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SISTEMA DE ESTUDIOS DE POSGRADO

APRENDIZAJE Y MADURACIÓN EN EL COMPORTAMIENTO
PREDADOR Y CONSTRUCCIÓN DE TELA SEGÚN EDAD Y SEXO EN LA
ARAÑA *PHYSOCYCLUS GLOBOSUS* (PHOLCIDAE)

Tesis sometida a la consideración de la Comisión del Programa de Estudios
de Posgrado en Biología para optar por el grado y título de Maestría
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Dedicatoria

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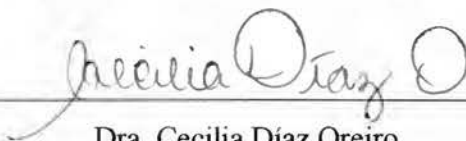
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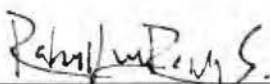
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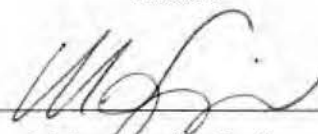
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RESUMEN

El comportamiento de ataque de las arañas está compuesto de muchas conductas, y sus secuencias varían según el tipo de presa en adultos de algunas especies. La variación y flexibilidad en el comportamiento predador de arañas inexpertas, recién emergidas son escasamente conocidas. Estudié arañas de *Physocyclus globosus* (Pholcidae), las cuales atacan presas que caminan o vuelan y que son retenidas en su tela. Dicha tela es una plancha en domo con una maraña arriba. Primero investigué cómo los detalles del ataque variaron en la primera experiencia de las arañas según el tipo de presa: moscas de la fruta u hormigas. Las transiciones de la secuencia entre comportamientos y el tiempo invertido en algunos de ellos (tocar, envolver, o manipular) difirieron con el tipo de presa. Algunas hormigas dañaron a las arañas, y cómo éstas fueron atacadas desde más lejos que las moscas, consideré que fueron presas más difíciles. Además, se demostró que el comportamiento predador es flexible desde su primera experiencia.

El aprendizaje permite a animales mejorar comportamientos como la predación con la experiencia. Sin embargo, en arañas el aprendizaje ha sido estudiado principalmente en adultos, por lo que el efecto de las experiencias previas se desconoce. Arañas recién emergidas de algunas especies incrementaron su éxito de captura con la experiencia, pero esto pudo deberse al aprendizaje o al desarrollo morfológico, neuronal y fisiológico (maduración). Por tanto, probé si el aprendizaje o maduración se asoció a cambios en el comportamiento predador de arañas *P. globosus*. Les di una presa cada tres días, y varié la secuencia de presas difíciles (hormigas) y fáciles (moscas). El apoyo a aprendizaje fue más fuerte que a maduración. El aprendizaje fue evidente cuando las primeras presas fueron difíciles y luego fáciles. La duración de seis comportamientos disminuyó cuando atacaron una presa fácil luego de difíciles, pero solo dos disminuyeron en la secuencia opuesta de presas, apoyando la hipótesis de aprendizaje. Una mayor cantidad de presas difíciles atacadas se asoció con mejoras en el ataque luego de mudar (envolver hormigas en menos tiempo, acercarse a moscas antes, etc), probablemente favorecido por la maduración.

Adicionalmente, probé si el comportamiento de construir tela varió con la edad o el sexo de *P. globosus*. Puse juveniles de quinto instar, machos adultos y hembras adultas en cajas con cuatro cámaras interconectadas. La mayoría de juveniles y hembras (pero sólo la mitad de machos) se establecieron en una cámara y tejieron su plancha. Los adultos inicialmente fijaron más hilos a las paredes que los juveniles. Comparativamente, los juveniles fijaron más hilos en la cámara de la plancha, machos fijaron más hilos fuera de esa cámara y hembras tejieron planchas más densas. Todas las arañas añadieron hilos durante 18 días. Los juveniles fueron poco exploradores, quizá se establecen rápido para asegurarse capturar presas y crecer. El patrón de construcción de tela y exploración de los juveniles no difirieron según sexo. Machos adultos exploraron y abandonaron su plancha más, quizá buscando pareja. Hembras adultas se establecieron y modificaron su tela posiblemente para mejorar su tasa de captura.

Los hilos con pie de goma fijados al sustrato ayudan a las arañas a capturar presas. En Pholcidae, recientemente se descubrió que hembras adultas de algunas especies construyen estos hilos en sus telas. Por primera vez se buscaron esos hilos en juveniles de Pholcidae, y en *P. globosus* hubo pies de goma en telas de juveniles de quinto instar, pero no en telas de primer instar o machos adultos. Las bandas de hilos adhesivo fueron continuas, contrario a las gotas discretas de otros Pholcidae. Se observó en las hileras una fúsula que contiene glándulas que se considera asociada a la producción de goma en Pholcidae. Esta fúsula fue relativamente más ancha en hembras y juveniles que en machos. Por lo que la aparición ontogenética de los pies de goma parece no asociarse con cambios en el desarrollo de dichas fúsulas en esta especie.

ABSTRACT

Spider predatory behavior is composed of many different tasks, and their sequences vary with the prey type in adults of some species. The variations and flexibility in predatory behavior of newly emerged, inexperienced spiderlings are scarcely known. I studied spiderlings of *Physocyclus globosus* (Pholcidae), which attacks walking and flying prey in its web. The web of this species is an irregular dome sheet web with an upper tangle in man-made structures. First, I investigated how behavioral units varied in prey attack in the first experience when attacking two prey types: fruitflies and ants. The sequence transitions between attack tasks and the time spent in certain behaviors (touching, wrapping, or handling) differed with prey type. Because ants were attacked further away than flies, and ants damaged spiderlings I considered that ants were a much more difficult prey to than flies. My findings suggest that the predatory behavior of spiders is flexible since their first experience.

Learning allows animals to improve behaviors such as predation with experience. However, in spiders learning has been studied mostly in adults, which mask the effect of previous experience. Newly emerged spiderlings of a few species tested increased their capture success with experience, but this could have resulted from either learning or body maturation. Here, I tested whether learning or maturation was associated with changes in predatory behavior by *P. globosus* spiderlings. I gave them one prey every three days, and varied the sequence of difficult (ants) and easy (fruitflies) prey. The support for learning was stronger than that for maturation. Learning was evident when difficult prey preceded easy prey. The durations of six behaviors decreased when an easy prey followed difficult prey, but only two decreased in the opposite prey sequence, supporting the learning hypothesis. A greater number of difficult prey previously attacked was associated with an improvement in predatory behavior after molting (wrapping ants quicker, approaching flies earlier and decreasing the proportion of flies that escaped the web). These changes may have occurred due to maturation.

Additionally, I tested if the web construction behavior varied with age and / or sex in *P. globosus*. I placed fifth instar juveniles, adult males, and adult females in cages with four interconnected chambers. Most juveniles and adult females (but only half of males) wove a sheet in one chamber, and rested there. Initially, adults laid more threads to the walls than juveniles in that chamber. Compared to the other groups, juveniles laid more threads in the sheet chamber, adult males attached more threads outside the sheet chamber and adult females built more dense sheets. All spiders continuously added threads throughout 18 days. Juveniles were the fewer explorers, perhaps they establish quickly to ensure capture prey and grow. Adult males explored more and abandoned their sheet more often, perhaps looking for mates. Adult females established and then modified their sheet structure possibly to improve the prey capture rate.

Gumfoot threads attached to the substrate help spiders to capture prey. In Pholcidae, these threads were recently discovered in adult females of some species. In *P. globosus* gumfoot threads were present in the webs of fifth instar juveniles, but absent in webs of first instar juveniles and adult males. The adhesive silk bands were continuous, in contrast to the discrete droplets of adult females of two other pholcids. In the spinnerets of this species we observed a spigot which is thought to be associated with the production of glue droplets. This spigot was relatively thicker in adult females and juveniles than in adult males. Therefore, the ontogenetic appearance of gumfoot threads is apparently not associated with changes in the morphology of that spigot.

PREFACIO

Aprendizaje y maduración en la ontogenia del comportamiento. El aprendizaje es un cambio o alteración en el comportamiento de un animal basado en sus experiencias previas. El individuo utiliza información sobre el efecto positivo o negativo de un estímulo para emitir una respuesta, tomar una decisión o resolver una tarea ante una situación nueva, similar o idéntica a la anterior (Hinde 1970, Pearce 1997, Alcock 2005, Shettleworth 2010). El desempeño y las tareas de un animal que involucran alta coordinación entre los sistemas sensores, neuronales y motores se mejoran con la edad, gracias al aprendizaje (revisión en Hinde 1970). La capacidad de aprender e incorporar información en las respuestas de un animal es útil en situaciones donde la incertidumbre sobre el ambiente es intermedia, así como cuando la variación ambiental en aspectos como disponibilidad de presas también es variable (Revisión en Morse 2007). Si el ambiente es predecible, entonces los comportamientos basados en su mayor parte en el componente innato se ven favorecidos. Por el contrario, si el ambiente y su variabilidad son poco predecibles (ej. tipo, cantidad y morfología de presas que un animal puede cazar), el aprendizaje favorece más al animal (Alcock 2005, Westneat y Fox 2010). La presencia de una recompensa o un castigo claro y asociable con un estímulo favorece el aprendizaje. Por su parte, el animal debe responder positivamente, pues dicha respuesta puede influir en su supervivencia y valor adaptativo (Mackintosh 1974, Moore 2004). Además, el animal debe tener criterios cognitivos para discriminar consecuencias, y disponer de memoria para retener las mejores alternativas de comportamiento y en el futuro tomar la decisión de emitir la respuesta que le favorezca (Ades 1989, Shettleworth 2010), en términos de valor adaptativo.

El desarrollo, crecimiento y diferenciación (“ontogenia”) de los sistemas sensoriales y motores, así como del sistema nervioso central puede continuar y completar su maduración aún después de las primeras etapas de la vida de un animal (inclusive aquellas de vida libre, cuando el animal ya se desplaza, caza, etc.). Esto podría influir en su conducta inicial y el desempeño de sus tareas vitales (Hinde 1970,

Forster 1977, Shettleworth 2010). Por ejemplo, Edwards y Jackson (1994) sugieren que arañas Salticidae jóvenes de menos de diez días no han terminado de madurar sus órganos sensoriales y motores, por lo que sus primeros ataques son poco efectivos durante este período. Además, la ontogenia puede influir en otros rasgos del fenotipo, por ejemplo la construcción de telas en arañas, sobre todo aquellas recién emergidas. Dicho comportamiento cambia a lo largo de la vida del animal (Eberhard *et al.* 2008, Hesselberg 2010), y son interpretados como maduración. Otros comportamientos pueden cambiar drásticamente conforme la edad del animal, por ejemplo, abejas sociales cambian las actividades que realizan durante su vida conforme envejecen, pasando de cuidadoras de larvas a forrajeras activas (revisión en Alcock 2005). Sin embargo, estudios donde distinguen entre los efectos de maduración o aprendizaje son muy escasos. Uno de los pocos ejemplos mostró que el llamado para pedir alimento a los padres en los pichones del ave *Turdoides bicolor* se debe a la experiencia de asociar la llegada de los padres con un llamado que los adultos hacen, en lugar de la maduración. Esto porque sometieron a pichones a la vocalización de los padres días antes de lo que se sabe que los pichones emiten el llamado de pedir comida, y los pichones realizaron el llamado antes en su vida (Raihani y Ridley 2008).

El comportamiento es influido tanto por un componente hereditario como por el ambiente (Alcock 2005). Dicho ambiente puede estar compuesto por estímulos que pueden ser o no frecuentes según la cantidad de veces que un animal los encuentre en su medio (Westneat y Fox 2010). Por ejemplo, un depredador puede enfrentarse muchas veces a la misma presa. Además, durante el desarrollo del animal, las secuencias de comportamiento a veces son se ejecutan incompletas (Hinde 1970), por lo que pueden ser un entrenamiento y una retroalimentación para que la coordinación en la respuesta generada se vuelva más precisa (Ades 1989), como por ejemplo aumentar el éxito de captura de presas conforme avanza la edad y la maduración del animal.

El desempeño de tareas complejas (aquellas que involucran alta coordinación de sistemas sensores y motores, como cazar) es variable y a través de la experiencia las respuestas se vuelven más eficientes (Ades 1989). Las habilidades necesarias para un efectivo desempeño en alimentación a veces se desarrollan en las primeras etapas de la

vida del animal (Ades 1989), y su efectividad puede aumentar con la experiencia (aún siendo adultos) de enfrentarse a presas variables en tamaño, masa, forma, etc. Por ejemplo, en aves y mamíferos las actividades depredadoras iniciales tienden a ser variables y sin un patrón distintivo (Schneirla 1964). Además, el desempeño predatorio de reptiles jóvenes hacia una misma presa es más variable en sus primeras etapas de vida, y su desempeño mejora con la experiencia (Desfilis y Font 2002, Mehta 2009).

Aprendizaje en insectos y arañas. La capacidad de aprender es un componente importante de la eficiencia en el forrajeo de insectos (Kamil 1983). Por ejemplo, avispas parásitas pueden aprender la ubicación y los olores de su hospedero (Collet 2008). Hormigas obreras de *Themnothorax albipennis* golpean en la cabeza a otras obreras y modifican su velocidad y dirección de movimiento, con lo que al parecer les enseñan a localizar fuentes de comida (Franks y Richardson 2006). También, hormigas cortadoras de hojas *Atta colombica* aprenden a no forrajear en plantas que podrían ser dañinas (con compuestos fungicidas) para el hongo que cultivan en el nido, y que pueden retener eso en la memoria a largo plazo (Saverschek *et al.* 2010).

En arañas, los principales hallazgos sobre la capacidad de aprendizaje y memoria se han enfocado en el éxito de captura, los comportamientos de desplazamiento en la tela y las modificaciones de la estructura, diseño y sitio de colocación de la tela. Por ejemplo, Bays (1962) demostró que *Araneus diadematus* luego de repetidas exposiciones a estímulos vibratorios en su tela aprende a asociarlos con un sabor agradable o desagradable que poseían. Luego respondieron solamente a las vibraciones del sabor agradable. En otro aranéido, *Zygiella x-notata*, se encontró que el tiempo que tardó en volver al escondite de su tela orbicular luego de que se le hizo salir y se cambió la orientación (horizontal y vertical) de su tela disminuyó conforme aumentaba el número de experiencias que había tenido. Ese tiempo también disminuía con la edad de la araña y con el número de telas previamente tejidas (LeGuette 1969). Para *Z. x-notata* también se encontró que el tamaño y la estructura de sus telas cambiaron con la experiencia. Hicieron telas de mayor tamaño y de un diseño más eficiente para capturar

presas luego de experiencias positivas en las cuales detectó, capturó e ingirió presas (Venneret *al.* 2000). Para otras arañas que construyen tela orbicular (*Argiope keyserlingi* y *Larinioides sclopetarius*) se encontró que las arañas que construyeron telas, al contrario de aquellas que fueron aisladas y no construyeron telas durante varios meses, hicieron telas más asimétricas hacia la parte inferior y con mayor área de espiral pegajosa para captura, lo cual se cree que aumenta el éxito de captura de la araña (Heiling y Herberstein 1999). Sin embargo, en el experimento no se controló por el tamaño de las glándulas de seda de las arañas, lo cual puede afectar la cantidad de seda producida y por ende el tamaño de la tela, además de que puede variar según la condición nutricional de la araña. Por otra parte, adultos de *Misumenia vatia* (Thomisidae) incorporaron información de su última experiencia para tomar la decisión de abandonar o no la planta en la cual forrajearon. Abandonaron plantas en las que usualmente no forrajeaban (*Rosa carolina*) con mayor frecuencia que las plantas donde usualmente forrajeaban (*Asclepias syriaca*), independientemente de si capturaron presas o no (Morse 2000a), lo que sugiere que estas arañas utilizan la experiencia para elegir el parche que satisface su condición de hambre. Esta evidencia muestra que la experiencia de largo y corto plazo influye en el comportamiento de caza.

Como requisito para ciertos tipos de aprendizaje, las arañas deben tener memoria espacial y temporal (Moore 2004). Por ejemplo, varias especies de arañas (*A. argentata*: Araneidae, *Nephila clavipes*: Nephilidae y *Neriene peltata*: Linyphiidae) buscan más tiempo cuando les robaron de su tela presas grandes o frescas luego de capturadas, comparadas con presas pequeñas y menos frescas (Rodríguez y Gamboa 2000). De igual forma, los adultos de la araña tejedora de tela orbicular *Cyclosa argenteoalba* aumentaron su éxito de captura cuando volvieron a poner su tela en sitios donde habían tenido grandes éxitos de captura (Nakata *et al.* 2003). Esto demuestra que estas arañas pueden retener la información acerca de un buen sitio de forrajeo.

Aprendizaje en arañas recién emergidas. Los experimentos sobre el aprendizaje en arañas han sido en su mayoría con arañas adultas, por lo que no toman en cuenta el

efecto de los eventos que el animal ha tenido durante su vida. Hay poca evidencia sobre el efecto de la experiencia o maduración en el desempeño de arañas recién emergidas luego de sus primeras experiencias, así como en el cambio en las tareas predatorias conforme avanzan en edad. Por ejemplo, para el saltícido *Phidippus regius* (quienes cazan activamente buscando presas en la vegetación) se encontró que la experiencia, y en menor medida la maduración de la araña, hizo que el éxito de captura de las ninfas aumentara (Edwards y Jackson 1994). En otro Salticidae, *Tritea uricoma* las arañas recién emergidas tienen mayor éxito de captura luego de sucesivas capturas (Forster 1977). En esa especie el 44% de las arañitas se orientaron a su primera presa de *Drosophila* correctamente, y sólo el 5% la perseguían. Hacia una segunda presa, casi todas se orientan y la perseguían, y algunas sí lograban capturarlas. Su tasa de orientaciones incorrectas hacia la presa y el número de saltos para someterla disminuía con experiencias sucesivas, hasta que alcanzaron una tasa de captura alta y estable (Forster 1982). La autora sugiere que, aunque las arañas pueden aprender, quizá sus sistemas sensoriales y motores deben madurar antes capturar eficientemente (Forster 1977), sin embargo sus datos no permitieron diferenciar entre estos dos posibles efectos. En arañitas recién emergidas y de segundo y tercer instar de *Misumenia vatia* (Thomisidae), una araña que caza acechando la llegada de moscas y abejas en flores, el efecto de experiencias previas varía en diferentes contextos. Por ejemplo, las arañitas permanecieron más tiempo en flores que en botones florales cuando fueron colocadas en dichos sustratos luego de haber estado en flores (Morse 2000b). También, las arañas de segundo instar se orientaron más rápido hacia presas de *Drosophila* en un plato Petri conforme aumentó el número de experiencias que tuvieron (Morse 2000c). Sin embargo, el tiempo que duraron para capturar la presa fue muy variable y no disminuyó con la experiencia. Ambos resultados (Morse 2000b, c) demostraron que ni la edad (cantidad de días entre eventos de alimentación que había pasado una araña), ni la condición energética o el hambre (número de días sin comer) que tenían las arañas afectaron su desempeño predatorio. En general, estos trabajos con arañas recién emergidas demuestran la capacidad de las arañas de incorporar experiencias en su futuro desempeño de alimentación, y que los cambios ontogénicos pueden afectar el

comportamiento predatorio, y posiblemente influir en su supervivencia y posterior reproducción.

Ontogenia de los hilos de goma en telarañas. Algunas arañas a través de su vida cambian la estructura y el diseño de las telas (sean telas orbiculares, tipo planchas o tridimensionales), un comportamiento que podría estar relacionado y favorecer el aumento en la destreza predatoria (Araneidae: Eberhard 1985, Nephilidae: Japyassú y Ades 1998, Tengellidae: Barrantes y Madrigal-Brenes 2008, Theridiosomatidae: Eberhard 2000, y Theridiidae: Eberhard *et al.* 2008). A través de los instars aparecen los hilos pegajosos previamente ausentes en el ataque o en sus telas. Las telas de las ninfas de *Uloborus* (Uloboridae) en su primer y segundo instar carecen del espiral pegajoso. En el tercer instar gradualmente empiezan a tejer telas más similares a las adultas, con espiral pegajoso presente (Eberhard 1977). *Mastophora* (Araneidae), en sus estadios ninfales capturan presas directamente con las patas, no cazan sosteniendo una línea con una bola de hilo de goma al final como lo hacen las hembras adultas (Eberhard 1980). Las ninfas de *Tengella radiata*, producen hilos pegajosos en sus telas a partir del sétimo instar, pese a que desde el tercer instar tienen las estructuras necesarias para producir este hilo (el cribelo), y para manipularlo con las patas IV (el calamistro) (Barrantes y Madrigal-Brenes 2008). Esto sugiere que la maduración de dichas estructuras o las limitaciones energéticas que enfrentan las ninfas generan que los hilos pegajosos aparezcan hasta cierto instar. Sin embargo, se desconoce si la aparición de hilos pegajosos podría aumentar eficiencia predatoria en alguna araña, o si las arañas jóvenes compensan la carencia de hilos pegajosos con una mayor velocidad, u otro mecanismo que favorezca su desempeño.

Biología de Pholcidae y de Physocyclus globosus. Con poco menos de mil las arañas de la familia Pholcidae habitan en la hojarasca, el interior de troncos huecos, bajo piedras u hojas e inclusive en edificios y otras estructuras construidas por el hombre. Tejen una tela tipo plancha cóncava e inician el ataque inmovilizando a sus presas con hilos

adhesivos (Foelix 1996, Barrantes y Eberhard 2007). *Physocyclus globosus* Taczanowski 1874 es ampliamente distribuida y abundante en América. Es común en casas, edificios, mercados e inclusive túneles y cañerías (Eberhard 1992a, Huber 1997, Huber 2000, Peretti *et al.* 2006). Allí construye su tela en forma de plancha cóncava irregular con una maraña sobre la plancha, usando como soporte la unión entre paredes y techos, los espacios entre cuadros y la pared, o entre estantes y muebles (González 2007). Su tela captura presas caminantes como hormigas, milpiés y otras arañas, y también captura presas voladoras como termitas y una amplia variedad de insectos (Eberhard 1992a). Los adultos de *P. globosus* y otras especies de Pholcidae usan líneas de hilo pegajoso en sus telas para capturar presas (Briceño 1985, Eberhard 1992b, Japyassú y Macagnan 2004), y al inicio del ataque de envoltura inmovilizante (por lo menos los adultos) aplica un hilo pegajoso a sus presas (Barrantes y Eberhard 2007). La secuencia predatoria sigue más o menos el siguiente orden (una descripción más detallada se encuentra en Japyassú y Macagnan 2004). La araña detecta el movimiento de la presa sobre la tela, se acerca rápidamente y la toca, luego empieza a envolverla, añadiendo mucha seda con un movimiento alternado de las patas IV, mientras sostiene la presa con las patas II o III y la araña (por lo menos en estado adulto) balancea el abdomen (Barrantes y Eberhard 2007). Luego de envolverla, corta hilos para tener mejor acceso a la presa, manipularla, liberarla de la tela y darle vueltas, y después sigue envolviéndola. A veces la lleva a otro lado de la tela diferente al lugar donde la envolvió. Luego empieza a buscar las uniones entre las extremidades de la presa para morderla (agarrar y penetrar con los quelíceros). En este punto a veces sigue manipulando la presa o la envuelve más, o le da una mordida (puede dar varias mordidas antes de que la araña deje de envolverla o que la presa esté completamente inmóvil); y empieza a consumir la presa (Fig. 1), por 10 - 40 minutos, según el tamaño de la misma (Kirchner y Opderbeck 1990, Jakob 1994, Japyassú y Macagnan 2004, Barrantes y Eberhard 2007, I. Escalante obs. pers.). Posiblemente consume la presa regurgitando fluido digestivos en la superficie de la presa, el cual penetra la presa y es re ingerido posiblemente por capilaridad (Eberhard *et al.* 2006).

Las hembras adultas de Pholcidae, que miden alrededor de 5 mm de largo, sostienen con sus quelíceros la masa de huevos, la cual consiste de un grupo de huevos rodeado de algunos hilos. La hembra construye pequeñas telas en domo donde permanece hasta que nacen las ninfas, y éstas permanecen allí hasta su primera muda (Sedey y Jakob 1998). Las hembras de *P. dugesi* producen de dos a cuatro masas de huevos por año (Rodríguez-Márquez y Peretti 2010). Las ninfas salen del huevo y se terminan de desarrollar en la masa de huevos, donde se mantienen inmóviles y a veces se designan como un estado post-larval (Eberhard 1992a, Foelix 1996). Luego, emergen $30,6 \pm 10,5$ arañas de primer instar (Peretti y Eberhard 2010), las cuales son ahora móviles y se dispersan. Aproximadamente cinco días después, cada una teje su plancha y empieza a alimentarse.

Resultados y conclusiones destacadas

A continuación presento un resumen de cada uno de los artículos que componen esta tesis. Resalto los resultados y las conclusiones más destacadas.

1. El comportamiento predador varía según el tipo de presa en arañas recién emergidas de *Physocyclus globosus* (Araneae: Pholcidae). El comportamiento predador de las arañas incluye muchas tareas diferentes. La secuencia del ataque se sabe que varía según el tipo de presa que ataquen, lo cual se ha demostrado en arañas adultas de algunas especies. Sin embargo, se desconoce si existe variación en el comportamiento predador de arañas inexpertas recién emergidas del huevo. No se sabe si este comportamiento es flexible cuando atacan diferentes tipos de presas. Estudié arañas recién emergidas de *Physocyclus globosus* (Pholcidae, Araneae), las cuales atacan presas caminantes o voladoras en su tela tipo plancha (Fig. 1). Esto para determinar si los detalles de su primer ataque varían cuando atacan dos tipos de presas: moscas de la fruta y hormigas obreras. Encontré que aunque las tareas del ataque que usaron las arañas fueron las mismas, las secuencias y sus transiciones difirieron según el tipo de presa. Las arañas atacaron, tocaron, envolvieron e inmovilizaron a las hormigas en períodos de tiempo más largos que a las moscas. Las arañas hicieron contacto directo más

frecuentemente y duraron más tiempo manipulando a las moscas que a las arañas. Ambos tipos de presas escaparon de la tela de las arañas en altas proporciones. Las hormigas a veces dañaron alguna pata de las arañas. Estos resultados sugieren que las hormigas fueron una presa más difícil de atacar que las moscas. Además, como las hormigas se movieron más en la tela probablemente eso causó que las arañas duraran más tiempo atacándolas, comparado con los ataques a moscas. Las arañas recién emergidas de Pholcidae viven en altas densidades, y tener un ataque exitoso aumentaría la probabilidad de consumir una presa, de sobrevivir y crecer. En resumen, el comportamiento predador de las arañas es flexible inclusive desde su primer ataque.

2. Aprendizaje y maduración del comportamiento de ataque a presas en arañas recién emergidas de *Physocyclus globosus* (Araneae: Pholcidae). El aprendizaje permite a los animales mejorar la eficiencia de comportamientos como la predación. Sin embargo, el aprendizaje en arañas se ha estudiado mayormente en adultos, lo que enmascara el posible efecto del número y tipo de experiencias que un individuo ha tenido. En algunas especies se ha visto que las ninfas recién emergidas aumentaron la velocidad de orientación hacia la presa y el éxito de captura luego de consecutivos ataques. Estos cambios pudieron deberse a aprendizaje o maduración de los sistemas sensores, motores o neurales. Investigué si el aprendizaje o la maduración se asociaron con cambios en el comportamiento predador por arañitas de *Physocyclus globosus* (Pholcidae). Puse arañas en vasos individuales (Fig. 3) y les di cuatro presas, variando la secuencia de presas difíciles (hormigas) y fáciles (moscas de la fruta). En general, más cambios pudieron deberse a aprendizaje que a maduración. La evidencia contra ambos factores fue escasa. La duración de seis comportamientos disminuyó cuando atacaron una presa fácil luego de presas difíciles, lo que apoya la hipótesis de aprendizaje, mientras que sólo dos comportamientos disminuyeron cuando una presa difícil fue precedida por fáciles. Independientemente de la secuencia de presas, luego de consecutivos ataques las arañas disminuyeron el tiempo en acercarse, tocar y añadir hilos a la presa. Esto es consistente con ambas hipótesis, pero no distingue entre ambas. Haber atacado más presas difíciles se asoció con mejoras en varios comportamientos luego de mudar, luego que las arañas tuvieron patas más largas, quelíceros más grandes,

hilos más gruesos, etc. Arañas de primer instar no disminuyeron la duración de ningún comportamiento luego de sucesivos ataques al mismo tipo de presa, pero luego de mudar envolvieron a las hormigas en menos tiempo, se acercaron a las moscas más rápido, y redujeron la proporción de moscas que escaparon de la tela. Estos cambios pudieron ocurrir gracias a la maduración. El comportamiento depredador estuvo compuesto de varias unidades de comportamiento independientes, algunas asociadas a aprendizaje y otras a maduración.

3. La exploración y la ontogenia de la construcción de tela difieren con la edad y sexo en la araña *Physocyclus globosus* (Pholcidae). Los diferentes intereses reproductivos entre sexos pueden afectar el comportamiento de construcción de tela. Este comportamiento también puede variar a través de la ontogenia. Este estudio probó si la exploración y la construcción de tela variaron con la edad o el sexo en la araña *Physocyclus globosus* (Pholcidae). Esta araña teje una tela tipo plancha irregular, con forma de domo, con una maraña arriba (Fig. 2). Juveniles de quinto instar, machos adultos y hembras adultas fueron puestos en cajas con cuatro cámaras interconectadas (Fig. 4). La mayoría de los juveniles y hembras adultas (pero sólo la mitad de los machos) tejieron una plancha en sólo una cámara, se establecieron ahí y descansaban ahí durante el día. Los machos adultos y las hembras adultas fijaron una cantidad mayor de hilos a las paredes, comparado con los juveniles, pero los juveniles añadieron más hilos a través de los días. Los juveniles fijaron el 91% de sus hilos en la cámara de la plancha; y comparativamente las hembras adultas pusieron 55% y machos adultos 41%. Los machos adultos fijaron más hilos fuera de la cámara de la plancha que los juveniles, y todas las arañas fijaron hilos fuera de dicha cámara durante todo el período de observación (18 días). Esto sugiere que abandonan la tela para explorar el ambiente. Los juveniles fueron criados para conocer su sexo. No hubo diferencias en el comportamiento de los juveniles según su sexo. Las hembras adultas construyeron planchas más densas que los machos adultos o los juveniles, lo cual podría mejorar su tasa de captura de presas. La reducida tasa de exploración de los juveniles sugiere que ellos se establecen rápidamente para aumentar la posibilidad de capturar presas y crecer rápido. Los machos adultos exploraron más frecuentemente, quizá en busca de hembras.

4. Ausencia de hilos pie de goma en las telas de juveniles recién emergidos y machos de *Physocyclus globosus* (Araneae: Pholcidae) puede asociarse a diferencias en morfología de las hileras. Los hilos pie de goma fijados al sustrato pueden ayudar a las arañas a retener presas. Estos hilos fueron recientemente descubiertos en arañas Pholcidae, y son similares a los que presentan las arañas Theridiidae. Reportamos por primera vez la presencia de hilos pie de goma en las telas juveniles de quinto instar de la araña *Physocyclus globosus* (Pholcidae), así como la ausencia de estos hilos en telas de conspecíficos de primer instar y de machos adultos. Las bandas de hilo adhesivo en fueron más cortas en los juveniles de quinto instar que en las hembras adultas, y fueron continuas en lugar de tener gotas discretas como en las telas de hembras adultas de otros dos géneros de Pholcidae. En las arañas de esta familia, una fusula grande en las espineretas laterales anteriores al parecer está conectada con la glándula piriforme altamente modificada, la cual se cree que es la que produce la goma. Esta fusula fue relativamente más ancha en hembras adultas y juveniles de primer instar que en los machos adultos. Por tanto, el origen ontogenético de los pies de goma parece no estar asociado a los cambios en morfología de estas fusulas.

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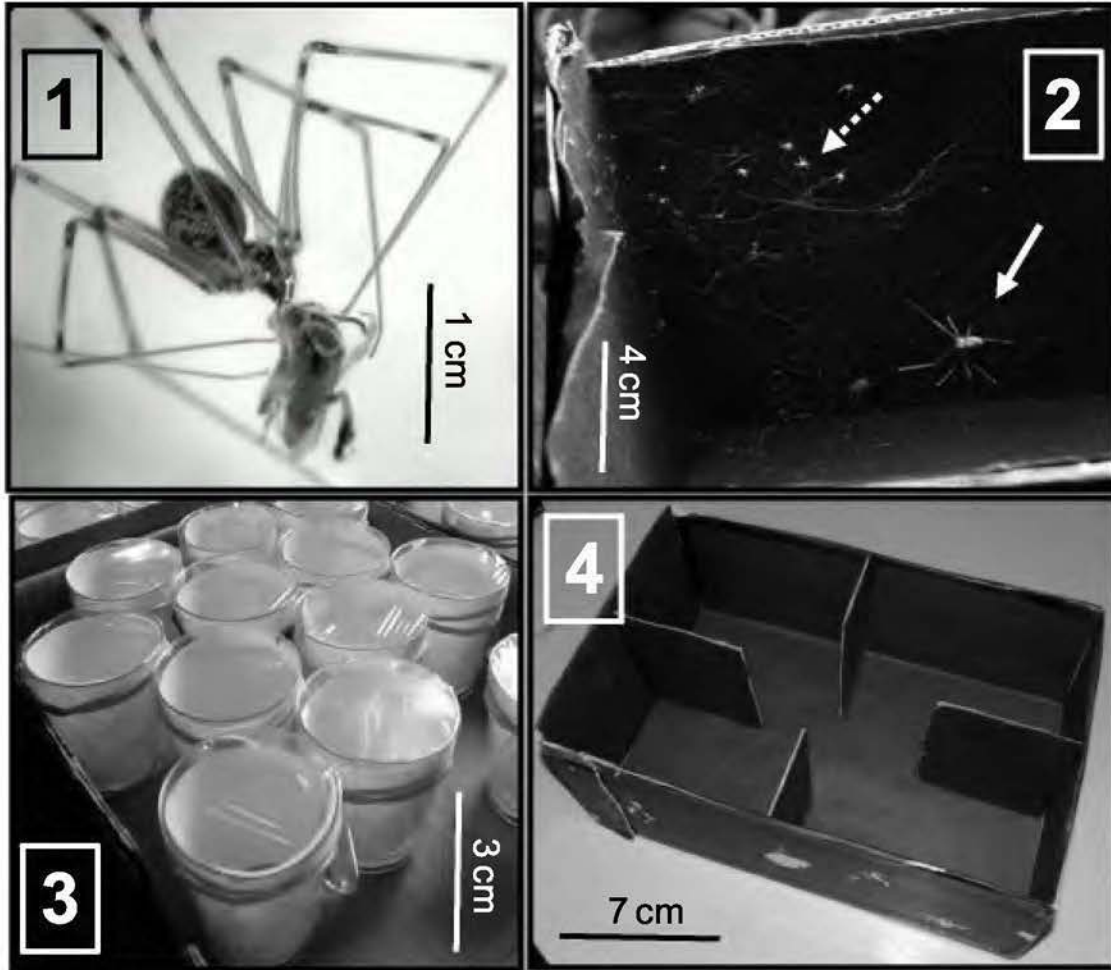
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Figs. 1 – 4. Arañas *Physocyclus globosus* (Pholcidae) y los contenedores usados durante esta investigación. 1) Hembra adulta alimentándose de una abeja sin agujón (*Tetragonisca* sp.). 2) Caja experimental donde las arañas tejieron tela, para medir la construcción y estructura de la tela durante los días. 3) Vasos donde las arañas de primer instar tejieron su tela y donde se grabó su ataque a dos tipos de presas. 4) Hembra adulta boca abajo en la plancha de su tela (flecha continua) y ninfas de primer instar (flecha punteada) cerca de los hijos fijados al sustrato.

ARTÍCULO 1

Predatory behavior differs with prey type in newly emerged spiderlings of *Physocyclus globosus* (Araneae: Pholcidae)

El comportamiento predador varía según el tipo de presa en arañas recién emergidas de *Physocyclus globosus* (Araneae: Pholcidae)

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Abstract. Spider predatory behavior includes many different units. Attack sequences vary with the prey type in adults of some spider species, but the variation in the predatory behavior of newly emerged inexperienced spiderlings is scarcely known. It is not known whether their behavior is flexible when attacking different prey types. I studied *Physocyclus globosus* (Pholcidae), which attacks walking and flying prey in its sheet and tangle web to determine whether the details of the first attacks of spiderlings vary when they attacks two prey types: fruitflies and ants. Even though the attack behavioral units employed were the same, the sequences of transitions between attack units differed with prey type. Spiderlings attacked, touched, wrapped, and immobilized ants for longer periods of time than flies. Spiderlings made more direct contact and spent more time handling flies than ants. The two prey types escaped in equally high proportions. Ants sometimes damaged a leg of the spiderling. These results suggest that ants were a more difficult prey than flies. Also, the greater movement of ants in the web probably caused that spiderlings spent more time attacking ants, compared with flies. Pholcid spiderlings live in high density of individuals, and a successful attack would increase the possibility of consuming a prey, survival and growth. In sum, the predatory behavior of spiders is flexible even in their first attack.

Key words: Attack flexibility, *Drosophila melanogaster*, ethograms, *Paratrechina longicornis*, prey wrapping.

Spiders prey on a wide variety of animals that vary in ecology, morphology and behavior (Foelix 1996). Spiders adjust to this variation with the plasticity in predatory tactics, which facilitates prey capture (Jakob *et al.* 2011, Nelson & Jackson 2011). Even so, the rate of success for many spider species in capturing prey in their webs is low (Eberhard 1990). Predatory behavior is thus crucial throughout an individual's life. When attacking its first prey a spider needs to decrease the possibility of losing a prey. In those initial experiences flexibility to attack different types of prey would increase the probability of retaining and consuming prey, as the spiderling needs to acquire energy quickly survive and grow (as suggested by Morse 2000).

Spider attack and prey consumption are elaborate phenotypes that sum many biologically different morphological, physiological, ecological and behavioral processes (Robinson & Olizarri 1971, Viera 1995, Japyassú & Macagnan 2004). The attack

behavior is likely to vary with the prey type mainly due the morphology of each prey, its defense behavior, chemical defenses and how easy is for spiders to detect and capture them. The features of each prey type could also affect how cautious an individual is. For example *Nephila clavipes* (Nephilidae) can start the attack wrapping or giving a long bite (Higgins 2007). Old juveniles and females of *Metepeira seditiosa* (Araneidae) took longer to locate, identify, and immobilize *Acromyrmex* ants than *Musca* flies, and the sequence of transitions differed between prey types (Viera 1995). More attacks by *Argiope argentata* (Araneidae) adult females on flies started with seizing and pulling than attacks on bees and butterflies (Robinson & Olizarri 1971). Adult females of *Theridion evexum* (Theridiidae) performed more bites and transported *Atta* ants more often than *Tenebrio* beetle larvae (Martins-García & Japyassú 2005). Adult females of the spitting spider *Scytodes pallida* (Scytodidae) regulates its spit expenditure, and spits more adhesive fluid toward larger and more energetically vibrating prey (Clements & Li 2005). In general, different prey types elicit different responses by spiders.

Most research on predatory behavior has been done with adult spiders. For pholcid spiders extensive descriptions of the predatory behavior are available for adult females of *Pholcus phalangioides*, *Holocnemus pluchei* and *Physocyclus globosus* (Kirchner & Opderbeck 1990, Jakob 1994, Japyassú & Macagnan 2004, Barrantes & Eberhard 2007). However, predatory behavior for pholcid spiderlings is only known for *H. pluchei* (Jakob 1994). In spiderlings in general the fine detail descriptions of their attack behavior are scarce. Larger spiderlings of *N. clavipes* capture *Drosophila melanogaster* fruitflies faster, and they capture larger prey more often in the field than smaller ones (Brown & Christenson 1983). Spiderlings of *N. clavipes* attacked their first prey by throwing silk, and after some experience they changed tactics, and delivered a long bite to the same prey type (Higgins 2007).

The Neotropical *P. globosus* Taczanowski 1874 (Pholcidae: Araneae) is an abundant inhabitant of houses, buildings, markets, and even tunnels and pipes (Eberhard 1992, Huber 1997, Huber 2000, Peretti *et al.* 2006). This species weaves a loosely meshed irregular domed sheet web with a tangle above, in the angles where walls and ceilings meet, behind furniture and paintings (González 2007). Their webs capture walking prey such as ants, millipedes and other spiders (even conspecifics), but they also feed on flying prey such as termites, flies, and a wide variety of other insect taxa

(Eberhard 1992, Escalante pers. obs.). *P. globosus* attack prey by wrapping with alternating leg IV movements until the prey is immobilized, and then a number of bites (Japyassú & Macagnan 2004, Barrantes & Eberhard 2007). In natural conditions *P. globosus* seldom have prey in their web, in 603 visits to 68 webs of mature males and females, only 53 captured prey were seen (Eberhard 1992). Also, in a building 15 % of adult females ($n = 14$), but only 4 % of advanced instar spiderlings ($n = 21$) were eating prey (Escalante, pers. obs.). Adults of *P. globosus* and other pholcids use sticky silk, both on lines in their webs (Briceño 1985, Japyassú & Macagnan 2004) and in their wrapping silk (Barrantes & Eberhard 2007).

In this paper I present detailed descriptions of the attack behavior of newly emerged spiderlings of *P. globosus*, emphasizing on the behavioral units employed and the most common transitions when spiders attack one of two prey types: ants and flies. I tested the hypothesis that differences in predatory behavior result from previous experiences. Under this hypothesis I expected to find no differences between attacks on different prey types in the first attack of the spiderlings. A contrasting hypothesis suggests that predatory versatility is present on emergence, and predicts that spiderlings would behave differently towards a prey type in their first attacks. An additional hypothesis suggests that differences in the morphology and defensive behavior of the prey are adaptive, and would be associated with particular differences in the prey. This predicted that spiderlings attacking ants would take longer attacking, wrapping, and handling the prey than spiderlings that attacked flies. Mainly because the ants have a long body, long antennae, strong mandibles, and they struggle more strongly in the web than do flies.

MATERIALS & METHODS

I collected adult spider of both sexes in buildings in the Central Valley of Costa Rica, and reared them in the lab (mean of 20 °C and 80% relative humidity) to induce mating and obtain egg sacs. Spiderlings emerge from the eggs and cluster in the female's web until they moult and disperse. In a natural setting, spiderlings emerged and five days after their first molt 90 % ($n = 30$) of them were within a 5 cm radius near the mother; 9 days after molting 53 %, and 30 % in a 5 – 15 cm radius ring. Finally, 16 days later no spiderling was near the web of the mother. After the spiderlings emerged

from the eggs, I placed them in plastic 50 ml cups (4 cm height, 3 cm upper diameter, and 2.5 cm base diameter). The inner wall of the cup was covered with white paper to allow spiderlings to walk and attach threads. The opening was covered with a clear plastic sheet with a small (0.5 cm) longitudinal opening to introduce prey. Spiderlings measured approximately 2.0 mm long (from mouth to abdomen tip). Ten days after emergence spiderlings had already moulted, built a sheet web and were hanging upside down in the center of the sheet, so I proceeded to feed them their first prey.

Prey items were worker ants of *Paratrechina longicornis* (Formicidae) which measured approximately 2.5 mm long, or wild type fruitflies *D. melanogaster* (Drosophilidae), approximately 2.4 mm long. I did not observe these species as prey items in *P. globosus* webs in natural conditions, although some species of ants and flies are common prey for this and other pholcids such as *P. phalangoides* (Netwing 1983, Kirchner & Opderbeck 1990, Eberhard 1992).

Using forceps, I induced the prey to walk onto the wall of the cup, and recorded the attack of the spiderlings with a SONY HandiCAM DCR-VX 1000 video camera, using three macro lens (+ 4 X each), and recording on mini-DV tapes at a 30 frames per second speed. I digitalized the videos using Microsoft Movie Maker, and analyzed them with the software Etholog 2.2 (Ottoni 2000). This allowed me to obtain frequency and time of the different attack behavioral units and prey behavior. I measured the delay between detecting the prey and first touching it, and the time to finally immobilize the prey (beginning with the moment when the spider started wrapping the prey, and ending with the moment when the prey stop moving and remained immobile for more than 20 s). I counted the number of transitions between each pair of attack units, to construct a transition matrix (similar to DeVries *et al.* 1993) for each prey type. Finally, whenever it was possible to see, I noted the appendage segment joint or the site of the prey's body where the spiders delivered the final bite and started feeding.

In an additional pilot study I narrated audio recordings of attacks on ants (n = 29) while observing them through a 20x dissecting microscope. I could not measure the duration of most handling behaviors in these attacks, but did include the frequency and timing of the rest of the attack units.

I tested whether attacks differed between ants and flies by comparing the medians of each response variable with Mann-Whitney U tests in STATISTICA 8.0

(StatSoft, Inc. Tulsa, Oklahoma, USA, 2007). I analyzed two qualitative attack variables (occurrence of pull-prey or not; at least one prey escape or none) with chi square proportion tests (Zar 1999). Qualitative analyses were appropriate because 0 and 1 frequency values accounted for 79% and 72% of the cases, respectively. I also used this test to determine whether spiderlings performed their final bite in a particular body part or segment more frequently, one test for every prey type. Finally, I compared if one behavioral unit was followed more frequently by any other particular unit with Chi² tests, one for each unit in each prey type.

I did not correct my alpha values with the sequential Bonferroni correction (suggested by Rice 1989) because that method neglects the probability of finding a general pattern in significance in a data matrix with many response variables. Additionally, even though my comparisons came from the same dataset, every test compared biologically and statistically different behavioral units with independent subjects. There are also mathematical and practical objections to the Bonferroni correction (Moran 2003, Nakagawa 2004). To avoid accepting spurious significance I separated the comparisons in different attack modules (Moran 2003), to highlight the processes in which differences in predatory behavior were associated with prey type.

RESULTS

The attacks of first instar spiderlings included 11 behavioral units, which I grouped in four stages (in approximate chronological order or execution): detecting, wrapping, biting, and handling (Table 1). I grouped the units in a certain module based on the chronology of the attack, represented related behaviors, and also because all units were performed discreetly and there was no overlap between behavioral units. The transitions between biting, wrapping and handling module units were common, especially after the prey was immobile (Fig.1).

Detecting module. The behavioral units in this module were defined as follows: 1) Detect. The time elapsed between when the prey fell into the sheet or touched the threads in the walls and when the spiderling changed its body orientation and began moving. This delay is a minimum estimate of when the spider sensed the presence of the prey. 2) Approach. The spiderling walked towards the prey in the sheet, threads and

mesh of the web. 3) Touch. The time spent making the initial taps on the prey before wrapping. Spiderlings touched the prey three to five times with their first and second pair of legs. 4) Prey pull. After touching the prey, the spiderling turned approximately 180° to face away from the prey. Then it pulled threads or the legs of the prey with flexing movement of one or both legs IV that lasted approximately 0.23 s. This pulled the prey from the wall toward the center of the sheet, and got 0.5 – 1.0 cm closer to the spider. An entire pull prey sequence lasted 0.30 – 0.37 s. Four to eight s later (Table 1), the spider continued wrapping or cutting threads near the prey. Sometimes the prey was not pulled (see Fig. 4), but the spiderling started the attack in the substrate, where in most cases the prey was tangled in the threads. Afterwards the spiderling cut the threads around the prey and lifted it up, away from the wall, toward the center of the sheet.

Wrapping module. 5) Wrap. The spider rapidly moved both legs IV alternately while pulling silk from the spinnerets and laying it onto the prey (as noted in Barrantes & Eberhard 2007). The spiderling often held the prey with legs II and /or III while wrapping. Spiderlings moved their abdomens from side to side, but did not swing or incline it as do adults of the same species (Barrantes & Eberhard 2007, pers. obs.).

Biting module. 6) Short bite. The spider touched the prey with its chelicerae for less than 1 min. This happened several times during the attack, and short bites sometimes were performed when the prey was still moving actively. 7) Feeding. Spiderling did a final bite and its chelicerae were more separated than during short bites; small dorso-ventral rhythmic pumping movements of the chelicerae were sometimes noticeable. The whole feeding process lasted 30 – 300 min (up to 14 hrs. in *P. phalangioides*: Kirchner & Opderbeck 1990).

Handling module: 8) Cut threads. The spider cut threads or silk at a variety of distances from the prey. It lowered its cephalothorax and brought it close to threads, which then broke. About one third of the time the spiderling cut threads by grabbing and pulling them near its mouth with its leg II or III. The spiderling cut up to four to six threads in each burst of thread cutting. The spiderling often moved away from the prey to cut threads, whether the prey was in the center of the sheet or near a wall. 9) Touch prey. The spiderling interrupted wrapping to touch the prey. Touching occurred while the spider was hanging in the web or on top of the prey. The spiderling touched the prey with the legs II and III; these taps seemed slower than the initial ones (third behavioral

unit, above). 10) Add threads. The spiderling added new threads to the prey package after the prey was already immobilized. The spiderling brought the tip of its abdomen into contact with the prey and attached a thread without using its legs (this movement thus differed from wrapping. 11) Move prey. Adding new threads, the spiderling moved the prey from one place to another, usually located closer to the center. Occasionally a spider carried the prey holding it with one or both legs IV.

Different prey elicited different attacks. Attacks on ants and flies differed (Table 1, Fig. 2). The total duration of attack was approximately 100 s longer in attacks on ants than in attacks on flies (Table 1). In the detecting module only 1 of 8 of the comparisons was different; flies were initially touched longer (Table 1, Fig. 2). In the wrapping module 3 of 4 comparisons were significantly different: flies were wrapped faster and in marginally shorter intervals, and were immobilized in less time than ants (Table 1, Fig. 2). In the biting module (and the wrap/bite transitions) none comparison was different. In the handling module 5 of 11 comparisons were different: in attacks on flies, the durations of cutting threads around, adding threads, and moving prey were shorter (Table 1, Fig. 2). Additionally, spiderlings seemed to initiate attacks on ants from a greater distance than from flies, always approximately at least one spider body length away. However, I was not able to measure the attack distance.

The attack sequence had a mean of 38.2 ± 19.3 transitions, and was similar regardless prey type. Spiderlings attacking flies or ants performed the same attack units. However, the number of times a unit was repeated varied with prey type (Table 1), as did the proportion of transitions from one unit to other or others (Fig. 1). The following transitions were significantly more frequent ($P < 0.05$) in attacks on flies than on ants: approach – wrap (without touching the prey), prey escape – touch, wrap – add threads, cut – add threads, cut – touching prey, and short bite – final bite (Fig. 1). In addition, attacks on flies more frequently had the sequence of cut threads – add threads – move prey (thicker arrows in Fig. 1) than attacks on ants. Spiderlings attacking ants performed the transitions prey escape – prey pull, add – cut threads, add threads – touching prey, and add threads – final bite more frequently than spiderlings attacking flies (Fig. 1).

The site on the prey's body that the spiderlings bit varied with prey type. Most final bites on ants were on articulations of the antenna or the legs. The femur / tibia joint was the most common segment in which spiderling bit ants ($\chi^2 = 12.67$; $df = 3$; $P =$

0.005). In contrast, spiderlings showed no clear preference for one site over another in attacks on flies ($\chi^2 = 1.25$; $df = 6$; $P = 0.97$; Table 2).

Defensive behavior by prey. Qualitative observations suggested that ants were more difficult prey. Ants moved more often and more rapidly during the first stages of attack in the web than flies. They made more strong movements with their long legs and antennae than flies, even when the wrapping process was nearly finished. The round bodies and shorter appendages of the flies seemed to be wrapped more easily by the spiderling than the long and slender body of the ants.. Five spiderlings terminated their attack on ants 3 – 5 minutes after starting and moved away from the prey. Two of these spiderlings had a fourth leg broken in the tarsus. These spiders did not approach the prey again and did not resume their attack.

Both flies and ants commonly escaped from the spider's web (64% of encounters by flies and 71% of encounters by ants) (Proportion $\chi^2 = 0.16$, $df = 1$; $P = 0.69$; Fig. 1 & 3). During the first 60 s of the attack, the prey struggled and often freed itself at least partly from the sheet, and resumed walking on the wall of the cup. In many cases the prey still had a thread attached to its body, so the spiderling followed it. If not, the prey encountered another thread attached to the substrate, and the spiderling then reached the prey again and resumed its attack. The prey commonly escaped the web, even though the spiderling pulled it from the walls. The flies escaped after a pull in 18 of 27 cases, and ants 18 of 24 cases.

Additional observations. The percentage of individuals that pulled ants (79%) or flies (64%) was the not statistically different ($\chi^2 = 2.03$; $df = 1$; $P = 0.15$). Spiderling repeated the prey pull (Fig. 4) in several contexts: when the prey escaped the sheet; when it was pulled from the substrate to the web but its movements released it partially or completely from the web; and when the spiderling pulled the prey several times towards the center of the web.

On a few occasions (< 15%) the spiderling moved to the center of the sheet after the prey pull, and only after 4 – 8 s it “recovered” and started to wrap or cut threads near the prey again (Table 1). In one uncommon variant, the spiderling did not turn in front of the prey before pulling, and was thus still facing the prey, and pulled it toward itself flexing its legs I. Another spiderling started attacking the prey, and after began wrapping it the prey started moving and the spider stopped the bite, moved towards the

center of the web, where it did a quick prey pull. I saw four spiderlings reeling in threads as described by Japyassú & Macagnan (2004). The spiderling held a thread attached to the prey with the legs III or IV, and after bending the legs, the prey started going upwards towards the spiderling.

The duration of wrapping did not differ with prey types ($U = 1189.0$; $P = 0.34$), and the longest wrapping session was approximately one third of the total wrapping time (mean \pm SD: 34.7 ± 18.1 s for flies, and 40.4 ± 24.4 s for ants). The wrapping sessions become shorter towards the end of the attack. The longest wrapping session was the first one in 30% of the attacks (median: 4th of 37 sessions for spiders that attacked flies and 3rd of 26 sessions for ants). Additionally, for both groups, 59.0 ± 26.0 % of the total time wrapping happened before attempting the first short bite (Table 1).

Additional behavioral observations suggest changes as the attack progressed. Even though I was not able to quantify it, spiderling seemed to attack prey at a larger distance during the first stages of the attack than towards the end. After prey immobilization, at approximately 70 % of the attack duration, the spiderling wrapped, touched, and cut threads apparently closer to the prey than at the beginning (two spider body lengths away). It held the prey package with its legs II and III bent towards the prey (which did not happen at the beginning of the attack).

The spiderling rested still in the web away from the prey for 4.5 ± 2.1 s after the prey was immobile (Fig. 1) (“pause” in Japyassú & Macagnan 2004). After that time the spiderling often went to the wall of the cup and attached threads close to where it attack the prey (perhaps to repair it), and cut others there and in the sheet. Then, the spiderling went to the prey and did handling behaviors and bit the prey.

DISCUSSION

The predatory behavior of *P. globosus* spiderlings was flexible in certain respects in the spider’s very first experience with prey. One third of the aspects of attack behavior studied here differed in attacks on flies and ants, suggesting that predatory differences are present since emergence. The lack of differences in the other aspects of behavior could be because those behaviors change after consecutive trials, or because both prey types elicit the same behavior in the spiderlings. The first alternative is less

probable because after consecutive attacks on the same prey type, *P. globosus* spiderlings did not decrease the time spent in detecting or handling behaviors (I. Escalante in prep.). The later possibility could occur because the morphology and behavior of each prey type could affect behaviors such as detecting and handling. Additionally, detecting a prey could be related with the web structure. However, any prey type was detected faster than the other. In general, only certain behaviors in the attack sequence differed with prey type. This is congruent with what was found in a theridiid (*A. digitus*), in which the sequence and duration of certain behavioral units differed with prey type (*Tenebrio* beetle larvae or *Atta* ant), and the immobilization phases were more variable than the detecting and biting (Japyassú & Caires 2008).

Spiderlings attacked flies and ants using the same types of behavior, but their frequencies and the transition sequences differed. The worker *P. longicornis* ants were a more difficult prey than *D. melanogaster* fruitflies: spiderlings spent more time wrapping, immobilizing and handling ants than flies. Additionally, the spiderlings seemed to make more frequent direct contact with flies than with ants. Nevertheless, ants did not escape more often than flies. Ants had long antennae, large mandibles, and moved actively in the web. Contrary, fruit flies were an easy prey to attack, probably because of the round and compact body of flies, their lack of long antenna, and the fact that they moved less in the web (compared with ants). Spiderlings seemed more cautious with ants than with flies because they apparently wrapped ants further away than flies. Also, spiderlings perhaps identified the prey type when they touched it initially. This could have promoted more cautiousness with ants, as suggested for the theridiid *Achaearanea tessellata* (Barrantes & Weng 2006). Especially, since ants could harm spiderlings: two individuals were injured during attacks on ants.

My finding that *P. longicornis* ants were more difficult prey than *D. melanogaster* fruitflies is in accord with several previous studies. Differences in predatory behavior of spiders according to prey type and its difficulty have been found in adults and advanced instar juveniles (Viera 1995, Barrantes & Weng 2006, Kosiba *et al.* 2012). Several species of ants were more difficult prey to attack, and spiders of different families spend more time wrapping, biting and handling them when compared to other prey types (Salticidae: Edwards & Jackson 1994, Araneidae: Viera 1995, Zodariidae: Pekár 2004, 2009, Theridiidae: Martins-García & Japyassú 2005). Additionally, fruitflies were an easier prey in two spider species because they were

attacked in shorter time and were subdued faster than house flies (for *Argiope argentata*: Robison & Olizarri 1971, and *H. pluchei*: Jakob 1994). What is novel here is that the differences in predatory behavior are present in the very first attacks of the spiderlings.

The attack behavior of *P. globosus* spiderlings includes many different, discrete and probably independent behavioral units elicited by different decisions, such as retaining, wrapping, biting, and moving prey to a safer location in the web, etc. The units of the attack behavior of *P. globosus* differed in the type of movement, and the probably in the physiological and neural mechanisms involved while detecting or touching prey are thought to be different in certain species of spiders (Ctenidae; Barth 2002).

It is reasonable to hypothesize that the variations in attack behavior toward different types of prey should increase the chances of a successful attack. Some behavioral units are in clear accord with this expectation. I frequently observed prey escaping the web, so putting it far away from the substrate, with a prey pull or moving it to the center of the sheet would increase the probability of retaining a prey. However, I observed that prey did not escape less frequently after the spiderlings pulled it, so wrapping it is also necessary to prevent escapes. Cutting threads near the prey and other handling units may help the spiders maneuver the prey better, though this variable was not measured. Subduing the prey quickly is crucial for pholcid spiderlings, since during their first stages this species was seen in high density, and in another species (*H. pluchei*) grouped spiderlings feed less often than the ones living alone (Jakob 1991). So if a prey escapes it would probably get snared in the web of another spiderling, reducing the possibility for the spiderling to recovering it. In this project spiderlings recovered prey because they were isolated in enclosures containing the prey. Also, in this species prey capture is known to be infrequent (Eberhard 1992, Escalante pers. Obs.), even in adults, which can presumably attack a wider variety of prey sizes. The first stages are critical to *P. globosus* spiderlings, which die 28 – 34 days after emerging if they are not fed (Escalante, pers. obs.). Therefore, subdue a prey would increase the probability of feeding, surviving and growing.

The behavioral units used by *P. globosus* spiderlings were similar to those described for the adults of other pholcid species (Kirchner & Opderbeck 1990, Jakob

1994, Japyassú & Macagnan 2004), and adult individuals of this species (Barrantes & Eberhard 2007, Escalante pers. obs.). The prey pull differed slightly from the “pull-out-prey” behavior of *P. phalangioides* adult females (Japyassú & Macagnan 2004). Spiderling of *P. globosus* used the legs IV to pull the prey, instead of legs I and III, and they did not always pull the prey while wrapping it (see Fig. 1), as in *P. phalangioides* (Japyassú & Macagnan 2004). Additionally, I saw 4 of 7 adult males and 9 of 9 adult females pulling a *Tetragonisca aungustula* (Meliponinae) worker stingless bees with their leg IV.

The site on the prey where spiderling performed the final bite differed between ants and flies. In ants, more final bites were in the legs and antennae, where the prominent articulations between segments allowed the chelicerae of spiderlings reach (Kirchner & Opderbeck 1990). Also, in insects the femur – tibia joint is more mobile and projects farther from the body than some others and have two point of articulation, instead of only one as in the tibia – tarsus joint (Chapman 1998). This greater disposition of leg joint and possible more flexed membrane area could favor biting and injecting. Adult females of *P. phalangioides* preying on *Formica* sp. ants also frequently bit leg joints (Kirchner & Opderbeck 1990). In *D. melanogaster*, there were more bites on the head and thorax, probably because their legs were less prominent and active, and/or because their cuticle is thinner.

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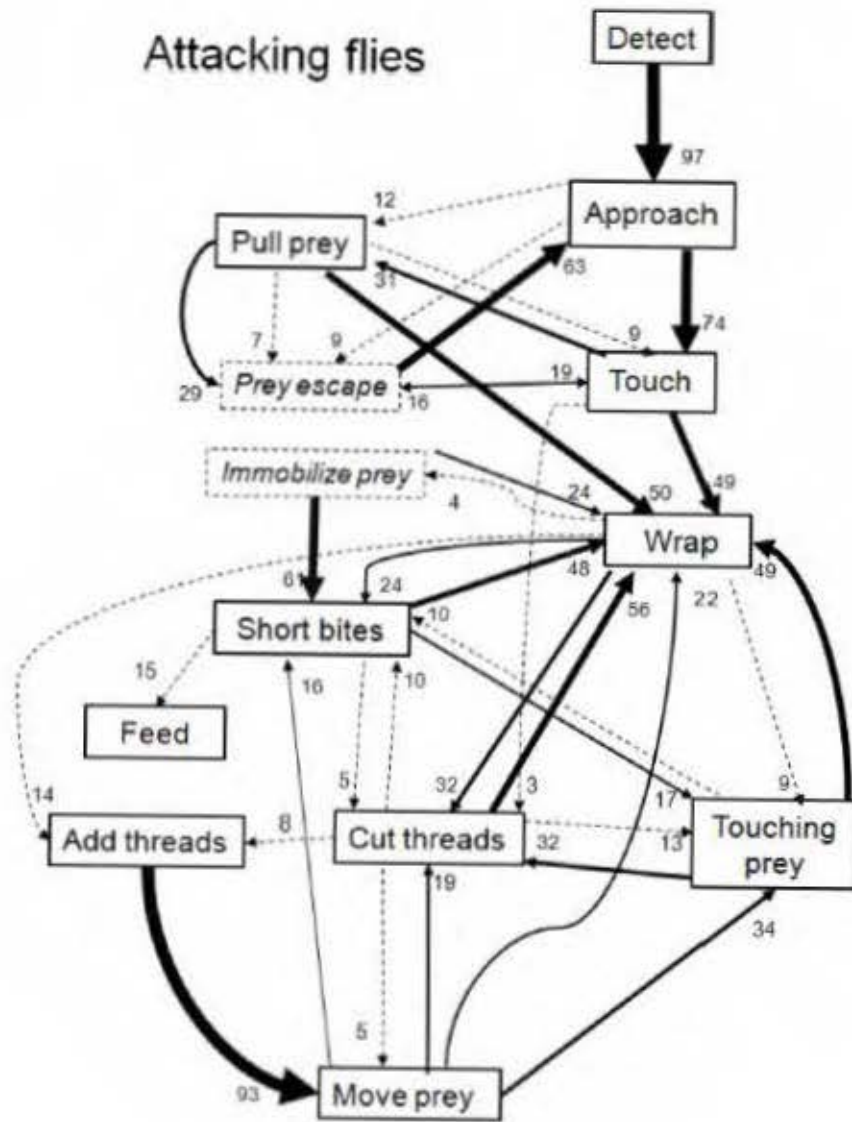
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Table 1. Summary values for the attack behavior variables of newly emerged spiderlings of *Physocyclus globosus* (Pholcidae) on two prey types. (+) = behavioral unit of the attack, shown in Fig. 1. Units are seconds, unless otherwise noted. Means \pm one standard deviation, minimum and maximum range, n = sample size. Differences are the result of Mann-Whitney tests when compared the behavior of spiderlings treatments according to the prey type they attacked. Some other comparisons are shown in Fig. 2.

MODULE / Variable	Fruitflies (<i>Drosophila melanogaster</i>)			Ants (<i>Paratrechina longicornis</i>)			Differences	
	Mean \pm SD	Range	n	Mean \pm SD	Range	n	U	P
Time spent attacking ¹	271.41 \pm 166.01	54.14 - 848.75	43	372.06 \pm 170.84	114.45 - 772.96	31	463	0.02
DETECT MODULE								
Detect prey +	2.51 \pm 2.85	0.54 - 16.50	45	2.54 \pm 3.06	0.37 - 21.50	63	1297	0.45
Approach prey +	2.77 \pm 2.27	0.31 - 9.43	44	2.96 \pm 2.78	0.23 - 14	58	3032	0.76
Detect - touch prey delay ²	5.35 \pm 7.32	1.27 - 39.10	44	3.18 \pm 2.28	0.50 - 11.41	60	1074	0.11
Detect - wrap prey delay ³	8.52 \pm 8.86	2.16 - 44.15	44	8.13 \pm 8.37	1 - 61.98	62	1339	0.87
Touch - wrap delay ⁴	3.58 \pm 5.28	0.68 - 29.26	44	4.09 \pm 4.25	0.64 - 23.77	58	1043	0.11
Prey pulls (n) +	1.46 \pm 2.17	0 - 12	43	1.60 \pm 1.63	0 - 7	30	560	0.34
Pull - touch / wrap delay ⁵	8.39 \pm 31.21	0.16 - 151.34	23	4.01 \pm 6.53	0.27 - 30.52	22	226	0.53
WRAP MODULE								
Wrap session length (mean)	10.98 \pm 4.55	5.08 - 21.22	44	13.53 \pm 8.16	5.50 - 54	62	1094	0.08
Wrapping sessions (n) ⁶	12.50 \pm 11.13	4 - 74	44	12.03 \pm 6.84	1 - 34	62	1304	0.70
Wrapping time +	137.32 \pm 138.3	20.30 - 663.66	44	231.95 \pm 131.01	29 - 631.32	30	553	<0.001
WRAP / BITE MODULES TRANSITION								
Wrap - first bite delay ⁷	140.79 \pm 139.70	9.72 - 646.40	42	121.81 \pm 98.51	16 - 470.98	60	1224	0.80
Wrap after first bite ⁸	77.06 \pm 120.22	0 - 601.80	41	78.95 \pm 93.72	0 - 542.69	60	1216	0.76
BITE MODULE								
Short bites length (mean)	15.59 \pm 26.51	1.03 - 169.78	41	10.62 \pm 8.66	3.50 - 40.46	28	498	0.92
Biting time ⁹	81.93 \pm 51.28	4.12 - 201	41	88.21 \pm 60.84	17.40 - 283.24	28	568	0.94
HANDLING MODULE								
Touching sessions (mean)	2.07 \pm 1.31	0 - 7.40	43	2.50 \pm 2.05	0 - 11.20	30	571	0.41
Touching total time +	10.31 \pm 11.44	0 - 60.28	43	12.00 \pm 10.90	0 - 38.50	30	545	0.27
Touching sessions (n)	4.65 \pm 3.36	0 - 14	43	5.30 \pm 4.12	0 - 17	30	586	0.51
Cut threads (mean)	4.24 \pm 2.26	1.02 - 9.99	43	3.16 \pm 1.13	1.53 - 5.92	30	469	0.05
Cut threads total time +	24.48 \pm 19.46	1.02 - 94.89	43	26.20 \pm 28.16	4.59 - 128.60	30	599	0.61
Cut threads (n)	6.25 \pm 4.52	1 - 24	43	7.40 \pm 5.93	2 - 30	30	591	0.54
Add threads to prey (n)	2.16 \pm 2.05	0 - 7	24	0.47 \pm 0.61	0 - 2	19	106	0.003
Move prey (mean)	3.33 \pm 2.12	0 - 9.40	43	2.81 \pm 2.90	0 - 11.90	30	492	0.09
Move prey total time +	12.90 \pm 9.37	0 - 38.62	43	10.00 \pm 22.08	0 - 121.60	30	392	0.004
Move prey (n)	3.55 \pm 2.42	0 - 10	43	2.30 \pm 3.24	0 - 15	30	377	0.002

Footnotes: 1) The total attack time (sum of all the behavioral units). 2) The delayed time after the detection and when spiderling touched the prey. 3) The delay time after the prey detection and the wrapping start. 4) The delay time between touching the prey and the start of the wrapping process. 5) The delay between a prey pull and when the spiderling began wrapping the prey or cutting threads around it again. 6) Sessions were the number of times a unit is repeated, with a start and an end, and which were separated by more than a 2 s pause, the spiderling changed its position or remained immobile. (n) = the number of times each unit was repeated in the attack. 7) The delay time between the wrapping start and the first short bite. 8) The time invested in wrap prey after the first short bite. 9) The sum of all the times spent in short bites in the attack.

Attacking flies



Attacking ants

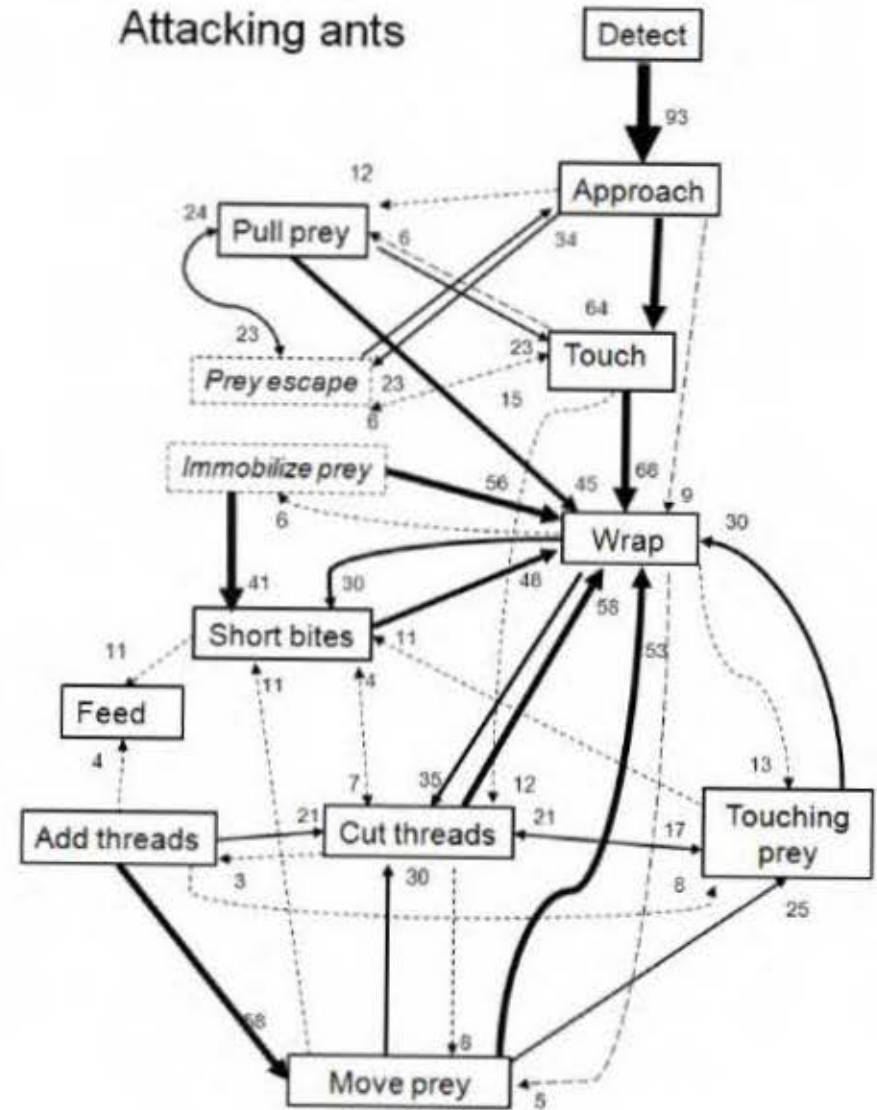


Fig. 1. Behavioral units and their sequence in the first attack of newly emerged spiderlings of *Physocyclus globosus* (Pholcidae) on fruitflies *Drosophila melanogaster* (left), and worker ants of *Paratrechina longicornis* (right). Solid boxes = behavior units, dashed boxes = processes related to prey behavior and condition. Dashed lines = transitions that happened less than 15% of the times. Most transitions that occurred less than 5% of the times are not shown. Numbers near the arrows' tip are the percentage of the total number of transitions from a unit to the next one(s), in all the attacks analyzed. Arrows' thickness reflects the percentage of those transitions. The attack started with the detection and ended in the final bite, following this sequence in a chronological order. In all transitions a unit was more frequently followed by the unit with the thicker arrow (χ^2 significant at the $P < 0.05$ level), except in the units following the escape of ants.

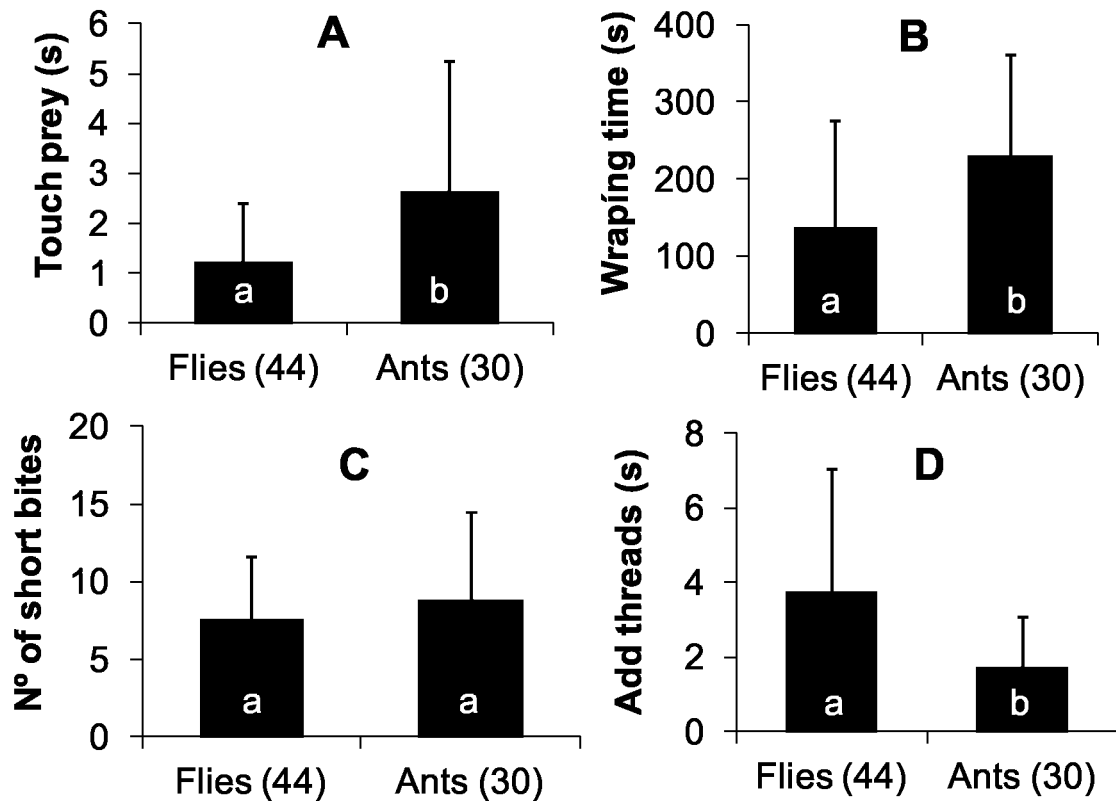


Fig. 2. Mean (+ one standard deviation) of four attack variables by two groups of *Physocylus globosus* (Pholcidae) newly emerged spiderlings that attacked two prey types. Flies were *Drosophila melanogaster* and ants *Paratrechina longicornis*. Sample size is shown in parenthesis. Letters in columns represent statistically different groups. Mann-Whitney U comparisons: A) Touch prey (detecting module), $U = 624.0$ $P < 0.001$, B) Immobilize prey (wrapping module), $U = 147.0$ $P < 0.001$, C) Number of short bites (Biting module), $U = 1148.5$ $P = 0.37$, D) Add threads (Handling module), $U = 364.0$ $P = 0.005$. Other comparisons of behaviors are shown in Table 1.

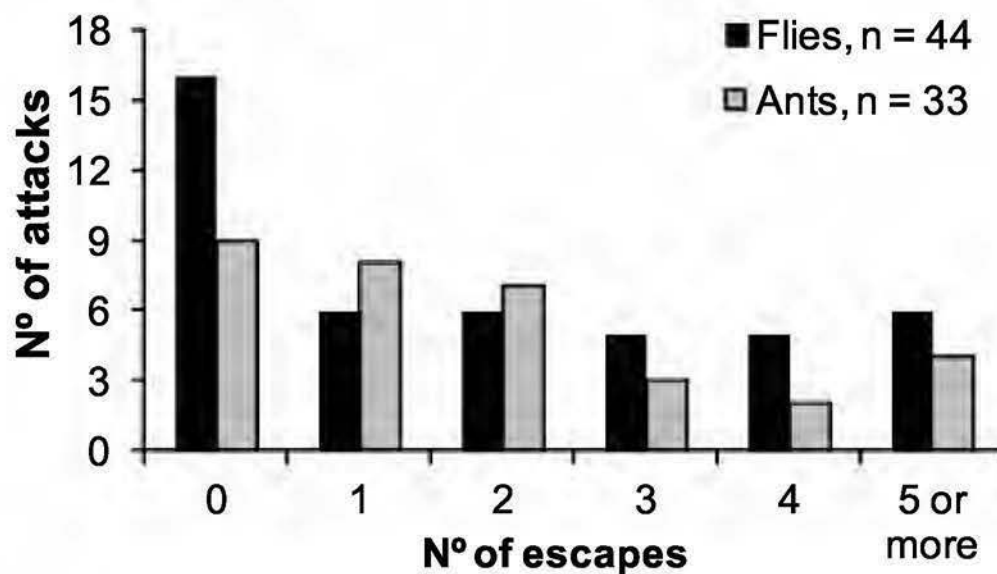


Fig. 3. Frequency of times a prey escaped the attack of *Physocyclus globosus* (Pholcidae) first instar spiderlings, in their first attack experience. Flies were *Drosophila melanogaster*, and ants *Paratrechina longicornis*. Sample size (n) is shown in the legend.

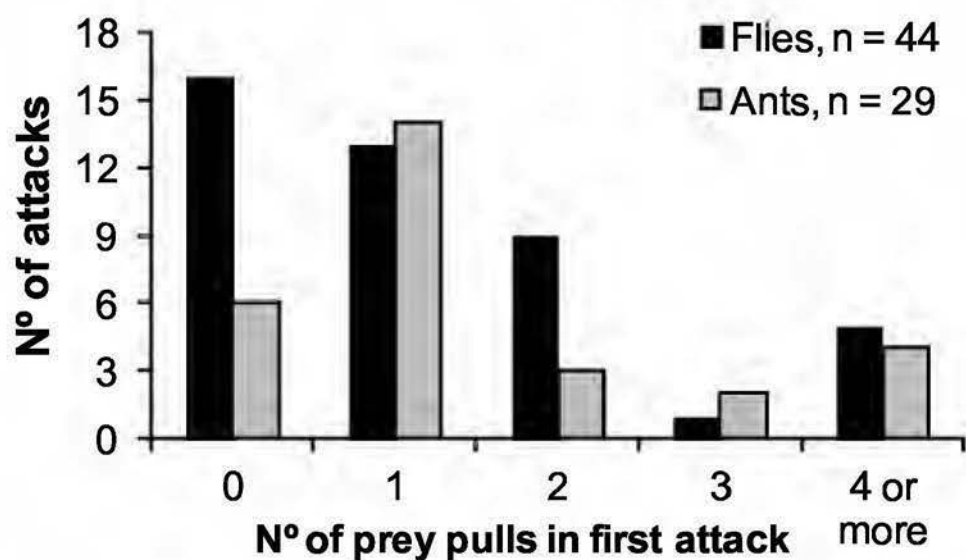


Fig. 4. Frequency of *Physocyclus globosus* (Pholcidae) first instar spiderlings that did a prey pull (or not = 0) in their first attack, according to the prey type they attacked. Flies were *Drosophila melanogaster* and ants *Paratrechina longicornis*. Sample size (n) is shown in the legend.

Table 2. Frequency of attacks according to the prey's segment or part where first instar spiderlings *Physocyclus globosus* (Pholcidae) performed their final bite and fed on two prey types.

Body part of the prey	Prey segment or part of final bite	Fruitflies (<i>Drosophila melanogaster</i>)	Ants (<i>Paratrechina longicornis</i>)
Antenna	Radicule / scape joint		2
	Scape / pedicel joint		3
Head	Mouth parts	3	
	Head, front	6	
	Head, dorsal	1	
	Head, lateral	5	
	Nape (head / thorax joint)	1	
Thorax	Thorax, dorsal	4	
	Abdomen, dorsal	5	
Abdomen	Abdomen, ventral	1	1
	Trochanter	5	
Leg	Femur / tibia joint	4	11
	Tibia / tarsus joint		2

ARTÍCULO 2

Learning and maturation of prey attack behavior in
Physocyclus globosus spiderlings (Araneae: Pholcidae)

Aprendizaje y maduración del comportamiento de ataque
a presas en arañas recién emergidas de *Physocyclus*
globosus (Araneae: Pholcidae)

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Abstract. Learning allows animals to improve the efficiency of behaviors such as predation. However, learning in spiders has been mostly studied only in adults, which mask the possible effects of the number and type of previous experiences. In a few species tested, newly emerged spiderlings increased their speed of orientation toward a prey and capture success with successive prey. These changes could have resulted from either learning or from maturation of motor, sensory and other neural systems. I tested here whether learning or maturation was associated with changes in predatory behavior by spiderlings of *Physocyclus globosus* (Pholcidae). I gave them four prey and varied the sequence of difficult (ants) and easy prey (fruitflies). In general, more changes appeared to be due to learning than to maturation. Evidence against both processes was scarce. The durations of six behaviors decreased when an easy prey followed difficult prey, supporting the learning hypothesis for these behaviors, while only two decreased when a difficult prey followed easy prey. Regardless the prey sequence, spiderlings decreased the time approaching, touching and adding threads to the prey in successive attacks. This is consistent with both hypotheses and did not distinguish between them. Having previously attacked more difficult prey was associated with improvements in several behavioral units after molting, when spiderlings have longer legs, larger chelicerae, thicker silk, etc. First instar spiderlings did not decrease the duration of any behavior in successive attacks on the same prey type, but after molting they wrapped ants more quickly, approached flies earlier, and reduced the proportion of flies that escaped from the web. These changes may occur due to maturation. Predatory behavior was composed of several independent behavioral units, some associated with learning and others with maturation.

Key Words. Attack behavior, nymphs, ontogeny, predatory sequence, prey difficulty.

The experiences that an animal has can promote adaptative changes in its behavioral phenotype via learning (West-Eberhard 2003). Individuals use information about previous negative or positive stimuli, to make subsequent decisions and solve new tasks (Hinde 1970, Pearce 1997, Alcock 2005, Shettleworth 2010). In order to do this, animals must have both cognitive criteria to discriminate consequences and memory mechanisms (Shettleworth 2010) to perform favorable responses in the future (Ades

1989, Shettleworth 2010). The capacity of recognizing stimuli as possible rewards or punishments can promote with learning. The animal can then respond accurately to favor survival, growth, and fitness (Mackintosh 1974, Moore 2004).

Behaviors such as predation, which require high coordination of sensory, neural, and motor systems, can improve with experience (summary in Hinde 1970). During development, behavioral sequences are often fragmented or incomplete (Hinde 1970). So, in the first stages of the life of an animal consecutive attacks could increase their effectiveness (Ades 1989), until coordination of responses becomes more precise (Ades 1989). For example, in vertebrates such as snakes, birds and mice the first predatory activities are more variable (Schneirla 1964, Sullivan 1988), and their effectiveness improves with experience (Desfilis & Font 2002, Mehta 2009).

Maturation as well as learning can also affect behavior. Maturation, or the development, growth and differentiation of sensory, motor and nervous systems, is a process that continues even after an animal has emerged, is moving, hunting, etc. (Foelix 1996). For example, setae and spigot morphology and number are known to change ontogenetically in the spider *Antrodiaetus unicolor* (Antrodiaetidae; Bond 1994), and in cribellar spinning spigots (Uloboridae; Opell 1995). Also, in the spider *Cupiennius salei* (Ctenidae) newly emerged spiderlings have 6 or 7 sensory trichobothria per walking leg, as compared with around 100 as adults (Barth 2002). The maturation effect was been reported mostly between instar, but development within an instar is poorly known. Ontogenetic changes in sensory and motor systems have been suggested to affect both predatory behavior (Forster 1977, Edwards & Jackson 1994), and orb web construction (Hesselberg 2010). Even after spiders reach adulthood, maturation of their gonads days after molting (Klein *et al.* 2012) may affect their foraging and reproductive behavior. However, studies which distinguish the effects of maturation versus learning in spiders are scarce. Learning, instead of maturation, was associated with the fact that nestlings of the bird *Turdoides bicolor* performed food request calls earlier in life when submitted to the vocalizations of their parents a few days before food request calls normally begin (Raihani & Ridley 2008).

Learning has also been studied in spiders in adult orb web construction, predation, spatial movements, orientation, and mate choice (reviews in Punzo 2004 and Jakob *et al.* 2011). In most cases adults learned to associate cues from past experiences

to respond or not to certain stimuli, or to improve their orientation (Bays 1962, Skow & Jakob 2005, Hoefler & Jakob 2006). LeGuette (1969) documented a possible effect of learning rather than maturation by rearing *Zygiella x-notata* (Araneidae) spiders without allowing them to build their orb web. Compared with spiders of the same age that had built many webs, the experimental spiders took longer to return to the retreat after they captured a prey on the web and the frame in which the web was built was rotated. After learning experiences, spiders changed their web size, structure or location to capture more prey (Heiling & Herberstein 1999, Venner *et al.* 2000, Nakata *et al.* 2003), and moved to sites where they increased their capture success (Vollrath 1984, Whitehouse 2011).

Spider learning has been investigated mostly in adults, therefore the effects of the number and types of previous prey throughout their life is unknown (Higgins 2007, Shettleworth 2010), and not controlled for in most studies. The few studies performed with spiderlings have found that experience affects their behavior. In *Phidippus regius* both experience and to a lesser extent the maturation of spiderlings correlated with increased capture success (Edwards & Jackson 1994). In *Trite auricomma* the incorrect orientations to prey and the number of jumps needed to capture it decreased with maturation and successive experiences, until they reached a high and stable capture rate (Forster 1977). The author suggested that, even though spiderlings can learn to improve their hunting success, a critical threshold of sensory and motor maturation is required before effective hunting behavior is initiated (Forster 1977). Newly emerged *Misumenia vatia* spiderlings orientated faster to a *Drosophila melanogaster* prey in a Petri dish after previous attacks, but capture time was variable and did not decrease (Morse 2000). Morse suggested that the behavior of spiderlings was affected by experience, and not by age (time interval between feeding events), or energetic condition (number of days without eating). Inexperienced spiderlings of two species of *Nephila* attacked stingless bees by throwing silk, but after successive attacks they performed a long bite, as adults did with the same prey type (Higgins 2007). Larger, older spiderlings of *N. clavipes* captured prey faster and with greater success than smaller individuals (Brown & Christenson 1983). In the lynx spider *Oxyopes salticus* the first prey presented to a spiderling created a preference for this type of prey in the future (Punzo 2002), a learning phenomenon also observed in *Linyphia triangularis* (Turnbull 1960). Therefore, young spiders can incorporate past experiences in their future feeding

behavior, and in at least some cases ontogenetic changes also occur and affect their predatory behavior.

I addressed two questions. The first was whether efficiency and effectiveness in handling prey improves depending on the number of attacks a spiderling has made. The first prediction stated that spiders will gradually spend less time in detecting, wrapping, biting and handling prey, to favor a successful attack. This prediction was tested in *P. globosus* spiderlings by giving them a constant number of trials with a single prey type in a standard time period, including attacks during the first instar and the first attack following the moult to the second instar.

Secondly, I investigated the possible causes of the changes seen in the first part, trying to separate learning and maturation, whose effect on predatory behavior has not been addressed. If maturation is the major factor associated with changes in the behavior of spiderlings, the effectiveness of predatory behavior would increase with increased experience, but would not be affected by the difficulty with which prey were subdued (this assumes that rates of maturation are not altered by experience). On the other hand, learning would be a major factor affecting behavior if variations in prey type resulted in different changes in attack behavior. I gave wild type fruit flies *Drosophila melanogaster*, which are easier prey for these spiders and worker ants of *Paratrechina longicornis* as difficult prey (see below, and I. Escalante in prep.). The learning hypothesis predicted that by alternating the prey difficulty, spiderlings should decrease the time wrapping, give fewer short bites, handling, etc. a difficult prey after having attacked other difficult prey. Spiderlings that attacked a difficult prey after having attached easy prey should not decrease the time spend in those behaviors. On the other hand, the maturation hypothesis predicted decreases in the time spent in those behaviors regardless the prey type sequence.

A further prediction of the learning hypothesis is that comparing a third experience spiderlings that had attacked more individuals of a difficult prey should perform better than spiderlings that attacked previously easy prey. In contrast, the maturation hypothesis would predict that the behavior of spiderlings would be similar in both groups. Also, spiderlings that attacked more difficult prey in the first instar should perform better in their first attack after molting, compared with individuals that had attacked fewer difficult prey. This comparison in second instar would also indicate if

molting as a major step in maturation (increase in leg length, chelicerae size, etc.) is associated with an improvement in attack behavior

I tested this hypothesis on newly emerged spiderlings of *Physocyclus globosus* Taczanowski 1874 (Pholcidae, Araneae), a widely distributed and abundant in the New World. It is common in houses, buildings, markets, and even tunnels and pipes (Eberhard 1992, Huber 1997, 2000, Peretti *et al.* 2006). Its web is a dome-shaped irregular sheet with a mesh above, often using as support a joint between two vertical substrates, such as walls, furniture and paintings (Huber 1997, González 2007). The web captures both walking prey such as ants, millipedes, and even conspecifics, and flying prey such as termites, flies, and other insects (Eberhard 1992). In natural conditions, spiders only seldom have prey in their webs, and spiderlings tend to aggregate near the mother's web during their first stages and built webs (I. Escalante in prep.). The overall process is known in detail for this species (Barrantes & Eberhard 2007, I. Escalante in prep.) and other pholcids (Kirchner & Opderbeck 1990, Jakob 1994, Japyassú & Macagnan 2004). The attack behavior of *P. globosus* includes four main behavioral stages or modules: detecting prey and initial attack, wrapping, handling, and biting. A spider detects a prey as it moves in the web or along the substrate (probably when it contacts the web), then approaches and touches it. Sometimes the spider quickly pulls the prey from the substrate into its web. Then the spider wraps the prey, applying silk lines with alternate movements of legs IV. Next the spider cuts threads around the prey, adds threads to it and moves it to the sheet, and during that process can give a series of approximately a dozen of short bites to inject digestive enzymes and venom. Then, the spider bites a joint between appendages segments of the prey and feeds.

MATERIALS & METHODS

Study population. I collected both mature male and female *P. globosus* houses and buildings in the Central Valley of Costa Rica. I kept them in laboratory conditions (about 20 °C and 80% relative humidity) and bred them. After the pre-nymphs hatched from the egg sac, each was placed in an individual plastic 50 ml round cup (4 cm tall, 3 cm upper diameter, and 2.5 cm base diameter). The inner walls and the floor were covered with bond white paper so the spiderling could walk and attach threads. A clear

plastic sheet with a small (0.5 cm) longitudinal opening through which prey were introduced formed the upper wall. Each cup was covered by a Petri dish to ensure that the plastic sheet was free of dust when the spiderlings moulted and their attack behavior was videotaped.

Nymphs emerge from the egg sac and remain more or less immobile near the female while they finish their development. This stage has been designated as post-larval stage following Foelix (1996). The “first instar” of spiderlings began after their first molt away from the egg sac. I waited ten days after placing the post-larval spiderling in the cup before beginning trials. During this period the spiderlings moved, molted to the first instar, and built their first prey capture webs. I gave each spiderling one prey every three days; I gave them their fourth prey seven days after the third prey; they molted from first to second instar during the period following the third prey.

Behavior trials. I fed a spiderling by placing a prey in its cup. One type of prey was a worker of the ant of *Paratrechina longicornis* (Formicidae; hereafter “ant” or “A”). The other type of prey was a wild fruit fly (*Drosophila melanogaster*; hereafter “fly” or “F”). Both prey measured approximately 2.5 mm long, but they differed in the difficulty that spiderlings had in subduing them; ants are more difficult prey than flies. Spiderlings last longer to touch, wrap, and immobilize ants than flies. Also, ants can damage legs of the spiderlings (I. Escalante in prep.). The same differences in difficulty have been found in several spider species (Robison & Olizarri 1971, Edwards & Jackson 1994, Jakob 1994, Viera 1995, Martins-García & Japyassú 2005, Pekár 2004, 2009). Even though I did not observe either of these two species as prey items in *P. globosus* sheet webs during incidental observations in natural conditions, other species of ants and flies are common prey for pholcids (*P. phalangioides*: Netwing 1983, Kirchner & Opderbeck 1990, and *P. globosus*: Eberhard 1992).

After introducing the prey into the cup, I used a SONY HandiCAM DCR-VX 1000 camera with three macro lens (+4 X each) to record onto mini-DV tapes at 30 frames per second speed. In an pilot group (see below), I taped a narration of the attack with a voice recorder while watching through a 20X Olympus SZ30 dissecting scope, and did not make video recordings. Some of the fine scale details and duration of the handling behaviors (see below) were not available in the voice recordings.

Treatments. I established five different treatment groups, each one with a different sequence of prey types. In the first, preliminary group I fed spiderlings with four ants in their first instar and two after molting, but only 15 of 32 spiderlings attacked a fourth prey in the first instar before molting. Therefore, to standardize the number of attacks I only gave three prey during the first instar in all four of the other groups. Treatment groups are indicated by the prey sequence, with capital letters representing preys attacked during first instar, and lower caps letters representing prey attacked in second instar: AAAf treatment was fed three ants during the first instar, and one fly during the second instar. The other groups had the following prey sequence: AAFA, FFAa, and FFFf. Spiders were randomly assigned to treatments after hatching; they came from ten broods (11 ± 9 per brood).

Video analyses. I analyzed the videos with the software Etholog 2.2 (Ottoni 2000) to obtain the duration, order and frequency of each behavioral unit in attacks as well as events involving the prey themselves (prey fell in sheet, prey is immobile, etc.). I measured 11 attack behavior units as qualitative response variables, in four different attack modules (see below, and I. Escalante in prep. for detailed descriptions). These variables were not strongly correlated ($r < 0.50$) within each other, and were from apparently independent and discrete biological processes in the attack. The variables in the detecting module were the times to detect the prey, to approach it, the delay between those two events, the delay between touch and initiation of wrapping, and the duration of the initial bout of touching the prey. The variables in the wrapping module the total time spent wrapping the prey, and the number of bouts of wrapping. The variables in the biting module were the total number of short bites, and their durations (excluding the final bite). The variables in the handling module were the time spent adding threads, the time spent touching prey after the first bout of wrapping had occurred, the time spent cutting threads, and the time spent moving the prey in the web. Qualitative variables included whether the spiderling performed a prey pull during the attack, and if the prey escaped from the web; both of these behaviors were grouped in the detecting prey attack module.

Statistical analysis. To investigate if the attack behavior of spiderlings changed during the series of attacks I performed generalized linear model analyses (GLM) in which I used the attack number as a fixed factor predictor ordinal variable, and spiderling identity as a random factor predictor to investigate if there were individual

differences. As response variables I used the 11 continuous predatory variables of the first three attacks, one model for each attack variable, in every treatment group. For these analyses I pooled the values of the first two attacks in the AAAf and AAFA groups, since those spiderlings had the same experience before the attack, giving me a larger sample size to test the GLMs. I also joined the first two attacks in the FFAa and FFFf groups. I did not use a multivariate statistical analysis because the fine scale behaviors represented different biological process, and most of them resulted in different patterns (see Results). Also, some analyses were not appropriate for this data. For example, in a Principal Component Analysis the first three components explained >60 % of the total variance.

To investigate whether the number of previously attacked ants correlated with subsequent behavior I compared each attack variable in the third attack of the group AAAf versus FFAa, and FFFf versus AAFA. Also, to test for changes after molting I compared the fourth attack of spiderlings, comparing AAAf versus FFFf, and AAFA versus FFAa. Due to the small sample size and the substantial variance, I also performed Mann-Whitney U analyses, one for each prey type in all the 11 quantitative attack variables.

In the groups that attacked the same prey type in the first and second instar (AAAa and FFFf) I tested whether the attack behavior changed from the first to the third attack, with Mann-Whitney U tests. Then, I compared the behavior in the third and fourth attack, to test whether molting was associated with changes in predatory behavior.

I also analyzed two qualitative attack variables: whether pull-prey occurred (1) or not (0); and whether there was one prey escape from web (1) or none (0). Qualitative analyses were appropriate because 0 and 1 frequency values accounted for 79% and 72% of the cases, respectively. For these variables I made the same comparisons mentioned above, but used proportion chi square tests (Zar 1999).

To test whether the attack behavior became more homogeneous with increasing numbers of attacks I performed Levene's tests for homogeneity of variances in the AAAa group. Additionally, I calculated the slope of the linear regression of the time to wrap prey in the AAAa group, to see if the durations decreased (negative slope), increased

(positive slope) or did not change (slope statistically not different than 0). All analyses were performed with STATISTICA 8.0 (StatSoft, Inc. Tulsa, Oklahoma, USA, 2007).

I did not correct my alpha values with the sequential Bonferroni correction (Rice 1989) because that method would reduce the probability of finding a general pattern in significance in this complex data analysis, with so many response and predictor variables. Additionally, even though my comparisons came from the same dataset, every test compared biologically and statistically different behaviors as independent subjects. There are also mathematical and practical objections to the Bonferroni correction (Moran 2003, Nakagawa 2004).

RESULTS

The general patterns of attack behavior are shown in Fig. 1 and Tables 2 & 3. The statistical comparisons of those data are summarized in Table 1, which is described in the following subsection. Color codes are used to indicate statistically significant evidence for or against the learning or maturation hypotheses, or both (see Table 1 for two exceptions) by meeting the original prediction(s).

1. Changes between attacks and treatments. The general patterns from Table 1 indicate that there were multiple effects of experience with previous difficult prey, most of which supported the learning hypothesis. The effects that most consistently favored learning were in the third attacks of the spiderlings in which the prey type changed in their third attack (AAFa and FFAa; blue boxes in Table 1). This because in the third attack AAFa decreased the prey pull proportion, the duration of wrapping, and the number of short bites, but those changes did not occur in FFAa (Fig. 1, Tables 1 & 3). In contrast, there were few changes in the behavior of spiderlings that attacked the same prey type repeatedly, and nearly all were only weakly significant (Table 1). Therefore, the alternation of easy and difficult prey sequence was associated with changes in predatory behavior, and the difficulty of prey seemed to be associated with more increases in efficiency.

The predatory behavior of spiderlings throughout attacks in the first instar also changed in ways that supported both the learning and the maturation hypotheses. However, no distinction between hypotheses was possible (green boxes in Table 1). For

example the proportion of prey that escaped decreased in spiderlings in which prey type was changed (AAFa and FFAa), the duration of initial touches to the prey decreased in AAAf and AAFa, as well as the touch –wrap prey delay; and FFFf decreased the duration to approach towards the third prey. AAFa and FFAa decreased only the prey escape proportion and the duration adding threads to the prey (Fig. 1, Tables 1 & 2). Therefore, the changes in only certain prey sequences made not possible to separate learning or maturation.

Another general trend was the lack of evidence against both hypotheses (grey boxes in Table 1). In a few cases (yellow boxes) the high P values (> 0.95) suggests significant confidence to accept the hypothesis that behavior did not change in certain behaviors (mostly in detecting and handling behaviors) throughout attacks in the same treatment group.

2. *The effect of many previous difficult prey.* When comparing the third attack of the learning hypothesis was mostly favored, because after having attacked a great amount of difficult prey, spiderlings tended to improve the predatory behavior after molting (but not in the first instar). For instance, comparing the groups that attacked a fly in their third attack AAFa and FFFf did not differ in any of the 13 behavior variables (Fig. 1, Table 1). However, comparing the groups that attacked an ant in their third attack AAAf spent more time in the initial prey touches, had a marginally significant higher touch – wrap delay and a higher prey escape proportion than FFAa (Fig. 1, Table 1). In the fourth attack, comparing the behavior towards an ant AAFa approached ants faster and had a marginally smaller delay between detection and touching than FFAa (Fig. 1, Table 1). Also, AAFa wrapped the ant marginally faster, spent less time adding threads, cutting threads around the prey and moving the ant than FFAa. When attacking a fly in the fourth attack AAAf wrapped a fly as fourth prey faster, and spent more time adding threads to it than FFFf (Fig. 1, Table 1). The proportion of prey escapes in the fourth attack was the same in spiderlings that attacked ants ($P = 0.24$) or flies ($P = 0.64$) (Table 2). Spiderlings that attacked an ant in their fourth attack had the same prey pull proportions ($P = 0.63$). However, ants were pulled in greater proportions than flies in the fourth attack of ($P = 0.05$; Table 3), when comparing the two treatments that attacked an ant as fourth prey versus a fly.

3. *Changes with molting.* Most of the behaviors analyzed did not change in the spiderlings that attack the same prey type in their first and second instar (AAAa and FFFf). However, some changes supported the maturation hypothesis, because the behavior improved after molting (comparing 3rd and 4th attack) but not before (comparing 1st and 3rd attack). After molting, AAAa showed a statistically marginal increase in the duration of wrapping the prey (Table 1 & 4). Also, FFFf after molting had a statistically marginal decrease in the duration to approach prey, and they decreased the prey escape and prey pull proportion (Table 2 & 3).

Additional observations. Individually, spiderlings differed in nine of the 11 behavioral units of the attack (at least one treatment in every behavior; Table 5). Therefore there were individuals that consistently spent more time in most variables than other spiders. Additionally, 13 spiderlings did prey pulls in all the four attacks, 6 individuals did not any prey pull, and 30 individuals had intermediate proportions (Fig. 2).

The prey did not escape from the web in 54% of the attacks ($n = 289$) In 17 % of the times the prey escaped once, 10 % twice, and the remaining 19 % the prey escaped from three to ten times (Table 2). Spiderlings did not pull the prey in 45% of the attacks ($n = 283$), in 34% they pulled once, in 12% twice, and in the remaining 9% they pulled three or more times (range: 3 – 12). Only in the first attack did spiderlings pull more than four times.

The variances in the attacks decreased towards the third prey compared with the first prey in the mean duration of the touch – wrap prey delay, the duration of initial prey touches, the duration of wrapping prey, and the number of short bites (Table 5). The wrapping time decreased in five spiderlings, did not change in 14, and increased in eight.

Spiderlings did their final bite and fed on fruitflies more commonly between the eyes (20 – 45%); and that proportion did not change throughout attacks ($X^2 = 3.79$; $df = 3$; $P = 0.29$; Table 6). In contrast, spiderlings performed their final bite and fed on ants most often (60 – 84%) in a femur / tibia joint; and that proportion did not change in the four attacks ($X^2 = 4.08$; $df = 3$; $P = 0.25$; Table 6). Therefore, spiderlings did not seem to change the part of the prey where they more commonly fed.

DISCUSSION

All attack behavior units of *P. globosus* spiderlings were present in the first attacks of inexperienced spiderlings on prey, indicating that learning is not necessary for their expression. The durations and frequencies of different aspects of predatory behavior differed in subsequent attacks, and the changes generally showed stronger effects of learning than of maturation. Learning was evident when the first prey were relatively difficult and were followed by relatively easier prey. Spiderlings decreased the time spent in six behaviors when an easy prey followed difficult prey, but only two decreased when a difficult prey followed easy prey, supporting the learning hypothesis. Behavior did not change when spiderlings attacked the same prey repeatedly. Apparently, spiderlings had a hidden learning (Shettleworth 2010) that promoted decreases in the duration of certain behaviors only when prey type was changed. The contrast in the stimuli that spiderling received through the prey type could cause this learning to become evident.

Learning seemed to increase the efficiency of predation, and this would be favored by natural selection. This is particularly important in contexts where the stimuli that an animal receive in its first stages vary in quantity and quality, such as the prey type availability for spiders. The learning hypothesis received support because in the AAFA group durations decreased in attacks on the third prey in more aspects of behavior compared to the treatment groups in which prey type was constant or the FFAA (in which they attacked only one previous difficult prey). The attack behavioral units that met the prediction of the learning hypothesis were the prey pull proportion, the duration of wrapping and the number of short bites. This suggests that having more previous difficult experiences (attacking ants) improves future attack behavior. Ants were a more valuable lesson, probably because ants were a more difficult prey type compared with fruit flies (I. Escalante in prep.). They made larger and stronger movements in the web, they attempted to bite with their mandibles, and their antennas and legs were more active compared with those of flies. Therefore, in subduing a more difficult prey (an ant), a spiderling may learn to improve its behavior (as suggested by Ades 1989).

The maturation hypothesis received support for a few aspects of the attack: after molting spiderlings wrapped ants more quickly, approached flies earlier and the

proportion of flies that escaped the web decreased. Changes in the body in the second instar may be associated with this pattern. The increase of their body size, leg length (Foelix 1996), and probably the thickness of silk lines after molting may have contributed to the improvement of predatory skills. After molting, spiderlings retained information (Shettleworth 2010) on the previous prey they had attacked to decrease the time spent in predatory behaviors, as noted for *M. vatia* spiderlings (Morse 2000). This is not surprising, given that molting is a phenomenon involving the cuticle, not the central nervous system.

The durations of some behaviors decreased in later attacks in certain groups, therefore supporting both learning and maturation hypothesis, but the combinations of prey did not allow these hypotheses to be distinguished. There were decreases towards the third attack in the durations of three attack behaviors in the AA Af, AA Fa, and FFF f treatments. This left uncertain whether the changes were associated with the change of prey type or the effect of accumulated attacks. Additional changes of the same sort occurred in the proportion of prey that escaped and the duration of adding threads in AA Fa and FF Aa. Therefore, one noticeable pattern is that the predatory behavioral units (and modules) were associated differently with learning or maturation, or none. However, in certain behaviors the variance was high, and my sample size was not large, so the power of the statistical analyses was not high.

Some behaviors did not change after consecutive attacks in any group: the time to detect, the delay between detection and touching the prey, and some handling behaviors. Those behaviors that did not change, and in which neither learning nor maturation seemed to occur, may be associated with the numbers and lengths of silk threads attached to the substrate, for example the duration to detect prey. The small number of short threads in the cups was apparently adequate for detecting prey, because detecting time did not decrease even though substrate thread number tended to increase with time (I. Escalante in prep.). Perhaps, some of those behaviors are related with the morphology and defensive behavior of each prey type. Also, increased speed in accomplishing some behaviors like touching prey, cutting threads near it or moving it in the web may not be crucial to improve if reducing the time spent does not make a difference in whether the spiderling subdues it or not.

The effect of previous attacks on difficult prey seemed to be more strongly associated with an improvement in predatory behavior after molting. Second instar spiderlings that had attacked three or two ants previously spent less time in eight behaviors in their fourth attack than spiderlings that attacked 0 or 1 ants. These findings support the hypothesis of learning in the attack behavior. In contrast, the lack of differences in five (of 13) attack behaviors in the fourth attack (first after molting) between different prey sequence groups do not support any hypothesis. That condition does not support the maturation hypothesis because the similarities were not strong enough ($P < 0.95$) to accept statistically that behavior was equal between spiderling treatments.

Wrapping is the most crucial behavior to successfully subdue a prey by *P. globosus* spiders (Japyassú & Macagnan 2004, Barrantes & Eberhard 2007), especially a difficult prey like ants. In the project the wrapping duration decreased after consecutive attacks. I assume that a greater time wrapping a prey would correlate with a greater amount of protein spent in the silk, and ATP metabolized (Jakob 1991, Venner *et al.* 2000). In Pholcidae there are no indications that wrapping silk is subsequently ingested, as it remains on the outer surface of the prey and the spider ingests only the contents of the prey. Hence, wrapping in less time could be beneficial to economize on those resources if they are substantial.

Successful attacks are obviously crucial for a spiderling to ensure feeding. In 45% of the attacks the prey escaped at least once. The escape rate of prey from adults in eight orb weaving spider ranges from 17 to 82% (Eberhard 1990), and it is 44% in early instar *N. clavipes* (Brown & Christenson 1983). It would be advantageous for spiderlings to decrease the possibility of losing the prey, especially since pholcids live in high density in their first instars (and as I noted for *P. globosus*) and sometimes are aggressive and eat each other (A. Peretti, pers. com.). In another pholcid (*H. pulchei*) nymphs in the field reach adulthood faster when fed with more prey, suggesting that they are food-limited (Jakob & Dingle 1990).

It is advantageous for spiderlings to avoid being bitten or damaged by sudden movements of the prey, which might occur if the prey is not immobilized quickly enough or is left with poorly wrapped appendages (pers. obs, and Morse 2010 for *M. vatia*). The two spiders which were injured while attacking ants document this danger,

as well as the advantage of more secure wrapping. The time the spiderling is wrapping the prey would also make the individual very conspicuous for predators and parasites, and constitute a selective force favoring spiderlings able to decrease wrapping times.

Spiderlings differed individually in their attack behavior in all the attack modules, the spider identity accounted for a significant part of the variance in predatory behavior at least in one group. Some spiderlings decreased, some increased, and others showed no change in the time spent wrapping their prey. Hence, some spiderlings were more skilled since emergence, while others improved. This variation was also present when the mean group variance significantly decreased in successive attacks in six behaviors, so in general spiderlings spent a similar (and lower) time wrapping prey after consecutive attacks. *P. globosus* spiderlings also showed individual differences because more than half of the individuals were consistent in doing a prey pull in all the attacks or not doing any. This individual variation suggest repeatability in the behavior of spiderlings (Bell *et al.* 2009, Rodriguez & Gloudeman 2011) since the moment they moult to the first instar, as has also been noted in *N. clavipes* (Higgins 2007).

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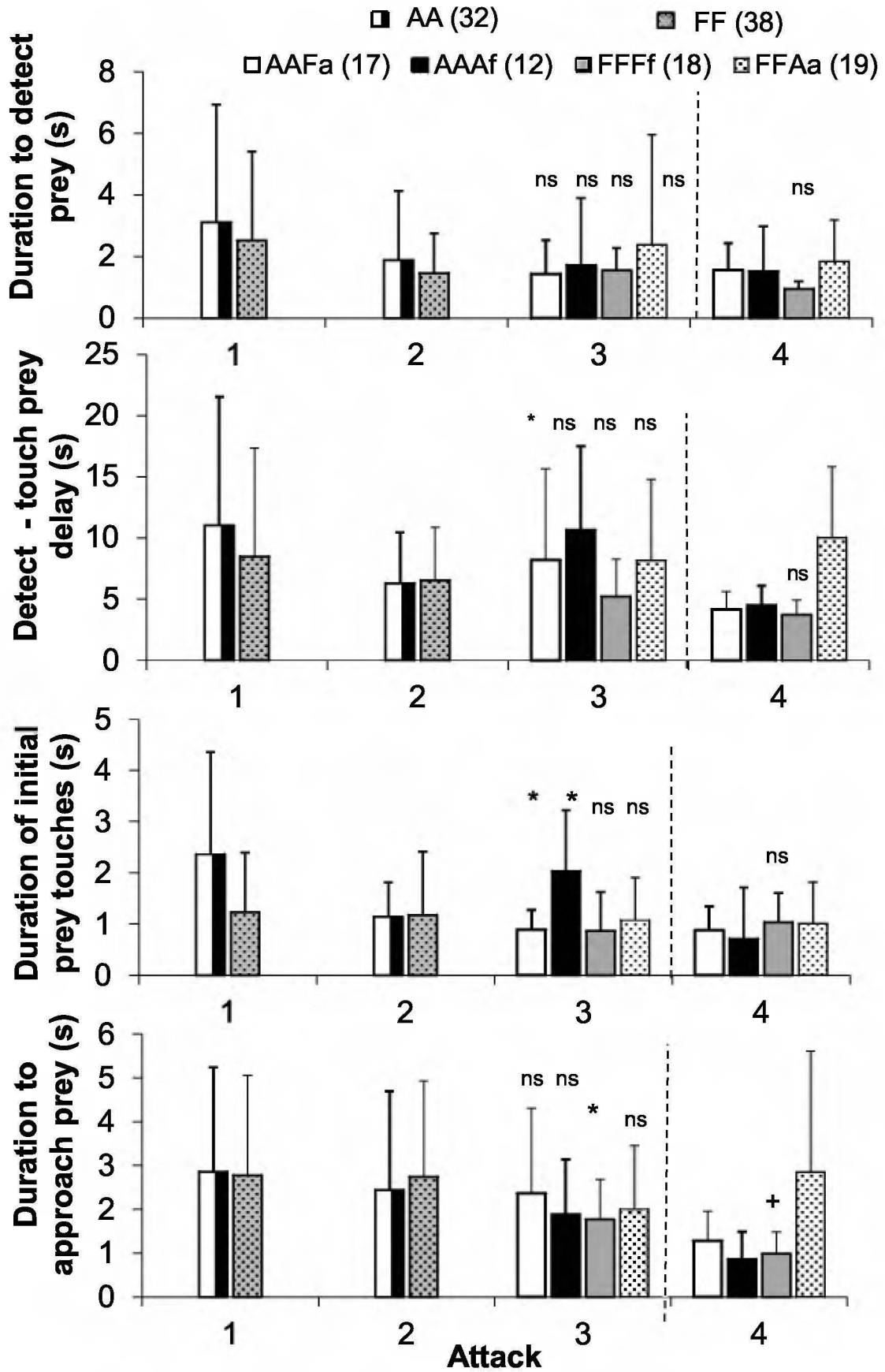
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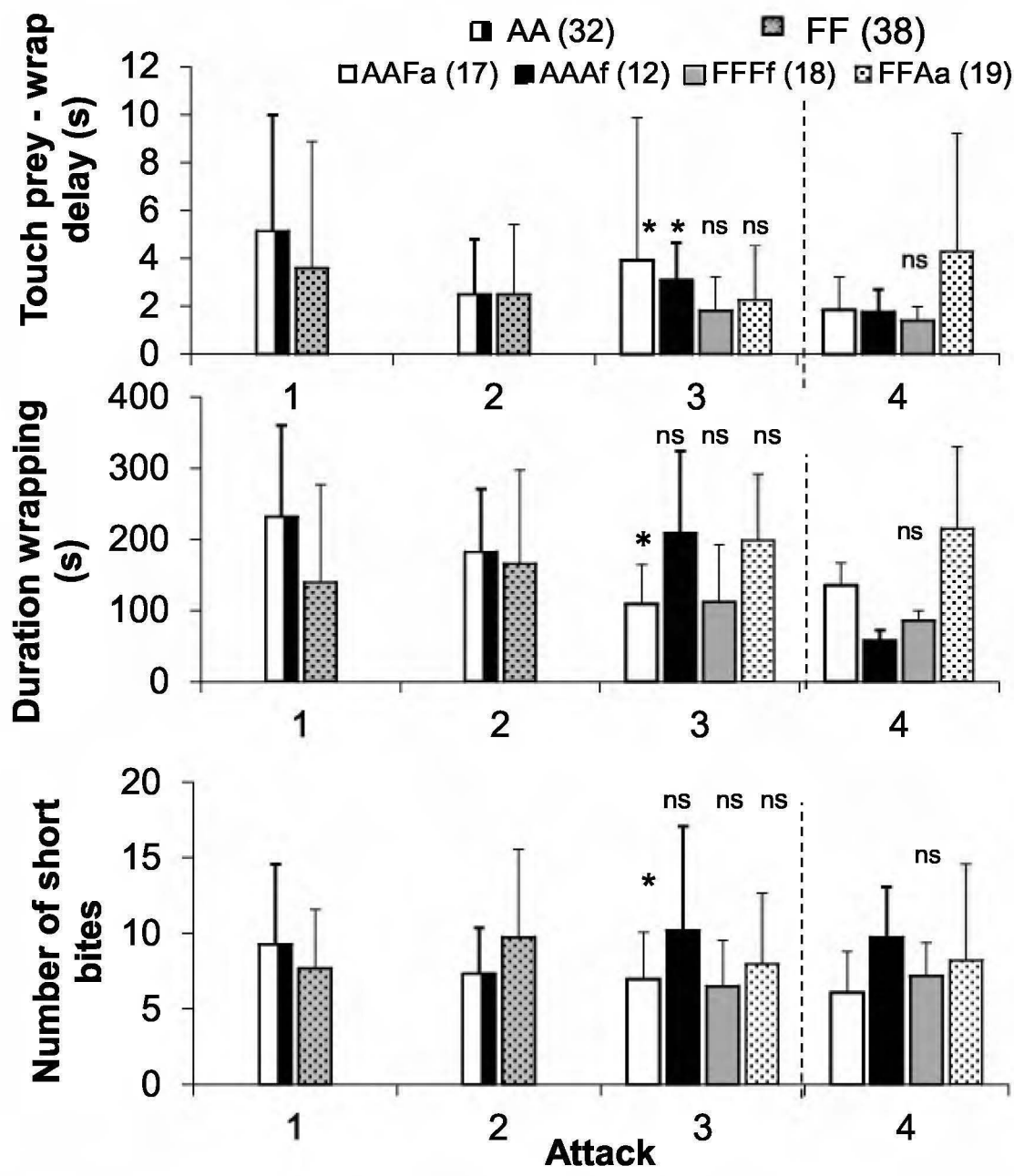
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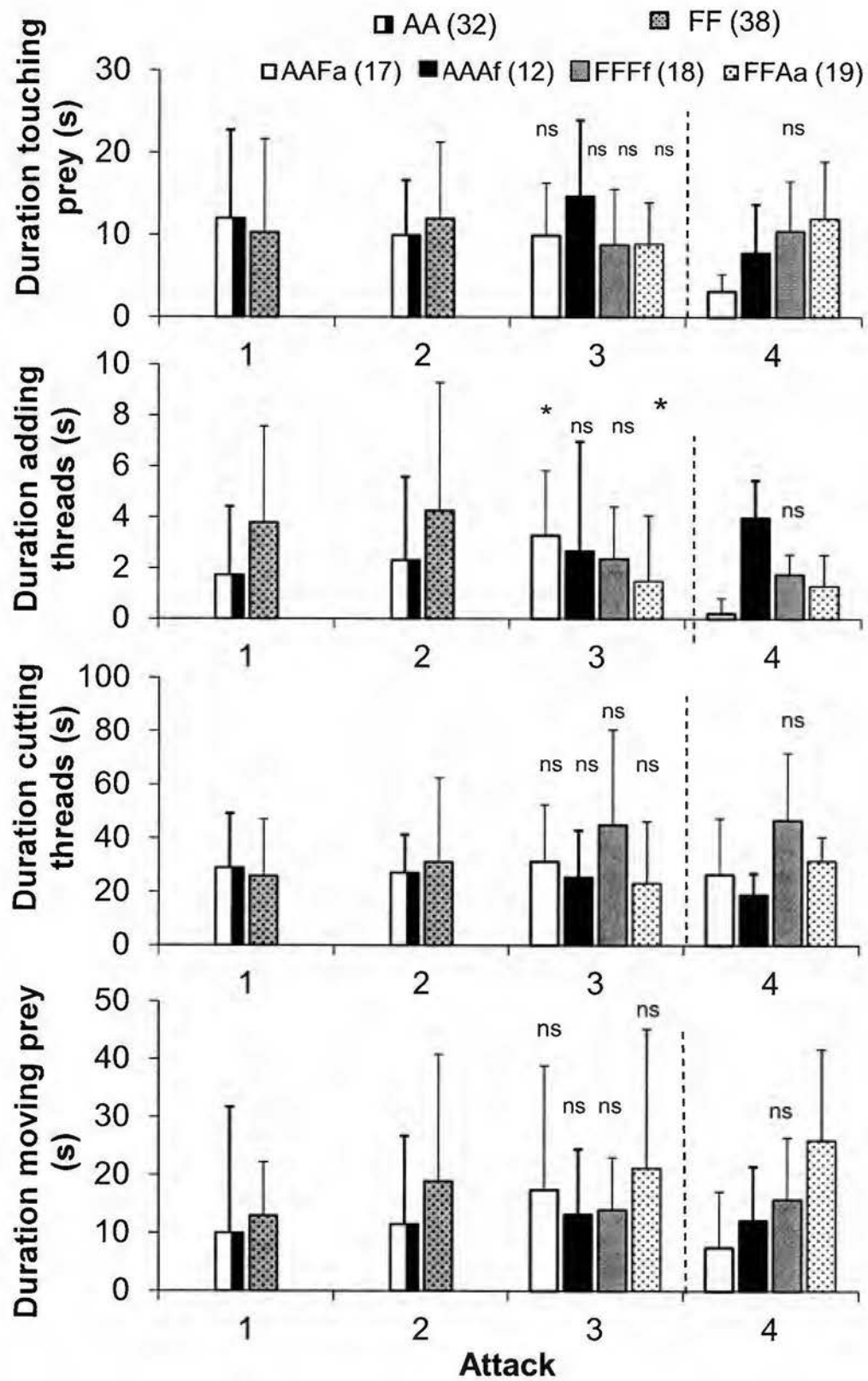


Fig. 1. Mean + one standard deviation of the quantitative response variables in four attacks to ants and flies by spiderlings during their first and second instar. The dashed vertical line separates instars. Each treatment involved a different sequence of prey types (noted in the legend above). The bars in the first two attacks represent two treatment groups joined because they attacked the same prey type, and in all cases there were no differences in between the spiderling treatments that attacked the same prey type ($P > 0.05$). In the third attacks the groups are separated on the basis of their prey sequence (note the colors and pattern merged). The sample size for the first attack is in parenthesis. Above each bar in the third attack is noted if the behavior of each treatment group differed throughout the first three attacks. * = significant change throughout attacks ($P < 0.05$), + = marginal significance ($0.10 < P > 0.05$), and ns = not significant changes ($P > 0.05$). The symbols above the FFFf bar in the fourth attack represent the comparison between the third and fourth attack. Exact P values are shown in Table 1.

Table 1. The P values of the generalized linear models (GLMs) for the change of behavioral units throughout attacks of *P. globosus* spiderlings to ants (A) or flies (F). Different comparisons are shown, see text for further details. Color codes are shown above the table, and the description of their logic is presented in the text. The vertical pair of comparisons in a box with black outline means that color given is based on both comparisons: the comparison for one group being significant and non-significant for the other one.

Support to none	Against both	Only maturation	Only learning	Support to both. No distinction is possible
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Comparison / data analysis	Detecting							Wrapping	Biting	Handling			
	Duration to detect prey	Detect - touch delay	Duration of initial prey touches	Approaching prey	Touch - wrap prey delay	Prey - pull prop.	Prey escape prop.			Duration wrapping	Number of short bites	Duration touching prey	Duration adding threads
Changes throughout attacks													
AAAf	0.10	0.11	0.005	0.72	0.004	0.11	0.65	0.18	0.24	0.47	0.90	0.97	0.89
AAFa	0.11	0.07	< 0.001	0.76	0.04	0.04	0.01	0.007	0.01	0.30	0.02	0.96	0.36
FFAa	0.13	0.26	0.61	0.85	0.47	0.30	0.03	0.58	0.14	0.37	0.01	0.25	0.27
FFFF	0.22	0.10	0.97	0.04	0.51	0.22	0.50	0.41	0.14	0.87	0.90	0.69	0.16
Effect of previous difficult prey													
Third attack: AAAf vs FFAa	0.37	0.20	0.05	0.97	0.07	0.56	0.01	0.80	0.37	0.13	0.49	0.39	0.80
Third attack: AAFa vs FFFF	0.78	0.11	0.19	0.15	0.22	0.10	0.16	0.71	0.74	0.98	0.50	0.55	0.80
Fourth attack: AAAf vs FFFF	0.80	0.76	0.23	0.37	0.62	0.05	0.64	0.01	0.15	0.31	0.004	0.31	0.37
Fourth attack: FFAa vs AAFa	0.60	0.08	0.79	0.02	0.80	0.63	0.24	0.07	0.48	< 0.001	0.02	0.005	0.004
Behavior with same prey type													
AAAa: 1st vs 3 rd	0.94	0.58	0.79	0.35	0.79			0.93	0.92				
AAAa: 3rd vs 4 th	0.35	0.25	0.51	0.68	0.50			0.06	0.58				
FFFF: 1st vs 3rd	0.11	0.22	0.86	0.22	0.55	0.65	0.31	0.42	0.12	0.36	0.96	0.92	0.33
FFFF: 3rd vs 4 th	0.14	0.14	0.47	0.07	0.47	0.001	0.03	0.47	0.72	0.80	0.47	0.78	0.72

Table 2. Proportions of attacks in which the prey escaped from the web during the first interaction with the spiderlings (n) in different treatments and attacks. The chi square *P* value from the comparison of the proportion of the first three attacks (in the first instar) is shown after the third attack.

Treatment	Proportion of attack in which prey escaped								
	First	n	Second	n	Third	n	<i>P</i>	Fourth	N
Ant-Ant-Ant-fly	0.90	10	0.67	12	0.73	11	0.65	0.09	11
Ant-Ant-Fly-ant	0.60	20	0.57	21	0.28	18	0.01	0.36	14
Fly-Fly-Ant-ant	0.61	23	0.47	19	0.26	19	0.03	0.50	10
Fly-Fly-Fly-fly	0.65	23	0.57	21	0.50	20	0.50	0.17	6

Table 3. Proportions of attacks in which prey was pulled from substrate by spiderlings (n) in different treatments and attacks. The chi square *P* value from the comparison of the proportion of the first three attacks (in the first instar) is shown after the third attack.

Treatment	Proportion of attacks with prey pull								
	First	n	Second	n	Third	n	<i>P</i>	Fourth	n
Ant-Ant-Ant-fly	0.80	10	0.50	12	0.45	11	0.11	0.45	11
Ant-Ant-Fly-ant	0.80	20	0.64	21	0.29	18	0.04	0.71	14
Fly-Fly-Ant-ant	0.68	23	0.69	19	0.57	19	0.30	0.60	10
Fly-Fly-Fly-fly	0.59	23	0.20	21	0.67	20	0.22	0.00	6

Table 4. Mean \pm one standard deviation of the behavior by first and second instar spiderlings of *Physocyclus globosus* (Pholcidae) in three attacks on workers of *Paratrechina longicornis*. n = sample size. I report the F and P value for the Levene's test for homogeneity of variances in the three attacks of the first instar. These results have the same pattern when the fourth attack was included (tests not shown). * = significant change from third to fourth attack, at the $P < 0.05$ level.

Attack	n	Duration to detect prey (s)	Detect - touch prey delay (s)	Duration approaching prey (s)	Touch - wrap prey delay (s)	Duration of initial prey touches (s)	Duration wrapping (s)	Number of short bites (s)
First	13	1.78 \pm 1.25	2.10 \pm 1.76	2.63 \pm 2.81	2.52 \pm 2.71	2.52 \pm 2.71	87.33 \pm 72.05	8.30 \pm 6.03
Third	13	1.78 \pm 0.93	2.12 \pm 2.82	2.33 \pm 2.36	2.60 \pm 2.55	2.60 \pm 2.55	83.19 \pm 34.57	9.85 \pm 5.96
Fourth	13	1.89 \pm 0.97	1.92 \pm 1.26	2.58 \pm 2.21	2.58 \pm 2.18	2.54 \pm 2.16	108.93 \pm 62.81 *	8.96 \pm 6.74
Levene's homogeneity of variances test	F	0.74	0.91	0.99	3.24	2.44	4.06	5.66
	P	0.48	0.41	0.38	0.04	0.09	0.002	0.001

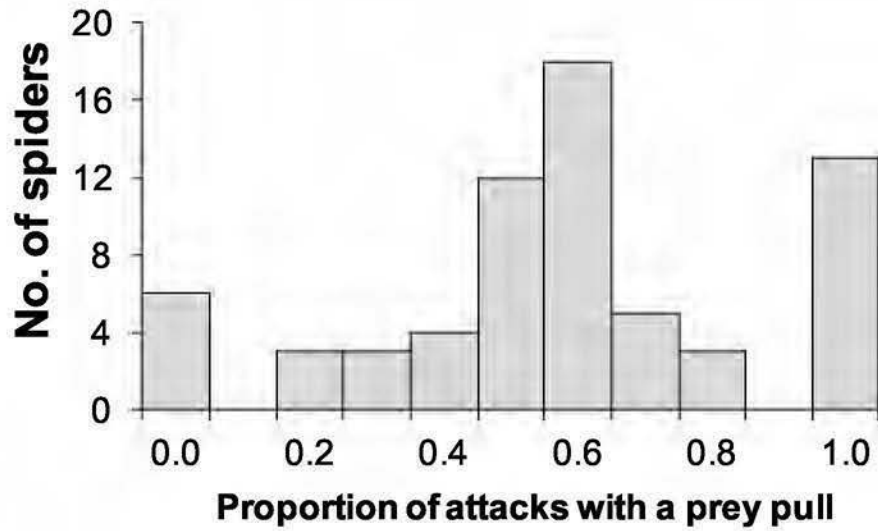


Fig. 2. Numbers of spiderlings with different proportion of attacks in which they performed at least one prey pull, combining all attacks (range: two to six attacks; 74 spiderlings, 283 attacks). Frequency of proportions differed from an even distribution ($\chi^2 = 25.69$; $df = 5$; $P < 0.001$).

Table 5. Statistical results showing individual differences in the attack behavior, with the spiderling identity factor in the GLM in Table 1. Differences in three attacks in four spiderlings treatments with different prey sequence. * = significant effect, $P < 0.05$.

Attack variable		Treatment			
		AAAf	AAFa	FFAa	FFFf
	DF	32/39	32/45	44/57	44/56
Duration to detect prey	F	1.2	1.12	1.01	1.05
	<i>P</i>	0.29	0.36	0.49	0.42
Detect - touch prey delay	F	0.64	1.07	0.88	1.84
	<i>P</i>	0.9	0.41	0.66	0.02*
Duration approaching prey	F	1.3	1.01	1.63	1.24
	<i>P</i>	0.22	0.47	0.04*	0.22
Duration of initial prey touches	F	1.03	1.58	0.84	1.09
	<i>P</i>	0.45	0.08	0.72	0.37
Touch - wrap prey delay	F	1.1	1.34	2.27	2.76
	<i>P</i>	0.39	0.18	0.002*	<0.001*
Duration wrapping	F	0.94	0.77	1.55	1.35
	<i>P</i>	0.56	0.75	0.06*	0.15
Number of short bites	F	1	2.93	1.07	1.37
	<i>P</i>	0.49	<0.001*	0.41	0.14
Duration adding threads	F	2.08	2.3	1.29	1.73
	<i>P</i>	0.02*	0.001*	0.19	0.03*
Duration touching prey	F	1.17	1.79	1.12	0.94
	<i>P</i>	0.33	0.04*	0.35	0.58
Duration cutting threads	F	1.93	1.72	0.77	1.38
	<i>P</i>	0.03*	0.05*	0.82	0.14
Duration moving prey	F	1.26	1.07	1.17	1.94
	<i>P</i>	0.25	0.41	0.3	0.01*

Table 6. Frequency of attacks in which *Physocylus globosus* (Pholcidae) first and second instar spiderlings fed from certain segments or body parts of the prey along four attacks.

Prey's body segment	Prey segment or part of final bite	Fruitflies (<i>Drosophila melanogaster</i>)				Ants (<i>Paratrechina longicornis</i>)			
		1 st	2 nd	3 rd	4 th	1 st	2 nd	3 rd	4 th
Antenna	Radicule / scape joint					2	6	3	2
	Scape / pedicel joint					3	8	5	3
	Mouth parts	3	2	2	2				1
	Eye		2						
Head	Between eyes (frontal)	6	6	13	4				
	Head, dorsal	1		1					
	Head, lateral	5	1	1	2				
Thorax	Nape (head / thorax joint)	1	3	1	3		1		
	Thorax, dorsal	4	6	1	3				
Abdomen	Abdomen, dorsal	5	1	3	1		1		
	Abdomen, ventral	1		1		1			1
	Trochanter	5	1	1	1		1		1
Leg	Femur / tibia joint	4	3	4	1	11	13	18	3
	Tibia / tarsus joint		3	1		2	2	1	5

Artículo 3

Ontogeny of exploration and web construction differ with age and sex in the spider *Physocyclus globosus* (Pholcidae)

Ontogenia de la exploración y la construcción de tela difieren con la edad y sexo en la araña *Physocyclus globosus* (Pholcidae)

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Abstract. Differences in reproductive interests between the sexes can affect web construction behavior. This behavior is known to vary also across ontogeny. This study tested whether exploration and web building behavior varied with age or sex in the spider *Physocyclus globosus* (Pholcidae), which weaves an irregular, domed sheet web with a tangle above. Fifth instar juveniles, adult males, and adult females were placed in cages with four interconnected chambers. Most of juveniles and adult females (but only half of adult males) wove a sheet in only one chamber, and rested there during the day. Adult males and adult females attached larger numbers of threads to the walls than did juveniles in the sheet chamber, but juveniles added more new threads there over time. Juveniles laid 91% of their threads in the sheet chamber; comparable numbers were 55% and 41% for adult females and adult males, respectively. Adult males attached more threads outside the sheet chamber than adult females or juveniles, and all spiders added threads outside the sheet chamber throughout the observation period, suggesting repeated abandonments of the web there for exploration. Adult females built more dense sheets than adult males or juveniles. The reduced rate of exploration by juveniles suggests that they establish webs more quickly to increase the possibility of capture prey and rapid growth. Juveniles did not differ in their exploration or web construction patterns based on sex. Adult males explored more often, perhaps looking for mates. Adult females perhaps modified their web structure after establishing, possibly to improve the prey capture rate.

Key words. Gumfoot, thread attachment, web density, web abandonment.

Differences in the costs of gametes (anisogamy) in males and females explain differences not only in the sexual secondary characters, but also in behavior and physiological activity (Andersson 1994). In spiders sexual size dimorphism is common in many spider families, and size differences between males and females reach up to two or three orders of magnitude (Foelix 1996). Adult males and females also differ in behavior (Walker & Rypstra 2002, Li & Kuan 2006). Adult female spiders are generally less mobile and more likely to stay in the same place for longer periods than males, which are more

mobile, frequently do not build webs after maturity, and feed less than females (Foelix 1996). In the spitting spider *Scytodes pallida* for instance, males relocated their web more often than adult females (Li & Kuan 2006).

On the other hand, in some families with little size dimorphism (such as Pholcidae) males continue building webs, foraging, and hunting after maturity (Eberhard & Briceño 1983, Edwards 2011). Adult pholcid females receive adult males, copulate, and oviposit in the same web; and spiderlings even stay a few days in the maternal web after emerging (Eberhard 1992a, Jakob 1991). In a population of *Physocyclus globosus* in large, underground tunnels, adult females stayed in the same site for several (2 – 8) days, but adult males stayed in the web of adult females less than two days, and one adult male moved 50 – 60 m (Eberhard 1992a). In *Pholcus manueli* a high dispersal of adult males and adult females in the first 24 hours was found when they were released at a new site (Edwards 2011). Both males and females dispersed in the same proportion, especially when the sex ratio of the population was biased toward males; and the density of individuals had no effect (Edwards 2011).

Web construction and exploration of an environment differ during the ontogeny of some species. This behavioral shift between juveniles and adults may be due to previous experience (Shettleworth 2010), the costs of finding another appropriate site and of building another web in terms of energy and nutrients to attach silk relative to body size (Foelix 1996), or delayed dispersal from the maternal web in juveniles (Jakob 1994). Traditionally, spider juveniles are thought to perform behaviors similarly between sexes until they reach sexual maturity (Foelix 1996). However, behavioral differences have been found between male and female juvenile spiders (Singer & Riechert 1994, Persons 1999). In the pholcid *Pholcus phalangioides* newly emerged juvenile males were active for a longer time than female juveniles. Also, juvenile females ate more prey than juvenile males when prey was offered daily (Hoefler *et al.* 2010). Hoefler *et al.* (2010) suggest that males disperse more to avoid inbreeding, and to increase the number of females with which they can mate in the future. The logic of these ideas is not convincing, because it is likely that newly emerged spiderlings would aim to feed on as many prey as possible, and differences in reproductive behaviors would not necessarily affect their decisions during prior to

maturity. Hence, I decided to look for differences in behavior between sexes and juveniles of a different species of pholcid, *Physocyclus globosus* Taczanowski 1874. I did so by analyzing exploration and web construction, which may be different between sexes in adults, in a way that allowed me to determine gender differences in behavior among juveniles.

The web of *P. globosus* is an irregular and concave dome-shaped horizontal sheet that is approximately horizontal, with a tangle of abundant threads attached to the substrate that are mostly above the sheet (Eberhard 1992b). Webs occur in man-made structures such as houses, buildings, markets and even pipes and tunnels (Eberhard 1992a, Huber 1997, Peretti *et al.* 2006). Webs are often built in the intersections between walls and ceilings, as well as in gaps between furniture and walls (González 2007).

Little is known about web construction behavior in pholcids. In *Modisimus guatuso*, the spider begins by attaching threads to the substrate to form a web skeleton. The skeleton is extended and filled, and the sheet is completed by adding more lines (Eberhard 1992b). Some pholcid species builds gumfoot threads in the substrate (Japyassú & Macagnan 2004, I. Escalante in prep.). After reaching adulthood, both male and female pholcids continue to weave webs (Kirchner 1986, Eberhard & Briceño 1983) and capture prey. The web of *P. globosus* captures both walking and flying prey (Eberhard 1992a).

Most previous studies of webs compared finished webs (mostly orb webs, and mostly of mature females). However, pholcids and other sheet weaving spiders keep adding new lines attached to the substrate and threads with glue drops in the sheet gradually over time, even after the sheet is already functional (Pholcidae: Kirchner 1986, Japyassú & Macagnan 2004. Theridiidae: Blackledge & Zevenbergen 2007. Agelenidae: Rojas 2011). To my knowledge, no previous study has addressed the changes in web structure over several days between juveniles and adults females and adult males. Since sex and age affect behavior, investigating both factors would elucidate the plasticity of spiders in exploration and web construction during different life stages, in the two sexes.

I tested the hypothesis that the exploration of an environment and the sheet construction is associated with age and sex in *P. globosus*. This hypothesis predicted that

juveniles would explore less than adults, because they would aim to establish quickly and build a sheet web to capture prey and mature faster. Additionally, I tested if exploration and web structure change throughout the days, reflecting a continuous process in expanding the web and maintaining it. Also, differences were expected in the exploration and web construction between sexes in adults, but not in juveniles. Females were predicted to be more sedentary, while juvenile males or females were not expected to differ, as both would aim to feed and grow. Additionally, I asked whether the spiders would chose to build their sheets in large or smaller spaces when both options were provided.

MATERIALS & METHODS

Adult males and adult females were collected in buildings of the campus of Universidad de Costa Rica in San Pedro de Montes de Oca, San José, Costa Rica, 1160 m in elevation. I kept adult spiders in round plastic containers (7 cm upper diameter, 5 cm base diameter and 14 cm height), and fed them once a week with a stingless bee (*Tetragonisca* sp.). I bred them to obtain juveniles and I reared the juveniles under in laboratory conditions (mean of 20 °C and 80% relative humidity) until the fifth instar (out of 7 – 9 instars prior to maturity). The juveniles were then reared to maturity to determine their sex. I used virgin and already mated adult males and adult females; and some females had already oviposited. Mating status was not distinguished, however, so it was not possible to test for possible effects of mating status. Specimens were deposited in the Museo de Zoología of the Escuela de Biología, Universidad de Costa Rica.

Cages and observational design. Spiders were placed in one of 20 cages, made of cardboard shoe boxes lined with black matte construction paper. The cages had a mean (\pm 1 unit standard deviation) of 31.6 ± 1.3 cm long, 20.9 ± 1.2 cm wide and 11.6 ± 0.6 cm tall (Fig. 1A, B), with an average volume of 7650 ± 810 cm³. The top of the cages was formed by a plastic transparent sheet, to which spiders seldom attached lines. Each cage was divided into four chambers by adding four cardboard walls (7 cm long, 12 cm tall and 0.5 cm wide) covered with black paper. Two walls were placed in the middle of the narrow sides of the cage, and two on the wider sides, in which the position of the wall varied (Figs.

1A-1C). I tested whether the spiders preferred spaces of certain sizes to build their sheets. Cages with design A) had four chambers of the same size (Fig. 1A). Spaces were asymmetric in cages with design B), in which diagonally opposite corners had chambers of the same size (small or large chambers; Fig. 1B). One wall was at 70% and the other at 30% of the length of the wider side. Cages of design C) had adjacent chambers of the same size, where both walls were at 70% of the wider side length (Fig. 1C). The small chambers had an area of $140 \pm 15 \text{ cm}^2$, and large chamber were $193 \pm 14 \text{ cm}^2$. The sizes of the chambers were designed to bracket web measurements in natural conditions: adult female ($n = 14$) sheet webs were $202 \pm 25 \text{ cm}^2$, and sheet webs of unknown (but advanced) instar juveniles ($n = 13$) were $115 \pm 54 \text{ cm}^2$. The cages were always stored in a bookshelf, hence two chambers were always next to the wall and two closer to the front of the shelf, where the chambers had more exposure to light (from artificial and natural sources).

Measurements of exploration and web structure. Once a spider was placed in a cage, I observed it for 5 minutes. I observed three adult males and three adult females for a longer period of time (30 minutes) and then for five minutes every hour for six hours during the day. I noted their general body and legs positions and movements, and the thread attaching behavior.

I analyzed the exploration by counting the number of substrate threads the spider attached, which reflects their past activity (Vollrath 1992, Eberhard 1992b). In all of the spiders I made an estimate of the lines they had laid every three days over 18 days. I counted the number of threads attached (as seen in Fig. 12 & 13 of Schütt 1996) to the walls of the cage (most common) and also to the floor (Fig. 1E, G), and the chamber in which these lines occurred. To do so I lifted the plastic sheet and illuminated the cage with a mobile fluorescent light so that I could see each silk thread (following Blackledge & Zevenbergen 2007). All spiders eventually built a sheet web in one of the four chambers, and rested in it during the day (Fig. 1D, F). I named this the “sheet chamber”. Threads were added to the sheet in the following days. Most spiders also added lines in the other three chambers during the next 18 days, but there was never a complete sheet web in another chamber. Thus I considered all the threads outside the sheet chamber as “exploration” threads, and each set of new exploration threads to represent the abandonment of the sheet

(at least once in those three days; this could be an underestimate the number of times the spider abandoned the sheet). These new threads represent a combination of the number of explorations outside the sheet, and the amount of movement made by the spider (assuming that spiders did not remove previous threads as they moved). To quantify exploration behavior I calculated the proportion of threads in the sheet chamber over the total number of threads attached in the cage. A proportion of 1.0 meant that all threads a spider attached were in the sheet chamber; if a spider placed equal number of threads in all four chambers, the proportion was 0.25.

To measure the density of lines in the sheet, I placed four small round wood sticks (2 mm in diameter, 14 cm long) on the top of every chamber, separated 3 cm from each other, and placed at the same points each time a given sheet was measured. Observing from directly above, I counted the number of times that every stick was “crossed” by a silk line. I took the average number of lines crossing the four sticks in the sheet chamber as a measure of sheet density. Also, I checked whether the spider had built a sheet in its web. Hereafter “web” refers only to the irregular group of threads attached to the substrate, mostly in the chambers other than the sheet chamber; and “sheet” refers only the dense, domed structure where the spiders rested (Eberhard 1992b).

I made a preliminary test to determine whether feeding had an effect, comparing adult males that were fed versus unfed adult males ($N = 6, 5$) that were fed a stingless bee (*Tetragonisca aungustula*; Meloponinae) nine days after entering the cage differed in their thread numbers and sheet density. These features did not differ between the two groups of males. The fed adult males had 49.3 ± 9.7 threads attached in the sheet chamber, proportion of those threads in the cage 0.5 ± 0.1 , and sheet density 20.7 ± 12.3 threads. For unfed males, the corresponding numbers were: threads = 35.0 ± 15.4 , proportion = 0.5 ± 0.3 , sheet density = 16.4 ± 17.9 threads (Mann-Whitney U comparisons $P > 0.10$ for all comparisons). Therefore I decided not to feed the juveniles, adult females or the rest of adult males during the 18 day observation period in the other experiments.

Statistical analyses. To investigate if the age and sex of the spiders affected the exploration and web structure throughout the days I used generalized linear models (GLM). I used the spider groups (adult males, adult females and juveniles) and the days after

entering the cage as fixed factor predictor variables, and the spider identity as a random factor to test whether individuals performed differently. As response variables I used the number of threads in the sheet chamber, their proportion, the number of exploration threads and the sheet density, with one model for each response variable. I performed four additional GLM analyses that were constructed as above, but comparing juvenile female versus juvenile males.

I tested whether the number of spiders that built a sheet in large or small chambers was different, with one chi-square test for adult females, one adult males and one for juveniles. I did the similar analyses to test the effect of the light versus dark locations. I compared the proportion of adult females, adult males and juveniles that had built sheet within three and within 15 days after entering the cage with an independence chi-square test comparing proportions (Zar 1999), and used another proportion chi-square to test whether the spider groups differed in the number of individuals that attached exploration threads after three days. I also tested whether the proportion of spiders in each group that had abandoned their sheets changed between observation periods with proportion chi-squares. I tested whether the age and sex groups differed in the number of times spiders abandoned their web with a one-way analysis of variance. Finally, I performed three sets of simple linear correlations between the four response variables, for juveniles, for adult females, and for adult males. I used the values of nine days old sheets, since most spiders had already built a sheet by this time. All analyses were done using STATISTICA 8.0 (StatSoft, Inc. Tulsa, Oklahoma, USA, 2007).

RESULTS

All spiders attached at least some threads to the walls during the first three days, but some spiders did not build a sheet, initially and instead rested in a chamber corner (Fig. 1D). After 6 – 9 days most spiders had built a clearly identifiable sheet, with a dome shape with a high density of threads, with silk lines in many directions (as described for *Modisimus* in Eberhard 1992b). Spiders usually built the sheet in the center of the chamber, or half way between the center and a corner. Before building the sheet, the spiders rested in the walls

joint. When the sheet was present the individual rested under it, upside down (Fig. 1F). Juveniles and adult females in a room made sheets that were apparently similar in size and in general structure to those in the cages (pers. obs.). The proportion of spiders that built a sheet was greater in adult females and juveniles than in males, both after 3 days and after 15 days (Fig. 2).

Almost all adult males (97%, of 28) and adult females (90% of 30), but only 45% of 39 juveniles attached exploration threads in the first three days (proportion $X^2 = 28.41$; $df = 2$; $P < 0.001$). Therefore, either the adults explored more thoroughly before building a sheet, or were more likely to explore after having built a sheet than juveniles. After six days, the percentage of adult females that abandoned their sheet and built new exploratory threads in another chamber was 57%, and then increased to 80% after 18 days ($X^2 = 12.78$; $df = 4$; $P = 0.01$). In adult males that percentage remained between 35 – 67 % and did not change ($X^2 = 6.58$; $df = 4$; $P = 0.16$). Juveniles also abandoned their sheets often (41 – 58%), but that proportion did not change significantly during this period ($X^2 = 3.52$; $df = 4$; $P = 0.48$). Finally, the mean number of three day periods in which an individual abandoned its sheet at least once was not different between adult females (2.9 ± 1.5 times), adult males (2.4 ± 0.9 times) or juveniles (2.3 ± 1.5 times) ($F_{2/92} = 2.40$; $P = 0.10$).

Age /sex differences and changes through time. Juveniles differed from adults in the web building behavior and web density pattern, but did not differ according to the juvenile's sex. The number of threads in the sheet chamber after three days was greater in adult females and adult males than in juveniles (Fig. 3). However, after 15 days, juveniles and adult females had attached more new threads to the sheet chamber than had adult males (Fig. 3). The proportion of threads in the sheet chamber (relative to the total of threads in the entire cage x) was greater in juveniles than in adult males and adult females (Fig. 4). In juveniles and adult females that proportion decreased with time, but did not change in adult males (Fig. 4). In total, juveniles and adult females laid in proportion more threads outside of the sheet chamber, but juvenile exploration was delayed because the average thread proportion attached in the sheet chamber was high (over 0.60) during the first 12 days (Fig. 4). Adult males attached most of their threads outside the sheet chamber, and the total number of these threads was greater in adult males than in adult females and juveniles (Fig.

5). This pattern remained constant throughout the observation period, but juveniles and adult females attached more exploration threads after 15 days than adult males (Fig. 5). The sheet density was greater in juveniles and adult females than in adult males after three days in the cage (Fig. 6). After 15 days, the sheet density of adult females and adult males increased slightly so that adult females had significantly greater sheet densities than either juveniles or adult males (Fig. 6).

All the exploration and web structure variables varied between individuals as follows (spider identity factor in the GLMs) threads in sheet chamber: $F_{82/450} = 7.42$; $P < 0.001$; proportion of threads in the sheet chamber, $F_{82/450} = 13.97$; $P < 0.001$; exploration threads, $F_{82/450} = 4.17$; $P < 0.001$; and sheet density, $F_{82/450} = 6.93$; $P < 0.001$).

Juvenile behavior did not differ according to sex. Juvenile females ($n = 23$) and juvenile males ($n = 13$) did not differ in the total number of threads attached in the sheet chamber during 18 days (GLM juvenile sex*days interaction: $F_{5/186} = 1.79$; $P = 0.12$), the proportion of threads in the sheet chamber ($F_{5/186} = 1.49$; $P = 0.20$), the exploration threads ($F_{5/186} = 0.61$; $P = 0.69$), or the sheet density ($F_{5/186} = 0.42$; $P = 0.84$). It was noticeable that throughout the days the proportion of threads in the sheet chamber decreased in juvenile females but did not decrease in juvenile males.

Correlations between threads numbers and sheet density. The three spider groups had a very different sheet structure (Table 1). Denser sheet correlated (positively) only with the number of exploration threads only in adult males (Table 1). Also, adult males that had most of their threads in the sheet chamber had less dense sheet (Fig. 7). Only in adult females denser sheets had greater number of threads in the sheet chamber (and its proportion) (Fig. 8). In adult males and juveniles, denser sheets did not have more threads attached (Table 1).

Original exploration and thread attachment behavior. When released into the cage, adult male and female ($n = 12$) walked on the floor and walls. Eventually the spider encountered the plastic “ceiling”, on which it could not walk easily, and it stopped. Most spiders visited two chambers in the first 5 min. and then remained immobile in a corner, with its ventral side touching the wall. This position was also frequent in juveniles. After 10

– 15 min the spider rose and started attaching threads to the wall of the cage. For each attachment the spider lowered the tip of its abdomen to contact the substrate, then raised the abdomen quickly and walked and attached it to another wall. As the spider walked it extended one of its legs I forward, and touched the substrate when it was nearby. The spider also explored by moving their extended leg from side to side. Movements that appeared to be exploratory were performed alternately mostly with both legs I, but also with legs II. The third pair of legs was either still or it moved with the body's advance. The fourth pair of legs held threads by which the spider supported its weight (similar to Fig. 8 in Eberhard 1992b).

Chamber selection. Adult spiders build their sheets equally in large or small chambers (Females: $n = 16$; $X^2 = 1.00$; $df = 1$; $P = 0.32$. Males: $n = 15$; $X^2 = 1.67$; $df = 1$; $P = 0.20$). However, more juveniles built their sheet in small chambers (14 of 19 individuals that built sheets) ($X^2 = 4.26$; $df = 1$; $P = 0.04$). About half of the spiders that built sheets built them in the dark chambers (farther from direct exposure to light): 16 of 30 adult females ($X^2 = 0.13$; $df = 1$; $P = 0.72$), 15 of 38 juveniles ($X^2 = 1.68$; $df = 1$; $P = 0.19$), and 18 of 27 adult males ($X^2 = 3.00$; $df = 1$; $P = 0.08$). There was thus no apparent effect of illumination, as was found in the theridiid *Archaeearanea tepidariorum* (Turnbull 1964).

DISCUSSION

Web building in *P. globosus* continued over several days. Juveniles, adult males, and adult females all added new threads to the sheet chamber and outside of it and increased their sheet density even 18 days after being placed in the cage. Therefore, adding new gumfoot threads and increasing the line density in the sheet may reflect the expansion of the capture area of the web to favor higher prey capture rates (reviewed in Blackledge & Zevenbergen 2007). Also, spiders may add new threads to the tangle to renew sticky gumfoot lines, because some break (pers. obs.) or dry after a few days, as reported for *P. phalangoides* (Kirchner 1986). The relation of these data with behavior in nature is not clear, however, because increases in sheet threads that occurred following the first exploration after the sheet was built may be an artifact of enclosing the spiders in small containers where they

eventually encountered their original webs after leaving them to explore. Nevertheless, *P. globosus* adult females reoccupied abandoned webs, however it was not possible to determine if a spider encounter its own web (Eberhard 1992).

Adding lines outside the sheet chamber once the sheet was built suggest that the spiders abandoned their sheet and were exploring the environment. In natural conditions they would presumably have to spend time and silk in weaving a new sheet after abandoning their sheet unless they are able to return to sheets following explorations (no data are available on this point). In the cages where these spiders were kept they reencountered their own webs after moving. Spiders probably reencounter their own web only seldom in the field, although sometimes they do occupy abandoned webs (Eberhard 1992a). The high rate of exploration and the frequent abandonment pattern found in *P. globosus* presumably balances the benefit of increased capture and the cost of abandoning their web (Switzer 1993). It could have resulted from the cages providing only unattractive websites.

Male *P. globosus* were the more active and exploratory. Most adult males laid threads in all four chambers in the first three days, and laid them in an almost even numbers inside and outside the sheet chamber; adult males also made sheets later. Therefore, even though adult males continue to build sheets and feed when adult, they seem to be more disposed to abandon their sheets and explore more than juveniles and adult females. In the field, adult males *P. globosus* remained fewer days in the same web than adult females (Eberhard 1992a). In addition, adult males of two species of *Modissimus*, and one *Blechnroscelis* also changed sites more often (Eberhard & Briceño 1983). After spiders were placed in already built webs, adult males of *P. manueli* dispersed earlier than adult females (Edwards 2011). This suggests that males disperse more to look for mates, since they visit adult females in their webs to copulate (Uhl 1998). Building a sheet and increasing its density would probably not happen all the times in males, since they could also capture prey in abandoned webs they encounter, or even in female webs (Eberhard & Briceño 1983). In an opposite pattern, most adult females seemed to establish in the first three days. Their proportion of threads in the sheet chamber positively correlated with denser sheets, as

noted for *P. phalangioides* webs (Kirchner 1986). This web structure could increase the possibility of capturing prey.

The age also was associated with the exploration and the sheet structure throughout the days. Juvenile *P. globosus* spiders tended to establish in a chamber and concentrate the threads they added in the sheet chamber (and building dense sheets), rather than in exploration; abandoning the sheet to explore was delayed, compared with adults. Subsequent exploration may have occurred because the spiders had not fed. Juveniles showed a preference not seen in adults for smaller chambers. These findings are in accord with the likely hypothesis that spider body size affects the site in which they build webs, as also occurs in orb weavers (Waldorf 1976, Gunnarson 1992).

The ontogenetic changes in the exploration behavior and sheet density of *P. globosus* were only subtle, compared to species of a wide variety of families in which structural, behavioral and functional web features differ between juveniles and adults (Uloboridae: Eberhard 1977, Araneidae: Eberhard 1985, Hesselberg 2010, Nephilidae: Japyassú & Ades 1998, Tenggellidae: Barrantes & Madrigal-Brenes 2008, Theridiosomatidae: Eberhard 2000, Theridiidae: Eberhard *et al.* 2008, Barrantes & Eberhard 2010, Pholcidae: Japyassú & Macagnan 2004).

The sex of juveniles was not associated with the thread attachment pattern, the sheet density, site tenacity or web density. This is contrary to Hoefler *et al.* (2010) who found that *P. phalangioides* juvenile males were more active, and less willing to hunt for as many prey as juvenile females. Juvenile *P. globosus* of an advance fifth instar did not differ in their exploration or sheet construction, so it is unlikely that they behave different since their first instar. Also, it is unlikely that juvenile males capture a suboptimal number of preys in the 5th instar, since immature spiders would be expected to eat as much as possible in order to mature sooner (Foelix 1996). The other variable investigated by Hoefler *et al.* (2010) was the amount of time during a 15 min interval that spiderlings spent in walking, building web, or other activities after being placed in plastic containers. The time spiderlings were moving in this context could be caused by their reaction after being released in an artificial substrate and needed to establish and build silk threads to secure themselves, behaviors which were observed in *P. globosus* after spiders were placed in

cages. Therefore, my results are congruent with the expectation that the sex of immature pholcid spiders would not affect their behavior, especially since in Pholcidae (as in other spider taxa) juveniles do not show exaggerated morphological sexual dimorphism.

Individual differences were present during exploration and web building, suggesting individual differences (and repeatability) in these two biologically different behaviors (Bell *et al.* 2009). For example, I found that adult males with denser sheet webs produced a higher proportion of exploration threads. In contrast, some adult males built their sheet 15 days in the cage only after exploring many times, and some individuals did not build a sheet web even though they attached many exploration threads. In adult females and juveniles individual differences were more quantitative (more threads attached to the walls, or denser sheets). These differences and repeatability may be caused by genetic and environmental factors (as suggested by Rodríguez & Gloudeman 2011), but my design could not identify them. However, differences in the condition of each spider before entering the cage, the accumulation of past experiences in explorations of the environment, differences in prey capture success of their sheet webs or sites, an unknown number and type of prey attacked, reproduction stage differences in adults (see below), and brood specific variation could also account for the behavioral differences found.

Accumulated hunger affects the pattern of web construction, exploration and web abandonment in other spiders. In *Latrodectus hesperus* (Theridiidae) fed adult females built a cloud of silk to avoid predation instead of adding new gumfoot lines to increase prey capture, when compared with unfed adult females (Blackledge & Zevenbergen 2007). Hunger could also make spiders abandon their sheet sooner, especially because web relocation is energetically costly for sheet weaving spiders (Tanaka 1989). In this project fed and not fed adult males did not differ in their exploration or web construction, but my sample sizes were very small, and probably the starvation time was not large enough to cause differences in behavior. The reproductive stage of adults can also affect a spider's behavior. Adult females of *L. hesperus* after copulation did the similar changes to their webs as mentioned above (Blackledge & Zevenbergen 2007). In this project I included virgin and non-virgin adults, and adult females that had oviposited or not. However, even

though hunger and reproductive status could affect the behavior of *P. globosus*, in this project those factors would have a greater within than between group effect.

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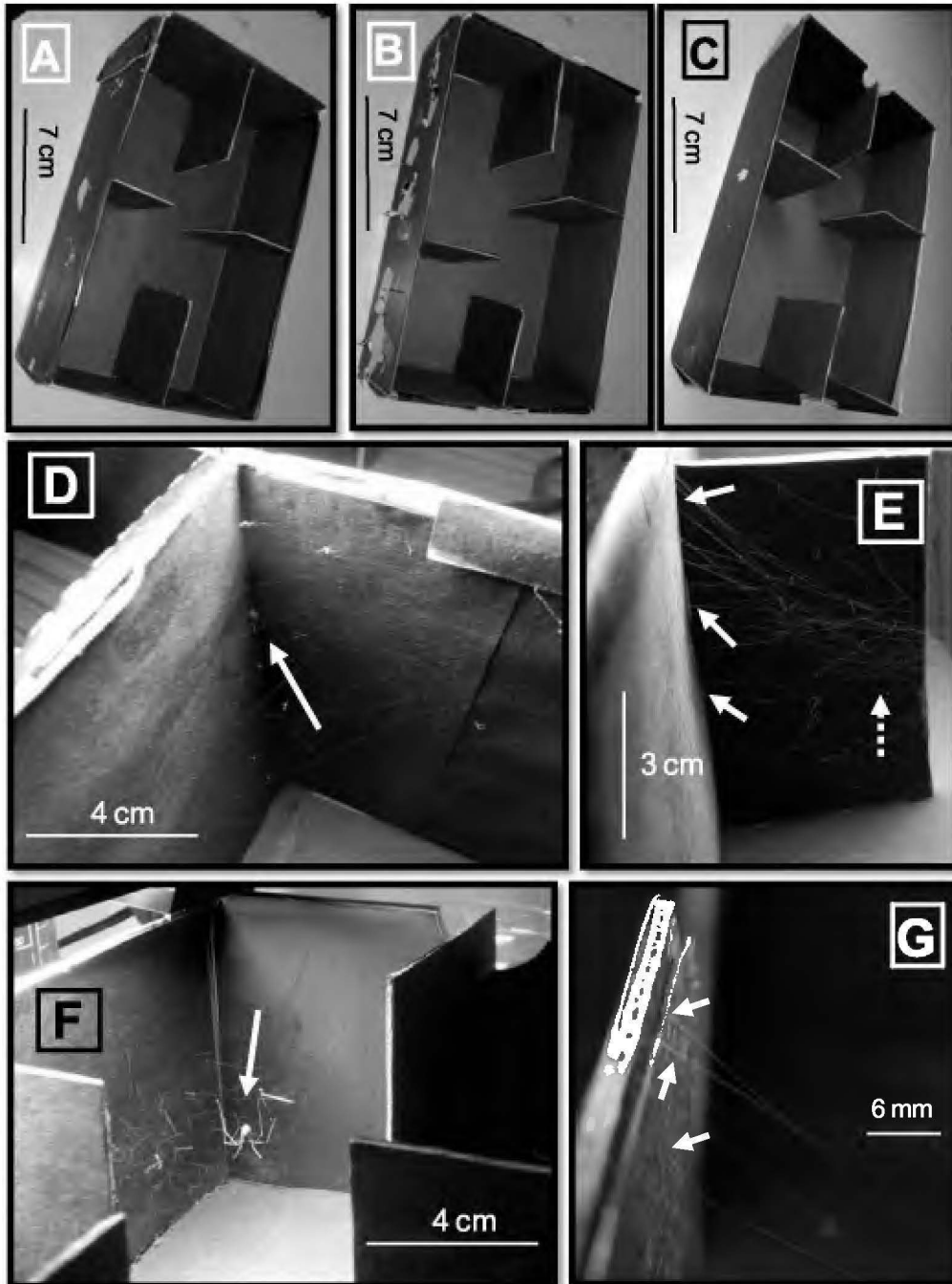


Fig. 1. Distribution of chambers for *Physocyclus globosus* (Pholcidae). A) Symmetric chambers cage. Asymmetric chambers cages, B) with diagonally opposite or C) adjacent chambers of the same size. D) Female (arrow) resting in a corner prior the sheet construction. Note the threads attached to the walls. E) Threads attached to the wall (left; solid arrows) and a sheet (right; dashed arrow). F) Female (arrow) resting upside down in the center of a sheet web. G) Close up of the threads attached to the wall (arrows).

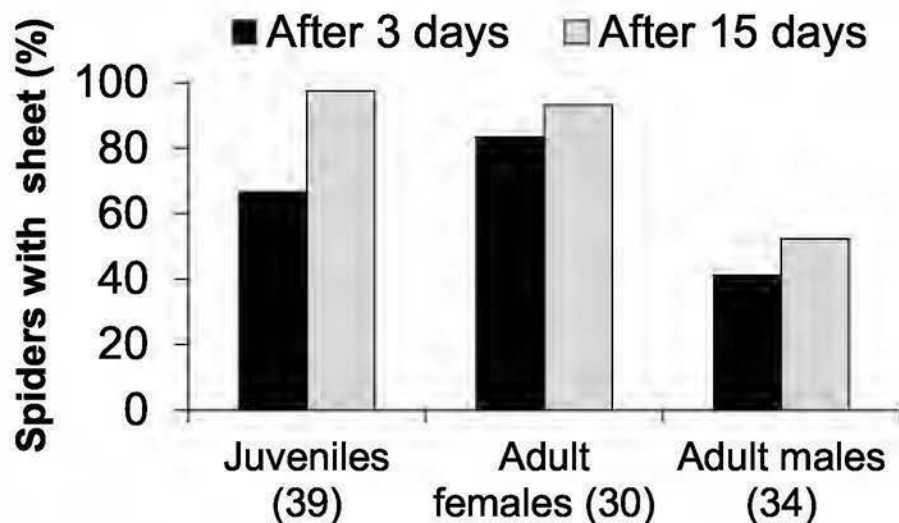


Fig. 2. Percentage of *Physocyclus globosus* (Pholcidae) spiders that had built a sheet three and 15 days after entering a cage. For 5th instar juveniles, adult females and adult males. Sample size is shown in parentheses. Groups were different three days later (proportion $X^2 = 12.51$; $df = 2$; $P = 0.002$) and also 15 days later ($X^2 = 25.53$; $df = 2$; $P < 0.001$).

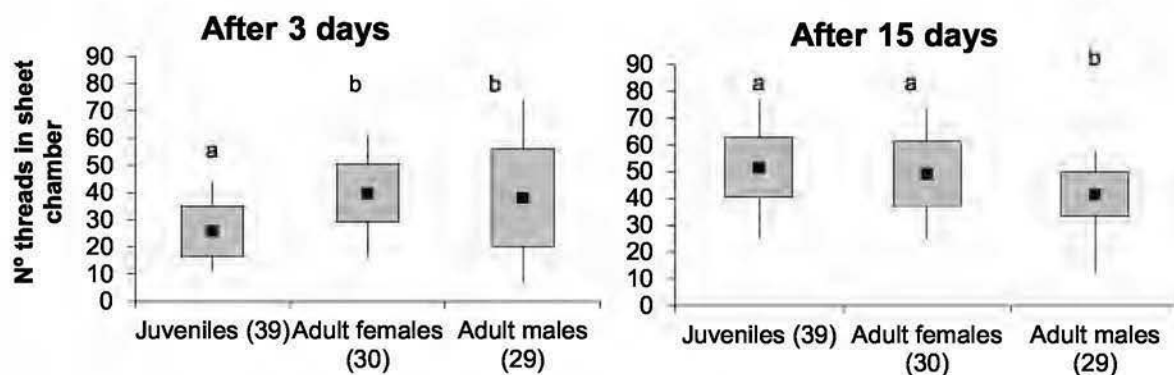


Fig. 3. Threads attached to the walls in the chamber where *Physocyclus globosus* (Pholcidae) spiders built their sheet, after three days (left), and after 15 days (right). Central filled square = mean, upper box limit = mean + one standard deviation, lower limit = mean - SD, line ends = maximum and minimum value. Sample size is shown in parenthesis. Letters above bars represent statistically significant different groups. GLM spider group*days interaction: $F_{10/450} = 10.42$; $P < 0.001$.

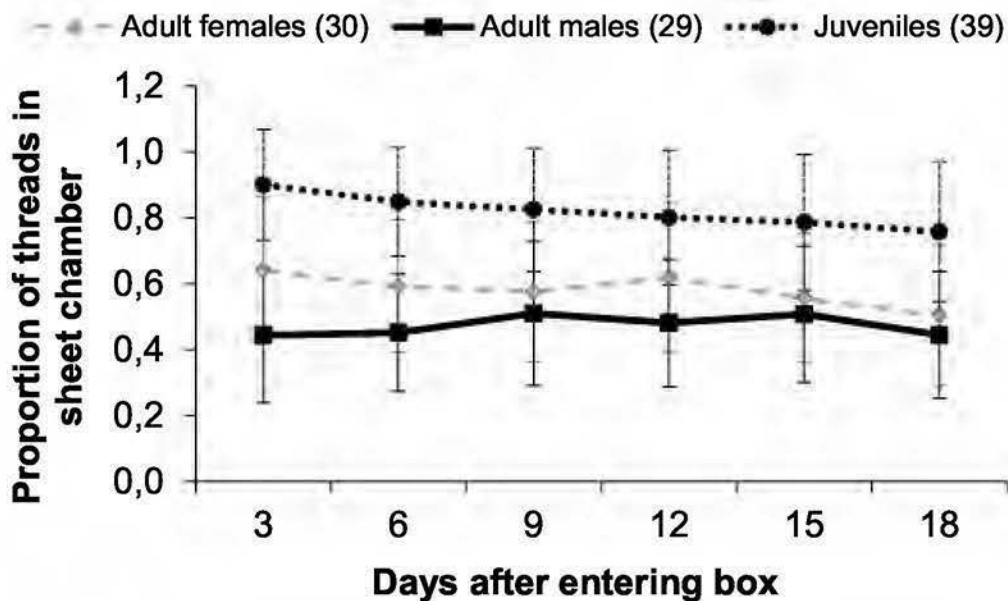


Fig. 4. Mean proportion (\pm one standard deviation) of threads attached to the walls of the chamber where *Physocyclus globosus* (Pholcidae) spiders built their sheet, relative to the total threads in cages, throughout 18 days. GLM spider group*days interaction: $F_{10/450} = 2.66$; $P = 0.004$.

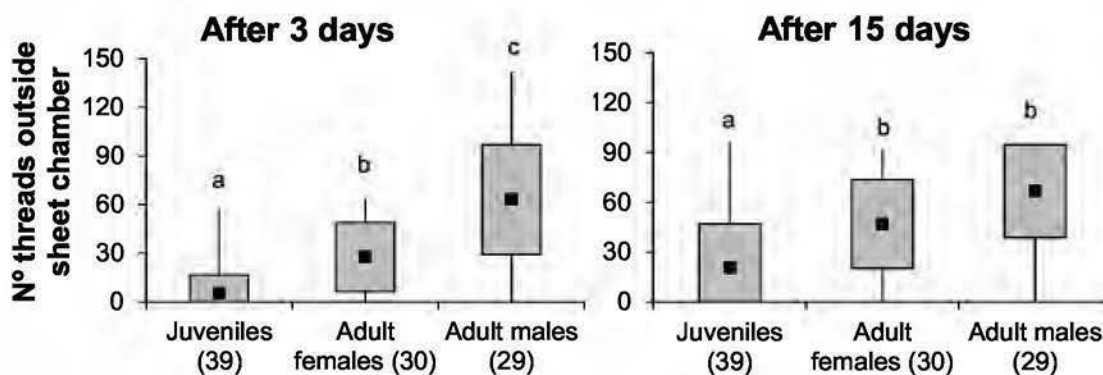


Fig. 5. Exploration threads, attached in the three chambers other than the sheet chamber where *Physocyclus globosus* (Pholcidae), after three days (left), and after 15 days (right). Central filled square = mean, upper box limit = mean + one standard deviation, lower limit = mean - SD, line ends = maximum and minimum value. Sample size is shown in parenthesis. Letters above bars represent statistically significant different groups. GLM spider group*days interaction: $F_{10/450} = 4.18$; $P < 0.001$.

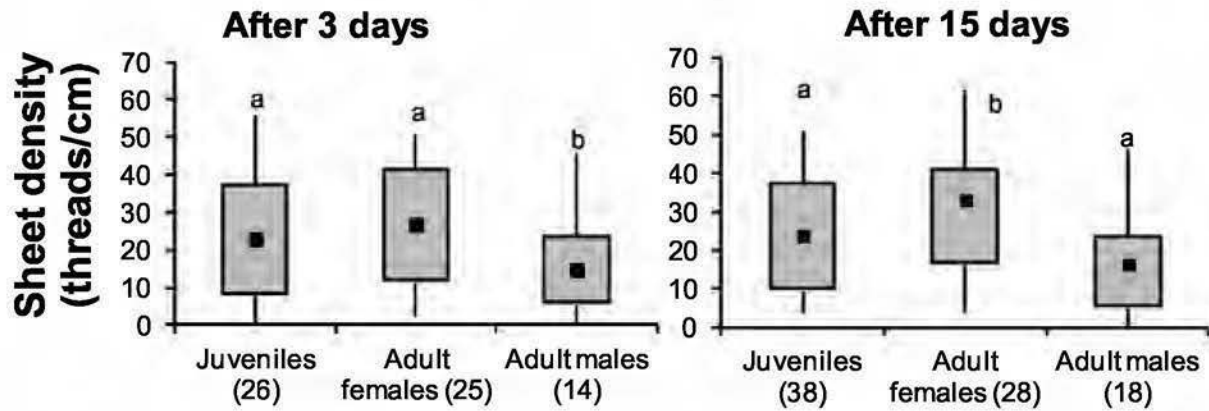


Fig. 6. Sheet density (number of silk lines that crossed sticks above the sheet) in the chamber where *Physocyclus globosus* (Pholcidae) spiders built their sheets. Central filled square = mean, upper box limit = mean + one standard deviation, lower limit = mean - SD, line ends = maximum and minimum value Sample size is shown in parenthesis. Note changes in pattern between the observations three (left) and 15 days (right) after spiders entered the cage. Letters above bars represent statistically different groups. GLM spider group*days interaction: $F_{10/450} = 1.423$; $P = 0.17$.

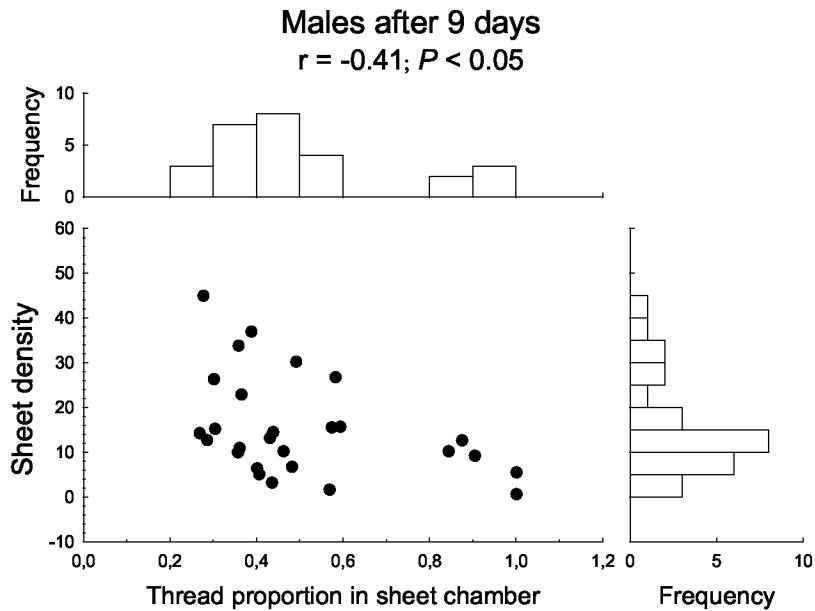


Fig. 7. Simple linear correlation between the thread proportion in the cage chamber where spiders built their web and the sheet density for 34 adult males of *Physocyclus globosus* (Pholcidae) nine days after entering the cage.

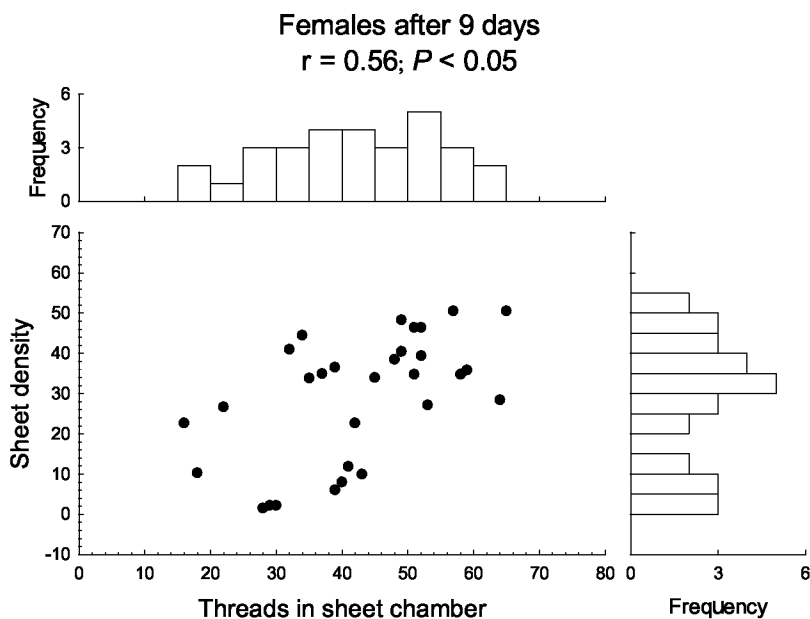


Fig. 8. Simple linear correlation between the number of threads in the sheet chamber and the sheet density for 30 adult females of the spider *Physocyclus globosus* (Pholcidae) nine days after entering the cage.

Table 1. Correlation coefficient (r) for simple linear correlations between the four response variables, analyzed separately by groups (adult females, adult males or juveniles) of *Physocyclus globosus* (Pholcidae) spiders. I used data from the third session (nine days after spiders entered a cage). Valid sample size for this analysis is shown in parenthesis. * = significant correlation at the $P < 0.05$ level.

Group	Variables	Exploration threads (outside sheet chamber)	Threads in sheet chamber	Proportion of thread in sheet chamber
	Threats in sheet chamber	0.14		
Adult females (30)	Proportion of thread in sheet chamber	-0.83 *	0.36 *	
	Sheet density	0.15	0.56 *	0.15
	Threats in sheet chamber	0.03		
Adult males (27)	Proportion of thread in sheet chamber	-0.89 *	0.13	
	Sheet density	0,50 *	0.07	-0.41 *
	Threats in sheet chamber	0,09		
Juveniles (39)	Proportion of thread in sheet chamber	-0,95 *	-0.01	
	Sheet density	-0.04	0.08	0.06

CAPÍTULO 4

The lack of gumfoot threads in the webs of early juveniles and males of *Physocyclus globosus* (Araneae: Pholcidae) is not associated with differences in spigot morphology

Ausencia de hilos pie de goma en telas de juveniles recién emergidos y machos de *Physocyclus globosus* (Araneae: Pholcidae) no se asocia a diferencias en morfología de las hileras

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Abstract. Gumfoot threads attached to the substrate may help spiders retain prey. These threads were recently discovered in Pholcidae spiders, and are similar to those in Theridiidae. We report for the first time the presence of gumfoot threads in the webs of immature spiders (fifth instar juveniles) of the pholcid *Physocyclus globosus*, and their absence in the webs of conspecific first instar juveniles and adult males. The bands of adhesive silk were shorter in fifth instar nymphs than in adult females, and were continuous rather than having discrete adhesive droplets as in the webs of adult females of two other pholcid genera. In Pholcidae a large spigot on the anterior lateral spinnerets is presumably connected with the highly modified pyriform gland, which is thought to produce glue. However, we found that this spigot was relatively thicker in adult females and first instar juveniles than in adult males. Therefore, the ontogenetic origin of gumfoot threads may not be associated with changes in the spigot morphology. The delayed production of gumfoot threads in this species is contrary to Theridiidae spiders, in which newly emerged spiderlings build gumfoot.

Key word: Adhesive silk, ontogeny, spinneret, spigots.

Gumfoot lines, with accumulations of adhesive material close to the point where the line is attached to substrate, are present in the webs of spiders in the related Entelegyne families Theridiidae, Synotaxidae and Nesticidae (Eberhard *et al.* 2008a). These lines help spiders to retain and capture prey (Sahni *et al.* 2011). Recently, apparently convergent gumfoot threads were found in the distantly related haplogyne family Pholcidae (Japyassú & Macagnan 2004). The general structure of these gumfoot lines, their construction and how spiders use them to capture prey are similar in these families.

Adhesive droplets that presumably help retain prey have been found in the webs of adult females of several species of pholcids. Glue drops are scattered in the sheet of *Modisimus guatuso* (Briceño 1985), *Pholcus phalangioides* (Kirchner 1986), *Physocyclus globosus* and *Mesabolivar cyaneotaeniatius* (Japyassú & Macagnan 2004). They also occur in the wrapping silk of *P. globosus* (Barrantes & Eberhard 2007). Gumfoot threads occur in

Trichocyclus arawari (F. Soley, pers. com.), *P. globosus*, *P. phalangioides*, and *Smeringopus pallidus*, but are absent in two species of *Mesabolivar* (Japyassú & Macagnan 2004).

To date, only webs of mature females have been checked in Pholcidae for adhesive silk. In this project we looked for gumfoot lines in the webs of early and late juveniles, as well as in adult males of *P. globosus*. We also checked the general morphology of the spinnerets for differences.

MATERIALS & METHODS

We placed four fifth instar *P. globosus* spiders, four adult females and six adult males in shoe boxes (31 cm long, 21 cm wide and 12 cm tall) to build their webs. Additionally, we placed four first instar individuals in small plastic 50 ml round cups (4.0 cm height, 3.0 cm upper diameter, and 2.5 cm base diameter). Both types of container were lined with black matte paper to facilitate the observation of the silk threads. In addition, four first instar spiderlings were placed in rectangular glass cages built with four microscope slides oriented vertically and a cover slip on the top (2.6 cm x 2.6 cm x 7.6 cm height). Spiders were kept in laboratory conditions (approximately 20 °C and 80% relative humidity). After 7 – 10 days we looked for gumfoot lines by directly lighting the webs with a fluorescent light, and checking the glass cages under a dissecting microscope. The lines were attached approximately perpendicular to the glass slides, and we collected them for observation under a compound microscope by moving a cover slip carefully from below along the length of a wall of the cage. Instar numbers followed the system recommended by (Foelix 1996) in which the “first instar” began after the first molt of the spiderlings away from the egg sac.

To study the anatomy of spinnerets in the scanning electron microscope, we preserved in 70% ethanol 15 individuals of the first and 15 of the second instar, one adult male and two adult females. The juveniles were fixed in modified Karnovsky solution (Glutaraldehyde 2.5 %, Formaldehyde 2 %, phosphate buffer 0.1 M and Sucrose 5 %) for one week, after which we washed the samples three times with a phosphate buffer 0.2 M.

Post fixation with Osmium tetroxide 4 % was performed for 1 hour, followed by three washes with distilled water. Fixation and post fixation were not performed for adult specimens. All samples were dehydrated twice in an alcohol gradient (30, 50, 70, 80, 90, 95 and 100 %), 15 minutes each and vacuum dried from a frozen tertbutyl alcohol step (two washes of 20 min. prior dry) using a Vacuum Freeze Dryer (VFD – 20). Samples were mounted on double sided adhesive carbon tape on aluminum stubs and coated with a Pt/Pd sputter coater GIKO-IB3 (coating thickness 35 nm for juveniles, 70 nm for adults) after 24 hours of stove storage at 40°C. Images were obtained using a Hitachi S-570 SEM. We assigned names to different spigots on the basis of comparisons with published figures with spigots labeled by Platnick *et al.* (1991).

RESULTS

Fifth instar nymphs and adult females of *P. globosus* built many gumfoot threads attached to the walls of the containers (Figs. 1 – 4). However, we were unable to find gumfoot threads in the webs of first instar spiderlings or those of adult males. The webs of first instar spiderlings had many tangled threads attached to the substrate, similar to those reported for adult females of the pholcid *P. phalangioides* (Fig.12 in Kirchner 1986). One adult female and one adult male of *Mesabolivar charrua* from Uruguay also did not have gumfoot threads in their lines to substrate (I. Escalante, pers. obs.).

In contrast to discrete droplets on the gumfoot lines of the pholcids *S. pallidus* and *P. phalangioides* (Fig. 3 in Japyassú & Macagnan 2004) and *T. arawari* (F. Soley, pers. com.), the glue on each sticky line seemed formed a long extended continuous band of adhesive silk near the tip of the thread, with small round thickenings (Fig. 2 inset, Fig. 3). The bands of adhesive of mature females were approximately 3 mm longer than those of juveniles (Figs. 2 & 3). The gumfoot lines were built below the tangle of dry silk lines in scattered groups of 4 – 6 threads whose attachments to the walls formed an approximately straight line on the wall. This linear arrangement in small groups of lines resembles the organization of the gumfoot threads built by certain theridiid spiders (Benjamin & Zschokke 2003, Eberhard *et al.* 2008b).

The general spinneret morphology of first and second instars were similar to that described for *P. phalangoides* (Platnick *et al.* 1991) (Fig. 5). The ALS had a group of small spigots that occupied most of the spinning field (Figs. 6 – 8), which are thought to be associated with the modified pyriform glands (PG: Platnick *et al.* 1991). There were five spigots per AL spinneret in juveniles, and seven in adult females (Figs. 5, 12, 13). However, the opening of the largest PG spigot in the AL spinnerets was relatively thicker in adult females (2.0 % of the maximum cephalothorax width, Fig. 13) and juveniles (1.9%, Fig. 6) than in adult males (0.6%, Fig. 14). In addition, there was one smaller spigot next to the HPG (Fig. 6 – 8), which is probably connected to the major ampullate glands (AG, Kovoov 1986).

The posterior median spinneret (PMS) had two spigots of different size, with fused bases (Figs. 9, 11, 15 – 17). The larger of the two may correspond to the minor ampullate glands (AGm, Fig. 9) (Kovoov 1986), while the smaller (Fig. 9) may be connected to the modified aciniform glands (ACG: Platnick *et al.* 1991). The posterior lateral spinnerets (PLS) lacked spigots in juveniles and adults (Fig. 10, 15).

DISCUSSION

The webs of first instar spiderlings and adult males lacked gumfoot lines, but gumfoot lines were present in webs of fifth instar spiderlings and adult females. This pattern suggests that gumfoot threads appear in the third or fourth instar of *P. globosus*, and are secondarily lost in mature males. Ontogenetic changes in silk occur in a few phylogenetically scattered families. In *Tengella radiata* (Tengellidae) the cribellated threads appear in the seventh instar (Barrantes & Madrigal-Brenes 2008), while in *Uloborus diversus* (Uloboridae) appeared in the third instar (Eberhard 1977). On the other hand, adhesive gumfoot threads are present in the first web of the first instar in several genera of theriidids (*Latrodectus*, *Steatoda*, *Archeearanea*, and *Tidarren*), and adult males of a few species of those genera lack gumfoot threads but have sparse sticky balls in their wrapping silk (Eberhard *et al.* 2008a, G. Barrantes, unpubl.). The ontogenetic appearance of gumfoot threads in the webs of later instars of pholcids may represent a case of the pattern

of ontogeny repeating phylogeny that is common in spider webs (Barrantes & Eberhard 2010, Kunter *et al.* 2010).

The webs of adult male *P. globosus* and early instar juveniles lacked gumfoot threads. This pattern is superficially similar to some species of Uloboridae, in which adult males weave webs similar to newly emerged spiderlings, but involves different glands (Eberhard 1977). On the other hand, adult males of Anapidae and Mysmenidae build webs with sticky spiral to capture prey (Eberhard 2007). Pholcid adult males also build webs to capture prey (Eberhard & Briceño 1983, Kirchner 1986), and the sheet webs of adult male *P. globosus* do not differ in general structure from those of adult females and fifth instar juveniles (I. Escalante in prep.), except for the absence of the gumfoot threads.

Preliminary indications from the phylogeny of pholcids (Huber 2011) suggest that the three species of *Mesabolivar* known to lack gumfoot threads probably lack the small set of pyriform glands that produce them in other pholcids (Dimitrov *et al.* in press.). Producing gumfoot threads is a widespread feature in the Pholcidae phylogeny, and since clades of less derived species (*Physocyclus* and *Trichocyclus*) as well as in more derived genera (*Smeringopus* and *Pholcus*) (Fig. 18) probably the glands that produce gumfoot threads did not evolved (or were secondary lost) in the *Mesabolivar* clade. Additionally, the gumfoot thread morphology seems to vary between *P. globosus* and two species of Pholcidae: *P. phalangoides* and *S. pallidus* (Japyassú & Macagnan 2004). Much further data on the behavior and spigot morphology of pholcids and of their immature stages, as well as in other families will be needed to determine the evolution of sticky threads in pholcid webs.

We did not find major morphological differences in the spigots of first instar spiderlings and adults. However, the PG spigots, which are thought to be used to attach draglines together or to the substrate (Coddington 1989), are known to increase in number on the ALS throughout the ontogeny (Yu & Coddington 1990). The HPG on the ALS in Pholcidae may be associated with producing the adhesive silk on gumfoot threads (Japyassú & Macagnan 2004). We report a difference in the relative sizes of HPG spigots in first instar and adult female with those of adult males. It may be that differences in relative

spigot size may be related with changes in the production of glue droplets on wrapping silk or gumfoot lines, at least in the case of adult males.

ACKNOWLEDGEMENTS

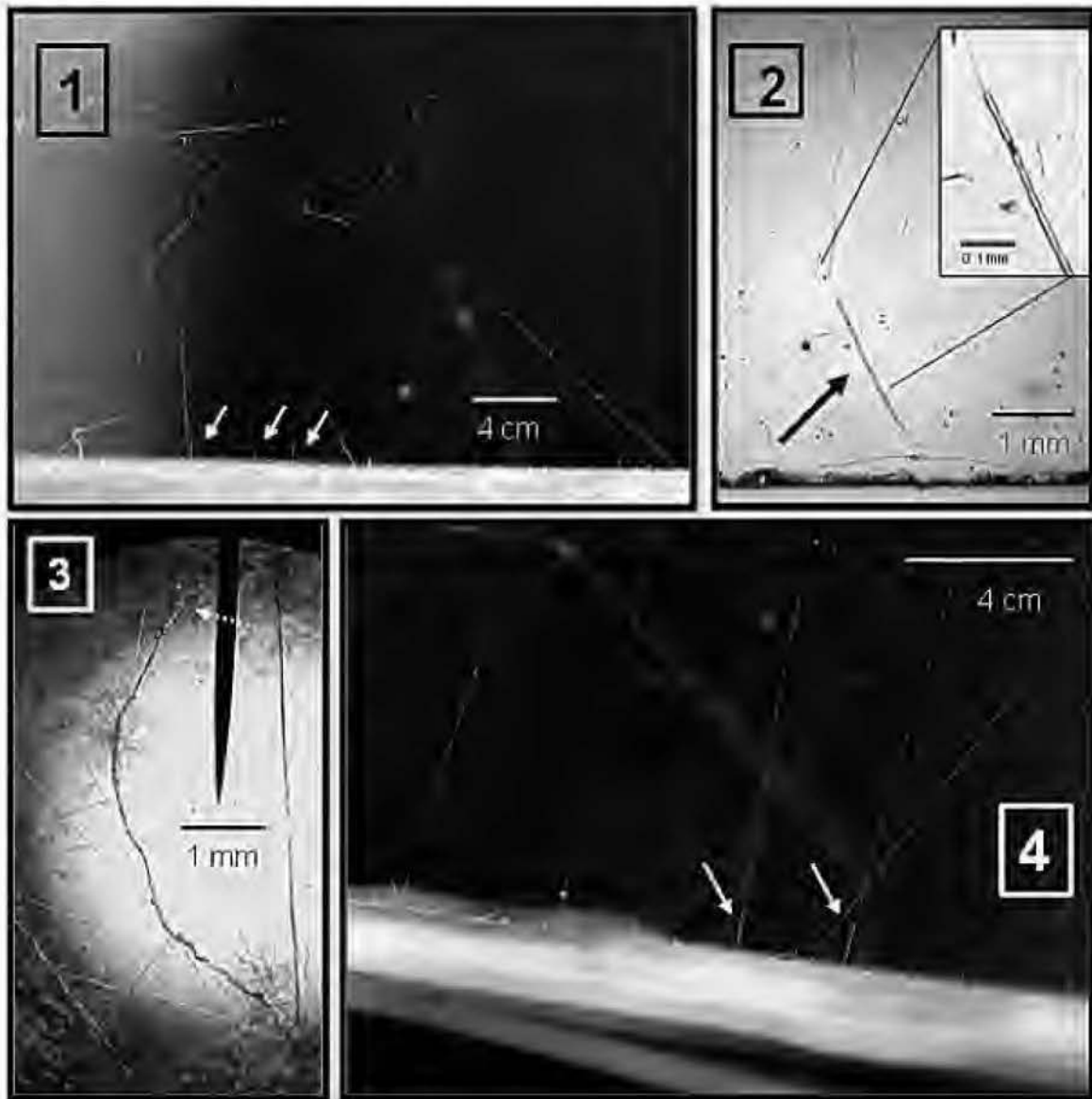
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Figs. 1 – 4. *Physocyclus globosus* (Araneae: Pholcidae) gumfoot threads attached to the wall substrate 1) by a fifth instar spider, three pointed by white arrows. 2) Compound microscope image of the gumfoot (arrow) of a fifth instar juvenile and a close up of the same thread (inset). Gumfoot threads of an adult female: 3) through compound microscope (the gumfoot starts in the lower right corner and ends at the arrow); and 4) in the walls of a box (arrows).

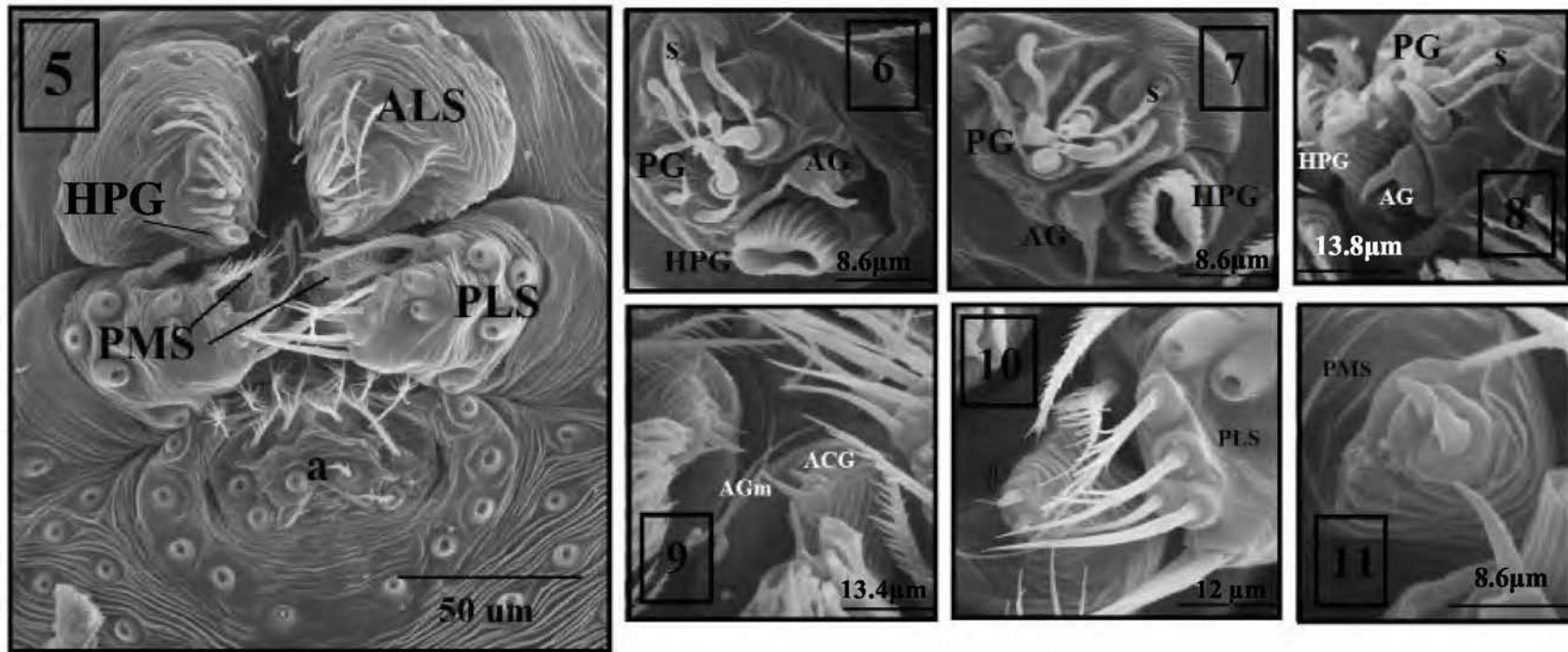
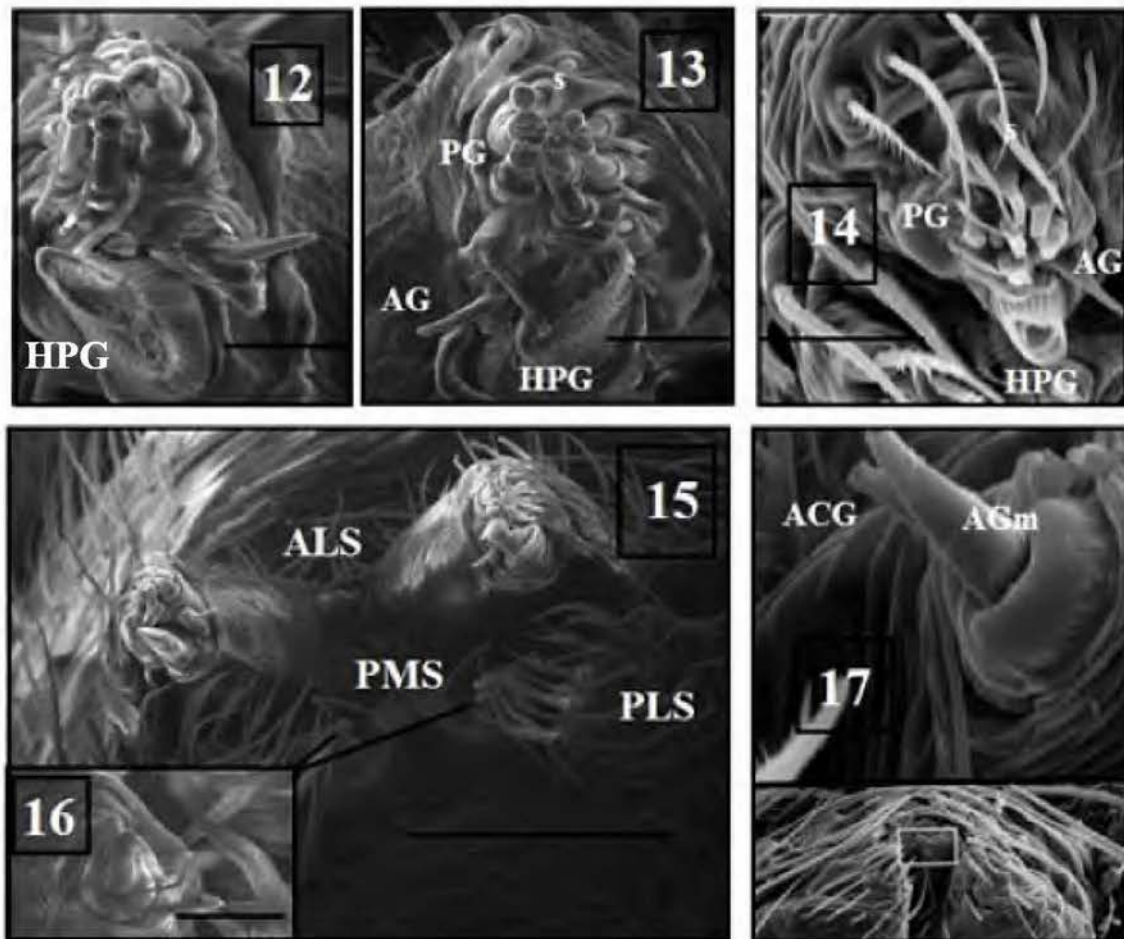


Fig. 5 – 11. SEM images of first instar (5 – 7) and second instar (8 – 11) juveniles of *Physocyclus globosus* (Araneae: Pholcidae). 5) General view of spinnerets (magnification: 600X): anterior (ALS), posterior lateral (PLS), posterior median (PMS) and anus (a). 6) Right and left (7) ALS: PG pyriform gland, AG major ampullate gland, HPG high modified pyriform gland, s setae (3500X, scale bar 8.6 µm on 6 and 7). 8) Right ALS, lateral view (2200X, scale 13.8 µm). 9) Both PMS, the view of minor ampullate gland (AGm) and aciniiform gland (ACG) (2200X, scale 13.4 µm). 10) PLS detail of setae, black arrow point the silk coming out from the ACG (2500X, scale 12 µm). 11) Detail of PMS of second instar (3500X, scale 8.6 µm).



Figs. 12 – 17. SEM image of the spinnerets of *Physocyclus globosus* (Araneae: Pholcidae) adult females (12, 13, 15, 16) and an adult male (14, 17). 12 – 13 right and left ALS (magnification: 1500X), 14) ALS (1300X). 15) General view of spinnerets (198X). 16) Close up of PMS (1500X). 17) A magnified view of the PMS from the gray box below) (2200X).

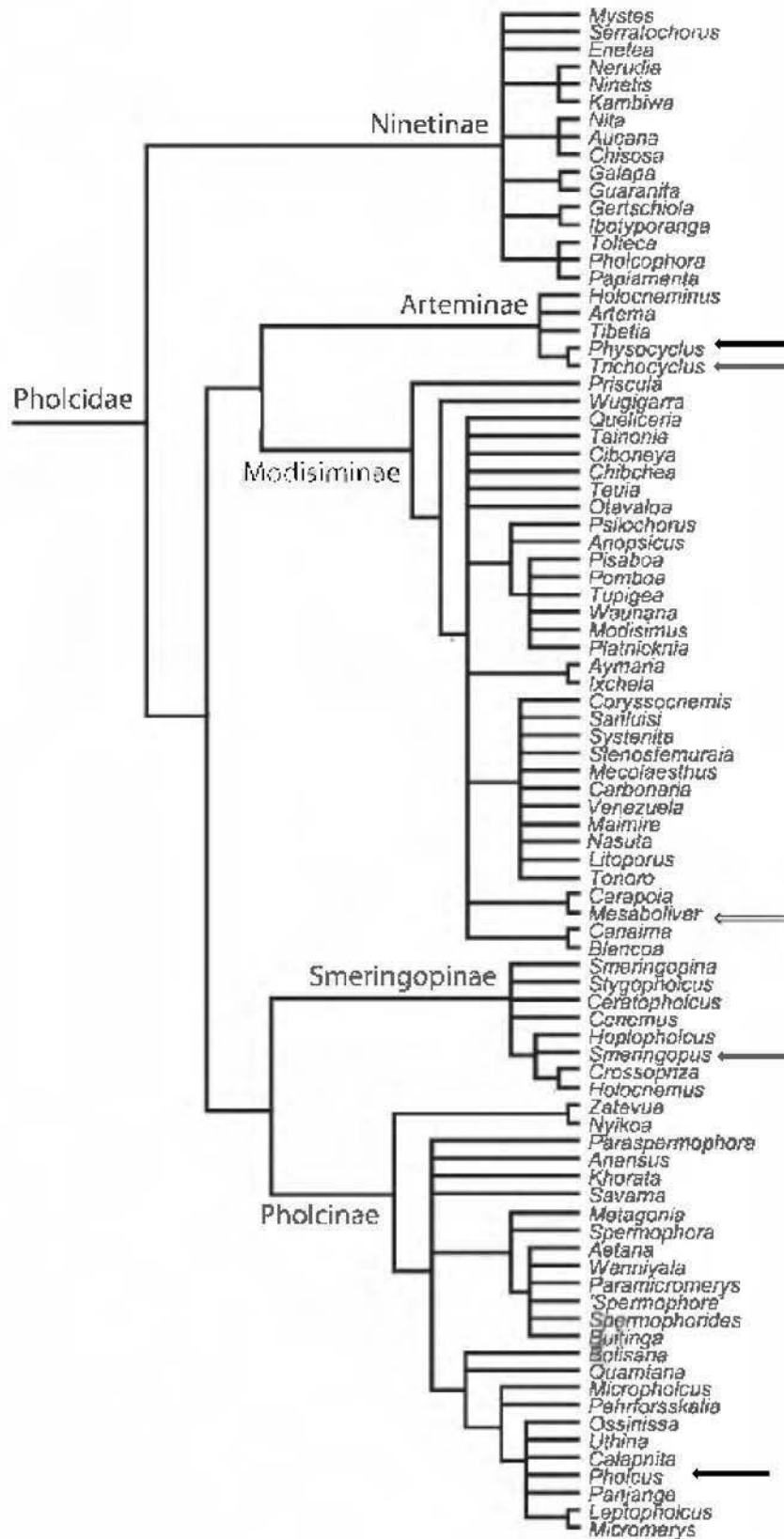


Fig. 18. Phylogeny of Pholcidae, with the genera in which species have been found to produce gumfoot threads (solid black arrows), and one genus in which species gumfoot threads were not found (white arrow). Cladogram modified after Huber (2011).