). Likelihood scores were computed using the "best" base tree topology search method (from both "Nearest Neighbour Interchange" and "Subtree Pruning and Regrafting"). MEGA-X 10.0.5 (Kumar et al. 2018) was used to calculate nucleotide composition, nucleotide pair frequencies, and transition and transversion rates (R ratio) according to the chosen nucleotide substitution model. A cytochrome-*b* sequence of *Lacerta bilineata* from Krbavica (Croatia) was added as outgroup before the analysis (Table S1).

Phylogenetic trees were generated using three methods: maximum parsimony (MP) and maximum likelihood (ML) both implemented in PAUP 4.0a (Swofford & Sullivan, 2003), and a Bayesian inference analysis implemented in MrBayes 3.2.7a (Ronquist et al. 2012). All analyses were performed after setting the calculated nucleotide substitution model and R ratio. Maximum parsimony and ML analysis were performed with full heuristic search and allowing tree-bisection-reconnection (TBR) branch-swapping. Maximum parsimony starting trees were obtained via stepwise addition with 10 replicates of each random addition sequence, bootstrap replicates were set to 1000 for MP trees. Maximum likelihood starting trees were obtained by neighbour-joining (NJ), bootstrap replicates were set to 100. Because the computational power and time required for ML is greater, number of rearrangements was limited to  $8 \times 10^6$ , and time per replicate was limited to 2000 minutes. For the Bayesian inferred trees  $6 \times 10^6$  generations were run, sampled every 100 (60000 trees) and the first 6000 trees were discarded (burn).

The maximum parsimony tree was preferred when correcting for the non-independence of the data in subsequent statistical analyses because this method maximizes the resolution of the relationships between *P. sicula* populations (Figure 2). The trees which were generated by the other two methods are provided in Table S2. The influence of phylogenetic heritage on the variation in bite force and every head dimension was investigated through the calculation of the Pagel's lambda (Pagel, 1999), using the "phylosig" function from "phytools" package. This measure of phylogenetic signal was calculated on both raw and residual head dimensions.



**Figure 6:** Phylogenetic relationships among the populations sampled in our study with one outgroup, generated using maximum parsimony (MP). Branch lengths are proportional to the number of changes in the DNA sequences. Bootstrap values for topology support are indicated and rounded (\* = 96; \*\* = 79. For precise values, see Figure S2a).

### Proxies for intraspecific competition

For both species separately, a principal component analysis (PCA) was run on head dimensions using the function “prcomp” from the “stats” package, allowing a reduction of dimensionality. The contribution of each specimen along the three first principal components (PC) was extracted and used to calculate the mean contribution of each sex of each population on these axes. The sexual dimorphism in head dimensions (SD) for each site was determined as follows:

$$SD = \sqrt{(m_1 - f_1)^2 + (m_2 - f_2)^2 + (m_3 - f_3)^2}$$

where  $m_i$  and  $f_i$  refer respectively to the mean contribution of the males and the females of the population of interest along with the PC<sub>*i*</sub>.

Two other proxies for intraspecific competition were estimated: the proportion of individuals which missed the longest toe on one of the hind feet (Vervust et al. 2009) and the proportion of individuals with a regenerated tail (Brock et al. 2014).

### **Statistical analyses**

All analyses were performed in R (v3.5.1, R Core Team 2018). To assure normality, the proportion data, which values are comprised between 0 and 1, were arcsin-transformed. For the same reason prey dimensions, head dimensions, hardness, disparity, distance variables and island area were  $\log_{10}$ -transformed. A Shapiro test and a Bartlett's tests ("shapiro.test" and "bartlett.test" functions from "stats" package) were used to test the normality and the homogeneity of residuals in each sub-dataset.

A two-way univariate analysis of covariance (ANCOVA) with SVL as co-variable was carried out on the data for both species separately to test for a possible effect and interaction of sex and island on bite force. Similarly, a two-way multiple analysis of covariance (MANCOVA) with SVL as co-variable was performed to detect a possible effect of sex and island on head dimensions. Phylogenetic univariate analyses of variance, using Holm-Bonferroni's correction for multiple comparisons, were carried out to test for an effect of sex and species on residual bite force and head dimensions (obtained subsequently to phylogenetic linear regressions using the function "phylolm" from the "phylolm" package), by using the function "phylANOVA" from the package "phytools".

Next, we explored associations between bite force and variables such as disparity, prey hardness or proxies for intraspecific competition through linear regressions (function "lm" in the package "stats"). Three different stepwise regressions (function "stepAIC" in the package "MASS") computed on prey dimensions, mass proportions or head dimensions enabled us to detect possible associations between bite force and these data. The best model was selected based on the minimal AIC value, and variables in the model were selected using both forward and backward procedures. A phylogenetic multiple regression was carried out to estimate the relationship between bite force and head dimensions when considering phylogeny (function "phylstep" in the package "phylolm"). Since a Shapiro test revealed diversity to be non-normally distributed, a Spearman-rank correlation test was used to detect possible relationship between bite force and prey diversity. For this analysis the Shannon index of diversity was averaged by population to avoid *ex-æquo* rankings in the Spearman test. Mean values of absolute and residual (against head length) bite force were also tested against island size, distance from the mainland, and distance from the nearest large island using linear regressions. Other stepwise regressions were performed to test for a relationship between isolation metrics and food items proportions and dimensions. Additionally, a simple regression between isolation metrics and prey mean hardness was carried out. Finally, the ecological variables that were demonstrated to drive variation in bite force in these statistical analyses were combined in a final stepwise regression to

compare the contribution of each relevant parameter to bite force. Standardized estimates of the stepwise regressions were given with the function “summ” from “jtools” to compare the relative contribution of each variable to the final model. Each one of these statistical analyses include all populations, except when excluding mainland populations was necessary (testing island area effect for example).

## RESULTS

When considering the whole dataset, bite force appears to be influenced by phylogeny (Table 2). When considering sexes separately, phylogenetic relationships affected bite force, raw, and residual head dimensions in females only. At the intraspecific level, phylogeny influenced raw head dimensions (but not residuals) in females of *P. melisellensis* only. No signal was detected in raw or in residual data in males of *P. melisellensis*. The phylogenetic signal was not calculated for *P. sicula* separately due to the very weak differentiation among populations.

The two-way univariate analysis of covariance reveals that in *P. sicula*, bite force was influenced by SVL ( $P < 0.001$ ), sex ( $P < 0.001$ ), and island identity ( $P < 0.001$ ) but there was no interaction between sex and island ( $P = 0.249$ ). This was also the case in *P. melisellensis*, but the interaction between sex and island identity was just significant ( $P = 0.048$ ). The two-way multivariate analysis of covariance indicated that head dimensions also differed between sexes and islands in both species. An interaction between sex and island was observed for *P. melisellensis* only ( $P = 0.004$ ). The phylogenetic ANOVAs showed that bite force and head dimensions differed between sexes but not species (Table S3).

### ***Morphological traits underlying variation in bite force***

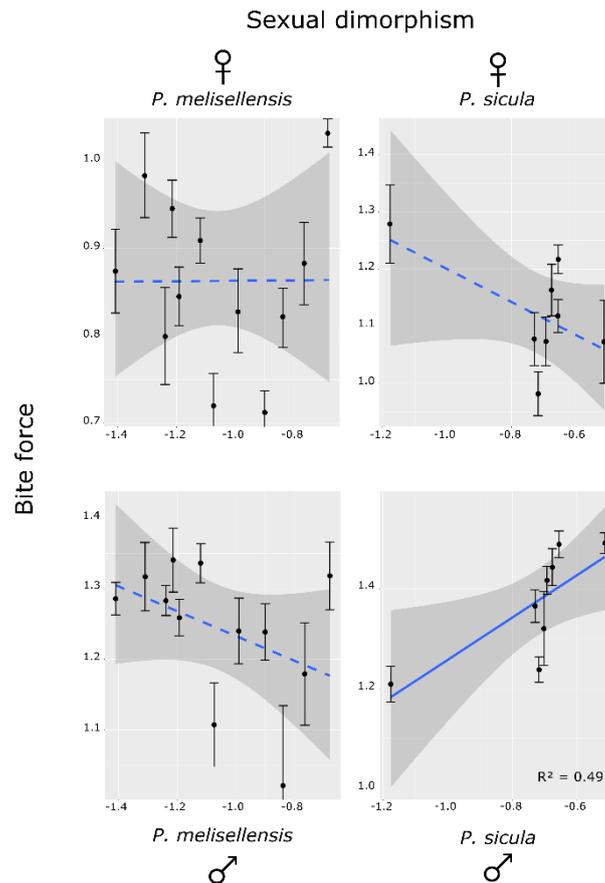
Bite force was highly correlated with body size (SVL) for each sex and species ( $P < 0.001$ ). The stepwise regressions between bite force and residual head dimensions further showed that head shape also predicted bite force in each species and each sex. In males of *P. sicula* ( $P = 0.002$ ,  $R^2 = 0.087$ ,  $F_{1,112} = 350.1$ ), head height and lower-jaw length were correlated with bite force ( $\beta$  coefficients: 1.13, -0.69). In females of *P. sicula* ( $P < 0.001$ ,  $R^2 = 0.187$ ,  $F_{2,90} = 11.59$ ), the in-lever for jaw-opening and the lower jaw length were correlated with bite force ( $\beta$  coefficients: -0.19, 1.26). In males of *P. melisellensis* ( $P < 0.001$ ,  $R^2 = 0.211$ ,  $F_{3,172} = 16.56$ ), head height, head width and lower-jaw length were related to bite force ( $\beta$  coefficients: 1.29, 1.27, -2.17). In females of *P. melisellensis* ( $P < 0.001$ ,  $R^2 = 0.280$ ,  $F_{3,148} = 20.57$ ), head height, head width and quadrate-to-tip length correlate with bite force ( $\beta$  coefficients: 1.84, 0.91, -1.98). The two latter results still hold when correcting for the phylogenetic relationships among populations of *P. melisellensis* (Table S4).

**Table 4:** summary of the Pagel's lambda ( $\lambda$ ). Asterisks indicate significant results ( $P < 0.05$ ). SVL: snout-vent length, HL: head length, HW: head width, HH: head height, LJJ: law-jaw length, QT: quadrate-to-tip length, CT: coronoid-to-tip length.

			SVL	Bite force	HL	HW	HH	LJJ	QT	CT	
Raw data	All dataset	$\lambda$	0.554	0.556	0.732	0.486	0.244	0.828	0.784	0.766	
		P	0.078	0.001*	0.004*	0.019*	0.189	0.003*	0.003*	0.004*	
	All females	$\lambda$	0.930	0.822	0.944	0.915	0.955	0.950	0.948	0.942	
		P	0.008*	0.001*	<0.001*	<0.001*	0.003*	<0.001*	<0.001*	<0.001*	
	All males	$\lambda$	0.275	0.244	0.567	0.173	<0.001	0.670	0.637	0.608	
		P	0.592	0.160	0.171	0.509	1	0.128	0.131	0.178	
	Females <i>P. melisellensis</i>	$\lambda$	0.991	<0.001	0.976	0.980	<0.001	0.970	0.972	0.959	
		P	0.016*	1	0.020*	0.081	1	0.018*	0.020*	0.026*	
	Males <i>P. melisellensis</i>	$\lambda$	0.122	<0.001	0.300	<0.001	<0.001	0.389	0.376	0.337	
		P	0.791	1	0.485	1	1	0.432	0.424	0.418	
	Residual data	All dataset	$\lambda$	-	-	0.310	0.671	0.896	0.203	0.299	0.258
			P	-	-	0.075	0.009*	0.049*	0.211	0.082	0.166
All females		$\lambda$	-	-	0.523	0.912	0.891	0.388	0.502	0.369	
		P	-	-	0.010*	<0.001*	0.005*	0.041*	0.017*	0.044*	
All males		$\lambda$	-	-	0.014	<0.001	<0.001	<0.001	0.025	<0.001	
		P	-	-	0.921	1	1	1	0.866	1	
Females <i>P. melisellensis</i>		$\lambda$	-	-	<0.001	0.831	0.634	<0.001	<0.001	<0.001	
		P	-	-	1	0.340	0.105	1	1	1	
Males <i>P. melisellensis</i>		$\lambda$	-	-	<0.001	<0.001	0.420	<0.001	<0.001	<0.001	
		P	-	-	1	1	0.317	1	1	1	

### Intraspecific competition

The three first axes of a PCA used to calculate sexual shape dimorphism jointly explained over 90% of the variance. In females, bite force did not correlate with the degree of sexual dimorphism in head dimensions, used here as a proxy for intraspecific competition and aggression (*P. melisellensis*:  $P = 0.121$ , *P. sicula*:  $P = 0.985$ ). In males, bite force increased with the degree of sexual dimorphism in *P. sicula* ( $P = 0.030$ ,  $R^2 = 0.498$ , slope = 0.423; Figure 3), but not in *P. melisellensis* ( $P = 0.185$ ). Except in females of *P. melisellensis* where a correlation between the proportion of individuals with missing toes was positively associated with bite force ( $P = 0.031$ ,  $R^2 = 0.394$ , slope = 0.96) the proportion of individuals with missing toes and the proportion of individuals with regenerated tails did not predict bite force (all  $P > 0.05$ ).



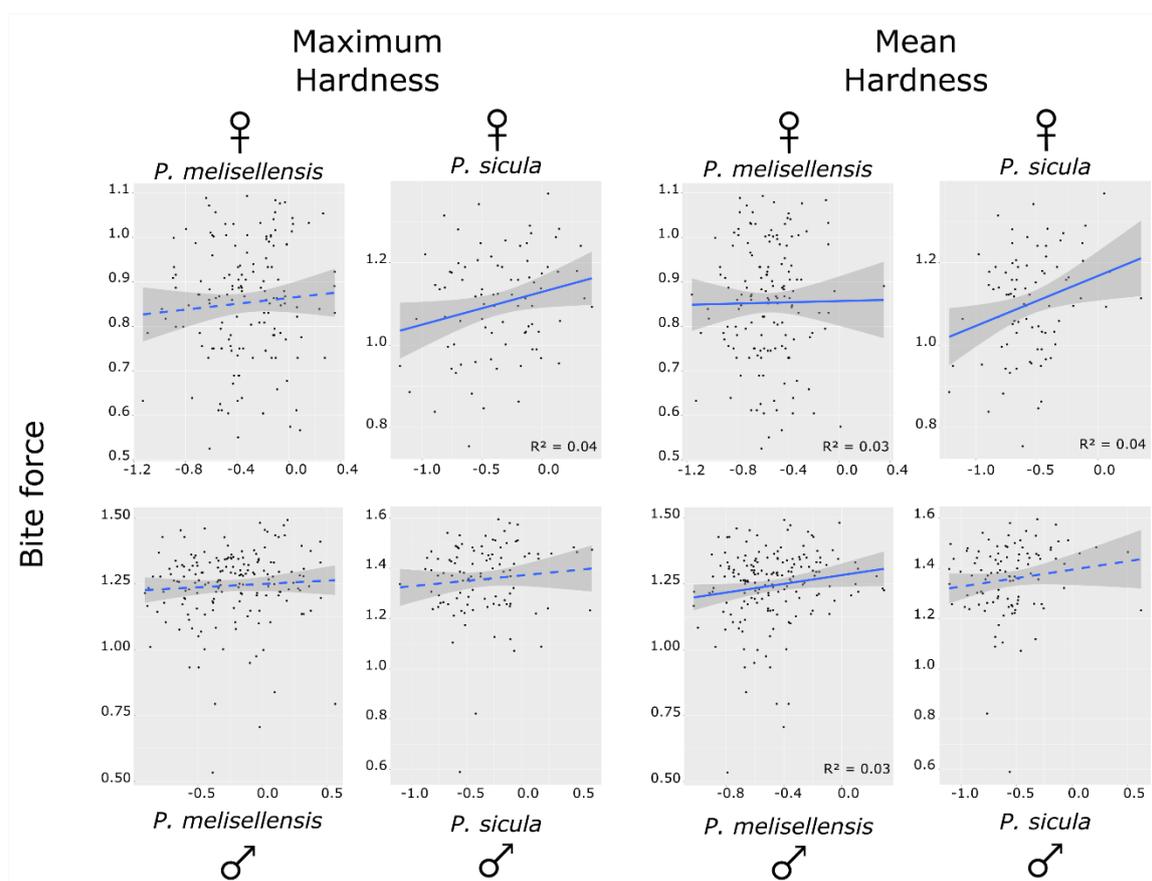
**Figure 7:** Linear regressions between bite force and sexual dimorphism in head dimensions at the population level. Full lines represent significant correlations ( $P < 0.05$ ). Dashed lines represent non-significant correlations. Determination regression coefficients are also indicated when appropriate.

### **Relationships between bite force and food properties**

The linear regression between bite force and maximum prey hardness was significant in females of *P. sicula* only ( $P = 0.039$ ,  $R^2 = 0.04$ , slope = 0.079) but explained little of the total variance (Figure 4, Table 3). Bite force was also positively associated with mean prey hardness in females of *P. sicula* ( $P = 0.014$ ,  $R^2 = 0.053$ , slope = 0.149), females of *P. melisellensis* ( $P = 0.034$ ,  $R^2 = 0.025$ , slope = 0.161), and males of *P. melisellensis* ( $P = 0.026$ ,  $R^2 = 0.024$ , slope = 0.180). A stepwise regression on food item dimensions revealed that an increase in bite force in females of *P. sicula* was associated with greater minimum prey width ( $P = 0.019$ ,  $R^2 = 0.048$ ,  $F_{1,91} = 5.665$ ), and that greater bite force was associated with greater maximum prey length in females of *P. melisellensis* ( $P = 0.006$ ,  $R^2 = 0.052$ ,  $F_{2,149} = 5.216$ ). Stepwise regressions on the proportion of different food items in the diet also provided statistically significant models in *P. sicula* (males:  $P = 0.010$ ,  $R^2 = 0.080$ ,  $F_{4,109} = 3.481$ ; females:  $P = 0.021$ ,  $R^2 = 0.081$ ,  $F_{4,88} =$

3.040). In males, bite force was explained by the proportion of plant matter, the proportion of hard prey and the proportion of prey of intermediate evasiveness consumed ( $\beta$  coefficients: 0.05, 0.13 and -0.15). Similarly, in females an increase in bite force was associated with an increase in the proportion of plant matter, medium prey, and hard prey ( $\beta$  coefficients: 0.16, 0.23, and 0.23). No statistically significant model was detected for regression between bite force and the proportions of different prey types in *P. melisellensis*.

No statistically significant correlation was observed between bite force and dietary diversity, nor between bite force and diet disparity (all  $P > 0.05$ , see Table 3).



**Figure 8:** Linear regressions between bite force and prey hardness variables at the individual level. Full lines represent significant correlations ( $P < 0.05$ ). Determination regression coefficients are also indicated when appropriate. Dashed lines represent non-significant correlations.

**Table 5:** Summary of the *p*-values and regression coefficients for each of the variables tested against bite force. Asterisks indicate significant values ( $P < 0.05$ ).

	Simple regressions															
	Mean hard.		Max. hard.		Diversity		Disparity		Island area		Dist. Island		Dist. Main.		Head dim.	
	<i>p</i>	R <sup>2</sup>	<i>p</i>	R <sup>2</sup>	<i>p</i>	R <sup>2</sup>	<i>p</i>	R <sup>2</sup>	<i>p</i>	R <sup>2</sup>	<i>p</i>	R <sup>2</sup>	<i>p</i>	R <sup>2</sup>	<i>p</i>	R <sup>2</sup>
Females meli.	<b>0,034</b>	<b>0,03</b>	0,366	-	0,852	-	0,978	-	0,66	-	<b>0,009</b>	<b>0,54</b>	0,865	-	0,985	-
Males meli.	<b>0,026</b>	<b>0,03</b>	0,436	-	0,142	-	0,326	-	0,166	-	<b>0,001</b>	<b>0,70</b>	<b>0,031</b>	<b>0,35</b>	0,185	-
Females sicula	<b>0,014</b>	<b>0,04</b>	<b>0,039</b>	<b>0,04</b>	0,405	-	0,156	-	0,822	-	0,333	-	0,421	-	0,121	-
Males sicula	0,097	-	0,338	-	0,293	-	0,623	-	0,854	-	0,747	-	0,351	-	<b>0,03</b>	<b>0,50</b>

	Stepwise regressions					
	Head shape		Prey dim.		Prey prop.	
	<i>p</i>	R <sup>2</sup>	<i>p</i>	R <sup>2</sup>	<i>p</i>	R <sup>2</sup>
Females meli.	<b>0,001</b>	<b>0,28</b>	<b>0,006</b>	<b>0,05</b>	0,899	-
Males meli.	<b>0,001</b>	<b>0,21</b>	0,126	-	0,103	-
Females sicula	<b>0,001</b>	<b>0,19</b>	<b>0,019</b>	<b>0,05</b>	<b>0,021</b>	<b>0,08</b>
Males sicula	<b>0,002</b>	<b>0,09</b>	0,147	-	<b>0,01</b>	<b>0,08</b>

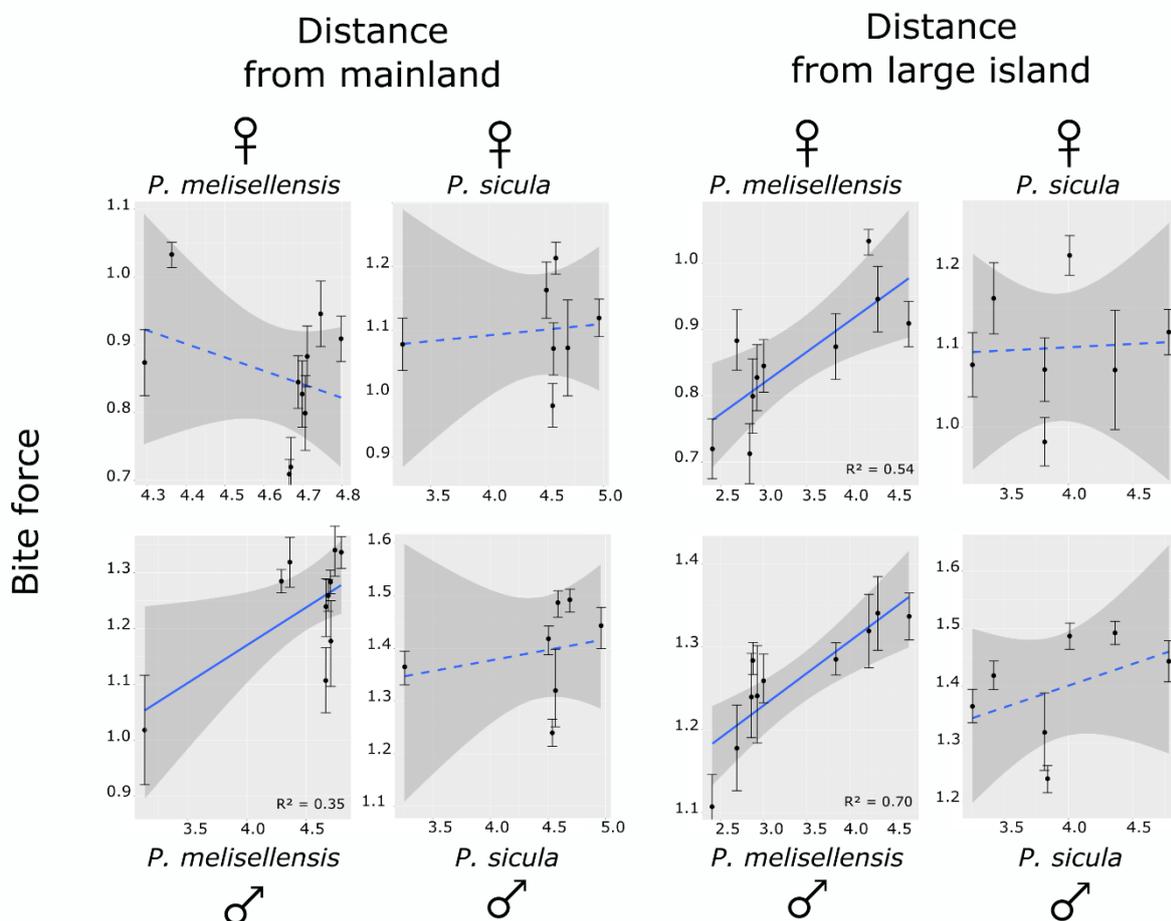
**Relationships between bite force, diet and island characteristics**

Linear regressions showed that in *P. melisellensis* absolute bite force increased when the island was located further from the nearest large island (males:  $P = 0.001$ ,  $R^2 = 0.695$ , slope = 0.077; females:  $P = 0.009$ ,  $R^2 = 0.540$ , slope = 0.094) (Figure 5, Table 3). In males of *P. melisellensis* absolute bite force also increased when the island was located further from the continent ( $P = 0.031$ ,  $R^2 = 0.348$ , slope = 0.135). This no longer held when considering residual bite force. Moreover, in neither species was absolute or residual bite force were correlated with island area (all  $P > 0.05$ , see Table 3).

In female *P. sicula* only a significant model was retained for the stepwise regression between the distance from the nearest large island and prey proportions ( $P = 0.01$ ,  $R^2 = 0.99$ ). In *P. melisellensis* only a significant model was retained for the stepwise regression between the distance from the mainland and prey dimensions (females:  $P = 0.001$ ,  $R^2 = 0.732$ ; males:  $P = 0.009$ ,  $R^2 = 0.462$ ). Simple regressions carried out between isolation metrics and mean prey hardness revealed marginally significant results in *P. sicula* (females:  $P = 0.08$ ; males:  $P = 0.06$ ).

### Main drivers of the variation in bite force

The final stepwise regressions using distance metrics, island area, proportion of plant matter, proportion of hard prey as well as mean prey hardness did not result in a statistically significant model in *P. sicula*. However, in males of *P. melisellensis*, the retained model ( $P = 0.004$ ,  $R^2 = 0.803$ ,  $F_{3,6} = 13.29$ ) indicates that the proportions of plants and of hard prey, and the distance to the nearest large island drive variation in bite force ( $\beta$  coefficients: 0.30, -0.08, 0.08). In females of *P. melisellensis*, the retained model ( $P = 0.008$ ,  $R^2 = 0.674$ ,  $F_{2,7} = 10.3$ ) reveals that the proportion of hard prey and the distance to a large island are the main drivers of bite force ( $\beta$  coefficients: 0.30, 0.12).



**Figure 9:** Linear regressions between bite force and island isolation metrics. Full lines represent significant correlations ( $P < 0.05$ ). Determination regression coefficients are also indicated when appropriate. Dashed lines represent non-significant correlations.

## DISCUSSION

### ***Variability across populations and phylogenetic influence***

We first investigated the effect of sex and island on the variation in bite force across populations. In *P. sicula* the lack of an interaction between the factors (sex and island) in the two-way ANCOVA and the two-way MANCOVA suggests that variation in bite force and head dimensions differs by island, irrespective of the sex considered. Differences between males and females are further independent of the variation between islands. In *P. melisellensis* the significant interaction between these factors suggests different effects of island and sex on the variation in bite force and head dimensions, and justifies the subsequent statistical analyses performed by sex and species separately.

Next, we investigated the influence of phylogenetic relationships between populations. The consistency of the results obtained with the phylogenetic ANOVAs suggest that differences in bite force and head dimensions between males and females are little impacted by phylogeny. On the contrary, differences between species are impacted by phylogeny. At the interspecific level, bite force variation across populations can be partially explained by the history of colonization of the islands given that the divergence between the two species occurs relatively deep in the tree. The impact of phylogeny on interspecific levels of performance has, for example, also been demonstrated in *Anolis* lizards (Wittorski et al. 2016). However, at the intraspecific level, bite force and head dimensions seem to be largely independent from the phylogeny, suggesting that local environmental characteristics strongly constrain these traits. This statement is supported by the fact that phylogenetic multiple regression of residual head dimensions on bite force provided similar results (Table S3). As for *P. sicula* no phylogenetic structure was detected among the examined island populations we could not test for phylogenetic effects (Figure 2).

### ***Proximate drivers of variation in bite force***

Although morphology can sometimes be decoupled from performance (Wainwright et al. 2005), the literature provides strong evidence that head shape and size are good predictors of bite force in vertebrates (Van Daele et al. 2008; Chazeau et al. 2013; Marshall et al. 2014). In squamates (Penning, 2017), and

especially in lizards (Herrel et al. 2001a; Herrel & Holanova, 2008), dimensions such as height and length drive much of the variation in performance. The present study confirms this and shows that variation in bite force is mostly driven by variation in head height in both species and both sexes. Not only may a taller head allow the insertion of larger jaw adductor muscles (Herrel et al. 2007), but it might also enhance bite force by increasing the moment arms of the muscles or by increasing the vertical component of the line of action of jaw muscles (Herrel et al. 2002). In males, wider heads are also associated with higher bite forces, probably by providing more space for muscles. Some studies have suggested that bite force may trade-off with speed of prey capture (Herrel et al. 2002; Herrel et al. 2009). This trade-off is also found in lizards where fast jaw closing benefits from longer jaw out-levers as well as longer in-levers for jaw opening, whereas bite force benefits from longer in-levers for jaw closing and shorter out-levers (Herrel et al. 2001; Vanhooydonck et al. 2007). Our results illustrate this trade-off between force and speed, as the length of the in-lever for jaw opening in females of *P. sicula* is negatively correlated with bite force. This particularity may confer to the females the ability to capture faster and more evasive prey than males do, as has been previously suggested for *Anolis carolinensis* (Herrel et al. 2007). Accordingly, males of both *Podarcis* species with relatively shorter lower jaws had higher bite forces as this optimizes the out-lever for biting.

The present results show that relevant head dimensions correlated with bite force, yet accounted for a relatively low amount of the total variance (from 9 to 28%). Unexpectedly, in both species head dimensions are stronger predictors of bite force in females than in males. This is possibly because head width is strongly influenced by pterygoid muscles that are proportionally larger in males (Herrel et al. 1996). Wider heads are thought to be a determinant factor in the outcome of male-male encounters (Molina-Borja et al. 1997; Huyghe et al. 2005). We here propose that head dimensions possibly are poorer predictors of bite force in males than in females because some traits like head width are shaped by sexual selection resulting in the investment in the size of the pterygoid muscles, which conversely contribute relatively little to the bite force for its size (Herrel et al. 1999).

### ***Ultimate drivers***

We tested whether sexual selection (i.e. the degree of sexual dimorphism) and resource use (i.e. functional properties of food items and food niche breadth) are drivers of variation in bite force. Sexual dimorphism in head dimensions may allow for food partitioning between males and females (Schoener,

1967; Herrel et al. 1999) but is generally considered to be maintained through male-male competition in lizards (Vincent & Herrel, 2007; Vanhooydonck et al. 2010). Dimorphism in head dimensions was previously demonstrated to be decoupled from dimorphism in diet in this study system (Taverne et al. 2019), hence suggesting that niche partitioning for limited resources likely does not explain the observed head dimorphism. This makes sexual dimorphism in head dimensions a good proxy for sexual selection, however. We found that the intensity of intra-sexual competition impacted bite force in males of *P. sicula*. This makes sense because with greater bites often win male-male interactions (Lailvaux et al. 2004; Huyghe et al. 2005; Husak et al. 2006; Lailvaux & Irschick, 2007). Additional information on other populations are needed, however, because extreme populations appear to be driving the correlation between sexual dimorphism and bite force. Moreover, other proxies for intraspecific competition including the proportion of individuals which missed the longest toe on the hind feet and the proportion of individuals with regenerated tails did not explain variation in bite force across populations in contrast to what has been observed in other systems (Lailvaux et al. 2004; Lailvaux & Irschick, 2007; Donihue et al. 2016). Data on population density would be informative in better understanding the relationship between intraspecific competition and performance in this system.

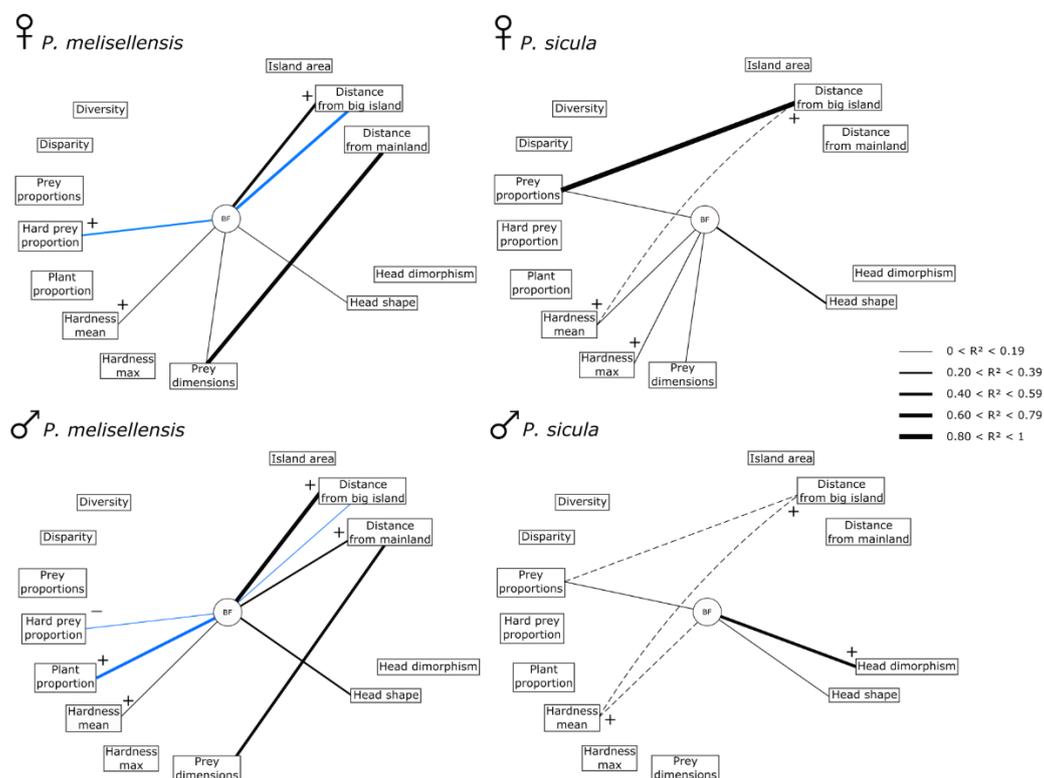
A previous study revealed a great diversity and disparity in the functional properties of food items eaten by insular Croatian *Podarcis* lizards (Taverne et al. 2019). Whereas this was not the case for diet diversity, diet disparity was shown to be impacted by island area, possibly because competition for food and population density tend to increase on smaller islands (Case, 1975; Buckley & Jetz, 2007). Variation in bite force has further been demonstrated to be associated with competition in similar insular systems (Donihue et al. 2016). For these reasons, a greater bite force can be expected to enable lizards to widen their dietary breadth, but this is not confirmed by the present study. However, our results do show that females of *P. sicula* that incorporate more plant items in their diet also bite more forcefully. Plants are considered very tough and fibrous (Lucas & Luke, 1984; Hiimae & Crompton, 1985), requiring greater bite force to reduce (Herrel et al. 1998a,b). A greater bite force thus enables lizards to rely on alternative food sources in case of resource scarcity. Moreover, in both sexes of *P. sicula*, animals that bite harder also include a proportionally greater proportion of hard prey items in their diet. Higher bite forces would thus enable them to gain access to other, harder resources such as beetles or snails.

The bite force of all individuals typically exceeds the maximum hardness of any prey item eaten, yet mean hardness is generally correlated with bite force (with the exception of male *P. sicula*). In bats (Aguirre et al. 2003) maximum food size and hardness corresponds well to the maximum bite force of an

individual, suggesting that functional prey properties may be important drivers of bite force in some cases. In lizards and bats the mechanisms underlying variation in bite force thus seem to differ. On one hand, bats are endotherms that need to maintain a high metabolic rate (Pough, 1980; Nagy, 2005). To fulfill their energetic needs, they select large food items to optimize their food intake and reduce foraging costs (optimal foraging, Stephens & Krebs, 1986, see also Hawlena & Pérez-Mellado, 2009). Prey hardness increases with size and thus may become a limiting factor driving an increase in bite force. In ectothermic organisms such as lizards, maximum hardness does not appear to drive an increase in bite force. Rather a higher level of performance allows them to eat more hard items on average (see also Verwaijen et al. 2002). From an energetic point of view, processing large or tough items requires more time and costs more energy than processing soft and small items (Preest, 1994; Herrel et al. 1999b; Herrel et al. 2001; Verwaijen et al. 2002). During this period, lizards are exposed to predation (Hawlena & Pérez-Mellado, 2009) and competition. Reducing handling time while foraging is consequently likely important for these lizards. A greater bite force enables them to reduce the intraoral transport time (Verwaijen et al. 2002) which may provide enough of an advantage to select for higher bite forces. However, the mechanical resistance of plant material was not considered here. Since a previous study (Herrel et al. 1999b) established that the hardness of plant material was far beyond those of arthropods of comparable size, including this quantitative data into account in future studies would be of particular interest because 1) it might reveal some direct correlation between maximum hardness and bite force in our study system, and 2) it might explain the low yet significant amount of variance in bite force explained by prey hardness (Figure 4).

We expected island area to be correlated with variation in bite force since this is an important feature that impacts the ecology of insular ecosystems (Hamilton & Armstrong, 1965; Whittaker & Fernández-Palacios, 2006; Santos et al. 2016; Itescu et al. 2019). First, islands are isolated environments hosting simpler communities that often lack top-predators (Losos & De Queiroz, 1997). Ecological release then often favors higher densities of meso-predators such as lizards (Litvaitis & Villafuerte, 1996; Ritchie & Johnson, 2009). Higher rates of intraspecific competition (Itescu et al. 2017; Pafilis et al. 2009) for territories and resource access due to food scarcity are also observed on the smallest islands. In contrast to a previous study that demonstrated a direct relationship between island area and bite force in *Podarcis* lizards (Donihue et al. 2016), our results did not show this pattern. Why this is the case remains unclear, but possibly the proximity of many of these islands to large islands or to the mainland may perturb the expected relationships.

Island isolation (linear distances of the island from the nearest large island and/or from mainland) was also predicted to be correlated with bite force since the most remote islands are thought to host the poorest species diversity (Whittaker et al. 2008; Santos et al. 2016). Distance from the mainland is a very common isolation metric because the mainland is assumed to be the richest source (Weigelt & Kreft, 2013), whereas the distance from the nearest large island is typically used when predicting plant species richness (Diver, 2008). We did find a positive correlation between island isolation and absolute bite force, but only in *P. melisellensis*. Stepwise regressions further suggested a direct relationship between isolation metrics and diet variables (Figure 6). The combined results reveal that island remoteness is correlated with the functional characteristics of diet in both species (marginally significant in *P. sicula*) including prey dimensions, mean prey hardness, and the proportions of different prey. Subsequently, these correlate with variation in bite force. The present results suggest that island remoteness may impact bite force by constraining the functional properties and abundance of the available resources to mesopredators like lizards, and that lizards can access these resources by increasing bite force along with their head size.



**Figure 10:** Summary of the significant correlations between the different factors tested and bite force (BF). Factors are grouped by diet, morphological, and habitat features. Line thickness is proportional to the determination coefficient ( $R^2$ ), except for the blue lines which summarize the results of the final stepwise regression and for which line thickness is proportional to the standardized  $\beta$  coefficients. Dashed lines represent marginally significant correlations ( $P < 0.1$ ). Direction of correlations found with simple regressions are indicated with plus and minus symbols (+, -).

**Conclusion**

Variation in bite force appears to be driven by different factors in the two species considered here. Indeed, whereas head width seems to be a fair predictor of bite force in *P. melisellensis*, it appears to be decoupled from bite force in *P. sicula*. Moreover, whereas patterns are consistent between males and females within *P. melisellensis*, this does not hold in *P. sicula*. Given that the intensity of sexual competition in this study system was shown to impact bite force in males of *P. sicula* only, we assume that strong competition between males in this species may be driving the different evolutionary response. Greater bite forces were not associated with a wider niche breadth (estimated by the prey taxonomic diversity and the dietary disparity) in either of the two species. Nevertheless, greater bite force enables the inclusion of greater amounts of difficult-to-reduce items (i.e. mean prey hardness, the proportion of hard prey, or plant material) in males and females of both species, suggesting that diet is an important driver of variation in bite force. The results of the present study also show that in *P. sicula* island remoteness indirectly impacts bite force by influencing food resource availability. On the other hand, a more direct correlation between island isolation and bite force was demonstrated in *P. melisellensis*. In summary, the present study suggests that different evolutionary responses between species might originate from different levels of intrasexual competition. Future studies exploring these patterns in other island systems with other lizards are, however, needed to confirm the generality of these observations.



# **Chapter 3**

## **Kinematics**

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**Relationships between dietary breadth and jaw movement flexibility:  
a case study of two recently diverged insular populations of *Podarcis* lizards.**

**ABSTRACT**

The kinematics of lizard feeding are the result of complex interactions between the craniocervical, the hyolingual, and the locomotor systems. The coordinated movement of these elements is driven by sensory feedback from the tongue and jaws during intraoral transport. The kinematics of jaw movements have been suggested to be correlated with the functional characteristics of the prey consumed, such as prey mobility and hardness. However, whether and how dietary breadth correlates with the flexibility in the behavioral response has rarely been tested, especially at the intraspecific level. Here we tested whether an increase in dietary breadth was associated with a greater behavioral flexibility by comparing two populations of insular *Podarcis* lizards differing in dietary breadth. To do so, we used high-speed cameras to analyze the three-dimensional jaw kinematics while offering lizards different prey types. Our results show that prey type impacts kinematics, especially maximum gape and maximum opening and closing speeds. Furthermore, the behavioral flexibility was greater in the population with the greater dietary breadth, suggesting that populations which naturally encounter and feed on more diverse prey items show a greater ability to modulate their movements to deal with variation in prey properties including hardness and mobility. Moreover, the more generalist population showed more stereotyped movements suggesting a finer motor control.

**Keywords:** dietary generalist, dietary specialist, jaw kinematics, prey type, stereotyped movements.

## INTRODUCTION

Resource use in depauperate environments can be challenging. In insular populations of lizards, where food is typically scarce and intraspecific levels of competition are often high (Pafilis et al. 2009; Itescu et al. 2017), dietary shifts towards generally unexploited resources are often observed (Van Damme, 1999; Bolnick, 2001; Herrel et al. 2008). Changes in diet in insular populations are often accompanied with anatomical specialisations compared to their mainland counterparts (Schoener, 1977; Perry, 1996; Thomas et al. 2009). However, changes in the environment are likely to impose selection primarily on performance and behavior rather than on anatomy (Arnold, 1983; Irschick et al. 2008). Because foraging and feeding are undoubtedly among the most critical aspects of an organism's daily activities, these traits have been widely investigated (see Schwenk, 2000; Reilly et al. 2007 for an overview).

Behavior associated with resource use is typically quantified by focusing on jaw and tongue kinematics, as this allows to link between anatomy, bite force, and diet (Herrel, Timmermans & De Vree, 1998; Meyers, Herrel & Birch, 2002; Curtis et al. 2009, Herrel & De Vree, 2009). Feeding consists of four major components: prey location, capture, intraoral transport, and swallowing. These tasks are assured by the interplay between the craniocervical, the hyolingual and the locomotor systems (Montuelle et al. 2009; Montuelle et al. 2012). Each of these apparatuses has to work together, yet is differentially involved depending on the step of resource acquisition and on the taxon considered. For example, the tongue is used to capture prey in some taxa (see in Chameleontidae: Bels & Baltus, 1987; Wainright et al. 1991; Agamidae: Schwenk & Throckmorton, 1989; Kraklau, 1991; Iguanidae: Schwenk & Throckmorton, 1989, Bels, 1990; Scincidae: Smith, Kardong & Bels, 1999; Cordylidae: Braeckhoven & Mouton, 2013) and plays an important role in intraoral transport and swallowing in most lizards. The role of the tongue has drawn much attention since it has been suggested to provide sensory feedback during intraoral transport, especially during the slow-opening phase (Herrel et al. 2001; Schaerlaeken, Herrel & Meyers, 2008).

Lizards are able to modulate their tongue and jaw kinematics in response to prey characteristics, including prey type (Herrel, Cleuren & De Vree, 1996; Schaerlaeken et al. 2011), hardness (Herrel, Verstappen & De Vree, 1999; Metzger, 2009), mobility (Schaerlaeken, Herrel & Meyers, 2008; Montuelle et al. 2010), and size (Montuelle et al. 2009; Broeckhoven & Mouton, 2013). It is often assumed that the flexibility of the behavioral repertoire and the diversity of the prey included in the diet are correlated, yet this remains rarely tested. Hence, animals which encounter a wide variety of prey in their environment (generalists) are thought to be

more likely able to modulate their behavioral response than specialists (e.g. in fishes, see Liem, 1978; Van Wassenberg et al. 2006; in lizards: Herrel et al. 1999).

Despite the relatively thorough understanding of the factors driving variability in feeding behavior and kinematics, they have rarely been investigated at the intraspecific level. Yet, diet can be highly variable among populations of the same species, especially in insular systems (Schoener, 1967; Brown & Pérez-Mellado, 1994; Sagonas et al. 2014; Donihue et al. 2016; Taverne et al. 2019). How intraspecific variability in diet might affect the behavioral response remains largely unknown to date. We here test how a change in diet affects the jaw kinematics and kinematic flexibility in two recently diverged populations of *Podarcis sicula* (Rafinesque-Schmaltz, 1810). Whereas one of these populations is insectivorous, the other includes a large proportion of plants into its diet (over 60%) and shows an increased dietary breadth (see Chapter 1).

The aim of this study was to test whether the increased dietary breadth observed in the omnivorous population is associated with an increase in the flexibility (*sensu* Wainwright, Mehta & Higham, 2008) in jaw kinematics when feeding on insect prey differing in their properties and size. Moreover, we explored whether males and females differ in the kinematics of intraoral transport given their difference in head morphology, bite force, and diet (Herrel et al., 2008; Taverne et al., 2019, 2020). We specifically predict that: 1) the type of prey should impact the jaw kinematics, 2) males and females should respond differently to changes in prey type with males showing lower absolute gape angles and shorter cycle times given their relatively larger heads and bite forces, 3) the generalist omnivorous lizards from Pod Mrčaru will show a greater flexibility in their feeding behavior compared to the insectivorous lizards from Pod Kopište.

## MATERIAL & METHODS

### *Specimens*

Adult specimens from the islets of Pod Kopište and Pod Mrčaru were captured during the summer 2018 and maintained in captivity in the Zagreb Zoo until the end of winter 2019. Individuals from both populations were fed crickets and maintained on a 12h light / dark cycle. Upon emergence from hibernation in 2019 animals were moved to the animal care facility of the Museum in Paris. In total, six individuals were included in the present study: one female and three males from Pod Kopište and one female and one male from Pod Mrčaru. The lizards were not fed one week prior to filming.

### *Experimental set-up*

Each lizard was introduced in a wooden box with an enclosed Plexiglas corridor (Figure 1). A prey was fixed at the end of the corridor to standardize the position of the lizard while feeding. Each individual was proposed two types of prey (crickets and mealworms), the size of which was adjusted to the size of the individual. We attempted to record, when possible, three trials per type of prey, per lizard. Two high-speed Phantom Miro R311 cameras with a 50 mm focal length lens were used to record the feeding bouts. Both cameras were positioned at an acute angle and in a different plane to facilitate the three-dimensional tracking of the jaw movements (Theriault et al. 2014). The two cameras were synchronized and were set to a recording rate of 250Hz. We ensured that each trial included at least five intraoral transport cycles.

### *Calibration of the cameras*

The cameras were calibrated using a structure-from-motion approach with sparse bundle adjustment optimization. To do so a 22.5mm wand was moved through the recording volume. The resulting 3D calibration was rotated such that the +X axis pointed along the length of the chamber, +Y along the width and +Z along the height. The average reprojection error (the difference between fitted and observed wand end point locations) was 0.175 pixels, and the average variation in the 3D length of the calibration wand was 0.7%; the quantities indicate a high-quality camera calibration. Calibration point

locations were collected using DLTdv8 Release 8.1.7 (Hedrick, 2008) and the structure-from-motion calibration was performed using easyWand (Theriault et al. 2014).

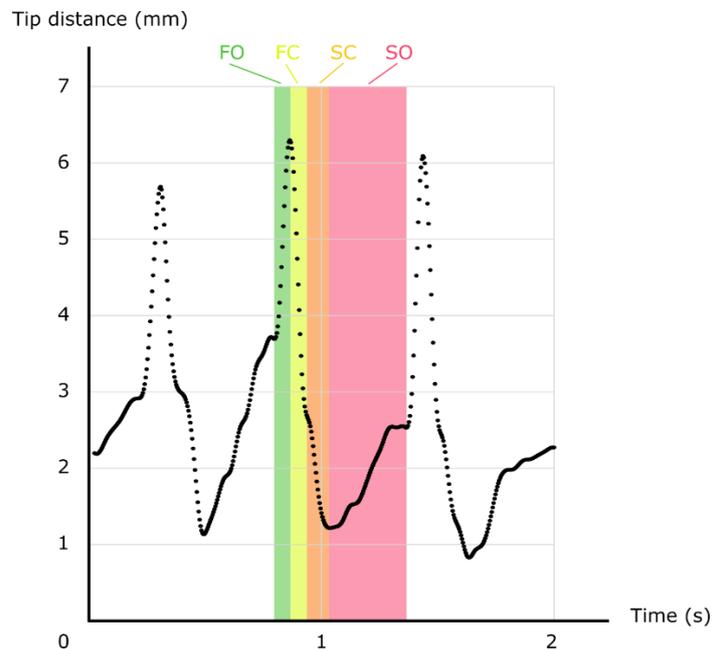


**Figure 1:** Picture of the experimental set-up showing the two high-speed cameras pointing to the end of the tunnel where the prey is positioned. Note the relative position of the cameras which are oriented to form an acute angle allowing to optimize camera calibration and to enhance the accuracy of the three-dimensional tracking.

### **Digitization of the videos**

Jaw movements were tracked manually using DLTdv8. Three points were tracked: the tip of the mandible, the tip of the snout, and the *anguli oris* or mouth corner. Each video thus provided over time the (u,v) coordinates of each point, which were ensured to be visible on each view by the relative position of the cameras. Thanks to the DLT coefficient obtained by the calibration procedure, the (u,v) coordinates extracted from the two views were compiled into three dimensional (x,y,z) coordinates that were subsequently used in the analyses. The gape angle and the distance between the tips of the jaw were calculated from these coordinates. The gape angle and distance curves were smoothed using the function “smooth.spline” of the package “stats” in R (R Core Team 2019). The new (x,y,z) coordinates generated by the curve smoothing were used to calculate the following 13 variables. We calculated the maximum distance between the tips of the jaw (in millimeters), the maximum gape angle (in degrees), the maximum opening speed, the maximum closing speeding (in  $\text{mm}\cdot\text{s}^{-1}$ ), the duration of the transport cycle (in seconds), and the absolute and the relative durations of each of the four phases of the jaw cycle (Bramble and Wake, 1985): the slow opening (SO), the fast opening (FO), the fast closing (FC)

and the slow closing (SC) (Figure 2). Each of these variables was obtained for each of the 166 complete cycles recorded (Table 1).



**Figure 2:** Smoothed displacement curve illustrating the changes in gape distance over three consecutive intraoral transport cycles. Colors illustrate the four kinematic phases of a gape cycle (Bramble and Wake, 1985). Slow opening (SO) generally takes the longest and is associated with the tongue moving under the prey. Next the jaws are opened rapidly during the fast-open phase (FO) allowing the tongue with adhering prey to be retracted in the oral cavity. At maximal gape the fast-closing phase (FC) starts until the jaws hit the prey which determines the onset of the slow-close/power stroke phase (SC) where the prey is crushed.

### Statistical analyses

All variables were log<sub>10</sub>-transformed to assure the normality of the data, except for the relative durations which were arcsin-transformed. To reduce the dimensionality of the dataset we performed a factor analysis with varimax rotation and extracted all factors with eigenvalues greater than one. A multivariate analysis of variance (MANOVA) was performed on the factors which cumulatively explained 85% of the variance to investigate the effects of island, sex, prey type and their interactions on the kinematics of feeding. Subsequent univariate analyses of variances (ANOVAs) were performed on each factor to test which variables drove the observed variation. The feeding repertoire was estimated by calculating the kinematic disparity based on the 13 kinematic variables, using the function “disparity.per.group” (package “dispRity”, Guillerme, 2018). The disparity metrics was compared between populations, sexes, and prey types through Wilcoxon tests including a Bonferroni correction for multiple testing (function “test.dispRity”).

## RESULTS

The factor analysis extracted five factors which cumulatively explained: 93.4% of the variance (Table 2). The first factor was mainly determined by the duration of the phases and of the overall cycle; factor 2 by the maximum gape distance and angle as well as the maximum opening speed; factor 3 by the absolute and relative durations of the SC phase; factor 4 by the duration of the FC phase; factor 5 by the absolute duration of the FO phase.

**Table 1:** Mean and standard errors (SE) associated with the tested variables (Dist: maximum gape distance, Angle: maximum gape angle, Open: maximum opening speed, Close: maximum closing speed, SO/FO/FC/SC: absolute durations of the slow opening / fast opening / fast closing / slow closing phases, r: relative duration, Cycle: duration of a cycle) for each population (PK: Pod Kopiste, PM: Pod Mrcaru), each sex (f: females, m: males), and for each prey type (C: cricket, MW: mealworm). Distances are in millimeters, durations in seconds, and speeds in  $\text{mm}\cdot\text{s}^{-1}$ . The number of cycles considered (Nb) is also indicated.

Island	Sex	Prey	Nb	Dist	Angle	Open	Close	SO	FO	FC	SC	rSO	rFO	rFC	rSC	Cycle	
PK	f	C	18	6.45	32.28	68.00	71.13	0.42	0.08	0.09	0.15	0.56	0.11	0.13	0.21	0.75	Mean
				1.17	5.76	14.58	11.80	0.15	0.02	0.07	0.06	0.10	0.02	0.08	0.09	0.20	SE
		MW	15	5.54	32.30	60.73	82.90	0.36	0.08	0.08	0.11	0.56	0.13	0.13	0.18	0.63	Mean
				0.91	5.26	15.25	15.21	0.12	0.02	0.01	0.07	0.08	0.03	0.03	0.08	0.16	SE
	m	C	23	7.09	31.18	68.96	69.74	0.47	0.08	0.08	0.13	0.60	0.12	0.12	0.17	0.76	Mean
				0.96	4.41	14.49	13.66	0.18	0.01	0.02	0.06	0.11	0.03	0.05	0.07	0.20	SE
		MW	23	5.27	23.69	51.88	67.92	0.44	0.09	0.07	0.15	0.57	0.13	0.10	0.20	0.75	Mean
				1.05	4.94	16.25	27.04	0.16	0.03	0.02	0.07	0.10	0.06	0.03	0.08	0.18	SE
PM	f	C	18	6.90	37.33	72.82	71.55	0.58	0.09	0.09	0.10	0.63	0.12	0.12	0.14	0.86	Mean
				0.65	3.13	13.88	21.60	0.33	0.02	0.03	0.03	0.13	0.04	0.05	0.06	0.36	SE
		MW	12	4.86	24.83	56.96	60.69	0.54	0.08	0.09	0.10	0.65	0.10	0.12	0.13	0.80	Mean
				0.67	3.54	15.99	17.69	0.22	0.02	0.03	0.05	0.14	0.03	0.05	0.08	0.22	SE
	m	C	45	7.87	31.86	75.57	76.95	0.55	0.09	0.08	0.18	0.58	0.11	0.11	0.21	0.89	Mean
				1.31	5.67	17.30	25.74	0.31	0.03	0.03	0.12	0.15	0.04	0.05	0.11	0.37	SE
		MW	12	5.23	21.46	40.89	55.50	0.48	0.09	0.06	0.12	0.62	0.12	0.09	0.17	0.74	Mean
				0.81	3.35	12.42	20.23	0.20	0.03	0.01	0.04	0.10	0.04	0.03	0.06	0.21	SE

**Table 2:** Results of the factor analysis with VARIMAX rotation carried out on the kinematic variables.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Eigenvalue	3.81	2.67	2.21	1.79	1.66
% variance	29.31	20.50	17.03	13.76	12.80
Max. distance	0.104	<b>0.873</b>	0.147	0.104	0.251
Max. gape angle	0.045	<b>0.891</b>	0.029	0.134	0.248
Max. opening speed	-0.077	<b>0.798</b>	0.001	-0.106	-0.418
Max. closing speed	-0.023	0.647	-0.163	-0.625	-0.137
Duration SO	<b>0.969</b>	0.03	-0.194	0.041	0.124
Duration FO	0.135	0.139	0.091	0.182	<b>0.941</b>
Duration FC	0.046	0.134	0.014	<b>0.949</b>	0.164
Duration SC	0.219	0.032	<b>0.935</b>	0.071	0.114
Rel. duration SO	<b>0.767</b>	-0.037	-0.604	-0.16	-0.114
Rel. duration FO	<b>-0.801</b>	-0.006	-0.063	-0.049	0.563
Rel. duration FC	<b>-0.764</b>	0.022	-0.115	0.601	-0.117
Rel. duration SC	-0.323	0.058	<b>0.924</b>	-0.063	-0.052
Duration of one cycle	<b>0.930</b>	0.087	0.097	0.156	0.245

The variables with loading greater than 0.7 are indicated in bold.

The MANOVA showed significant sex and prey effects as well as significant interactions between island and prey, and between sex and prey (Table 3). This suggests that the kinematics of feeding differ between prey type and sexes but also that the way individuals of the two populations deal with different prey differs. Finally, males and females also differed in the way they processed mealworms versus crickets.

**Table 3:** Results of the MANOVA performed on the five first factors testing the effects of island, sex, and prey type on the jaw kinematics.

Effect		Value	F	df hypothesis	df error	P
Intercept	Wilks' Lambda	0.931	2.26	5	153	0.052
Island	Wilks' Lambda	0.955	1.46	5	153	0.208
<b>Sex</b>	<b>Wilks' Lambda</b>	<b>0.862</b>	<b>4.92</b>	<b>5</b>	<b>153</b>	<b>&lt; 0001</b>
<b>Prey</b>	<b>Wilks' Lambda</b>	<b>0.553</b>	<b>24.72</b>	<b>5</b>	<b>153</b>	<b>&lt; 0001</b>
Island * sex	Wilks' Lambda	0.951	1.56	5	153	0.174
<b>Island * prey</b>	<b>Wilks' Lambda</b>	<b>0.888</b>	<b>3.87</b>	<b>5</b>	<b>153</b>	<b>0.002</b>
<b>Sex * prey</b>	<b>Wilks' Lambda</b>	<b>0.870</b>	<b>4.57</b>	<b>5</b>	<b>153</b>	<b>0.001</b>
Island * sex * prey	Wilks' Lambda	0.959	1.29	5	153	0.269

The significant results are indicated in bold. F: F-statistic, df: degrees of freedom, P: P-value.

Specifically, differences between males and females were driven by variation in gape and jaw opening speed with males showing higher gapes and greater speeds than females (Tables 4 and 5).

**Table 4:** Results of the ANOVAs illustrating the significant effects.

Source	Variable	df	F	P
Sex	Factor 2	1, 157	9.75	0.002
	Factor 3	1, 157	5.11	0.025
Prey	Factor 2	1, 157	104.12	< 0.001
Island * prey	Factor 2	1, 157	17.84	< 0.001
Sex * prey	Factor 2	1, 157	12.18	0.001

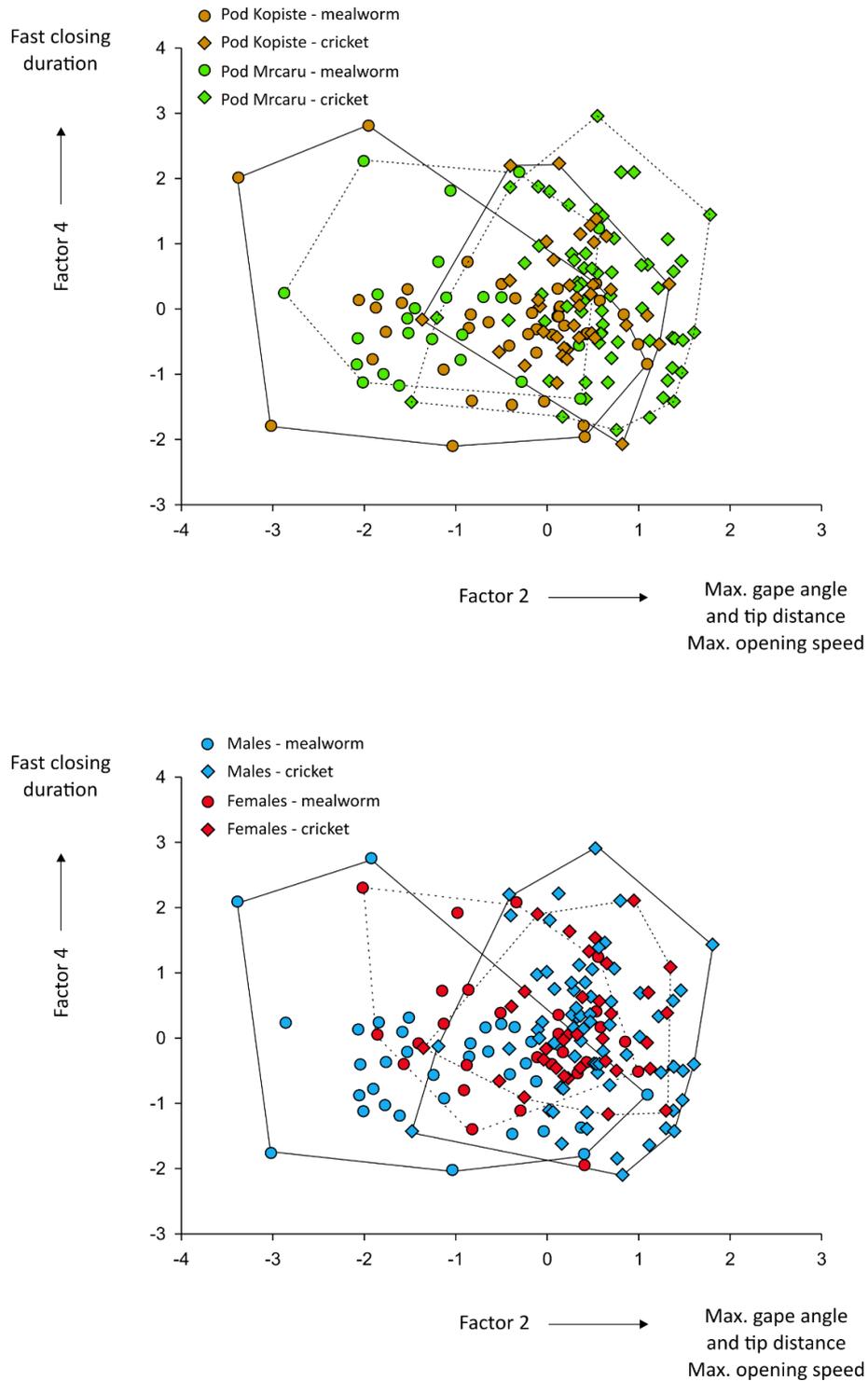
F: F-statistic, df: degrees of freedom, P: P-value.

**Table 5:** Mean  $\pm$  standard errors (SE) for the variables that differ between the factors tested.

Variable	Island	Sex	Prey	Mean	SE
Factor 2	Pod Kopište	male	cricket	0.324	0.151
			mealworm	-0.945	0.151
	Pod Mrčaru	female	cricket	0.202	0.176
			mealworm	0.006	0.187
		male	cricket	0.600	0.108
			mealworm	-1.485	0.209
Factor 3	Pod Kopište	male	cricket	-0.118	0.203
			mealworm	0.202	0.203
		female	cricket	0.211	0.236
			mealworm	-0.259	0.251
	Pod Mrčaru	male	cricket	0.311	0.145
			mealworm	-0.015	0.281
		female	cricket	-0.459	0.229
			mealworm	-0.598	0.281

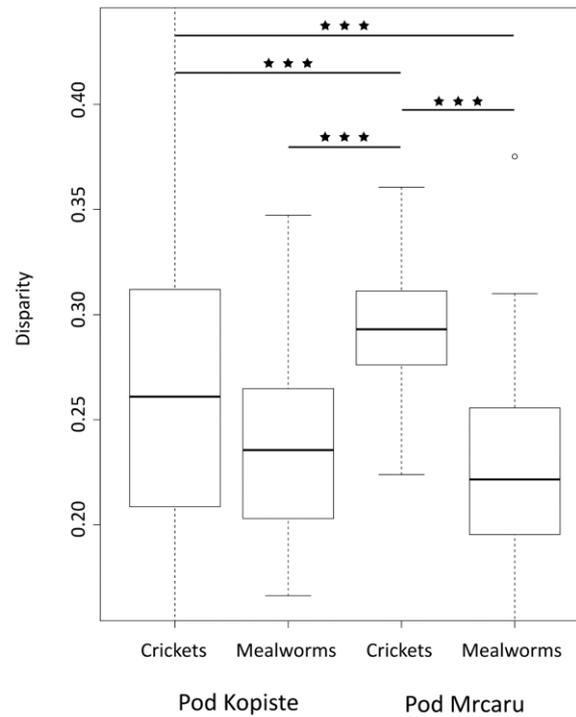
However, whereas females showed greater gapes and jaw opening speeds when consuming mealworms, males showed higher gapes and opening speeds when eating crickets (Figure 3). Similarly, whereas individuals from Pod Kopište used larger gape angles when consuming mealworms, individuals from Pod Mrčaru used larger gapes when consuming crickets (Figure 3). The kinematic disparity was significantly higher in individuals of Pod Mrčaru (PK: 0.326, PM: 0.368,  $P < 0.001$ ), higher

when crickets were consumed (crickets: 0.341, mealworms: 0.291,  $P < 0.001$ ), but did not differ between males and females (females: 0.359, males: 0.334,  $P = 0.062$ ).



**Figure 3:** Plot of the factors two and four illustrating differences between prey types, populations (top) and sexes (bottom). Circles: mealworms; diamonds: crickets, dark red: Pod Kopršte; green: Pod Mrčaru; blue: males; red: females.

Interestingly, no difference in disparity was detected between prey types for individuals from Pod Kopište (crickets: 0.263, mealworms: 0.236,  $P = 0.116$ ), whereas in Pod Mrčaru, a greater disparity was observed during the consumption of crickets (crickets: 0.299, mealworms: 0.238,  $P < 0.001$ ) (Figure 4). Furthermore, whereas both populations were equally disparate when eating mealworms ( $P = 0.367$ ), individuals of Pod Mrčaru showed an increase in disparity when eating crickets ( $P < 0.001$ ).



**Figure 4:** Plot of the disparity metrics illustrating the differences in kinematic flexibility between the two populations depending on the prey consumed (results of the Wilcoxon's tests for comparison of the means are indicated with three stars, representing a  $p$ -value  $< 0.001$ ).

## DISCUSSION

One of the major drawbacks of the current data set is that only few individuals from each population and sex are included in the study. In particular, because the variability in jaw kinematics of an individual or individuals within species can sometimes be high (Sanderson, 1988), more individuals should be included to conclude on the differences between prey type, sex, and populations. Moreover, because the population of Pod Mrčaru naturally includes plant matter into its diet, it would be of interest to record the jaw kinematics of the individuals while eating plant matter to better mimic the food items they typically encountered under natural conditions. Although the present results are mostly preliminary and should be discussed carefully, we believe that they are promising and insightful.

### *Effect of prey characteristics*

Our results show that prey type is associated with a change in the jaw kinematics, irrespective of the island or sex. In all cases, the consumption of crickets, which are harder and also bigger and more mobile than mealworms, is associated with a greater maximum gape and a maximum opening speed (Figure 3). The relationship between prey size and maximum gape was also described for *Agama stellio* (Herrel et al. 1996). Intuitively, jaws need to be more widely opened to let the prey move through the oral cavity during intraoral transport. Additionally, the ingestion of more evasive prey is usually thought to be accompanied with a decrease in the duration of the fast opening and closing phases, and with an increase in jaw velocity (Montuelle et al. 2010; Montuelle et al. 2012). This is partly confirmed by our results which demonstrate that individuals increase the maximum opening speed when dealing with the mobile prey (cricket) rather than the slower prey (mealworm). Moreover, on average, feeding disparity increased when consuming crickets compared to mealworms. Our results thus suggest that prey size and evasiveness are important characteristics affecting kinematics as demonstrated previously for other lizards (Schaerlaecken, Herrel & Meyers, 2008; Metzger, 2009; Montuelle et al. 2010; Montuelle et al. 2012).

### ***Effect of sex***

As revealed by the ANOVAs, males and females differed in maximum gape distance and angle, the maximum opening speed, and the absolute and relative durations of the slow-closing phase. Whereas the effect of sex on kinematics also depended on the prey, no interaction between sex and island was detected, suggesting that the differences in feeding kinematics between sexes are similar on Pod Kopište and Pod Mrčaru. Contrary to our prediction, and despite the fact that males are larger than females, males used wider gapes when feeding on crickets. Although we are not certain why this pattern was observed, maybe differences in motor control exist between sexes. Alternatively, our data set may be too limited and these results may not reflect reality. Studies comparing jaw kinematics between males and females are scarce, and more effort should be undertaken to tackle this question.

### ***Behavioral flexibility and stereotypy***

The question on how trophic breadth correlates with feeding flexibility has been mostly addressed in fishes (Ralston & Wainwright, 1997; Sanderson, 1988; Sanderson, 1990; Van Wassenbergh et al. 2016; Moran et al. 2018). On the contrary, it has rarely been investigated in tetrapods (but see Herrel, Verstappen & De Vree, 1999). We predicted that the individuals of the generalist and omnivorous population of Pod Mrčaru should show a greater flexibility in their feeding behavior than individuals of the insectivorous population of Pod Kopište. Consequently, the disparity in jaw kinematics should be greater in the generalist population. Our results confirmed this prediction, with individuals of the generalist population of Pod Mrčaru increasing their behavioral variability when facing a mobile prey like a cricket. This suggests that the unpredictability of the movements of the prey were overcome by an increased variety of jaw movements and speeds, i.e. an increased flexibility.

Interestingly, when considering each prey type, the overall variance in the disparity metrics was also lower in the population of Pod Mrčaru (Figure 4), suggesting that under similar circumstances (i.e. feeding on the same prey type), the generalist lizards did not vary their movement repertoire much. This observation might reveal an increased behavioral stereotypy (*sensu* Wainwright, Mehta & Higham, 2008). A study on the motor pattern in archer fish (Wöhl & Schuster, 2007) suggests that a high flexibility combined with stereotyped movements may reveal a high degree of movement control on the part of the individual. Although the present study does not allow us to draw the same conclusions, our results suggest that the omnivorous population of Pod Mrčaru might benefit from a finer motor control

allowing the specimens to modulate their behavioral response when facing different prey types, whilst opting for more stereotyped movements associated with specific prey types.

### ***Conclusion***

By focusing on the jaw kinematics, the present study investigated how rapid changes in diet between two recently diverged populations impacted their behavioral response during feeding. Under natural conditions, the two populations feed on items differing in hardness and mobility, one being insectivorous (specialist) and the other one omnivorous (generalist). Our results suggest that these prey characteristics influence the way individuals modulate their jaw movements, especially the gape and the opening speed. Prey mobility also seems to be associated with an increase in the behavioral repertoire, probably because the unpredictability of the prey movements forces the individual to modulate its jaw kinematics. However, differences between males and females cannot be explained by the present study and would require further investigation. Most importantly, our results suggest that the individuals from the generalist population, characterized by a greater trophic breadth, showed greater behavioral flexibility in response to a change in prey type. Also, they showed an increased movement stereotypy possibly suggesting a finer motor control in the generalist population. Since the individuals included in the present study were kept in captivity and fed solely with crickets for a year and a half preceding the experiment, we suspect that these traits are hard wired and may be under selection. Whether the observed differences in feeding kinematics are adaptive, and underlying feeding specialization would be worth investigating in the future.



# **Chapter 4**

## **Morphology**

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## Drivers of head shape variation in an island radiation:

### *Podarcis* lizards in the Adriatic

- submitted to *Functional Ecology* -

#### ABSTRACT

The evolution and radiation of animals on small islands is often associated with shifts in anatomy, physiology, performance, and ecology due to the strong selective pressures exerted in these resource-limited environments. At an interspecific scale, variation in head dimensions has been shown to evolve in response to changes in the environment. However, whether and how subtle differences in musculoskeletal anatomy at the intraspecific level can lead to significant changes in performance and ecology remains unclear. Here, we tested for the evolutionary covariation between diet, bite force, jaw muscle architecture, and the shape of the cranium and mandible in insular lizard populations of the species *Podarcis melisellensis* and *Podarcis sicula*. We found strong patterns of covariation between resource use, bite force, and jaw muscle architecture. Depending on the type of food consumed (plants, hard prey) different muscle groups were differentially developed and associated with changes in the cranium and mandible shape. Whereas the covariation between bite force and anatomy was explained primarily by allometry, subtle allometry-free shape changes appear to take place in response to space and mechanical constraints associated with variation in muscle architecture. Our findings suggest that diet is an important factor driving the evolution of phenotypic diversity among populations of insular *Podarcis* lizards resulting in distinct changes in skull shape and jaw muscle architecture in response to the functional constraint imposed by the food items consumed.

**Keywords:** bite force, geometric morphometrics, head shape, intraspecific variation, island, lizards, resource use.

## INTRODUCTION

Any biological structure is the result of the interplay between the phylogenetic heritage of the organism, its function, and its development (D'Arcy Thompson, 1942; Gould & Lewontin, 1979; Goodwin & Trainor, 1980; Pigliucci & Kaplan, 2000). The morphology of an organism thus reflects the constraints imposed by the physical characteristics of the environment (Sagnes et al. 1997; Fish, 1998; Fish et al. 2008; Segall et al. 2019; Hedenström, 2002; Altshuler et al. 2015; Hedenström & Johansson, 2015) within the limits imposed by its genetic and developmental repertoire. Moreover, functional and constructional trade-offs may limit the expression of a given phenotype (Cheverud, 1982; Barel et al. 1989; Herrel et al. 2009). Consequently, complex integrated systems such as the vertebrate feeding or locomotor systems may not represent mechanically optimized structures (Zweers, 1979; Wake & Roth, 1989).

The skull has been studied extensively because it appears to be under strong selective pressure. Indeed, it fulfills many essential tasks including feeding, protection of the sensory organs and the brain, interacting with conspecifics or other species, and locomotion (Wake, 2003; Herrel et al. 2007). Consequently, the vertebrate skull likely evolves in response to a variety of factors including physical constraints (Segall et al. 2020), locomotion (Roscito & Rodrigues, 2010; Rodrigues et al. 2015; Da Silva et al. 2018), activity pattern (Martin & Ross, 2005), and foraging strategies (Reilly, Miles & McBrayer, 2007). The skull is composed of multiple bones which protect vital organs like the brain and the major sensory organs, provide attachment areas for the masticatory muscles, and resist the external forces generated during feeding or locomotion. Bone is a living tissue that is remodeled by the magnitude and the direction of the forces it experiences (Currey, 2002; Renaud et al. 2010). Consequently, it can be expected that the shape of cranium and mandible are strongly integrated with the jaw musculature (Fabre et al. 2014a; Cornette et al. 2015; Fabre et al. 2018).

Here, we studied insular populations of two species of lacertid lizards, *Podarcis melisellensis* and *Podarcis sicula*, in order to understand the relationships between skull shape, muscle architecture, bite force, and diet. Insular systems have been suggested to impose strong ecological constraints, thus favoring the emergence of adaptive responses in morphology (Baeckens & Van Damme, 2020). Moreover, insular systems provide relatively simple and replicated ecosystems allowing the drivers of variation in form and function to be teased apart (Losos, 2009; Losos & Ricklefs, 2009; Kueffer, Drake & Fernandez-Palacios, 2014). A previous study (Taverne et al. 2019) highlighted variation in the diet of insular populations of *Podarcis* lizards living on small islands in the Adriatic. These populations range from insectivorous to

omnivorous in the smallest and most depauperate environments, resulting in strong selection on morphology. Additionally, a recent study demonstrated that the inclusion of mechanically resistant items in the diet (i.e. plant material and hard prey) as well as island remoteness are important drivers of variation in bite force in these lizards (Taverne et al. 2020). Variation in bite force is partly driven by variation in head shape (Herrel et al. 2001, 2010; Verwajen et al. 2002; Lappin et al. 2006; Huyghe et al. 2009; Wittorski et al. 2016), as taller and wider heads likely provide more space for muscles (Herrel et al. 2007). However, relatively weak correlations between bite force and external head dimensions were detected in these insular *Podarcis* lizards (Taverne et al. 2020), suggesting that variation in bite force is probably driven more by variation in muscle architecture. Additionally, subtle morphological differences between populations are likely not quantifiable through external and linear measurements (Lappin & Husak, 2005; Fabre et al. 2014b). Three-dimensional geometric morphometrics (Bookstein, 1997; Klingenberg, 2002, 2011; Gunz et al. 2005; Kaliontzopoulou, 2011; Adams, 2013) represents a powerful alternative to linear measurements for quantifying subtle morphological variation, and determining how it relates to variation in performance and diet. Despite the availability of this tool, surprisingly few studies have quantified intraspecific morphological variation in skull shape in association with variation in muscles and bite force (but see Herrel et al. 2007; Fabre et al. 2014a). Even fewer have attempted to quantify the intraspecific variation in skull shape in 3D in relation to variation in muscle architecture and bite force (but see Cornette, Tresset & Herrel, 2015).

Here, we investigated the relationships between diet, bite force, muscle architecture, and the three-dimensional morphology of the skull in 139 specimens across 16 populations of two species of *Podarcis* lizards. We first tested for the covariation between muscle architecture, the three-dimensional morphology of both the cranium and mandible, and bite force. Next, we tested whether cranial shape and musculature co-vary with diet. We predicted that: 1) the increase in bite force will be associated with an increase in the cross-sectional area of the adductor muscles; if this is the case, then 2) the shape of the skull should be modified either by enlarging attachment areas, or by showing shape variation in regions that need to withstand feeding or muscle loads; 3) jaw musculature, skull shape and mandible shape will reflect dietary specialization. Specifically, we predict that constraints associated with the ingestion of plant material and hard prey will impact jaw muscle architecture and skull shape, albeit in different ways.

## MATERIAL AND METHODS

### *Specimens, ecological, and bite force data*

The 16 populations of interest were sampled across 14 islands in the Adriatic and two mainland sites. Adult lizards were captured by noose or by hand at the end of the summer of 2016. In total, 455 specimens were captured (Table S1). All individuals were stomach flushed right after capture using a syringe with ball-tipped steel needle (Herrel et al. 2006). Stomach contents were preserved in individual vials containing a 70% aqueous ethanol solution and analyzed as described in Taverne et al. (2019). *In-vivo* bite force was measured for all individuals as described by Taverne et al (2020). In brief, we made lizards bite on the plates of a bite set-up containing an isometric Kistler force transducer (type 9203) connected to a Kistler charge amplifier (type 5995, Kistler Inc., Winterthur, Switzerland; see Herrel et al. 1999 for a detailed description of the set-up) while standardizing gape and bite point.

### *CT scanning*

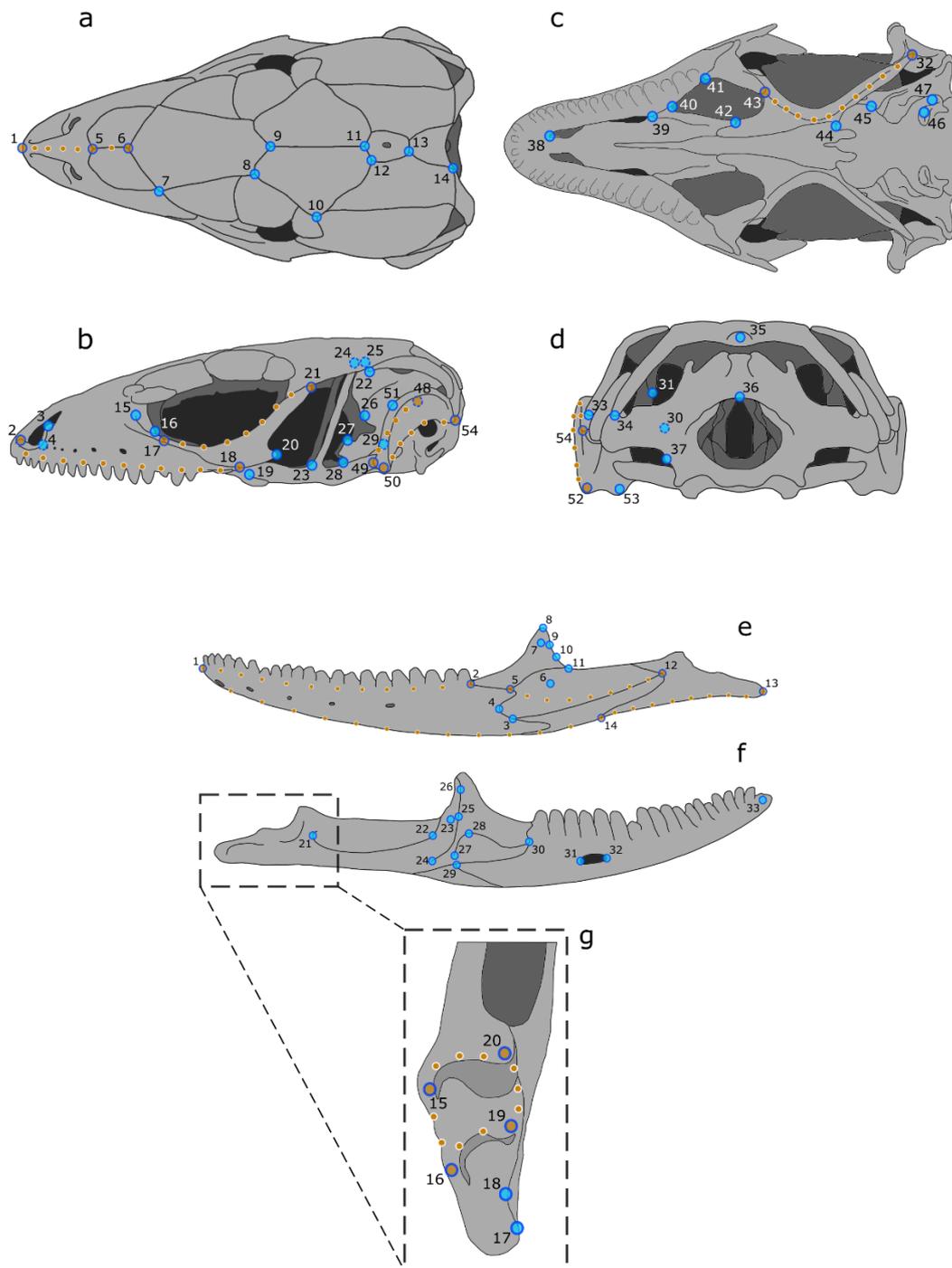
We sacrificed five male and five female lizards of each population (where authorized; see Table S1) by means of an intramuscular injection of pentobarbital. Lizards were fixed in a 10% aqueous formaldehyde solution for 48h, rinsed and transferred to a 70% ethanol solution. Specimens were scanned using an X-Tek HMX 160  $\mu$ CT system (Nikon, X-Tek Systems Ltd, UK) at a voxel size of 24.90  $\mu$ m with the following parameters: X-ray voltage, 90 kV; X-ray intensity, 70  $\mu$ A; exposure time, 2000 ms; number of projections, 2500. Scans were segmented using Avizo 9.0 (Thermo Fischer Scientific) and 3D surfaces of the cranium and mandible were reconstructed and exported separately.

### *Geometric morphometrics*

Anatomical landmarks were placed on the left side of the skull and mandible in Idav Landmark 3.6 (Institute for Data Analysis and Visualisation, University of California, Davis). Each hemi-mandible was defined by 33 anatomical landmarks, and each half of the cranium by 47 landmarks (Table 1). In addition, 54 and 49 semi-landmarks on curves were digitized on the cranium and mandible, respectively (Figure 1). Sliding semi-landmarks were projected onto the surface using a thin-plate spline deformation (Gunz & Mitteroecker, 2013) and slid. Then, three iterations of thin-plate spline

**Table 6:** List of the anatomical landmarks

Skull	LM n°	Mandible
Anterior tip of the premaxillar	1	Anterior tip of the dentary
Most medial anterior part of nasal opening	2	Antero-lateral tip of the coronoid
Dorsal point of nasal at the midline	3	Antero-lateral junction between the angular and the surangular
Lateral dorsal protuberance of nasal	4	Antero-lateral tip of the surangular
Anterior end of the joint between the internasal scales	5	Junction between the dentary, the coronoid and the surangular
Anterior corner of the frontal scale	6	Posterior border of the angular foramen below the coronoid
Antero-lateral corner of the frontal scale	7	Dorso-lateral edge of the coronoid
Postero-lateral corner of the frontal scale	8	Dorsal tip of the coronoid
Posterior corner of the frontal scale	9	Dorsal posterior most constriction of the coronoid
Lateral corner of the fronto-parietal scale	10	Mid distance between landmarks 9 and 11
Anterior corner of the interparietal scale	11	Posterior junction between the coronoid and the surangular
Antero-lateral corner of the interparietal scale	12	Junction between the surangular, the angular and the articular
Postero-lateral corner of the interparietal scale	13	Posterior tip of the retroarticular process
Most post. part of the border between parietal & occipital scales	14	Antero-ventral junction between the angular and the articular
Junction between lacrimal, maxillar and prefrontal bones	15	Antero-lateral corner of the articular surface
Ventro-medial tip of the frontal bone	16	Postero-lateral corner of the articular surface
Anterior tip of jugal	17	Medial edge of the retroarticular process
Posterior tip of the maxillar, at the junction with the jugal	18	Maximum of curvature between the points 17 and 19
Antero-lateral tip of the pterygoid bordering the ectopterygoid	19	Postero-medial corner of the articular surface
Posterior tip of the jugal	20	Antero-medial corner of the articular surface
Dorsal tip of the jugal	21	Posterior edge of the adductor fossa
Anterior tip of the squamosal	22	Anterior edge of the adductor fossa
Anterior part of the junction between epipterygoid and pterygoid	23	Hollow between the post. and the medial ridges of the coronoid
Dorsal tip of the epipterygoid	24	Postero-ventral tip of the medial ridge of the coronoid
Maximum of curvature of the alar process of prootic	25	Dorsal tip of the medial ridge of the coronoid
Maximum of curvature of the anterior semi-circular canal	26	Dorso-medial tip of the coronoid
Anterior tip of the alar process of sphenoid	27	Antero-ventral tip of the medial ridge of the coronoid
Dorsal tip of the alar process of sphenoid	28	Max. of curvature of the ventro-medial hollow of the coronoid
Lateral maximum of curvature of the crista prootica	29	Junction between the prearticular, the angular and the splenial
Medial tip of the jugal, at the junction with the ectopterygoid	30	Antero-medial tip of the coronoid
Ventral tip of the postorbital	31	Posterior edge of the Meckelian foramen
Posterior tip of the pterygoid	32	Anterior edge of the Meckelian foramen
Posterior tip of the squamosal	33	Dorso-anterior tip of the dentary
Posterior tip of the paroccipital process of the parietal	34	
Posterior most point of the parietal at the midline	35	
Maximum of curvature of the posterior ridge of the occipital	36	
Ventral bead surrounding the fenestra ovalis	37	
Junction between the vomer and the premaxillar	38	
Anterior junction between the palatin and the maxillar	39	
Posterior junction between the palatin and the maxillar	40	
Anterior tip of the ectopterygoid, at the junction with the maxillar	41	
Posterior tip of the palatin, at the junction with the pterygoid	42	
Postero-medial tip of the ectopterygoid bordering with the pterygoid	43	
Anterior tip of the basiptyergoid process	44	
Posterior tip of the basiptyergoid process	45	
Lateral process of the basioccipital	46	
Lateral process of the basioccipital	47	
Top of the medial parasagittal bead of the quadrate	48	
Antero-ventro-medial tip of the quadrate	49	
Antero-ventro-medial tip of the quadrate	50	
Maximum of curvature of the anterior face of the quadrate	51	
Postero-ventro-lateral tip of the quadrate	52	
Postero-ventro-medial tip of the quadrate	53	
Postero-dorsal tip of the quadrate bordering the supratemporal	54	



**Figure 1:** illustration of the landmarks taken to quantify the shape of the cranium and mandible. Large blue circles represent anatomical landmarks and small orange circles represent sliding landmarks on curves (a-b-c-d: dorsal, left lateral, ventral, caudal views of the skull. e-f-g: left lateral and medial views of the left mandible, and dorsal focus on the retro-articular process).

relaxation were performed against a Procrustes consensus, using the library “Morpho” (Schlager, 2013). Anatomical landmarks and curves of the skull were mirrored across the sagittal plane (“mirrorfill” function from “paleomorph” package) (Cardini, 2016; 2017).

### **Musculature**

After scanning, cranial muscles were dissected on the left side of the skull of each specimen, blotted dry and weighed using a digital balance (Mettler AE100;  $\pm 0.1$  mg). Muscle volume was obtained by dividing muscle mass by density ( $1.06 \text{ g.cm}^{-3}$ ; Mendez & Keys 1960). Muscles were immersed in an aqueous solution of nitric acid (30%) for 20 to 24 hours to digest the connective tissues and to separate muscle fibers. Muscles were then transferred into a 50% aqueous glycerol solution to stop the reaction. Approximately 10 muscle fibers per muscle were randomly selected, and drawn using a camera lucida mounted on a Leica binocular scope. Drawings including a scale bar were scanned and muscle fiber lengths were measured using Image J 1.52 (National Institutes of Health, USA). The physiological cross-sectional area (PCSA) of each muscle was calculated by dividing muscle volume by the mean fiber length. We identified 12 jaw muscle bundles representing five functional groups. The jaw openers included the *m. depressor mandibulae* (mDM) only. The group of the external adductors gathered *m. adductor mandibulae externus pars superficialis anterior* (mAMESA) and *posterior* (mAMESP), *pars medialis* (mAMEM) and *profundus* (mAMEP). The *M. adductor mandibulae posterior* (mAMP) was considered part of this group although it is not an external adductor *sensu stricto*. The group of the pseudotemporalis was composed of *m. pseudotemporalis superficialis* (mPSTS) and *profundus* (mPSTP). The pterygoids included *m. pterygoideus pars lateralis* (mPTL) and *medialis* (mPTM), while the constrictor dorsalis muscles encompassed *m. levator pterygoidei* (mLPT) and *m. protractor pterygoidei* (mPPT).

### **Statistical analyses**

All statistical analyses were performed using R (R Core Team, 2020). Bite force and muscle data were log-10 transformed, proportions were arcsine-transformed, and the homogeneity of variances and normality of the distribution of the residuals were verified using Bartlett and Shapiro tests, respectively. The effect of sex and species on the muscle architecture (i.e. muscle mass, fiber length, and PCSA of each muscle group) was investigated through a two-way multivariate analysis of covariance (MANCOVA) with snout-vent length (SVL) as co-variable. Two additional MANCOVAs with SVL as co-variable were performed to test for differences between sexes and localities for each species separately. Similarly, three MANOVAs were performed to examine the effect of sex and species, and the effect of sex and locality on the mandible and skull shape. The latter analyses focused on the axes of a principal component analysis (PCA) cumulatively explaining at least 85% of the total variance.

Next, muscle and morphological data were averaged by population and by sex. Relationships between each muscle variable (mass, fiber length and PCSA), bite force, the proportion of plants consumed, the proportion of hard prey, and mean prey hardness were investigated for males and females separately. To do so, stepwise regressions were performed either on raw or on residual muscular data (generated by regressing traits against SVL) using the function “stepAIC”, or using the function “phylostep” (“phylolm” package) when accounting for phylogeny. Similarly, the relationships between mandible or skull shape and muscle variables (excluding fiber length), bite force and diet were assessed by running a two-block partial least squares regressions using the function “pls2B” (“morpho” package), or using the function “phylo.integration” (“geomorph” package) when accounting for phylogeny. For analyses including phylogeny we used a previously published tree describing the relationships between the populations in this study system (Taverne et al. 2020).

The contribution of allometry to the observed shape changes was estimated using a Procrustes ANOVA with permutation (“procD.lm” function, “geomorph” package) which tested the relationship between the Procrustes coordinates and the centroid size of each specimen (the “procD.pgls” function was used when including phylogeny). Finally, additional two-block partial least square regressions were used to investigate the relationships between the residual muscular variables (obtained by a regression on SVL), diet variables, and the allometry-free (AF) mandible and skull shape obtained with the functions “CAC” and “showPC” (“morpho” package). The shape changes associated were extracted using the function “tps3d” (“morpho” package).

## RESULTS

### *Inter-population variability in muscle architecture and shape*

The two-way MANCOVAs revealed that muscle architecture differed between sexes (Wilk's Lambda = 0.28;  $F_{14,120} = 21.85$ ;  $P < 0.001$ ) and species (Wilk's Lambda = 0.49;  $F_{14,120} = 8.77$ ;  $P < 0.001$ ). The effect of snout-vent length was also significant (Wilk's Lambda = 0.34;  $F_{14,120} = 16.50$ ;  $P < 0.001$ ). A significant interaction between sex and species was also detected (Wilk's Lambda = 0.78;  $F_{14,120} = 2.38$ ;  $P = 0.006$ ) prompting us to run analyses for each species separately. A subsequent MANCOVA found significant sex (Wilk's Lambda = 0.11;  $F_{14,50} = 29.10$ ;  $P < 0.001$ ), locality (Wilk's Lambda = 0.005;  $F_{14,50} = 3.17$ ;  $P < 0.001$ ), and SVL effects (Wilk's Lambda = 0.64;  $F_{14,50} = 2.01$ ;  $P = 0.036$ ) for *P. melisellensis*. However, no interaction between sex and locality (Wilk's Lambda = 0.22;  $F_{14,50} = 1.30$ ;  $P = 0.080$ ) was detected. Similarly, for *P. sicula* the effects of sex (Wilk's Lambda = 0.24;  $F_{14,32} = 7.33$ ;  $P < 0.001$ ), locality (Wilk's Lambda = 0.013;  $F_{14,32} = 2.76$ ;  $P < 0.001$ ), and SVL (Wilk's Lambda = 0.35;  $F_{14,32} = 4.24$ ;  $P < 0.001$ ) were significant. The interaction between sex and locality was not significant (Wilk's Lambda = 0.19;  $F_{14,32} = 0.96$ ;  $P = 0.61$ ), however.

The MANOVA on mandible shapes performed on the first 12 axes of the PCA revealed significant effects of sex (Wilk's Lambda = 0.204;  $F_{1,12} = 38.42$ ;  $P < 0.001$ ), species (Wilk's Lambda = 0.18;  $F_{1,12} = 48.00$ ;  $P < 0.001$ ), and the interaction between sex and species (Wilk's Lambda = 0.83;  $F_{1,12} = 2.08$ ;  $P = 0.023$ ). For *P. melisellensis*, a MANOVA performed on the first 10 axes of the PCA describing mandible shape showed an effect of sex (Wilk's Lambda = 0.10;  $F_{1,10} = 50.08$ ;  $P < 0.001$ ) and locality (Wilk's Lambda = 0.001;  $F_{9,90} = 7.39$ ;  $P < 0.001$ ), but not their interaction (Wilk's Lambda = 0.35;  $F_{5,50} = 1.32$ ;  $P = 0.090$ ). Similarly, for *P. sicula* a MANOVA on the 12 first axes of the PCA detected significant sex (Wilk's Lambda = 0.16;  $F_{1,12} = 16.03$ ;  $P < 0.001$ ), locality (Wilk's Lambda = 0.007;  $F_{5,60} = 5.35$ ;  $P < 0.001$ ) but not interaction effects (Wilk's Lambda = 0.33;  $F_{5,60} = 0.77$ ;  $P = 0.88$ ). The MANOVA performed on the first 13 axes of the PCA describing skull shape showed an effect of sex (Wilk's Lambda = 0.19;  $F_{1,13} = 52.50$ ;  $P < 0.001$ ), species (Wilk's Lambda = 0.15;  $F_{1,13} = 41.218$ ;  $P < 0.001$ ), as well as the interaction between sex and species (Wilk's Lambda = 0.73;  $F_{1,13} = 3.47$ ;  $P < 0.001$ ). In *P. melisellensis*, the MANOVA performed on the first 11 axes of the PCA showed an effect of sex (Wilk's Lambda = 0.053;  $F_{1,11} = 88.39$ ;  $P < 0.001$ ) and locality (Wilk's Lambda = 0.010;  $F_{9,99} = 7.35$ ;  $P < 0.001$ ), but not their interaction (Wilk's Lambda = 0.33;  $F_{5,55} = 1.24$ ;  $P = 0.138$ ). The MANOVA performed on the 11 first axes of the PCA in *P. sicula*

detected significant sex (Wilk's Lambda = 0.11;  $F_{1,11} = 26.81$ ;  $P < 0.001$ ), locality (Wilk's Lambda = 0.004;  $F_{5,55} = 7.53$ ;  $P < 0.001$ ), and interaction effects (Wilk's Lambda = 0.17;  $F_{5,55} = 1.50$ ;  $P = 0.026$ ).

### **Allometry**

In females, allometry explained 12.1 and 18.0 % of the total variation in mandible and cranium shape, respectively (all  $P < 0.001$ ). In males, allometry explained 9.6 and 7.1 % of the total variation in mandible and cranial shape (all  $P < 0.001$ ). When accounting for phylogeny, allometries were no longer significant (all  $P > 0.05$ ).

### **Relationships between muscle architecture, bite force and ecology**

The physiological cross-sectional area (PCSA) of the jaw muscles explained variation in bite force (Table 2). In females, greater absolute ( $R^2 = 0.85$ ,  $P < 0.001$ ) and residual ( $R^2 = 0.71$ ,  $P = 0.001$ ) bite force was associated with relatively stronger external adductors and pseudotemporalis muscles. In males an increase in absolute bite force ( $R^2 = 0.30$ ,  $P = 0.04$ ) was associated with stronger external adductors and weaker pterygoid muscles. These results held when accounting for phylogeny (Table 2).

The proportion of plants consumed was also significantly correlated with the absolute and relative PCSA of jaw adductor muscles in both females and males (absolute females:  $R^2 = 0.54$ ,  $P = 0.012$ ; males:  $R^2 = 0.53$ ,  $P = 0.013$ ; residual females:  $R^2 = 0.42$ ,  $P = 0.013$ ; males  $R^2 = 0.42$ ,  $P = 0.037$ ). In females, a higher proportion of plants in the diet was associated with relatively stronger pseudotemporalis muscles and weaker pterygoids. In males an increase in the amount of plant matter in the diet was associated with relatively stronger jaw openers and external adductors, and relatively weaker pterygoids and constrictor dorsalis muscles. These results were largely upheld when accounting for phylogeny (Table 2).

The multiple regressions also revealed a significant correlation between the proportion of hard prey items consumed and the PCSA of the jaw muscles in females ( $R^2 = 0.43$ ,  $P = 0.033$ ) and the residual PCSA in both females and males (females:  $R^2 = 0.44$ ,  $P = 0.030$ ; males:  $R^2 = 0.57$ ,  $P = 0.008$ ). In females, a greater proportion of hard prey was associated with stronger pterygoids and relatively weaker external adductors. In males, the same pattern was observed but pseudotemporalis muscles also had a higher PCSA. Despite some small differences, the results of these regressions remained consistent

**Table 7:** Results of the regressions between bite force (BF), the proportion of plants (PLANT), the proportion of hard prey item (HARD), and the PCSA of the 5 muscle groups (DM: jaw opener, ADD: external adductors, PSEU: pseudotemporalis, PTG: pterygoids, CONST: constrictor dorsalis muscles). *s*: slope,  $\beta$ : standardized coefficient,  $R^2$ : regression coefficient, *P*: p-value. Bold values indicate retained models. Values in blue and red indicate a negative and a positive correlation, respectively. Note that the rest of the results, concerning muscle mass and fiber length, is compiled in the Suppl. Material S2.

		Females				Males				
		Raw		Residuals		Raw		Residuals		
No correction for phylogeny	Model	<b>P &lt; 0.001</b> <b>R<sup>2</sup> = 0.854</b>		<b>P = 0.001</b> <b>R<sup>2</sup> = 0.71</b>		<b>P = 0.04</b> <b>R<sup>2</sup> = 0.3</b>		P = 0.084 R <sup>2</sup> = 0.212		
	BF	DM								
		ADD	<i>s</i> = 2.838 $\beta$ = 0.35	<i>s</i> = 3.06 $\beta$ = 0.16	<i>s</i> = 1.629 $\beta$ = 0.16					
		PSEU	<i>s</i> = -1.384 $\beta$ = -0.20	<i>s</i> = -1.212 $\beta$ = -0.07						
		PTG			<i>s</i> = -1.231 $\beta$ = -0.13					
		CONST								
No correction for phylogeny	Model	<b>P = 0.012</b> <b>R<sup>2</sup> = 0.541</b>		<b>P = 0.013</b> <b>R<sup>2</sup> = 0.422</b>		<b>P = 0.013</b> <b>R<sup>2</sup> = 0.53</b>		<b>P = 0.037</b> <b>R<sup>2</sup> = 0.423</b>		
	PLANT	DM			<i>s</i> = 2.635 $\beta$ = 0.23	<i>s</i> = 2.524 $\beta$ = 0.16				
		ADD			<i>s</i> = 7.716 $\beta$ = 0.76	<i>s</i> = 7.479 $\beta$ = 0.40				
		PSEU	<i>s</i> = 7.550 $\beta$ = 1.07	<i>s</i> = 3.966 $\beta$ = 0.24						
		PTG	<i>s</i> = -6.912 $\beta$ = -0.88		<i>s</i> = -6.656 $\beta$ = -0.70	<i>s</i> = -6.713 $\beta$ = -0.38				
		CONST			<i>s</i> = -2.644 $\beta$ = -0.23	<i>s</i> = -2.578 $\beta$ = -0.19				
No correction for phylogeny	Model	<b>P = 0.033</b> <b>R<sup>2</sup> = 0.425</b>		<b>P = 0.030</b> <b>R<sup>2</sup> = 0.439</b>		P = 0.113 R <sup>2</sup> = 0.175		<b>P = 0.008</b> <b>R<sup>2</sup> = 0.574</b>		
	HARD	DM								
		ADD	<i>s</i> = -4.607 $\beta$ = -0.56	<i>s</i> = -4.455 $\beta$ = -0.23			<i>s</i> = -3.955 $\beta$ = -0.21			
		PSEU					<i>s</i> = 2.729 $\beta$ = 0.15			
		PTG	<i>s</i> = 4.167 $\beta$ = 0.53	<i>s</i> = 3.592 $\beta$ = 0.15			<i>s</i> = 3.485 $\beta$ = 0.20			
		CONST					<i>s</i> = -0.703 $\beta$ = -0.05			
With correction for phylogeny	Model	<b><math>\sigma^2 &lt; 0.001</math></b>		<b><math>\sigma^2 = 0.001</math></b>		<b><math>\sigma^2 = 0.001</math></b>		<b><math>\sigma^2 = 0.001</math></b>		
	BF	DM								
		ADD	<i>s</i> = 3.451 <i>p</i> = 0.003	<i>s</i> = 3.493 <i>p</i> = 0.003	<i>s</i> = 2.568 <i>p</i> = 0.013	<i>s</i> = 3.193 <i>p</i> = 0.007				
		PSEU	<i>s</i> = -2.065 <i>p</i> = 0.023	<i>s</i> = -2.097 <i>p</i> = 0.024						
		PTG			<i>s</i> = -1.934 <i>p</i> = 0.029	<i>s</i> = -2.009 <i>p</i> = 0.020				
		CONST								
	With correction for phylogeny	Model	<b><math>\sigma^2 = 0.011</math></b>		$\sigma^2 = 0.021$		<b><math>\sigma^2 = 0.008</math></b>		<b><math>\sigma^2 = 0.009</math></b>	
		PLANT	DM			<i>s</i> = 1.768 <i>p</i> = 0.052	<i>s</i> = 1.395 <i>p</i> = 0.132			
			ADD			<i>s</i> = 7.384 <i>p</i> = 0.015	<i>s</i> = 5.595 <i>p</i> = 0.049			
			PSEU	<i>s</i> = 5.709 <i>p</i> = 0.043						
			PTG	<i>s</i> = -3.333 <i>p</i> = 0.156		<i>s</i> = -5.523 <i>p</i> = 0.027	<i>s</i> = -4.222 <i>p</i> = 0.101			
			CONST			<i>s</i> = -2.771 <i>p</i> = 0.011	<i>s</i> = -2.797 <i>p</i> = 0.017			
With correction for phylogeny	Model	<b><math>\sigma^2 = 0.003</math></b>		<b><math>\sigma^2 = 0.003</math></b>		<b><math>\sigma^2 = 0.008</math></b>		<b><math>\sigma^2 = 0.002</math></b>		
	HARD	DM	<i>s</i> = -1.891 <i>p</i> = 0.025	<i>s</i> = -1.852 <i>p</i> = 0.037			<i>s</i> = -0.964 <i>p</i> = 0.066			
		ADD	<i>s</i> = -2.840 <i>p</i> = 0.139	<i>s</i> = -2.752 <i>p</i> = 0.104	<i>s</i> = -2.506 <i>p</i> = 0.092	<i>s</i> = -4.177 <i>p</i> = 0.003				
		PSEU			<i>s</i> = 3.245 <i>p</i> = 0.039	<i>s</i> = 4.345 <i>p</i> = 0.002				
		PTG	<i>s</i> = 3.920 <i>p</i> = 0.040	<i>s</i> = 3.951 <i>p</i> = 0.042			<i>s</i> = 2.599 <i>p</i> = 0.004			
		CONST								

when accounting for phylogeny. The correlations between musculature characteristics and mean prey hardness were marginal (Table S2).

### ***Co-variation between ecology, muscle architecture and head shape***

For both sexes of each species, the 2b-PLS revealed a significant covariation between mandible and cranial shapes, muscle PCSA, muscle mass, and the proportion of plants in diet. However, the proportion of plants consumed no longer covaried with head shape for either sex when accounting for phylogeny (Table 3). Head shape in females only also covaried with bite force, and male head shape also covaried with the proportion of hard prey even after phylogenetic correction.

In females only, changes in mandible shape along the axis of the 2b-PLS showed that an increase in bite force was associated with a thickening of the joint surface area, a wider and larger area for the insertion of the external adductors, a more pronounced curvature, and a posterior enlargement of the coronoid (Figure 2). Changes in skull shape were characterized by an increase in the size of the temporal fenestra, which was associated with a more pronounced curvature of the jugal, the pterygoid and quadrate. The tip of the snout was also more elongated, the tip of the epipterygoid and the wings of the prootic were positioned more anteriorly. Similar morphological changes were observed with an increase in the proportion of plants in diet (Figures S12-14).

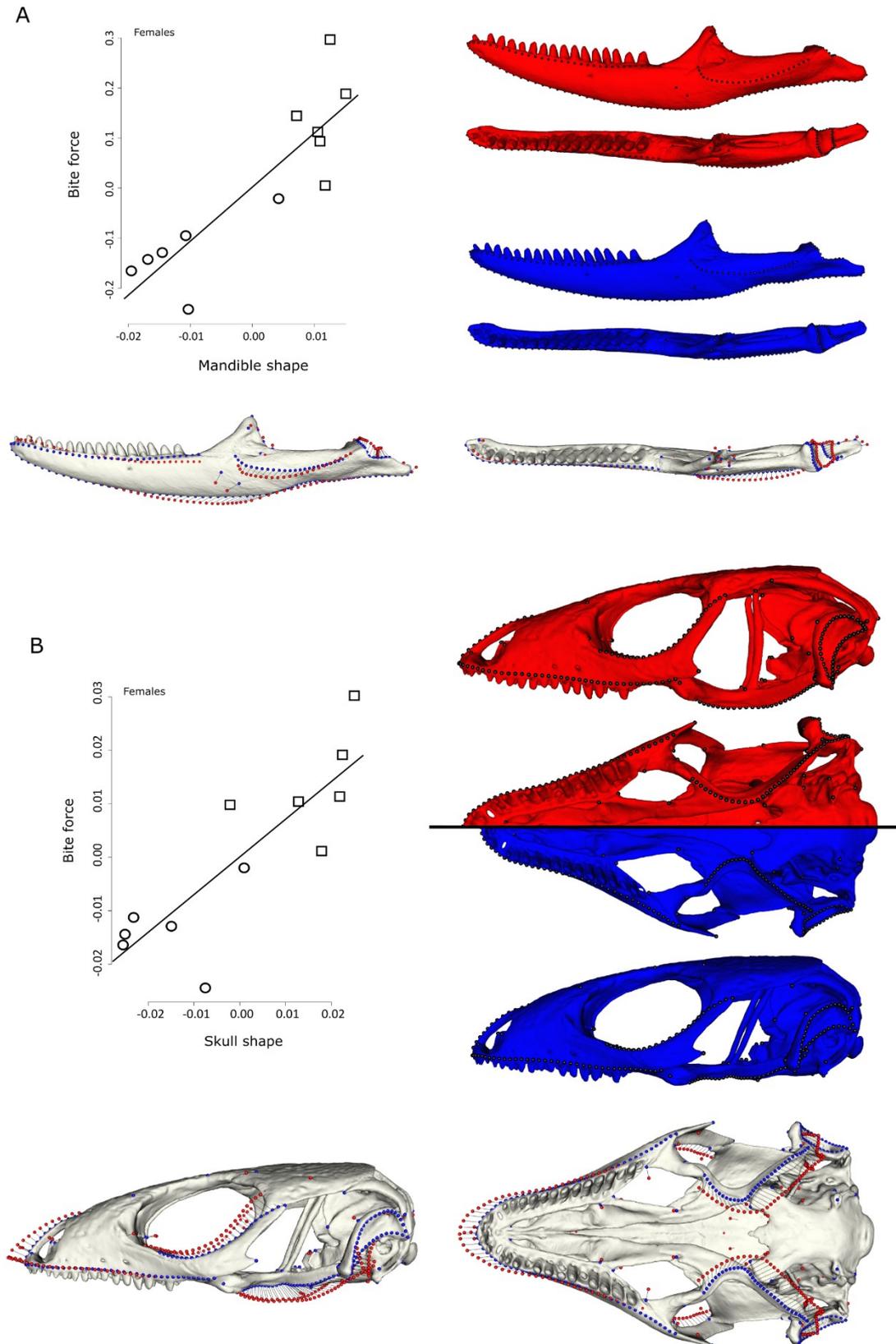
Residual data of muscles variables and bite force against body length only rarely covaried with absolute head shape (Table 3), but often covaried with allometry-free head shape. In females, covariations between residual PCSA, residual muscle mass and allometry-free shape were detected both with and without taking phylogeny into consideration (Table 3). In males, allometry-free shape covaried with residual muscle mass, and with the proportion of plants and hard prey. After phylogenetic correction, allometry-free shape of the mandible covaried with residual PCSA and the proportion of hard prey.

In males, variation in the proportion of hard prey consumed was associated with an increase in mandible robustness, with an enlargement of the coronoid, and with a more pronounced transversal curvature of the mandible (Figure 3). The proportion of hard prey consumed was also associated with a shorter and more rounded cranium, and with an increase in the size of the temporal fenestra resulting from a higher skull vault, a more curved quadrate, and a jugal that was more vertically orientated and positioned more anteriorly. In females, the same pattern was observed for the

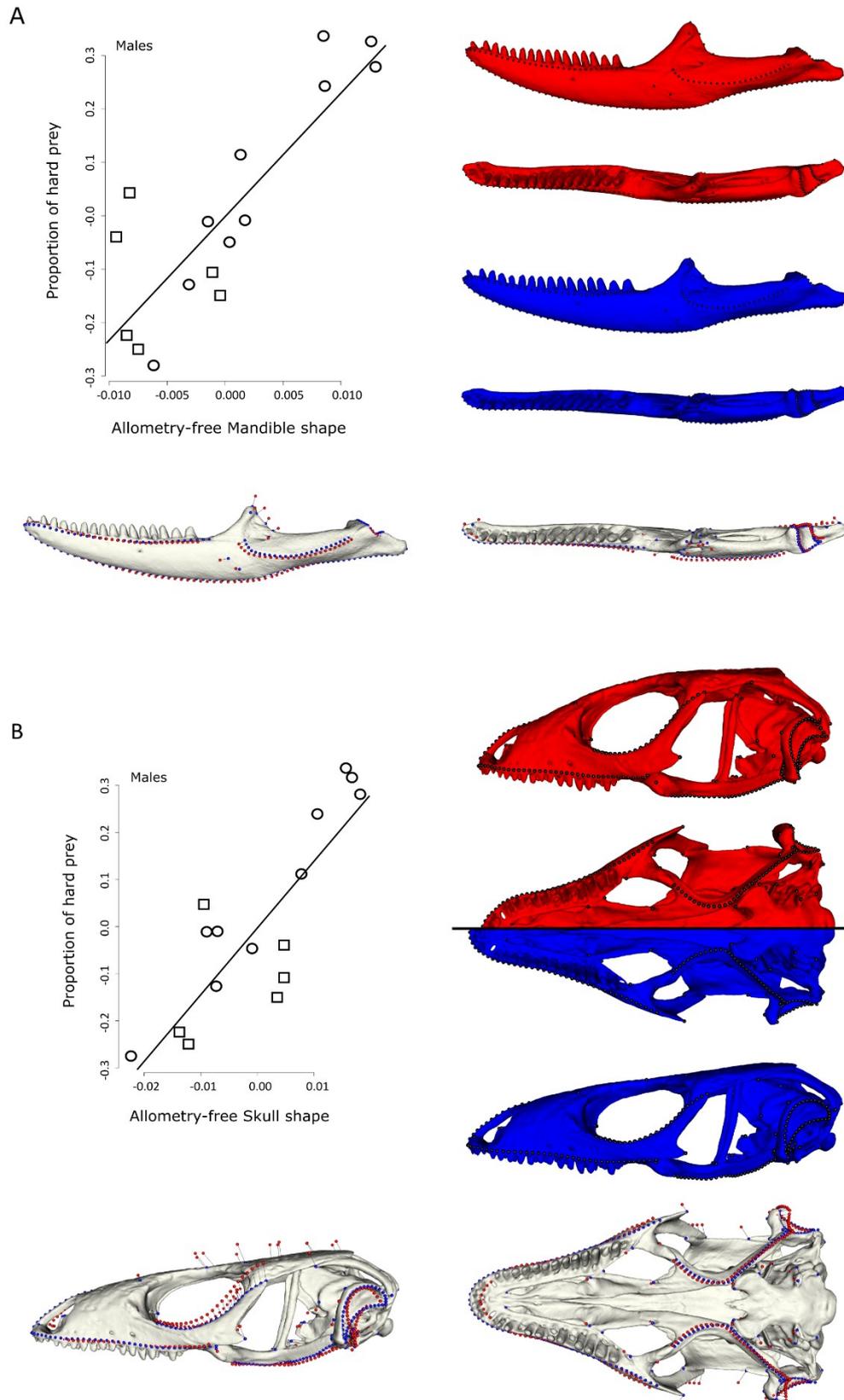
covariation between allometry-free head shape and residual PCSA and residual muscle mass (Figures S18-21).

**Table 8:** Results of the 2b-PLS carried out between the bite force (BF), the muscular data (muscle PCSA and MASS), resource use (PLANT: proportion of plants, HARD: proportion of hard prey items in the diet), and residual data (r) against Raw shapes and Allometry-free shapes. P: P-value, rPLS: coefficient of determination of the PLS axis, % covar: percentage of covariance explained by the PLS axis considered. Bold values are statistically significant ( $P < 0.05$ ).

Dependent variable	Statistic	Without correction for phylogeny				With correction for phylogeny				Morphology
		Females		Males		Females		Males		
		Skull	Mandible	Skull	Mandible	Skull	Mandible	Skull	Mandible	
BF	P	<b>0.004</b>	<b>0.002</b>	0.151	0.342	<b>0.036</b>	0.445	0.398	0.465	vs. Raw Shape
	rPLS	0.831	0.867	0.637	0.655	0.756	0.616	0.598	0.590	
	% covar	100	100	100	100	-	-	-	-	
PCSA	P	<b>0.001</b>	<b>0.001</b>	<b>0.003</b>	<b>0.048</b>	0.051	<b>0.042</b>	<b>0.021</b>	<b>0.039</b>	
	rPLS	0.882	0.913	0.808	0.743	0.787	0.824	0.802	0.766	
	% covar	97.560	97.304	92.055	91.926	-	-	-	-	
MASS	P	<b>0.003</b>	<b>0.001</b>	<b>0.022</b>	0.058	<b>0.001</b>	<b>0.034</b>	0.258	0.141	
	rPLS	0.816	0.889	0.746	0.710	0.918	0.812	0.646	0.695	
	% covar	96.966	97.493	93.309	93.332	-	-	-	-	
PLANT	P	<b>0.031</b>	<b>0.011</b>	0.073	<b>0.019</b>	0.598	0.463	0.696	0.306	
	rPLS	0.708	0.756	0.667	0.846	0.531	0.615	0.522	0.637	
	% covar	100	100	100	100	-	-	-	-	
HARD	P	0.136	0.085	<b>0.004</b>	0.072	0.101	0.141	0.084	<b>0.009</b>	
	rPLS	0.618	0.662	0.892	0.839	0.715	0.719	0.712	0.828	
	% covar	100	100	100	100	-	-	-	-	
rBF	P	0.313	0.196	0.491	0.266	0.148	0.527	0.151	0.402	
	rPLS	0.527	0.576	0.646	0.652	0.688	0.598	0.688	0.612	
	% covar	100	100	100	100	-	-	-	-	
rPCSA	P	0.157	0.135	0.064	0.122	<b>0.006</b>	0.110	0.165	<b>0.016</b>	
	rPLS	0.829	0.688	0.719	0.671	0.863	0.779	0.694	0.794	
	% covar	20.738	72.023	11.816	4.229	-	-	-	-	
rMASS	P	<b>0.040</b>	0.176	0.089	<b>0.030</b>	<b>0.001</b>	0.110	0.166	0.101	
	rPLS	0.712	0.661	0.598	0.800	0.933	0.806	0.685	0.724	
	% covar	28.780	72.237	32.814	33.715	-	-	-	-	
rBF	P	0.318	0.380	0.255	0.125	0.322	0.534	0.108	0.271	vs. Allometry-free Shape
	rPLS	0.824	0.685	0.738	0.642	0.659	0.594	0.720	0.705	
	% covar	100	100	100	100	-	-	-	-	
rPCSA	P	<b>0.022</b>	<b>0.028</b>	0.061	0.080	<b>0.005</b>	0.058	0.132	<b>0.045</b>	
	rPLS	0.704	0.716	0.800	0.648	0.893	0.811	0.722	0.810	
	% covar	13.399	35.257	75.146	5.391	-	-	-	-	
rMASS	P	<b>0.009</b>	<b>0.019</b>	<b>0.003</b>	0.081	<b>0.002</b>	<b>0.047</b>	0.179	0.067	
	rPLS	0.824	0.781	0.607	0.777	0.943	0.851	0.689	0.798	
	% covar	30.617	31.444	36.156	28.263	-	-	-	-	
PLANT	P	0.627	0.311	0.171	<b>0.002</b>	0.535	0.306	0.623	0.512	
	rPLS	0.889	0.720	0.782	0.826	0.604	0.656	0.548	0.650	
	% covar	100	100	100	100	-	-	-	-	
HARD	P	0.543	0.192	<b>0.003</b>	<b>0.013</b>	<b>0.032</b>	0.110	0.082	<b>0.001</b>	
	rPLS	0.846	0.836	0.854	0.847	0.805	0.736	0.730	0.891	
	% covar	100	100	100	100	-	-	-	-	



**Figure 2:** results of the 2b-PLS analysis exploring the covariation between bite force and mandible (A) and cranial (B) shape in females (circles: *P. melisellensis* populations, squares: *P. sicula* populations). Cranial and mandible shapes in blue are associated with low bite force, and cranial and mandible shapes in red are associated with high bite force. Note the differences in the adductor chamber size, snout length, and the ventral curvature of the mandible.



**Figure 3:** results of the 2b-PLS analysis exploring the covariation between the proportion of hard prey and allometry-free mandible (A) and cranial (B) shape in males (circles: *P. melisellensis* populations, squares: *P. sicula* populations). Cranial and mandible shapes in blue are associated with a low proportion of hard items in the diet, and cranial and mandible shapes in red are associated with a high proportion of hard items in the diet. Note the differences in the adductor chamber size, the curvature of the snout, and the increase in the insertion area of the external adductor on the lateral side of the mandible.

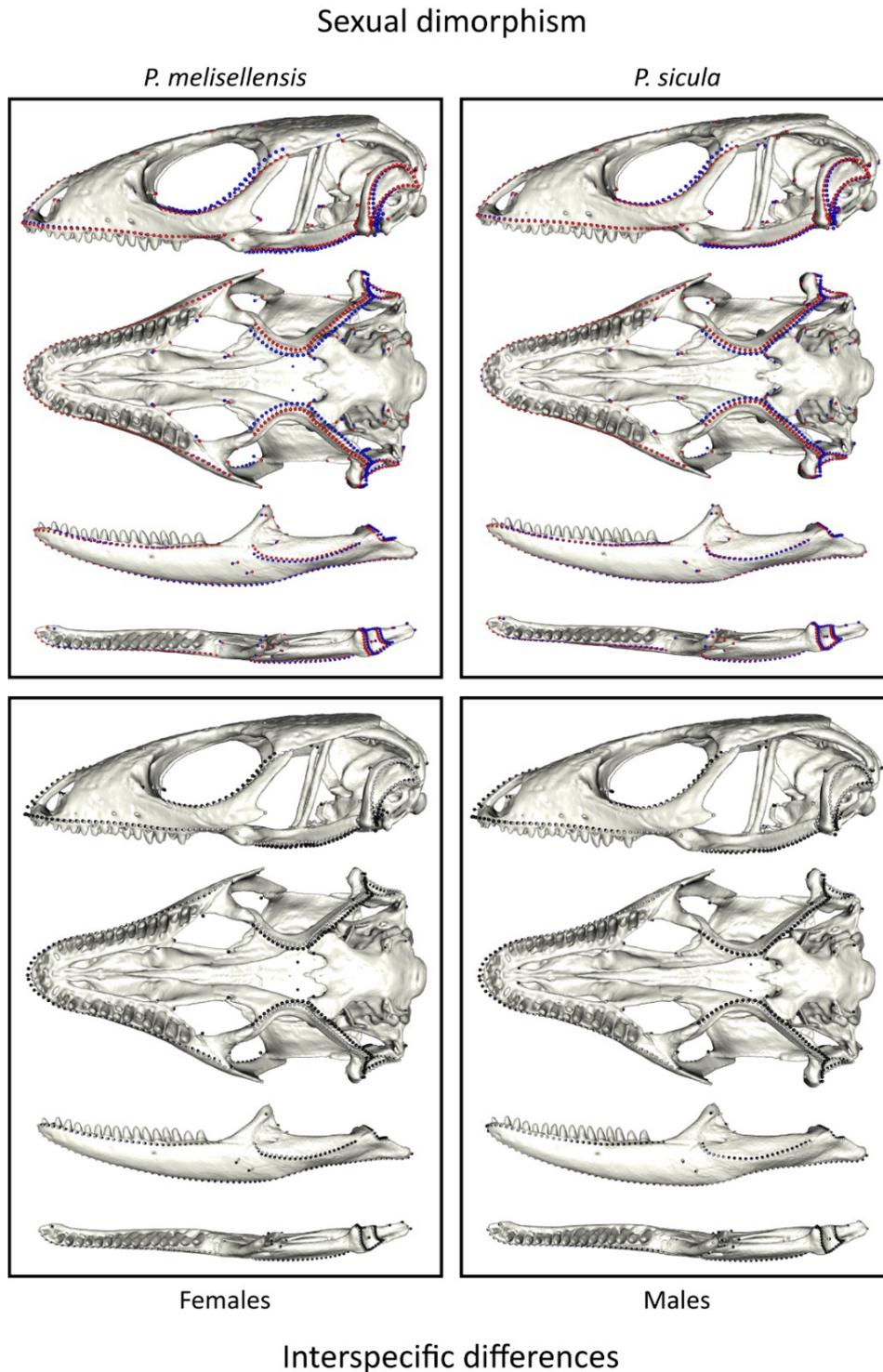
## DISCUSSION

### *Analyses of variance*

Muscle architecture differed significantly between species and sexes. The PCSA of the adductors, the pseudotemporalis, and the pterygoids are greater in males than in females for both species in accordance with the known differences in head size between males and females (Brecko et al. 2008; Sagonas et al. 2014; Lopez-Darias et al. 2015). These muscles are also stronger in *P. sicula* compared to *P. melisellensis*. The latter observation is congruent with the competitive superiority of *P. sicula* as demonstrated previously (Nevo et al. 1972; Downes & Bauwens, 2001; Nikolic et al. 2019). Females of *P. sicula* have heavier jaw openers, adductors, pseudotemporalis and pterygoids than females of *P. melisellensis*. Surprisingly, males of *P. melisellensis* have heavier muscles than the males of *P. sicula*, suggesting that the way to achieve an increase in muscle force differs between the two species. Moreover, muscle architecture varied between localities for both *P. melisellensis* and *P. sicula*. MANOVAs performed on the residuals revealed that in *P. melisellensis* the localities differed in every muscle variable, whereas this was not the case in *P. sicula* (fiber lengths, and the PCSA of the jaw openers and of the adductors significantly varied across the populations only). This implies that in *P. sicula*, changes in the musculature are partly explained by variation in SVL. This is in accordance with the fact that changes in diet are often associated with changes in overall size in lizards (Schoener, 1982; Van Damme, 1999; Cooper & Vitt, 2002; Meiri, 2008).

The MANOVAs performed on mandible and cranial shape showed similar results suggesting strong differences between species, sexes and localities (Figure 4). Individuals of *P. melisellensis* have a more rounded skull than individuals of *P. sicula*, which possess a more elongated skull with a more pointed snout due to a straighter premaxilla, nasal and frontal bones. The mandible in *P. sicula* is more robust than that of *P. melisellensis*, with a narrower and more vertically oriented coronoid. However, males of *P. melisellensis* have a wider lateral attachment area for the external adductors on their mandible than males of *P. sicula*. This morphological peculiarity might enable the heavier adductor muscles in males of *P. melisellensis* to attach. In both species, the differences between males and females are obvious. The adductor chamber is wider in males than in females, mostly because of the shape of the pterygoid whose ventral and medial curvature is more pronounced. The quadrate of the females is less curved than in males and seems to be associated with an anterior translation of the jaw joint. This configuration likely increases the jaw opening in-lever in females, resulting in higher jaw opening

velocity for the capture of more evasive prey, as has been previously suggested for some lizards (Herrel et al. 2001; Herrel, McBrayer & Larson, 2007; Vanhooydonck et al. 2007). The mandible of males is also wider and possesses a more strongly developed coronoid and a larger insertion area for the external adductors.



**Figure 4:** illustration of the shape differences between species (in males and in females) and between males and females (in both species). In the case of a comparison between sexes, the female shape is represented and characterized by red dots, whereas the male shape is indicated by blue dots. In the case of a comparison between species, the shape of *P. melisellensis* is represented and characterized by gray dots, whereas the shape of *P. sicula* is illustrated by black dots.

***Ecology, Musculature and Phylogeny***

We found that muscle PCSA, a proxy for muscle force, is strongly correlated with bite force in the two species studied. Moreover, our analyses suggest that this relationship is not purely allometric, and is retained after taking into account the phylogenetic relationships between populations and species. We therefore interpret the patterns of morphological variation in *P. melisellensis* and *P. sicula* as indicative of adaptive changes in response to local environmental conditions. An increase in bite force was associated with an increase in the absolute and the relative PCSA of the external jaw adductors in both males and females (Table 2), suggesting that increasing the force of this muscle group is the most effective way to induce variation in bite force. This assumption is corroborated by previous studies that pointed out that the external adductor muscles are the primary drivers of variation in bite force (Wittorski et al. 2016). In males, the variation in the contribution of the external adductors is largely the result of the variation in muscle volume; since they are positioned laterally in the head, their volume might be less constrained by other cranial structures than deeper muscle bundles (Rieppel & Gronowski, 1981; Herrel et al. 1998; Herrel et al. 2007).

The investigation of the relationships between musculature and the proportion of plants consumed reveals that in females an increase in the PCSA of pseudotemporalis muscles is linked directly to variation in diet. However, this variation is mostly due to variation in overall size. In contrast, in males an increase in the proportion of plants ingested is always accompanied with an increase in the PCSA of the pseudotemporalis irrespective of variation in size. Interestingly, this is achieved by decreasing fiber length rather than by increasing volume thus possibly allowing to circumvent the spatial packing constraints imposed on this deep muscles. An increase in the proportion of hard prey is associated with an increase in the PCSA of the pterygoids in females, and of the pterygoids and pseudotemporalis in males. The increase in the PCSA of the pterygoids is achieved by increasing muscle volume in females, and a shortening of muscle fibers in males. By contrast, in males the increase in the PCSA of the pseudotemporalis associated with an increase in the proportion of hard prey is driven by an increase in muscle volume. Short muscle fibers appear to constrain jaw gape, and larger muscle volume rather than shorter fiber length is thus expected to be privileged for optimizing bite force at large gape. In addition, the pterygoids are more efficient at generating bite force at large gape as their moment arm increases significantly with gape (Herrel et al. 1999 a,b). Therefore, the presence of larger pterygoids and the maintenance of short muscle fibers in the pseudotemporalis suggest that an increase in the proportion of hard prey accompanies an increase in prey size.

***Ecology, Morphology and Phylogeny***

Our analyses of covariance revealed strong associations between: 1) diet, bite force and muscle architecture, and 2) mandible and cranial shape. However, the fact that residual bite force and muscle variables only rarely showed significant covariation with shape highlights the influence of size, as corroborated by the significant allometry in the shape of the mandible and cranium in both males and females. Yet, residual data showed covariations with allometry-free mandible and cranial shapes, indicating that variation in shape is not explained by allometry alone. Instead, it appears that the covariation between bite force and morphology is explained primarily by size effects and allometry, whereas muscle forces covary with shape corrected for allometry. Thus, shape variation beyond that imposed by overall size variation seems to reflect local constraints imposed by the development of more forceful jaw muscles in these lizards.

The fact that the results are largely unchanged after phylogenetic correction suggests that much of the variation in shape and muscle architecture is driven by local selective regimes in these insular ecosystems. Unexpectedly, the co-variation between morphology and the proportion of plants consumed was no longer significant when including phylogeny in our analyses. This may be due to the fact that populations of *P. sicula* consume significantly more plants than those of *P. melisellensis* (Taverne et al. 2019). Additional populations would need to be sampled to assess the link between skull shape and the proportion of plants in the diet in *P. sicula* only as currently we have too few populations in our data set to do so with confidence. Overall, our results suggest that lizard populations in depauperate environments are under strong selection for bite force. They also suggest that the most effective way to increase bite force is through allometry-dependent changes in muscle architecture and cranial and mandibular shape. Allometry-free morphology covaried with muscle architecture and diet, however. This implies that selection favors bite force in the context of resource use (Taverne et al. 2020) through absolute changes in muscle architecture, whereas variation in cranial and mandibular shape associated with variation in ecology is the result of variation in the forces and space constraints imposed by the jaw musculature. Moreover, the relative investment in functional muscle groups may depend on resource use. Head shape thus appears to reflect diet with different patterns of covariation being associated with the type of resources consumed (plants or hard prey), and the functional constraints these impose upon the head.

**Conclusion**

The associations between head dimensions, bite force, and diet have been well characterized at the interspecific level, but evidence for these relationships remains scarce at the intraspecific or intra-genus level. The weakness or the lack of associations between morphology and diet at the intraspecific level has been linked to intraspecific competition and the need for food partitioning (Schoener, 1967; Herrel et al. 1999), or to sexual selection through male-male combat (Sagonas et al. 2014; Lopez-Darias et al. 2015; Donihue et al. 2016). Using insular *Podarcis* lizards as a model system, we here show that diet can be an important driver of phenotypic diversity at the intraspecific level. Our results highlight the importance of combining information on ecology and performance with quantitative shape analyses to understand how subtle morphological variation relates to patterns of ecological divergence such as dietary shifts. Further efforts should be undertaken to characterize population density and the degree of intraspecific competition in the populations under study to better understand the interplay between form, function and ecology in driving phenotypic variation.



# **Chapter 5**

## **Biomechanics**

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## INTRODUCTION

The first chapter described how populations of lizards in the Adriatic that occupy habitats that differ in size, structure and isolation rely on different food resources. We observed a significant variability among populations and species which differed in the proportion of plant items consumed with populations ranging from insectivorous to omnivorous, but also in the food niche breadth characterized by the functional properties of the food items. Changes in diet and island characteristics subsequently impacted bite force (Chapter 2). In particular, the inclusion of difficult-to-reduce items (gastropods, plant matter, or arthropods with well-developed exoskeletons such as beetles), as well as island isolation were associated with populations of lizards showing greater bite forces. However, external head dimensions explained variation in performance to a relatively small degree suggesting that further efforts should be undertaken to understand the morphological basis of variation in bite force. Considering that the link between performance and fitness is modulated by behavior, we explored how changes in diet affect the way lizards handled prey items. The third chapter provided insights in how animals are able to modulate their jaw kinematics as a behavioral response to changes in prey characteristics like hardness and mobility. The fourth chapter demonstrated that variation in bite force was enabled by differential investment in muscle groups reflecting dietary specialization. Furthermore, muscle architecture covaried with underlying 3D morphology of the cranium and mandible. However, how small changes in the morphology can give rise to large changes in bite force and subsequently diet remains to be answered. What is the functional relationship between morphology and performance? Is variation in morphology solely a by-product of space requirements for muscles, or is it somehow under selection itself? Can the evolution of phenotypic variation be explained by the way it interacts with the mechanical constraints imposed by agents of selection such as diet and competition?

To answer those questions, we need to focus on microevolutionary scales, and a comparison of two populations of *P. sicula* seems particularly appropriate to tackle this task. The two populations of Pod Kopište and Pod Mrčaru, in contrast to the other populations studied (approximately 18.000 years of divergence), have diverged very recently. The time of divergence is known since one of these populations is the result of an experimental introduction that took place in 1971 with animals from Pod Kopište being released on Pod Mrčaru. Whereas the original population remained strictly insectivorous, the introduced population of Pod Mrčaru not only outcompeted the local population of

*P. melisellensis*, but also rapidly shifted towards an omnivorous diet. This dietary shift was accompanied with important anatomical changes. In particular, lizards on Pod Mrčaru evolved caecal valves in their digestive tract, a trait typically observed in herbivorous animals that help them slow down intestinal transit, and thus increases the digestive efficiency (Wehrle et al. 2020). They also have longer digestive tracts and are more robust, and have shorter limbs and larger heads (Vervust, Grbac & Van Damme, 2007). The increase in the proportion of plant items included in their diet is also associated with greater bite forces (Herrel et al. 2008). The social structure also changed and population density drastically increased (Vervust et al. 2009), likely impacting the ecological (resource partitioning) and functional (biting, aggression) constraints.

The aim of this last chapter is to investigate whether the divergence in morphology which occurred between these two populations can be explained by the mechanical constraints imposed by differences in diet or other ecological pressures such as intraspecific competition. To do so, we first quantified differences in skull and mandible shape between lizards of Pod Kopište and Pod Mrčaru. Indeed, although differences were observed in the comparative study included in Chapter 4, we here used an extended set of  $\mu$ CT-scans of these two populations. Second, we performed multibody dynamics analyses (MDA) to understand the functional role of each muscle group, and to understand how changes in morphology resulted in *in silico* bite force changes. Finally, we carried out finite element analyses (FEA) to quantify the mechanical advantage provided by the observed morphological differences between these two populations. We specifically predict that 1) morphological differences observed between the insectivorous and the omnivorous lizards are in line with the previously-described covariation pattern between head shape and the proportion of plants in the diet (Chapter 4), 2) the skull conformation of the individuals of Pod Mrčaru provides an advantage in generating bite force, 3) the skull morphology of the omnivorous population is designed so that it can resist greater amounts of stress and strain, 4) both modelling approaches will reveal that subtle morphological variation reflects the functional constraints imposed upon the feeding system and may thus be the result of selection. A discussion of the results is included in this chapter, which then finishes with a wider and integrative discussion of the results of the present thesis, in the light of this biomechanical modelling study.

## MATERIAL AND METHODS

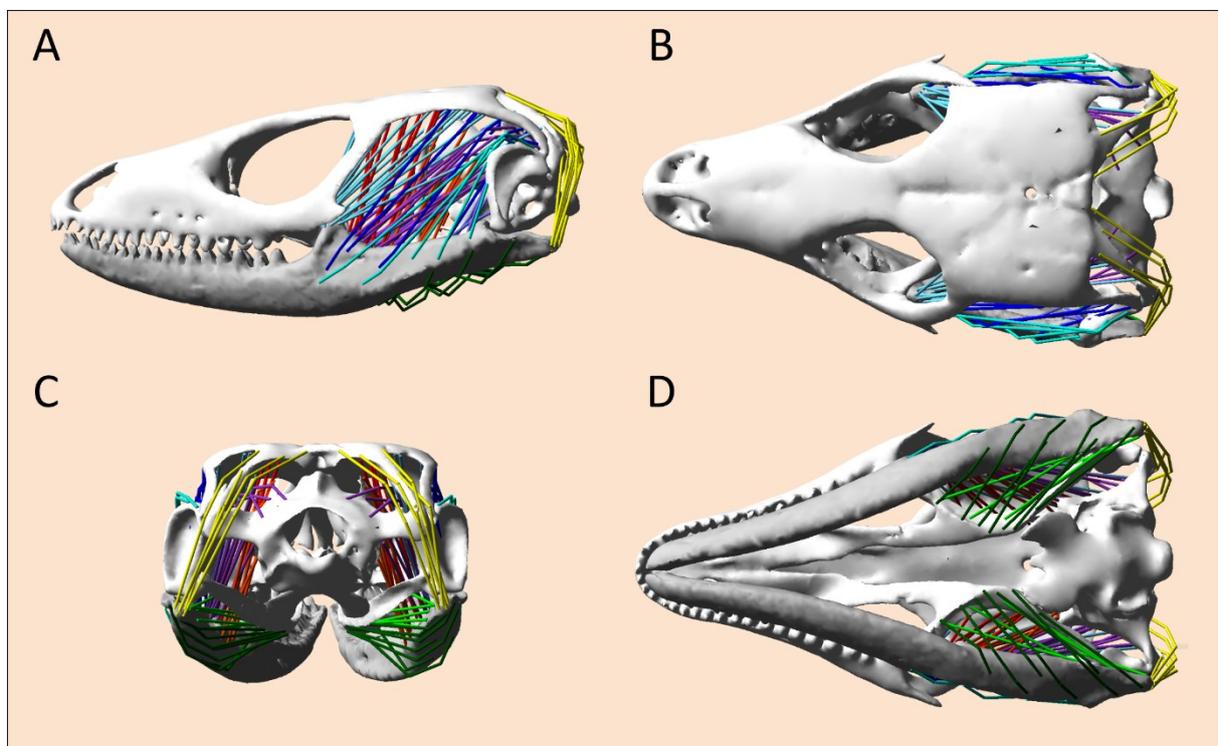
### ***Morphology***

Thirteen male specimens from Pod Kopište and 14 male specimens from Pod Mrčaru were captured by hand or by noose at the end of the summer 2013. They were sacrificed with permission of the Croatian Ministry of the Environment by an intramuscular injection of 0.3mL of pentobarbital. Specimens were preserved in a 10% formaldehyde solution for 48h then stored in a 70% aqueous ethanol solution. The heads of these 27 specimens were scanned at the university of Poitiers on an Easytom micro CT by Renaud Boistel. Scans were imported into AVIZO to segment the mandible and skull and these objects were subsequently exported as PLY files. The same set of landmarks and semi-landmarks on curves as used in Chapter 4 were used to quantify the shape variation between individuals from the two islands. Sliding landmarks on curves were slid as detailed in Chapter 4 and submitted to a Procrustes analysis. The function “plotTangentSpace” was used to run a Principal Component Analysis (PCA) on the Procrustes coordinates obtained with the function “gpagen” (“Geomorph” package). A MANOVA on the principal components (PC) cumulatively explaining at least 85% of the variance was run to test for shape differences between the populations. The theoretical shapes corresponding to the extremes of the PCs that distinguish populations were visualized using the functions “tps3d” and “shade3d” (“Morpho” package).

### ***Multibody dynamics analysis model design***

Two multibody dynamics models were developed for the purpose of the present study. Morphological and muscular data were extracted from the comparative dataset of Chapter 4. The skull and the mandible of two males of *P. sicula*, one from the island of Pod Kopište (PK), one from Pod Mrčaru (PM), were segmented using Avizo 9.0, and served to reconstruct the 3D isosurfaces. The origin and insertion of each jaw muscle was assessed based on dissections and each muscle was virtually divided into a number of distinct muscle strands based on muscle volume and area of insertion. The coordinates of the sites of origin and insertion of each muscle bundle were determined using Avizo. The mobile parts of the head (mandible) were converted into Parasolid format and were given a mass. The skull surface, the mandible parasolid and the muscle bundle coordinates were imported into MSC ADAMS motion simulation software (Santa Ana, CA, USA). The muscle bundles were modelled as contractile springs.

Where necessary, muscle bundles were wrapped around the bone to increase accuracy of the modelling (Gröning et al. 2013) (Figure 1). The muscle input forces in the simulations (Table 1) were calculated as the muscle PCSA corrected by the pennation angle, corrected for the typical 10% loss in muscle volume subsequent to tissue preservation (Kikuchi & Kuraoka, 2014) and multiplied by an intrinsic muscle stress value of 40 N/cm<sup>2</sup> (Gröning et al. 2013). A kinematic model was created to open and to close the jaw ensuring maximal activation of the bundles when the system is at the equilibrium. A food particle was created and aligned perpendicularly to the tooththrow of the upper jaw to generate a reaction force due to jaw closing (simulated bite force). To validate the output force generated by the model, the location of the contact between the food particle and the teeth was prescribed to match the location of the contact between the teeth and the plates of the force transducer used to measure *in vivo* bite force in the field (see Chapter 2). To run further simulations, the location of the item was then standardized at the middle of the maxillary tooththrow, as our video analyses showed that lizards typically reduced prey at that location (see Chapter 3). The stiffness of the food particle was intentionally beyond the hardness of prey typically consumed by the lizards to obtain maximal bite force estimates.



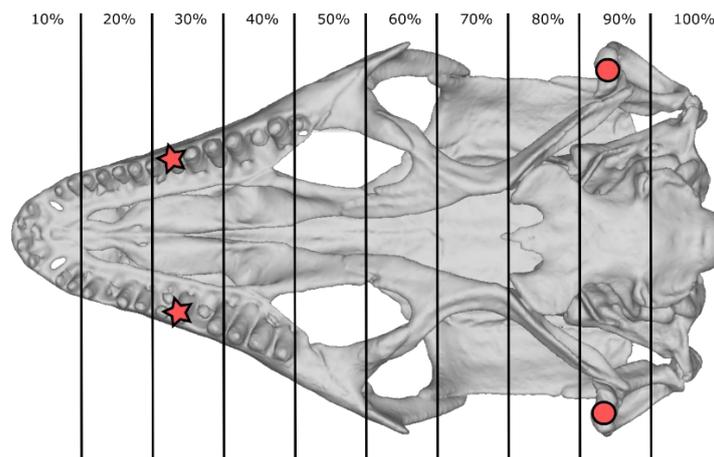
**Figure 1:** MDA model design illustrating the muscle wrapping around the bone. A: left lateral view of the skull, B: dorsal view, C: caudal view, D: ventral view. The jaw opener is represented in yellow, the external adductors in shades of blue, the pseudotemporalis in red and orange and the pterygoids in green.

**Table 9:** Input variables used to build the MDA model for each jaw muscle.

Muscle	Pennation	Number of bundles	Pod Kopište			Pod Mrčaru		
			PCSA (mm <sup>2</sup> )	Muscle force (N)	Force by bundle (N)	PCSA (mm <sup>2</sup> )	Muscle force (N)	Force by bundle (N)
AMESA	15°	5	2.33	0.93	0.19	4.95	1.98	0.40
AMESP	18°	5	2.71	1.09	0.22	3.51	1.40	0.28
AMEM	11°	11	2.80	1.12	0.10	2.84	1.13	0.10
AMEP	41°	9	0.82	0.33	0.04	0.79	0.32	0.04
AMP	21°	4	0.99	0.40	0.10	1.61	0.64	0.16
PSTS	27°	5	1.70	0.68	0.14	2.20	0.88	0.18
PSTP	0°	6	4.13	1.65	0.28	4.65	1.86	0.31
PTL	30°	7	2.70	1.08	0.15	4.44	1.77	0.25
PTM	0°	5	4.93	1.97	0.39	5.47	2.19	0.44

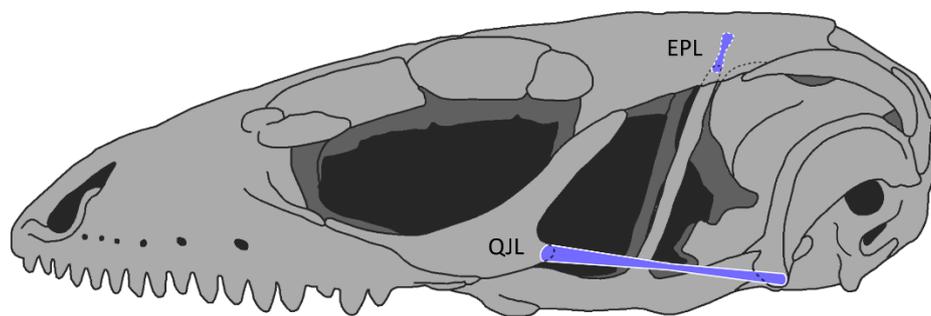
### Finite element analysis model design

The segmentation labels generated by Avizo and used to reconstruct the skull surfaces used in the MDA were converted into tetrahedral-elements meshes (PK: 1.527.268 elements, PM: 1.321.278 elements) and imported into ANSYS Mechanical (ANSYS, Inc., Canonsburg, PA, USA). Bone was considered to be an isotropic material with a Young's modulus of 17 GPa and a Poisson's ratio of 0.3. The models were constrained at the two joints and at two symmetrical bite points (Figure 2).



**Figure 2:** segments of the skull used to investigate the variation in stress accumulation over the structure (here represented by a ventral view). Note that the 30% section includes the bite points (red stars), the 60% and the 90% sections include the insertion sites of the quadratojugal ligament, the 80% section contains the anterior part of the neurocranium, and the 90% section contains the posterior part of the neurocranium and the quadrate (the joint surfaces are indicated with red circles).

The coordinates of these nodes were directly exported from ADAMS. The three-dimensionally resolved forces exported from the MDA solutions were directly applied onto the skull model. The quadrato-jugal and the epipterygoid-parietal ligaments were modelled as tension-only links with a stiffness of  $250 \text{ N/mm}^2$  and a cross-sectional area of  $1 \text{ mm}^2$  (Figure 3). Sensitivity tests were carried out using the Pod Kopište model biting at a  $20^\circ$  gape to quantify the impact of the presence / absence and of the variation in the resistance to stretch of the ligaments ( $50$ ,  $250$ , and  $500 \text{ N/mm}^2$ ) on the stress distribution. The stress values associated with each element of the mesh were exported into an element table for postprocessing.



**Figure 3:** Illustration of the origin and insertion sites of the epipterygoid-parietal ligament (EPL) and the quadrato-jugal ligament (QJL). Note that the EPL inserts on the ventral side of the parietal and the most dorsal tip of the epipterygoid; the QJL inserts on the most caudal and ventral tip of the jugal and the most lateral and ventral tip of the quadrate.

### **MDA and FEA simulations**

The bite force generated by a model with known muscular data was calculated during each MDA simulation. Four different models were tested: two “natural” models (“PK\_PK”: the morphology of Pod Kopište with its own musculature, and “PM\_PM”: the morphology of Pod Mrčaru with its own musculature), and two “hybrid” models in which musculature data were exchanged (“PK\_PM”: the morphology of Pod Kopište with the musculature of Pod Mrčaru, and “PM\_PK”, the morphology of Pod Mrčaru with the musculature of Pod Kopište). Hence, we were able to test the impact of changes in morphology on the simulated bite force by comparing the results of the simulations: PK\_PK with PM\_PK, and PM\_PM with PK\_PM. The simulated bite force in each of the four models was calculated for 10 different gape angles, from  $0^\circ$  (closed jaw) to  $45^\circ$  (maximum gape typically observed at the onset of fast closing, see Chapter 3). For each model, the conversion rate of the total muscle force into bite force was calculated at every gape simulated. The total amount of change between the two original

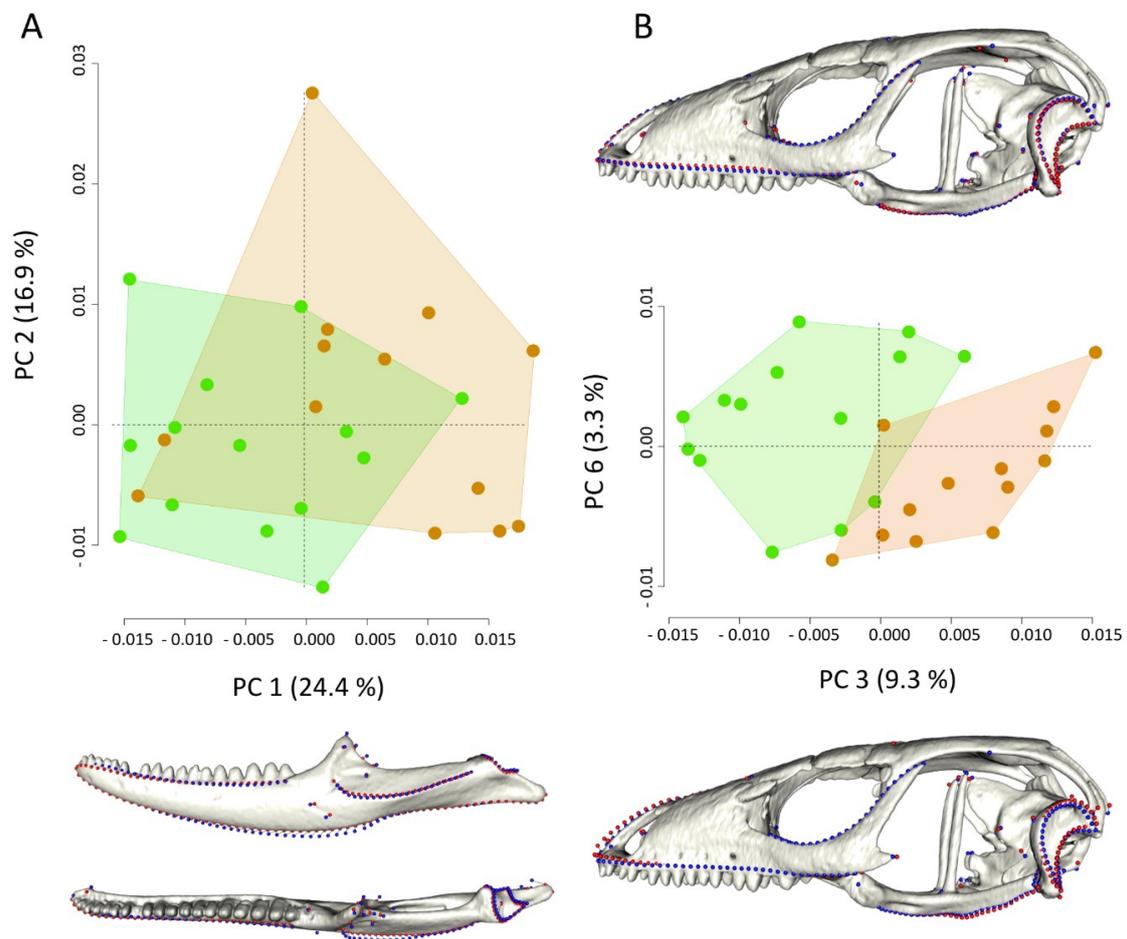
models was quantified, and the relative contributions of the changes in muscular data or in morphology were calculated. To understand the functional significance of each muscle group and how their function is impacted by variation in gape and by morphology, we ran simulations with only one muscle activated at a time. Its contribution to the total simulated bite force and its conversion rate were calculated.

The results of the MDA were then imported into ANSYS after being all multiplied by 5 to obtain more realistic forces (see Results). For the FEA simulations performed on the four different models only three gape values were retained that mimicked three ecologically relevant scenarios: biting a thin object like a leaf ( $0^\circ$ ), biting a prey of medium size ( $20^\circ$ ) and biting a large prey item or biting a conspecific during male-male interactions ( $45^\circ$ ). Values of stress in each skull element were saved. Specifically, we focused on the first principal stress (the most tensile stress), the third principal stress (the most compressive stress), and the Von Mises stress (VMS), which provides a summary of the overall loading, by recording the mean stress values for the whole skulls and the variation of the stress along the skull. To do so we divided the mesh into 10 segments of equal length (Figure 2). We then calculated the 'advantage' of the PM morphology relative to the PK morphology (for example) as a percentage increase/decrease of the mean VMS accumulation along the skull. Finally, we estimated the homogeneity of the distribution of stress by calculating the Shannon's entropy (Shannon, 1948) based on the density distribution of VMS values. Originally, this index was designed for the telecommunication field to describe the quantity of information delivered by a source of information, in other words to estimate the redundancy in values of data series. Since then it has been applied to different ends, including spatial and medical imagery (concept of Mutual Information, see Pluim et al. 2003), ecology (the Shannon's index, see Poole, 1974; Taverne et al. 2019), and evolutionary biology (Toussaint et al. 2020). The higher the value of the entropy is, the lower the redundancy is, so the higher the disparity in the data. As previously, the 'advantage' provided by the PM morphology was estimated as the proportional difference between the two models.

## RESULTS

### *Morphological divergence between the two populations*

The MANOVA including the first nine axes of the PCA carried out on skull shape (cumulative variance: 85.2%) revealed an effect of island (Wilks lambda = 0.22;  $F_{1,25}=6.861$ ;  $P < 0.001$ ), especially along PC3 (variance=9.3%;  $F_{1,25}=26.48$ ;  $P < 0.001$ ) and PC6 (variance=3.3%;  $F_{1,25}=4.83$ ;  $P = 0.037$ ). The theoretical shape deformation along PC3 indicated that the population of PM had a slightly shorter snout and a more curved quadrate (Figure 4A).

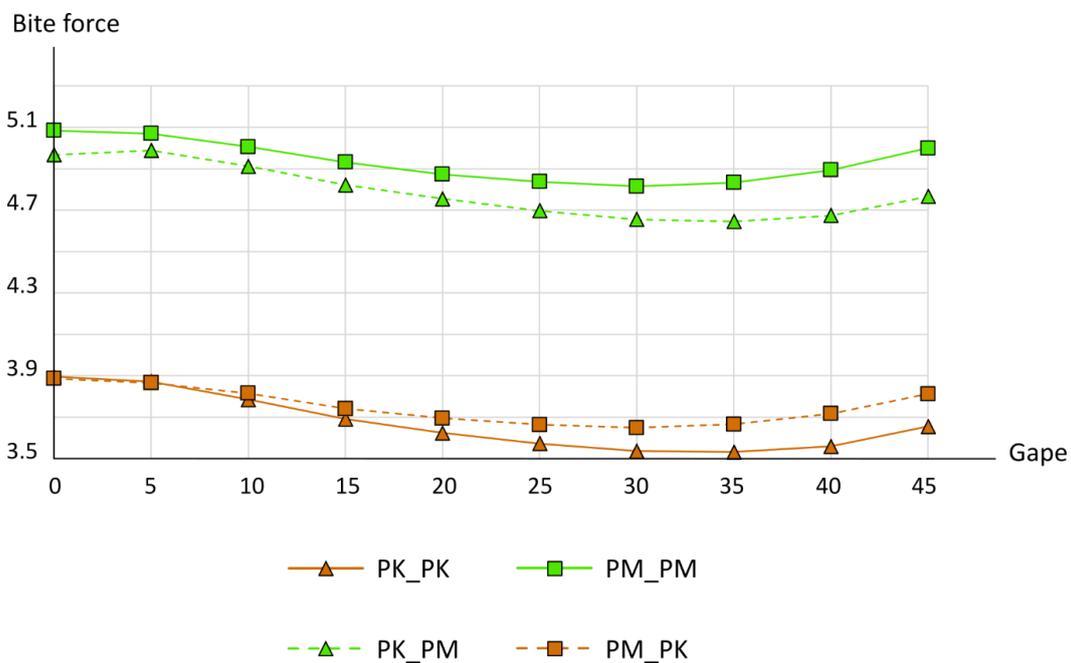


**Figure 4:** results of the principal component analysis performed on mandible (A) and skull (B) shape of the specimens of *Pod Kopište* (brown circles) and *Pod Mrčaru* (green circles). The theoretical shapes corresponding to the negative (blue spheres) and positive (red spheres) sides of the principal components are represented on A) the lateral (top) and the dorsal views (bottom) of the mandible shape deformation along the PC1, and in B) the lateral view of the skull shape deformations along PC3 (bottom) and PC6 (top of the figure).

The MANOVA including the first 9 axes of the PCA carried out on mandible shape (cumulative variance: 85.0%) also revealed an effect of island (Wilks lambda = 0.40;  $F_{1,25}=2.86$ ;  $P = 0.029$ ), especially along PC1 (variance=24.4%;  $F_{1,25}=7.50$ ;  $P = 0.011$ ). The theoretical shape deformation along PC1 showed that the mandible of the PM population was overall more ventrally curved (Figure 4B). The coronoid was medially and caudally thicker, the lateral crest which serves as the insertion site of external adductors was wider, the retroarticular process shorter, and the joint surface area larger.

### MDA simulations

The MDA simulations carried out on the two natural and the two hybrid models revealed that models built with the musculature of PM always resulted in higher bite forces, and that interestingly, models built with the morphology of PM resulted in higher bite forces than PK irrespective of muscle architecture (Figure 5, Table 2).

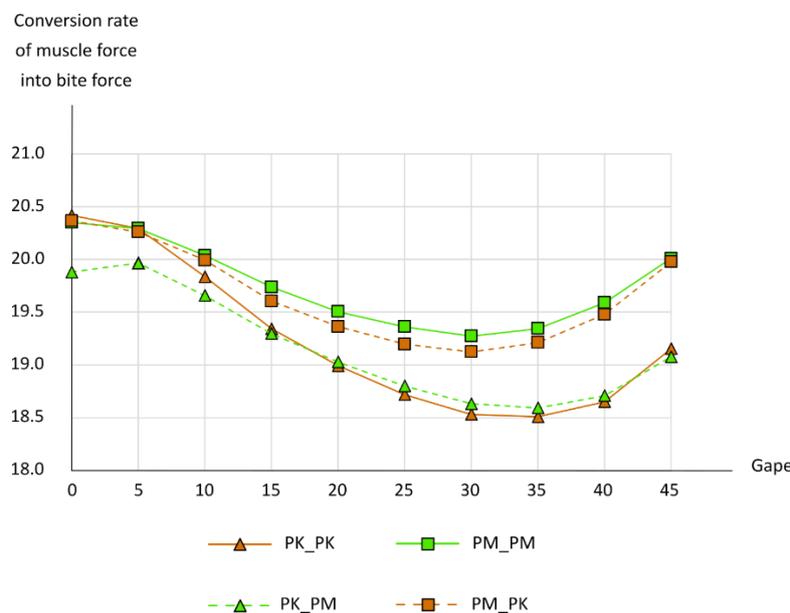


**Figure 5:** Relationship between bite force (in Newton, in ordinate) estimated by the MDA in the four models and the gape (in degrees, in abscissa). The two natural models (PK\_PK: including the morphology and the musculature of Pod Kopište, PM\_PM: including the morphology and the musculature of Pod Mrčaru) are represented with full lines, whereas the two hybrid models (PK\_PM: including the morphology of Pod Kopište and the musculature of Pod Mrčaru, PM\_PK: including the morphology of Pod Mrčaru and the musculature of Pod Kopište) are represented with dashed lines. Note the false origin on the y-axis.

**Table 10:** Results of MDA simulations including the values of simulated bite force and the conversion rate of muscle force into bite force for each of the four models tested, relative to gape angles (PK\_PK and PM\_PM are the phenotypes of Pod Kopište and Pod Mrčaru, respectively; PK\_PM is the hybrid model including the morphology of Pod Kopište with the morphology of Pod Mrčaru, PM\_PK is the hybrid model with the opposite configuration).

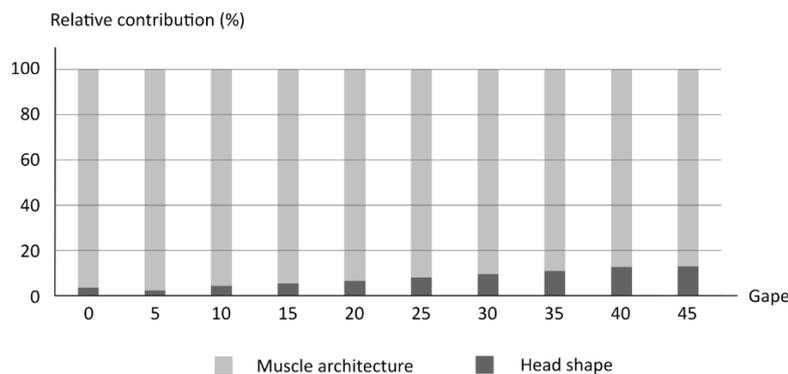
Gape angle	Simulated Bite Force (N)				Force conversion rate (%)			
	PK_PK	PM_PM	PK_PM	PM_PK	PK_PK	PM_PM	PK_PM	PM_PK
0°	3.90	5.08	4.97	3.89	20.42	20.35	19.88	20.37
5°	3.87	5.07	4.99	3.87	20.29	20.29	19.97	20.26
10°	3.79	5.01	4.91	3.82	19.84	20.04	19.66	19.99
15°	3.69	4.93	4.82	3.74	19.34	19.74	19.30	19.61
20°	3.62	4.87	4.75	3.70	18.99	19.51	19.03	19.36
25°	3.57	4.84	4.70	3.66	18.72	19.36	18.80	19.20
30°	3.54	4.82	4.66	3.65	18.53	19.27	18.63	19.12
35°	3.53	4.83	4.65	3.67	18.51	19.35	18.59	19.21
40°	3.56	4.89	4.67	3.72	18.65	19.59	18.71	19.48
45°	3.66	5.00	4.77	3.81	19.16	20.01	19.08	19.98

This trend further held irrespective of the gape tested, and even increased with gape. We also noticed that simulated bite force reached two peaks in the four models, at closed gapes (0° or 5° in PK\_PM) and wide gapes (45°) and was minimal at a gape of around 30° / 35°. Knowing that the total amount of intrinsic muscle force of the PK and the PM jaw system is 19.08 N and 24.98 N respectively, the simulations showed that the conversion rate allowed by the PM morphology is always higher than the PK morphology. This trend also tends to increase with gape (Figure 6).



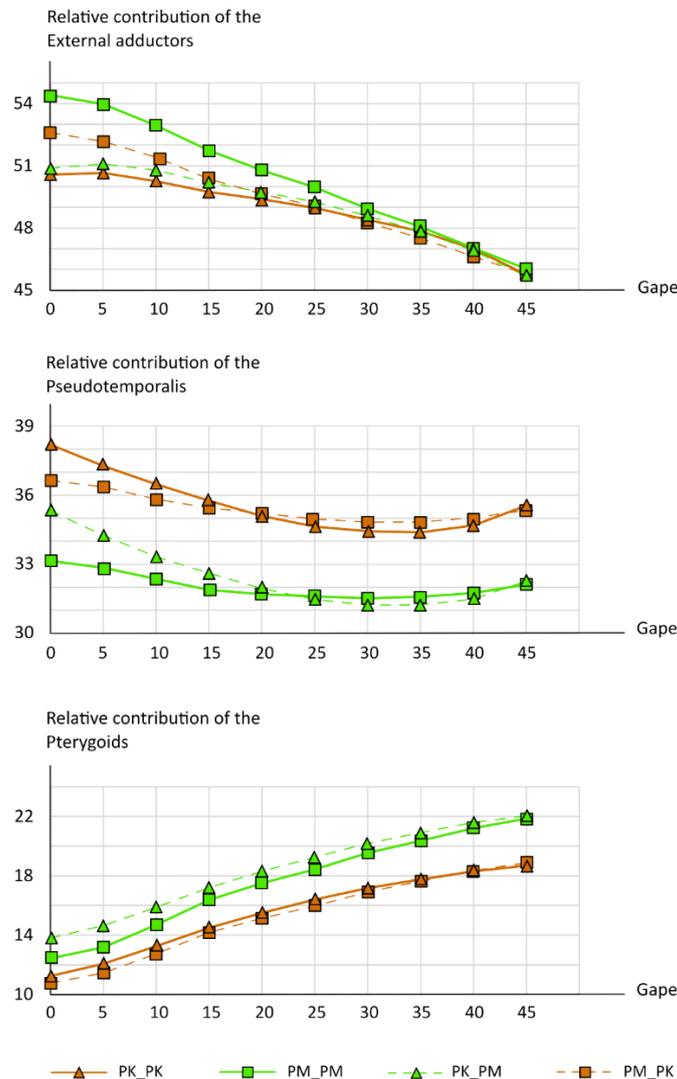
**Figure 6:** Relationship between the conversion rate of the total muscle force into simulated bite force (in percentage, in ordinate) for all models at different gape angles (in degrees, in abscissa). The two natural models (PK\_PK: including the morphology and the musculature of Pod Kopište, PM\_PM: including the morphology and the musculature of Pod Mrčaru) are represented with full lines, whereas the two hybrid models (PK\_PM: including the morphology of Pod Kopište and the musculature of Pod Mrčaru, PM\_PK: including the morphology of Pod Mrčaru and the musculature of Pod Kopište) are represented with dashed lines. Note the false origin on the y-axis.

The total amount of change in simulated bite force between the two natural models varied between 30.49% (0°) and 37.51% (40°). Based on this, the bite force variation due to a change in morphology and to a change in musculature was assessed, and the relative contribution of each was calculated. On average, the relative contribution from the change in shape to total variation in bite force increased with gape and varied between 2.41% (5°) and 12.49% (45°), the remainder being explained by variation in muscle architecture (Figure 7).



**Figure 7:** Relative contribution (in percentage) of the variation in musculature (in light gray) and of the variation in morphology (in dark gray) to the total amount of simulated bite force divergence between the two phenotypes (Pod Kopište and Pod Mrčaru) relative to gape (in degrees).

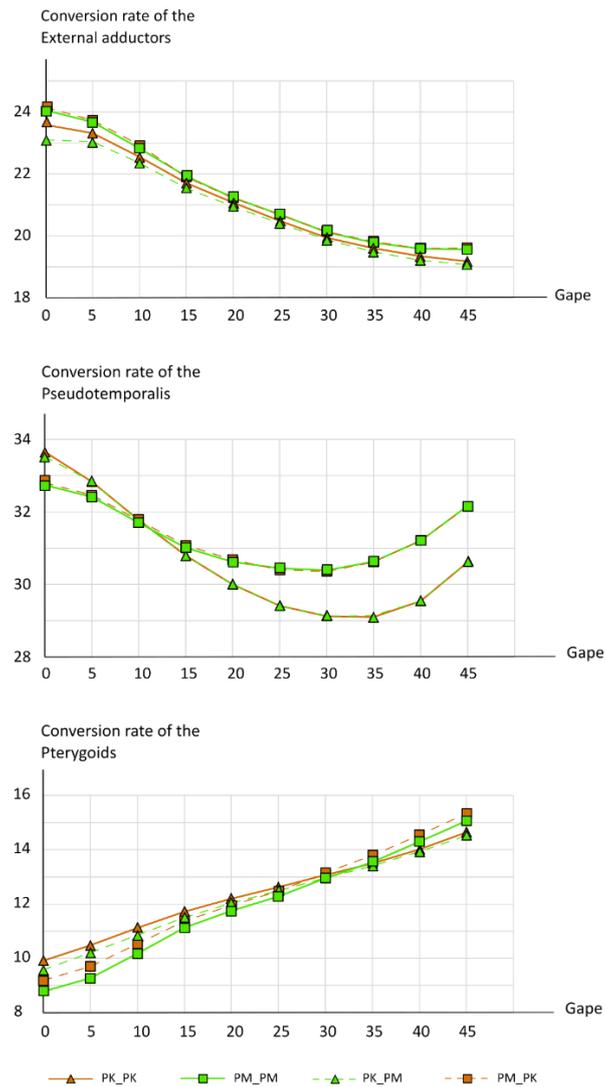
The simulations including only one muscle allowed us to calculate the absolute and the relative contributions of each muscle to the total simulated bite force (Figure 8, Table S1). In all four models, the group of the external adductors contributed the most to bite force (on average approximately 50%), then the pseudotemporalis (35%) and the pterygoids (15%). Their contribution was also impacted by gape, with the functional contribution of the external adductors decreasing with gape, and role of the pterygoid muscles increasing. In contrast to muscle contribution, the calculation of the conversion rate of each muscle group revealed that the pseudotemporalis group is the most efficient in converting muscle force into bite force (approximately 30% in average), while external adductor and pterygoid conversion rates were only approximately 22% and 12%, respectively (Figure 9). Each muscle group seemed to react differently to variation in gape: the conversion rate of the adductors decreased with gape in all models, while the conversion rate of the pterygoids increased, and that of the pseudotemporalis decreased from gape 0° to 30° and increased from 30° to 45°. Compared to the morphology of PK, the PM morphology was associated with higher conversion rates of the adductors for all gapes, of the pseudotemporalis for gapes wider than 10°, and for the pterygoids for gapes wider than 30°.



**Figure 8:** Relative contribution of each muscle group to the total simulated bite force (in percentage, in ordinate) in all models, depending on gape (in degrees, in abscissa). The two natural models (PK\_PK: including the morphology and the musculature of Pod Kopašče, PM\_PM: including the morphology and the musculature of Pod Mrčaru) are represented with full lines, whereas the two hybrid models (PK\_PM: including the morphology of Pod Kopašče and the musculature of Pod Mrčaru, PM\_PK: including the morphology of Pod Mrčaru and the musculature of Pod Kopašče) are represented with dashed lines. Note the false origin on the y-axis.

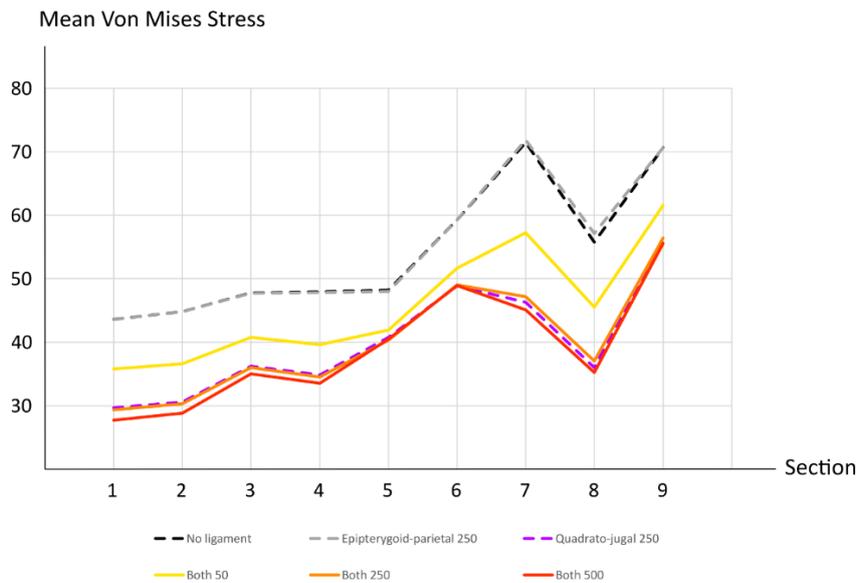
### FEA simulations

**Ligament sensitivity tests** – These tests revealed that the inclusion of an epipterygoid-parietal ligament (EPL) into the model has little or no effect on the mean amount of VMS across the skull, whereas the inclusion of a quadrato-jugal ligament (QJL) could decrease the mean VMS by up to approximately 26% (Figure 10). The importance of the resistance to stretch of the ligaments was also assessed. The inclusion of ligaments with a stiffness of 50, 250 and 500 N/mm<sup>2</sup> decreased the mean amount of stress of approximately 16%, 26% and 28%, respectively. Increasing the stiffness of ligaments above 250 N/mm<sup>2</sup> only had a minor impact on the mean accumulation of stress across the skull.



**Figure 9:** Conversion rates of muscle force into simulated bite force of each muscle group (in percentage, in ordinate) in all models depending on gape (in degrees, in abscissa). The two natural models (PK\_PK: including the morphology and the musculature of Pod Kopište, PM\_PM: including the morphology and the musculature of Pod Mrčaru) are represented with full lines, whereas the two hybrid models (PK\_PM: including the morphology of Pod Kopište and the musculature of Pod Mrčaru, PM\_PK: including the morphology of Pod Mrčaru and the musculature of Pod Kopište) are represented with dashed lines. Note the false origin on the y-axis.

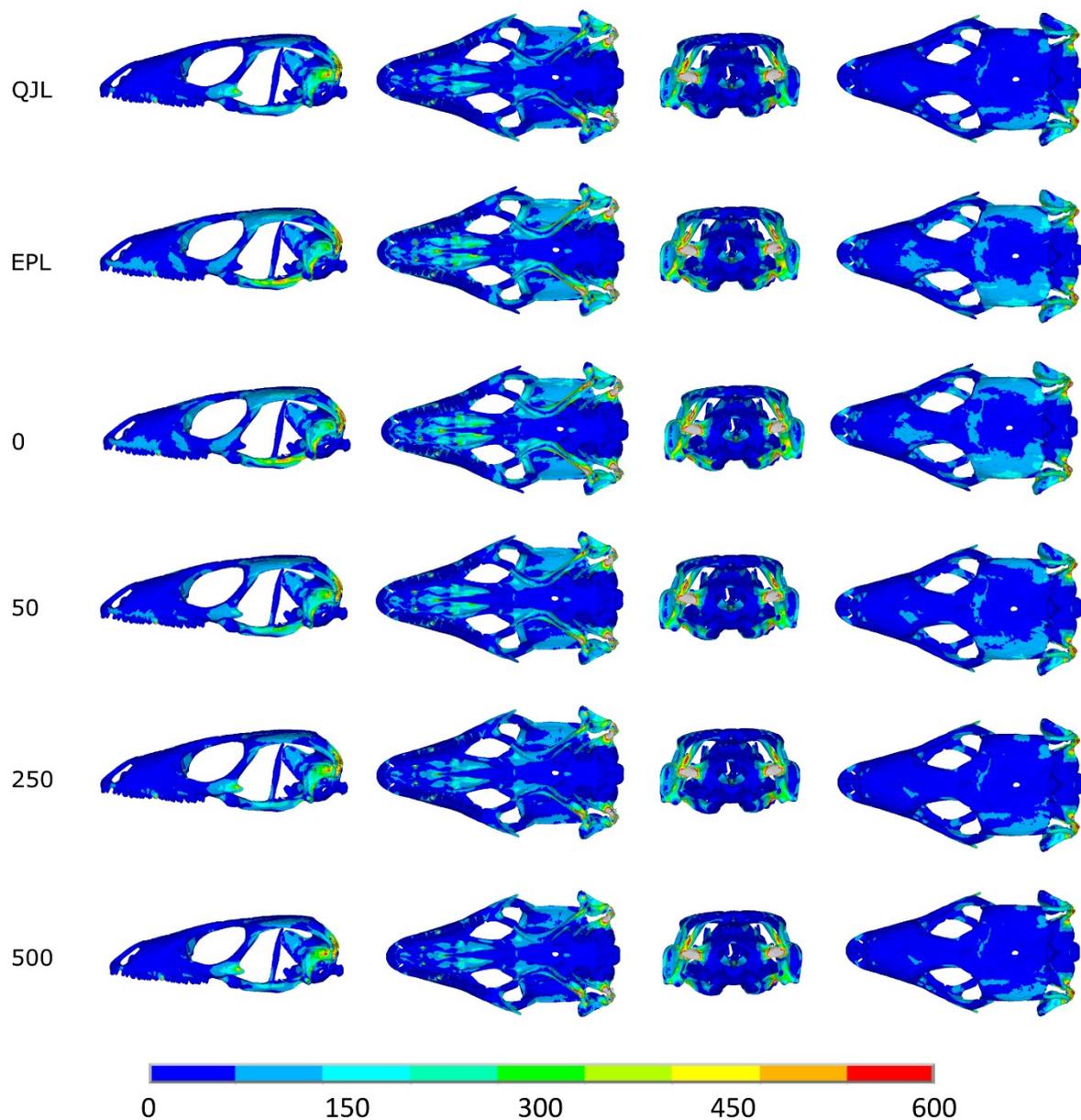
Specifically, including ligaments in the FEA model induced a decrease in the amount of VMS accumulated in the snout (Figure 11), but also unloaded the palatine, the vomer, the pterygoid, the cranial vault, and the paraoccipital process of the parietal. The ligaments (especially the QJL) seemed to distribute the VMS on the posterior part of the jugal and the dorsal part of the quadrate, which did not show high stress concentrations otherwise. This trend is emphasized by the simulated increase in the stiffness of the ligaments.



**Figure 10:** Results of the ligament sensitivity test. The graph is representing the mean amount of Von Mises stress calculated along the different sections of the skull, depending on the way to model the ligaments. The dashed lines represent the situations where no ligament was modelled (black), only the epipterygoid-parietal (gray) or only the quadrato-jugal ligament (violet) was modelled. The full lines represent the situations where both ligaments were modelled and were assessed a stiffness of 50 (yellow), 250 (orange) or 500 (red) N/mm<sup>2</sup>. Note the false origin on the y-axis.

We also noticed that at 45° in every model, the stress accumulation in the region of the jugal decreased but increased on the posterior part of the quadrate, and that this pattern was associated with a decrease in the ligament tension values (Table S2). The most posterior section of the skull was not included in the following results, since abnormally high stress values were detected in this region.

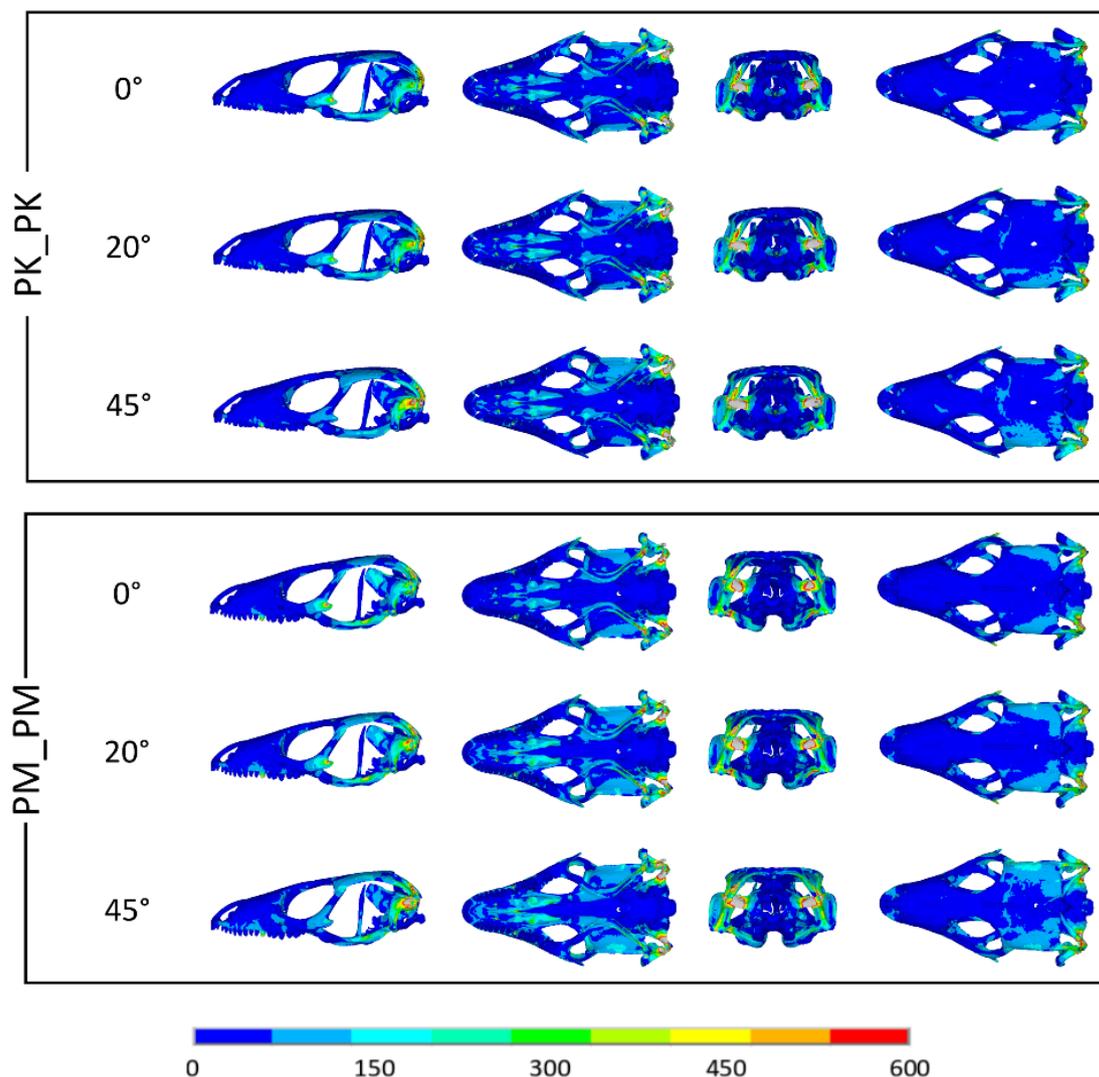
*Comparison between natural models* – The comparison of the two natural models showed that on average, the level of VMS in PM\_PM was higher (from 6% to 17%) than that of PK\_PK (see the graph of Figure 12). Although PM\_PM showed lower level of VMS (except for the two most posterior sections of the skull) at a 0° gape, more stress was detected at 20° and 45°. Interestingly, the amount of VMS observed significantly increased with gape in PM\_PM, whereas this was not the case in PK\_PK. Specifically, the increase in VMS in the PM\_PM natural model affected the whole skull, as shown in the Figure 12. The skull presented higher levels of VMS at the snout, the palatal region, and the quadrate compared to the rest of the skull. The less impacted regions were the jugal, the pterygoid and the wings of the prootic. An increase of 2% to 6% was also observed in the overall entropy in the PM\_PM model (Table 3).



**Figure 11:** Von Mises stress distribution across the skull testing for the impact of ligaments and their isotropic properties (on the left of the figure, 0: no ligament, QJL: only the quadratojugal with a stiffness of 250 N/mm<sup>2</sup> is modelled, EPL: similarly, only the epipterygoid-parietal ligament, 50: both ligaments included with a stiffness of 50 N/mm<sup>2</sup>, 250: both ligaments with a stiffness of 250, 500: both ligaments with a stiffness of 500). The left lateral, ventral, caudal and dorsal views of the skull are represented (from left to right). The scale bar at the bottom indicates that hotter colors are associated with higher levels of accumulation of Von Mises stress.

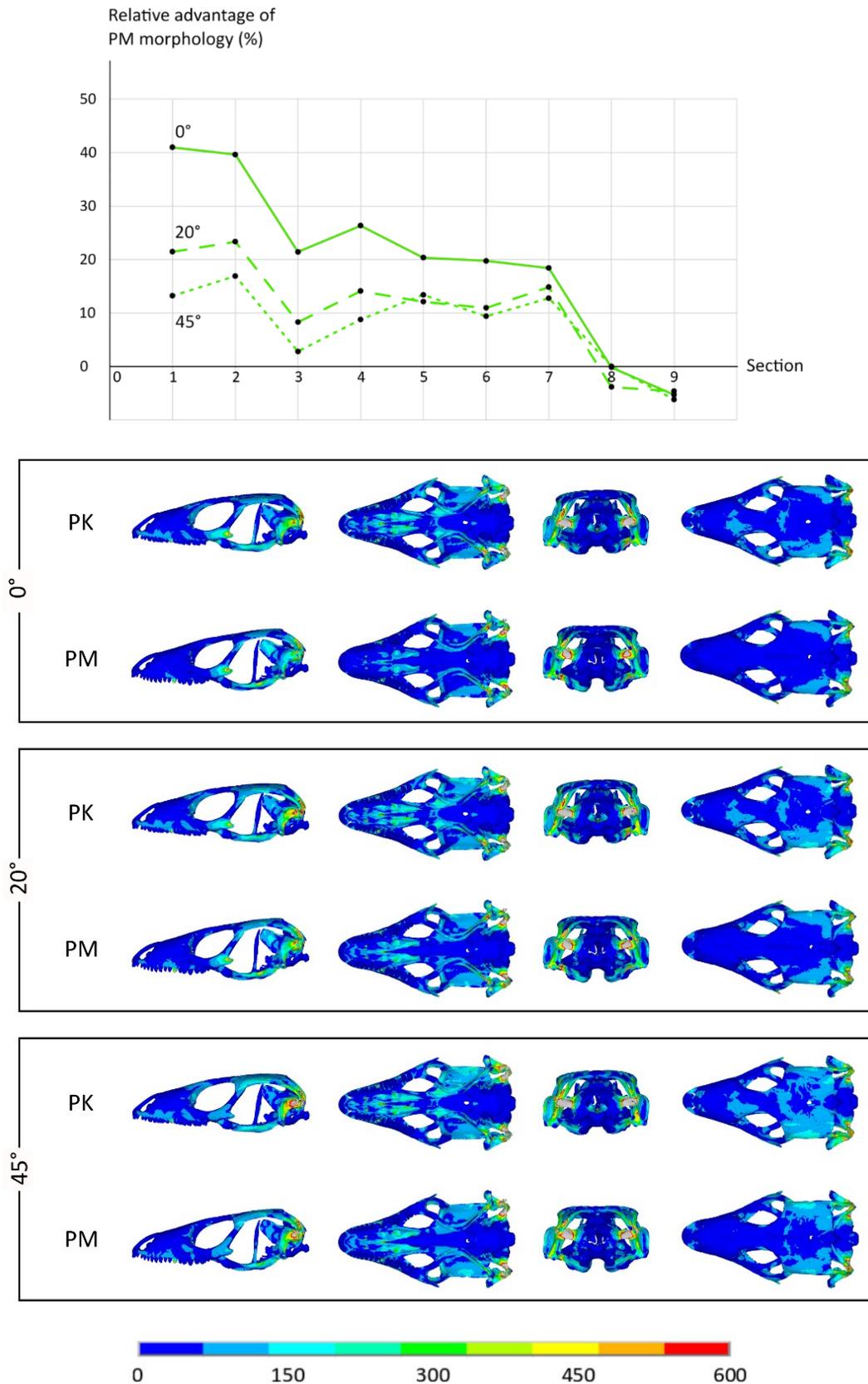
**Table 11:** Differences in the values of Shannon's entropy index, in percentage. The first column shows the increase of overall entropy from the Pod Kopiste phenotype (PK\_PK) to the Pod Mrcaru phenotype (PM\_PM), the second shows the increase in overall entropy when applying a PM morphology rather than a PK morphology on a given morphology, the third gives the relative advantage provided by PM morphology in reducing entropy.

Gape angle	PK_PK vs. PM_PM	Effect of musculature	Advantage of PM morphology
0°	1.84	5.51	3.74
20°	5.28	6.89	1.71
45°	6.58	7.35	0.82



**Figure 12:** Von Mises stress distribution in the two natural models (Brown lines: PK\_PK, green lines: PM\_PM) for the three gapes tested (in the graph: full lines: 0°, dashed lines: 20°, dotted lines: 45°). These are plotted relative to the skull's section number. Note the false origin on the y-axis. The left lateral, ventral, caudal and dorsal views of the skull are represented (from left to right). The scale bar at the bottom indicates that hotter colors are associated with higher levels of accumulation of Von Mises stress.

*Impact of morphology and musculature* – When considering the whole skull, the PM morphology decreased the mean VMS by 6 to 15%, the first principal stress by 7 to 16%, and the third principal stress by 5 to 14%. The advantage provided by the PM morphology, estimated in percentage of loss in VMS, is inversely correlated with gape and is more pronounced in the anterior part of the skull than the posterior part (see the graph of Figure 13). Indeed, the advantage of a PM morphology is 13% to 41% in the snout, 9% to 20% in the region of the jugal, and becomes null or slightly negative in the two most posterior regions. Specifically, the unloading allowed by the PM morphology concerns the maxilla, the vomer, the frontal, the anterior part of the pterygoid, the epipterygoid, the parietal and the quadrate. On the contrary, areas that seem to show higher loads include the wings of the prootic and the posterior tip of the pterygoid. Although less obvious when reaching a 45° gape, this pattern of stress distribution was similar at all gapes (Figure 13). On average, the level of VM stress increased (from 19% at 0° to 27% at 45°) when a PM musculature rather than a PK musculature was applied on a given morphology. In all cases, the overall level of entropy increased when a PM musculature rather than a PK musculature was applied on a given morphology (from 6% at 0° to 7% at 45°). However, the PM morphology provided only a poor advantage in reducing overall entropy (observed differences all <5%) (Table 3).



**Figure 13:** Consequences of morphological variation on the Von Mises stress distribution. The graph represents the mean advantage (in %) provided by the PM morphology along the skull (full line: 0°, dashed line: 20°, dotted line: 45°). Here the models PK\_PM and PM\_PM (PK and PM in the figure) were compared for each of the three gapes tested (see Figure S1 for the alternative combination PM\_PK vs. PK\_PK). The scale bar at the bottom indicates that hotter colors are associated with higher levels of accumulation of Von Mises stress.

## DISCUSSION

### *Morphological divergence between the populations*

The morphological divergence between the populations of Pod Kopište and Pod Mrčaru was significant in terms of cranial and mandibular shape. Because a dietary shift from insectivory to omnivory occurred after lizards from Pod Kopište were introduced onto Pod Mrčaru (Herrel et al. 2008), the morphological changes were expected to be similar to the ones described by the patterns of covariation between head shape and the proportion of plants consumed (see Chapter 4). That is to say, we expected the skulls of Pod Mrčaru to have a larger temporal window, a longer and more flattened snout, a pterygoid that is more curved, and a mandible that is more ventrally curved, with a wider lateral insertion area for adductor muscles, and a more robust coronoid. The results on mandible shape are relatively clear since the principal component analysis which discriminates both populations explains more than 24% of the total variance in morphology. The associated theoretical shape confirms our prediction and includes all the morphological changes expected. The results on skull morphology, however, are more subtle. The two PCs which discriminate the two populations cumulatively explain 13% of the total variance, which means that the morphological changes observed is less striking. Of the predicted morphological changes expected, only the curvature of the quadrate was observed. In contrast to our predictions the snout of Pod Mrčaru specimens appeared to be shorter. The fact that the morphological divergence of the skull is less clear than in the mandible is likely a consequence of the multiple functions that the skull fulfills, and the associated constraints and trade-offs it might face. Also, the patterns expected were based on a comparative study (see Chapter 4) including populations that diverged several thousands of years ago (Podnar et al. 2004; Podnar et al. 2005) and might not take into account the specific local ecological and social context of these insular populations or the possible role of founder effects (Kolbe et al. 2012).

### *Validation of the models*

The simulated bite forces generated by the two MDA natural models were significantly smaller than the *in vivo* bites measured for the same individuals on the field (5 times smaller on average). Indeed, the bite forces measured for the individual of Pod Kopište and Pod Mrčaru were of 15.0 N and 20.6 N, respectively. This surprising result could be explained by several factors, such as abnormal muscular

masses, a low value of intrinsic muscle stress, both possibly resulting in inaccurate values of muscle force, or a non-optimal level of muscle activation in the MDA model. However, all these possible biases were discarded. First, the muscle masses obtained in the present study through quantitative dissections are similar to muscle masses calculated for *P. melisellensis* specimens by Huyghe et al. (2009). Second, the value of intrinsic muscle stress used here (40N/cm<sup>2</sup>) was high considering the values available in the literature (Medler, 2002; Anderson & Roberts, 2020; but see Hatze, 1981 who calculated 87.1N/cm<sup>2</sup> in other muscles). Finally, each muscle bundle was fully activated during biting simulations at equilibrium. It is thus unlikely that the methodology used to simulate *in silico* bite force is responsible for underestimation of the *in vivo* bite force. Rather, when measuring *in vivo* bite force the lizard's body is maintained by the operator. We hypothesize that maintaining the animals during bite force measurement allows them to recruit axial muscles and hence to increase *in vivo* bite force values. Underestimating bite force through the use of models has been reported before in squamates (see Curtis et al. 2010 for a review), although to our knowledge no experimental test of this hypothesis exist in the literature. Nevertheless, the comparative aims of the present study should not be impacted by the inconsistency between the absolute *in vivo* and *in silico* data. We were careful to discuss the results of one model relatively to another only, rather than to compare the absolute values of *in silico* forces. When exporting the results from ADAMS to ANSYS, I multiplied the intrinsic muscle stress by 5 so that the simulated bite force roughly matched the *in vivo* bite force, in order to load the skull with more realistic amounts of force.

### ***Mechanical basis for the variation in bite force***

The *in silico* measures of bite force generated by the MDA simulations confirmed that the lizards from Pod Mrčaru bite harder than that of Pod Kopište. Although the two specimens tested were of a comparable size, this difference is principally explained by the difference in the total muscle PCSA between them (Table 1). Beyond that, the cranium and mandible shapes also contributed to the observed variation in bite force. The comparison of the natural and hybrid models showed that the morphology of Pod Mrčaru allows them to generate greater bite forces in all cases. Moreover, the correlation between bite force and gape seems to covary with that of the conversion rate and gape (Figures 4,5). Hence, the results suggest that variation in bite force along with gape depends on the proportion of muscle force converted into bite force, which in turn seems to be impacted by head shape. Indeed, this assumption was further confirmed by the calculation of the relative contribution

of musculature and morphology to the differences observed between the two phenotypes. Although total muscle force was mainly responsible for the difference in bite force, head shape also contributed to it. We thus demonstrate that rather subtle shape changes between the two recently diverged populations are responsible for a notable part of the variation in bite force, likely by modulating the lever-arms associated with the muscle bundles. We posit that the influence of shape on performance demonstrated here can be generalized to the other populations of the Adriatic archipelago studied in the present thesis. Moreover, considering that the magnitude of the shape changes observed here is relatively moderate compared to the maximum shape changes previously described between the most extreme populations of the comparative dataset (Chapter 4), the contribution of shape might even be greater when comparing two populations with extreme morphologies, although this remains to be tested.

Because jaw muscles were previously suggested to have different functional importance in jaw movements and in the generation of bite force, we investigated the functional role of each muscle group, and how it varied with gape. Whereas the conversion rate and the relative contribution of the external adductors to bite force decreased with gape, they increased with gape in pterygoids. The pseudotemporalis showed a singular pattern, with a sub-optimal gape for force production at around 30°. Previous studies on lizards have investigated the functional role of muscles (Throckmorton, 1978; Smith, 1982; Herrel et al. 1999a,b). These studies have suggested that the pterygoids which, despite their relatively low efficiency in converting muscle force into bite force compared to other groups, tend to increase their efficiency with gape because their moment arm increases. On the other hand, external adductors and pseudotemporalis muscles are activated when maximum gape angle is reached and remain activated during jaw closing. Considering the patterns of conversion, one might suggest that bites occurring at lower gapes should be facilitated by a preferential investment in external adductors and pseudotemporalis, whereas bites occurring at wider gapes should be facilitated by the investment in pseudotemporalis and pterygoids. The consumption of plants can be expected to be associated with low gape angles, the consumption of arthropod prey with intermediate gape angles, and male-male combat with high gapes. In males, the consumption of plants was demonstrated to be associated with more forceful external adductors, whereas the consumption of hard prey was in line with more forceful pseudotemporalis and pterygoids. The results of the biomechanical models are congruent and suggest that depending on the functional demands of each biting situation, investment in muscle groups can be predicted by the intrinsic characteristics of the system which determines the efficiency of the muscles in generating bite force. In females, different muscle groups are associated with variation in

diet but since the MDA model was built based on male specimens only, we are unable to further discuss the differences between males and females.

### ***Structural performance of the skull***

The comparison of the natural models PK\_PK and PM\_PM illustrated differences in structural performance between the phenotypes of the two populations. It revealed that the phenotype of Pod Mrčaru accumulates more stress on average than the phenotype of Pod Kopište. Given that the PM model includes a greater total muscle PCSA, this is expected. This is further confirmed by the present results which show that applying an overall stronger musculature on a given morphology increases the level of VM stress over the skull. But the magnitude of differences in stress due to musculature is greater (19% to 27%) than that observed between the natural models (from 6% to 17%), suggesting that morphology itself might help dissipate stress. Indeed, the PM morphology conferred an advantage in dissipating stress (Figure 12). Similarly, the comparison of the natural models revealed that the phenotype of Pod Mrčaru had a greater level of overall entropy than its counterpart of Pod Kopište (from 2% at 0° to 6% at 45°). Again, we can assume that this is mainly due to an increase in the total muscle force and the associated greater levels of stress that this phenotype elicits. We indeed observed that applying a PM musculature rather than a PK musculature on a given morphology increased the overall entropy (from 6% at 0° to 7% at 45°). Interestingly, the present results suggest that an increase in the total muscle force is accompanied with an increased disparity in the distribution of the stress over the skull, yet the phenotype of Pod Mrčaru maintained a similar level of homogeneity of the stress distribution.

Surprisingly, the PM morphology is more advantageous than the PK morphology in producing bite force, especially at wide gapes (as revealed by the MDA simulations), whereas it is more advantageous in dissipating stress at lower gapes (as revealed by the FEA simulations). That said, even if at wide gape where the advantage of PM morphology is lower, it remains slightly more advantageous than the PK morphology. Although this might first seem contradictory, we propose that these two results are not incompatible. We rather think that selection likely primarily acts upon performance that the musculoskeletal system is able to produce (bite force) within the limits imposed by the intrinsic capacity of the system to dissipate the associated stress and strain. Therefore, the MDA results suggest that the evolution of skull morphology towards a Pod Mrčaru phenotype enabled an optimization of bite force at wide gapes. This is likely driven by intraspecific competition which often leads to male-

male aggression and involves biting congeners (Donihue et al. 2016) thus requiring large gapes. However, data on population density and levels of intraspecific aggression are needed to explore this further (**short-term Perspective 1**). The consumption of hard and fibrous material like plant matter was demonstrated to require repeated and frequent bites over a longer period of time (Herrel, Verstappen & De Vree, 1999) to reduce the item into smaller and more easily digestible bite-size pieces (Bjorndal et al. 1990; Bjorndal & Bolnick, 1992). The structural advantage provided by the PM morphology at low gapes might be important in this context. Indeed, the repeated loading occurring at low gapes experienced by the skull while feeding on plants is likely particularly constraining and might represent a second selective agent favoring a “shock-absorbing” skull. A scenario in which head shape in insular *Podarcis* lizards is primarily driven by intraspecific competition, and secondly maintained by functional demands associated with resource use, would be in line with the results of Donihue and collaborators (2016) and by the preliminary results of the follow-up experiment in the Aegean system, presented in the General Introduction.

## GENERAL DISCUSSION

### *Synthesis of our current understanding of the Adriatic system*

The Adriatic archipelago was formed at the end of last glacial age, approximately 18.000 years ago, and is composed of 698 land-bridge islands and islets that differ in size and structure, making it the second largest archipelago in the Mediterranean. The elevation of the sea level after the ice melt resulted in the fragmentation of the habitat for the local flora and fauna. The body of published work available on this system includes studies on the terrestrial snail (Štamol, Vuković & Kletečki, 2012) and butterfly (Koren & Lauš, 2020) diversity, the diversity and the biogeography of small vertebrates (Kryštufelek & Kletečki, 2007; Vervust et al. 2009) and of lizards in particular (Podnar, Mayer & Tvrtković. 2004, 2005; Senczuk et al. 2017). The biology of *Podarcis* lizards in the Adriatic has been investigated by focusing on their behavior (Biaggini et al. 2010), their competitive interactions (Nevo et al. 1972), and their morphology (Ljubisavljevic et al. 2005). Studies dealing with the ecomorphology of lizards in this archipelago are mainly represented by studies focusing on the divergence between Pod Kopište and Pod Mrčaru. These studies have investigated gut anatomy and function (Wehrle et al. 2020), head dimensions and bite force (Herrel et al. 2008a), limb dimensions and locomotor performance (Vervust, Grbac & Van Damme, 2007), or a set of morphological and performance variables (Vervust et al. 2008, 2010). However, no comparative study of the ecomorphology of lizards at the inter-population level exists. Despite the presence of *Podarcis* lizards on numerous islands and islets of the archipelago (in particular *P. melisellensis* and *P. sicula*), their relatively recent divergence, and the undeniable value of this system for addressing evolutionary questions, the divergence of these species on these islands remains to be thoroughly described (but see Kammerer, 1926). Before being able to tackle the central question of the present thesis, a comprehensive investigation of the ecological and morphological diversity of the populations inhabiting the archipelago was needed, and constituted the main work undertaken in the Chapters 1,2 and 4. Below is a summary of what we now know about the ecomorphology of these *Podarcis* lizards in the Adriatic.

- *Ecology*: The comparative work presented in this thesis enabled us to describe the intraspecific variability in the ecology of the populations studied. Specifically, the resource use and the dietary niche breadth of each population was estimated through the calculation of the disparity of the prey items consumed. Additionally, the resource use at the individual level was estimated by the calculation of a diversity index. *Podarcis melisellensis* and *P. sicula* differed in their dietary niche breadth and how this varied with island size and thus with levels of intraspecific competition. The latter

species increased its dietary breadth when competition becomes more intense, possibly explaining its invasive success in the Balkan region. Interestingly, the investigation of niche breadth suggested that variation among populations was permitted by individual specialization, resulting in an increase in the between-individual variation. However, the relevance of the inter-individual variation in diet remains to be investigated further (**short-term Perspective 2**). The sexual dimorphism in resource use was not explained by sexual differences in head dimensions, and contradicts the theory of food partitioning as a driver of sexual shape dimorphism. Finally, ecological release due to a lack of top predators typically described in island systems may promote dietary diversification and the shift towards alternative resources such as plant matter. Indeed, several populations included significant amounts of plants in their diet. However, the reasons for these dietary shifts are not fully understood, with quantification of resource availability needed to test for electivity in these populations (**short-term Perspective 3**).

- *Performance*: Variation in the type of resource consumed by the lizards, described in Chapter 1, depended on the island isolation (distance to the nearest large island), which was suggested to be a key factor influencing the diversity and abundance of prey available for the animals to eat. The consumption of difficult-to-reduce items such as plant matter and hard prey was associated with greater bite forces, essential to increase digestive efficiency and reduce intraoral transport time. Greater bite forces were in turn associated with functionally relevant morphological characteristics, including body size and linear head dimensions, which is congruent with the patterns previously observed at the interspecific level in other lizards. However, the head dimensions that correlated with bite force differed in both species, suggesting that proximate drivers of bite force are species-specific. As expected, the phylogenetic relationships had very little effect on the observed patterns, suggesting that local constraints including resource use are strong and favor convergence patterns in head dimensions and performance. Although variables used as proxies for intraspecific interactions and aggression did not correlate with bite force, more effort should be undertaken to characterize the social interactions in these populations, especially by quantifying population densities (**short-term Perspective 4**).

- *Behavior*: Maximum levels of performance in themselves are informative but in an attempt to make the link between function and fitness, the actual use of this function (here, biting) needs to be replaced in a behavioral context. Indeed, if evolutionary pressures imposed by the environment fluctuate, selection might not directly operate on maximal performance but rather on the ability of animals to modulate their behavioral response, thus slightly changing the context in which performance is relevant. Investigating this behavioral flexibility was thus a logical step to understand

the relationships between morphology and ecology in this system. A study on the jaw kinematics of lizards from Pod Kopište and Pod Mrčaru (Chapter 3) revealed that prey characteristics such as hardness and size impacted the movements used by the lizards while feeding. Both sexes differed in the way they modulated their movements to deal with differences in prey mobility and hardness, but differences were consistent in the two populations. Interestingly, the population which showed a greater dietary niche breadth (Pod Mrčaru) also showed a greater kinematic flexibility as well as more stereotyped movements, suggesting a finer motor control. If we think about this in an ecological context of resource use, the array and diversity of prey items that animals can have access to depends on their ability to deal efficiently with them. Since the divergence between these two populations is marked by a shift towards omnivory, additional data should be gathered on jaw kinematics while feeding on plant matter to explore this further (**short-term Perspective 5**).

- *Morphology*: The investigation of the morphological basis of variation in bite force and diet focusing on the muscle architecture and head shape revealed that an important part of the total variance in bite force and diet observed among populations was explained by muscle architecture. Whereas investing in external adductors seemed to be the most effective way to increase bite force, differential investment in muscle groups was observed reflecting dietary specialization. This investment together with the consumption of specific food items, reflects their functional characteristics and the local island-specific constraints on the underlying morphology. In this sense, muscle force and head shape covary locally, whereas the covariation between head shape and bite force is mainly due to allometry.

- *Biomechanics*: Because diet, and in particular the type of food item ingested (plant matter or hard arthropods) and male-male interactions seem to impose a significant constraint on the feeding system, we tested how the musculoskeletal system would respond to these constraints. Three situations were tested, each corresponding to a functionally relevant behavior: biting a plant item (gape of 0°), an arthropod (gape of 20°), or a congener (gape of 45°). We were able to describe the function of each muscle group, and demonstrated that head shape variation was responsible for a notable part of the variation in bite force, by modulating the efficiency of the system to convert muscle force into bite force. Furthermore, we demonstrated that an increase in muscle force, needed in the case of a dietary shift towards more difficult-to-reduce material, imposes greater levels of stress on the skull, and that the evolution of morphological variation enabled dissipation of stress and a more uniform distribution.

Most interestingly, the pattern of morphological divergence between the two populations Pod Kopište and Pod Mrčaru was congruent with that expected by the results of the comparative study. Moreover, within-population pattern of correlation between musculature and resource use lies in line with what would be expected if investment in muscle groups followed the *in silico* estimations of muscle efficiency. In other words, patterns observed at a microevolutionary scale (the recent divergence between two populations) and experimentally tested through *in silico* simulations were consistent with the patterns at a macroevolutionary scale (at the level of the archipelago). The observation that small-scale microevolutionary variation in phenotype and performance in an ecological context is translated into macroevolutionary variation suggests that the Adriatic archipelago is an illustration of an adaptive radiation in its initial stages.

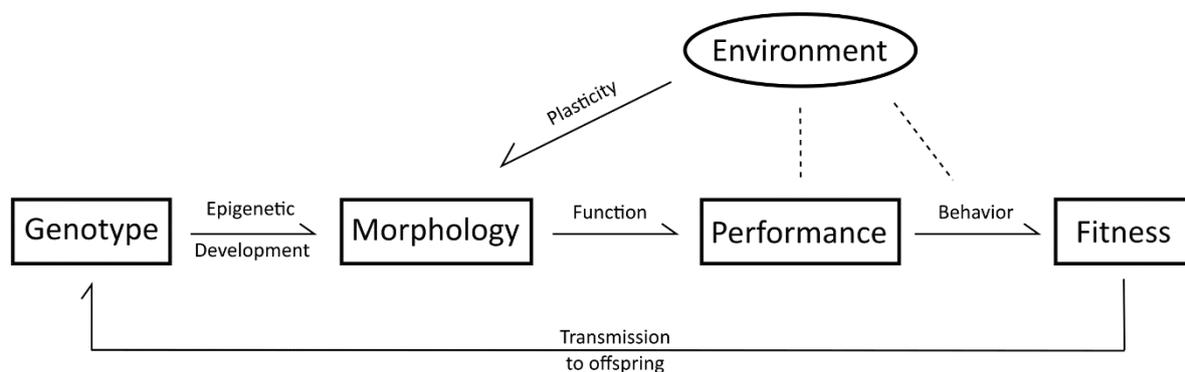
### ***The Adriatic archipelago, a unique model-system?***

Whereas the Adriatic archipelago has been studied to some extent, other island systems are considered “classics” in evolutionary biology and have drawn particular attention over the last decades.

The *Anolis* radiation in the Caribbean is one of these “classic systems” where the combined effort by the research community over the past 50 years has accumulated in a solid knowledge on the phylogeny, the physiology, the behavior, the habitat, the morphology, and the evolution of this group of lizards (see Losos, 2009 for a review). Furthermore, this has involved both observational and experimental approaches. The radiation of Darwin’s finches in the Galapagos is also a classic model which has been studied for a long time (Bowman, 1963; Schluter et al. 1997; Grant & Grant, 2003). One limitation in studying this system is that the archipelago is a protected area and thus does not allow field experiments to be carried out. Finally, the radiation of the cichlid fishes in the Eastern African lakes also belongs to these amazing systems where an exceptional diversification occurred subsequently to the invasion of limited and isolated environments (Fryer & Iles, 1972; Rossiter, 1995; Seehausen, 2006).

As suggested by Arnold (1983), the understanding of the emergence of phenotypic diversity and its maintenance cannot be fully understood if not in the light of performance. The link between morphology and performance can be understood by investigating function, and the influence of performance on associated fitness depends on the behavioral responses and adaptability (Figure 14). Despite the impressive amount of studies focusing on the three systems listed above, to date no study

has linked the morphology and shape of a musculoskeletal system, with the associated performance and behavior in a context of habitat use. Few attempts have been made though, including an investigation of the relevance of beak shape in cracking seeds in Darwin's finches (Soons et al. 2010; Genbrugge et al. 2011; Soons et al. 2015); a study on the relationships between head dimensions, bite force and ecomorphs in *Anolis* lizards (De Meyere et al. 2019); a study on the relationships between limb anatomy and locomotor performance (Foster & Higham, 2012; Anzai et al. 2014) or between locomotor behavior and habitat use (Vanhooydonck et al. 2006; Herrel et al. 2008b); or between trophic anatomy and ecological divergence (Cooper et al. 2010). Each of these otherwise very insightful study lacks the systematic comparative approach necessary to take advantage of the replicates provided by insular systems, or a quantitative description of the relationship between musculature and performance. So surprisingly all of these classic model systems lack a complete and integrative approach proposed by the ecomorphological framework.



**Figure 114:** Ecomorphology framework.

The present thesis aimed to understand how morphological variation can result in significant changes in ecology. To do so, we have taken advantage of the replicates provided by the Adriatic archipelago to describe morphology through the quantification and the systematic comparison of the shape of the feeding apparatus; quantify the muscle architecture variation in relation to variation in shape; estimate the relationships between head shape and musculature with performance through both a correlational comparative approach and a biomechanical modelling approach; and assessed the link between performance and ecology (resource use, intraspecific competition) through the scope of a study on feeding behavior (Figure 14).

***Bridging the gap between micro and macroevolution***

The aim of the present thesis was to understand the link between micro and macroevolution in association with ecology by taking advantage of an island system. To do so, we asked whether and how small-scale changes in morphology could result in significant changes in performance, ultimately enabling behavioral and ecological changes at larger scale. We here demonstrated that this is indeed the case.

In an attempt to understand how evolution works, four levels of integration need to be tackled. First, the exploration of interspecific patterns at a macroevolutionary level (i.e. what is usually done in classic systems like the Caribbean or the Galapagos), in other words to look for convergent patterns of evolution among distinct species that evolved independently towards similar phenotypes under similar environmental conditions. Second, one can focus on the intraspecific patterns of diversity. The case study of the *Podarcis* lizards in the Adriatic archipelago fits this level of integration, with one species spreading on isolated environments being separated for a relatively short time and showing contrasted phenotypes, yet also converging under similar circumstances. Below this is the level of the intraspecific patterns of variation where populations have recently diverged and show distinct phenotypes in relation with environmental characteristics. The divergence between Pod Kopište and Pod Mrčaru belongs to this level. Finally, one can focus on the processes of evolution themselves, that is to say to quantify how and to what extent processes such as natural selection, sexual selection, phenotypic plasticity, genetic drift or founder effects operate. The present work focused on the two intermediate levels. The description of intraspecific macroevolutionary patterns of covariation between morphology, musculature, performance, behavior and ecology (comparative study) was corroborated by the intraspecific patterns of microevolution investigated through the biomechanical modelling (recent divergence between two populations), suggesting that functional constraints imposed by diet favor the evolution of biomechanically performant musculoskeletal systems, and that this translates to the whole archipelago system. Moreover, although the present study does not directly allow us to infer how and whether this could translate to variation at the interspecific macroevolutionary scale, similar patterns are likely to be observed.

To go one step further and to fully conclude the ultimate objective presented in the present work, some final questions remain to be answered. Is the morphological variation observed really under selection? How is morphological diversity passed on to the next generation? Are these changes adaptive or mainly the result of phenotypic plasticity? If divergent selection really occurs, then we could consider the Adriatic system to be in the initial stages of an adaptive radiation.

### ***On-going and long-term future work directions***

- *Quantifying phenotypic plasticity*: during the present project, we started to conduct a study on the seasonal changes on the island of Pod Kopište. Because food availability and thus diet often changes between spring and summer in Mediterranean habitats, we first explored whether diet and bite force significantly varied between seasons on Pod Kopište. Whereas this was the case on Pod Kopište this was not the case on Pod Mrčaru. We also noticed that jaw musculature and underlying head shape changed seasonally at levels of magnitude greater than that observed between the two populations. The question we asked in this project was: are seasonal changes in morphology due to phenotypic plasticity or differential survival of certain phenotypes causing the mean population to change over time? Additionally, we wondered whether these changes are heritable and accumulate year after year. In other words, is seasonal variation a key agent of selection driving the evolution of this insular population? We thus started a field experiment during which we captured lizards in spring, measured them and marked them with pit tags and released them. By comparing recaptured specimens between seasons, we should be able to assess the role of phenotypic plasticity in driving seasonal changes. We are currently still gathering data in order to characterize this seasonal variation over several years to check whether this is a repeatable pattern.

A better understanding of the relative contribution of plasticity and genetic variation in driving morphological variation in these lizards can also be estimated with lab experiments. In one unpublished experiment (Vanhooydonck & Herrel pers. comm.) lizards were maintained in captivity and mated. The genealogy of the progeny was recorded, and different prey types were offered to the lizards (soft and hard prey). The idea was to follow the morphological, anatomical and performance trajectories of these lizards with different treatments throughout ontogeny, while knowing the parental relationships between them. Analyzing the data provided by this experiment would be very insightful to understand how plastic is morphological variation in relation to diet.

- *Exploring the performance landscape*: The present work has paved the way to the quantification of mechanical and structural performance of the feeding musculoskeletal system through biomechanical modelling. One interesting aspect of phenotypic diversification would be to explore the morphospace built from the comparative study and the associated levels of performance which could be estimated by the validated models developed in the present thesis. Is the phenotypic diversity observed in our system, at the macroevolutionary scale, predicted by the performance landscape? Addressing this type of question is the very point of developing models that can be manipulated. For example, we could change the shape of the skull to test whether unrealistic phenotypes are mechanically relevant or inexistant because mechanically inferior or developmental or constructional constraints.

- *Intra-population variability*: Because the divergent evolution of each population of the island system is likely enabled by the natural variation in phenotype and performance within each population, it would be interesting to investigate the relevance of between-individual variation, through observational, experimental and modelling approaches.

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## General introduction

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## Chapter 1: Ecology

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# Appendices

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## APPENDICES – Chapter 2: Performance

**Table S1:** Population, sequence origin, species, and GenBank accession number of the samples used in the analyses.

<b>Name</b>	<b>Population(s)</b>	<b>Species</b>	<b>Sampled</b>	<b>Accession no.</b>
<i>Bijelac 1</i>	Bijelac	<i>P. sicula</i>	2016	MN995801
<i>Bijelac 2</i>	Bijelac	<i>P. sicula</i>	2016	MN995802
<i>Kopiste 1</i>	Kopište	<i>P. sicula</i>	2016	MN995805
<i>Kopiste 2</i>	Kopište	<i>P. sicula</i>	2016	MN995806
<i>Pod Mrčaru 1</i>	Pod Mrčaru	<i>P. sicula</i>	2016	MN995803
<i>Pod Mrčaru 2</i>	Pod Mrčaru	<i>P. sicula</i>	2016	MN995804
<i>Split</i>	Split <sup>#</sup>	<i>P. sicula</i>	GenBank	AY185094
<i>Susac</i>	Sušac	<i>P. sicula</i>	GenBank	AY770889
<i>Pod Kopiste</i>	Pod Kopište,	<i>P. sicula</i>	GenBank	AY770890
<i>Pijavica</i>	Pijavica			
<i>Mala Palagruza</i>	Mala Palagruža	<i>P. sicula</i>	GenBank	AY770891
<i>Sinj 1</i>	Sinj <sup>#</sup>	<i>P. melisellensis</i>	2016	MT010548
<i>Sinj 2</i>	Sinj <sup>#</sup>	<i>P. melisellensis</i>	2016	MT010549
<i>Veli Tajan 1</i>	Veli Tajan	<i>P. melisellensis</i>	2016	MT010550
<i>Veli Tajan 2</i>	Veli Tajan	<i>P. melisellensis</i>	2016	MT010551
<i>Veli Barjak 1</i>	Veliki Barjak	<i>P. melisellensis</i>	2016	MT010552
<i>Veli Barjak 2</i>	Veliki Barjak	<i>P. melisellensis</i>	2016	MT010553
<i>Jabuka 1</i>	Jabuka	<i>P. melisellensis</i>	GenBank	AY185097
<i>Jabuka 2</i>	Jabuka	<i>P. melisellensis</i>	GenBank	AY185066
<i>Glavat</i>	Glavat	<i>P. melisellensis</i>	GenBank	AY185042
<i>Brusnik 1</i>	Brusnik	<i>P. melisellensis</i>	GenBank	AY185057
<i>Brusnik 2</i>	Brusnik	<i>P. melisellensis</i>	GenBank	AY185058
<i>Brusnik 3</i>	Brusnik	<i>P. melisellensis</i>	GenBank	AY185059
<i>Korčula</i>	Korčula	<i>P. melisellensis</i>	GenBank	AY185028
<i>Mali Paržanj 1</i>	Mali Paržanj	<i>P. melisellensis</i>	GenBank	AY185069
<i>Mali Paržanj 2</i>	Mali Paržanj	<i>P. melisellensis</i>	GenBank	AY185070
<i>Veli Budikovac</i>	Veli Budikovac	<i>P. melisellensis</i>	GenBank	AY185063
<i>Ravnik</i>	Ravnik	<i>P. melisellensis</i>	GenBank	AY185071
<i>Greben</i>	Greben	<i>P. melisellensis</i>	GenBank	AY185065
<i>Mali Barjak</i>	Mali Barjak	<i>P. melisellensis</i>	GenBank	AY185068
<i>Krbavica</i>	Krbavica <sup>x</sup>	<i>Lacerta bilineata</i>	GenBank	LN834641

<sup>#</sup> mainland populations; <sup>x</sup> outgroup used for phylogenetic analysis

**Table S2:** List of primers used for cytochrome *b* sequence analysis. Primer use: A – PCR amplification, R – PCR reamplification, and S – sequencing.

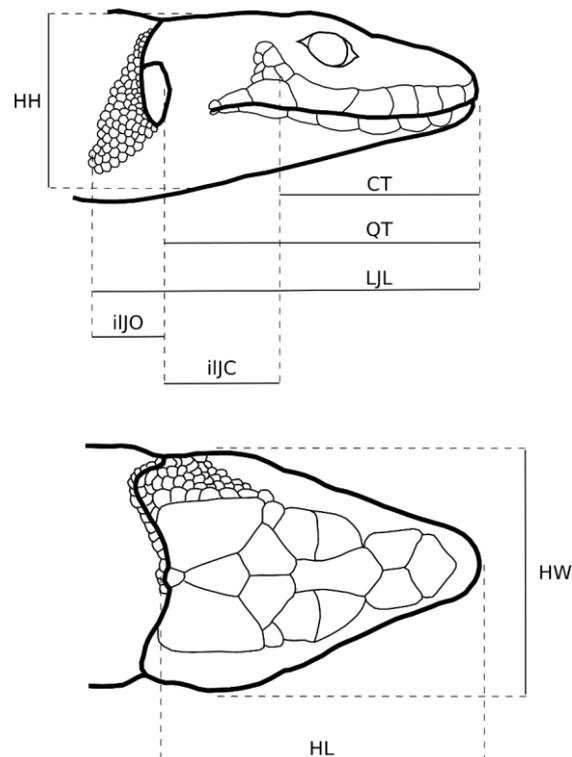
Species	Primer name	Primer sequence 5' 3'	Primer use
<i>P. sicula</i>	L-14253	TTT GGA TCC CTG TTA GGC CTC TGC C	A, R
	H-15425	GGT TTA CAA GAC CAG TGC TTT	A
	H-15150	ATA ATA AAG GGG TGT TCT ACT GGT TGG CC	R, S
	H-14776	GGT GGA ATG GGA TTT TGT CTG	S
<i>P. melisellensis</i>	L-14132	ATT CAA CTA TTA AAA CCT CTA ATG	A
	H-15425	GGT TTA CAA GAC CAG TGC TTT	A
	H-15150	ATA ATA AAG GGG TGT TCT ACT GGT TGG CC	S
	H-14776	GGT GGA ATG GGA TTT TGT CTG	S

**Table S3:** Results of the phylogenetic univariate analyses of variances (BF: bite force, HL: head length, HW: head width, HH: head height, LJJ: law-jaw length, QT: quadrate to tip length, CT: coronoid to tip length; P: P-value, F: F-statistic).

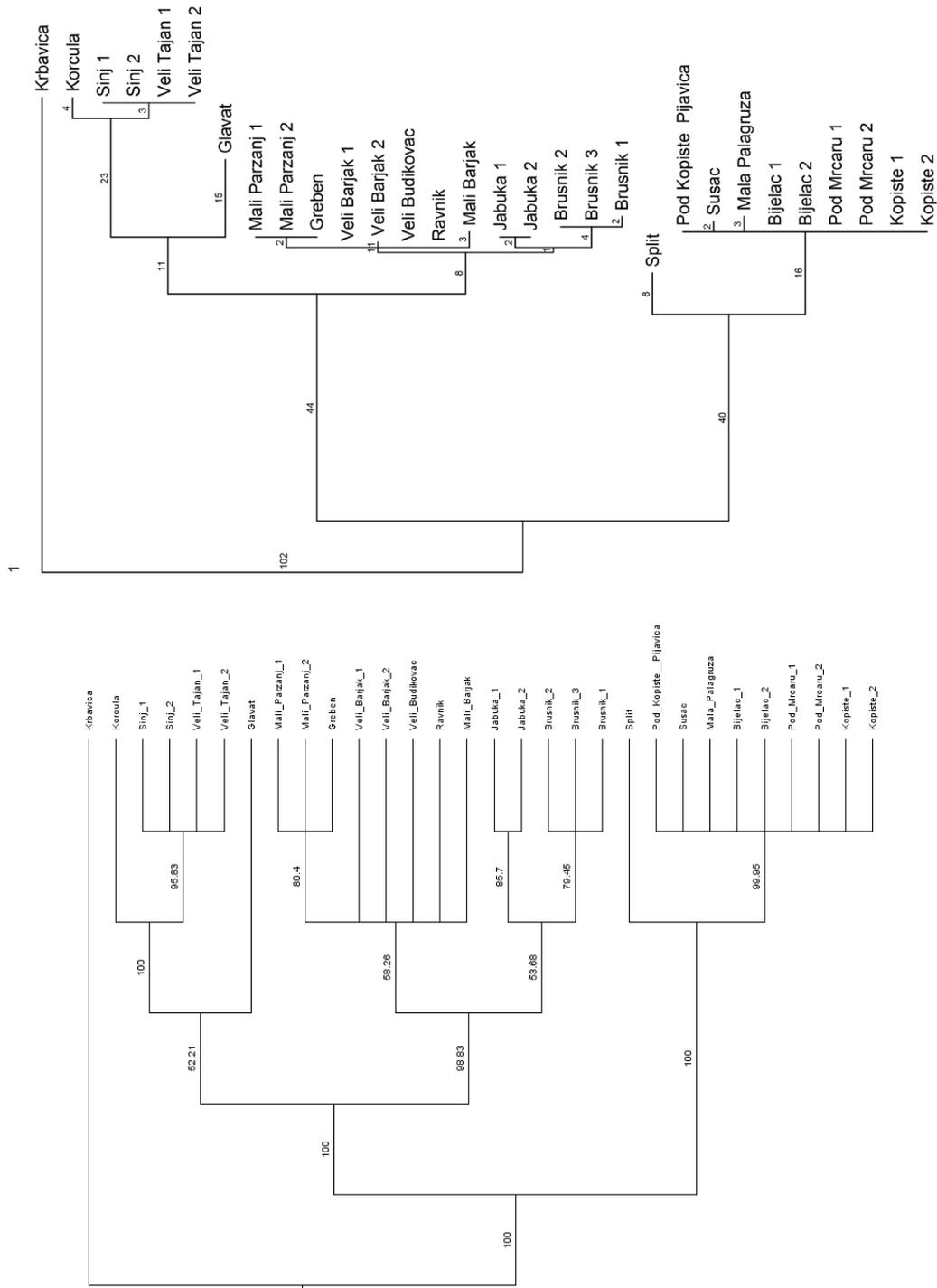
	Sex	Species		
		males	females	
BF	0.010*	0.962	0.840	P
	0.205	0.055	1.343	F
HL	0.013*	0.732	0.483	P
	1.003	2.838	17.540	F
HW	0.022*	0.966	0.604	P
	0.548	0.084	9.040	F
HH	0.086	0.749	0.881	P
	0.256	2.844	0.698	F
LJJ	0.009*	0.812	0.601	P
	1.060	1.479	8.921	F
QT	0.01*	0.740	0.539	P
	1.029	2.798	13.032	F
CT	0.019*	0.828	0.587	P
	0.935	1.270	9.762	F

**Table S4:** Results of the phylogenetic multiple regression of residual head dimensions on bite force (HL: head length, HW: head width, HH: head height, LJL: law-jaw length, QT: quadrate to tip length, CT: coronoid to tip length).

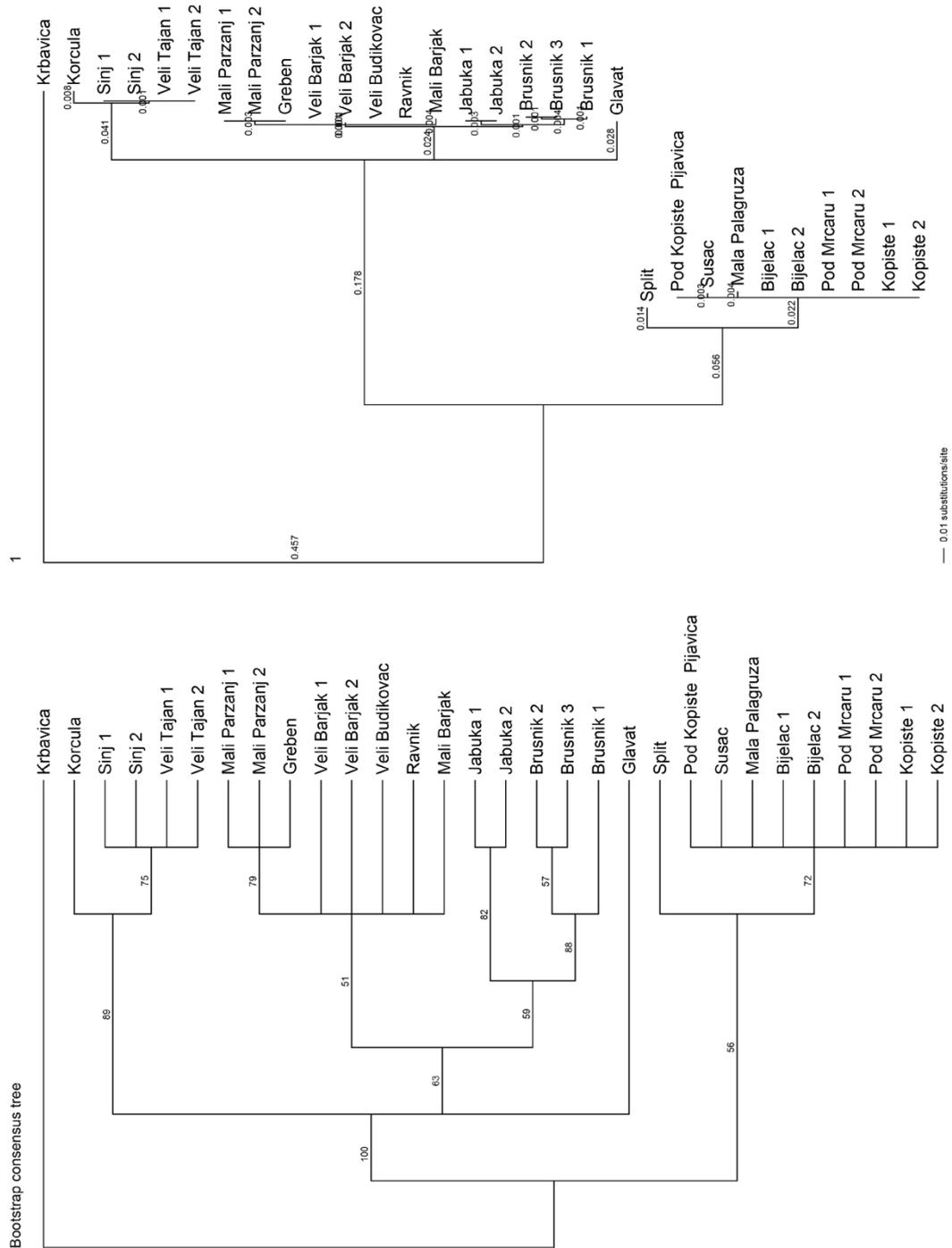
	$\sigma^2$	Model retained						Slope coefficient
		hl	hw	hh	ljl	qt	ct	
Females <i>P. melisellensis</i>	<0.001	-11.82	5.32	-	-17.78	23.21	-	
Males <i>P. melisellensis</i>	<0.001	-	3.32	12.05	-26.27	21.15	8.01	



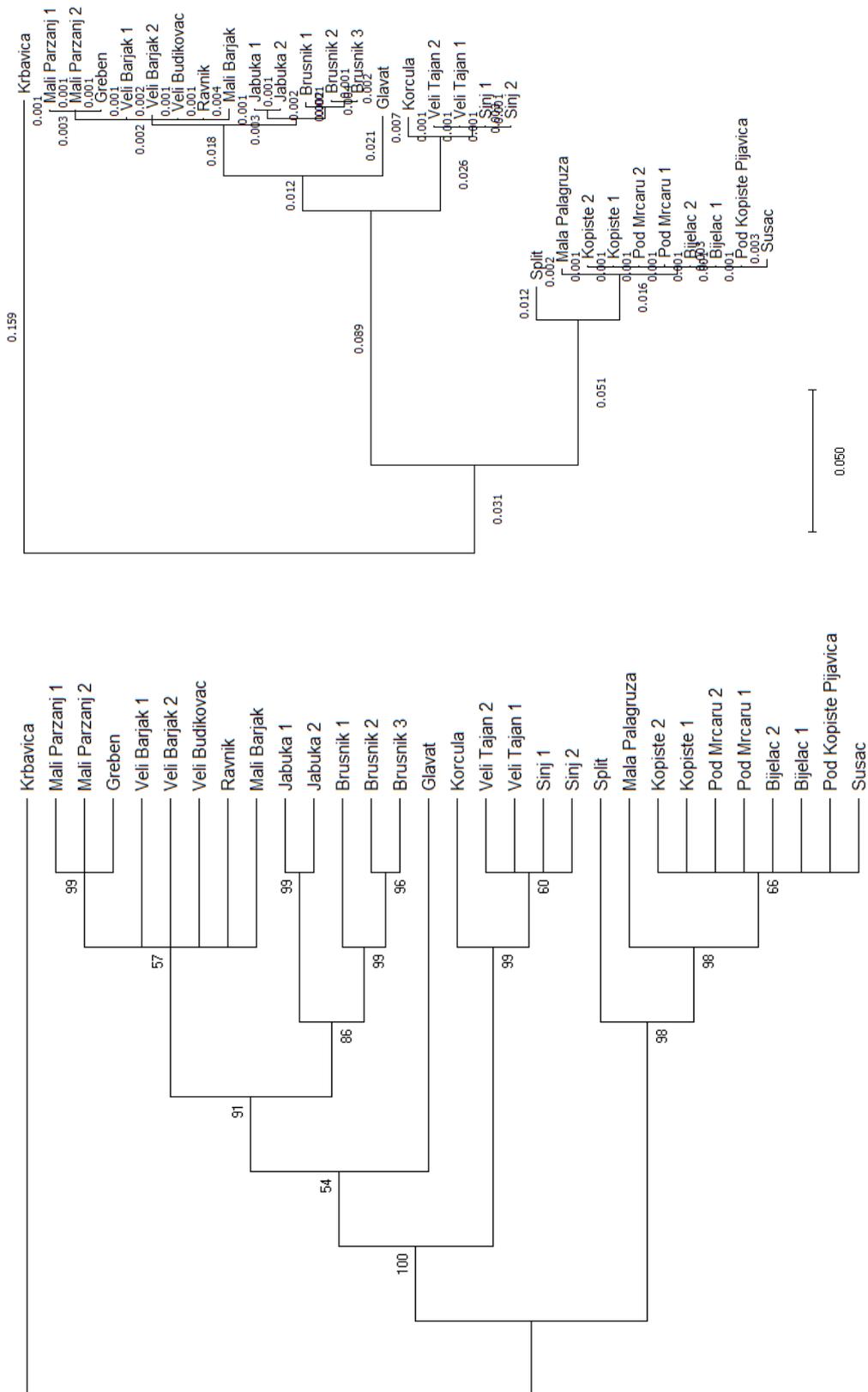
**Figure S1:** Linear head dimensions. HL= head length, HW= head width, HH= head height, CT= coronoid to tip length, QT= quadrate to tip length, LJL= lower-jaw length, ilJO= in-lever for jaw opening, ilJC= in-lever for jaw closing.



**Figure S2a:** *Podarcis* MP bootstrap 50% majority-rule consensus phylogram tree (top) with length of each branch according to the number of changes, and consensus cladogram tree (bottom) with percentage of trees supported (1000 bootstrap repetitions).



**Figure S2b:** *Podarcis* ML bootstrap 50% majority-rule consensus phylogram tree (top) with length of each branch according to the substitution rate, and consensus cladogram tree (bottom) with percentage of trees supported (100 bootstrap repetitions).



**Figure S2c:** *Podarcis* 50% majority rule phylogram tree calculated with MrBayes with length of each branch according to the substitution rate (top); and consensus cladogram tree (bottom) with posterior probability associated to each clade.

## APPENDICES – Chapter 4: Morphology

**Table S1:** List of the specimens included in the present study.

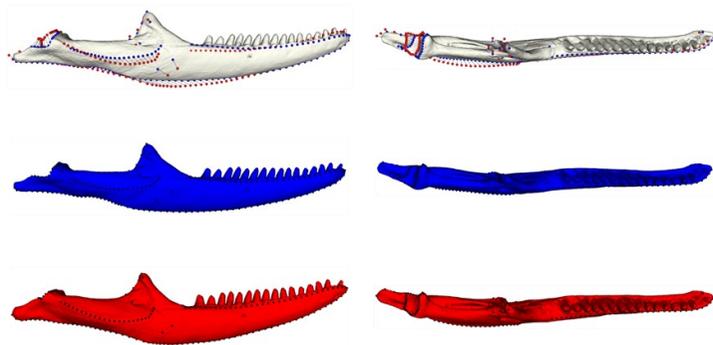
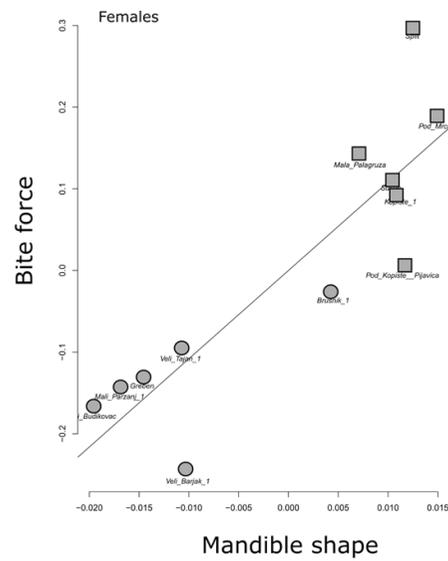
Genus	Species	Site	Ecological data		Morphological data	
			Females	Males	Females	Males
<i>Podarcis</i>	<i>melisellensis</i>	Brusnik	22	20	5	5
		Glavat	9	12	-	5
		Greben	12	18	5	5
		Jabuka	21	17	-	5
		Mali Barjak	14	18	-	5
		Mali Paržanj	13	12	5	5
		Sinj *	5	11	-	5
		Veli Barjak	19	21	5	5
		Veli Budikovac	17	17	5	5
		Veli Tajan	7	8	5	5
	<i>sicula</i>	Kopište	14	19	5	5
		Mala Palagruža	8	12	5	4
		Pod Kopište	15	22	5	5
		Pod Mrčaru	12	24	5	5
Split *		9	7	5	5	
Sušac		15	5	5	5	

**Table S2:** Results of the regressions between bite force, the proportion of plants, the proportion of hard prey items, mean prey hardness, and the muscular characteristics including the PCSA, muscle mass, and the mean fiber length of the 5 muscle groups (DM: jaw opener, ADD: external adductors, PSEU: pseudotemporalis, PTG: pterygoids, CONST: constrictor dorsalis muscles). S: slope,  $\beta$ : standardized coefficient,  $R^2$ : regression coefficient, P: p-value. Bold values indicate retained models. Values in blue and red indicate a negative and a positive correlation, respectively.

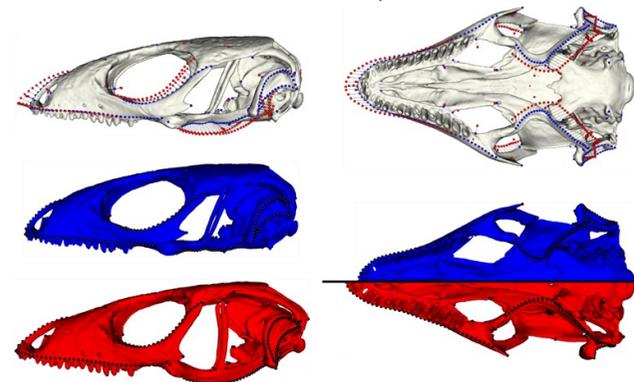
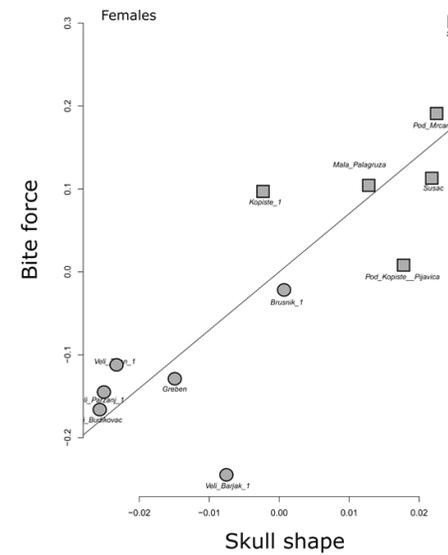
		No correction for phylogeny				With correction for phylogeny				
		Females		Males		Females		Males		
		Raw	Residuals	Raw	Residuals	Raw	Residuals	Raw	Residuals	
Bite Force	PCSA	Model	<b>P &lt; 0.001</b> <b>R<sup>2</sup> = 0.854</b>	<b>P = 0.001</b> <b>R<sup>2</sup> = 0.71</b>	<b>P = 0.04</b> <b>R<sup>2</sup> = 0.3</b>	<b>P = 0.084</b> <b>R<sup>2</sup> = 0.212</b>	<b><math>\sigma^2 &lt; 0.001</math></b>	<b><math>\sigma^2 = 0.001</math></b>	<b><math>\sigma^2 = 0.001</math></b>	<b><math>\sigma^2 = 0.001</math></b>
		DM								
		ADD	<b>s = 2.838</b> $\beta = 0.35$	<b>s = 3.06</b> $\beta = 0.16$	<b>s = 1.629</b> $\beta = 0.16$		<b>s = 3.451</b> $p = 0.003$	<b>s = 3.493</b> $p = 0.003$	<b>s = 2.568</b> $p = 0.013$	<b>s = 3.193</b> $p = 0.007$
		PSEU	<b>s = -1.384</b> $\beta = -0.20$	<b>s = -1.212</b> $\beta = -0.07$			<b>s = -2.065</b> $p = 0.023$	<b>s = -2.097</b> $p = 0.024$		
		PTG			<b>s = -1.231</b> $\beta = -0.13$				<b>s = -1.934</b> $p = 0.029$	<b>s = -2.009</b> $p = 0.020$
	CONST									
	Mass	Model	<b>P &lt; 0.001</b> <b>R<sup>2</sup> = 0.812</b>	<b>P = 0.01</b> <b>R<sup>2</sup> = 0.559</b>	<b>P = 0.212</b> <b>R<sup>2</sup> = 0.09</b>	<b>P = 0.355</b> <b>R<sup>2</sup> = 0.016</b>	<b><math>\sigma^2 &lt; 0.001</math></b>	<b><math>\sigma^2 &lt; 0.001</math></b>	<b><math>\sigma^2 &lt; 0.001</math></b>	<b><math>\sigma^2 = 0.001</math></b>
		DM								<b>s = 1.604</b> $p = 0.006$
		ADD							<b>s = 0.961</b> $p = 0.033$	<b>s = 5.477</b> $p = 0.002$
		PSEU	<b>s = 2.640</b> $\beta = 0.36$	<b>s = 2.689</b> $\beta = 0.15$			<b>s = 1.268</b> $< 0.001$	<b>s = 2.300</b> $p = 0.029$	<b>s = -4.053</b> $p = 0.016$	<b>s = -5.599</b> $p = 0.001$
		PTG	<b>s = -1.466</b> $\beta = -0.22$	<b>s = -1.333</b> $\beta = -0.08$						
	CONST							<b>s = 1.312</b> $p = 0.005$		
Fiber length	Model	<b>P = 0.001</b> <b>R<sup>2</sup> = 0.7</b>	<b>P = 0.054</b> <b>R<sup>2</sup> = 0.359</b>	<b>P = 0.027</b> <b>R<sup>2</sup> = 0.34</b>	<b>P = 0.057</b> <b>R<sup>2</sup> = 0.256</b>	<b><math>\sigma^2 = 0.001</math></b>	-	<b><math>\sigma^2 = 0.002</math></b>	-	
	DM	<b>s = 4.204</b> $\beta = 0.14$								
	ADD	<b>s = -1.039</b> $\beta = -0.04$		<b>s = -2.175</b> $\beta = -0.10$						
	PSEU									
	PTG			<b>s = 1.7</b> $\beta = 0.08$						
CONST					<b>s = 2.483</b> $p = 0.010$					
PLANT	PCSA	Model	<b>P = 0.012</b> <b>R<sup>2</sup> = 0.541</b>	<b>P = 0.013</b> <b>R<sup>2</sup> = 0.422</b>	<b>P = 0.013</b> <b>R<sup>2</sup> = 0.53</b>	<b>P = 0.037</b> <b>R<sup>2</sup> = 0.423</b>	<b><math>\sigma^2 = 0.011</math></b>	<b><math>\sigma^2 = 0.021</math></b>	<b><math>\sigma^2 = 0.008</math></b>	<b><math>\sigma^2 = 0.009</math></b>
		DM			<b>s = 2.635</b> $\beta = 0.23$	<b>s = 2.524</b> $\beta = 0.16$			<b>s = 1.768</b> $p = 0.052$	<b>s = 1.395</b> $p = 0.132$
		ADD			<b>s = 7.716</b> $\beta = 0.76$	<b>s = 7.479</b> $\beta = 0.40$			<b>s = 7.384</b> $p = 0.015$	<b>s = 5.595</b> $p = 0.049$
		PSEU	<b>s = 7.550</b> $\beta = 1.07$	<b>s = 3.966</b> $\beta = 0.24$			<b>s = 5.709</b> $p = 0.043$			
		PTG	<b>s = -6.912</b> $\beta = -0.88$		<b>s = -6.656</b> $\beta = -0.70$	<b>s = -6.713</b> $\beta = -0.38$	<b>s = -3.333</b> $p = 0.156$		<b>s = -5.523</b> $p = 0.027$	<b>s = -4.222</b> $p = 0.101$
	CONST			<b>s = -2.644</b> $\beta = -0.23$	<b>s = -2.578</b> $\beta = -0.19$			<b>s = -2.771</b> $p = 0.011$	<b>s = -2.797</b> $p = 0.017$	
	Mass	Model	<b>P = 0.019</b> <b>R<sup>2</sup> = 0.578</b>	<b>P = 0.033</b> <b>R<sup>2</sup> = 0.428</b>	<b>P = 0.208</b> <b>R<sup>2</sup> = 0.094</b>	<b>P = 0.061</b> <b>R<sup>2</sup> = 0.174</b>	<b><math>\sigma^2 = 0.006</math></b>	<b><math>\sigma^2 = 0.006</math></b>	<b><math>\sigma^2 = 0.009</math></b>	<b><math>\sigma^2 = 0.010</math></b>
		DM						<b>s = -3.406</b> $p = 0.093$	<b>s = 3.053</b> $p = 0.026$	<b>s = 2.815</b> $p = 0.049$
		ADD	<b>s = -15.265</b> $\beta = -1.85$	<b>s = -13.382</b> $\beta = -0.68$			<b>s = -13.131</b> $p = 0.002$	<b>s = -12.304</b> $p = 0.005$		
		PSEU	<b>s = 15.440</b> $\beta = 2.13$	<b>s = 14.422</b> $\beta = 0.81$			<b>s = 7.117</b> $p = 0.047$		<b>s = -7.595</b> $p = 0.035$	<b>s = -7.883</b> $p = 0.047$
		PTG					<b>s = 4.850</b> $p = 0.064$	<b>s = 10.253</b> $p = 0.007$	<b>s = 4.669</b> $p = 0.086$	<b>s = 4.748</b> $p = 0.170$
	CONST	<b>s = -1.677</b> $\beta = -0.18$					<b>s = 1.034</b> $p = 0.118$			
Fiber length	Model	<b>P = 0.004</b> <b>R<sup>2</sup> = 0.785</b>	<b>P = 0.003</b> <b>R<sup>2</sup> = 0.668</b>	<b>P = 0.002</b> <b>R<sup>2</sup> = 0.606</b>	<b>P = 0.009</b> <b>R<sup>2</sup> = 0.512</b>	<b><math>\sigma^2 = 0.003</math></b>	<b><math>\sigma^2 = 0.010</math></b>	<b><math>\sigma^2 = 0.003</math></b>	<b><math>\sigma^2 = 0.005</math></b>	
	DM	<b>s = -1.239</b> $\beta = 0.19$	<b>s = 9.247</b> $\beta = 0.16$			<b>s = 11.252</b> $p = 0.012$		<b>s = -2.382</b> $p = 0.044$	<b>s = -1.790</b> $p = 0.110$	
	ADD	<b>s = -7.048</b> $\beta = -0.28$					<b>s = -6.326</b> $p = 0.071$		<b>s = 3.912</b> $p = 0.064$	
	PSEU			<b>s = -7.905</b> $\beta = -0.34$	<b>s = -8.290</b> $\beta = -0.35$	<b>s = -8.854</b> $p = 0.001$		<b>s = -9.162</b> $p = 0.001$	<b>s = -9.000</b> $p = 0.003$	
	PTG	<b>s = 5.707</b> $\beta = 0.23$		<b>s = 3.540</b> $\beta = 0.17$	<b>s = 3.949</b> $\beta = 0.17$	<b>s = 6.344</b> $p = 0.004$	<b>s = 6.767</b> $p = 0.078$	<b>s = 3.688</b> $p = 0.017$		
CONST	<b>s = -7.860</b> $\beta = -0.24$	<b>s = -15.008</b> $\beta = -0.32$	<b>s = 3.042</b> $\beta = 0.08$	<b>s = 3.654</b> $\beta = 0.08$	<b>s = -7.162</b> $p = 0.079$	<b>s = -6.345</b> $p = 0.137$	<b>s = 7.815</b> $p = 0.001$	<b>s = 7.608</b> $p = 0.002$		

		No correction for phylogeny				With correction for phylogeny												
		Females		Males		Females		Males										
		Raw	Residuals	Raw	Residuals	Raw	Residuals	Raw	Residuals									
HARD	PCSA	Model	P = 0.033 R <sup>2</sup> = 0.425		P = 0.030 R <sup>2</sup> = 0.439		P = 0.113 R <sup>2</sup> = 0.175		P = 0.008 R <sup>2</sup> = 0.574		σ <sup>2</sup> = 0.003		σ <sup>2</sup> = 0.003		σ <sup>2</sup> = 0.008		σ <sup>2</sup> = 0.002	
		DM							s = -1.891 p = 0.025	s = -1.852 p = 0.037			s = -0.964 p = 0.066					
		ADD	s = -4.607 β = -0.56	s = -4.455 β = -0.23			s = -3.955 β = -0.21		s = -2.840 p = 0.139	s = -2.752 p = 0.104	s = -2.506 p = 0.092	s = -4.177 p = 0.003						
		PSEU					s = 2.729 β = 0.15				s = 3.245 p = 0.039	s = 4.345 p = 0.002						
		PTG	s = 4.167 β = 0.53	s = 3.592 β = 0.15			s = 3.485 β = 0.20		s = 3.920 p = 0.040	s = 3.951 p = 0.042		s = 2.599 p = 0.004						
	CONST					s = -0.703 β = -0.05												
	Mass	Model	P = 0.374 R <sup>2</sup> = 0.082		P = 0.324 R <sup>2</sup> = 0.130		P = 0.006 R <sup>2</sup> = 0.471		P < 0.001 R <sup>2</sup> = 0.692		σ <sup>2</sup> = 0.003		σ <sup>2</sup> = 0.003		σ <sup>2</sup> = 0.003		σ <sup>2</sup> = 0.003	
		DM							s = -1.991 p = 0.015	s = -1.859 p = 0.057								
		ADD			s = -10.662 β = -1.05	s = -5.573 β = -0.23						s = -4.718 p = 0.116	s = -4.637 p = 0.120					
		PSEU			s = 9.583 β = 1.05	s = 7.604 β = 0.37						s = 10.667 p = 0.001	s = 10.336 p = 0.001					
		PTG							s = 1.698 p = 0.009	s = 1.741 p = 0.011	s = -4.637 p = 0.090	s = -4.072 p = 0.157						
	CONST					s = -1.108 β = -0.07					s = -1.788 p = 0.042	s = -1.686 p = 0.049						
	Fiber length	Model	P = 0.095 R <sup>2</sup> = 0.426		P = 0.038 R <sup>2</sup> = 0.568		P = 0.194 R <sup>2</sup> = 0.104		P = 0.212 R <sup>2</sup> = 0.091		σ <sup>2</sup> = 0.005		σ <sup>2</sup> = 0.003		σ <sup>2</sup> = 0.008		σ <sup>2</sup> = 0.010	
		DM		s = -5.240 β = -0.09							s = 3.142							
		ADD											s = 3.698 p = 0.206					
PSEU			s = -4.522 β = -0.19							s = -2.475								
PTG			s = 3.458 β = 0.13					s = 1.483 p = 0.073			s = -4.593 p = 0.092	s = -1.393 p = 0.153						
CONST		s = 10.926 β = 0.23																
Mean HARD	PCSA	Model	-		P = 0.123 R <sup>2</sup> = 0.143		-		-		σ <sup>2</sup> = 0.002		σ <sup>2</sup> = 0.003		σ <sup>2</sup> = 0.006		σ <sup>2</sup> = 0.006	
		DM							s = -1.223 p = 0.060	s = -1.676 p = 0.021								
		ADD																
		PSEU							s = -2.482 p = 0.072									
		PTG							s = 2.186 p = 0.097									
	CONST																	
	Mass	Model	P = 0.237 R <sup>2</sup> = 0.113		P = 0.244 R <sup>2</sup> = 0.107		-		-		σ <sup>2</sup> = 0.002		σ <sup>2</sup> = 0.002		σ <sup>2</sup> = 0.003		σ <sup>2</sup> = 0.003	
		DM							s = -1.382 p = 0.024		s = 2.799 p = 0.004	s = 2.046 p = 0.018						
		ADD							s = 4.290 p = 0.024	s = 4.249 p = 0.022	s = 5.212 p = 0.054	s = 5.424 p = 0.133						
		PSEU							s = -3.358 p = 0.067		s = -3.629 p = 0.132							
		PTG								s = -3.011 p = 0.043	s = -2.596 p = 0.161	s = -5.723 p = 0.042						
	CONST								s = -0.572 p = 0.092		s = -1.040 p = 0.235							
	Fiber length	Model	P = 0.109 R <sup>2</sup> = 0.328		P = 0.034 R <sup>2</sup> = 0.425		P = 0.138 R <sup>2</sup> = 0.090		P = 0.155 R <sup>2</sup> = 0.134		σ <sup>2</sup> = 0.003		σ <sup>2</sup> = 0.003		σ <sup>2</sup> = 0.006		σ <sup>2</sup> = 0.006	
		DM		s = 3.216 β = 0.06														
		ADD							s = -2.856 p = 0.209									
PSEU								s = 3.712 p = 0.081										
CONST			s = 2.225 β = 0.05							s = 4.021 p = 0.0125								

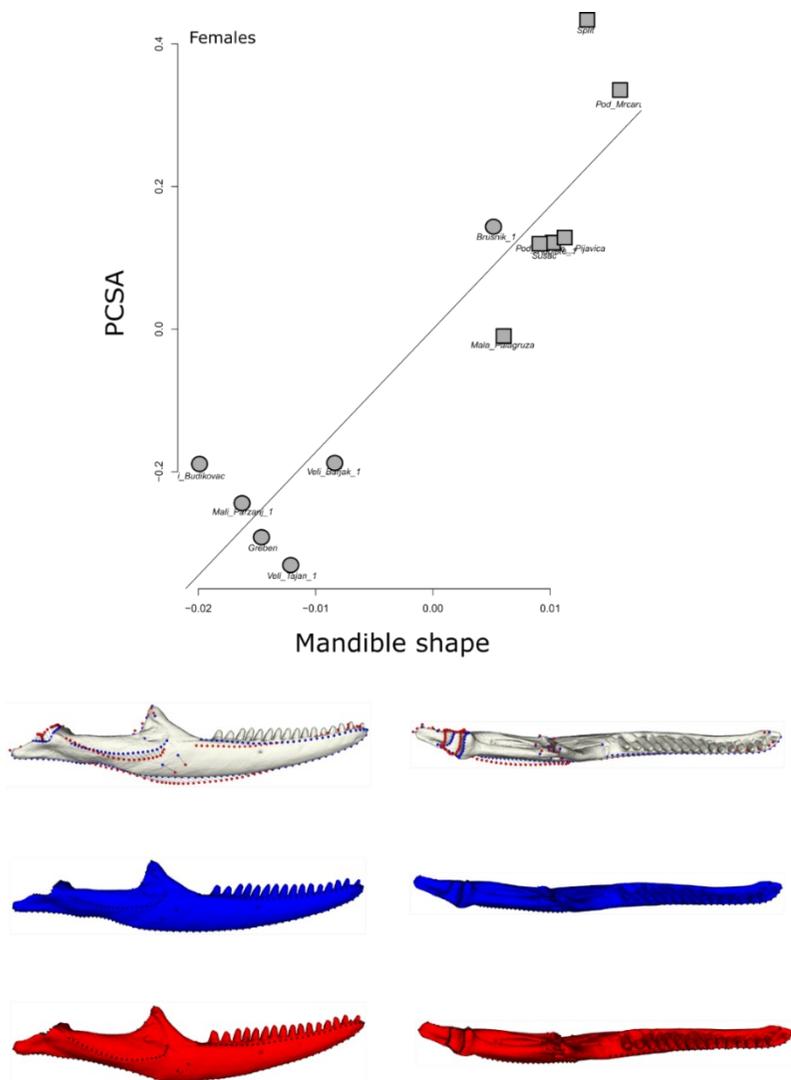
**Figure S1 - 23:** Results of the 2b-PLS analyses exploring the covariation patterns between morphology and variables including muscle architecture, bite force, and resource use. Blue and red dots illustrate the conformation associated with the extreme negative and positive sides of the covariation axis, respectively. Squares and circles in the graph represent *P. sicula* and *P. melisellensis*.



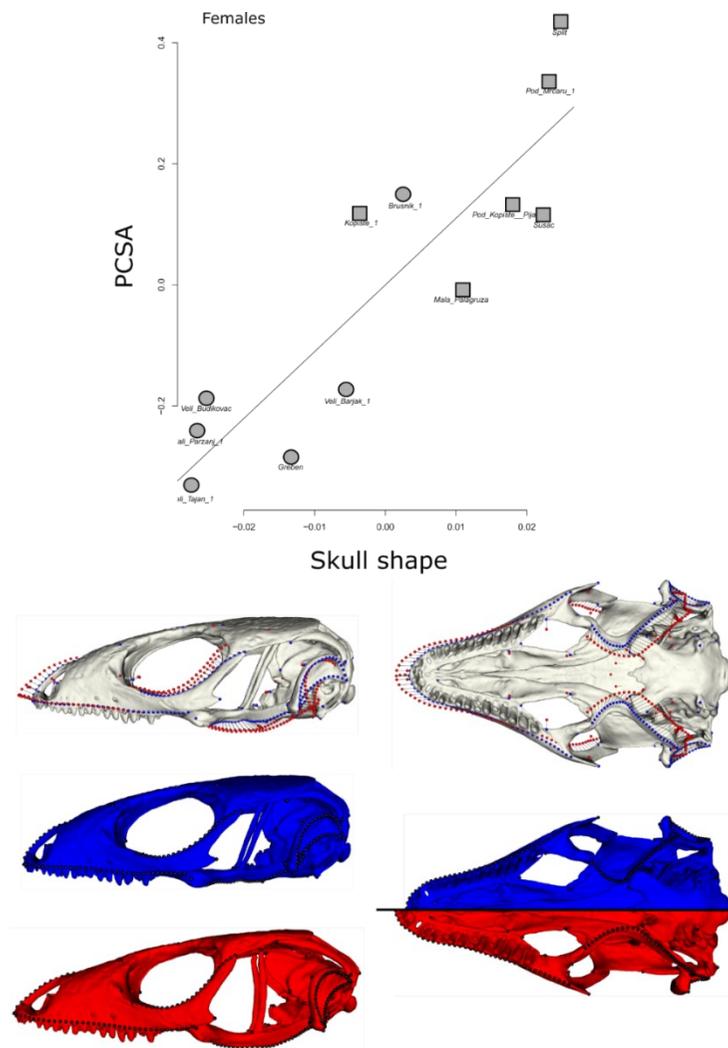
**Fig. S1:** Covariation between mandible shape and bite force in females.



**Fig. S2:** Covariation between skull shape and bite force in females.

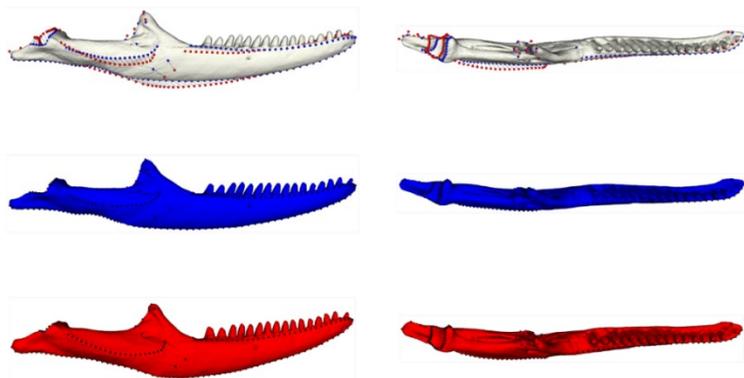
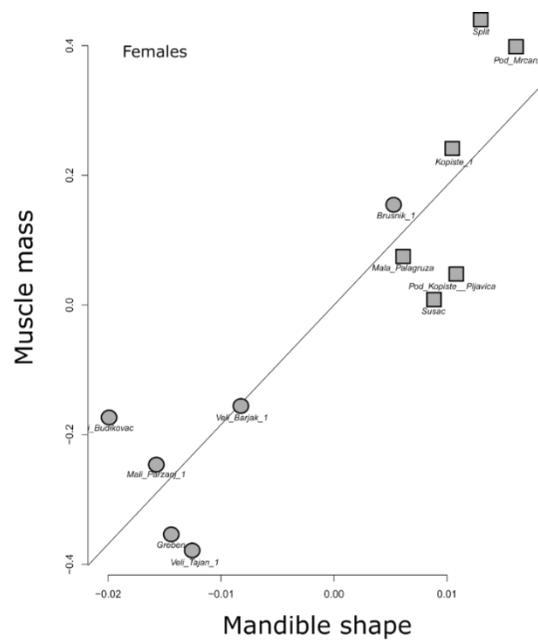


**Fig. S3:** Covariation between mandible shape and muscle PCSA in females.

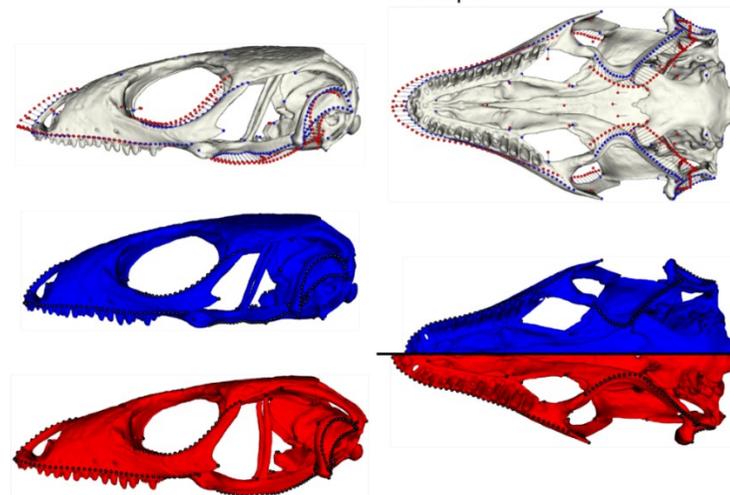
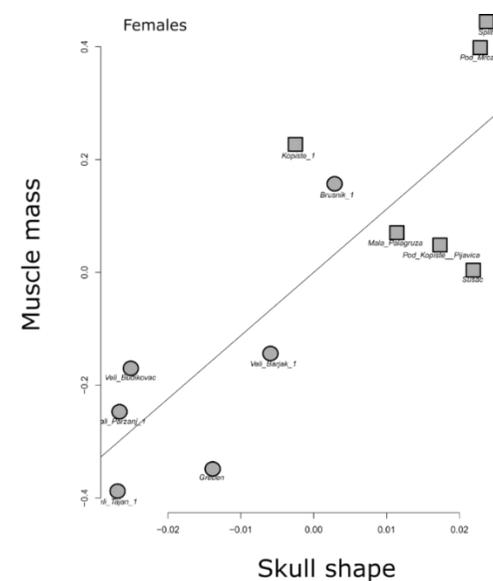


**Fig. S4:** Covariation between skull shape and muscle PCSA in females.

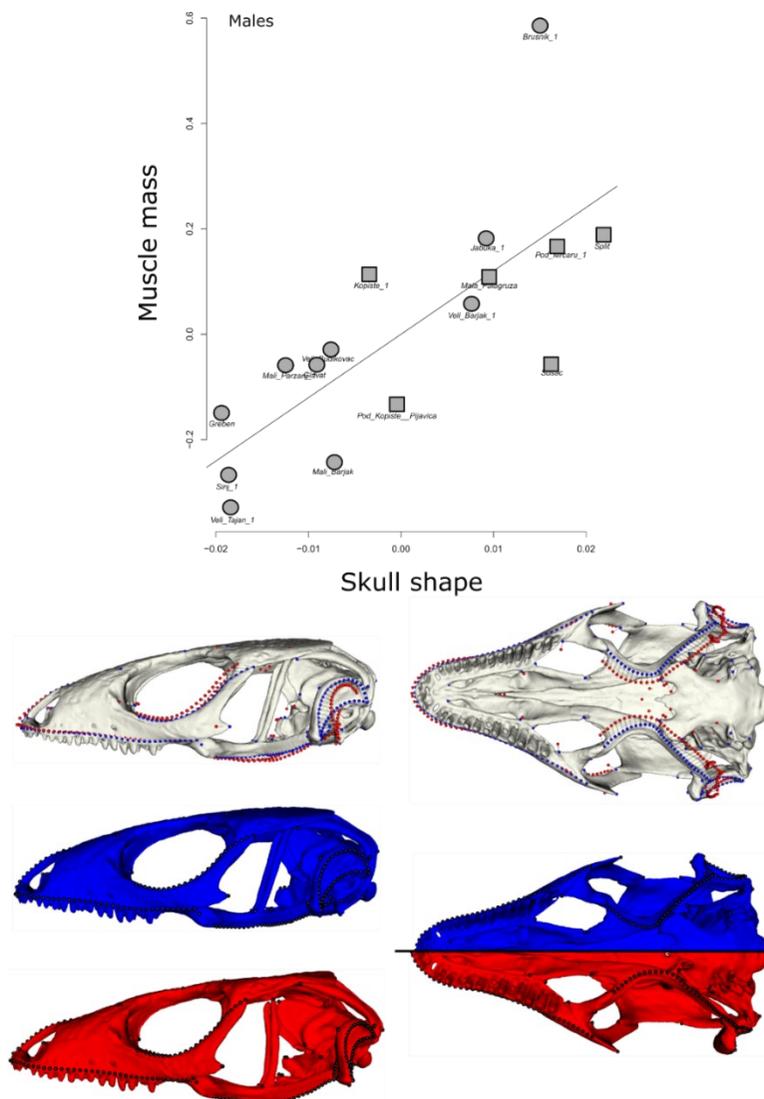


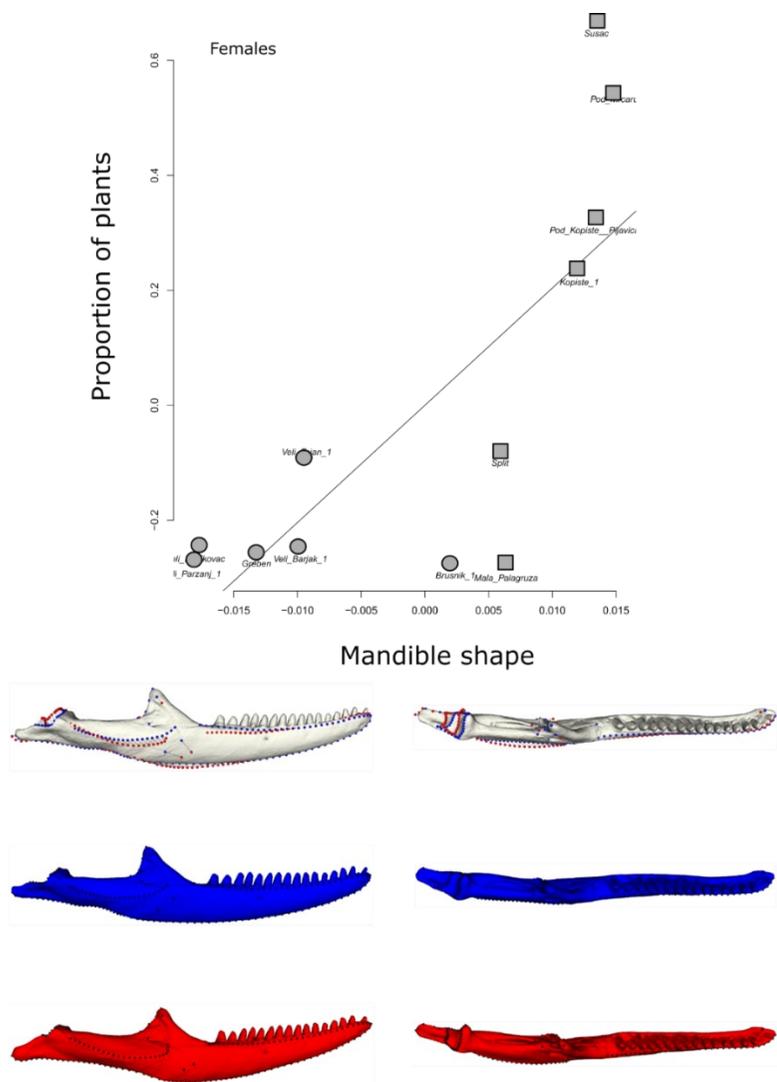


**Fig. S7:** Covariation between mandible shape and muscle mass in females.

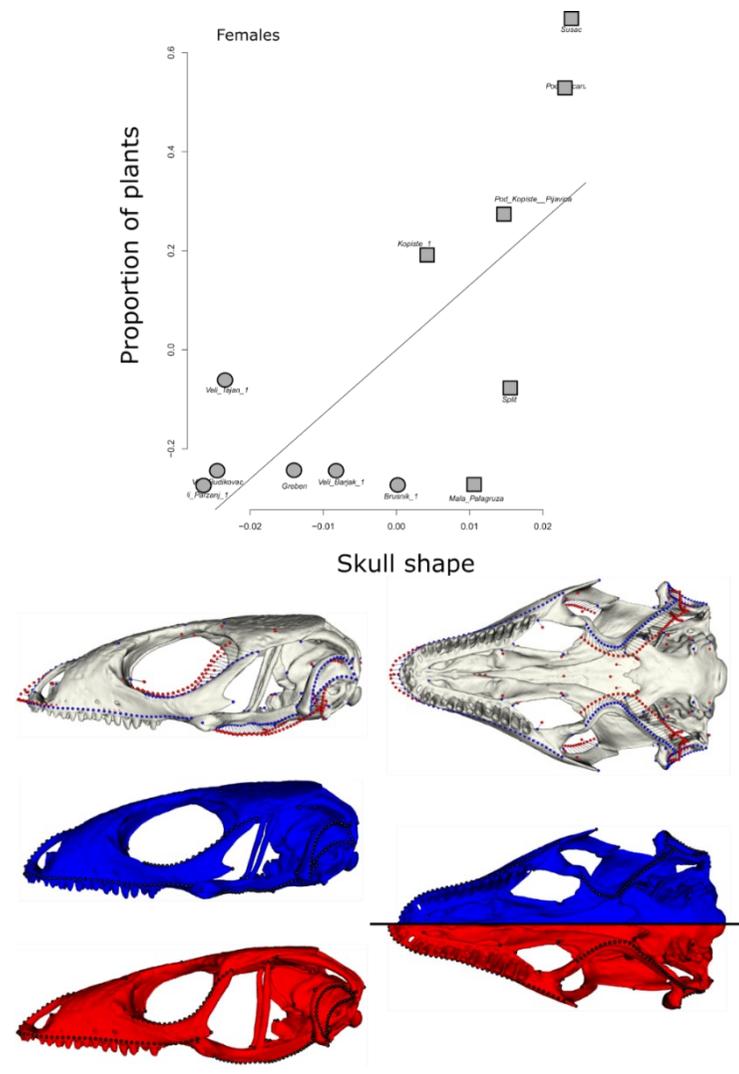


**Fig. S8:** Covariation between skull shape and muscle mass in females.

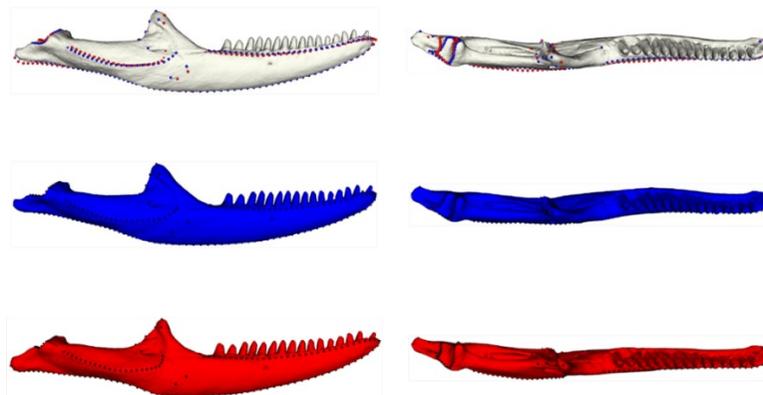
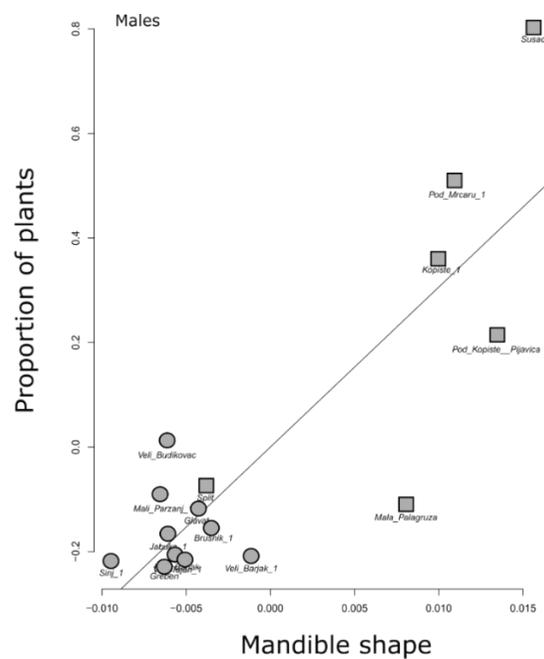




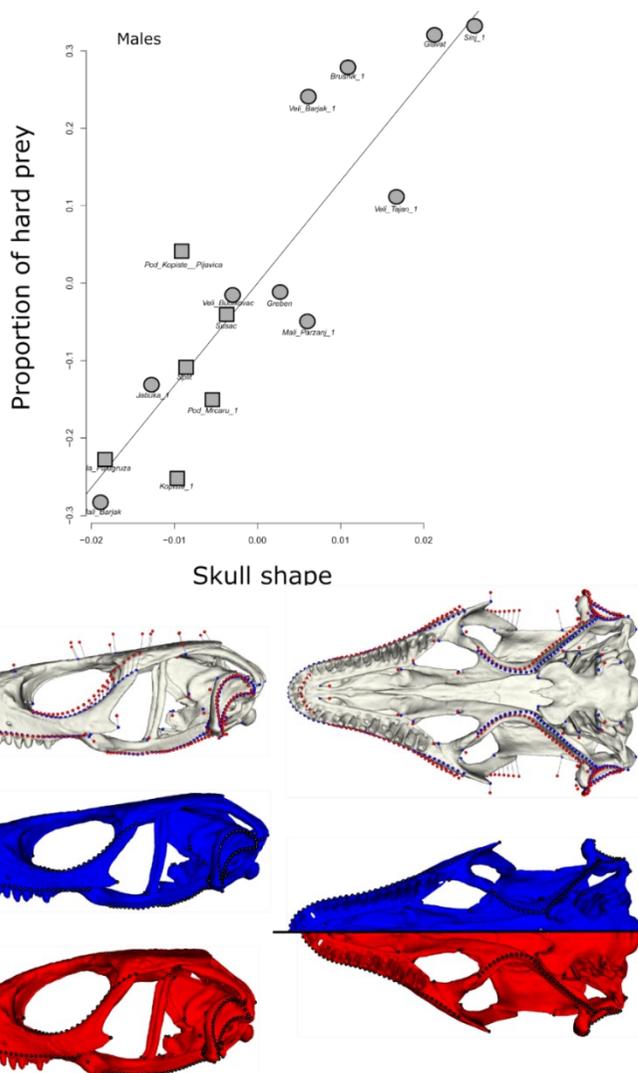
**Fig. S10:** Covariation between mandible shape and the proportion of plants in the diet in females.



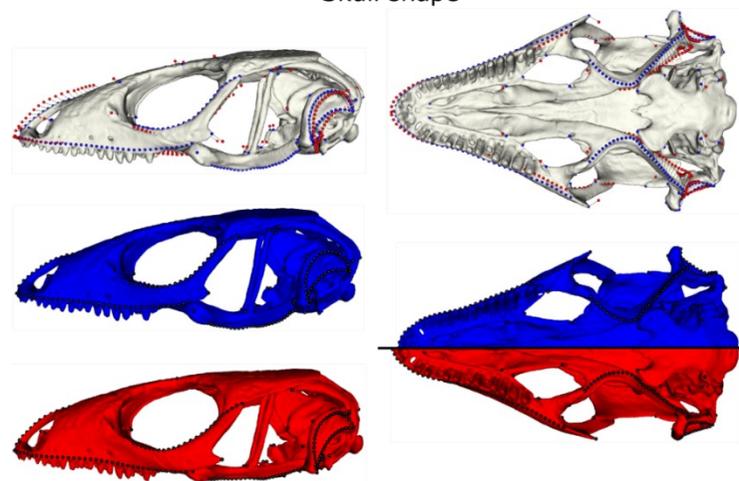
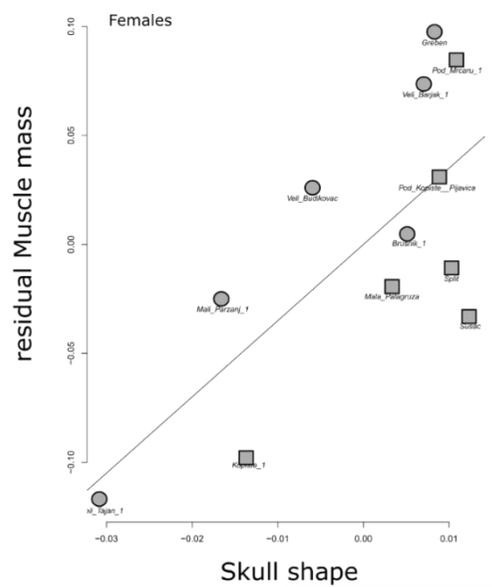
**Fig. S11:** Covariation between skull shape and the proportion of plants in the diet in females.



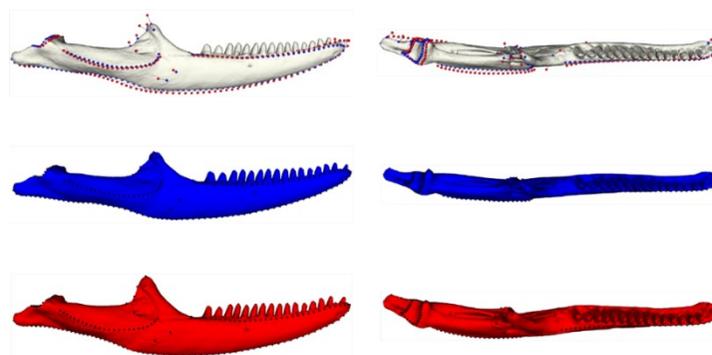
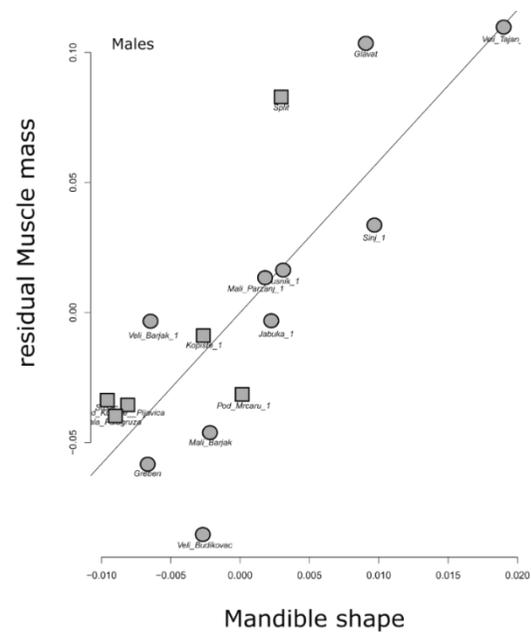
**Fig. S12:** Covariation between mandible shape and proportion of plants in the diet in males.



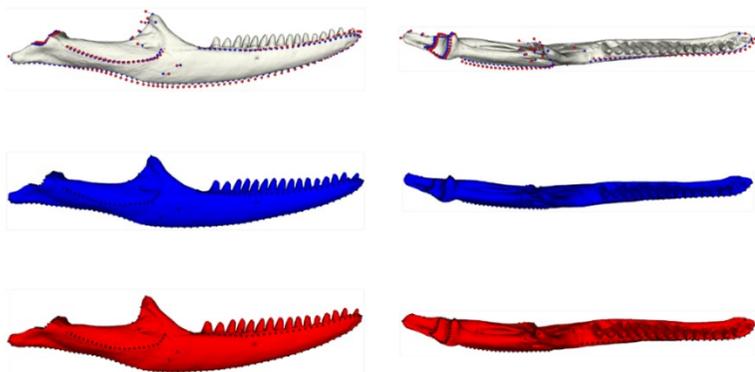
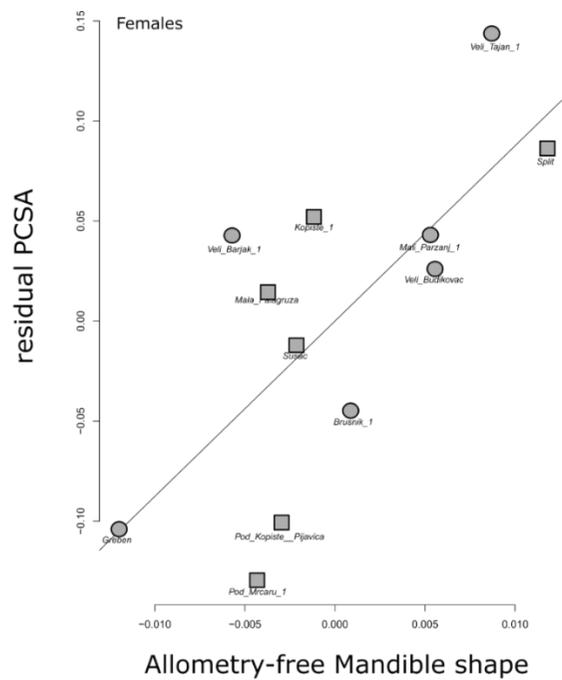
**Fig. S13:** Covariation between skull shape and the proportion of hard prey in the diet in males.



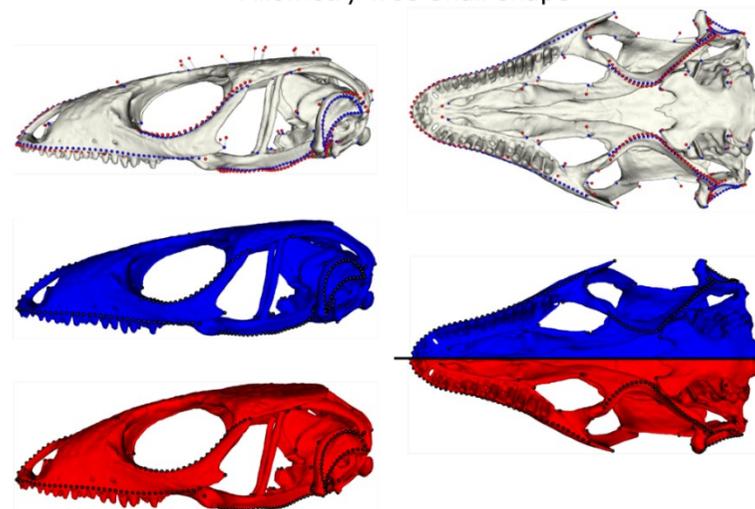
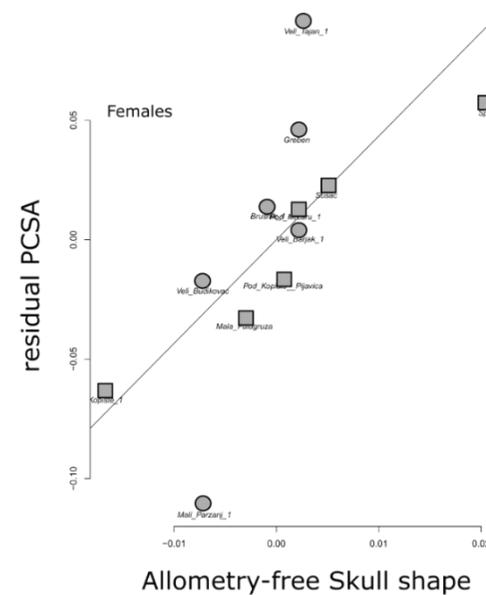
**Fig. S14:** Covariation between skull shape and residual muscle mass in females.



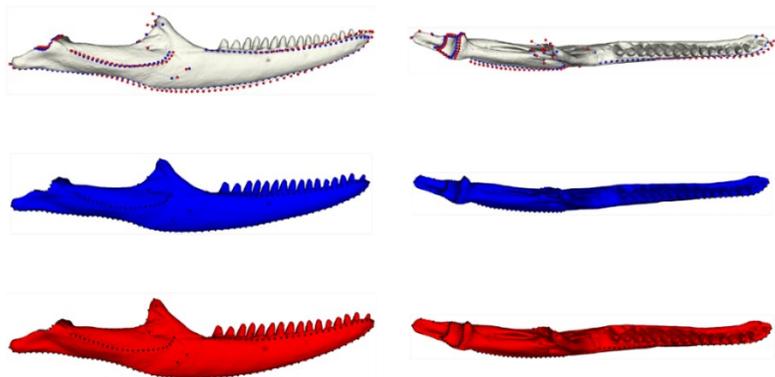
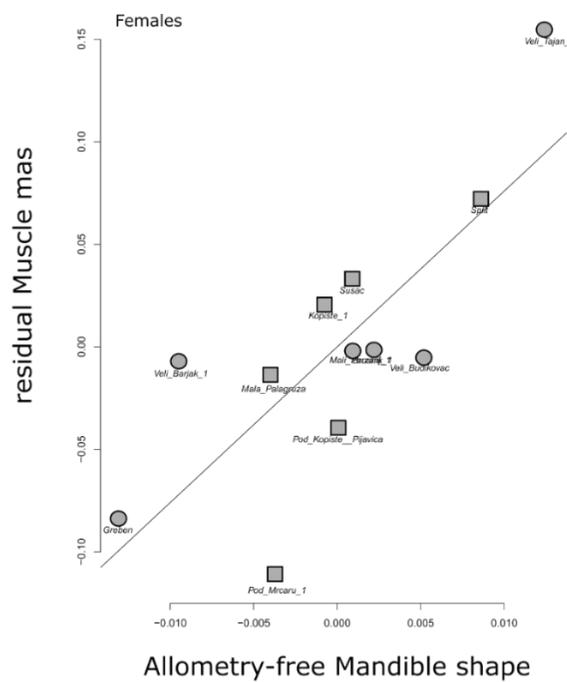
**Fig. S15:** Covariation between mandible shape and residual muscle mass in males.



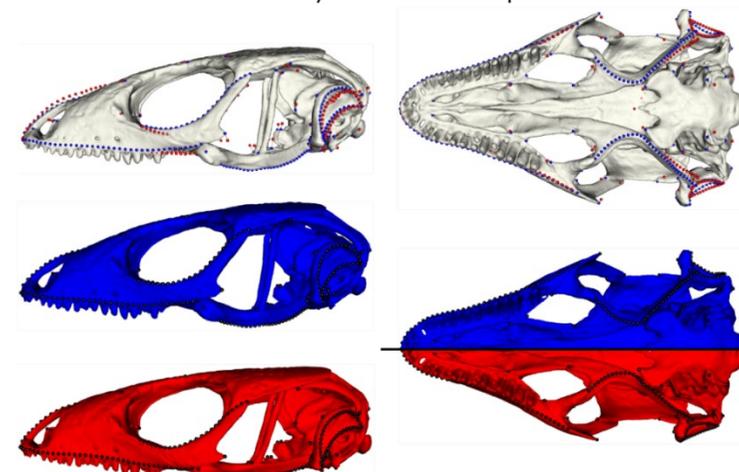
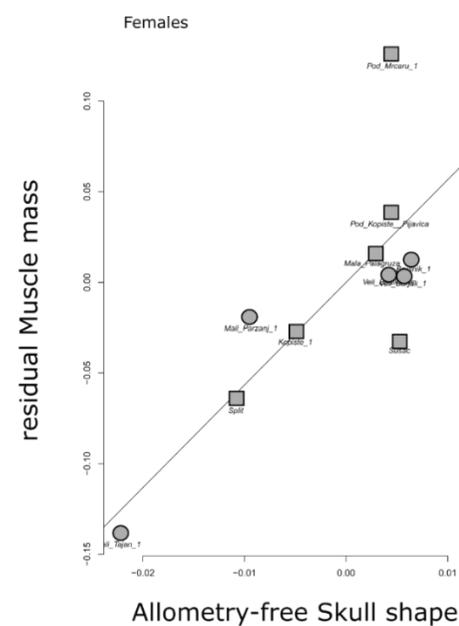
**Fig. S16:** Covariation between allometry-free mandible shape and residual muscle PCSA in females.



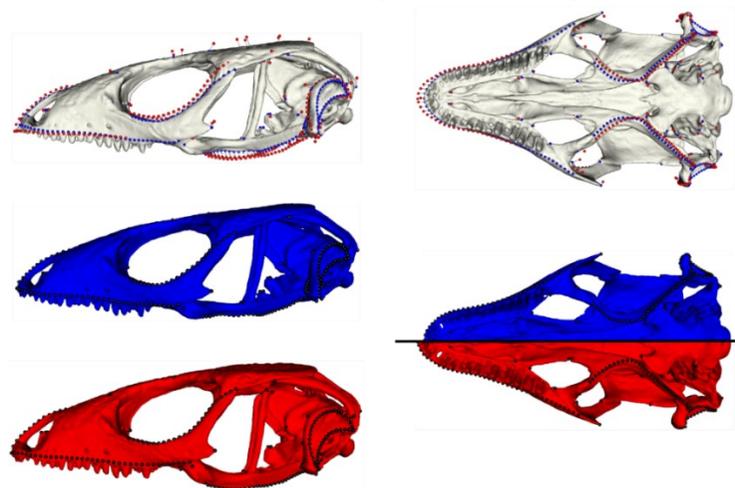
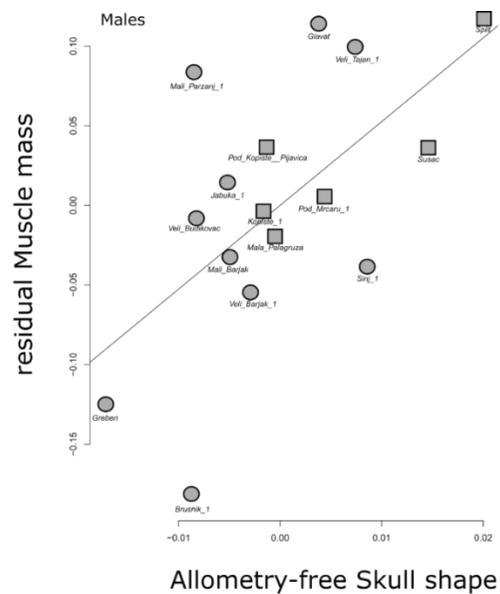
**Fig. S17:** Covariation between allometry-free skull shape and residual muscle PCSA in females.



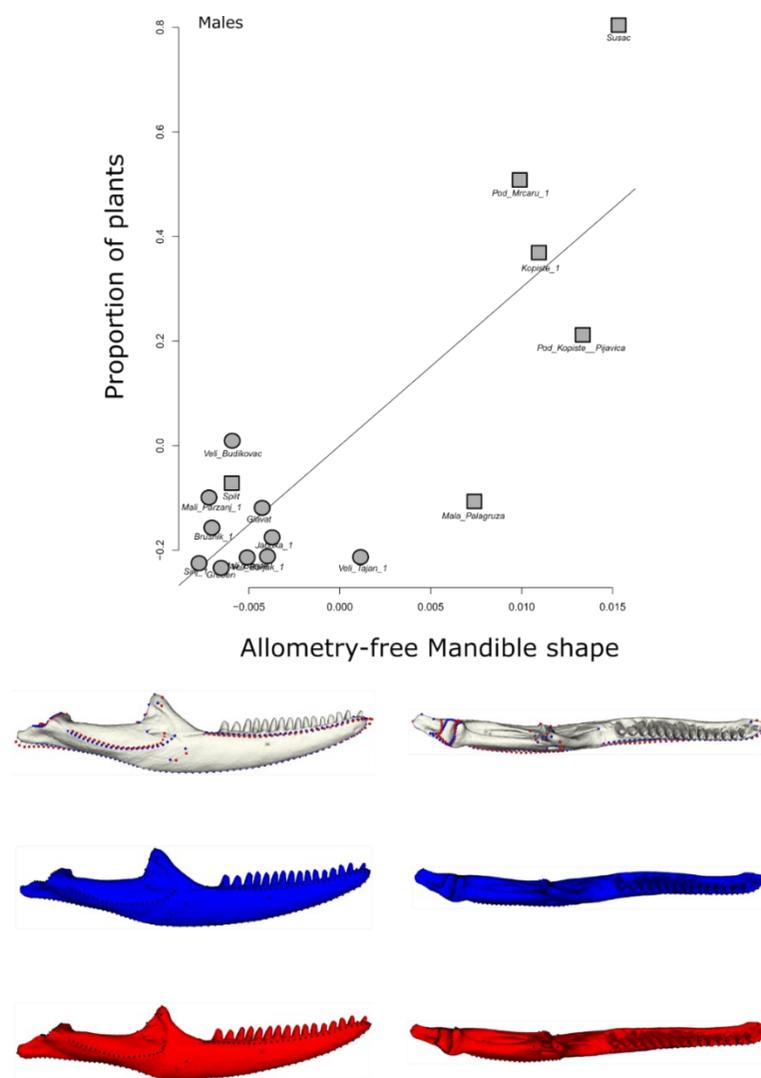
**Fig. S18:** Covariation between allometry-free mandible shape and residual muscle mass in females.



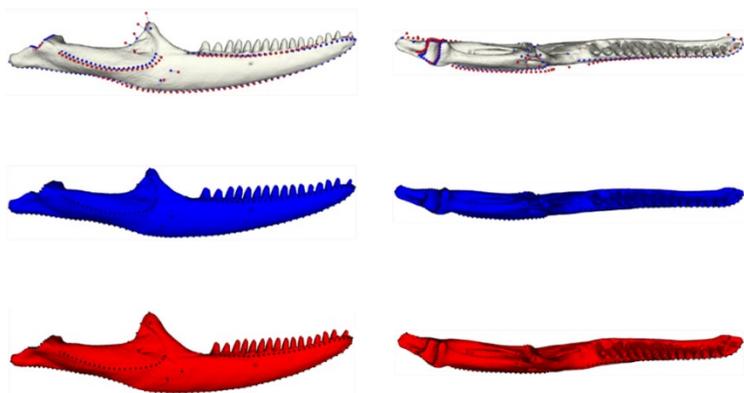
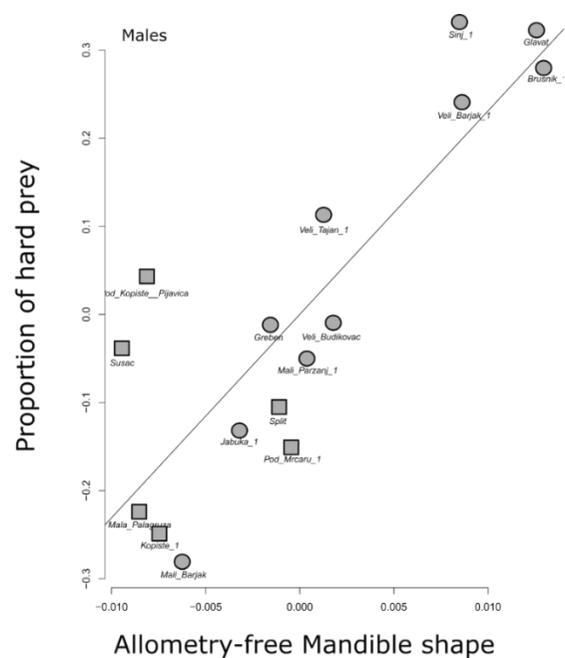
**Fig. S19:** Covariation between allometry-free skull shape and residual muscle mass in females.



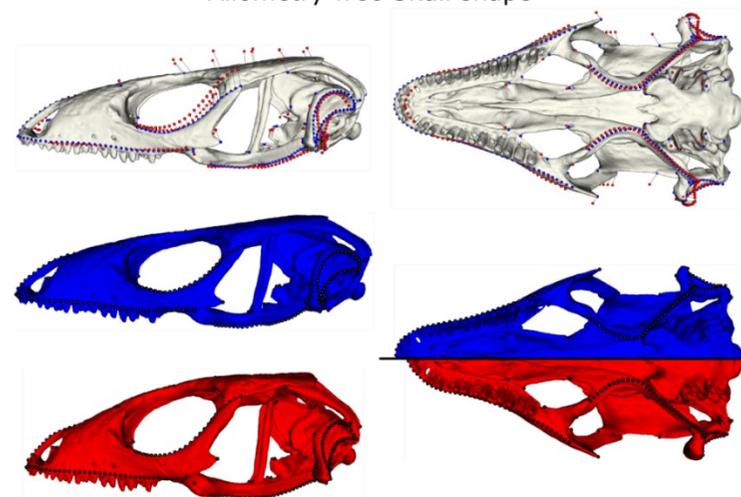
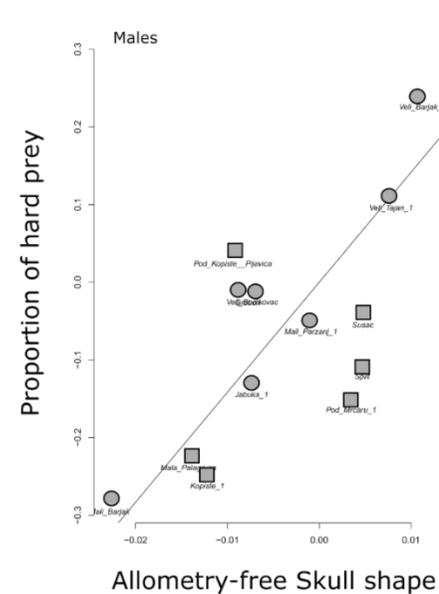
**Fig. S20:** Covariation between allometry-free skull shape and residual muscle mass in males.



**Fig. S21:** Covariation between allometry-free mandible shape and the proportion of plants in the diet in males.



**Fig. S22:** Covariation between allometry-free mandible shape and the proportion of hard prey in the diet in males.



**Fig. S23:** Covariation between allometry-free skull shape and the proportion of hard prey in the diet in males.

**APPENDICES – Chapter 5: Biomechanics**

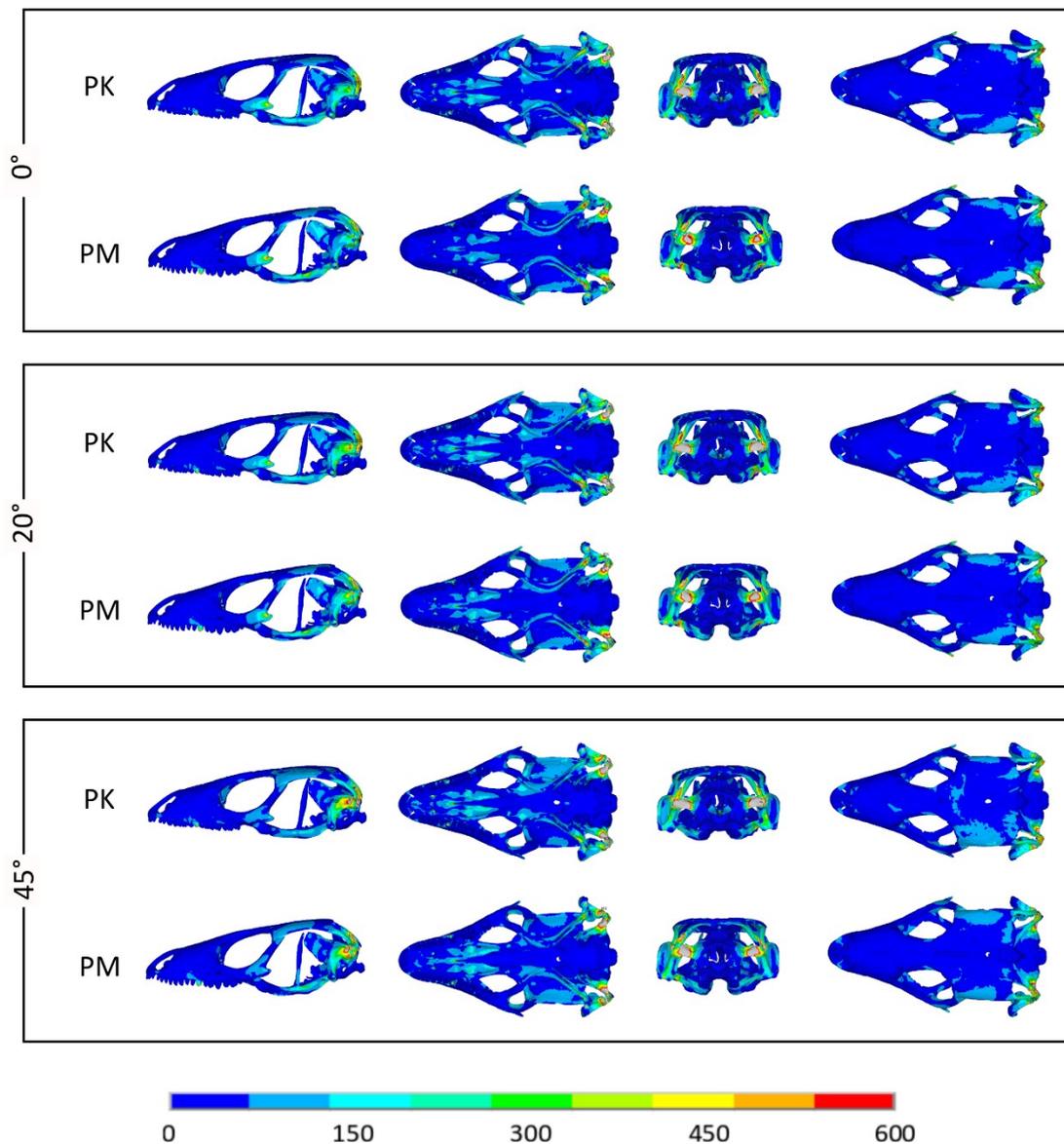
**Table S1:**

*Relative contribution (%) of each muscle to the in silico bite force calculated for each model using MDA, depending on gape angle.*

Model	Muscle	0°	5°	10°	15°	20°	25°	30°	35°	40°	45°
PK_PK	AMESA	10.8	11.0	11.4	11.4	11.3	11.2	11.0	10.9	10.7	10.4
	AMESP	10.1	10.2	9.4	8.9	8.6	8.2	7.8	7.5	7.0	6.4
	AMEM	23.1	23.1	23.2	23.4	23.5	23.7	23.9	24.0	24.0	23.8
	AMEP	4.6	4.5	4.3	4.1	3.9	3.7	3.5	3.4	3.2	3.0
	AMP	2.0	2.0	2.0	2.0	2.1	2.1	2.1	2.1	2.1	2.1
	PSTS	13.3	13.3	13.4	13.6	13.7	13.9	14.1	14.2	14.3	14.2
	PSTP	24.9	24.0	23.1	22.2	21.4	20.7	20.3	20.2	20.4	21.3
	PTL	10.8	11.3	12.1	12.8	13.4	14.0	14.6	15.1	15.7	16.0
	PTM	0.5	0.7	1.2	1.7	2.1	2.4	2.6	2.6	2.7	2.6
	PM_PM	AMESA	21.7	21.6	21.0	20.2	19.7	19.2	18.6	18.1	17.5
AMESP		9.2	9.0	8.7	8.3	7.9	7.5	7.0	6.6	6.2	5.8
AMEM		17.9	17.9	17.9	18.0	18.2	18.4	18.6	18.8	18.9	19.0
AMEP		3.3	3.2	3.0	2.8	2.6	2.5	2.3	2.2	2.0	1.9
AMP		2.3	2.3	2.3	2.4	2.4	2.4	2.4	2.4	2.4	2.4
PSTS		13.3	13.3	13.4	13.5	13.6	13.8	13.9	14.1	14.2	14.2
PSTP		19.8	19.5	19.0	18.4	18.1	17.9	17.6	17.5	17.6	17.9
PTL		11.6	12.2	13.4	14.7	15.6	16.4	17.4	18.1	18.9	19.5
PTM		0.8	1.0	1.3	1.7	1.9	2.1	2.2	2.3	2.3	2.3
Relative contribution (%) PK_PM		AMESA	17.3	17.4	17.6	17.7	17.7	17.7	17.4	17.1	16.7
	AMESP	9.2	9.7	9.4	8.8	8.4	8.0	7.6	7.2	6.8	6.3
	AMEM	18.3	18.1	18.1	18.1	18.1	18.2	18.3	18.4	18.4	18.3
	AMEP	3.6	3.4	3.2	3.0	2.9	2.8	2.6	2.5	2.4	2.2
	AMP	2.6	2.5	2.6	2.6	2.6	2.6	2.6	2.6	2.7	2.6
	PSTS	13.5	13.3	13.4	13.5	13.6	13.7	13.8	13.9	14.0	14.0
	PSTP	21.8	20.9	20.0	19.1	18.4	17.8	17.4	17.3	17.5	18.3
	PTL	13.4	14.1	15.0	15.9	16.6	17.4	18.1	18.8	19.4	19.9
	PTM	0.4	0.5	0.9	1.3	1.7	1.9	2.1	2.2	2.2	2.2
	PM_PK	AMESA	13.5	13.4	13.0	12.6	12.3	12.0	11.6	11.3	10.9
AMESP		9.5	9.4	9.1	8.5	8.1	7.7	7.2	6.8	6.4	6.0
AMEM		23.2	23.2	23.3	23.6	23.8	24.0	24.3	24.5	24.7	24.7
AMEP		4.4	4.3	4.0	3.8	3.6	3.4	3.1	2.9	2.7	2.6
AMP		1.9	1.9	1.9	1.9	1.9	1.9	2.0	2.0	1.9	2.0
PSTS		13.5	13.5	13.6	13.8	13.9	14.0	14.2	14.4	14.5	14.5
PSTP		23.1	22.8	22.3	21.7	21.3	20.9	20.6	20.5	20.6	20.9
PTL		9.6	10.2	11.0	12.0	12.7	13.3	14.1	14.7	15.3	15.8
PTM		1.1	1.3	1.7	2.2	2.4	2.6	2.8	2.9	3.0	3.1

**Table S2:** Values of the most tensile stress associated with the elements (at the origin and insertion) where the ligaments (QJL: quadrato-jugal ligament; EPL: epipterygoid-parietal ligament) are inserted, in each model and for each gape angle tested.

		PK_PK			PM_PM			PK_PM			PM_PK		
		0°	20°	45°	0°	20°	45°	0°	20°	45°	0°	20°	45°
QJL	Origin	9,37	8,24	2,86	12,48	11,72	4,92	11,56	9,65	3,02	11,66	9,75	3,79
	Insertion	9,72	8,85	4,19	12,71	11,86	4,09	12,32	10,43	4,65	11,62	9,13	2,74
EPL	Origin	0,91	0,59	0,06	0,61	0,77	0,01	0,97	0,61	0,01	0,66	0,61	0,01
	Insertion	1,12	0,05	0,01	0,66	0,91	0,01	0,57	0,01	0,01	0,88	0,98	0,01



**Figure S2:** Consequences of morphological variation on the Von Mises stress distribution. Here the models PK\_PK and PM\_PK (PK and PM in the figure) were compared for each of the three gapes tested (see Figure 12 for the alternative combination PM\_PM vs. PK\_PM). The scale bar at the bottom indicates that hotter colors are associated with higher levels of accumulation of Von Mises stress.





The emergence of phenotypic diversity can be the result of evolutionary processes including natural and sexual selection, founder effects and stochastic processes such as genetic drift. A significant part of the current phenotypic and specific diversity is thought to originate from radiation events where the colonization of novel environments and unexploited niches is associated with phenotypic variation. Island systems are considered natural laboratories offering replicates of natural populations, making them the perfect venue to address key questions on evolution and organismal biology. Radiation events on islands provide opportunities to understand how and whether variation in morphology evolves along with environmental constraints as suggested by ecological theory. However, how changes in morphology can give rise to significant changes in performance and subsequently ecology remains unclear and needs to be studied. To tackle this question, we used an island system, the Adriatic archipelago, where numerous populations of *Podarcis* lizards (Sauria, Lacertidae) have remained isolated since the end of the last glacial epoch. We carried out a comparative study combining field observations, quantitative dissections and three-dimensional geometric morphometric analyses to investigate how macroevolutionary variation in bite force, behavior, head shape and the underlying musculature correlates with environmental constraints including resource use and intraspecific competition. We then utilized computational engineering methods (Multibody Dynamics Analyses, and Finite Element Analyses) to test for the mechanical significance of subtle morphological variation observed between populations. The present study suggests that head shape variation impacts the mechanical efficiency of the musculoskeletal system of the feeding apparatus. Moreover, variation in morphology, musculature, behavior, and performance reflect dietary specialization among populations. Our results provide evidence of how microevolutionary changes in morphology can ultimately result in macroevolutionary patterns of ecological variation. They further suggest the Adriatic system to be in the initial stages of an adaptive radiation.

L'émergence de la diversité phénotypique peut résulter de processus évolutifs tels que la sélection naturelle ou sexuelle, les effets de fondations, ou des processus stochastiques comme la dérive génétique. On considère aujourd'hui qu'une part non-négligeable de la diversité actuelle a pour origine des événements radiatifs pendant lesquels la colonisation de nouvelles niches écologiques est accompagnée d'une variation phénotypique. Les systèmes insulaires peuvent être comparés à des laboratoires à ciel ouvert parce qu'ils offrent des répliques de populations naturelles, essentiels pour répondre à des questions centrales en biologies des organismes et de l'évolution. Les radiations insulaires offrent des opportunités intéressantes d'étudier comment la variation morphologique évolue en relation avec les contraintes environnementales, comme le suggère la théorie écologique. Cependant, il reste encore à comprendre dans quelle mesure les variations morphologiques peuvent donner lieu à des changements significatifs dans l'écologie et la performance des organismes. Nous proposons de répondre à cette question en utilisant un système insulaire, l'archipel croate de la mer Adriatique, où sont établies des populations de lézards du genre *Podarcis* (Sauria, Lacertidae) qui sont restées isolées les unes des autres depuis la fin de la dernière glaciation. Pour ce faire, nous avons combiné des observations de terrain, des dissections quantitatives et des analyses en morphométrie géométrique 3D pour comprendre les relations entre les variations macroévolutives de la force de morsure, du comportement, de la forme du crâne et de sa musculature avec les contraintes environnementales, comme l'exploitation des ressources ou la compétition intraspécifique. Nous avons ensuite eu recours à des méthodes d'ingénierie (modélisation dynamique multicorps, et modélisation en éléments finis) pour tester les conséquences biomécaniques des variations morphologiques subtiles observées au sein des populations. La présente étude suggère que la variation de la forme du crâne influence l'efficacité mécanique du système musculosquelettique de l'appareil masticateur. De plus, les variations de morphologie, musculature, comportement et performance reflètent des spécialisations de régime alimentaire. Nos résultats apportent une démonstration de la façon dont les changements microévolutifs de la morphologie peuvent finalement aboutir à des patrons macroévolutifs de variation écologique. Ils suggèrent également que le système insulaire de l'Adriatique se trouve dans une phase initiale de radiation adaptative.