



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS

ESCUELA NACIONAL DE ESTUDIOS SUPERIORES UNIDAD MORELIA

Ecomorfología y Evolución de Tortugas

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

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MORELIA, MICHOACÁN, OCTUBRE, 2021.



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COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
ENTIDAD ESCUELA NACIONAL DE ESTUDIOS SUPERIORES UNIDAD MORELIA

OFICIO CPCB/912/2021

ASUNTO: Oficio de Jurado

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P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Subcomité de Biología Experimental y Biomedicina del Posgrado en Ciencias Biológicas, celebrada el día **21 de junio de 2021** se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **BUTTERFIELD TAGGERT GRAYSON** con número de cuenta **518490441** con la tesis titulada **“Ecomorfología y Evolución de Tortugas”**, realizada bajo la dirección del **DR. JORGE ALBERTO CONTRERAS GARDUÑO**, quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
“POR MI RAZA HABLARÁ EL ESPÍRITU”
Ciudad Universitaria, Cd. Mx., a 20 de septiembre de 2021

COORDINADOR DEL PROGRAMA



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AGRADECIMIENTOS INSTITUCIONALES

En primer lugar al Posgrado en Ciencias Biológicas por aceptarme como alumno. Agradezco al CONACyT por apoyarme con una beca (No. 863562) y también por su Beca de Movilidad en 2019. Agradezco a mi tutor principal, Jorge Alberto Contreras-Garduño por su apoyo y su paciencia durante mis estudios. Gracias a los miembros del mi comité tutor, Mark Olson-Zuñiga y Rodrigo Macip-Ríos. Gracias a Mark Olson-Zuñiga por exponerme a nuevas ideas y por ayudarme a moldear mi mente entorno a la evolución. Muchas gracias a Rodrigo Macip-Ríos por ser un mentor excepcional, por guiarme y entrenarme en la burocracia de México, por ser paciente, por apoyarme para realizar el proyecto de mis sueños, y por ser un buen amigo.

.

AGRADECIMIENTOS A TÍTULO PERSONAL

Gracias a la Escuela Nacional de Estudios Superiores (ENES) unidad Morelia en la UNAM por apoyarme a perseguir mi sueño de ser un científico. Desde el inicio, todas las personas con las que tuve el gusto de interactuar me han apoyaron de formas imaginables. Quiero agradecer a la Mtra. Janette Huante Pineda, la Asistente de la Secretaría de Investigación y Posgrado en ENES Morelia, sin su apoyo seguramente me hubieron sacado del posgrado en el primer año por no entender los tiempos y las fechas de los trámites. También agradezco a Lilia Judith Espinosa Sánchez y al Dr. Ernesto Armando Rodríguez Reyes por tomar su tiempo en contestar todas mis preguntas y ayudarme a navegar por el Posgrado de Ciencias Biológicas

Gracias a Martha Harfush, Carlos Verdugo, Abel Dominguez, James Callaghan, Mario Uc Uc, Félix García, Eduardo Grajales, Lydia Lozano, la comunidad de El Paso de Xonene, Oaxaca, la Estación de Biología Chamela, y Katherine Renton por todo su apoyo para realizar el trabajo de campo de esta tesis en Oaxaca, Sonora, y Yucatán. Gracias a Anthony Herrel por ser un excelente colaborador conmigo, por ayudarme a revisar mis artículos, y por aceptarme para realizar una estancia en su laboratorio.

Gracias a mi querida esposa Alejandra Monsiváis-Molina por apoyarme para estudiar para el examen de ingreso a la UNAM, su guía en los trámites de la UNAM, su apoyo en el campo, su apoyo revisando mi redacción en español, y por su cariño.

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RESUMEN

Entender los orígenes y los catalizadores de la diversidad ecológica es un objetivo central en la biología evolutiva. El estudio de la ecomorfología permite evaluar el vínculo entre la morfología y la ecología de los organismos, mientras que los estudios comparativos que utilizan ejemplares de museos tienen el objetivo de abordar preguntas evolutivas más amplias. En esta tesis nos preguntamos cuál es la relación entre la morfología de las tortugas y su ecología, y comparamos la morfología de las tortugas a lo largo de su filogenia para entender cómo covarían con su diversidad ecológica. Para abordar esta interrogante, se empleó tanto un estudio de campo ecomorfológico como un estudio basado en ejemplares de museo para entender con más detalle la ecología y la evolución de las tortugas terrestres y dulceacuícolas. Se utilizaron datos de catorce especies de tortugas en cuatro diferentes sitios de campo en los estados de Jalisco, Oaxaca, Yucatán y Sonora, México, y se encontraron fuertes correlaciones entre la morfología, el desempeño, y la ecología. Por ejemplo, el tamaño de la pata delantera, la cantidad de membrana interdigital y la altura del caparazón explican la variación de la velocidad máxima de natación y el porcentaje de tiempo en que una especie de tortuga fue encontrada sobre la tierra. Las especies de tortugas que se encuentran exclusivamente en agua tienden a tener patas delanteras más largas, mayor membrana interdigital, caparazones más planos, y mayor velocidad de natación máxima. Las especies de tortugas que se encuentran más frecuentemente sobre la tierra tienden a tener las patas delanteras más pequeñas, menos membrana interdigital, caparazones más altos, y menor velocidad de natación máxima. El continuo que existe entre los estilos de vida en las tortugas, desde completamente acuático hasta completamente terrestre, es el que parece caracterizar la variación morfológica a través de la diversidad de especies de tortugas. También se determinaron correlaciones entre la morfología de la cabeza, fuerza de mordida y la dieta, pero fue menos clara la relación entre la morfología de la cabeza y la dieta porque el nivel isotópico que utilizamos para medir la dieta puede estar influido por factores tanto alimenticios como por factores ambientales. Con los datos de los museos de historia natural se midieron características de las extremidades, caparazón y cabeza en diferentes especies de tortugas que representan el 69% de diversidad total de las especies terrestres y dulceacuícolas hasta ahora conocidas (246/360 de las especies). Los datos muestran que la variación morfológica caracteriza la variación ecológica tanto a través de la diversidad de las tortugas que observamos en los ejemplares como las que observamos en campo. Por ejemplo, en los datos de campo y del museo, se observó que el continuo de tortugas acuáticas a terrestres está caracterizado por el tamaño de la pata delantera, la cantidad de membrana interdigital y el alto del caparazón. Así, en el campo y en los ejemplares de museo se observa que las tortugas acuáticas tienen patas delanteras más largas, más membrana interdigital, y caparazones más planos. También preguntamos si hay modularidad en la covariación que se observa entre las patas delanteras, el caparazón y la cabeza, lo que reveló patrones significativos de modularidad a través de todas las especies de tortugas, los clados principales de las tortugas y en tortugas caracterizadas por diferentes ecologías (acuática, semi-acuática y terrestre). Por ejemplo, en el plan corporal de las tortugas, las variables que pertenecen a las regiones de las extremidades, el caparazón y la cabeza son módulos que covarían más entre ellos que con otras regiones. También se comparó la integración entre módulos a través de la diversidad de tortugas, en clados principales, y en tortugas con diferente ecología, lo cual mostró que hay una integración débil entre la cabeza y las extremidades/caparazón en los clados más diversos: Emydidae, Geoemydidae y Pelomedusoides. En contraste, se observó una integración significativa en la cabeza y las extremidades/caparazón

de los clados de tortugas que sólo son acuáticas: Trionychidae, Chelidae y Chelydroidea; y en el clado que es sólo terrestre: Testudinidae. Estos datos sugieren que el desacoplamiento de rasgos, es decir cuando la integración entre dos rasgos es débil, ha promovido potencialmente la diversificación ecológica de las tortugas.

ABSTRACT

Understanding the origins and catalysts of ecological diversity is a central goal in evolutionary biology. Ecomorphological studies often aim to understand the link between morphology and ecology in a particular group of organisms, whereas comparative studies using museum specimens often aim to address broader evolutionary questions. In this study, we employ both an ecomorphological field study and a museum-based study to better understand the ecology and evolution of freshwater and terrestrial turtles. We directly measured fourteen turtle species across four different field sites in Mexico, and found strong correlations between measures of morphology, performance, and ecology. For example, we found that hand size, interdigital webbing, and shell height, all correlated to maximum swimming speed and the percent time a turtle species occurs on land. Turtle species that are more aquatic tend to have longer hands, more interdigital webbing, shorter shells, faster maximum swimming speed, and are exclusively encountered in the water. Turtle species that are more terrestrial have smaller hands, less interdigital webbing, taller shells, slow maximum swimming speed, and are found on land more frequently. It is this continuum of turtle lifestyles from aquatic to terrestrial species that appears to characterize the morphological variation across the diversity of turtles. We also found correlations in head morphology, bite performance, and diet, but it is less clear if there is relationship between head morphology and diet because the isotope values that we used are not only influenced by diet, but also by the environment. Visiting natural history museums, we were able to measure limb, shell, and head variables of turtle species that represent 69% of the total species level diversity in freshwater and terrestrial turtles (246/360 species). These data show that the variation in morphology that was correlated to performance and ecology in the field, also characterizes the variation in morphology across the diversity of turtles. For example, in field and museum data we observe that the continuum of aquatic to terrestrial turtles is similarly characterized by hand size, interdigital webbing, and shell height. Furthermore, analysis of covariation in limb, shell, and head variables in museum data reveal significant patterns of modularity across all turtles, major turtle clades, and turtles with a different ecology (aquatic, semi-aquatic, and terrestrial). The limb, shell, and head regions of the turtle body plan are modular when measured in all turtles, turtle clades, and different ecologies. We also compare integration across turtles, major turtle clades, and turtles with different ecologies showing that there is weak integration between the head and limb/shell variables in the most diverse turtle clades, Emydidae, Geoemydidae, and Pelomedusoides. In contrast, significant integration was observed in the aquatic turtle clades, Trionychidae, Chelidae, and Chelydroidea; and the terrestrial clade Testudinidae. These data suggest that trait decoupling, in which the covariation between two traits is relaxed, has promoted the ecological diversification of turtles.

INTRODUCCIÓN GENERAL

Una de las preguntas básicas de la biología es entender cómo es que los organismos se diversifican con respecto a su ecología. Un primer paso para hacer esto es medir cómo es que la morfología de los organismos se correlaciona con el uso del hábitat y la dieta (Arnold, 1983). Los estudios ecomorfológicos en campo han sido fundamentales para explicar cómo los factores ecológicos pueden influir a la diversificación de varios grupos de organismos. Por ejemplo, en lagartijas (Losos, 2011), peces (Fulton *et al.*, 2005; Parsons *et al.*, 2012) y ranas (Gomes *et al.*, 2009; Moen, 2019) se ha mostrado que su diversificación tiene la tendencia de coincidir con sus nichos ecológicos. Un corolario importante de dichos estudios es que éstos permiten a los investigadores utilizar grandes bases de datos tomadas de especímenes de museos para poner a prueba preguntas más amplias sobre cómo es que los organismos se diversifican (Felice & Goswami, 2018; Moen, 2019; Stayton, 2019; Watanabe *et al.*, 2019; Navalón *et al.*, 2020). Por ejemplo, Mahler *et al.* (2013) utilizaron especímenes de museo para poner a prueba preguntas macroevolutivas en las lagartijas del género *Anolis* en las islas de las Antillas Mayores, donde encontraron que las especies han evolucionado independientemente hacia morfologías similares en las diferentes islas. Un estudio comparativo como el de Mahler *et al.*, (2013) no habría sido posible sin el trabajo de campo fundacional sobre la ecomorfología de las lagartijas *Anolis* que demostró que la morfología está correlacionada con el uso del hábitat (Losos, 1990).

Los estudios ecomorfológicos buscan medir la relación entre morfología y ecología, y una predicción que hacen dichos estudios es que la influencia de la morfología sobre el desempeño en tareas ecológicamente relevantes refleja la ecología del organismo y puede influir a su adecuación (Arnold, 1983; Losos, 1990; Wainwright, 1991; Irschick *et al.*, 2008). Las tareas ecológicamente relevantes son comportamientos específicos puestos a prueba experimentalmente que están diseñados por el experimentador para evaluar las variación en la habilidad de los organismos de utilizar algún aspecto del ambiente (Arnold, 1983). En las tortugas y otros organismos, las medidas de desempeño que han sido puestas a prueba incluyen natación (Pace *et al.*, 2001; Fulton *et al.*, 2005), fuerza de mordida (Herrel *et al.*, 2001; Hocking *et al.*, 2021), entre otros ejemplos (Ana *et al.*, 2015; Mayerl *et al.*, 2016; Xiao *et al.*, 2017; Tan *et al.*, 2020). Estos estudios son importantes porque documentan las formas en que la variación morfológica determina e influye sobre cómo los organismos pueden utilizar y manipular su ambiente. En caso de las tortugas dulceacuícolas y terrestres, los estudios de campo ecomorfológicos han recibido

poca atención, lo cual impide tener una comprensión completa de la diversificación ecológica de las tortugas. En particular, hace falta un entendimiento más profundo sobre cómo la morfología de las tortugas se correlaciona con diferentes aspectos de su ecología, como el porcentaje de tiempo que se observan en el agua y los recursos alimenticios que utilizan.

Un beneficio principal de los estudios ecomorfológicos, y de aquellos que buscan comprender cómo la morfología de un organismo se relaciona con diferentes aspectos de su ecología, es que los especímenes de museo pueden ser usados para abordar preguntas evolutivas más amplias. Frédérich *et al.*, 2014, por ejemplo, usó especímenes de museos para mostrar que el desacoplamiento de la parte inferior y superior de la mandíbula en los peces damisela está asociado con un incremento en la diversificación trófica de un gran número de especies. Este ejemplo y varios más (Sanger *et al.*, 2012; Mahler *et al.*, 2013; Sherratt *et al.*, 2017; Felice & Goswami, 2018; Watanabe *et al.*, 2019; Bardua *et al.*, 2020), resaltan el beneficio de utilizar especímenes de museo para estudiar la diversificación de diferentes clados. En el caso de las tortugas, varios estudios han utilizado especímenes de museo para estudiar cómo la forma del caparazón (Stayton, 2019), las extremidades (Joyce & Gauthier, 2004) o la cabeza (Foth *et al.*, 2017) están asociados con diferentes dietas, hábitats o riqueza de especies. Sin embargo, no hay estudios que hayan intentado entender cómo la relación covariante entre las extremidades, el caparazón y la cabeza de las tortugas ha influido sobre los patrones generales de diversificación de estos organismos.

Las tortugas son un grupo de organismos ideal para entender cómo los patrones ecomorfológicos influyen la diversificación ecológica. Las tortugas son idóneas porque no son tan diversas como los grupos de organismos que más se han utilizado en los estudios evolutivos (p. ej. las aves, de las que hay más que 10,000 especies). A diferencia de los estudios que analizan muchas especies, los estudios con menos especies permiten probar hipótesis a más detalle. Las 360 especies existentes de tortugas ocupan un amplio espectro de hábitats: marinos, dulceacuícolas y terrestres; y forman comunidades donde interactúan varias especies (Turtle Taxonomy Working Group *et al.*, 2017). Además, la distribución geográfica y la posición filogenética de la mayoría de las tortugas está relativamente bien resuelta y hay varios estudios independientes que han encontrado resultados similares (Pereira *et al.*, 2017; Turtle Taxonomy Working Group *et al.*, 2017; Thomson *et al.*, 2021). Por lo tanto, la información que ya está disponible sobre las tortugas proporciona una oportunidad única para estudiar a detalle la

diversificación ecológica de un orden completo. Sin embargo, para estudiar la diversificación evolutiva de las tortugas se necesita un buen entendimiento sobre cómo la morfología determina la ecología de las tortugas en campo.

En el caso de las tortugas, se han llevado a cabo una serie de estudios ecomorfológicos para entender el vínculo entre su morfología y ecología. Por ejemplo, hay estudios de laboratorio que han proporcionado evidencia detallada sobre cómo las extremidades y la morfología de la cabeza impactan en el desempeño al nadar y al morder, respectivamente, mostrando que la morfología está correlacionada con la ecología (Pace *et al.*, 2001; Herrel *et al.*, 2002, 2018; Rivera, 2008; Young *et al.*, 2017; Mayerl *et al.*, 2019). Una combinación de estudios de laboratorio y de campo ha mostrado que el caparazón de las tortugas está correlacionado con la velocidad a la que fluye el agua, y que las especies que viven en ambientes lóticos tienen caparazones más planos (Rivera, 2008; Stayton, 2019). Por otro lado, los estudios del comportamiento autocorrectivo han mostrado que las tortugas con caparazones más altos pueden voltearse más fácilmente (Domokos & Várkonyi, 2008; Ana *et al.*, 2015). Todos estos estudios ecomorfológicos han proporcionado una base sólida sobre cómo la morfología está correlacionada con algún aspecto de la historia natural de las tortugas, y, sobre cómo dichos patrones han evolucionado a lo largo del tiempo. Sin embargo, la mayoría de los estudios sólo han investigado un aspecto en particular de la morfología (p.ej. carapacho; Stayton *et al.*, 2018) o una sola medida del desempeño (p. ej. Herrel *et al.*, 2002). Además, hay pocos estudios de campo (pero ver Xiao *et al.*, 2017), y es por ello por lo que se necesitan estudios de campo que integren las medidas de las extremidades, el caparazón y la cabeza de las tortugas con diversos aspectos de su ecología para entender cómo diferentes regiones del plan corporal de una tortuga determinan cómo puede utilizar su ambiente.

El objetivo general de este trabajo fue llenar estos vacíos de información para las tortugas y de entender su evolución a lo largo de tres capítulos. El objetivo del Capítulo 1 fue analizar la relación entre morfología, desempeño y ecología en tres especies de tortugas simpátricas en Chamela, Jalisco, México. Propusimos la hipótesis de que el hábitat y la velocidad de natación de las tortugas se correlacionan con su morfología, y que las tres especies de tortugas en Chamela (*Rhinoclemmys pulcherrima*, *Rhinoclemmys rubida* y *Kinosternon chimalhuaca*) dividen los recursos de hábitat y dieta. Con base en un estudio previo que documentó que las tortugas más acuáticas tienen patas delanteras más largas (Joyce & Gauthier, 2004), la predicción

planteada fue que el largo de la pata delantera y el área de la membrana interdigital entre los dedos estarían positivamente correlacionados con la velocidad de natación. Asimismo, dado que se ha observado que las especies simpátricas dividen los recursos (Schoener, 1974), predijimos que las tortugas que se traslapan en dieta no se traslaparían en hábitat, es decir, que habría división de recursos dentro de esta comunidad de tortugas. Medimos la morfología de las patas, las extremidades y la cabeza, se estimó el uso de hábitat por el tipo de vegetación donde se encontraban las tortugas, y se determinó su dieta utilizando isótopos estables de nitrógeno y carbono colectados de la queratina del carapacho (p. ej. Murray & Wolf, 2012). Encontramos evidencia que corrobora nuestra hipótesis: por un lado, se encontró que el uso de hábitat está relacionado con la velocidad máxima de natación, la morfología de las extremidades y el carapacho; por otro lado, encontramos que las tres especies en Chamela dividen recursos. Las dos especies que se traslapan en dieta (*R. rubida* y *K. chimalhuaca*), no se traslapan en hábitat y que la especie que se traslapa en hábitat con las otras especies (*R. pulcherrima*) tiene una dieta distinta (Butterfield *et al.*, 2020). No obstante, este primer capítulo se basó solamente en tres especies, por lo que para tener un entendimiento más completo de la ecomorfología de las tortugas comparamos más especies en un contexto filogenético para tomar en cuenta la no independencia entre las especies, y con medidas adicionales que no fueron consideradas en el Capítulo 1.

El objetivo del Capítulo 2 fue evaluar la relación entre morfología, desempeño, y ecología en un contexto filogenético en 14 especies de tortugas en los estados de Oaxaca, Jalisco, Yucatán y Sonora en México. Propusimos la hipótesis de que las patas delanteras y el caparazón se correlacionan con la velocidad de natación y el uso de hábitat, y que la cabeza revela la fuerza de mordida y la dieta. La predicción fue que el largo de la pata delantera y la cantidad de la membrana interdigital estarían positivamente correlacionados con la velocidad de natación, y que el largo del plastrón y el alto del caparazón estarían negativamente correlacionados con la velocidad de natación. Igualmente, esperamos que las correlaciones entre morfología y natación se reflejarían en el uso de hábitat, y que las especies que nadan más rápido tendrían las patas delanteras más largas y se observarían más frecuentemente en el agua. Además, predijimos que el ancho de la cabeza estaría positivamente correlacionado con la variación que se observa en la fuerza de mordida y con su dieta. Estas predicciones se basaron en los resultados del Capítulo 1 y en la literatura que ha mostrado que la morfología de las patas, el caparazón, y la cabeza pueden

influir en los recursos que las tortugas utilizan (Pace *et al.*, 2001; Herrel *et al.*, 2002; Rivera, 2008). Se comparó la morfología de las extremidades, el caparazón y la cabeza; la velocidad máxima de natación y la fuerza de mordida; la frecuencia en la que se encontró a cada una de las especies sobre tierra y los isótopos estables de carbono, para determinar si hay correlaciones entre morfología, desempeño y ecología. Se analizaron los datos en un contexto filogenético y se encontró evidencia en favor de nuestra hipótesis según la cual, la morfología, el desempeño, y la ecología están altamente correlacionados a través de las 14 especies que consideramos. Por ejemplo, el largo de la pata delantera y la cantidad de la membrana interdigital estuvo positivamente correlacionados con la velocidad de natación y la frecuencia con la que encontramos en tierra a las 14 especies estudiadas. Igualmente, el ancho de cabeza estuvo positivamente correlacionado con la fuerza de mordida y el nivel trófico.

Por último, en el Capítulo 3 se recopilaron datos de especímenes de tortugas en museos de historia natural de México, Estados Unidos y Francia con el objetivo de comparar los patrones de variación y covariación (i.e. modularidad e integración) entre todas las tortugas, los principales clados de tortugas y tortugas con diferentes ecologías. La hipótesis propuesta fue que las características morfológicas que observamos en los Capítulos 1 y 2 también explicarían la variación a través de la diversidad de tortugas. Por ejemplo, predijimos que la variación en el uso de hábitat está caracterizada por las variables que encontramos correlacionadas con la velocidad de natación y la morfología (ej. tortugas acuáticas tendrían las patas delanteras más largas). Esta predicción está basada en estudios previos con otros grupos de organismos que muestran que hay correlaciones entre la morfología de un organismo y diferentes aspectos de su ecología (Moen, 2019; Larouche *et al.*, 2020; Navalón *et al.*, 2020). También propusimos la hipótesis de que los patrones de covariación explicarían cómo unos clados se han diversificado más que otros. Por ejemplo, predijimos que el aumento de la modularidad y la falta de integración entre las extremidades, el caparazón y la cabeza serían características de las familias de tortugas más diversas. Para hacer esto, medimos la morfología de las extremidades, el caparazón y la cabeza de especímenes de tortugas que representan el 69% de la diversidad de tortugas a nivel de especie (i.e. excluyendo subespecies). A partir de estos datos, encontramos apoyo para la hipótesis sobre que los principales ejes de variación morfológica a través la diversidad de las tortugas están caracterizados por el continuo de especies de tortugas que va desde las más acuáticas hacia la más terrestres. Igualmente, los patrones de covariación en las familias sugieren

que la falta de integración, o desacoplamiento, entre la cabeza y las extremidades/el caparazón ha promovido la diversificación en las familias de tortugas más diversas. En el anexo presentamos un trabajo que apoya la observación del desacoplamiento entre la cabeza y las extremidades/el caparazón en una especie (*Terrapene nelsoni*), y en dicha especie, los machos y hembras son similares en sus medidas de las extremidades/el caparazón, pero los machos tienen cabezas más grandes que las hembras. Así, nuestros datos sugieren que el dimorfismo sexual que se observa en *T. nelsoni* podría ser debido al desacoplamiento, o falta de integración, entre la cabeza y las extremidades/el caparazón que se observa en la familia Emydidae.

CAPÍTULO 1. MORPHOLOGY, PERFORMANCE, AND ECOLOGY OF THREE SYMPATRIC TURTLES IN A TROPICAL DRY FOREST

Butterfield, T., M. Olson, D. Beck, and R. Macip-Ríos. 2020. Morphology, Performance, and Ecology of Three Sympatric Turtles in a Tropical Dry Forest. *Copeia* 108 (4): 957-966.

Morphology, Performance, and Ecology of Three Sympatric Turtles in a Tropical Dry Forest

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Resource partitioning in communities is often achieved by sympatric species having different morphologies that allow them to access different resources. This is because differences in morphology influence an organism's capability to perform a task that is relevant to their ecology. Here, we compare limb, shell, and head morphology, swimming performance, habitat use, and diet of three species (*Rhinoclemmys rubida*, *R. pulcherrima*, and *Kinosternon chimalhuaca*) that co-occur in the tropical dry forest of Chamela, Jalisco, Mexico. We found that these species do not overlap in both habitat or diet, and the overlap that we observed in habitat was contrasted by differences in diet. We also found a consistent relationship among limb and shell morphology, swimming speed, and habitat. *Rhinoclemmys rubida* occupies the driest deciduous forest atop and along hills, has shorter hands, less interdigital webbing, longer plastrons, more-domed shells, and slower swimming speeds in proportion to body size. In contrast, *Kinosternon chimalhuaca* exclusively occupies arroyos or seasonal streams, has longer hands, more interdigital webbing, smaller plastrons, less-domed shells, and faster swimming speeds in proportion to its body size. *Rhinoclemmys pulcherrima* was found in all habitats and intermediate in morphology and swimming speed between the other two species. Therefore, in this study system, limb and shell morphology are good indicators of habitat differences between turtle species. These differences are likely due to the influence that limb and shell morphology have on swimming performance. Relationships between head morphology and diet were less clear, which might be the result of changes in behavior or habitat rather than morphology. Patterns of resource partitioning in Chamela seem to coincide with other studies of turtle communities, which suggests that relationships among morphology, performance, and ecology that we observe here might be a general pattern across turtles.

MOST of the ecological differences observed between species within biological communities are manifestations of morphological differences. This is because organisms evolve differences in morphology that maximize their ability to use a certain range of resources, and these differences permit species to partition resources (Schoener, 1974). For example, Darwin's finches (*Geospiza* sp.) have evolved beak morphologies that enhance their ability to crush or manipulate different types of seeds (Schluter and Grant, 1984; Herrel et al., 2005). Because these morphological differences correspond to differences in performance, sympatric species of Darwin's finches can coexist in sympatry without competitively excluding one another (Lack, 1947; Grant, 1968; Grant and Grant, 1982). In turtles, scientists have observed that morphology can influence performance of different tasks such as swimming, biting, self-righting, and climbing (Herrel et al., 2002; Rivera et al., 2006; Domokos and Várkonyi, 2008; Rivera, 2008; Xiao et al., 2017; Young et al., 2017), and that morphological differences exist between sympatric turtle species (Lindeman, 2000). Yet, the role that morphology and performance play in determining the structure of turtle communities has not been investigated.

In turtle community ecology, documenting how sympatric turtle species differ in habitat and diet has been the main approach to understanding how turtle species partition resources in communities. For example, DonnerWright (1999) compared distribution and abundance of sympatric turtle species in the St. Croix River and found that species

partition habitats along a continuous gradient ranging from deep-fast water to slow-mucky water. Similarly, Vogt and Guzman (1988) compared diets of sympatric Neotropical turtles and revealed that there are distinct differences in feeding habitats between sympatric species. These two examples, and many others (Dreslik and Phillips, 2005; Luiselli, 2008; Anthonysamy et al., 2014; Welsh et al., 2017), provide strong support that sympatric turtle species partition habitat and diet within communities. However, a question that remains is how differences in morphology and performance contribute to resource partitioning in turtle communities.

In other systems, such as anole lizard communities of the Caribbean (Losos, 1990), cichlid fish communities of African rift lakes (Carroll et al., 2004), or anuran communities in different regions of the globe (Moen, 2019), investigators have found close relationships between morphological traits and the ability of an organism to perform a task that is directly related to their ecology. For example, limb size in *Anolis* influences their ability to jump from tree limb to tree limb, escape predators, and grasp tree branches (Losos, 2011). This relationship allows species with different morphologies to use distinct microhabitats without competing for the same resources. If turtle communities are structured similarly, it should be expected that the ecological differences between turtles are manifestations of differences in morphology and performance. For example, habitats in which turtles occur throughout the landscape should be directly related to limb and shell morphology and ability to swim (or walk), whereas

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Submitted: 6 December 2018. Accepted: 10 August 2020. Associate Editor: J. W. Snodgrass.

© 2020 by the American Society of Ichthyologists and Herpetologists DOI: 10.1643/CE-18-165 Published online: 28 December 2020

a turtle's diet should be related to head morphology and ability to bite.

To determine if morphology and performance play important roles in structuring turtle communities, we compare morphology, performance, and ecology of three sympatric turtle species (*Rhinoclemmys rubida*, *R. pulcherrima*, and *Kinosternon chimalhuaca*) that co-occur in the coastal tropical dry forest of Chamela, Jalisco, Mexico. This system is ideal for studying this question because these species co-occur in similar areas but seem to occupy different habitats and little information exists on their diet. For example, *Rhinoclemmys rubida* is considered terrestrial because it is mainly found atop and along hills, *R. pulcherrima* is considered semi-terrestrial because it can be found in the water and on land, and *K. chimalhuaca* occurs mostly in the water but can also be considered semi-terrestrial because it estivates during the dry season (Legler and Vogt, 2013; Butterfield et al., 2018). Anecdotal observations of diet in captive individuals and wild specimens suggest that all species are omnivorous (Legler and Vogt, 2013). However, substantial overlap in diet seems unlikely given that it could lead to intense competition for the same resources (Fredrickson and Stephanopoulos, 1981). Because turtles have a rigid shell they are restricted to a narrow range of terrestrial or aquatic resources. Therefore, even though there are only three species in Chamela, they should exhibit morphological, performance, and ecological differences that are sufficient to permit their co-existence.

To understand if such differences exist, we first explored differences in habitat and diet to determine if Chamela species partition resources. Then, we explore the relationship among morphology, swimming performance, habitat use, and diet to determine if the resource use in this community is a manifestation of morphological and performance differences.

MATERIALS AND METHODS

Study site.—We conducted this study at the Chamela Biological Field Station located within the Chamela-Cuixmala Biosphere Reserve, near Chamela, Jalisco, Mexico. Vegetation in Chamela is characterized as lowland tropical deciduous “dry” forest with marked seasonality in precipitation. About eighty percent (800.5 mm) of the annual precipitation falls during the four-month wet season from June to September, and mean annual temperature is 24.9°C and ranges from 14.8–32°C (Bullock, 1986; Maass et al., 2018). The landscape throughout Chamela consists of small undulating hills with small, hydrologically distinct arroyos that cut through the hills. These elevational differences throughout the landscape create continuous changes in the structural and floristic diversity of the forest from the hilltops to the arroyos (Lott et al., 1987; Durán et al., 2002). These structural and floristic differences in the forest have been used to categorize two distinct forest types, deciduous and semi-deciduous forest (Durán et al., 2002). Deciduous forest is found along and atop hills and can be identified by its dense understory and smaller trees (majority < 10 cm diameter at breast height [DBH]; Durán et al., 2002). The semi-deciduous forest is associated with the larger drainages, has a less dense understory, and has many trees that exceed 10 cm DBH (Durán et al., 2002). Arroyos are the stream beds found within the semi-deciduous forest that only receive

water during the four-month wet season. Depending on amount of precipitation, arroyos they can vary from dry washes, to scattered ephemeral pools, and flowing water.

Turtle species in Chamela.—Three turtle species exist in Chamela, *Rhinoclemmys rubida perixantha*, *Rhinoclemmys pulcherrima rogerbarbouri*, and *Kinosternon chimalhuaca* (Legler and Vogt, 2013). All three are distributed along the west coast of Mexico and Central America. *Rhinoclemmys rubida* is distributed from the states of Nayarit to southern Chiapas, *R. pulcherrima* from Sonora, Mexico to Costa Rica, and *K. chimalhuaca* is microendemic and only distributed from the Cihuatlán River at the Colima-Jalisco state border, then north to the San Nicolás River (Turtle Taxonomy Working Group, 2017). Whenever the ranges of *R. rubida* and *R. pulcherrima* overlap, their ranges also overlap with a species of *Kinosternon* (Turtle Taxonomy Working Group, 2017). For example, the ranges of *R. rubida* and *R. pulcherrima* overlap with *K. oaxaca* in the state of Oaxaca, *K. integrum* from the states of Colima to Guerrero, and *K. scorpioides* from southern Oaxaca to Chiapas (Turtle Taxonomy Working Group, 2017). Therefore, a community of three species similar to the one at Chamela can be found throughout the entire range of *R. rubida*. It is possible that *Trachemys grayi* also occurs with these species from Oaxaca to Chiapas, but it is unclear to what extent (Turtle Taxonomy Working Group, 2017). The little information that exists on the natural history of Chamela species suggests that *R. rubida* is terrestrial and omnivorous, *R. pulcherrima* is semi-terrestrial and omnivorous, and *K. chimalhuaca* is semi-terrestrial and omnivorous (Legler and Vogt, 2013; Butterfield and Rivera-Hernandez, 2014; Butterfield et al., 2018). Semi-terrestrial is synonymous with semi-aquatic and can mean that turtles spend significant time on land and in water (*R. pulcherrima*) or that species estivate on land for long periods of time (*K. chimalhuaca*).

Locating turtles and habitat use.—Turtles were located during the wet season (June–September) between November 2014 and September 2017 by searching potential turtle habitat throughout the Chamela field station. Data were collected during an ongoing study of *R. rubida* during 2014–2017; therefore, more *R. rubida* are included in our sample than *R. pulcherrima* and *K. chimalhuaca*. All turtles were marked with unique numbers using a triangle file to ensure that we did not have repeated samples. Thus, unique individuals encountered throughout this entire period were used to quantify habitat use. Habitat use was quantified by recording the frequency that turtles were located in deciduous forest, semi-deciduous forest, and arroyos. If an individual was found atop or along a hill it was considered deciduous forest, if in a larger drainage but not in water it was considered semi-deciduous, and if in the water it was considered arroyo.

Stable isotope analyses.—Turtle shells are made up of bone, a thin layer of epidermal tissue, and a layer of keratin. Shell keratin is relatively inert and the stable isotope values of the keratin can be used as an index of the foods consumed during the time of development (Murray and Wolf, 2012). Consequently, carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values of shell keratin can be used to estimate the proportion of food resources consumed during development. Turtles grow rings on their shell similar to a tree, and the isotope values of a particular ring reflects a weighted average of

resources that were consumed during its development (Murray and Wolf, 2012). Thus, the isotope values of a single ring depend on how fast it develops (Murray and Wolf, 2012). Samples in this study were taken from several growth rings at the peripheral edge of the costal or pectoral scute. Recapture data suggest that *R. rubida* grow very slowly. For example, some individuals that have been captured four years after their initial capture have no observable growth rings added (unpubl.). *Rhinoclemmys pulcherrima* and *K. chimalhuaca* likely grow differently than *R. rubida*, but both species are exposed to the same eight-month dry season where little growth likely occurs. Thus, keratin samples in this study likely represent the weighted average of stable isotopes values over several years. In terrestrial ecosystems, isotopic values of nitrogen reflect trophic level, with carnivorous predators having the highest values (e.g., $\delta^{15}\text{N} = 7.6\%$ in wolves, *Canis lupus*; Szepanski et al., 1999), herbivores low-intermediate values (e.g., $\delta^{15}\text{N} = 1.6\%$ in moose, *Alces alces*; Szepanski et al., 1999), and primary producers with the lowest (e.g., $\delta^{15}\text{N} = 0.18\%$ in C_3 plant, *Achatocarpus gracilis*; present study). Carbon isotope values reflect photosynthetic pathway, with C_3 plants having the lowest values (e.g., $\delta^{13}\text{C} = -29\%$ in C_3 plant, *Achatocarpus gracilis*; present study) and C_4 and CAM having the highest (e.g., $\delta^{13}\text{C} = -12\%$ in cactus, *Opuntia excelsa*; present study).

Keratin sampling of adult turtles took place during the 2015 and 2017 field seasons. Approximately 1 mg squares of keratin were cut with a razor saw from the peripheral edge of the 2nd or 3rd costal scutes or the peripheral edge of the pectoral scute, and then removed by lifting off the square with a razor blade. This process took approximately five minutes. Upon removal, keratin samples were washed with 99% isopropyl alcohol then placed into tin capsules. Tin capsules were loaded into a 96-well plate and sent to the Center for Stable Isotopes (CSI) at the University of New Mexico. In 2015, we sampled 12 *R. rubida* and 1 *R. pulcherrima*, and, in 2017, we sampled 3 *R. rubida*, 11 *R. pulcherrima*, and 13 *K. chimalhuaca*. This sampling resulted in 15 total samples for *R. rubida*, 12 for *R. pulcherrima*, and 13 for *K. chimalhuaca*.

The main objective of using stable isotopes in this study was to determine whether species have overlapping diets, as opposed to a detailed analysis of the exact resources incorporated into the diet. One reason we did not estimate diet for all species was that this would have required extensive sampling of potential food resources in the environment and we did not sample potential food resources for *K. chimalhuaca*, which inhabits ephemeral streams and pools where isotope values of resources are likely different than terrestrial resources. Nonetheless, we did sample resources of *R. rubida* in 2015 and we used these data to determine what proportion of available resources are incorporated into the diet of *R. rubida* and *R. pulcherrima*. We included *Rhinoclemmys pulcherrima* because they are suspected to be terrestrial foragers and consume similar resources to *R. rubida* (Legler and Vogt, 2013).

We collected plant and arthropod tissues in the Chamela forest during the wet season from July and August 2015. The isotope values of resources in this system are likely different in dry and wet seasons, but it is unlikely that isotope values observed during the wet season change significantly year to year (e.g., Ehleringer et al., 1992). We could not identify all species sampled, so plants were identified based on their

photosynthetic pathway “ C_3 , CAM, and C_4 ” and arthropods by their taxonomic order. Leaves were collected with the exception of cacti, for which we sampled stem tissue. Most plants (include cacti, herbaceous plants, vines, and saplings) were sampled immediately above the ground. We collected arthropods by hand and with Berlese funnel traps. We did our best to sample widely so as to obtain a representative sampling of all the different species that are available to turtles, but we were also biased towards what we have observed *R. rubida* foraging on in the wild. Resources that *R. rubida* forage on included many unknown species of herbaceous plants and saplings, prickly pear cacti (2 *Opuntia* sp.), and fruits from *Guapira macrocarpa* (Butterfield and Rivera-Hernandez, 2014). All plant tissues were dried in an oven, then homogenized with a mortar and pestle. Arthropods were only dried, not homogenized. For most insects we sampled chitin, but the entire abdomen or entire individual was sampled for very small insects. It has been shown that there is some variation in the isotope ratios of different body parts on insects which could potentially bias our results (Gratton and Forbes, 2006). After drying, samples were divided into several aliquots (e.g., 1.0 mg) and loaded into tin capsules. Capsules were loaded into 96-well plates along with the keratin samples for analysis at CSI.

At CSI, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values were determined using a Thermo Scientific Delta V mass spectrometer (Thermo Scientific, Bremen, Germany) interfaced with a Costech 4010 (Costech, Valencia, CA) elemental analyzer (<http://csi.unm.edu/instrumentation>). All sample runs included regularly spaced lab standards to monitor precision of the readings. The CSI uses internal protein standards when analyzing protein samples, and based on the repeated measurement of these lab standards the precision of measurement was 0.1‰ SD for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Stable isotope results are expressed as δ values, $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = ([\text{R}_{\text{sample}}/\text{R}_{\text{standard}}] - 1) * 1000$. The R_{sample} and $\text{R}_{\text{standard}}$ represent ratios of heavy to light isotopes for $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ for the sample and standard. Stable isotope units are expressed as parts per thousand, or per mil (‰).

One caveat of using stable isotopes is that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the food resources change during tissue synthesis (Caut et al., 2009). This is called trophic discrimination and results in consumer tissue that does not perfectly match the isotopic values of the resources it consumed. Controlled diet-switch experiments with known isotopic values are used to understand how much the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values change from the food resource to the consumer tissue (e.g., Murray and Wolf, 2012). Since we did not conduct a diet-switch experiment to measure trophic discrimination of the turtles in Chamela we correct our data using the carbon (0.8‰) and nitrogen (2.55‰) trophic discrimination factors that have been experimentally determined for keratinous tissue in Desert tortoises (*Gopherus agassizii*; Murray and Wolf, 2012, 2013).

Morphology and maximum swimming speed.—Adult turtles that were encountered in the field during August and September 2017 were brought to the field station laboratory to record morphological measurements and perform swimming trials. For each individual, we measured straight-line carapace length (CL, measured from nuchal scute to cleavage between supracaudal scutes), straight-line plastron length (PL, measured from intergular scute to cleavage between anal

scutes), carapace width (CW, measured between 5th and 6th marginal scutes), shell height (SH; maximum vertical height from plastron to carapace), hand length (Hand, measured from the middle of the wrist crease to distal end of 3rd digit where skin meets the nail), and the surface area of interdigital webbing (Webbing). The surface area of interdigital webbing was calculated by multiplying the width and length of the interdigital webbing between the 2nd and 3rd front digits and dividing by two. All measurements were taken to the nearest 0.1 mm using dial calipers.

After taking morphological measurements, turtles were housed overnight in individual plastic containers and swimming trials were performed the following morning at approximately 0830 hr. Turtles were exposed to ambient air temperatures (typically range between 25°C and 30°C) throughout all trials. Although kinosternids are generally nocturnal, all turtle species seemed to respond equally to being housed in plastic containers overnight by walking around and scratching up against the container at intermittent intervals. This behavior after being placed in containers was known *a priori*; for that reason we were consistent in conducting the trials the day following capture so that all turtles were exposed to similar conditions. Swimming trials were conducted inside a custom-built 150x15x30 cm glass aquarium. This aquarium was filled with approximately 15 cm of water and allowed to equilibrate to ambient temperatures at least 24 hr before each trial began. Thin strips of opaque tape placed vertically at 10 cm intervals across the back of the tank were used as reference points when recording swimming speed.

Each turtle was encouraged to swim across the aquarium five times at one-hour intervals. The fastest speed of the five trials was used as an individual's maximum swimming speed. Different stimuli were used, such as tapping on the glass or manually stimulating the carapace to encourage turtles to swim across the tank. Trials were recorded with a Nikon AW120 camera that was placed 110 cm perpendicular from the front glass of the tank. The camera was placed in the same position for each trial. Swimming speed data were recorded as the elapsed time that turtles took to swim across the reference lines at the back of the tank. Start and stop times were recorded when the front of the carapace passed one of the spaced reference lines on the back of the tank. Recording started as turtles began swimming uninhibited, i.e., turtles were not scratching or bumping up against the glass. Recording stopped when the front edge of the carapace arrived at the 120 cm reference line. Swimming data were recorded in iMovie (Apple, Inc.; <https://www.apple.com/lae/imovie/>). See Supplemental Video 1–3 for sample trials for each species (see Data Accessibility).

We did not measure head dimensions in the field. Therefore, liquid-preserved specimens of the three Chamela turtle species were measured at two natural history collections, IBUNAM and MZFC, in Mexico City. Museum abbreviations follow Sabaj (2020). These specimens were originally collected in the Chamela region. Head length (HL, measured from the premaxilla to supraoccipital), head width (HW, measured at the widest part of the skull), and head height (HH, measured at highest part of the skull at posterior end of jaw) were measured using dial calipers (± 0.1 mm). There is sexual dimorphism in relative head size in *K. chimalhuaca* that we do not consider here; we measured one male and three females at IBUNAM and MZFC.

Statistical analyses.—All analyses were carried out in R statistical software (R Core Team, 2018) at the significance level of $\alpha = 0.05$. Normality was assessed using a Shapiro-Wilk test, and homogeneity of the residuals was assessed visually. In all cases, residuals followed a normal distribution, and variances among groups were relatively homogeneous.

Habitat overlap was tested using a chi-square goodness of fit test. Diet overlap was measured by calculating the percent of overlap observed in size-corrected standard ellipse areas (SEAc) for each species. Standard ellipse areas (SEA) are a new extension of the convex hull area, which was originally developed to describe the isotopic niche of a group of organisms by encompassing all of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within a convex polygon (Layman et al., 2007; Jackson et al., 2011). The convex hull method can overestimate the isotopic niche by encompassing outliers; therefore, the Bayesian inference was used to calculate SEA because it is less affected by outliers (Jackson et al., 2011). Moreover, the SEAc is SEA-corrected for sample size and is found to perform better than SEA and unbiased to small samples (Jackson et al., 2011). The SEAc was calculated for each species in the package 'SIBER' using the 'createSiberObject' function and overlap of these ellipses were calculated using the 'maxLikOverlap' function (Jackson et al., 2011). The 'maxLikOverlap' function uses the maximum likelihood estimated means and covariances matrices of two specific groups to calculate the area of overlap. Overlap was quantified by measuring the amount of area (%²) that the SEAc of two species overlaps.

The proportion of food resources that are incorporated into the diet of *R. rubida* and *R. pulcherrima* were measured using a mixing model (function 'simmr_mcmc', package 'simmr'; Parnell, 2016). A mixing model uses a Markov chain Monte Carlo fitting algorithm that uses iterations (1,000 in this study) to repeatedly estimate the values of the dietary proportions of the consumers to find values that best fit the data, while discarding values that are not consistent with the data. New estimates are required to be closer to the old estimates, creating a Markov chain. At the end of the run, a sample of the posterior diet proportions is produced (Parnell, 2016). One caveat of this analysis is that it assumes that every resource included in the analysis makes up some proportion of the consumer's diet. Because of this, *a priori* knowledge of what resources were consumed in the diet is important to avoid erroneously estimating a resource that consumers do not eat. We excluded gastropods as a potential resource when estimating the diet for *R. pulcherrima* because there are no reports of this species eating gastropods and we have not found anything besides plant material in their feces (Legler and Vogt, 2013; unpubl.). Including gastropods in the analysis of diet in *R. pulcherrima* does not change the results that we present here. C_4 plants were removed from all analyses because they are very rare throughout the Chamela reserve and are likely not consumed by either species.

We use a one-way analysis of variance (ANOVA) to compare quantitative differences in morphology and swimming speed between Chamela species. Before carrying out these analyses, size-free morphological variables were calculated by regressing $\log(\text{CL})$ on the \log of each morphological variable separately (function 'lm'; package 'stats'), and residuals from these linear models were used for analyses. The effect of body size on swimming speed was removed by calculating swimming speed as carapace lengths swum per second (carapace s^{-1} ; Stephens and Wiens, 2008). Using size-

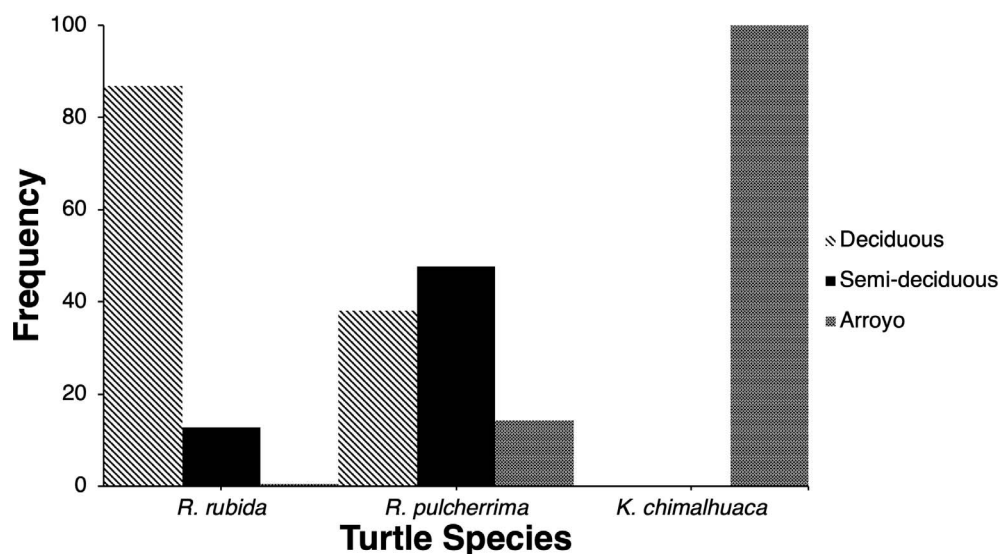


Fig. 1. Frequency that each turtle species was observed in deciduous forest, semi-deciduous forest, and arroyos in Chamela with respect to the total number of observations for each species. *Kinosternon chimalhuaca* ($n = 18$), *R. rubida* ($n = 220$), *R. pulcherrima* ($n = 21$).

free variables, six separate ANOVAs were calculated to test whether PL, CW, SH, Hand, Webbing, and carapace s^{-1} differed among Chamela species. To account for multiple testing with these six separate ANOVAs, we present P -values for uncorrected (P -value) and sequential Bonferroni corrected values (P -adjusted; function 'p.adjust', package 'stats'). Then, *post hoc* Tukey honest significance tests were used to test pairwise comparisons between species (function 'TukeyHSD'; package 'stats'). This same procedure (body size correction, ANOVA, then *post hoc* Tukey test) was conducted separately for data taken on museum specimens to test whether HL, HH, and HW differed among Chamela species.

We use linear mixed models (LMM) with species as a random effect to test whether there was a correlation between carapace s^{-1} and morphology. Five individual models were fit with carapace s^{-1} as the dependent variable and residual PL, CW, SH, Hand, and Webbing as the independent variables. Significance of these models was

tested using likelihood ratio tests (function 'drop1'; package 'stats'). Likelihood ratio values follow a chi-squared distribution and are used to test the significance of the full model (with independent variable) compared to the nested model (without independent variable). We report LRT test statistic values and their associated P -values for each morphological variable.

RESULTS

Habitat and diet overlap.—There was very little overlap in either habitat or diet among Chamela turtles ($X^2 = 233.45$, $P < 0.001$). *Kinosternon chimalhuaca* and *R. rubida* differed greatly in habitat, whereas *R. pulcherrima* overlapped in habitat with both species (Fig. 1). *Kinosternon chimalhuaca* was observed in arroyo habitats 100% of the time ($n = 18$). *Rhinoclemmys rubida* was found in arroyo, semi-deciduous, and deciduous habitats 0.5, 13, and 89% of the time, respectively ($n = 220$). *Rhinoclemmys pulcherrima* was found in arroyo, semi-deciduous, and deciduous habitats 14, 48, and 38% of the time, respectively ($n = 21$). High overlap was observed in diet between *K. chimalhuaca* and *R. rubida*, and no overlap was observed between *R. pulcherrima* and other species. More specifically, size-corrected standard ellipse areas (SEAc) showed that there is $0.93\%^{0.2}$ overlap between *R. rubida* and *K. chimalhuaca*, $0.00\%^{0.2}$ between *R. rubida* and *R. pulcherrima*, and $0.00\%^{0.2}$ between *K. chimalhuaca* and *R. pulcherrima* (Fig. 2).

In total, we collected 83 C_3 plants, 36 CAM plants, 16 C_4 plants, and 48 arthropods. The species of the majority of these plants are unknown. Results from the stable isotope mixing model suggests that *R. rubida* is omnivorous and *R. pulcherrima* is mainly herbivorous. The diet of *R. rubida* is estimated to be composed of $36 \pm 6\%$ C_3 plants, $7 \pm 4\%$ CAM plants, $15 \pm 10\%$ diplopods, $33 \pm 7\%$ isopods, and $9 \pm 6\%$ gastropods. The diet of *R. pulcherrima* is estimated to be composed of $88 \pm 4\%$ C_3 plants, $4 \pm 2\%$ CAM plants, $5 \pm 3\%$ diplopods, and $3 \pm 2\%$ isopods. See Table 1 for summary isotope values of resources and turtle species.

Morphology and swimming speed.—Morphology and swimming speed were measured for 12 *K. chimalhuaca* (9 males and 3 females), 5 *R. pulcherrima* (5 males), and 11 *R. rubida* (7

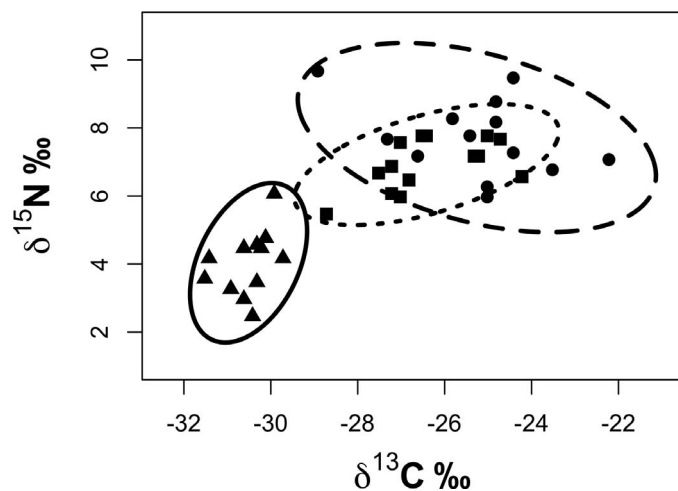


Fig. 2. Stable isotope composition of the three Chamela turtle species as indices of dietary overlap. Shapes correspond to the three different species, and each individual shape represents the isotopic value for an individual turtle. Ellipses represent corrected standard ellipse areas for each species (SEAc). Triangle and solid line: *R. pulcherrima*; square and dotted line: *R. rubida*; circle and dashed line: *K. chimalhuaca*.

Table 1. Summary (mean \pm SD) of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) values of data that were used with the mixing model to estimate diet of *R. pulcherrima* and *R. rubida*. *Kinosternon chimalhuaca* was not included in the mixing model because we did not sample potential diet sources.

Isotope source	<i>n</i>	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
C ₃ plants	83	2.95 \pm 1.82	-31.91 \pm 1.96
CAM plants	36	2.74 \pm 2.03	-14.78 \pm 1.73
Diplopoda	13	5.73 \pm 2.21	-23.37 \pm 1.74
Isopoda	7	8.84 \pm 0.47	-24.57 \pm 1.13
Gastropoda	16	1.21 \pm 3.82	-25.60 \pm 1.37
<i>R. pulcherrima</i>	12	4.04 \pm 0.96	-30.51 \pm 0.55
<i>R. rubida</i>	15	6.92 \pm 0.73	-26.41 \pm 1.24
<i>K. chimalhuaca</i>	13	7.72 \pm 1.14	-25.26 \pm 1.68

males and 4 females). Five of these individuals (5 *K. chimalhuaca*) were too large for the aquarium or slid against the bottom of the aquarium while trying to swim rather than swimming freely in the water column. Therefore, we first analyzed interspecific differences in morphology with all individuals (*n* = 28), then analyzed interspecific differences in swimming speed only with individuals from which we obtained uninhibited swimming trials (*n* = 23). Average CL (\pm SD) for individuals used to analyze swimming data were: *K. chimalhuaca*: 120.1 \pm 8.8 mm, *R. pulcherrima*: 115.1 \pm 6.6 mm, and *R. rubida*: 109.3 \pm 15.8 mm.

Differences observed in habitat use are consistent with the quantitative differences in morphology. *Rhinoclemmys rubida* had a longer PL, taller SH, smaller Hand size, and less Webbing than other species (Table 2). *Rhinoclemmys pulcherrima* was intermediate for all morphological variables except CW, which was wider than either *R. rubida* or *K. chimalhuaca* (Table 2). One-way ANOVAs supported these observations and detected significant differences among species in PL ($F_{3,28} = 5.42$, $P = 0.01$; P -adjusted = 0.06), SH ($F_{3,28} = 5.34$, $P = 0.01$; P -adjusted = 0.06), CW ($F_{3,28} = 21.42$, $P = 2.8 \times 10^{-6}$; P -adjusted = 1.8×10^{-5}), Hand ($F_{3,28} = 11.66$, $P = 0.0003$; P -adjusted = 0.002), and Webbing ($F_{3,28} = 20.56$, $P = 5.4 \times 10^{-6}$; P -adjusted = 3.2×10^{-5}). *Post hoc* pairwise comparisons showed that *R. rubida* and *K. chimalhuaca* were different in all standardized morphological variables (Table 3). *Rhinoclemmys pulcherrima* differed from *R. rubida*, but not *K. chimalhuaca* in Webbing (Table 3). All species differed in CW, with *R. pulcherrima* having the greatest CW, followed by *R. rubida* and *K. chimalhuaca* (Table 3).

We obtained uninhibited swimming trials from 6 *K. chimalhuaca* (4 males and 2 females), 5 *R. pulcherrima* (5 males), and 12 *R. rubida* (7 males and 4 females). Significant differences in swimming speed (carapace s^{-1}) were found among turtle species ($F_{3,23} = 9.5$, $P = 0.001$; P -adjusted = 0.007). *Post hoc* analysis showed that these differences were only significant between *K. chimalhuaca* and *R. rubida* ($P =$

0.0008), and that there were no differences between *R. pulcherrima* and *R. rubida* ($P = 0.20$) or between *R. pulcherrima* and *K. chimalhuaca* ($P = 0.15$).

Linear mixed models detected a positive relationship between carapace s^{-1} and Webbing (LRT = 23.74, $P = 8.1 \times 10^{-5}$) and a negative relationship between carapace s^{-1} and both Plastron (LRT = 7.75, $P = 0.014$) and SH (LRT = 7.22, $P = 0.01$; Fig. 3). There was no relationship between swimming speed and standardized hand size (LRT = 0.06, $P = 0.08$).

Size-free head measurements taken on museum specimens show that Chamela species differ in HL ($F_{3,12} = 8.7$, $P = 0.008$), HH ($F_{3,12} = 16.9$, $P = 0.0009$), and HW ($F_{3,12} = 48.9$, $P = 1.469 \times 10^{-05}$). *Post hoc* Tukey tests showed that differences were only significant between *K. chimalhuaca* and each of the species of *Rhinoclemmys* (Table 4).

DISCUSSION

In this study, we found that Chamela turtle species partition both habitat and diet resources in a way that results in no overlap in either resource between two species. For example, *R. pulcherrima* overlaps in habitat with both *R. rubida* and *K. chimalhuaca* but has a significantly different diet than both species (Figs. 1, 2), whereas, *R. rubida* and *K. chimalhuaca* use very different habitats but overlap in diet (Figs. 1, 2). This separation in both habitat and diet suggests that competition is important in structuring turtle communities, and patterns observed in other turtle communities support this conclusion. For example, in Australia, Welsh et al. (2017) documented the frequency that turtles were found in different aquatic habitat types and analyzed fecal samples to find that turtles that are found in similar habitats have very different diets. This example and other community-level studies (Moll, 1990; DonnerWright et al., 1999; Lindeman, 2000; Dreslik and Phillips, 2005) are consistent with the idea that sympatric turtle species partition resources to avoid competition for the same resource. Moreover, resource partitioning between co-occurring turtle species seems to exist even when distantly related turtle species are included in the study (DonnerWright et al., 1999; Lindeman, 2000; Welsh et al., 2017; this study), which might be unexpected based on the expectation that interactions are strongest between closely related species (Schoener, 1974). Because differences in habitat and diet are consistently observed between sympatric turtle species, a future priority should be to understand if these patterns can be linked to general morphological patterns across turtles.

Linking differences observed in morphology to a turtle's ecology could provide additional tools needed for scientists to understand general patterns in phenotypic evolution of turtles and the formation of turtle communities. For example, if we know that there is a consistent relationship between morphology and ecology, then measurements from

Table 2. Mean \pm SD limb and shell characteristics standardized for body size of the three species of turtle in Chamela. Plastron: standardized straight-line plastron length, Hand: standardized hand length, SH: standardized shell height, CW: standardized carapace width, Webbing: standardized area of interdigital webbing.

Species	<i>n</i>	Plastron	Hand	SH	CW	Webbing
<i>R. rubida</i>	7	0.02 \pm 0.03	-0.04 \pm 0.04	0.01 \pm 0.02	0.01 \pm 0.02	-0.44 \pm 0.29
<i>R. pulcherrima</i>	5	0.01 \pm 0.01	0.0005 \pm 0.03	0.01 \pm 0.02	0.04 \pm 0.01	0.10 \pm 0.16
<i>K. chimalhuaca</i>	11	-0.02 \pm 0.02	0.06 \pm 0.04	-0.02 \pm 0.03	-0.03 \pm 0.02	0.36 \pm 0.35

Table 3. Results from the *post hoc* Tukey test comparing pairwise differences in shell and limb morphology between turtle species in Chamela. Plastron: standardized straight-line plastron length, Hand: standardized hand length, SH: standardized shell height, CW: standardized carapace width, Webbing: standardized area of interdigital webbing.

	Species	Mean difference	Lower bound	Upper bound	P-adjusted
Plastron	<i>R. pulcherrima</i> – <i>K. chimalhuaca</i>	–0.49	–1.11	0.14	0.15
	<i>R. rubida</i> – <i>K. chimalhuaca</i>	–0.89	–1.41	–0.37	8.0*10^{–04}
	<i>R. rubida</i> – <i>R. pulcherrima</i>	–0.41	–0.98	0.17	0.2
Hand	<i>R. pulcherrima</i> – <i>K. chimalhuaca</i>	–0.04	–0.09	0.02	0.2
	<i>R. rubida</i> – <i>K. chimalhuaca</i>	–0.08	–0.12	–0.04	2.0*10^{–04}
	<i>R. rubida</i> – <i>R. pulcherrima</i>	–0.04	–0.10	–0.01	0.14
SH	<i>R. pulcherrima</i> – <i>K. chimalhuaca</i>	0.03	–0.01	0.06	0.12
	<i>R. rubida</i> – <i>K. chimalhuaca</i>	0.03	0.01	0.06	0.01
	<i>R. rubida</i> – <i>R. pulcherrima</i>	0.01	–0.03	0.04	0.92
CW	<i>R. pulcherrima</i> – <i>K. chimalhuaca</i>	0.06	0.04	0.09	6.00*10^{–06}
	<i>R. rubida</i> – <i>K. chimalhuaca</i>	0.04	0.02	0.06	6.00*10^{–04}
	<i>R. rubida</i> – <i>R. pulcherrima</i>	–0.03	–0.06	–0.002	0.03
Webbing	<i>R. pulcherrima</i> – <i>K. chimalhuaca</i>	–0.26	–0.66	0.14	0.25
	<i>R. rubida</i> – <i>K. chimalhuaca</i>	–0.80	–1.11	–0.49	4.00*10^{–06}
	<i>R. rubida</i> – <i>R. pulcherrima</i>	–0.54	–0.94	–0.13	7.9*10^{–03}

museum specimens could be used to represent the ecology of turtle species. This would open the door for more comprehensive analyses with species that cannot be studied in the wild. In other organisms, comparison of morphology, performance, and ecology has often been used to disentangle the relationship between morphology and ecology, e.g., birds (Grant, 1968), rodents (Price and Brown, 1983), lizards (Losos, 1990), fishes (Rüber and Adams, 2001), and bats (Cochran-Biederman and Winemiller, 2010). Similar to these studies, we found that differences in morphology and swimming performance coincide with the continuum of habitats used by Chamela turtle species. *Kinosternon chimalhuaca* has longer hands, more webbing, smaller plastrons, less-domed shells, faster swimming speeds, and is found in exclusively in water. *Rhinoclemmys rubida* has shorter hands, less webbing, longer plastrons, more-domed shells, is a slower swimmer, and occupies the driest habitats (deciduous forest). *Rhinoclemmys pulcherrima* is intermediate to both species in morphology and swimming speed and occurred in all habitat types. Head morphology did not correspond to differences observed in diet. *Kinosternon chimalhuaca* has a

greater relative head length, head width, and head height than both species of *Rhinoclemmys*, but overlaps in diet with *R. rubida* (Fig. 2). *Rhinoclemmys rubida* and *R. pulcherrima* did not differ in head morphology but had very different diets (Fig. 2). These data suggest that there is a relationship among morphology, swimming performance, and habitat use but that the link between head morphology and diet remains to be explored further.

The literature on turtle morphology is dominated by comparisons of body size and shell shape (Rivera et al., 2006; Rivera, 2008; Angielczyk et al., 2011; Stayton, 2011; Vega and Stayton, 2011), with a priority for future studies being the integration of these data with head and limb morphology and the functional significance of correlations among these variables. We show that species comparisons of performance in ecologically relevant tasks such as swimming can be used to measure the functional significance of morphology. For example, limb and shell morphology were correlated with maximum swimming speed and habitat use in Chamela turtles (Fig. 3). If this pattern is found in other turtle communities, then limb and shell morphology have

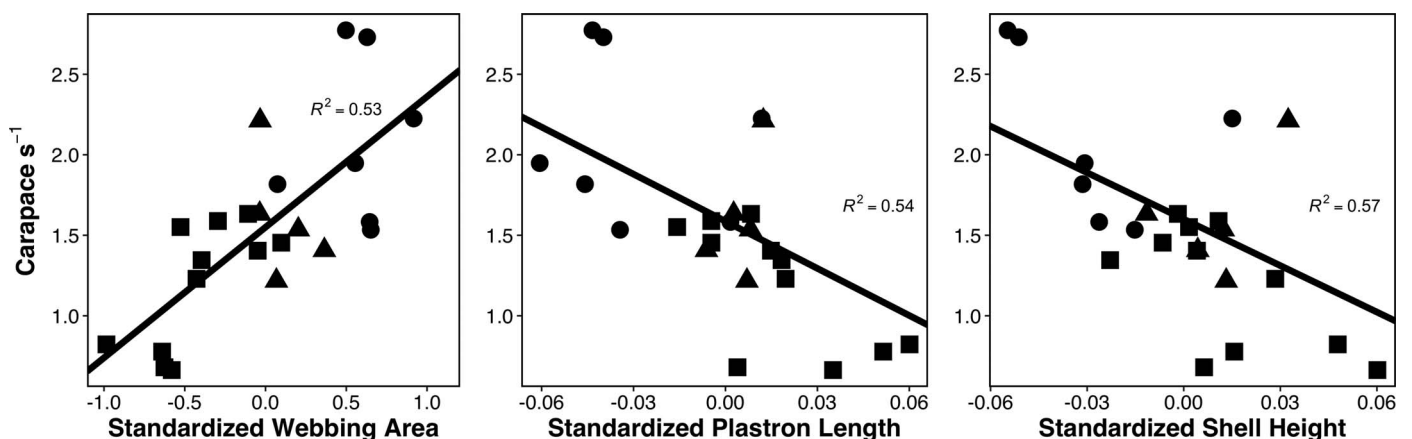


Fig. 3. Correlations between size-free swimming speed (carapace s^{–1}) and size-free morphological variables of *R. rubida* (squares), *R. pulcherrima* (triangles), and *K. chimalhuaca* (circles). Only relationships that were statistically significant are presented. Line represents slope of intercept of fixed effects. R² is correlation between fitted and observed values.

Table 4. Results from *post hoc* Tukey test comparing pairwise differences in head morphology between turtle species in Chamela. HL: standardized head length, HH: standardized head height, HW: standardized head width.

	Species	Mean difference	Lower bound	Upper bound	<i>P</i> -adjusted
HL	<i>R. pulcherrima</i> – <i>K. chimalhuaca</i>	–0.22	–0.37	–0.07	0.01
	<i>R. rubida</i> – <i>K. chimalhuaca</i>	–0.15	–0.3	0	0.05
	<i>R. rubida</i> – <i>R. pulcherrima</i>	0.07	–0.08	0.22	0.45
HH	<i>R. pulcherrima</i> – <i>K. chimalhuaca</i>	–0.32	–0.49	–0.15	0
	<i>R. rubida</i> – <i>K. chimalhuaca</i>	–0.29	–0.46	–0.12	0
	<i>R. rubida</i> – <i>R. pulcherrima</i>	0.03	–0.14	0.2	0.91
HW	<i>R. pulcherrima</i> – <i>K. chimalhuaca</i>	–0.37	–0.48	–0.26	0
	<i>R. rubida</i> – <i>K. chimalhuaca</i>	–0.27	–0.38	–0.16	0
	<i>R. rubida</i> – <i>R. pulcherrima</i>	0.1	–0.01	0.21	0.07

the potential to be used to represent turtle habitat use. This level of understanding could allow limb and shell morphology to be used as proxies for turtle habitat use to test broader evolutionary questions across the phylogeny of turtles. However, the relationship between head morphology and diet is less clear among Chamela turtle species because *R. rubida* and *R. pulcherrima* do not differ in head shape but have very different diets (Fig. 2; Table 4). Measuring bite performance in these two species may reveal that differences in jaw musculature, and not head shape, allow *R. rubida* to eat food items such as snails and beetles that *R. pulcherrima* cannot eat (Herrel et al., 2018). Furthermore, aside from swimming and bite performance, other measures like climbing and self-righting ability may also reveal important relationships between morphology and ecology (Muegel and Claussen, 1994; Herrel et al., 2002; Domokos and Várkonyi, 2008; Xiao et al., 2017).

We argue that a better understanding of the relationship among morphology, performance, and ecology can allow scientists to better understand broad evolutionary patterns in turtle communities and turtles in general. Morphological differences among Chamela turtles are related to habitat use but not diet. Other studies suggest that the relationship between limb and shell morphology and habitat use might be general across turtles. Aquatic turtles tend to have longer hands and more compressed shells than terrestrial turtles (Joyce and Gauthier, 2004; Rivera, 2008). Also, Stephens and Wiens (2008) compared swimming performance in emydid turtles, and although they did not measure morphological differences, they found a correlation between swimming performance and more aquatic turtles. The relationship between diet and morphology remains more obscure, and only weak non-significant patterns have been found among head size, bite force, and diet (Herrel et al., 2002; Claude et al., 2003; Foth et al., 2017). However, there seems to be a potentially important relationship between tongue morphology and diet in turtles (Josef-Beisser et al., 2004). Much remains to be studied regarding the functional significance of turtle morphology and future studies should aim to understand how the limb, shell, and head are related to a turtle's performance and ecology. Community studies offer much promise to address these questions because highly aquatic to highly terrestrial turtles often co-exist within the same area.

DATA ACCESSIBILITY

Supplemental information is available at <https://www.copeiajournal.org/ce-18-165>.

ACKNOWLEDGMENTS

This work was conducted under a permit issued by the Secretary of the Environment and Natural Resources (SEMARNAT) of the Mexican Government permit no. SGPA/DGVS/004756/18. We thank A. Herrel for instructive comments on this manuscript, the staff of the Chamela Biology Station for logistical support throughout the project, V. Reynoso-Rosales and Sr. Armando Borgonio from the National Reptile and Amphibian Collection, Instituto de Biología UNAM, in Mexico City, and Dr. Oscar Flores-Villa and Sr. Mundo Edmundo Pérez-Ramos from the Zoology Museum, Facultad de Ciencias, UNAM, in Mexico City. This project was funded by the Rufford Foundation, NSF award 1559447 (IRES SOBRE Mexico), and PAPIIT (IA200418). TB thanks the Consejo Nacional de Ciencia y Tecnología (CONACyT) for providing a scholarship (No. 863562). We express our appreciation for technical and logistic support from UNAM, especially the Escuela Nacional de Estudios Superiores (ENES) Unidad Morelia and the Posgrado de Ciencias Biológicas.

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CAPÍTULO 2. MORPHOLOGY OF THE LIMB, SHELL, AND HEAD EXPLAIN THE VARIATION IN PERFORMANCE AND ECOLOGY ACROSS 14 TURTLE SPECIES.

Butterfield, T., A. Herrel, M. Olson, J. Contreras-Garduño, and R. Macip-Ríos. 2021. Morphology of the limb, shell and head explain the variation in performance and ecology across 14 turtle taxa (12 species). *Biological Journal of the Linnean Society* XX:1-13.

Morphology of the limb, shell and head explain the variation in performance and ecology across 14 turtle taxa (12 species)

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Received 30 May 2021; revised 13 July 2021; accepted for publication 22 July 2021

Given that morphology directly influences the ability of an organism to utilize its habitat and dietary resources, it also influences fitness. Comparing the relationship between morphology, performance and ecology is fundamental to understand how organisms evolve to occupy a wide range of habitats and diets. In turtles, studies have documented important relationships between morphology, performance and ecology, but none was field based or considered limb, shell and head morphology simultaneously. We compared the morphology, performance and ecology of 14 turtle taxa (12 species) in Mexico that range in their affinity to water and in their diet. We took linear measurements of limb, shell and head variables. We measured maximum swimming speed, maximum bite force and how often turtles were encountered on land, and we used stable isotopes to assess trophic position. We used these data to test the following three hypotheses: (1) morphology, performance and ecology covary; (2) limb and shell variables, like hand length, are correlated with swimming speed and the percentage of time spent on land; and (3) head variables, such as head width, are correlated with bite force and stable isotopes. We find support for these hypotheses and provide the first evidence that morphology influences performance and ecology in turtles in the field.

ADDITIONAL KEYWORDS: bite force – evolution – habitat use – morphology – stable isotopes – swimming – turtles.

INTRODUCTION

Natural selection favours morphologies that maximize the ability of an individual to utilize a specific range of resources (Irschick *et al.*, 2008). For this reason, studies that test directly how morphology impacts the ability of an organism to utilize a resource provide key insights into how different morphologies arise (Arnold, 1983). Fulton *et al.* (2005), for example, demonstrated that pectoral fin aspect ratio in labriform fishes

is positively correlated with swimming speed and water velocity. This finding, and the fact that coral reef habitats are characterized by a mosaic of different water velocities, suggests that pectoral fin aspect ratios in coral reef fish evolve in response to variation in water velocity (Fulton *et al.*, 2005). Similar relationships have been observed in nearly all major vertebrate groups, including primates (Fabre *et al.*, 2019), bats (Aguirre *et al.*, 2002), lizards (Losos, 1990), frogs (Moen, 2019) and fishes (Wainwright, 1991; but see Schulte *et al.*, 2004), crystallizing the assertion that ecology drives the evolution of morphology through its impact on performance (Arnold, 1983).

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The ways in which ecology can impact morphology are complex, and measuring organismal performance in ecologically relevant tasks can help to strengthen the understanding of morphology by providing context on how morphology can affect the ability of an organism to utilize resources (Losos, 1990; Wainwright, 1991, 2007; Moen, 2019). Many studies have investigated morphology, performance and ecology in a diverse range of organisms, but few have investigated this in freshwater and terrestrial turtles in the wild.

Turtles are an ideal model organism to address questions that might not be feasible in other taxa. For example, there are fewer turtle species than most major vertebrate groups (365 turtle species vs. 10 000+ bird species; Turtle Taxonomy Working Group, 2017; Gill *et al.*, 2020), they exhibit surprisingly diverse ecologies (Pritchard, 1979; Ernst & Barbour, 1989), and there are communities of interacting species in nearly all regions of the world (Rodrigues *et al.*, 2019). Thus, if biologists aim to understand how ecology influences morphological evolution, turtles are ideal because they are ecologically diverse, there is abundant information that already exists on their ecology, and most species could be measured physically in natural history museums.

Morphology, performance and ecology have been investigated a number of times in freshwater and terrestrial turtles. Studies that use morphological measurements of museum specimens have shown that aspects of limb, shell and head morphology are associated with habitat use (Claude *et al.*, 2003, 2004; Joyce & Gauthier, 2004; Rivera & Claude, 2008; Stayton, 2019) and diet (Foth *et al.*, 2017). Laboratory studies have shown that there are fundamental differences in how terrestrial and aquatic turtles swim (Pace *et al.*, 2001; Stephens & Wiens, 2008; Young & Blob, 2015; Young *et al.*, 2017; Mayerl *et al.*, 2019), and in how head morphology is associated with bite force and diet (Herrel *et al.*, 2002, 2018; Pfaller *et al.*, 2011; Marshall *et al.*, 2012). These studies have paved the way for our understanding of how limb, shell and head morphology interact to influence performance and ecology, yet few studies are field based or have considered measurements of limb, shell and head in the same study or assessed these measurements with field-based measures of ecology.

In this study, we compare the morphology, performance and ecology of turtles from four different field sites in Mexico. The dataset comprised 14 taxa (12 species) that belong to four major turtle families, with species habitat use ranging from highly aquatic to highly terrestrial and diets ranging from herbivorous to carnivorous. Based on previous studies, we predicted that turtle morphology should be correlated with the continuum of aquatic–terrestrial habitats and herbivorous–carnivorous diets that are observed

across the diversity of turtles (Pace *et al.*, 2001; Joyce & Gauthier, 2004; Rivera & Claude, 2008; Foth *et al.*, 2017; Stayton, 2019). For instance, turtle species that are more frequently found in the water should have longer hands, a more streamlined carapace and faster swimming speeds (Pace *et al.*, 2001; Claude *et al.*, 2004; Joyce & Gauthier, 2004; Rivera, 2008), whereas turtle species with more carnivorous diets should have wider heads and stronger bite forces (Herrel *et al.*, 2001). In this study, we test the predictions of three different hypotheses. The first hypothesis is that morphology, performance and ecology should covary significantly. The second hypothesis is that there are specific limb (e.g. hand length) and shell variables (e.g. shell height) that contribute a disproportionate amount of the variation in maximum swimming speed and the percentage of time spent on land. The third hypothesis predicts that specific head variables (e.g. head width) will contribute more to variation in bite force and diet. We test these hypotheses in a phylogenetic framework using independent contrasts.

MATERIAL AND METHODS

STUDY SITES AND SAMPLING

This work was conducted by permission of the Secretary of the Environment and Natural Resources (SEMARNAT) of the Mexican Government under permits SGPA/DGVS/004756/18 and SGPA/DGVS/004756/19. We sampled at four different field sites in the states of Jalisco, Sonora, Oaxaca and Yucatan, Mexico. Turtle species at these sites consist of individuals that belong to four of the most diverse turtle families, Emydidae, Geoemydidae, Kinosternidae and Testudinidae (Fig. 1). Our field site in Jalisco was located at the Chamela biological station (19°29'55.05"N, 105°2'37.00"W) of the National Autonomous University of Mexico, within the larger Chamela-Cuixmala Biosphere Reserve. Our Sonora field site was located in the southeast corner of the state, within the Monte Mojino reserve (27°0'15.32"N, 108°48'34.05"W), within the larger Sierra de Alamos-Río Cuchujaqui natural protected area. The field site in Oaxaca was a network of different privately owned properties near Pochutla (15°46'43.40"N, 96°30'39.61"W). Our field site in Yucatan was located within the Kaxil Kiuc Biocultural Reserve (19°58'31.61"N, 89°11'38.35"W).

Fieldwork was carried out over the span of 4–6 weeks during the rainy season (June–October) from 2017 to 2019. More data were collected in Jalisco as part of a long-term study that began before the present study (Butterfield *et al.*, 2018). Our main methods of capturing turtles were visual encounter surveys and baited hoop

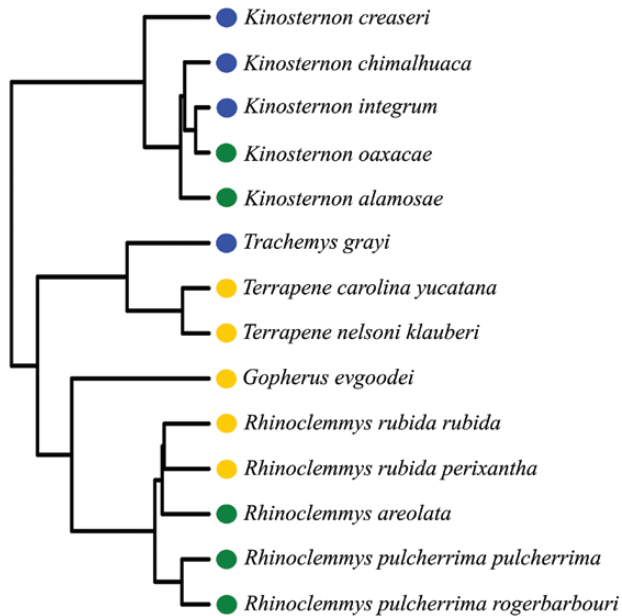


Figure 1. Phylogeny depicting the examined turtle species, based on the study by [Pereira et al. \(2017\)](#). Colours indicate the habitat categorization for each species ([Legler & Vogt, 2013](#); [Berlant & Stayton, 2017](#)), as follows: blue, aquatic; green, semi-aquatic; orange, terrestrial.

traps. Visual encounter surveys consisted of walking through potential turtle habitat with a group of three to ten people in a loose grid fashion to locate turtles on land. Hoop traps were baited with canned sardines and placed in rivers, small creeks and small ponds to trap mainly kinosternid species. In Oaxaca, *Trachemys grayi* was captured using a traditional cast net and by hand. The sampling effort of visual encounter surveys and trapping nights, including the number of people in the survey or number of traps, were recorded in Oaxaca, Sonora and Yucatan, but not in Jalisco.

MORPHOLOGY

We measured shell, limb and head characteristics of adult turtles that were captured in the field throughout the study. The majority of these turtles were used for swimming and/or biting performance trials, in which case turtles were measured after the experiments. The shell characteristics that we measured included straight-line carapace length (CL; cervical scute to cleavage between supracaudal scutes), straight-line plastron length (PL; symmetry axis from epiplastra to xiphiplastra), plastron width (PW; length of the seam that connects abdominal and pectoral plastral scutes), plastral lobe width (Lobe; the length of the seam that connects femoral and abdominal scutes), carapace width (CW; the width between fifth and sixth marginal scutes), shell height (SH; the maximum

vertical height from plastron to carapace) and bridge length (Bridge; the length from axilla to inguinal notch). The limb characteristics that we measured included antebrachium length (Ante; apex of elbow to wrist crease), manus length (Hand; middle of the wrist crease to distal end of third digit where skin meets the nail), surface area of interdigital webbing of manus (FrontWeb; the area of webbing between the second and third digit), crus length (Crus; apex of knee to apex of heel), pes length (Foot; apex of heel to distal end of third digit where skin meets nail) and surface area of interdigital webbing of pes (RearWeb; area of webbing between second and third toe). Interdigital webbing areas were calculated by multiplying the width and length of the interdigital webbing and dividing by two. The head characteristics that we measured included head width (HW; widest part of the skull), head length (HL; premaxilla to posterior edge of supraoccipital) and head height (HH; highest part of the skull at the posterior end of the jaw). All measurements were made to the nearest 0.1 mm using dial callipers.

PERFORMANCE

Maximum swimming speed was measured by filming turtles swimming in a custom-built aquarium. To ensure maximum swimming speed, turtles were motivated to swim by gently tapping on the glass of the aquarium and on the carapace. A black bag was placed around the finishing end of the aquarium to make it appear to the turtle to be a refuge. The fastest of five trials was taken as the ‘maximum swimming speed’ of an individual. In Chamela, the custom aquarium was made of glass and measured 150 cm × 15 cm × 30 cm; in Sonora it was acrylic and measured 200 cm × 30 cm × 30 cm, and in Oaxaca and Yucatán we used the same acrylic tank, which measured 150 cm × 30 cm × 30 cm. Each aquarium was filled with 15 cm of water and allowed to equilibrate to ambient temperature for ≥ 24 h before each trial began. Thin strips of opaque tape placed vertically at 10 cm intervals across the back of each tank were used as reference points when recording swimming speed. Trials were recorded in lateral view using a point-and-shoot camera (Nikon AW120) that was placed perpendicular in front of each tank such that the field of view in the camera encompassed only the entire tank. We made sure that the camera was placed in the same spot for every trial to avoid potential biases owing to parallax.

Swimming experiments took place at ~08.00 h on the day after the initial capture of the turtles that we subjected to the experiment. Turtles were housed overnight in plastic or semi-natural enclosures depending on each field site. During housing and experiments, turtles were exposed to ambient air

temperatures (typically ranging between 25 and 30 °C). All species seemed to respond in a similar manner to being housed overnight, by walking around and scratching the enclosure at intermittent intervals. Thus, the potential effects on energy expenditure that turtles endured before the experiment are assumed to be similar for all individuals subjected to the experiments.

Each turtle was encouraged to swim across the aquarium five times, at 1 h intervals. Using iMovie (Apple, Cupertino, CA, USA; <https://www.apple.com/lae/imovie/>), we analysed the video of each trial and recorded the fastest time that a turtle swam uninhibited between two reference lines. Recording started when the front edge of the carapace touched the first 10 cm reference line that was encountered after beginning to swim uninhibited, i.e. turtles were not scratching or bumping up against the glass. Recording stopped when the front edge of the carapace touched the last reference line that was encountered while swimming uninhibited. The fastest speed across the five trials was taken as the maximum swimming speed of an individual.

We measured bite force (in newtons) in the field using a custom isometric force transducer (type 9203, range ± 500 N; Kistler, Winterthur, Switzerland) that was mounted in a custom-built holder connected to a digital amplifier (type 5058A5; Kistler). Bite force was measured by coaxing turtles into biting thin metal plates that were part of the custom-built mount and noting the maximum force exerted (displayed on the hand-held digital amplifier) while a turtle was biting the plate (for more detail, see [Herrel *et al.*, 2001](#)). These experiments took place within 3 h after capturing an individual, and all individuals were subject to ambient air temperatures. Bite force has been shown to be relatively insensitive to variation in temperature in turtles ([Vervust *et al.*, 2011](#)). For each individual, we conducted three trials that were separated by ≥ 1 h, with the maximum force exerted in the three trials taken as the maximum bite force of an individual. All measurements were taken at the anterior point of the mandible. Bite force values were corrected by 0.67 to correct for the lever arms of the bite plates.

ECOLOGY

Habitat use and diet were the two aspects of ecology that we measured. Habitat use was measured by calculating the percentage of all our observations for each species that was composed of finding a unique individual on land (% land). Trophic position, as a proxy for diet, was assessed using stable isotopes.

During the fieldwork, we used visual encounter surveys and hoop traps to sample turtles, with the exception of *Trachemys grayi*, which we captured using

cast nets and by hand. We did not collect sufficient data to measure the differences in detectability of species during our visual encounter surveys, and it is possible that the % land measure could be biased, in particular for *Kinosternon* spp. A disproportionate number of kinosternids were captured using hoop traps instead of visual encounter surveys, but we choose to use % land because we believe that this measure captures more variation in habitat use than discrete habitat categorizations, such as terrestrial or aquatic.

The ratio between heavier/lighter isotopes of carbon, $^{13}\text{C}/^{12}\text{C}$ (denoted $\delta^{13}\text{C}$), has been used extensively in ecological studies to estimate patterns of resource use ([Ben-David & Flaherty, 2012](#)). This is because $\delta^{13}\text{C}$ values change in semi-predictable ways as they move through one biological system to the next owing to fractionation, which describes the change in stable isotope ratios that results from chemical reactions favouring the most abundant stable isotopes, such as ^{13}C , and ‘wasting’ uncommon isotopes, such as ^{12}C , resulting in a $\delta^{13}\text{C}$ that increases gradually from primary producers (plants or phytoplankton) to carnivores ([McConnaughey & McRoy, 1979](#); [Ben-David & Flaherty, 2012](#)). In this study, our aim was to understand where turtles fall on the herbivore–carnivore spectrum and not to identify specific food sources. For this reason, we sampled the $\delta^{13}\text{C}$ of turtle shell keratin and interpreted lower values as turtle species with more herbivorous diets and higher values as turtle species with more carnivorous diets.

Turtle shells are made up of bone, epidermal tissue and keratin, and the $\delta^{13}\text{C}$ values of keratin reflect the isotope values of the resources that were consumed during its development ([Murray & Wolf, 2012](#)). Keratin grows very slowly, and small (1 mg) pieces taken from adults can represent the isotope values of resources consumed over several years ([Murray & Wolf, 2012, 2013](#)). Previous studies have shown that the stable isotope turnover in shell keratin is very slow ([Murray & Wolf, 2012](#)). For this reason, we are confident that samples taken from individuals are likely to represent a weighted average of the resources consumed by individuals over several seasons and are not susceptible to seasonal variation in diet. In this study, we took 1 mg keratin samples from adults of each turtle species. These samples were cut with a razor saw from the peripheral edge of the second or third costal scutes or the peripheral edge of a pectoral scute, and then removed by lifting off the square with a razor blade. This procedure took only several minutes, and turtles showed minimal discomfort during the process. After removal, keratin samples were washed with 99% isopropyl alcohol and placed into tin capsules. Tin capsules were organized into a 96-well plate and sent to the Center for Stable Isotopes (CSI) at the University of New Mexico for analysis.

At the CSI, $\delta^{13}\text{C}$ values are determined using a ThermoScientific Delta V mass spectrometer (Thermo Scientific, Bremen, Germany) interfaced with a Costech 4010 (Costech, Valencia, CA, USA) elemental analyser (<http://csi.unm.edu/instrumentation>). All their sample runs include regularly spaced laboratory standards to monitor the precision of the readings. In our data measurement, the precision was 0.1‰ SD. Stable isotope results are expressed as δ values, $\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$. The R_{sample} and R_{standard} represent ratios of heavy to light isotopes, $^{13}\text{C}/^{12}\text{C}$, for the sample and standard. Stable isotope units are expressed as parts per thousand, or per mil (‰).

Keratin samples were collected in Jalisco during 2015 and 2017. Sonora, Oaxaca and Yucatán samples were collected in 2018. Given that $\delta^{13}\text{C}$ values are negative, we calculated their absolute value before analysing them so that we could \log_{10} -transform and analyse isotope values with the other morphology and performance variables. Lastly, in isotope studies $\delta^{13}\text{C}$ values are commonly adjusted by a trophic discrimination factor, which represents the percentage by which $\delta^{13}\text{C}$ values change from the resource to the consumer during tissue synthesis (Caut *et al.*, 2009). We did not determine trophic discrimination factors experimentally or adjust our values using previously reported values because trophic discrimination has been studied in only one species (*Gopherus agassizii*; Murray & Wolf, 2012). Adjusting all our data by the discrimination factor of this single species would not affect the interpretation of our data.

PHYLOGENY, PHYLOGENETIC INDEPENDENT CONTRASTS, AND BODY-SIZE CORRECTION

Given that turtle species in our study have a shared evolutionary history, the data cannot be treated as independent and need to be adjusted for phylogeny. Likewise, many data we collected are correlated with body size (e.g. head width, swim speed, bite force) and need to be corrected for body size so that interpretation is not biased by body size. To correct for both phylogeny and body size, we first pruned the phylogeny developed by Pereira *et al.* (2017) using the ‘drop.tip’ function in the ‘stats’ package in R to include the turtle species in this study (R Core Team, 2019). The phylogeny of Pereira *et al.* (2017) is fossil calibrated, and branch lengths are proportional to time. Subspecies were not included in the phylogeny published by Pereira *et al.* (2017). Therefore, we added sister taxa manually to *Rhinoclemmys rubida* and *Rhinoclemmys pulcherrima* to reflect the two subspecies of each that we included in the study. Subspecies were added using the ‘bindtip’ function in the R package ‘phytools’ (Revell, 2012). Using the edited phylogeny (Fig. 1), we calculated

phylogenetic independent contrasts (PICs) for all morphology, performance and ecology variables using the ‘pic’ function in the ‘ape’ package in R (Paradis *et al.*, 2004). We used PIC instead of other methods (e.g. phylogenetic residuals; Revell, 2009) because it was the only way we could correct for phylogeny and analyse variables related to body size (e.g. manus) with variables that are not related to body size (e.g. $\delta^{13}\text{C}$ values). All variables were \log_{10} -transformed before calculating PIC to minimize the differences among values attributable to measuring units.

After calculating PICs, we regressed the PICs of morphology and performance variables on the PIC of CL using a linear model forced through the origin by setting the intercept to zero (‘lm’ function, ‘stats’ package; R Core Team, 2019). The residuals of these linear models are referred to as ‘standardized PICs’ and represent size-free PICs of morphological and performance variables. Standardized PICs (morphology and performance variables corrected for CL) and PICs ($\delta^{13}\text{C}$ and % land) were used for all subsequent analyses.

To ensure robustness of our results using standardized contrasts, we also ran our analyses using phylogenetically independent contrasts of log-shape ratios (Mosimann, 1970; Larouche *et al.*, 2020), and we have included these results in the Supplementary Results section in Supporting Information. Contrasts of the log-shape ratios consist of dividing the variables of interest (e.g. SH) by the geometric mean of all variables that are correlated with body size (all morphological variables in this study), taking the \log_{10} , then calculating the contrast of this value.

STATISTICAL ANALYSIS

All statistical analysis was carried out in the statistical environment R v.3.6.1, and our analyses were scrutinized to a significance level of $\alpha = 0.05$ (R Core Team, 2019). To test hypothesis 1, that morphology, performance and ecology covary, we used a two-block partial least squares analysis (2B-PLS) in the R package ‘geomorph’ (Rohlf & Corti, 2000; Adams & Otárola-Castillo, 2013). The 2B-PLS analysis assesses the degree of covariation between two separate matrices or ‘blocks’ and calculates the correlation coefficient between scores of projected values between the first singular vectors of each block, defined as “ R_{PLS} ”. The 2B-PLS analysis is used primarily with landmarks and Procrustes-fitted coordinates but can be used for any matrix of variables (Adams & Felice, 2014). The 2B-PLS method compares the associations between vectors that contain singular value decompositions of variables in a matrix. In this study, we defined three blocks, with the first containing the standardized PICs of all morphological

variables (morphology), the second containing the standardized PICs of maximum swimming speed and bite force (performance), and the third containing PICs of $\delta^{13}\text{C}$ and % land (ecology). These blocks were used to compare covariation between: (1) morphology and performance; (2) morphology and ecology; and (3) performance and ecology. This was executed using the 'two.b.pls' function in the R package 'geomorph', which uses permutation (999 in this study) to assesses the significance of covariation between two blocks (Adams & Otárola-Castillo, 2013).

We fitted general linear models through the origin and used Akaike information criterion (AIC) selection and likelihood ratios to test hypotheses 2 and 3. For hypothesis 2, we tested for significant relationships between the PICs of maximum swimming speed and % land as dependent variables and standardized PICs of limb (Ante, Hand, FrontWeb, Crus, Foot and RearWeb), shell (SLP, PW, Lobe, Bridge and SH) and head (HW, HL and HH) variables as independent variables, resulting in six different linear models (three per dependent variable). Given that three tests were done for each dependent variable, we adjusted *P*-values for multiple comparisons using the Bonferroni correction. A full model with all limb, shell and head variables was not possible because when all morphological characters were included the number of independent variables exceeded the number of turtle species in this study, which led to model overfitting. We tested hypothesis 3, that head morphology is correlated with bite force and $\delta^{13}\text{C}$, with two different linear models, the first between bite force (dependent variable) and head variables (HW, HL and HH) as independent variables, and the second between $\delta^{13}\text{C}$ (dependent variable) and head variables (HW, HL and HH) as independent variables. We reduced each model by one independent variable at a time using the 'drop1' function in the 'stats' package (R Core Team, 2019). The models with the lowest possible AIC values (reduced models) were used to identify which morphological characteristics best explained the variation in performance and ecology of each hypothesis. A likelihood ratio test was used to test the significance of each model compared with a null model. This was done using the 'anova' function in the R package 'stats' (R Core Team, 2019).

To quantify the strength of variables and directions of relationships in the full models, we calculated beta coefficients for independent variables in each full model. Beta coefficients were calculated using 'Beta Coefficient Calculator' by (Gardener, 2015); this function calculates the beta coefficient of the independent variables in a linear model using the model generated with the lm() function.

We did not assess inter-individual variation in this study. However, the Supporting Information (Figs S1, S2)

illustrates intra-individual variation between swimming speed and shell height, and between bite force and head width.

RESULTS

SAMPLING SUCCESS

We gathered morphological, performance and ecological data on all species except for *Kinosternon alamosae* and *Trachemys grayi* (Table 1). For this reason, we decided to divide the data we had for each species into three subsets. The first dataset contains all species, the second contains all species except for *Trachemys grayi*, and the third contains all species except *Trachemys grayi* and *K. alamosae* (Butterfield *et al.*, 2021). Species means were used for all analysis and \log_{10} -transformed before analysis to minimize the variation resulting from different units of measure (the absolute value of $\delta^{13}\text{C}$ was calculated in order to calculate the \log_{10}). The % land variable was arcsine transformed before analysis because raw variables were not normally distributed.

To obtain our % land measurement, we placed four hoop traps on four nights in Yucatan in 2018 and none in 2019, resulting in 27 *Kinosternon creaseri*. Visual encounter surveys were conducted on 15 days in 2018 and 2019 in Yucatán, totalling 170 person-hours (\bar{x} = 2.07 persons per survey) and 16 *Terrapene carolina yucatanana*, 29 *Rhinoclemmys areolata*, and 17 *K. creaseri*. In Oaxaca, we trapped for three nights in 2018 and 2019, averaging 6.5 traps/night and resulting in 26 *Kinosternon oaxacae*. Visual encounter surveys in Oaxaca were conducted on 26 days in 2018 and 2019, totalling 700.5 person-hours (\bar{x} = 8.9 persons/survey) and 24 *Rhinoclemmys rubida rubida*, six *Rhinoclemmys pulcherrima pulcherrima* and 12 *K. oaxacae*. We spent only 2 days in Oaxaca manually trapping *Trachemys grayi* in 2019 with cast nets, resulting in 11 individuals. In Sonora, we placed hoop traps for six nights in 2018 and 2019, averaging 1.7 traps/night, which resulted in 49 *Kinosternon integrum* and 11 *K. alamosae*. Visual encounter surveys were conducted on 32 days in Sonora in 2018 and 2019, totalling 249.6 person-hours (\bar{x} = 2.2 persons per survey) and five *Gopherus evgoodei*, 41 *Terrapene nelsoni*, 11 *Rhinoclemmys pulcherrima rogerbarbouri* and six *K. integrum*. Sampling effort was not recorded in Jalisco, but hoop traps were used for only three nights to capture individuals for this study in 2019. In Jalisco, we did not record sampling effort, but we found unique turtles without traps on 219 days from 2015 to 2019, resulting in 351 *Rhinoclemmys rubida perixantha*, 13 *R. p. rogerbarbouri* and 42 *Kinosternon chimalhuaca*. Sampling effort estimates for Yucatan,

Oaxaca and Sonora are based only on hours that T.G.B. spent in the field, but additional individuals captured by locals and brought to T.G.B. were also used in the % land measure before being released back into the field; these individuals were not included in the sampling effort estimate.

RELATIONSHIP BETWEEN MORPHOLOGY, PERFORMANCE AND ECOLOGY

Using the 2B-PLS test to compare covariation between matrices or 'blocks' of the standardized PICs of morphology, performance and ecology variables,

we found partial support for hypothesis 1. The morphological block covaried significantly with the performance block ($R_{\text{PLS}} = 0.897$, $P = 0.003$; Fig. 2A) and the ecological block ($R_{\text{PLS}} = 0.62$, $P = 0.035$; Fig. 2B). The performance and ecological blocks did not covary significantly ($R_{\text{PLS}} = 0.63$, $P = 0.06$; Fig. 2C). The loadings from each of these analyses suggest that the majority of the variation between turtles is attributed to the affinity of each species for water (Supporting Information, Table S1), such that traits like FrontWeb, swim speed and % land load very strongly on the first vectors of each of the first two 2B-PLS blocks (Supporting Information, Table S1). Similar results were found when using

Table 1. Sample sizes of morphology, performance and ecology variables that were measured for each species

Species	Shell	Limb	Head	Bite force	Swim speed	% Land	$\delta^{13}\text{C}$
<i>Gopherus evgoodei</i>	7	7	5	3	7	10	7
<i>Kinosternon alamosae</i>	7	7	7	7	6	11	–
<i>Kinosternon chimalhuaca</i>	20	14	12	12	7	42	13
<i>Kinosternon creaseri</i>	18	15	18	9	10	44	12
<i>Kinosternon integrum</i>	12	12	11	6	6	55	11
<i>Kinosternon oaxacae</i>	21	20	15	10	11	47	7
<i>Rhinoclemmys areolata</i>	20	19	18	9	9	34	8
<i>Rhinoclemmys pulcherrima pulcherrima</i>	14	14	14	4	6	25	6
<i>Rhinoclemmys pulcherrima rogerbarbouri</i>	20	18	13	8	13	13	21
<i>Rhinoclemmys rubida perixantha</i>	17	13	5	6	11	351	15
<i>Rhinoclemmys rubida rubida</i>	18	18	18	7	15	39	7
<i>Terrapene carolina yucataca</i>	16	16	16	10	11	23	12
<i>Terrapene nelsoni klauberi</i>	17	17	17	9	5	57	11
<i>Trachemys grayi</i>	7	7	7	7	–	11	–

The shell, limb and head columns indicate the number of times we measured the morphological variables that correspond to these regions of the turtle body plan. These sample sizes vary because it was not possible to measure limb or head variables on some individuals. Bite force and swim speed indicate the number of individuals used in each experiment. The % land column indicates the number of times we observed a unique individual of each species. The majority of *Rhinoclemmys rubida perixantha* observations are from a previous study (Butterfield et al., 2018). The $\delta^{13}\text{C}$ indicates the number of keratin samples for each species that were processed by the Center of Stable Isotopes at the University of New Mexico. Males and females were represented equally in all samples except for *Gopherus evgoodei*, for which the sample is dominated by males ($N = 6$).

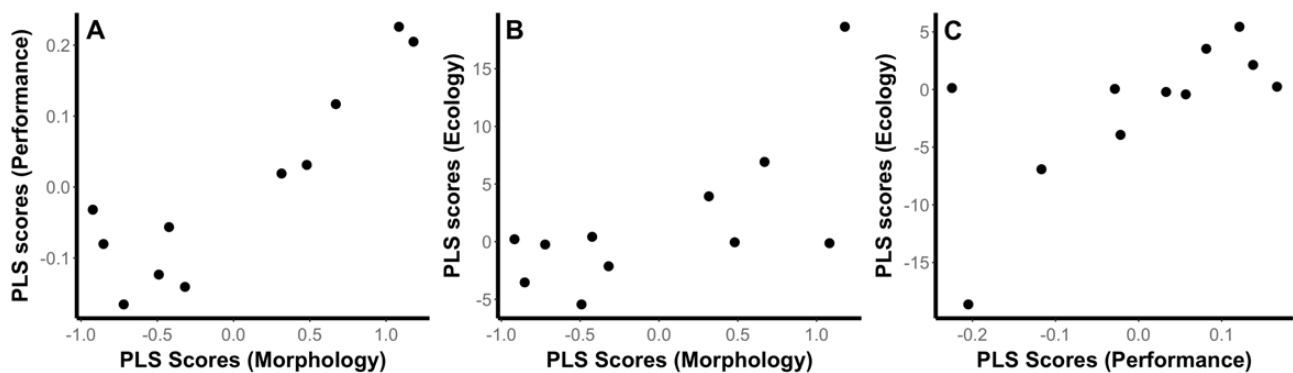


Figure 2. Results from two-block partial least squares (2B-PLS) test between morphology and performance blocks (A), between morphology and ecology blocks (B) and between performance and ecology blocks (C). These scatterplots illustrate the covariation between morphology, performance and ecology. Blocks represent matrices of phylogenetic independent contrasts (and standardized contrasts) of morphology, performance and ecology. *Kinosternon alamosae* and *Trachemys grayi* are not included in this analysis.

contrasts of log-shape ratios (Supporting Information, Supplementary Results; Fig. S3).

Linear models revealed significant support for hypotheses 2 and 3. Most reduced models were significantly different from the null model after using AIC selection, with the exception of the linear models between swimming speed and head variables and between $\delta^{13}\text{C}$ and head variables, which were not significant (Table 2). With respect to hypothesis 2, we found that PW, Lobe, SH and RearWeb were significantly correlated with swimming speed, whereas SLP, Lobe, HW and HL were significantly correlated with % land (Table 2). With respect to hypothesis 3, we found that HW was significantly correlated with bite force and that HL exhibited a marginally significant correlation with $\delta^{13}\text{C}$ (Table 2). The AIC values and R^2 values from these analyses suggest that swimming speed and % land are best explained by shell characteristics overall; most of the variation in bite force is explained by head characteristics, and $\delta^{13}\text{C}$ is explained only in part by HL (Table 2). Beta weights of morphological variables used in the linear models showed similar patterns in the proportion of the variance they explained in each model (Table 3). The results of the full models are presented in the Supporting Information (Table S2). Similar results were obtained when using contrasts of the log-shape ratios (Supporting Information, Supplementary Results; Table S3).

DISCUSSION

Understanding how the ecology of an organism covaries with morphology and performance provides insight

on how variation in ecology could impose selective pressures driving the evolution of morphology. In this study, we compared 14 turtle taxa (12 species) across Mexico to test the hypotheses that morphology, performance and ecology covary (hypothesis 1) and that specific limb, shell and head variables are correlated with our measures of performance and ecology (hypotheses 2 and 3). We found support for all three hypotheses, documenting significant covariation between morphology, performance and ecology and identifying specific morphological variables that correlated performance and ecology. These data are consistent with the notion that the selective pressures associated with habitat use and dietary regimes might be driving the evolution of morphology and performance in a consistent way in turtles.

One of the most valuable findings from this study is that direct measurements of limbs, shell and head reflect consistent and continuous differences in the ecology and diet of different turtle species, even if they belong to very different phylogenetic clades. Previous studies that have investigated the evolution of turtle lifestyles have used habitat or diet categories to depict turtle lifestyles (e.g. aquatic carnivorous). These categorizations have been extremely useful (e.g. Stephens & Wiens, 2003, 2008; McLaughlin & Stayton, 2016; Agha *et al.*, 2018; Stayton *et al.*, 2018; Stayton, 2019), but could conceal the possibility that turtle lifestyles form a continuum, as suggested by the present study. For example, habitat categories would not have detected the significant negative relationship between Hand, Foot, RearWeb and % land identified in the present study (Table 2). Likewise, DonnerWright *et al.*, (1999) demonstrated that continuous differences

Table 2. Summary of reduced linear models that compared performance and ecological variables with morphological variables

Hypothesis	Dependent variable	Independent variables	AIC	R^2	F	P -value
2	Swim speed	PW* + Lobe* + SH*	-88.28	0.93	56.39	< 0.001
	Swim speed	Hand + FrontWeb + RearWeb*	-80.33	0.87	27.62	< 0.001
	Swim speed	HL* + HH*	-62.92	0.41	5.12	0.43
	% Land	SLP* + Lobe*	37.68	0.80	27.45	< 0.001
	% Land	Hand + RearFoot + RearWeb	40.25	0.77	15.78	< 0.001
	% Land	HW* + HL + HH*	46.08	0.65	8.87	0.01
3	Bite force	HW* + HL + HH*	-87.21	0.95	78.36	< 0.001
	$\delta^{13}\text{C}$	HL	-89.96	0.23	4.33	0.06

All variables were corrected for phylogeny using independent contrasts (PIC), and variables that were correlated with body size were corrected by the independent contrast of body size (standardized PICs). The Akaike information criterion (AIC) and R^2 demonstrate the fit of models. The F values and P -values are results from likelihood ratio tests in which we compared the model with a null model. The P -values for hypothesis 2 were corrected for multiple comparisons using the Bonferroni correction.

The following definitions were used to represent standardized PICs or PICs for each variable: Ante, antibrachium length; bite force, maximum bite force; Crus, length of crus; FrontWeb, area of webbing between second and third front digits; Hand, hand length; HH, head height; HL, head length; HW, head width; Lobe, plastral lobe length; MW, width between fifth and sixth marginal scutes; PW, plastron width; RearFoot, rear foot length; RearWeb, surface area of webbing between second and third rear digits; SH, shell height; SLP, straight-line plastron; Swim speed, maximum swimming speed.

*Independent variables that explained a significant amount of the variation in each model.

Table 3. Beta weights, or standardized coefficients, from each of the full linear models before reducing the model via AIC selection

Independent variable	Hypothesis 2		Hypothesis 3	
	Swim	% Land	Bite	$\delta^{13}\text{C}$
SLP	-0.05	-0.11*	-	-
PW	-0.72*	-0.34	-	-
MW	0.04	0.12	-	-
Lobe	0.44*	0.75*	-	-
Bridge	0.07	0.27	-	-
SH	-1.30*	0.46	-	-
Antebrachium	-0.11	0.18	-	-
Hand	-0.18	-0.40	-	-
Front webbing	0.42	-1.3	-	-
Crus	0.07	-0.83	-	-
Foot	-0.10	0.24	-	-
Rear webbing	0.62*	-0.03	-	-
Head width	0.41	-1.10*	0.98*	-0.38
Head length	1.20*	-0.61*	0.006	-0.66
Head height	-1.50*	1.20*	-0.03	0.25

For the definitions of variables, see the footnote to Table 2.

*Independent variables that were significant in the linear models after Akaike information criterion (AIC) reduction.

in the geomorphology and stream velocity of the St. Croix river in Minnesota and Wisconsin, USA were correlated with the occurrence of different species throughout the watershed, suggesting that there are also continuous differences that permit multiple aquatic species to co-exist. Patterns of continuity have been observed in other studies, suggesting that such measures have the potential to provide a better understanding of turtle lifestyles (Lindeman, 2000; Welsh *et al.*, 2017). Future work should aim to use measurements of the limbs, shell and head together when investigating the evolutionary diversification of turtles. We also think that our findings here, and work by others (Pace *et al.*, 2001; Herrel *et al.*, 2002; Stayton, 2011, 2019; Ana *et al.*, 2015; Mayerl *et al.*, 2016; Young *et al.*, 2017), provide enough insight on how turtle morphology is related to ecology to enable the use of measurements of the limb, shell and head of museum specimens to address broader evolutionary questions.

The relationship between morphology, performance and ecology has been measured in many different contexts to address different ecological and evolutionary questions. Many studies focus on a single morphological feature (Collar *et al.*, 2014), a specific measure of performance (Herrel *et al.*, 2001) or a specific aspect of the ecology of an organism (Stephens & Wiens, 2003). In this study, we had a unique opportunity to include measurements of the entire turtle body plan, record two measures of performance and estimate two

aspects of ecology. This approach allowed us to use the 2B-PLS analysis, a multivariate analysis that was developed to test for significant covariation between different matrices of variables (Adams & Felice, 2014). The 2B-PLS analysis allowed us to consider all the variables together and make pairwise comparisons of singular value decompositions that represented the variation in matrices of morphological, performance and ecological variables, respectively. We found significant correlations between morphology and performance and between morphology and ecology variables (or 'blocks'), suggesting that ecology and performance both drive the morphological evolution of turtles. Moreover, the fact that we observed inconsistent relationships between morphology, performance and ecology variables suggests that evolution to habitats and diets could be mosaic in turtles. Mosaic evolution is the concept that evolutionary change can take place in some body parts without causing changes in other body parts (Clarke & Middleton, 2008; Felice & Goswami, 2018). With respect to hypothesis 1, we found significant covariation between morphology, performance and ecology blocks, but for hypotheses 2 and 3 we identified only a handful of traits that correlated significantly with performance and ecology. If the ecological evolution of turtles is mosaic, then limb, shell and head traits could contribute in semi-independent ways to determining the ecology of turtle species, resulting in turtles with similar ecologies but with different morphological traits that covary with performance and ecology. Nonetheless, it is possible that some of these correlations were not significant owing to the low interspecific sample size.

The percentage of time that turtles are found on land (% land) was negatively correlated with how fast turtles swim and with their limb, shell and head morphology (Tables 2 and 3). Turtles that spend more time on land are slower swimmers and are characterized by smaller hands, taller shells, more enclosed plastrons and smaller heads (Tables 2 and 3). This finding builds on previous museum-based studies (Claude *et al.*, 2003; Joyce & Gauthier, 2004; Stayton *et al.*, 2018) by demonstrating that a combination of limb, shell and head characteristics are correlated with swimming performance and habitat use in the wild.

Likewise, we showed that that head morphology (and other variables) directly influence bite force and $\delta^{13}\text{C}$, strengthening findings that head morphology, bite force and diet are correlated (Herrel *et al.*, 2002, 2018). Our study also suggests that increasingly high $\delta^{13}\text{C}$ values (or less negative values) correspond to turtle species that are more carnivorous, have stronger bite forces and wider heads (Tables 2 and 3). This observation is based on the interpretation that increasingly high $\delta^{13}\text{C}$ corresponds to an increasing level of carnivory and that lower $\delta^{13}\text{C}$ values correspond

to more herbivorous species. This relationship between $\delta^{13}\text{C}$ and diet has been observed in other studies, but was not investigated specifically here (Fry *et al.*, 1978; McConnaughey & McRoy, 1979; Ben-David & Flaherty, 2012; Murray & Wolf, 2013). Plants are the only source of carbon in terrestrial ecosystems, and every time carbon is processed by an organism the $\delta^{13}\text{C}$ value of its tissue increases owing to isotope fractionation (Smith & Epstein, 1971; Farquhar *et al.*, 1989; Ben-David & Flaherty, 2012). Thus, organisms that have higher $\delta^{13}\text{C}$ values have been observed to consume items that have processed carbon a greater number of times since it was first produced in a plant (Ben-David & Flaherty, 2012; the same is true of phytoplankton and marine organisms, McConnaughey & McRoy, 1979). The $\delta^{13}\text{C}$ values of species included in this study range from -30 to -20‰ (Butterfield *et al.*, 2021), making it possible that higher $\delta^{13}\text{C}$ values in this study are attributed to species that consume more plants with C_4/CAM photosynthesis, which are known to have average $\delta^{13}\text{C}$ values around -10‰ , compared with C_3 plants that average -27‰ (Smith & Epstein, 1971; Farquhar *et al.*, 1989). However, direct observation of turtles foraging in the field suggest that this is not the case because only two subspecies, *R. r. rubida* and *R. r. perixantha*, have ever been observed foraging on CAM plants in the wild. Three species have been observed consuming C_3 plants (*G. evgoodei*, *R. p. pulcherrima* and *R. p. rogerbarbouri*), and all other species that have been observed or are known to consume a mix of C_3 plants, insects, fungi and gastropods (T.G.B., personal observation; Legler & Vogt, 2013). If our findings can be corroborated in future studies, it would support the notion that evolutionary changes in the limb, shell and head are correlated with the continuum of aquatic to terrestrial and herbivorous to carnivorous ecologies.

There are several caveats that limit the interpretation of our results and that we hope will stimulate further investigation. One limitation to our study is taxonomic diversity. The turtles we studied belong to five different genera, and the species belonging to a genus all have similar ecologies (Fig. 1). Therefore, although we incorporated phylogeny in our analyses, the observations in our study could reflect the sampling of turtle lineages that have distinct ecologies. Future work should investigate whether the correlations between morphology, performance and ecology observed here hold true as more species from different families are incorporated into the analysis.

A second caveat of our study is the ecological diversity of the turtles at the field sites that we studied. Although each field site that we studied has a range of aquatic to terrestrial species, some localities across the globe are even more diverse; for example, there can be more than five aquatic species in the same locality (e.g.

turtle communities in India; Mital, 2016) or a locality can be dominated by terrestrial species (e.g. tortoise communities in Africa; Luiselli, 2003). Therefore, a future priority should be to compare morphology, performance and ecology in turtle communities that vary in their ecological diversity.

Lastly, the lack of biomechanical detail that we investigated limits our interpretation of swimming ability in different species of turtles. It has been shown that different turtle species have fundamentally different kinematic stroke patterns while swimming (Pace *et al.*, 2001; Young & Blob, 2015; Mayerl *et al.*, 2016, 2019; Young *et al.*, 2017). Our filming equipment was not sufficient to measure kinematics in detail, although one anecdotal observation we made that deserves further investigation occurred during swimming experiments in Sonora. During these experiments, it appeared that the rear foot in terrestrial emydid turtles (*Terrapene nelsoni klauberi*) might be more efficient at reducing drag during the recovery phase of the stroke in comparison to the semi-terrestrial geoemydid turtle (*R. p. rogerbarbouri*). In *Terrapene nelsoni klauberi*, during the recovery phase of the stroke, individuals maintained the rear foot in a plane parallel to the plastron, which appeared to reduce drag. In *R. p. rogerbarbouri*, the rear foot of individuals feathered during the recovery phase, which appeared likely to increase drag during the swimming stroke. This anecdotal observation suggests that comparison of kinematic patterns of the swimming stroke in emydid and geoemydid turtles could reveal fundamental differences in the swimming stroke between these families.

This study builds on previous work by combining multiple techniques and the is first field-based study aiming to understand the relationship between morphology, performance and ecology in turtles. Pace *et al.* (2001) and Joyce & Gauthier (2004) found that larger hands were associated with faster swimming speeds (increased thrust generation) and aquatic lifestyles. Stayton (2019) and Rivera (2008) demonstrated that turtles in more aquatic habitats had more streamlined and hydrodynamic shells. Herrel *et al.* (2002) and Claude *et al.* (2003, 2004) found that head morphology across turtles was significantly correlated with bite force and habitat. Our results tell a similar story to these previous studies and provide the first empirical evidence from the field that limb, shell and head morphology are correlated with turtle ecology and their ability to perform an ecologically relevant task. Furthermore, the significant results from 2B-PLS analysis and the patterns across linear models suggest that the evolution of turtles could occur along a specific continuum of variation, in which not all variables correlate significantly with ecology, but all make important contributions to the design of the overall organism.

ACKNOWLEDGEMENTS

This work was conducted under permits issued by the Secretary of the Environment and Natural Resources (SEMARNAT) of the Mexican Government (SGPA/DGVS/004756/18 and SGPA/DGVS/004756/19). For help throughout the course of this fieldwork, all authors would like to express deep gratitude to James Callaghan and Mario Uc Uc at Kaxil Kiuic Biocultural Reserve in Yucatán; Félix García, Abel Dominguez, Carlos Verdugo, Lydia Lozano, Alma Montano Hernandez, Martín Figueroa, Mercy Vaughn, staff at Nature Culture International and Comisión Nacional de Áreas Naturales Protegidas in Sonora; Martha Harfush, Eduardo Reyes, the communities of El Paso Xonene, Nopalera, El Aguacate and Cofradía, and the Centro Mexicano de la Tortuga in Oaxaca; and the staff at the Chamela field station, Katherine Renton, Abel Verduzco, Daniel Beck, Cameron Cupp, Jessica Luna, Nicholas DeHollander and José Garrido in Jalisco. The project was funded by the Rufford Foundation, the American Museum of Natural History (Theodore Roosevelt Grant) and Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (no. IA200418). T.G.B. thanks the Consejo Nacional de Ciencia y Tecnología (CONACyT) for providing a scholarship during his studies (no. 863562). T.G.B. and R.M.-R. express their appreciation for technical and logistic support from Universidad Nacional Autónoma de México, especially the Escuela Nacional de Estudios Superiores (ENES) Unidad Morelia (especialmente Jannette Huante) and the Posgrado de Ciencias Biológicas. Lastly, we sincerely thank all reviewers of this manuscript for their constructive comments that significantly improved this manuscript.

DATA AVAILABILITY

The data used in the analysis in this paper can be accessed at the Dryad Digital Repository ([Butterfield et al., 2021](#)).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Intra-individual variation between bite force and head width.

Figure S2. Inter-individual variation between swimming speed and shell height.

Table S1. Loadings from each two-block partial least squares (2B-PLS) analysis.

Table S2. Results from full and reduced linear models.

Supplementary Results

Figure S3. Two-block partial least squares (2B-PLS) test using contrasts of log-shape ratios.

Table S3. Results of linear models using contrasts of log-shape ratios.

CAPÍTULO 3. TRAIT COVARIATION AS A SOURCE OF ECOLOGICAL
DIVERSIFICATION AND BIAS IN TURTLES: COMPARATIVE EVIDENCE
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Manuscript
In preparation for: Journal of Evolutionary Biology

Article title:

Trait covariation as a source of ecological diversification and bias in turtles: comparative evidence across 272 species of freshwater and terrestrial turtles.

Short title:

Modularity and integration in turtles.

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Data Accessibility Statement:

Data can be found on Dryad: pending result of manuscript.

Acknowledgements:

This work would not have been possible without the enormous amount of help from A. Resetar, J. Mata, and K. Angielczyk (FMNH); D. Kizirian, M. Arnold, D. Dickey, L. Vonnahme, and C. Raxworthy (AMNH); O. Flores-Villela, M. Pérez-Ramos (MZFC); V. Reynoso, A. Borgonio (IBUNAM); T. Giermakowski, C. Loughran (Museum of Southwestern Biology); F. Xiao, H. Shi (Hainan Normal University); S. Rogers (CM); G. Schneider (UMMZ); and S. Robson (NHMU). We thank A. Monsiváis for helping produce the figures and D. Adams for reviewing the first version of this manuscript. We thank the Consejo Nacional de Ciencia y Tecnología for supporting TB with a scholarship (No. 863562) throughout his studies. This study was funded by PAPIIT project No. IA200418, and collection study grants from both AMNH and FMNH. Additional thanks to ENES Morelia and the Posgrado en Ciencias Biológicas at UNAM, especially J. Huante-Pineda.

Author contributions:

T.B., M.E., J.C.G, R.M.R designed the study. T.B. collected and analyzed the data. T.B., M.E., J.C.G, R.M.R wrote the manuscript.

Conflict of interest:

The authors have no conflict of interest to declare.

ABSTRACT

Identifying patterns of variation and covariation among traits can provide insight regarding the ways that development influences the range of morphologies that can be favored by selection, as reflected in patterns of modularity and integration. Modularity is observed when subsets of traits exhibit stronger covariation within one another than with traits in other subsets. Integration is observed when there are high levels of covariation between any two traits. Studies have shown that modularity and weak integration can lead to increased diversity and disparity. We tested this and explored patterns of variation in turtle morphology using limb, shell, and head measurements taken on 246 species of freshwater and terrestrial turtles. Phylogenetic principal component analysis shows that aquatic species tend to have larger hands, less domed shells, and heads that are more diverse in size. Terrestrial species tend to have smaller hands, more domed shells, and smaller heads. Semi-aquatic species are intermediate in morphology. We find support for the hypothesis that increased modularity and weak integration is associated with clades that have both aquatic and terrestrial species. However, modularity patterns are similar across all turtles and the most diverse clades were the least morphologically disparate. This observation suggests that weak integration between head and limb/shell characteristics, or trait decoupling, may have played an important role in the diversification of turtles. These data contribute to the broader understanding of how covariation influences evolutionary diversification, showing that the nature of covariation between traits can act as a source of innovation in some clades and ecologies, and bias in others.

KEY WORDS: variation, modularity, integration, disparity, turtles.

INTRODUCTION

A key discovery in evolutionary biology has been the recognition that developmental processes, physics, and physiology bias the range of morphologies that are accessible in development (Gould & Lewontin, 1979; Alberch, 1989; Losos, 2011). This discovery has transformed how biologists think about adaptation because instead of natural selection filtering out unfavorable variants from among a vast and continuous space of variants, inherent tendencies of development can limit the range of variants, even if these would be favored if they could be produced (Olson, 2012). As a corollary, understanding the limits within which organisms vary, and the ways that different morphological parts interact in producing different variants is a key starting point to understand how organisms adapt to their environment.

Organisms are coordinated systems and their traits covary. Identifying the variation of traits and the strength of covariation between them provides insight into which axes of morphological variation could represent inherent biases in developmental, or potential adaptations (Olson, 2012). Traits can be integrated, meaning there is strong covariation between traits, or they can be modular, meaning that there are high levels of covariation within some sets of traits but weak covariation among those sets of traits (Pigliucci & Preston, 2004; Callebaut & Rasskin-Gutman, 2005). One of the first studies to identify these patterns hypothesized that modularity should increase the range of developmentally accessible trait relationships (more variation) because weaker semi-autonomous relationships between parts during development implies that there are more developmentally accessible trait combinations (Wagner & Altenberg, 1996). Similarly, Felice et al. 2018 demonstrated with computer simulations that modularity can lead to increased morphological variation, and that integration can lead to less variation but more extreme morphologies due to the exaggerated evolution along specific axes of variation. These

hypotheses have been tested in a wide range of organisms, and in some instances, investigators have corroborated hypotheses that were proposed by Wagner and Altenberg 1996 and tested by Felice et al. 2018, showing that modularity leads to increased variation (Goswami & Polly, 2010; Felice & Goswami, 2018; Larouche *et al.*, 2018). However, the opposite pattern has also been observed, and Hu et al. 2016 attribute the exceptional diversity in Antarctic notothenoid fish to strong craniofacial integration (Evans *et al.*, 2021 find similar patterns of integration and increased diversification in flatfishes). Studies in amphibians and squamates have also found no correlation between modularity, integration, or diversity (Bardua, 2019; Watanabe *et al.*, 2019; Bardua *et al.*, 2020).

A majority of the studies on integration and modularity have focused on covariation patterns within individual traits (e.g. comparing different regions of the skull; (Bardua *et al.*, 2020), and it remains unclear how integration and modularity manifest in an organism's general body plan (e.g. head, torso, and limbs). Larouche *et al.*, 2018 studied integration and modularity between the head, trunk, and tail in ray-finned fish (Actinopterygii), showing that there are significant levels of covariation between the trunk and tail across species, and the interaction between these variables and head characteristics explain the major axes of morphological variation. This relationship between the trunk/tail and head that help explain the major axes of diversity in ray-finned fish highlights the important relationship between major regions and organisms body (Larouche *et al.*, 2018), such insights would not have been recognized in a study focusing solely on mandibles (Parsons *et al.*, 2012) or fins (Du *et al.*, 2019). Failing to investigate the ways that major components of an organism's body covary and interacted could lead to a misunderstanding of how organisms evolve and adapt to their environment (Olson, 2019). For this, understanding how major features of an organism covary, including head, torso, and limb characteristics is an ideal starting point to understand which traits might represent adaptations, and which might exist only due to correlations with other traits.

Freshwater and terrestrial turtles are an ideal group to test broad evolutionary questions because there are only 356 species, making it feasible to collect data on most extant species (e.g. Stayton et al. 2018), and they exhibit a wide range of lifestyles. For example, the two most diverse turtle families, Geoemydidae and Emydidae, contain species that have invaded terrestrial and aquatic habitats multiple times (Ernst & Barbour, 1989). The other major turtle clades are mostly restricted to aquatic (Kinosternidae, Trionychidae, and the suborder Pleurodira) or terrestrial habitats (Testudinidae; Ernst and Barbour 1989). This wide range of lifestyles observed across turtle families provides a wide range of trait variation for testing the ways that major axes of variation differ between families, and how traits covary.

In this study, we aimed to understand how traits vary across turtles and to test the hypothesis that patterns of covariation between traits could have played a role in guiding their ecological diversification. We expected that more ecological and species diverse turtle families (e.g. Emydidae and Geoemydidae) would exhibit higher levels of modularity, weak integration between traits, and increased morphological disparity because high levels of modularity and weak integration between traits has been shown to lead to increased ecological diversification and disparity (Felice & Goswami, 2018), To test this hypothesis, we measured limb, shell, and head characteristics of 246 turtle species, representing 69% of turtle species level diversity. We use phylogenetic principal component analysis to document the major axis of variation across all turtles, and each major turtle clade. Then, we tested three modularity hypotheses to identify modular organization of the turtle body plan, and we test for significant integration between the best supported modules. We carry out these analyses with the full data set, by family, and by

habitat categories (terrestrial, semi-aquatic, and aquatic). Our results support our hypothesis that weak integration is observed in the most ecologically and species diverse turtle families, suggesting that weak integration, or trait decoupling, has contributed to the ecological diversification of turtles. However, other patterns did not fit our hypothesis and modularity was similar across all turtle families and ecologies, not just the most diverse ones. We also find that the most diverse clades with weak integration between modules are the least disparate, not the most disparate as we expected.

METHODS

Specimens and morphological measurements

We gathered data from 1204 ethanol preserved and 165 wild caught turtle specimens, representing 246 species and 272 subspecies (see Supplementary Data). This covers 69% of the species level diversity and 56.9% of subspecies level diversity in extant turtles (Turtle Taxonomy Working Group *et al.*, 2017). We aimed to measure only adult specimens, which were identified based on expected straight-line carapace sizes of adults from the literature (e.g. Ernst and Barbour 1989) and ossification of the shell (adults have a completely ossified shell). However, we made exceptions to include sub-adults of rare species (e.g. Yunnan box turtle, *Cuora yunnanensis*), or sub-adults of species that are difficult to preserve in liquid (e.g. the very large Indian soft-shell turtle *Nilssonina gangetica*). When possible, three males and three females were measured for each species. Linear distances of limb, shell, and head characters were measured for each individual with a digital caliper to the nearest ± 0.5 mm. Limb characteristics measured included the manus (Hand; middle of the wrist crease to distal end of 3rd digit where skin meets the nail), antebrachium (Ante; apex of elbow to wrist crease), pes (Foot; apex of heel to distal end of 3rd digit where skin meets nail), and crus (Crus; apex of knee to apex of heel). Shell characteristics measured included straight-line carapace (SLC; nuchal scute to cleavage between supracaudal scutes), straight-line plastron (SLP; intergular scute to cleavage between anal scutes), bridge (Bridge; from axilla to inguinal pocket), plastral lobe (Lobe; maximal ventral width across femoral scutes where they fuse with abdominal scutes), plastron width (PW; length of seam that connects abdominal and pectoral plastral scutes), marginal width (MW; width between 5/6 marginal scutes), and shell height (SH; maximum vertical height from plastron to carapace). Head characteristics measured included head length (HL; premaxilla to posterior edge of supraoccipital), head width (HW; widest part of the skull), and head height (HH; highest part of the skull at posterior end of jaw).

Phylogeny

To incorporate phylogenetic relationships into our analyses, we relied on a published molecular phylogeny that includes 294 extant turtle species (Pereira *et al.*, 2017). This phylogeny was estimated using sequences from 13 mitochondrial ribosomal gene loci and maximum likelihood inference (Pereira *et al.*, 2017). Of the 272 species and subspecies in our full dataset, 221 of these are represented in the Pereira *et al.* (Pereira *et al.*, 2017) phylogeny. Therefore, to include all 272 species and subspecies from our full dataset, we manually inserted taxa that were in our dataset, but not the Pereira *et al.* 2017, as polytomies to their closest known relatives (Fig. S1). We did this first by removing species that were in the Pereira *et al.* 2017 phylogeny and not our data, then adding the 51 species and subspecies that were in our data but not Pereira *et al.* 2017 as polytomies to their closest known ancestors. This tree was modified using the Mesquite program version 3.5 (Maddison, 2008), and then exported to be used for subsequent analysis in R

version 3.11.1 (R Core Team, 2019). To ensure that manually adding species did not produce spurious results we run all analyses with the dataset that included all 272 species in our morphological dataset (referred to as ‘full dataset’), and a dataset that only included species that were in our data and the original Pereira et al. 2017 phylogeny (referred to as ‘Pereira only’). We rely on Turtle Taxonomy Working Group 2017 to follow the most up-to-date nomenclature (Turtle Taxonomy Working Group 2017).

Clade divisions

To analyze patterns of morphological variation, modularity, integration, and disparity across major turtle clades we partitioned the dataset and phylogeny into seven subsets that represent the major evolutionary lineages of freshwater and terrestrial turtles (see Adams *et al.*, 2009 for similar example in salamanders). Phylogenies were pruned using the *drop.tip* function in the *ape* package in R (Paradis & Schliep, 2019). These subsets were selected because they represent distinct and well-supported monophyletic lineages that differ in their ecological diversity. Geoemydidae (n of species in our dataset = 62) is the most species diverse family and is made up of turtles adapted to aquatic, semi-aquatic, and terrestrial habitats (Ernst & Barbour, 1989). Testudinidae ($n = 47$) is the family that comprises only terrestrial tortoises (Guillon *et al.*, 2012). Emydidae ($n = 58$) is the second most diverse turtle family, and is made up of aquatic, semi-aquatic, and terrestrial species (Stephens & Wiens, 2003). We include here the suborder Chelydroidea ($n = 36$) instead of Kinosternidae and Chelydridae families separately because there are only five extant species of Chelydridae, and these two families make up a monophyletic clade (Berlant & Stayton, 2017). Chelydroidea is comprised of aquatic bottom-walking turtles (Ernst & Barbour, 1989). Trionychidae ($n = 18$) is made up of aquatic soft-shell turtles and Chelidae ($n = 34$) aquatic side-neck turtles (Engstrom *et al.*, 2004); the suborder Pelomedusoides ($n = 22$), which we consider instead of Podocnemididae and Pelomedusidae separately because there are only eight extant species in Podocnemididae, is made up of aquatic and semi-aquatic side-neck turtles (Ernst & Barbour, 1989). To help visualize the range of lifestyles in each clade we assigned a habitat category, aquatic, semi-aquatic, and terrestrial in the full dataset only (Table S1). It should be noted that these categorizations are approximations, and the ecology of most freshwater and terrestrial turtle species remains unstudied.

Body-size correction

Morphological features that we measured of turtle limbs, shell, and head are influenced by body size and shared evolutionary history. To account for both, we used the *phyl.resid* function in the *phytools* package, which uses linear regression while controlling for phylogeny to produce residuals that are independent of body size and evolutionary history (Revell, 2009, 2012). Morphological variables were log transformed prior to analysis, SLC was used as the independent variable, and all other variables were included as dependent variables in the *phyl.resid* function. For the analysis of the seven major turtle clades, instead of calculating the residuals separately for each clade, we use residuals that were calculated for all 272 turtle species in the *phyl.resid* function and use partitions of these data to analyze the seven turtle clades separately in subsequent analyses.

Phylogenetic Principal Component Analysis

Our first goal of this study was to determine which morphological variables best explain the major axes of variation across turtles, and each turtle clade. To do this we conducted

phylogenetic principal components analyses (pPCA) of all 272 turtles and each of the seven turtle clades, resulting in eight separate pPCAs. These were calculated using the *phyl.pca* function in the *phytools* package in R (Revell, 2009). We used the loadings and scores of the first two principal components (PC) of each pPCA to visualize differences and indicate what traits explain the majority of the variation. pPCAs were also calculated with Pereira-only data.

Modularity

We tested three modularity hypotheses across all 272 turtle species, and each of the seven major turtle clades. These three modularity hypotheses were based on the configuration of six different modules that represent measurements from different regions of the turtle body plan (Table 1; Fig. 2). These hypothesis were tested using the *phylo.modularity* function in the *geomorph* package in R (Adams & Otárola-Castillo, 2013). The *phylo.modularity* function calculates a covariance ratio coefficient (CR), which represents the ratio of covariation between proposed modules to the covariation within modules and tests the significance of CR using permutation (999 in this study; Adams & Otárola-Castillo, 2013). Thus, we use *phylo.modularity* to test the significance of each modularity hypothesis for the full dataset and each clade, then we used the *compare.CR* function in the *geomorph* package to compare the strength of modularity hypotheses. The *compare.CR* function calculates effect sizes of CR coefficients (Z_{CR}) by subtracting the observed CR value by the expected value of CR under the null hypothesis of no modular signal, then dividing this value by the standard deviation of the empirical sampling distribution (Adams & Collyer, 2019). Thus, the resulting Z_{CR} is a standardized measure that can be used to make direct comparisons of the “strength” of modular signal, with negative values corresponding to stronger modular signal (Adams & Collyer, 2019). Because we made multiple comparisons of the same hypothesis across several datasets, we corrected *P*-values for multiple comparisons using Bonferroni correction using the *p.adjust* function in the *stats* package (R Core Team, 2019). *P*-values were corrected by the number of datasets that were used to test the same hypotheses, e.g., we tested modularity hypotheses in the full dataset and in each of the seven clades = 8 comparisons. We carried out both analyses with 272 species in full dataset and 222 species in Pereira-only data.

Table 1. Linear measurements that were included in each proposed module. A module in this study represents a matrix with measurements taken from a specific region of the body. For example, the head module is a “n x p” matrix in which “n” corresponds to species and “p” corresponds to three columns that include phylogenetic and size corrected head width, head height, and head length.

Proposed Module	Morphological traits included in matrix
Plastron	straight line plastron, bridge, lobe
Carapace	curved carapace, shell height, marginal width
Head	head width, head height, head length
Limbs	crus, antebrachium, manus, pes
Shell	straight line plastron, bridge, lobe, curved carapace, shell height, marginal width

ShellLimb	straight line plastron, bridge, lobe, curved carapace, shell height, marginal width, crus, antebrachium, manus, pes
Whole-body	straight line plastron, bridge, lobe, curved carapace, shell height, marginal width, crus, antebrachium, manus, pes, head width, head height, head length

Integration

Modularity and integration are not necessarily two ends of the same spectrum (Adams, 2016). Modularity compares the relative covariation observed among modules to the covariation within those modules, whereas integration only compares the covariation between proposed modules. We aimed to test where there is significant covariation between modules, because the support of a modular hypothesis does not necessarily mean there are low levels of integration between modules. Therefore, we tested for integration between proposed modules using a partial least squares analysis using the *phylo.integration* function in the package *geomorph* (Adams & Otárola-Castillo, 2013). This function quantifies the degree of phylogenetic morphological covariation between two modules, then uses permutation (999 in this study) to compare the empirical data to a null distribution to test whether integration is statistically significant (Adams & Felice, 2014). We calculated integration between Limbs, Shell, Head, and LimbShell modules for the full dataset, then for each the seven major turtle clades. We did not include the Plastron and Carapace modules in these comparisons because the only hypothesis that included these modules (hypothesis three) received little support. In these analysis we also make multiple comparisons and correct for this using the *p.adjust* function in the *stats* package (R Core Team, 2019). We do these same analyses using the Pereira-only data.

Disparity

Disparity of whole-body, Limb, Shell, and Head modules for all turtles and each of the seven turtle clades was defined as Euclidean variance, calculated using the *morphol.disparity* function in the *geomorph* package (Adams & Otárola-Castillo, 2013). We did not test disparity of Plastron or Carapace modules because they did not receive strong support in the modularity test. We also do these same tests with the Pereira-only data.

Integration, Modularity, and Disparity of Ecological Categories

We ran the same aforementioned analyses, modularity, integration, or disparity of species that were categorized as aquatic, semi-aquatic, and terrestrial. To do, this we split the phylogeny of the full data set by habitat and ran the analyses for each category individually. We did not run these analyses with the Pereira-data.

Statistical software

All statistical analyses were carried out using R statistical software version 2.6.3 (R Core Team, 2019). Significance was assessed at $\alpha < 0.05$. Bonferroni adjusted *P*-values are referred to as ‘*P* - adj’.

RESULTS

Phylogenetic principal component analysis

In the full dataset and each clade, the first five PCs explained between 76% and 90.1% of the variation in the data (Table S2). Turtle species with high PC1 scores and low PC2 scores tend to be terrestrial or semiterrestrial species and characterized by smaller manus/pes, longer antebrachium/crus, taller shell, more enclosed plastra, and smaller head (Fig. 1A; Table S2). Also, in the full dataset, species that were categorized as aquatic exhibited a wider range of morphologies, with lower PC1 and higher PC2 scores corresponding to turtle species with longer manus/pes, shorter antebrachium/crus, more compressed shell, smaller plastra, and larger head (Fig. 1A; Table S2). The pPCAs that were done separately for each clade revealed that the axis of variation that differentiates species varies across clades. Head characteristics (PC1) followed by limb and shell characteristics (PC2) explained the majority of the variation among species in Chelidae, Trionychidae, Chelydroidea, and Emydidae (Fig. 1B, D–F; Table S2), whereas the variation in the first two PCs in Testudinidae were distributed relatively equally across limb, shell, and head characteristics (Fig. 1G; Table S2). Shell and limb characteristics loaded strongest in Pelomedusidae, and limb characteristics loaded strongest on PC2 (Fig. 1C; Table S2). Limb and shell (PC1) followed by head (PC2) characteristics loaded strongest on the first two PCs in Geoemydidae (Fig. 1H; Table S2). Similar results were obtained when using Pereira-only data (Table S3).

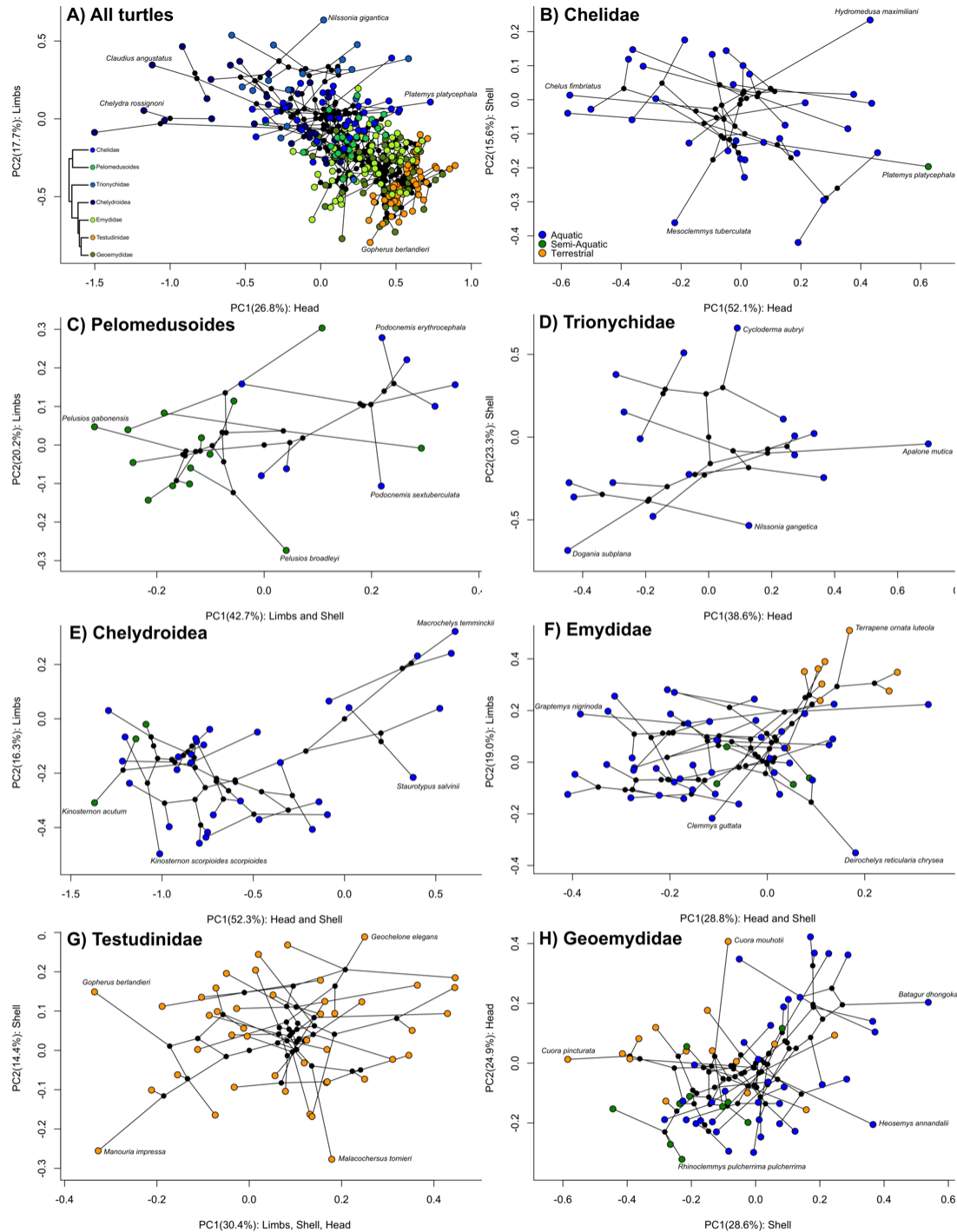


Figure 1. First two principal components of the phylogenetic principal component analysis (pPCA) of full dataset (A; label in left corner of graph) and each of the seven major turtle clades (B–H; labels in left corner of each graph). Axis labels indicate the percent of variance explained by each principal component (PC) and the body region corresponding to the location of morphological traits that load strongest on that axis. Colors correspond to ecological category. In A, color shades correspond to clades with predominantly aquatic (blue), terrestrial (orange), or a mix of aquatic, terrestrial, and semi-aquatic species (green). In B–H colors correspond to ecological categories aquatic (blue), semi-aquatic (green), and terrestrial (orange). Cl

Modularity

When testing the modularity hypotheses for the full dataset and each clade individually, we found that modularity hypothesis one significantly explained the covariation patterns in the proposed modules in the full dataset and each clade except Chelidae, Trionychidae, and Chelydroidea (Fig. 2). Modularity hypothesis two was significant in all clades except Trionychidae (Fig. 2). Modularity hypothesis three was only significant in Geoemydidae (Fig. 2). Pairwise comparisons of modular signal across clades revealed no significant pairwise difference in modular signal, Z_{CR} , between any clade in any of the hypotheses (Fig. 3A; Table S4). Despite the lack of significance, under hypothesis two clades tend to have the lowest Z_{CR} , or strongest modular signal, compared to the other hypotheses (Fig. 2;3). Similar results were obtained when doing these analyses with the Pereira-only data (Table S5; S6).

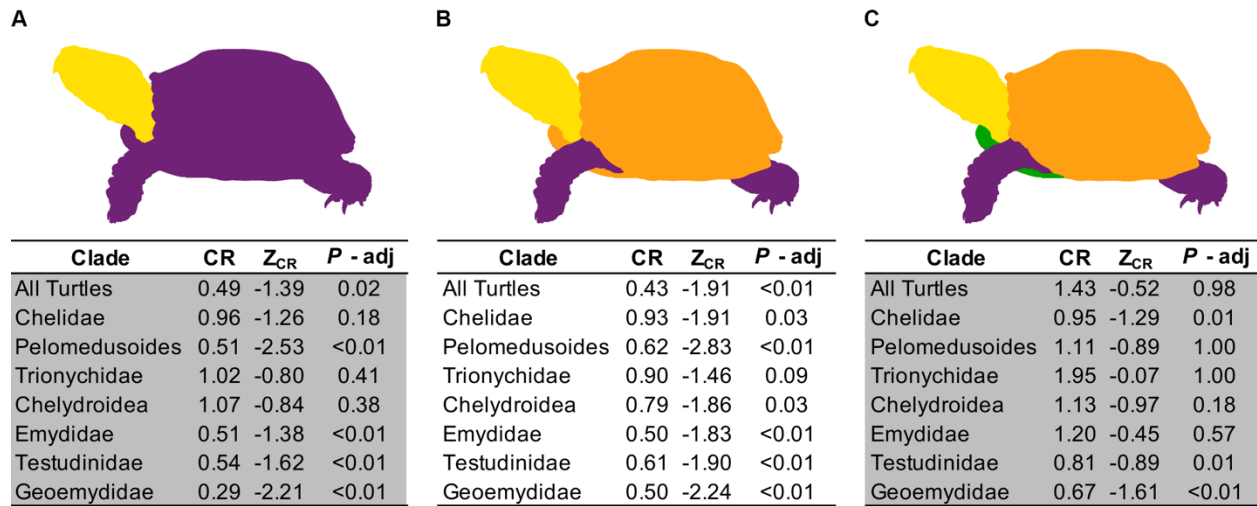


Figure 2. The three proposed modularity hypotheses that we tested. A) hypothesis one composed of LimbShell (purple) and head (yellow) modules, B) hypothesis two composed of Limbs (purple), Shell (orange), and Head (yellow) modules, C) hypothesis three composed of Limbs (purple), Plastron (green), Carapace (orange), and head (yellow) modules. Covariance ratio coefficient (CR) was generated using *phylo.modularity* function in *geomorph* and modular signal (Z_{CR}) was calculated using the *compare.CR* function in the same package (Adams & Otárola-Castillo, 2013).

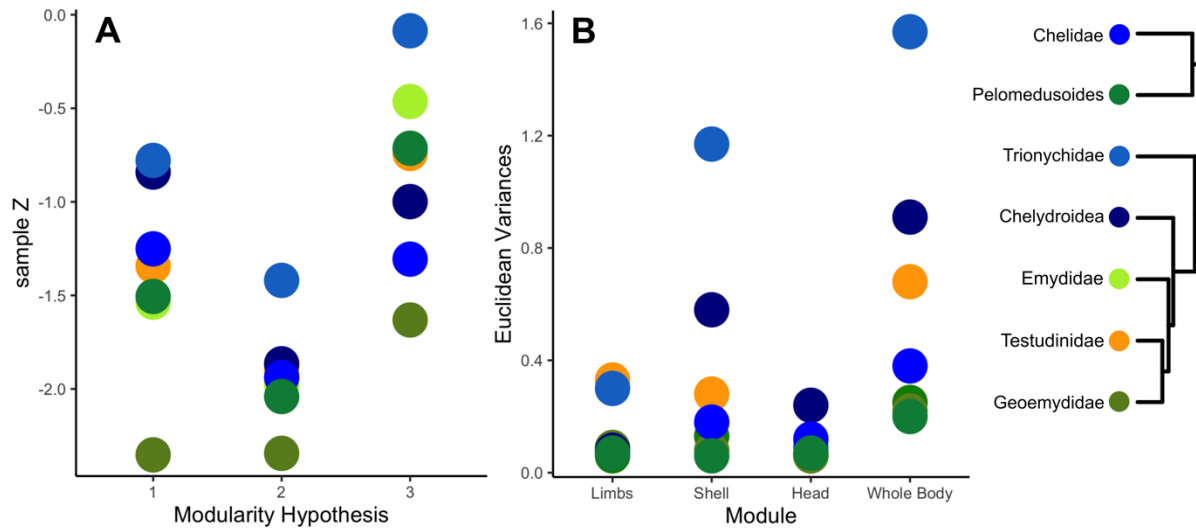


Figure 3. A) strength of modularity (Z_{CR}) compared across clades for the three modularity hypotheses, B) disparity of Limbs, Shell, Head, and whole-body for each clade.

Integration

Pairwise comparisons between modules reveal significant integration between Limbs - Shell modules in the full dataset, Chelidae, Pelomedusoides, Trionychidae, Chelydroidea, Emydidae, and Geoemydidae, but not Testudinidae (Table 2). Integration was significant between the Limbs-Head modules in Chelidae, Chelydroidea, and Testudinidae (Table 2). Shell - Head modules were significantly integrated in all groupings except for Trionychidae and Emydidae (Table 2). LimbShell-Head modules were significantly integrated in Chelidae, Trionychidae, Chelydroidea, and Testudinidae (Fig. 4; Table 2). Similar results were obtained when running these analyses with Pereira only data (Table S7).

Table 2. Summary of two block partial least squares results from pairwise comparisons of integration between the modules that were included in modularity hypothesis one and two. “Clade” corresponds to partition of the data that was used to make module comparisons, “Module comparison” corresponds to which modules were compared, “r-PLS” corresponds to the estimate of morphological integration, and “P-adj” indicates the significance of integration between two modules. The first two hypothesis of modularity had the strongest modular signal in each clade division except for Chelydroidea. Chelydroidea was the only clade in which modular hypothesis three displayed the strongest modular signal and pairwise comparisons for modules used in hypothesis 1-3 can be found in Table S3.

Grouping	Module comparison	r - PLS	Effect Size	P - adj
All Turtles	Limbs-Shell	0.309	4.320	<0.01
Chelidae	Limbs-Shell	0.646	2.953	<0.01
Pelomedusoides	Limbs-Shell	0.75	3.123	<0.01
Trionychidae	Limbs-Shell	0.456	-0.870	1
Chelydroidea	Limbs-Shell	0.58	3.323	0.016
Emydidae	Limbs-Shell	0.548	3.132	<0.01
Testudinidae	Limbs-Shell	0.555	2.253	0.084
Geoemydidae	Limbs-Shell	0.626	4.846	<0.01
All Turtles	Limbs-Head	0.152	0.964	0.616
Chelidae	Limbs-Head	0.72	4.776	<0.01
Pelomedusoides	Limbs-Head	0.358	-0.264	1
Trionychidae	Limbs-Head	0.565	1.172	0.576
Chelydroidea	Limbs-Head	0.615	4.266	<0.01
Emydidae	Limbs-Head	0.461	2.574	0.06
Testudinidae	Limbs-Head	0.514	2.868	0.028
Geoemydidae	Limbs-Head	0.271	0.335	1
All Turtles	Shell-Head	0.47	9.881	<0.01
Chelidae	Shell-Head	0.685	4.063	<0.01
Pelomedusoides	Shell-Head	0.351	-0.349	1
Trionychidae	Shell-Head	0.719	2.612	0.024
Chelydroidea	Shell-Head	0.707	4.996	<0.01
Emydidae	Shell-Head	0.468	2.938	0.028
Testudinidae	Shell-Head	0.509	2.258	0.076
Geoemydidae	Shell-Head	0.405	2.675	0.052
All Turtles	LimbShell-Head	0.474	8.325	<0.01
Chelidae	LimbShell-Head	0.795	4.774	<0.01
Pelomedusoides	LimbShell-Head	0.376	-0.679	1
Trionychidae	LimbShell-Head	0.895	3.171	<0.01
Chelydroidea	LimbShell-Head	0.727	4.689	<0.01
Emydidae	LimbShell-Head	0.499	2.154	0.136

Testudinidae	LimbShell-Head	0.587	2.631	0.024
Geoemydidae	LimbShell-Head	0.401	1.548	0.316

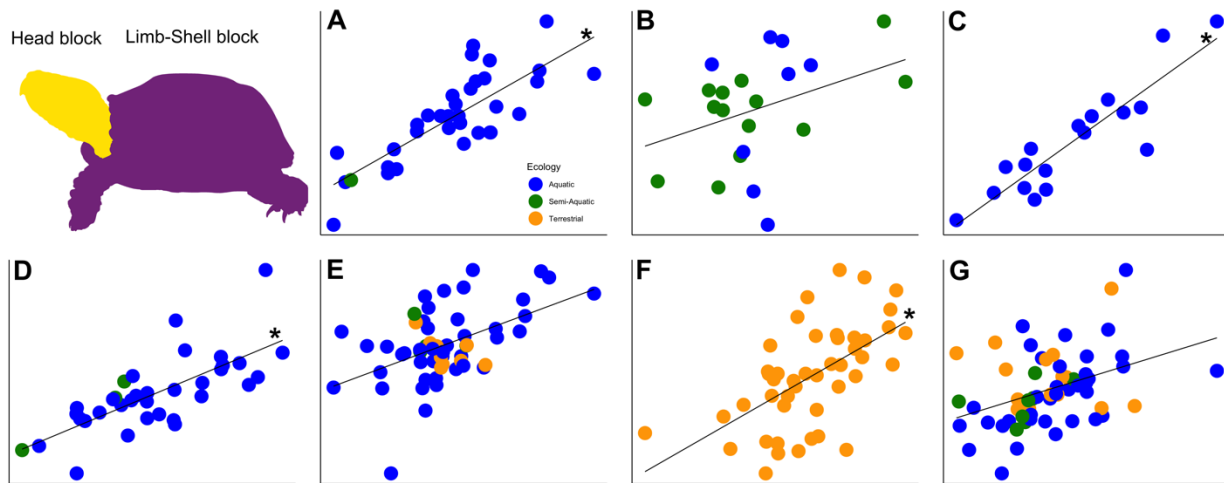


Figure 4. Integration between LimbShell-Head modules in each major turtle clade, modules pictures in turtle silhouette. Asterisk (*) indicates significant integration. A) Chelidae, B) Pelomedusoides, C) Trionychidae, D) Chelydroidea, E) Emydidae, F) Testudinidae, and G) Geoemydidae. Colors correspond to ecological classification (Table S1)

Disparity

Across all turtles, the lowest disparity was observed in the Limb and Head modules, and the greatest disparity was observed in the Shell module and Whole-Body (Fig. 3B; Table S8). Limb disparities in Trionychidae and Testudinidae were overlapping and higher than all other clades (Fig. 3B, Table S8), whereas highest disparities in the Shell module were observed in Trionychidae, followed by Chelydroidea and Testudinidae (Fig. 3B; Table S8). Overlapping values of Shell disparity were observed in Chelidae, Pelomedusoides, Emydidae, and Geoemydidae, and all clades had similar Head disparity (Fig. 3B; Table S8). When considering disparity of all measurements (Whole-Body), Trionychidae was the most disparate, followed by Chelydroidea, Testudinidae, and Chelidae. The least disparate clades were Emydidae, Geoemydidae, and Pelomedusoides (Fig. 3B; Table S8). Data were slightly different when using the Pereira-only data, with Chelidae and Testudinidae being more disparate than other clades (Table S9).

Modularity, integration, and disparity across aquatic, semi-aquatic, and terrestrial turtles

Modularity hypothesis two received significant support for each ecological category, and it also exhibited the lowest effect size Z_{CR} (Table 3). Significant integration was found between most modules in aquatic and terrestrial turtles, but significant integration was only observed between Limb-Shell modules in semi-aquatic turtles (Table 4). Aquatic and terrestrial turtles are more morphological disparate in each module and whole-body characteristics compared to terrestrial turtles (Table 5).

Table 3. Covariation ratio (CR), modular signal (Z_{CR}), and P -adj for the three modularity across each habitat use category.

Hypothesis	Habitat Category	CR	Z_{CR}	P -adj
1	Aquatic	0.5973	-1.4677	0.063
	Semi-Aquatic	0.6703	-1.5523	0.003
	Terrestrial	0.6984	-1.8584	0.021
2	Aquatic	0.4821	-2.135	0.003
	Semi-Aquatic	0.732	-1.8643	0.003
	Terrestrial	0.7092	-2.326	0.006
3	Aquatic	0.83	-1.1775	0.015
	Semi-Aquatic	1.0266	-0.7152	0.156
	Terrestrial	0.9128	-0.8072	0.024

Table 4. Integration tests via two block partial least squares test for each habitat category.

Habitat Category	Module comparison	r - PLS	Effect Size	P - adj
Aquatic	Limbs-Shell	0.291	2.3739	0.042
Aquatic	Limbs-Head	0.252	2.3449	0.066
Aquatic	Shell-Head	0.54	9.1088	0.003
Aquatic	LimbShell-Head	0.546	7.576	0.003
Semi-Aquatic	Limbs-Shell	0.596	2.2677	0.063
Semi-Aquatic	Limbs-Head	0.438	1.2672	0.342
Semi-Aquatic	Shell-Head	0.624	2.9711	0.015
Semi-Aquatic	LimbShell-Head	0.603	2.3679	0.066
Terrestrial	Limbs-Shell	0.505	3.0447	0.012
Terrestrial	Limbs-Head	0.419	2.7478	0.024
Terrestrial	Shell-Head	0.419	2.7478	0.024
Terrestrial	LimbShell-Head	0.463	2.428	0.054

Table 5. Results from disparity tests of Limbs, Shell, Head modules, and the Whole-Body across habitat categories.

Habitat category	Limbs	Shell	Head	Whole-Body
Aquatic	0.11	0.31	0.11	0.54
Semi-Aquatic	0.04	0.09	0.06	0.19
Terrestrial	0.24	0.24	0.06	0.54

DISCUSSION

Patterns of variation and covariation in organismal traits are central to understanding the limits within which organisms vary and how trait relationships might influence their evolution. Such an

understanding of variation and covariation is key, considering that variation is not unlimited, and is inherently biased by development, physics, and physiology (Klingenberg, 2008). Turtles are an ideal group to study such questions because they are ecologically diverse, but not as speciose as beetles (~350,000 species) or birds (~10,000 species). This makes it possible to take direct measurements on most of the 356 extant species and to provide insight on broader evolutionary questions. Our main objectives in this study were to 1) identify the major axes of variation across turtles, and 2) test the hypothesis that increased modularity and weak integration between traits is observed in the most ecologically and diverse turtle clades.

The two major axes of morphological variation observed in the pPCA of the full dataset demonstrated that head variables (PC1), followed by limb variables (PC2) explain most of the morphological variation across the diversity of turtles. One explanation of this finding is that head variables and limb variables are the primary morphological variables that define the continuum of diet and habitats observed across turtles. This finding was partially corroborated by a recent study that compared morphology, performance, and ecology of three sympatric turtle species. Butterfield *et al.* 2020 found that the amount of interdigital webbing and shell height were correlated to maximum swimming speed and habitat. The most aquatic species, *Kinosternon chimalhuaca*, had the most interdigital webbing, compressed shell, and faster maximum swimming speed, the terrestrial species *Rhinoclemmys rubida* was opposite in these measurements, and the semi-aquatic *Rhinoclemmys pulcherrima* was intermediate (Butterfield *et al.*, 2020). Additional studies on turtle limbs (Joyce & Gauthier, 2004), shell (Stayton *et al.*, 2018), and head (Foth *et al.*, 2017) find similar relationships between morphology and ecology, providing additional support that the variation in PC1 and PC2 reflects adaptation to habitat and diet.

Organisms are made up of quasi-autonomous parts or modules, and how these modules arise in evolution and how modularity facilitates or directs evolution are major issues in evolutionary biology (Klingenberg, 2008). Results from our modularity tests show that hypothesis two in which we considered Limb, Shell, and Head modules, followed by hypothesis one where we considered Head and LimbShell modules, represented the best explanation of modular organization across turtles, turtle clades, and habitat categories in our data (Fig 2, 3A). This modular organization suggests that the covariation within the limbs, shell, and head of turtles is stronger than the covariation between these traits, implying that limbs, shell, and head of turtles are modular units that vary, and can evolve semi-independently from each other (e.g. Felice & Goswami, 2018). Studies of covariation among traits in developing embryos are needed to link the patterns we observe using linear measurements.

Strong integration between traits provides evidence of potential biases to variation, and insight on the range of trait combinations that are favored by selection. For example, strong craniofacial integration between the beak and cranium in Darwin's finches and Hawaiian honeycreepers is suggested to bias the direction of morphological variation in a specific direction, leading to more extreme morphologies instead of more variable morphologies (Navalón *et al.*, 2020). However, the presence of this directional bias in Darwin's finches and Hawaiian honeycreepers also suggests that natural selection could favor a specific range of trait combinations (Navalón *et al.*, 2020). In the full dataset, we find that there are significant levels of integration between most module comparisons, suggesting that there are strong levels of integration between the major regions of the turtle body plan across the diversity of turtles. However, integration patterns of clades and habitat categories are more complex, and our results

seem to suggest that integration between the major regions of the turtle body plan has played a role in shaping the ecological diversity within turtle clades and ecological categories.

Significant integration between the Head, Limb, Shell, and ShellLimb modules was more common in turtle clades that are comprised of primarily aquatic (Chelydroidea, Trionychidae, and Chelidae) or terrestrial species (Testudinidae; Table 2). Non-significant integration was more frequent between modules in clades that have aquatic, semi-aquatic, and terrestrial species (Emydidae, Geoemydidae, Pelomedusoides; Fig. 4). We also found significant integration between most modules when comparing aquatic and terrestrial species, and non-significant integration between modules in semi-aquatic species (Table 4). These results suggest that integration (or lack thereof) between the head and limb/shell characteristics could have acted as a source of bias to variation in development. However, the variation that we don't observe in this study could also be accessible in development. For example, it is possible terrestrial traits are developmentally accessible in highly integration clades like aquatic Chelydroidea, but not observable because they were not selected for.

Trait decoupling, in which there is low integration between two traits, has been predicted to increase morphological diversity (e.g. Frédérich et al. 2014). Our data provide partial evidence for this, suggesting that trait decoupling in the ecologically diverse clades, and in semi-aquatic turtles between the Head and ShellLimb modules could have been a principal driver of their ecological diversity. This understanding of integration could also help solve questions in turtles, such how has sexual dimorphism in body size and head size evolved to extremes in the aquatic genus *Graptemys* (Lindeman, 2000). If traits were tightly correlated in *Graptemys*, then extreme morphological differences that exist in males and females might not be developmentally accessible. Similar patterns were also observed in the Sierra box turtle (*Terrapene nelsoni*), in which males and females were not significantly different in relative shell or limb characteristics, but males have significantly longer relative head length than females (Butterfield *et al.*, in press). Such variation in the Sierra box turtle may not be possible if there was tight integration between the head, shell, and limb variables (Butterfield *et al.*, in press). We do not consider sexual size dimorphism in this study because we did not have an even sample size of males and female for each species, but we believe this would be one avenue of investigation to understand how trait decoupling manifests in populations. Another promising avenue of research is understanding if there are inherent differences in development that allow semi-aquatic turtles to have different patterns of integration than aquatic or terrestrial turtles.

The highest levels of disparity when considering whole-body characteristics were observed in Trionychidae, Chelydroidea, Testudinidae, and Chelidae (Fig. 3B). Species belonging to these clades are categorized as aquatic or terrestrial, and we found strong integration between modules in these clades. This pattern, tight integration leading to extreme morphologies (i.e. higher disparity), supports previous work that has shown or predicted that integration leads to more extreme morphologies (Goswami et al. 2014). Navalón et al. 2020 found that craniofacial integration is correlated to extreme morphologies in Darwin's finches and Hawaiian honeycreepers, and that this integration has contributed to the ecological diversification of these clades. In contrast, our data do not suggest that integration has led to ecological diversification in turtles, instead suggesting that clades with the most integration are restricted to aquatic or terrestrial habitats. Geoemydidae, Emydidae, and Pelomedusoides which are more ecologically diverse, exhibit less integration between modules (Table 2). This finding provides an additional layer of evidence that integration, or the lack thereof (i.e. trait

decoupling), could have played an important role in the diversification of turtles to aquatic, semi-aquatic, and terrestrial habitats.

A deeper understanding of development and function are needed to understand if the patterns we observe are a result of developmental biases or selection. One strength of some studies that investigate patterns of modularity and integration, is that they are able to link their traits under study directly to development (e.g. Parsons et al. 2012). In turtles, developmental studies have documented anatomical and heterochronic differences throughout development (Werneburg et al. 2009; Werneburg 2013), but covariational patterns between limbs, shell, and head have not been explored. If patterns observed in this study correspond to turtle development, then it would be expected that similar patterns in modularity and integration we find would be observable throughout development. If such patterns are not present, then it would provide evidence that natural selection is favoring the patterns of modularity and integration we observe in this study and not developmental bias. For example, Sherratt *et al.*, 2017 studied covariation patterns in larvae and adult frogs, showing ecologically different frog species are similar to each other as larvae, but not adults, suggesting ecological differences in adults have arisen through natural selection and not developmental bias. Future studies of covariational patterns of turtles throughout development are needed to test if development or natural selection are the major drivers of morphological diversity in turtles.

Previous studies provide evidence that variation in the limbs, shell, and head of turtles correspond to habitat and diet. For example, head morphology has been shown to be largely influenced by habitat and diet, with aquatic and carnivorous turtles having larger and differently shaped skulls than terrestrial or herbivorous turtles (Claude *et al.*, 2004). Shells of aquatic turtles have been found to have more flat-hydrodynamic shells compared to terrestrial turtles (Claude *et al.*, 2003; Rivera, 2008; Stayton, 2011; McLaughlin & Stayton, 2016; Stayton *et al.*, 2018). Turtle limbs have been shown to be correlated to habitat, with aquatic turtles having longer hands and shorter antebrachium than terrestrial turtles (Joyce & Gauthier, 2004). Additional studies have linked morphology to performance, showing that morphology is correlated to bite force (Herrel *et al.*, 2001), swimming ability (Mayerl et al. 2019; Rivera and Stayton 2011), self-righting ability (Domokos & Várkonyi, 2008; Ana *et al.*, 2015), and predatory avoidance (Vega & Stayton, 2011; Dosik & Stayton, 2016; Polly *et al.*, 2016). In aggregate, these studies provide evidence that morphology is linked to performance, and ecology. However, it remains to be seen how the variation and covariation patterns we find in this study influence performance and ecology of turtles in the wild.

Conclusion

Using basic linear measurements taken on the limbs, shell, and head of museum preserved specimens, as well as wild caught turtles, we document the major axes of variation across turtles, turtle clades, and ecologies. We also document patterns of modularity, integration, and disparity across all turtles, clades, and ecological categories. Together, these data suggest two important patterns. The first, is that the major axis of morphological variation across turtles is defined by variation in limb, shell, and head morphology, such that aquatic turtles have tend to have larger hands, less domed shells, and heads that are more diverse in size. Second, patterns of integration suggest that trait decoupling between head and limb/shell characteristics may have played an important role in the ecological diversification of diverse turtle clades, and semi-aquatic turtles. These data contribute to the broader understanding of how covariational patterns influence

evolutionary diversification, supporting other notion that the nature of covariation between traits can act as a source of evolutionary innovation, or a source of bias (Felice *et al.*, 2018).

DATA AVAILABILITY STATEMENT

The data used to produce the results in this study can be accessed at Dryad: [here if accepted](#).

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DISCUSIÓN GENERAL

Los estudios ecomorfológicos brindan información sobre cómo la variación morfológica se relaciona con la ecología, y los estudios comparativos que utilizan especímenes de museo brindan oportunidades para entender cómo los organismos se diversifican. En esta tesis, los objetivos fueron estudiar diferentes especies de tortugas en campo para medir la relación entre morfología y ecología, y medir ejemplares de tortugas en museos para determinar cómo los patrones de covariación han influido en la diversificación de los clados principales de tortugas. Para esto, se comparó la morfología, el desempeño y la ecología de las tortugas en campo, y se colectaron datos de especímenes de museo que representan el 69% de la diversidad de tortugas. Estos datos permitieron documentar correlaciones significativas entre la morfología, el desempeño y la ecología, primero para tres especies que habitan una selva baja en Chamela, Jalisco, México (Capítulo 1), posteriormente para 14 especies distribuidas en diversos estados en México (Capítulo 2). También se encontró que los clados de tortugas más diversos ecológicamente (Emydidae, Geoemydidae, y Pelomedusoides), que incluyen a varias especies acuáticas, semi-acuáticas y terrestres, presentaron una covariación débil entre los caracteres de la cabeza y las variables de las extremidades/carapacho, lo cual sugiere que el desacoplamiento de rasgos pudo haber jugado un papel importante en la diversificación ecológica de dichas tortugas (Capítulo 3).

La relación entre morfología, desempeño y ecología ha sido investigada en un gran número de taxones para entender cómo los organismos interactúan con su ambiente y cómo la morfología se correlaciona con las variables ecológicas (Arnold, 1983; Losos, 1990; Wainwright, 1991; Moen, 2019). Se comparó la morfología, el desempeño y la ecología de las tortugas en vida silvestre y se proporcionó evidencia empírica de que la morfología de las tortugas se

correlaciona con la ecología. Quizá el hallazgo más destacado es que la morfología de las extremidades y del caparazón se correlacionan con la velocidad de natación y el porcentaje de tiempo que una especie tortuga es encontrada sobre tierra. Por ejemplo, las tortugas con patas delanteras más largas, con mayor área de membrana interdigital, y con caparazones más planos nadan más rápido y se encuentran en el agua un mayor porcentaje del tiempo. Esto se observó primeramente en Chamela, Jalisco, donde la especie terrestre Tortuga Casco Rojo (*Rhinoclemmys rubida perixantha*) tiene las patas delanteras más pequeñas y menos membrana interdigital, es la que nada más lento y se encuentra en la cima de colinas del bosque tropical caducifolio; la más acuática Tortuga de Pantano Jalisciense (*Kinosternon chimalhuaca*) tiene las patas delanteras más grandes, más membrana interdigital, nada más rápido y sólo se encuentra en agua; y la semi-terrestre Tortuga de Monte Occidental (*Rhinoclemmys pulcherrima rogerbarbouri*) es intermedia a las otras dos especies (Butterfield *et al.*, 2020). También se observó esto en el Capítulo 2, donde se identificaron variables específicas que correlacionan la velocidad de natación con el tiempo que pasan sobre tierra. Por ejemplo, el tamaño de la pata delantera, la cantidad de la membrana interdigital, la altura del caparazón y el ancho del plastrón se correlacionaron significativamente con la velocidad de natación y el porcentaje de tiempo observadas sobre tierra. Estos datos sugieren que las especies de tortugas existen a lo largo de un continuo de hábitats acuáticos a terrestres que está caracterizado por la variación en la morfología de las extremidades y del carapacho.

También documentamos correlaciones entre la morfología de la cabeza, el desempeño de la mordida y la dieta. De manera similar a estudios previos que han documentado correlaciones entre la morfología de la cabeza y la fuerza de mordida, en este trabajo se encontraron correlaciones significativas entre el largo, alto y ancho de la cabeza y la fuerza de mordida.

También se encontró una correlación marginalmente significativa entre la morfología de la cabeza y el $\delta^{13}\text{C}$. De cualquier forma, los estudios previos que han investigado la morfología de la cabeza y la dieta no han encontrado una relación clara entre la dieta y la morfología (Herrel *et al.*, 2002; Foth *et al.*, 2017), lo cual sugiere que la relación entre la morfología de la cabeza y la dieta podría ser más compleja que una simple correlación entre la cabeza y el $\delta^{13}\text{C}$. De hecho, un estudio que comparó la morfología de la cabeza, la dieta y el hábitat usando especímenes de museo encontró correlaciones más significativas entre la morfología de la cabeza y el hábitat que con la dieta (Claude *et al.*, 2004). Tomados en conjunto, estos antecedentes sugieren una hipótesis: el hábitat de las tortugas podría presentar un sesgo sobre la morfología de su cabeza y su dieta. Por ejemplo, la incapacidad de las tortugas de desplazarse a altas velocidades sobre el terreno para buscar comida limita severamente el tipo de recursos que pueden consumir, lo cual podría representar un sesgo en la variación morfológica que puede evolucionar en tortugas (Olson, 2012; Jablonski, 2020). La gravedad también podría representar un sesgo en la variación morfológica que podría evolucionar porque la gravedad impone una demanda física sobre la locomoción de la tortuga, y es poco probable que el desarrollo o la selección pudieran favorecer a las especies terrestres con una cabeza enorme, como aquellas observadas en las tortugas acuáticas (p. ej. Tortuga caimán, *Macrochelys temminckii*). Para entender mejor cómo en dichas instancias en que el desarrollo o la selección pudieran influir sobre la evolución de la morfología, se usaron las medidas de especímenes de museo colectadas en el Capítulo 3 para describir los patrones de variación y covariación a través de la diversidad de tortugas.

Se tomaron medidas lineales en especímenes de museos de historia natural que representan el 69% de las especies actuales de tortugas terrestres y dulceacuícolas. Dichos datos permitieron evaluar la variación y covariación morfológica en todas las tortugas, siete clados

principales de tortugas y tortugas con diferentes caracteres ecológicos. Se encontró que la variación en las extremidades, el caparazón y la cabeza de las tortugas está caracterizada por las mismas variables morfológicas que encontramos correlacionadas con el desempeño y la ecología en los Capítulos 1 y 2. También documentamos patrones significativos en la covariación a lo largo de todas las especies de tortugas, mostrando que las variables de las extremidades, el caparazón y la cabeza covarían entre ellos más que con otras variables. Este patrón de modularidad sugiere que las extremidades, el caparazón y la cabeza han evolucionado de manera semi-independiente uno respecto a otro.

Quizá el hallazgo más importante de los datos del museo fue que los clados con mayor diversidad ecológica (Emydidae, Geoemydidae y Pelomedusoides) son los clados en los que no existe una integración significativa entre las variables de la cabeza y las extremidades/carapacho. En contraste, los clados analizados de tortugas estrictamente acuáticas (Trionychidae, Chelydroidea y Chelidae) y el clado de tortugas terrestres (Testudinidae), tuvieron fuerte integración entre las variables de la cabeza y las extremidades/carapacho. Esto sugiere que el desacoplamiento de rasgos, en el cual hay poca covariación entre rasgos, podría haber sido un impulsor de la diversidad ecológica en las tortugas. Frédéricich *et al.* (2014) encontró que en los peces damisela el desacoplamiento de rasgos entre dos regiones de la mandíbula ha promovido la diversificación ecológica. Otros estudios no han encontrado relación entre la integración morfológica y la diversidad ecológica (Bardua *et al.*, 2019). También se han encontrado patrones opuestos: una integración fuerte entre dos rasgos promueve la diversificación ecológica (Hu *et al.*, 2016; Evans *et al.*, 2021). Por lo tanto, es plausible que la influencia de la integración en la diversificación ecológica pudiera depender del clado bajo estudio, y que pudiera haber una amplia variación en cómo la integración influye la diversificación ecológica.

En este trabajo encontramos que existe una integración significativa en las especies de tortugas que se clasificaron como acuáticas y terrestres, pero no en las especies de tortugas semi-acuáticas. Este resultado sugiere que los patrones de covariación que observamos en la integración podrían ser el resultado de limitaciones físicas impuestas a las tortugas por su morfología torpe y por la gravedad. Tales limitaciones físicas, como las ejercidas por la gravedad, han sido identificadas como factores que pueden sesgar la variación morfológica durante la evolución (Klingenberg, 2008; Olson, 2012; Jablonski, 2020). Los patrones observados en este estudio podrían reflejar limitaciones físicas que impiden a las tortugas caminar sobre tierra y no limitaciones del desarrollo ni fisiológicos. Por ejemplo, las tortugas del desierto (familia Testudinidae) tienden a tener cabezas más pequeñas que el resto de las especies. Es posible que la evolución de cabezas más grandes en Testudinidae esté accesible durante el desarrollo de los organismos, pero que la selección natural no las favorezca porque limitarían la capacidad locomotora de la tortuga. Trabajos futuros en este campo deberían indagar en cómo los posibles sesgos de la física, el desarrollo y la fisiología podrían influir en la adecuación y la diversidad ecológica en las tortugas.

CONCLUSIONES

A partir de datos de campo y de museo, esta tesis proporciona una explicación integral sobre la relación entre morfología, desempeño y ecología en las tortugas. Los datos de campo muestran que las tortugas existen a lo largo de un continuo de hábitats acuáticos a terrestres, el cual está caracterizado por su morfología (tamaño de mano, cantidad de membrana interdigital, largo de plastrón, alto de plastrón), desempeño de natación (velocidad de natación) y fuerza de mordida. Las tortugas que se encuentran en el agua con mayor frecuencia tienen manos más largas, más membrana interdigital, caparazones más planos, plastrones más cortos, y nadan más rápido.

Dado que utilizamos medidas continuas, como el porcentaje del tiempo que se les observa en tierra, nuestros resultados sugieren que las categorizaciones como “terrestre, “semi-terrestre”, o “acuática” podrían fallar en plasmar a toda la diversidad ecológica que se observa en las comunidades de tortugas. Más aún, las tortugas que tienen dietas más carnívoras tienden a tener cabezas más largas y fuerza de mordida más fuertes. No obstante, las correlaciones entre los isótopos estables y el tamaño de la cabeza no fueron significativas, por lo tanto, se necesitan más estudios para aclarar la relación entre morfología y dieta. Los datos de los especímenes de museo muestran que las variables de las extremidades, el caparazón y la cabeza caracterizan la diversidad de tortugas de una forma similar. Por ejemplo, las especies y los clados de tortugas acuáticas estuvieron caracterizados por patas delanteras más largas, caparazones más aplanados, y una mayor diversidad de tamaños de cabezas, mientras que las tortugas terrestres tuvieron las patas delanteras más cortas, caparazones más altos y tamaños de cabezas más pequeñas/menos diversas. Es a lo largo de este continuo, desde los estilos de vida altamente acuáticos hasta los terrestres, en el cual las tortugas han evolucionado. Por lo tanto, el análisis de covariación mostró que la falta de integración y modularidad entre la cabeza y las extremidades/caparazón se observa en las familias de tortugas más diversas (Emydidae, Geoemydidae y Pelomedusoides). Estos patrones de covariación sugieren que la falta de integración o desacoplamiento de rasgos ha promovido la diversificación ecológica de las tortugas. El trabajo futuro en este campo debe enfocarse en las poblaciones silvestres para entender cuáles rasgos, o cuáles patrones de covariación de rasgos, pudieran influir sobre la adecuación individual para entender a que nivel biológico se manifiestan los patrones que observamos en la diversidad de las tortugas.

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ANEXO 1. A FIRST LOOK INTO THE NATURAL HISTORY OF THE SIERRA BOX TURTLE (*TERRAPENE NELSONI KLAUBERI*) IN SOUTHEAST SONORA, MEXICO.

Butterfield, T., F. García-Caballero, A. Domínguez-Pompa, and R. Macip-Ríos. 2021. A First Look into the Natural History of the Sierra Box Turtle (*Terrapene nelsoni klauberi*) in Southeast Sonora, Mexico. *Chelonian Conservation and Biology* 20(1): 82-90.

A First Look into the Natural History of the Sierra Box Turtle (*Terrapene nelsoni klauberi*) in Southeast Sonora, Mexico

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ABSTRACT. – The Sierra Box Turtle, *Terrapene nelsoni*, comprises 2 recognized subspecies that are distributed across the Sierra Madre Occidental mountain chain in western Mexico and little is known about their natural history. We present new information on the natural history of *Terrapene nelsoni klauberi*. Our study area was the Monte Mojino reserve located within the larger Sierra de Alamos–Río Cuchujaqui federal protected area in southeastern Sonora. We located 49 individuals in 3 different vegetation types: 4 were found at 600 m above sea level (masl) in the tropical dry forest, 4 at 1000 masl in grassy oak (*Quercus* spp.) savannah, and 41 at 1300 masl in pine (*Pinus* spp.)–oak forest. Seven turtles were monitored using radiotelemetry and have the average home range size of 1.7 ha. The areas we sampled appear to consist of mainly adults and the sex ratio is female-biased (1♂:1.5♀). Cloacal temperatures of active turtles and their immediate surroundings suggest that *T. n. klauberi* actively maintain their body temperature above the surrounding temperatures. Males also have higher average body temperatures than females (29.58°C ± 2.26°C vs. 27.35°C ± 2.71°C). We found no significant patterns of sexual dimorphism in carapace length (i.e., sexual size dimorphism) but did find sexual dimorphism in head size. We also found that 44% of males and 22% of females had severe damage to their marginal scutes, possibly from a local predator or from enduring bites over time from other turtles. Lastly, we observed *T. n. klauberi* foraging on beetles, mushrooms, grass, and wildflowers. These findings provide a first insight into the natural history of *T. n. klauberi* and how this species is distributed across different elevations and vegetation types. This information provides a starting point to assess the conservation status of this species. However, this species remains unstudied throughout the rest of its geographic range.

KEY WORDS. – Sierra box turtle; *Terrapene nelsoni*; natural history; Sonora

North American box turtles belonging to the genus *Terrapene* have been icons of turtle research and conservation for more than a century (Surface 1908). These small terrestrial and omnivorous turtles are distributed in the United States and Mexico and occur in habitats ranging from moist humid tropical forests, deserts, to hardwood forests (Dodd 2002). Unfortunately, all extant species face serious conservation threats, and these threats are as diverse as the habitats they occupy. Population bottlenecks, illegal trafficking, and climate change are just a few (Kuo and Janzen 2004; Gong et al. 2009), and have elevated the conservation status of 3 species as vulnerable (*Terrapene carolina*; van Dijk 2011), near threatened (*Terrapene ornata*; van Dijk and Hammerson 2011), and endangered (*Terrapene coahuila*; van Dijk et al. 2007) by the International Union for Conservation of Nature

(IUCN) Red List of Threatened Species. The fourth species, *Terrapene nelsoni*, however, remains largely unknown to science and considered data deficient by IUCN (Tortoise and Freshwater Specialist Group 1966).

Terrapene nelsoni, or the Sierra box turtle, is 1 of only 3 *Terrapene* species that are distributed in Mexico and has 2 recognized subspecies, *T. n. nelsoni* and *T. n. klauberi* (Shaw 1952). These subspecies are distributed throughout the Sierra Madre Occidental mountain chain, with *T. n. klauberi* distributed in Sonora, southwestern Chihuahua, and Sinaloa, and *T. n. nelsoni* in Sinaloa, Nayarit, and Jalisco (Turtle Taxonomy Working Group 2017). Throughout this range, *T. nelsoni* has been documented at elevations ranging from 400 m above sea level (masl) in tropical dry forest to > 1500 m in pine–oak (*Pinus* spp.–*Quercus* spp.) forest (Legler and Vogt 2013). Beyond the

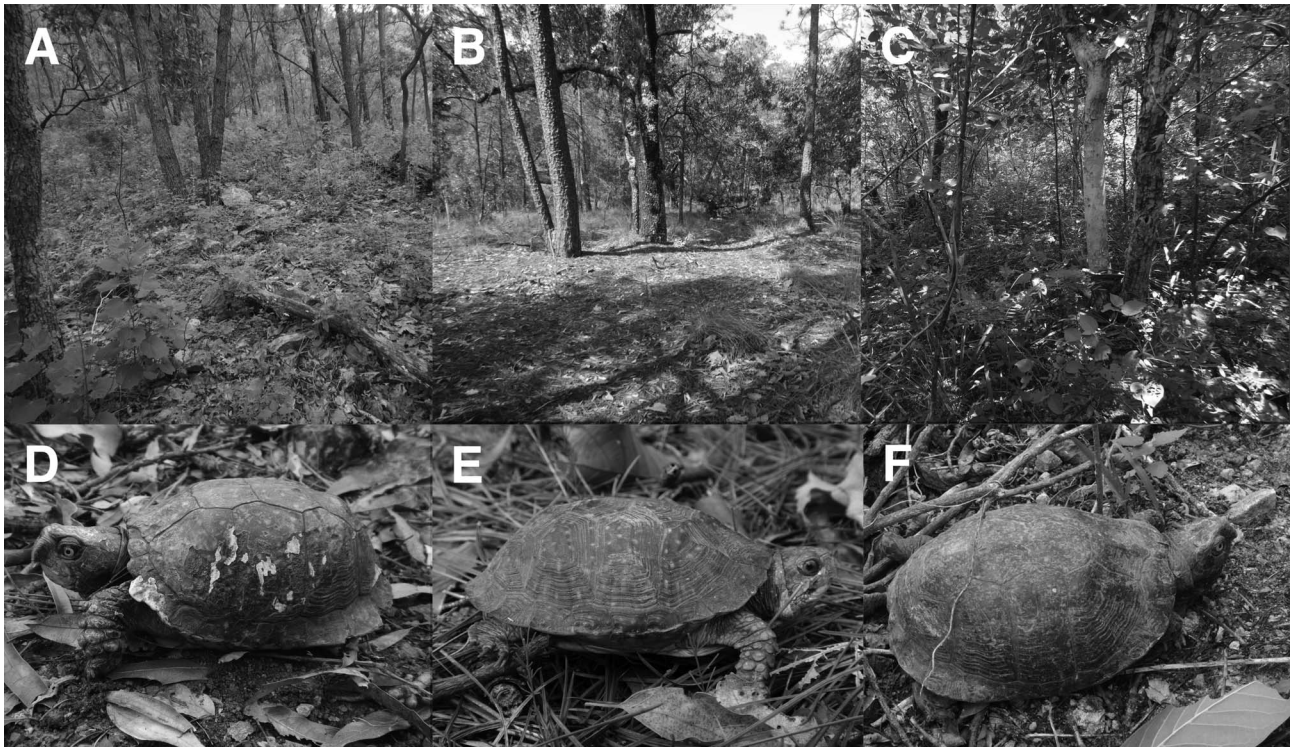


Figure 1. Vegetation types within Monte Mojino reserve that we surveyed and an individual *Terrapene nelsoni klauberi* that we captured in each site. (A and D) Pine–oak forest, (B and E) grassy oak savannah, and (C and F) tropical dry forest. Adult male (D and F) and juvenile (E) pictured. Photos by Taggert Butterfield.

handful of locality information that exist on *T. nelsoni*, little is known about their natural history.

Information on the natural history of *T. nelsoni* is needed because deforestation, climate change, and illegal trafficking are serious threats throughout the Sierra Madre Occidental mountain chain. For example, tropical dry forests in Mexico, which characterizes the lower elevations of the Sierra Madre Occidental, lost > 71% of its original vegetation cover by year 2010 and < 2% of the remaining TDF falls within protected areas (Portillo-Quintero and Sánchez-Azofeifa 2010). Future climate models predict that the forests of Sierra Madre Occidental will be significantly reduced by year 2070 (Prieto-Torres et al. 2016). In addition, illegally collecting and trading freshwater and terrestrial turtles is not heavily enforced in Mexico, and despite all Mexican box turtles being classified as appendix II CITIES (Macip-Ríos et al. 2015), permits to collect, reproduce, and legally sell turtles are easier to obtain for species like *T. nelsoni* because little is known about their natural history. Thus, an immediate priority is to understand the natural history of *T. nelsoni* and to assess their conservation status.

Our goal was to provide a first look into the natural history of *T. n. klauberi* in southeastern Sonora. To do this, we conducted visual encounter surveys for *T. n. klauberi* and tracked select individuals with radiotelemetry periodically between July 2018 and April 2020 in the Monte Mojino reserve in southeastern Sonora. We present data on the occurrence of turtles in different vegetation types,

home range size, demography, thermal biology, sexual dimorphism, and provide a preliminary assessment of their diet.

METHODS

Study Site. — This study was conducted in the Monte Mojino reserve, located within the Sierra de Alamos–Río Cuchujaqui federal protected area in southeastern Sonora, Mexico. This reserve and protected area represent a cross-section of the Sierra Madre Occidental mountain chain that runs from northern Mexico in the states of Sonora down south to Nayarit. Monte Mojino is representative of this mountain chain because it has the same elevational gradient that characterizes the western slope of the entire mountain chain, which starts at 300 masl in tropical dry forest vegetation, and gradually reaches to about 1300 masl where pine–oak forest is the dominant vegetation. *Terrapene nelsoni* has been observed along this gradient at elevations ranging from 400 to 1600 masl (Legler and Vogt 2013), so we sampled turtles in 3 vegetation types. Two sites were in the tropical dry forest at 300 masl (Figs. 1C and 2), another site was at 1000 masl in a grassy oak savannah (Figs. 1B and 2), and the third site was at 1300 masl in pine–oak forest (Figs. 1A and 2). These field sites have a prolonged dry season that can last 6–9 mo, followed by a short wet season (June–September; González-Elizondo et al. 2012). All field sites experience between 300 and 1200 mm of precipitation per year (González-Elizondo et al. 2012). Average monthly

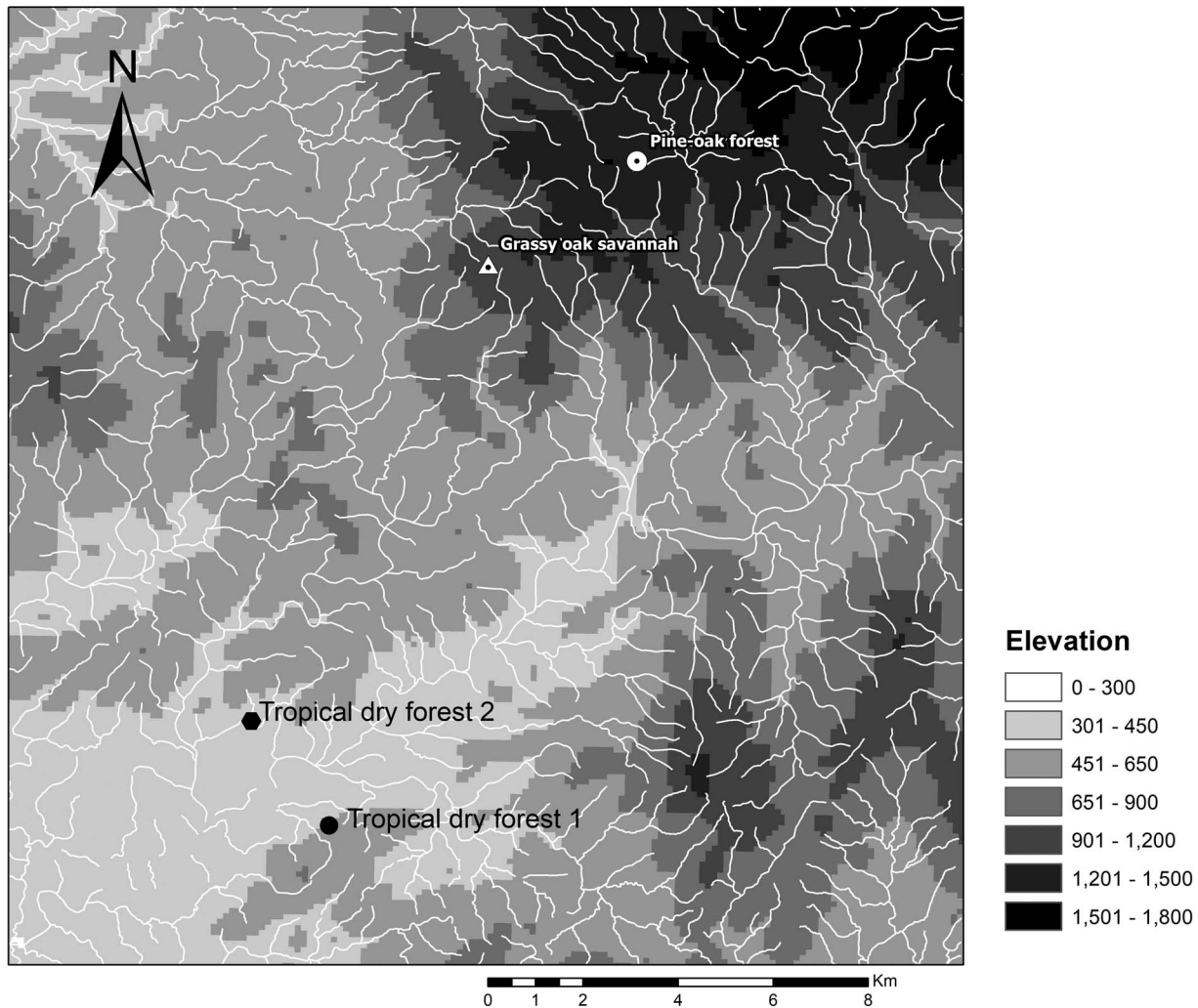


Figure 2. Map depicting the spatial extent of the 4 different field sites where we sampled turtles. Elevation scale corresponds to meters above sea level (masl). Tropical dry forest sites 1 and 2 were located at about 300 masl, grassy oak savannah was located at 1000 masl, and pine-oak forest was located at 1300 masl.

temperatures in the tropical dry forest averages 23.9°C–35.6°C (González-Elizondo et al. 2012). Monthly average temperatures of grassy oak savannah and pine-oak forest are not available, but temperatures recorded at the field station in pine-oak forest in 2017 ranged between 2.1°C and 32.7°C and averaged 16.6°C (L. Lozano, *pers. comm.*, September 2019). Understory vegetation in the tropical dry and pine-oak forests varies from open to very dense with many species of shrub, vines, and cacti. In grassy oak savannah, grass is the dominant understory vegetation with intermittent patches of agave plants. There are very few canopy gaps in tropical dry and pine-oak forest, but canopy gaps, or small grassy meadows, are a normal feature of grassy oak savannah.

Sampling Protocol and Sampling Periods. — We located turtles using visual encounter surveys, which consist of walking in a loose grid fashion with 2–5 people walking about 3 m apart from each other through potential turtle habitat. The total area surveyed encompassed an estimated area of 23 ha in the pine-oak forest, 17 ha in

grassy oak savannah, and 42 ha in tropical dry forest. The surveys in tropical dry forest were split between two localities that were 2.7 km apart. These surveys were conducted during the short rainy season that occurs from June to October and weather data were not recorded during surveys. The start and end times, number of people, and number of turtles encountered were documented for each survey. The sum of search hours for each survey was multiplied by the number of people to calculate total person-hours per survey. The sum of these person-hours per survey for each vegetation type was divided by the number of turtles found in each vegetation type to calculate the estimated number of person-hours needed to find one individual turtle (hrs/individual). We sampled each sampling site at irregular intervals between July and August 2018. Then, starting in May 2019 we began conducting searches in tropical dry forest at least twice per month to locate more individuals in lower elevations and these surveys continued through March 2020. Lastly, in August 2019, surveys were conducted over the course of 3

wks in grassy oak savannah and pine–oak vegetation types.

Home Range Size and Microhabitat Use. — To estimate home range and document microhabitat usage, we radio-fitted a subset of turtles found during surveys. Turtles were equipped with 15-g Holohil RI-2B radios (Holohil Systems, Ltd, ON, Canada) on their posterior costal scutes using epoxy putty, and then monitored with a Telonics TR-4 receiver and rubber ducky antenna (Telonics, Inc, AZ). Each time we located a turtle in the field, we recorded date, time, Global Positioning System (GPS) location, activity (e.g., active walking or inactive), and microhabitat when inactive. If the turtle was active, we did not record any microhabitat data. We also did not record macrohabitat data when tracking turtles. All GPS locations are omitted to protect the species from illegal poaching.

We tracked radio-fitted turtles between August 2018 and March 2020. We tracked these turtles sporadically because of our inability to arrive at field sites. Thus, our data can only be used to provide a baseline of home range for this species. In August 2018, we put a radio on 1 male (no. 5) in the tropical dry forest, 2 males (no. 2 and 8) in pine–oak forest, and 1 female (no. 3) in pine–oak forest. These first individuals were monitored over the course of 3 wks during August 2018, 3 d in April 2019, and 1 wk in August 2019. Three additional individuals (no. 27, 28, and 29) were located in the tropical dry forest in May 2019, equipped with radios, and then tracked until April 2020. Individual no. 29 was only tracked through July 2019 until his radiotransmitter fell off.

Home range was estimated using the 100% minimum convex polygon (MCP) technique and was executed using the ‘mcp’ function in the ‘adehabitatHR’ package in Program R (Calenge 2006). Home range size between vegetation type and sex was tested for significance using a Mann-Whitney U-test (or Wilcoxon ranked sum test) because these data were not normally distributed. This was executed using the ‘wilcox.test’ function in R Statistical Software version 3.6.1 (R Core Team 2019).

Body Temperature. — To gain insight into the thermoregulatory behavior of *T. n. klauberi*, we used a Schultheis “quickreading” reptile thermometer (Miller and Weber, Inc, NY) to take body temperatures of turtles and their immediate surroundings when encountered active in the field. Body temperatures (T_b) of turtles were taken with a quickreading thermometer by inserting it approximately 10 mm into the cloaca of the turtle for about 15 sec and noting the temperature. After taking T_b , 3 environmental temperatures were taken in a similar fashion at the location where the turtle was found. These temperatures were taken 1.5 cm below the surface of the soil (T_s), 10 cm above the surface of the soil (T_{10}), and 150 cm above the surface of the soil (T_{150}).

We used an independent sample Student *t*-test with the ‘t.test’ function in Program R to determine whether there are significant differences in T_b , T_s , T_{10} , and T_{150}

between males and females. The assumption of normality of each variable was assessed using the Shapiro-Wilks test with the ‘shapiro.test’ function in R, and the assumption of homoscedasticity was assessed visually using boxplots. If these assumptions were not met, then a Mann-Whitney U-test was used as a nonparametric alternative. Furthermore, to determine if turtles regulate their body temperatures or conform to ambient temperatures, we used paired Student *t*-tests with the ‘t-test’ function in R and test differences between $T_b - T_s$, $T_b - T_{10}$, and $T_b - T_{150}$ without considering sex. The assumptions of normality and homoscedasticity of these paired *t*-tests were also assessed using the Shapiro-Wilk test and visually inspecting boxplots.

Sexual Dimorphism in Morphology. — All turtles that were encountered in the field were given a unique number using the notch system (Cagle 1939) and then measured. The standard morphological measurements taken for each individual include carapace length (CL, nuchal scute to cleavage between supracaudal scutes), plastron length (PL, intergular scute to cleavage between anal scutes), plastral lobe width (Lobe, length of seam that connects femoral and abdominal scutes), carapace width (CW, width between 5th and 6th marginal scutes), and shell height (SH, maximum vertical height from plastron to carapace). Limb and head characteristics were measured for select adult individuals that were temporarily brought to the laboratory for a different study in August 2018 and August 2019. Limb characteristics measured include: antebrachium length (Ante, apex of elbow to wrist crease), manus length (Hand, middle of the wrist crease to distal end of 3rd digit where skin meets the nail), crus length (Crus, apex of knee to apex of heel), pes length (Foot, apex of heel to distal end of 3rd digit where skin meets nail), and surface area of interdigital webbing of pes (RearWeb, area of webbing between 2nd and 3rd toe). Interdigital webbing area was calculated by multiplying the width and length of the interdigital webbing and dividing by two. Head characteristics measured include head width (HW, widest part of the skull), head length (HL, premaxilla to posterior edge of supraoccipital), and head height (HH, highest part of the skull at posterior end of jaw). All measurements were measured to the nearest 0.1 mm using dial calipers.

To provide insight into the sexual differences of this species, we compared linear measurements of the limbs, shell, and head of males and females. Not all morphological measurements were taken on all individuals observed because characteristics of the limbs and head were only taken on select individual that were temporarily brought to the laboratory for a different study in August 2018 and August 2019. For this, there are different sample sizes among the morphological variables that we investigated. We used Student *t*-tests to compare significant differences of each morphological variable between males and females using the ‘t-test’ function in R (R Core Team 2019). The assumption of normality was measured using the Shapiro-Wilk test and homoscedasticity was visually assessed

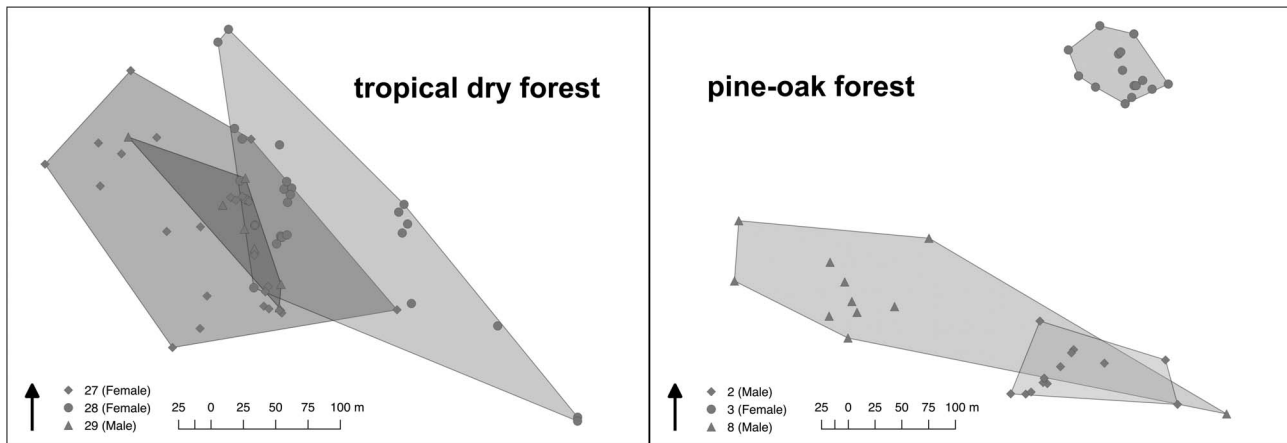


Figure 3. Maps depicting the relocations and 100% minimum convex polygon home ranges of 6 *Terrapene nelsoni klauberi* that we monitored with radiotransmitters in this study. Left panel depicts 3 turtles that were tracked in the tropical dry forest and right panel depicts 3 turtles we tracked in the pine–oak forest. One of the individuals (male no. 5) that we monitored is not depicted in these maps because his home range was 2.7 km from the individuals depicted in the left panel and could not fit onto the same map.

using boxplots. Most morphological variables are correlated to body size, so we also compared significant differences in each morphological variable after removing the effect of body size. To do this, we used the residuals calculated from regressing each morphological variable on CL. These linear regressions were calculated using the ‘lm’ function in R and are referred to as “size-free variables”. The assumptions (normality and homoscedasticity) of these linear models were assessed using the diagnostic plots for the ‘lm’ function in R before proceeding with Student *t*-test. The original nontransformed morphological variables are referred to as “original variables”. We also calculated the sexual size dimorphism index for *T. n. klauberi* using CL. Sexual size dimorphism index is a standard measure used to quantify deviations in sexual size dimorphism in a population, and is calculated by dividing the CL of the larger sex by the smaller sex and assigning a negative sign to these values when males are the larger sex (Lovich and Gibbons 1992). Lastly, many of the individuals we encountered in the field had severe damage to their marginal scutes, and to determine whether the frequency of damage is different between the sexes, we calculated the proportion of males and proportion of females that were observed with such damage.

Only descriptive statistics were used to assess abundance in different vegetation types, microhabitat use, and population structure. Whereas, direct observations were used to preliminarily assess diet. All statistical analyses were scrutinized to an α level = 0.05.

RESULTS

Sampling Effort in 3 Different Vegetation Types. — We conducted 89 surveys on 68 different days throughout the study period with groups of 1–5 people (\bar{x} = 2.6 people) and surveys lasting between 1 and 5 hrs (\bar{x} = 2.4 hrs). There are more surveys than days that we conducted surveys because when we were in the pine–oak vegetation type, we conducted 1–3 surveys/d. *Terrapene nelsoni*

klauberi are more common in pine–oak, then grassy oak savannah, followed by tropical dry forest vegetation type. The sum of our search effort totaled 144.2 hrs and 41 turtles in pine–oak, 29.75 hrs and 4 turtles in grassy oak savannah, and 397.1 hrs and 4 turtles in tropical dry forest. This indicates that it takes an estimated 3.5/person-hours (hrs/turtle) to find *T. n. klauberi* in pine–oak forest, 7.4 hrs/individual in grassy oak savannah, and 99.3 hrs/individual in tropical dry forest. An empty shell of an adult individual was found in the tropical dry forest in July 2018 and not included in this estimate.

Home Range Size. — Over the course of this study, 7 individuals that were located in tropical dry forest (4 males and 3 females) and pine–oak forest (2 males and 1 female) were equipped with radiotransmitters (Fig. 3). No individuals in grassy oak savannah were tracked because this vegetation type was not close enough to a field station where we could consistently go to this vegetation type. Home range sizes ranged from 0.41 to 3.09 ha (Table 1; Fig. 3). Average (\pm SD) home range size was 1.76 ± 1.2 ha for all individuals, 1.42 ± 1.4 ha in pine–oak forest, and 2.02 ± 1.2 ha in tropical dry forest. Average male and female home range size was 1.55 ± 1.2 and 2.05 ± 1.42 ha, respectively. There were no significant differences in home range size between vegetation type (Mann-Whitney U-test; $W = 5$, $p = 0.857$) or sex ($W = 6$, $p = 1.00$). Turtles that were observed inactive during telemetry surveys ($n = 40$) were found in rock shelters ($n = 14$), woody debris ($n = 10$), tree shelter ($n = 9$), leaf litter ($n = 6$), or soil burrows ($n = 1$). Microhabitat when turtles were active was not recorded.

Population Characteristics. — Our sample of *T. n. klauberi* in the Monte Mojino reserve is composed of mainly adults (Fig. 4). We captured 49 unique individuals—4 were juveniles, 27 were female, and 18 were male (1♂:1.5♀). Of the 49 turtles captured, only 5 adults were found in the tropical dry forest, 1 of these individuals was dead, and the rest had eroded growth rings suggesting that

Table 1. Summary of *Terrapene nelsoni klauberi* that we tracked with radiotransmitters between 2018 and 2019. Pine–oak = pine–oak forest; TDF = tropical deciduous forest; n = locations per individual; CL = carapace length (mm); MCP = 100% minimum convex polygon. No individuals were tracked in grassy oak savannah.

Vegetation	Individual	n	First date observed	Last date observed	Sex	CL	MCP (ha)
Pine–oak	2	17	25 Jul 18	15 Aug 19	Male	116.7	0.76
Pine–oak	3	17	25 Jul 18	15 Aug 19	Female	139.2	0.41
Pine–oak	8	11	2 Aug 18	11 Aug 19	Male	134.2	3.09
TDF	5	11	25 Aug 18	30 Aug 19	Male	132.8	1.91
TDF	27	28	24 Jan 19	3 Apr 20	Female	126.1	3.02
TDF	28	28	24 Jan 19	3 Apr 20	Female	146.0	2.72
TDF	29	7	2 Jan 19	13 Aug 29	Male	138.0	0.44

they were very old individuals (Fig. 1F). The remaining turtles were found in grassy oak savannah ($n = 4$) or pine–oak vegetation ($n = 41$). Only 1 juvenile was found in grassy oak savannah and 3 were found in pine–oak forest; the rest of the individuals we located in these vegetation types were adults.

Body Temperatures. — We collected 28 body temperatures of 10 males and 18 females and the environmental temperatures of the locations where they were encountered. All but one of these body temperatures (male no. 5) were taken on individuals in pine–oak vegetation. When comparing T_b , T_s , T_{10} , and T_{150} between males and females, all data were normal and homoscedastic except for the T_{150} measurements of males, which were not normally distributed. There was significant difference between sexes in T_b (Student t -test; $t = -2.21$, $p = 0.018$), but not T_s ($t = -1.56$, $p = 0.065$), T_{10} ($t = -1.58$, $p = 0.063$), or T_{150} (Mann-Whitney U-test; $W = 54.5$, $p = 0.267$; Table 2). Results from the paired t -tests show that in our observations, *T. n. klauberi* had significantly higher T_b than T_s ($t = 8.92$, $p < 0.001$), T_{10} ($t = 5.87$, $p < 0.001$), and T_{150} ($t = 5.63$, $p < 0.008$; Table 2). T_b was an average 3.34°C above T_s , 2.24°C above T_{10} , and 1.84°C above T_{150} .

Sexual Dimorphism in Morphology. — Male and female *T. n. klauberi* do not differ in CL (Table 3) and have a sexual size dimorphism index of 1.01. When comparing the nonstandardized morphological variables of the limbs, shell, and head, we found that males have a smaller plastral lobe, more compressed shell, and longer

head (Table 3). Removing the effect of CL from these variables showed similar results, and that males weigh less, have a smaller plastral lobe, more compressed shell, and longer head (Table 3). A total of 14/45 (31%) of the individuals that we observed had moderate to severe damage to their anterior marginal scutes. Of the male individuals, 8/18 (44%) had damage to their scutes, and 6/27 (22%) females had damage to their marginals. All of the turtles with damage to their scutes were located in pine–oak forest.

Diet Observations. — We observed turtles foraging 15 times in pine–oak forest and 5 times in tropical dry forest. Six of our observations consist of finding *T. n. klauberi* foraging in horse manure. Turtles that were found foraging in horse manure were located within the 26-ha tract of land that was surveyed in the pine–oak forest, and this horse manure is from four horses that are allowed to pasture in this area. It appears (not directly observed) that turtles are targeting several species of dung beetle that process the manure, one of which has been identified as *Phanaeus amithaon* (subfamily: Scarabaeinae). We also found turtles on 2 occasions at waste piles that are created by leaf-cutter ants (*Atta* sp.), apparently foraging on beetles. Patent-Leather beetles (subfamily: Passalinae) have been the only type of beetle observed at these waste piles. Furthermore, on 4 occasions we observed turtles foraging on 2 species of mushroom—*Rusela* sp. and possible *Amanita* sp.—and 2 of these observations were made on the same mushroom by 2 different individuals on consecutive days. The remaining 6 observations consist of observing turtles forage on unidentifiable herbaceous vegetation ($n = 4$), grass ($n = 1$), and wildflowers ($n = 1$).

DISCUSSION

Our study provides a preliminary assessment of the natural history of *T. n. klauberi* by providing baseline data on local abundance, demography, and home range. We also document sexual differences in morphology and behavior between males and females that have not been documented in other turtle species or populations. Lastly, we provide observations of diet items that *T. n. klauberi* was observed foraging on in the wild.

Finding *T. n. klauberi* at elevations ranging from 300 to 1300 masl corroborates previous observations (Legler

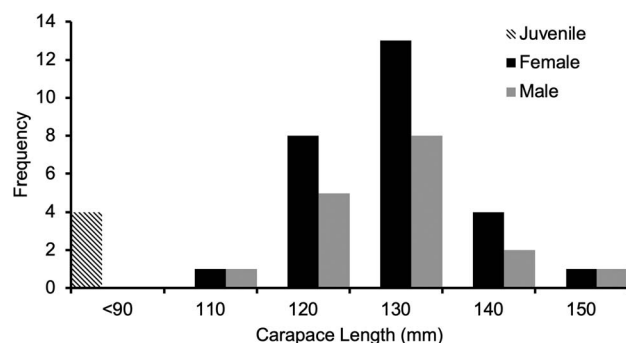


Figure 4. Size structure (CL) of *Terrapene nelsoni klauberi* population in Monte Mojino reserve.

Table 2. Mean (\pm SD) body and environmental temperatures ($^{\circ}$ C) for males, females, and all individuals. T_b = cloacal temperature; T_s = 15 mm below the surface of the soil; T_{10} = 10 cm above the surface of the soil; T_{150} = 150 cm above the surface of the soil.

Sex	<i>n</i>	T_b	T_s	T_{10}	T_{150}
Male	10	29.58 \pm 2.26	25.44 \pm 1.78	26.84 \pm 2.59	27.28 \pm 2.22
Female	18	27.35 \pm 2.71	24.48 \pm 1.44	25.4 \pm 2.14	25.68 \pm 2.35
All	28	28.15 \pm 2.74	24.82 \pm 1.60	25.91 \pm 2.37	26.25 \pm 2.39

and Vogt 2013), but finding that this species is extremely rare at lower elevations (300 masl) and more common at higher elevations (1000 and 1300 masl) has not been previously documented. This observation might represent the natural distribution and abundance of *T. nelsoni* at different elevations. However, an expected outcome of climate change is a shift in species distribution to higher elevations (Chen et al. 2011). Thus, it is possible that the rarity of *T. n. klauberi* at lower elevations could reflect population declines at lower elevations due to climate change. Although these differences in abundance at elevations could reflect their natural abundance, it is important to recognize this possibility because anecdotal

reports from local people in the Monte Mojino reserve suggest that prominent stands of oak trees (*Quercus tuberculata*) that are located at the top of some hills in the tropical dry forest have been disappearing over the past few decades. These oak stands are located in cooler microhabitats, and the locals attribute the disappearance of these trees to increasing temperatures and longer dry seasons. Similarly, locals attribute the establishment of several tropical dry forest tree species at higher elevations in the pine–oak forest to warming temperatures. Therefore, even if our findings represent the natural abundance of this species and not declining populations due to climate change, the observations from locals that climate change could be affecting vegetation in this area merits future research to determine whether populations of *T. n. klauberi* are being affected. Accomplishing this will require more surveys at intermediate elevations to determine how the abundance and distribution of *T. n. klauberi* changes from lower elevations in the tropical dry forest to pine–oak forest.

The average home range size of *T. n. klauberi* of 1.76 ha is similar to that observed in a population of *Terrapene ornata luteola* (1.6 ha; Nieuwolt 1996) and in a population of *T. c. carolina* (1.88 ha; Donaldson and Echternacht 2005), but smaller compared with other studies (2.68–4.04 ha; Refsnider et al. 2012; Kapfer et al. 2013). Therefore, even though our results are preliminary and might not be representative of this species, these results seem to fall in the range of observed home ranges in other species of *Terrapene*. Likewise, the population structure of *T. n. klauberi* seems to be similar to some populations of *Terrapene* in that it is dominated by adult individuals (Dodd 2002). The female-biased sex ratio of 1:1.5 that we observe in *T. n. klauberi* is similar to some populations of *T. ornata* (Legler 1960; Doroff and Keith 1990), but is uncommon among other populations of *Terrapene* that usually have sex ratios that are closer to 1:1 or male-biased (Dodd 2002). We hope that future investigation with *T. nelsoni* will provide a more comprehensive understanding of home range size and population structure, especially at lower elevations where turtles seem to be rare. An additional future priority of this species should be determining how weather, season, vegetation, age class, or sex could affect the detectability of this species during surveys.

We found that active *T. n. klauberi* had an average body temperature of 28.15 $^{\circ}$ C \pm 2.73 $^{\circ}$ C. Similar body temperatures of active turtles have been observed in *T. o.*

Table 3. Summary of the comparison of morphological variables between males and females of *Terrapene nelsoni klauberi* in Monte Mojino Reserve. Original variables correspond to raw measurements (mm) taken for each morphological variable and size-free variables represent residuals of variables after being regressed against CL to remove the effect of body size. Sample size, mean values for each variable in males and females, *t*-statistic, and *p*-values are displayed. Bold font indicates *p* < 0.05. CL = carapace length; PL = plastron length; Lobe = plastral lobe width; MW = marginal width; SH = shell height; Ante = antibrachium length; Hand = manus length; Crus = crus length; Rear foot = pes; HW = head width; HL = head length; HH = head height.

Variable	<i>n</i>	Male	Female	<i>t</i>	<i>p</i>
Original					
CL	51	132.70	133.93	0.50	0.62
Weight	51	375.20	401.55	1.81	0.08
PL	51	128.51	131.08	1.39	0.17
Lobe	17	73.61	74.97	2.14	0.05
MW	51	86.40	88.88	1.68	0.10
SH	51	60.38	63.46	3.40	< 0.01
Ante	17	19.60	20.50	1.22	0.24
Hand	17	14.40	14.40	0.01	0.99
Crus	17	27.90	28.90	0.77	0.45
Rear foot	17	27.46	26.20	−1.54	0.14
HW	27	27.07	25.57	−1.65	0.11
HW	27	41.79	38.72	−2.20	0.04
HW	27	24.75	23.53	−1.64	0.11
Size-free					
Weight	51	−0.033	0.025	2.10	0.04
PL	51	−0.008	0.006	1.49	0.14
Lobe	17	−0.002	0.015	2.36	0.03
MW	51	−0.013	0.010	1.68	0.10
SH	51	−0.026	0.020	3.40	< 0.01
Ante	17	−0.013	0.030	1.28	0.22
Manus	17	0.020	−0.070	−0.27	0.80
Crus	17	−0.040	0.020	0.67	0.51
Pes	17	0.010	−0.020	−1.93	0.07
HW	27	0.022	−0.032	−1.89	0.07
HL	27	0.030	−0.040	−2.33	0.03
HH	27	0.020	−0.030	−1.72	0.10

luteola in Arizona (Plummer 2003), and our observations also fall within the proposed optimum temperature for locomotor activity in *T. c. carolina* (24°C–31.9°C; Adams et al. 1989). That turtles regulate temperatures an average 1.84°C–3.34°C above environmental temperatures suggests that both males and females actively thermoregulate to maintain their body temperature above ambient temperatures (Huey and Slatkin 1976). The opposite has been observed in *T. c. carolina*, which have been shown to conform to environmental temperatures (Parlin et al. 2017), making it unclear what determines the tendency for turtles to actively thermoregulate or conform to surrounding environmental temperatures. Thermoregulation in *Terrapene* merits further research, and the observation that male *T. n. klauberi* have higher body temperatures than females suggest that there could be sexual dimorphism in thermoregulatory behavior.

One of the most interesting results of our study is that males and females are not significantly different in CL, but differ in other morphological characteristics, including head length. Such differences in head length have not been observed in other terrestrial turtle populations. However, it has been reported in the genus of aquatic turtles, *Graptemys*, in which some species have evolved megacephalic heads that are correlated to differences in diet (megacephalic species consume more mollusks; Lindeman 2000). Closer investigation of diet in *T. n. klauberi* is needed to determine if differences in head size are attributed to differences in diet or if there are other factors that explain the sexual differences in head size.

We found that 30% of all the turtles we observed, including 44% of all males and 22% of all females, had moderate to severe damage on their anterior or marginal scutes. All of the turtles with marginal damage were adults in pine–oak forest, which could indicate higher levels of predation in this vegetation type. However, the tendency for this damage to be on the anterior marginal scutes is inconsistent with the damage that we have observed in other terrestrial species that we study in the tropical dry forest ecosystem, in which damage seems to be randomly distributed across different regions of the shell (*Rhinoclemmys rubida*, *Rhinoclemmys pulcherrima*, *Terrapene yucatana*, and *Gopherus evgoodei*; T. Butterfield, pers. obs.). This high frequency of damage, and the fact that male *T. n. klauberi* have very large heads, makes it seem like this damage could be inflicted by other males over time if biting is an important part of their natural history. Whether this damage is from increased predation or from other turtles, determining the reason for a high frequency of damage to the marginals of *T. n. klauberi* and the reason that males have larger heads should be a research priority for this species.

The diet items that we observed *T. n. klauberi* foraging on (beetles, mushrooms, herbaceous vegetation, grass, and wildflowers) have also been observed in other studies in the *Terrapene* genus (Dodd 2002). Interestingly, however, the majority of our diet observations

were made in pine–oak vegetation of turtles apparently scavenging through horse manure, and it is unclear how an artificial food source such as manure could influence the dynamics of turtle populations. It is possible that this could contribute to *T. n. klauberi* being more abundant at our field site in pine–oak vegetation, but further investigation of diet at similar sites that are not regularly used by horses is needed to determine whether this is the case.

Together, these findings provide an outline of the natural history of *T. n. klauberi* and in many ways parallel findings from previous studies on other *Terrapene* sp. However, the wide range of elevations and vegetation types in which *T. nelsoni* occur seems to be a unique and important aspect of their natural history. For this reason, in order to assess the conservation of *T. nelsoni* in other parts of its geographic distribution, a priority should be providing a basic understanding of how their abundance and distribution changes from lower to higher elevations and among different vegetation types. Such an understanding could help prioritize where to conduct future surveys for this species in different parts of its geographic distribution. Our study suggests that locating field sites in pine–oak vegetation at elevations > 1000 masl is a good starting point for such surveys.

ACKNOWLEDGMENTS

All animal handling was done in compliance with ethical standards established by the Mexican government (Mexican universities do not have internal animal use and care committees) and approved under permits SGPA/DGVS/004756/18 and SGPA/DGVS/004756/19 issued by the Secretary of the Environment and Natural Resources (SEMARNAT). We would like to thank Lydia Lozano and staff of NCI for logistical help throughout this study, Ruben Alvarez for his local knowledge in Santa Barbara, Alma Montano-Hernandez from the local CONANP office in Alamos, Martín Figueroa for his local knowledge on turtles, and Mercy Vaughn for helping us begin studying turtles in Monte Mojino. We also thank Jose Carlos Verdugo for his help in the field during 2018 and Alejandra Monsiváis-Molina for her help in the field in 2019. Big thanks to Alejandro Montiel Ugalde for producing Fig. 2. This study was funded by PAPIIT (IA200418) and the Rufford Foundation and part of a bigger project that is monitoring the 6 species of turtles in the Sierra de Alamos protected area (studentsconservingnature.org). T.G.B. thanks the Consejo Nacional de Ciencia y Tecnología (CONACyT) for providing a scholarship during his studies (No. 863562) and to the Posgrado en ciencias Biológica, UNAM. T.B. and R.M.R. express their appreciation for technical and logistic support from UNAM, especially the Escuela Nacional de Estudios Superiores (ENES) unidad Morelia (especially Jannette Huante) and the Posgrado de Ciencias Biológicas.

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Received: 18 May 2020

Revised and Accepted: 31 July 2020

Published Online: 4 June 2021

Handling Editor: Joshua R. Ennen