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The utilization of visible implant fluorescent elastomers in spiders (Araneae: Theraphosidae)

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Abstract: The present study was designed to analyze the efficiency of visible implant fluorescent elastomers (VIFE) in five spiders of the family Theraphosidae. The individuals were marked on the ventral face of the articulations of the patella with the femur and of the metatarsus with the tibia on the first pair of legs. The animals were kept in individual terrariums while determining the duration of the markings. The markings had a maximum retention time of 316 days, which was longer than the duration of other markers described in the literature, and they demonstrated the additional advantage of remaining visible after ecdysis.
Key words: Araneae, Theraphosidae, tarantulas, VIFE, marking.

Uso de elastómeros fluorescentes visibles en arañas (Araneae: Theraphosidae)
Resumen: El presente estudio se diseñó para analizar la eficienca de los elastómeros fluorescentes visibles (VIFE) en cinco arañas de la familia Theraphosidae. Se marcó a los individuos en la cara ventral de la articulación de la patela con el fémur y la del metatarso con la tibia del primer par de patas. Los animales se mantuvieron en terrarios individuales mientras se determinaba la duración de las marcas. Las marcas tuvieron un tiempo máximo de retención de 316 días, que fue mayor que la duración de los otros marcadores descritos en la bibliografía, y además demostraron una ventaja adicional, el hecho de que permanecieron visibles después de la ecdisis.
Palabras clave: Araneae, Theraphosidae, tarántulas, VIFE, marcas.

Ecological and behavioral studies often require the recognition of individual organisms (Woods & Martin-Smith, 2004; Lipton & Thangaraj, 2007; Zambonato *et al*., 2010). Such studies have used natural and artificial markers to identify individual specimens, often combined with mathematical techniques to estimate a number of different biological parameters (e.g. population size, density, dispersal, behavior) (Willis & Babcock, 1998; Woods & Martin-Smith, 2004; Brennan *et al*., 2007; Mazlum, 2007; Lipton & Thangaraj, 2007). Ideally, artificial markings should be easily applicable, persistent, and recognizable during the entire study period, and should not affect the health, behavior, or survival of the animals (Evans & Gleeson, 1998; Zambonato *et al*., 2010).

Spiders have been found to be particularly difficult to mark (Evans & Gleeson, 1998), and more generally used techniques involving painting the exoskeleton are faced with problems related to the scarcity of localities for those markings as well as their premature loss due to environmental factors and during ecdysis (Zambonato *et al*., 2010). Internal markings, on the other hand, can avoid many of the problems related to external markings (Evans & Gleeson, 1998), although difficulties are present during field application and the markings may last for only short periods. As such, we investigated the efficiency of using visible implant fluorescent elastomers (VIFE) as a new methodology for marking spiders.

VIFE, from Northwest Marine Technology Inc., is a liquid fluorescent polymer that is easily injected into animals after mixing its components; the resulting material is a flexible plastic that appears to have minimal effects on animal survival and behavior. These compounds were originally designed for marking fish (Catalano *et al*., 2001; Olsen & Vøllestad, 2001; Roberts & Angermeier, 2004; Woods & Martin-Smith, 2004; Brennan *et al*., 2007; Bushon *et al*., 2007; Lipton & Thangaraj, 2007; Jensen *et al*., 2008; Carvalho-Souza *et al*., 2010) but are also used in other groups of animals such as: corals (Hoey & McCormlck, 2006; Zeeh & Wood, 2009), crustaceans (Willis & Babcock, 1998; Woods & James, 2003; Mazlum, 2007), worms (Butt & Lowe, 2007; Butt *et al*., 2009), squids (Replinger & Wood 2007), amphibians (Regester & Woosley, 2005), reptiles (Hutchens *et al*., 2008), and more recently with spiders (Melo *et al*., 2010) and scorpions (Chapin, 2011).

Marking tests were undertaken under laboratory conditions using five spiders of the family Theraphosidae (Table I). The spiders were captured on different dates, and marked on the ventral faces of the articulations of the patella with the femur and the metatarsal with the tibia on the first pair of legs (Figure 1). The marking sites were selected based on the fact that leg articulations are less sclerified in spiders than other parts of their bodies, which facilitated the application and visualization of the VIFE; it would also be expected that articulation marking would have a lower probability of affecting the health of these animals as compared to applications in the prosoma or opistosoma regions. The markings were applied using sterile insulin syringes (8 mm long needles x 0.03 mm caliber), inserting the needle at an approximately 45° angle to the articulations and injecting the VIFE just below the exoskeleton (Figure 1). After we applied the VIFE the marking was visible through ultraviolet light. After marking, the spiders were maintained in individual wooden cages (20 x 17x 15 cm) at temperature range. The duration of the experiment for each animal was dependent on the permanence of the marking and their survival under captive conditions. None of the spiders demonstrated apparent changes in their behavioral patterns during the entire duration of the experiment, and the markings remained on the animals throughout their lifetimes.

The shortest and longest durations of the experiments were 60 and 316 days respectively (Table I). The average duration of VIFE markings in spiders was approximately 149 days – which was superior to the maximum recorded time of 81 days for exoskeleton painting (Zambonato *et al*., 2010) or 21 days for internal markings (Evans & Gleeson, 1998), demonstrating that this technique will be adequate for long-term experiments.

Of the five spiders, only one underwent ecdysis during the course of the experiment, and the VIFE markings did not disappear after molting, remaining for 289 additional days and corroborating

Table I. Persistence of visible implant fluorescent elastomers (VIFE) in spiders. * Immatures spiders.

Specie	Marking date	Molting date	Loss of marking	Date of death	Marking duration
Lasiodora sp.*	13/04/10	None	No	27/07/10	105 days
Lasiodora sp.	13/04/10	None	No	06/10/10	176 days
Lasiodora sp.*	29/04/10	None	No	27/07/10	89 days
Aviculariinae sp.	29/04/10	None	No	28/06/10	60 days
Lasiodora sp.*	04/06/10	01/07/10	No	15/04/11	316 days

the findings of Chapin (2011). The conservation of the markings (even after ecdysis), associated with their long duration, will allow studies focusing on the spider ecology and development to be undertaken in the natural environment with greater data fidelity (Melo *et al*., 2010; Chapin, 2011) as their tags will not be lost.The advantages of VIFE as a new marking technique for spiders resides in its long duration time as well as its permanence after ecdysis. As there are many colors available with VIFE, and spiders have large numbers of articulations available for marking, large numbers of unique combinations of colors and marking localities can be created, thus permitting the identification of many individual animals.

In spite of the necessity of handling the animal, VIFE marking is simple enough to be applied rapidly and securely even in the uncontrolled conditions encountered in field environments. The principal limiting factor of this technique is the size of the animal. We believe the minimum size for this marking application are animals presenting a diameter of articulation of 1,5mm or more.

Our results indicate that VIFE is equal to or more adequate than external or internal marking techniques previously described in the literature, although it will still be necessary to undertake additional experiments with more spiders to ratify the advantages and efficiency of this technique, and confirm marking durations under natural conditions.

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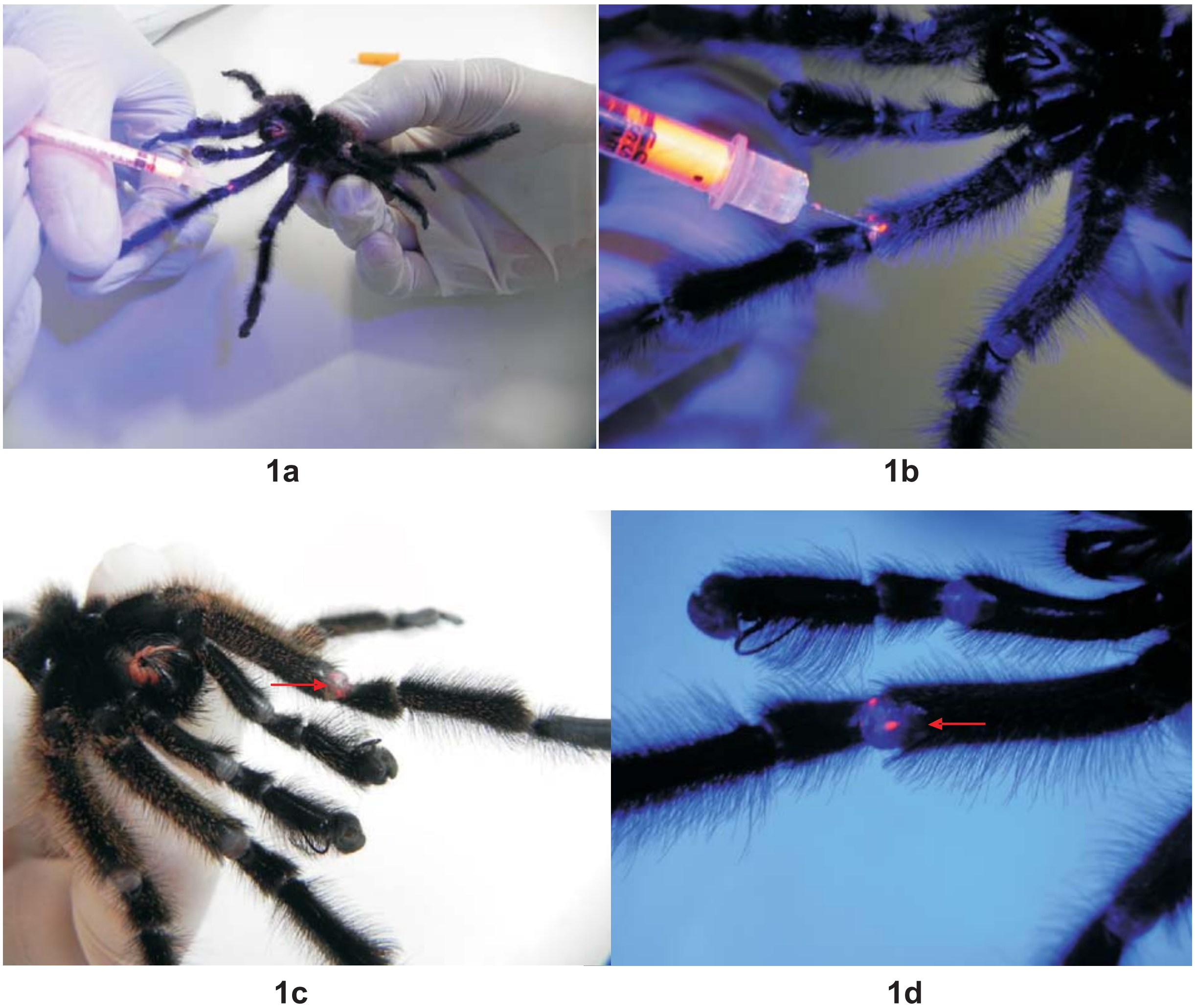

Fig. 1. Photographs demonstrating the application of VIFE markings. **A** – The application process of VIFE under ultraviolet light. **B** – Amplified image of the marking application. **C** – Visualization of the marking under visible light. **D** – Visualization of the marking under ultraviolet light. Arrow – Indication of the marking locality.