Ultrastructure of *Deinopis* egg sac (Araneae: Deinopidae)

Gilbert Barrantes*[1]
Olman Alvarado-Rodríguez[2]
Emilia Triana[1]
[1]Escuela de Biología,
Universidad de Costa Rica,
Ciudad Universitaria Rodrigo Facio,
San José, Costa Rica, CP 11501-2060
*corresponding author, email: gilbert.barrantes@gmail.com
[2]Centro de Investigación en Estructuras Microscópicas (CIEMIC),
Universidad de Costa Rica,
Ciudad de la Investigación,
San José, Costa Rica, CP 11501-2060

Summary

Attacks by predators and parasitoids on spider eggs are common. To counteract their negative effect, spiders have evolved a series of behaviours and features related to their egg sacs. In the genus *Deinopis* (Deinopidae) the tightly woven external layer of the egg sac seems to be an effective barrier. This layer consists of two sub-layers: the outer sub-layer is constructed of thin threads densely woven with an apparently hardened liquid that cements the threads together, and an inner sub-layer that seems to be even more compact and in which threads are not discernible. Threads of the external layer are presumably produced by the cylindrical glands, but other glands may be involved in production of the liquid substance.

Introduction

Numerous strategies have evolved in different groups of animals to protect their reproductive investments (Clutton-Brock 1991). Spiders, for instance, display a wide variety of behaviours which have likely evolved to maintain appropriate micro-environmental conditions inside the egg sac for embryo development, and to physically protect eggs and embryos from the attacks of predators and parasitoids (Austin 1985; Hieber 1985, 1992a; Triana, Barrantes & Hanson 2012). Different spiders accomplish these functions in different ways. For instance, the shapes of egg sacs and the coloration of their external silk layers, which presumably evolved to reduce conspicuousness of egg sacs, are extremely variable even between related genera within the same family (Barrantes *et al*. 2013). The construction of retreats in which to maintain egg sacs, and of silk stalks with which to attach the egg sac to a substrate, are also characteristics and behaviours that vary widely in spiders (Nielsen 1932; Bristowe 1958; Coddington 1986; Eberhard 1991; Hieber 1992b; Moya *et al*. 2010).

The external layer of the egg sac in many species acts as the main barrier against the attack of parasitoids and other arthropod predators (Foelix 2011), but it also has to allow an appropriate humidity, gas exchange, and thermal insulation for embryo development (Hieber 1985). Hence, a completely effective barrier against the attacks of parasitoids and predators may have detrimental effects on the environmental conditions inside the egg sac, affecting development of embryos. Consequently, the characteristics of the external layer of spiders' egg sacs are expected to result from trade-offs among multiple factors such as predators and parasitoids (e.g. parasitoid abundance) as well as external and internal environmental conditions (e.g. relative humidity).

The densities of the silk threads of the external layer of egg sacs vary widely across spider species but, in most cases, it is still possible to distinguish individual threads (under the dissecting microscope, by hand lens or the naked eye). However, in a few species the density of threads of the external layer is very high, making silk threads undetectable and creating a hard, compact external layer. Spiders in the family Deinopidae construct egg sacs with perhaps the most densely built and hardest external layer (Coddington, Kuntner & Opell 2012). The aim of this study is to describe the ultrastucture of the egg sac in *Deinopis* sp. MacLeay, 1839 and discuss the advantage of producing such a dense external layer.

Material and methods

Ultrastructure of egg sacs

We collected 34 empty egg sacs of *Deinopis* sp. in plantations of African oil palm (*Elaeis guineensis*) in Parrita, Puntarenas Province, Costa Rica (09º30′N 84º10′W; 10 m a.s.l.). All egg sacs collected were attached to rachises of dead fern leaves or dead twigs on plants that were growing on the trunk of oil palms. We collected one adult female in this plantation, but in Costa Rica six species of *Deinopis* occur, so species identification is difficult, even having both males and females (J. Coddington pers. comm.). Furthermore, we cannot be sure that all egg sacs belonged to the same species, so we have focused this study at the genus level.

We photographed two egg sacs under the dissecting microscope to obtain information on the characteristics of the external layer, and photographed two additional egg sacs with a Hitachi Model S-5700N scanning electron microscope (SEM) in the Centro de Investigación en Estructuras Microscópicas (CIEMIC), Universidad de Costa Rica. For the SEM, the egg sacs were covered with a 10 nm layer of gold at 15 mA for 2 min using a sputter coater EMS 550X. We dissected all 34 egg sacs to check for spider exuviae, pupae or dead bodies of parasitoids, and arthropod predators. Parasitoids and most arthropod egg predators (e.g. Mantispidae) moult to adulthood inside the egg sac, leaving the shed skin of the pupae inside the egg sac (Redborg & Macleod 1985; Hanson & Gauld 2006).

Results

Ultrastructure of egg sacs

The egg sacs of *Deinopis* are nearly spherical (width: 6.5 mm ± 0.08; height: 6.8 mm ± 0.29; n = 6) and have a stalk about 2 cm long. The brownish external layer has two sub-layers. Viewed externally it has a compact smooth appearance under the dissecting microscope (Fig. 1A). SEM images show that the external sub-layer had a thickness of

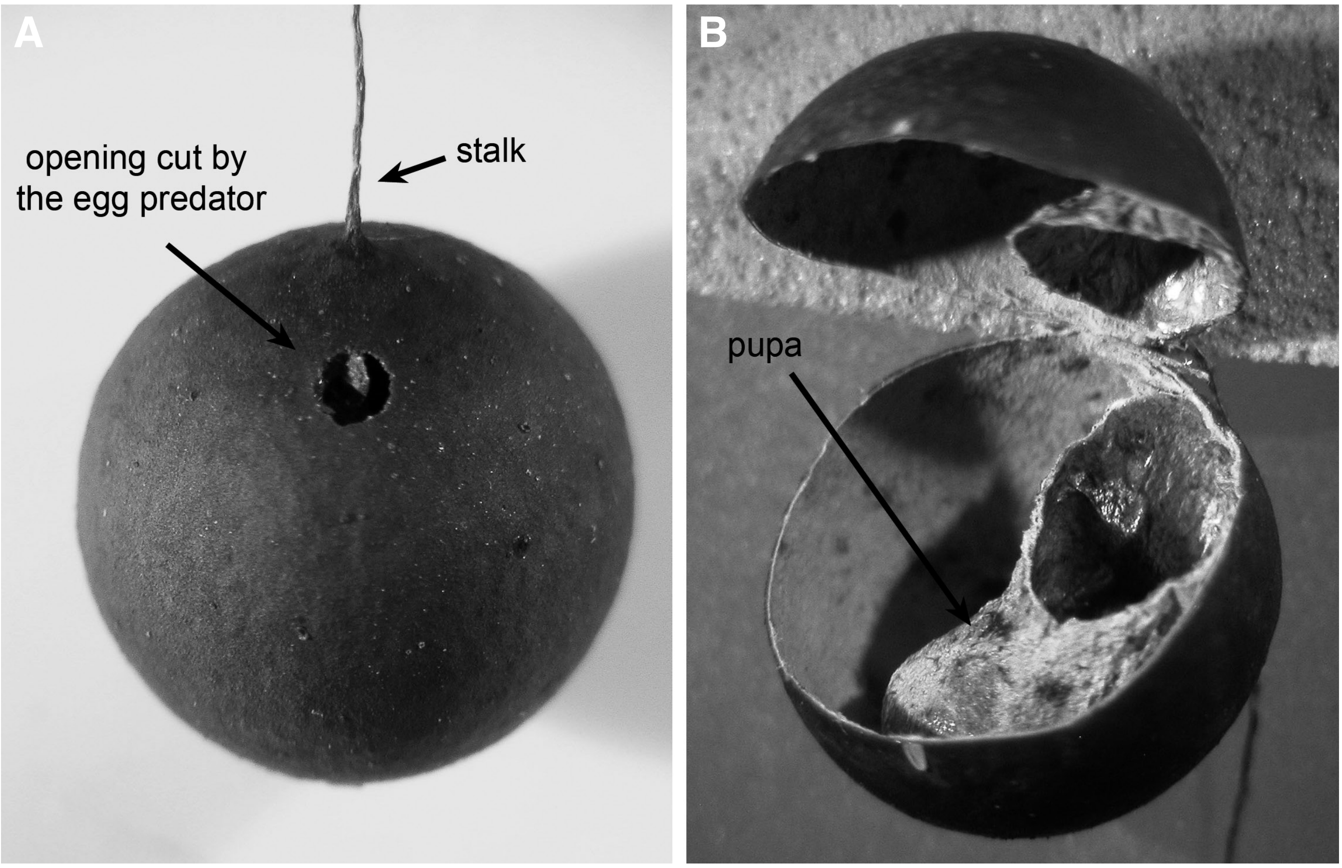

Fig. 1: Egg sac occupied by the egg predator. **A** opening cut by the egg predator; opening of the predator is cut closer to the stalk than that cut by spiderlings; **B** pupa of the egg predator; note the absence of whitish flocculent silk inside the egg sac.

60 µm and very compact consistency with both threads and an apparently hardened liquid (Fig. 2A–D). The threads were *c*. 1–2 µm in diameter, and were densely packed (Fig. 2D). This outer sub-layer has approximately one third of the total thickness of the entire layer. The internal sub-layer is much more compact and shows no signs of threads (Fig. 2C). Inside this compound outer layer is a mat of flocculent, whitish silk that probably is in contact with the eggs and spiderlings (Fig. 2C).

The stalk consists of silk threads that varied in thickness; most threads were *c*. 15 µm in diameter, but other threads were up to 30 µm in diameter. The core of the stalk consists of long, thick threads that the spider wraps with other threads in a circular fashion near the egg sac, giving the appearance of rings holding the threads of the core (Fig. 2E). The area in which the stalk connects to the egg sac consists of thinner threads (Fig. 2F).

Egg sac construction

The complete construction of egg sacs was not observed. We obtained a short video recording (provided by I. Escalante from a different locality) of the final stage of construction of a *Deinopis* egg sac, at 15 cm above the ground in a grassy area near a forest edge. The spider was applying the last layer to the outer surface of the egg sac (Fig 3). To apply this last layer she hung by her first legs from silk threads of a scaffold she had built across grass blades. All other legs and her palps were in contact with the egg sac, presumably holding the egg sac steady, while she curved her abdomen ventrally and, with rapid dorso-ventral movements of her abdomen, apparently applied silk or liquid to a reduced area on the outer surface of the egg sac (Fig 3). During 17 s she placed and raised her spinnerets 53 times; she placed her spinnerets consecutively on the same or nearly the same point of the outer surface.

Additional information

We cut open 34 egg sacs looking for the remains of parasitoids and egg predators. One had the pupa of a relatively large predator (Fig. 1B). This was the only egg sac that did not contain the shed skins of the spiderlings (spiderlings moult once before they abandon the egg sac). Strangely, this egg sac lacked the flocculent layer of whitish silk. The edge of the exit produced by this predator was roughly cut, compared with the smoother edges of exits produced by spiderlings (Fig. 2B).

Discussion

Deinopis sp. constructs what seem to be relatively unprotected egg sacs which are left alone without the mothers' active protection. However, the extremely dense, compound external layer of the egg sacs built by *Deinopis* may be an effective barrier against parasitoids and most arthropod predators. In addition to silk threads, this layer has what appears to be a hardened liquid like substance that cements

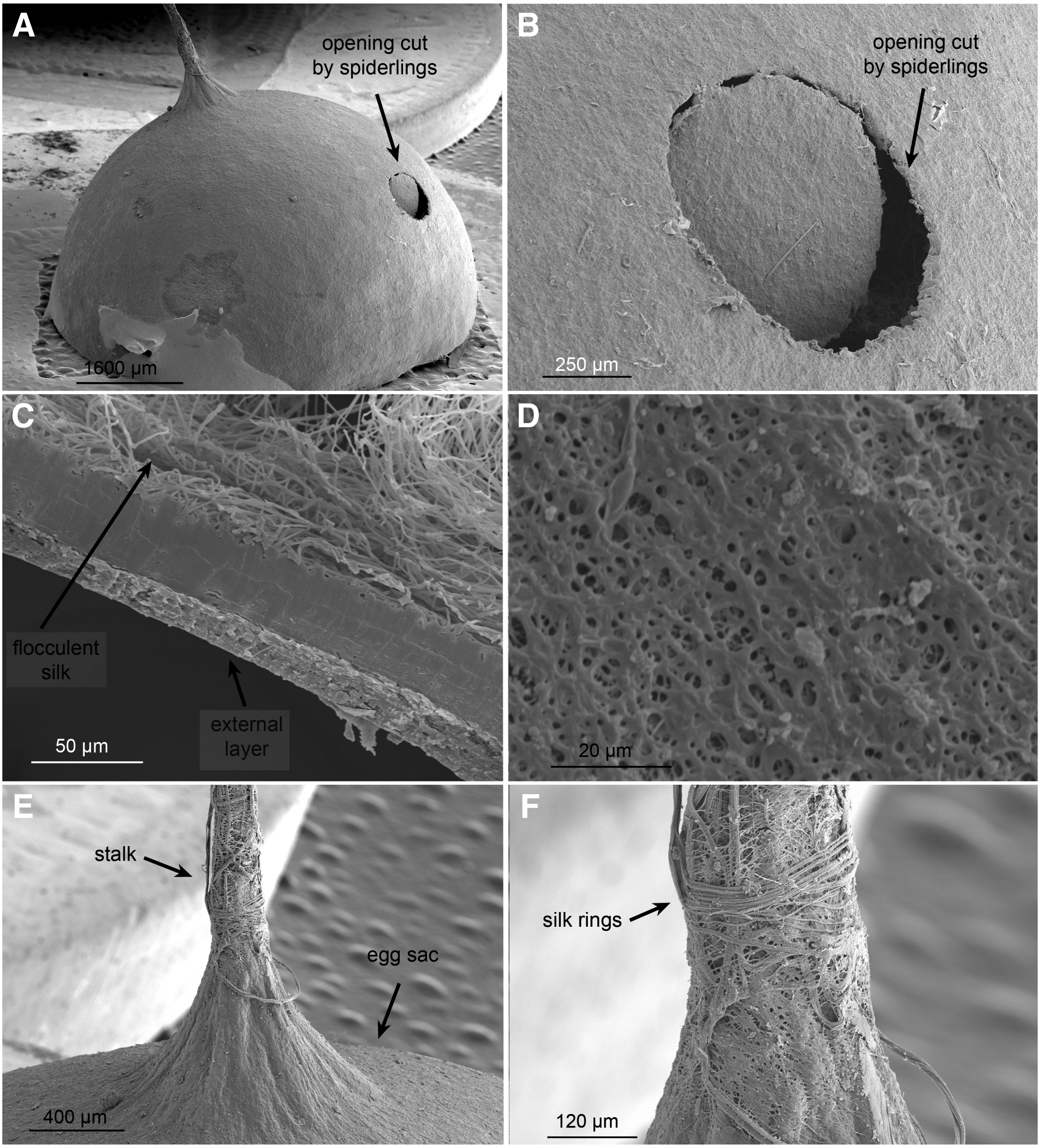

Fig. 2: Ultrastructure of the egg sac of *Deinopis* sp. **A** external layer of the egg sac; arrow points the opening cut by spiderlings to exit the egg sac; **B** detail of the opening cut by spiderlings; **C** external layer; note the two sections of the external layer and the flocculent silk in the interior of the egg sac; **D** silk threads of the external layer covered with a hardened liquid substance; **E** stalk of the egg sac; **F** silk threads laid by the spider around the stalk.

the threads, and perceptibly reduces the space between them.

It appears that *Deinopis* uses at least two types of material in the construction of its sacs. The external layer is presumably constructed with silk threads produced by the cylindrical (= tubiliform) glands (Foelix 2011), but it is uncertain whether the apparently liquid substance is produced by the same glands. In *Theridiosoma gemmosum* a silk/secretion, presumably from aciniform glands, produces a dense compact cover that resembles the outer layer of egg sacs of *Deinopis* (Hajer *et al*. 2009). However, further behavioural observations on egg sac construction and silk characteristics are necessary to know which silk types are involved in the construction of egg sacs in *Deinopis*, and whether this hardened liquid-like substance is produced by the same glands in *Deinopis* and *T. gemmosum*.

A major function that has repeatedly been attributed to the external layer of the spider egg sacs is protection against egg parasitoids and egg predators (Austin 1985; Hieber 1992b). Nevertheless, egg parasitoidism in spiders seems to be very high (Valerio 1971; Triana, Barrantes & Hanson 2012). At least some wasp parasitoids (e.g. *Baeus*,

Fig. 3: *Deinopis* sp. placing her spinnerets on the outer surface of the egg sac. Note the curvature of her abdomen to reach the egg sac.

Scelionidae) penetrate the external wall to reach the eggs inside the sac, and then lay their own eggs inside the spider's eggs (Pemberton & Rosa 1940). Other parasitoids introduce a long ovipositor through the external layer of the egg sac to lay their own eggs inside the spider's eggs (Austin 1985). In both cases, the woven silk with which most spiders built the external layer of egg sacs allows parasitoids to access the eggs inside the egg sac (see Foradori *et al*. 2002 and Agnarsson 2004 for some examples of the external layer of egg sacs).

The density of the external layer of the sacs of *Deinopis* seems to reduce the access of parasitoids and predators to their eggs. No sign of parasitoids was found in any of the egg sacs dissected, and only one egg sac had a pupa of an egg predator. This pupa possibly belonged to a Mantispinae of the genus *Zeugomantispa* (based on size and shape of the pupa), a mantidfly specialized on spider eggs. Larvae of different species of Mantispinae gain access to the spider eggs by burrowing through the external layer, or by entering the egg sac during its construction, or using both strategies (Redborg & MacLeod 1985; Guarisco 1998).

The egg sacs of *Deinopis* have several characteristics which may have evolved to reduce the attack of parasitoids and egg predators. The coloration of the external layer helps camouflage the egg sac against the background (Barrantes *et al*. 2013). The stalk, as has been demonstrated in other spiders (Hieber 1992b), probably reduces the access of predators to the eggs (e.g. ants). The hard, compact, external layer probably makes it more difficult for parasitoids and predators to gain access to the eggs.

Acknowledgements

We thank I. Escalante for kindly providing a video and pictures of the construction of an egg sac, and A. Retana-Salazar and A. Rodríguez-Arrieta for their technical support. Research by GB was supported by the Vicerrectoría de Investigación.

References

AGNARSSON, I. 2004: Morphological phylogeny of cobweb spiders and their relatives (Araneae, Araneoidea, Theridiidae). *Zoological Journal of the Linnean Society* **141**: 447–626.

AUSTIN, A. D. 1985: The function of spider egg sacs in relation to parasitoids and predators, with special reference to the Australian fauna. *Journal of Natural History* **19**: 359–376.

BARRANTES, G., SANDOVAL, L., SÁNCHEZ-QUIRÓS, C., BITTON, P.-P. & DOUCET, S. M. 2013: Variation and possible function of egg sac coloration in spiders. *Journal of Arachnology* **41**: 342–348.

BRISTOWE, W. S. 1958: *The world of spiders*. London: Collins.

CLUTTON-BROCK, T. H. 1991: *The evolution of parental care*. Princeton, NJ: Princeton University Press.

CODDINGTON, J. A. 1986: The genera of the spider family Theridiosomatidae. *Smithsonian Contributions to Zoology* **422**: 1–96.

CODDINGTON, J. A., KUNTNER, M. & OPELL, B. D. 2012: Systematics of the spider family Deinopidae with a revision of the genus *Menneus*. *Smithsonian Contributions to Zoology* **636**: 1–61.

EBERHARD, W. G. 1991: Notes on the natural history of *Wendilgarda galapagensis* (Araneae: Theridiosomatidae). *Bulletin of the British Arachnological Society* **8**: 246–248.

FOELIX, R. 2011: *Biology of spiders*. *Third edition*. New York: Oxford University Press.

FORADORI, M. J., KOVOOR, J., MOON, M.-J. & TILLINGHAST, E. K. 2002: Relation between the outer cover of the egg case of *Argiope aurantia* (Araneae: Araneidae) and the emergence of its spiderlings. *Journal of Morphology* **252**: 218–226.

GUARISCO, H. 1998: First observation of egg sac predation of the Mantisfly, *Mantispa interrupta* (Neuroptera, Mantispidae) upon the Wolf Spider, *Gladicosa gulosa* (Araneae, Lycosidae). *Transactions of the Kansas Academy of Science* **101**: 60–62.

HAJER, J., MALÝ, J., HRUBÁ, L. & REHÁKOVÁ, D. 2009: Egg sac silk of *Theridiosoma gemmosum* (Araneae: Theridiosomatidae). *Journal of Morphology* **270**: 1269–1283.

HANSON, P. E. & GAULD, I. D. 2006: *Hymenoptera de la región Neotropical*. Gainesville, FL: American Entomological Institute.

HIEBER, C. S. 1985: The "insulation" layer in the cocoons of *Argiope aurantia* (Araneidae: Araneae). *Journal of Thermal Biology* **10**: 171–175.

HIEBER, C. S. 1992a: The role of spider cocoons in controlling desiccation. *Oecologia* **89**: 442–448.

HIEBER, C. S. 1992b: Spider cocoons and their suspension systems as barriers to generalist and specialist predators. *Oecologia* **91**: 530–535.

MOYA, J., QUESADA, R., BARRANTES, G., EBERHARD, W. G., ESCALANTE, I., ESQUIVEL, C., ROJAS, A., TRIANA, E. & ARIAS, A. 2010: Egg sac construction in folded dead leaves by *Pozonia nigroventris* and *Micrathena* sp. (Araneae, Araneidae). *Journal of Arachnology* **38**: 371–373.

NIELSEN, E. 1932: *The biology of spiders*. Copenhagen: Levin & Munkgaard.

PEMBERTON, C. E. & ROSA, J. S. 1940: Notes on the life history of *Baeus californicus* Pierce, an egg parasite of the Black Widow spider. *Hawaiian Planter's Record* **44**: 73–80.

REDBORG, K. E. & MACLEOD, E. G. 1985: The developmental ecology of *Mantispa uhleri* Banks (Neuroptera: Mantispidae). *Illinois Biological Monographs* **53**: 1–130.

TRIANA, E., BARRANTES, G. & HANSON, P. 2012: Incidence of parasitoids and predators on eggs of seven Theridiidae species (Araneae). *Bulletin of the British Arachnological Society* **15**: 293–298.

VALERIO, C. E. 1971. Parasitismo en huevos de araña *Achaearanea tepidariorum* (Koch) en Costa Rica. *Revista Biología Tropical* **18**: 99–106.