OBSERVATIONS OF *THEOTIMA MINUTISSIMUS* (ARANEAE, OCHYROCERATIDAE), A PARTHENOGENETIC SPIDER

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ABSTRACT.   It has been suggested by several authorities that at least one species of spider of the genus *Theotima*, family Ochyroceratidae, occurring in tropical regions in South Africa, the Caribbean and Asia may be parthenogenetic. *Theotima minutissimus* is particularly abundant in the tropical rainforest leaf litter on El Yunque, Puerto Rico. While many hundreds of specimens have been collected over many years, none has been a male. To examine the possibility that this small species, ± 0.9 mm body length, is parthenogenetic, live specimens were collected and maintained in the laboratory. A second generation spiderling, raised separately, produced viable progeny.

Keywords: Parthenogenesis, spider, tropical rainforest, leaf litter, *Wolbachia*

The six-eyed spider family Ochyroceratidae is found in subtropical and tropical regions around the world and is especially species rich in the Indo-Pacific. There are ten genera found worldwide with four known from the western hemisphere; *Fageicera* and *Speocera* recorded only from Cuba, *Ochyrocera* in the Caribbean region and Brazil, and *Theotima*. *Theotima minutissimus*, originally described as *Oonopinus minutissimus* Petrunkevitch 1929, occurs in the Caribbean region, also in the Indo-Pacific and possibly Africa as well (Platnick 1997). Females of this family typically carry their eggs until they hatch in their chelicerae. Two undescribed sympatric species of *Ochyrocera* occur in forest leaf litter in Puerto Rico. There is little published information available on the life history of any of these spiders.

Theotima minutissimus is abundant in the Caribbean National Forest, El Yunque, Luquillo, Puerto Rico. Its preferred habitat is litter composed of smaller leaves in second growth forests with understory shrubs or in those forests with damper and more easily decayed leaves such as mahogany (*Swietenia macrophylla* King) or bamboo (*Bambusa vulgaris* Schrad.). *T. minutissimus* prefers elevations less than 800 m. From 1992–2001, we collected more than 700 0.25 m^2 litter sam-

ples. These samples were taken in all the principal habitats on El Junque. The abundance of *T. minutissimus* in the samples ranged from 5–460 individuals m^{-2}. An earlier study in the Tabonuco forest (*Dacryodes excelsa* Vahl), demonstrated a range of abundance of 43–166 individuals m^{-2}, with a mean annual density of 74 (Pfeiffer 1996). Only in the Tabanuco forest was the abundance of *T. minutissimus* consistently exceeded by another litter spider species, *Modisimus montanus* Petrunkevitch 1929.

The number of penultimate and adult *Theotima minutissimus* specimens examined to date exceeds 1,000. No male has ever been found. The male palpi of other members of the family Ochyroceratidae are distinctively different from those of the female (see for example fig. 1, p. 83 in Emerit & Lopez 1985) and are readily distinguishable even in the penultimate instar. The absence of any *T. minutissimus* showing such palpi leads to the supposition that this spider could be parthenogenetic. Machado (1964) became convinced that a species of the genus *Theotima* of the feminina group in Africa in the Congo and Angola was parthenogenetic. Deeleman-Reinhold (1995) subsequently reported what appears to be the same species of *T. minutissimus* from Panama, the Philippines, Borneo, Indonesia,

Sumatra, Thailand and Guam. She noted, in addition to not finding males, that "The most salient feature is the paired lightly sclerotized arch on the lateral margin of the epigastric opening: the "spermathecae" are very thin walled and easily shift position." This report implies a potentially faulty genital apparatus. Deeleman-Reinhold also suggested that this spider may be parthenogenetic.

To determine whether or not *Theotima minutissimus* was in fact parthenogenetic, on 8 February 2001, 22 females judged to be either mature or penultimate were collected and placed separately in small petri dishes (Fisher No. 09-75-53C, 50 mm diameter Petri Dish with absorbent pad). Each pad was moistened with two drops of water, and 10 or more entomobryid springtails (*Sinella curviseta* Brook) were added as food. The dishes were then placed in a larger plastic container containing a moistened sponge to help maintain humidity. The temperature was maintained between 19–22 °C.

Unless the spider positioned itself on the top of the petri dish, venter side up, it was not possible to determine whether or not it was an adult. There appear to be at least 5 instars that gradually increase in pigmentation from the third instar on, but with no obvious changes in pigmentation, body structure or the appearance of the epigynum between the penultimate and adult stage. Twenty adults averaged slightly less than 1 mm (0.79–0.98) and 20 penultimate 0.76 mm (0.69–0.83) in body length. In the petri dish any exuviae or remains of deceased spiders were quickly scavenged by the springtails and seldom seen.

Female *Theotima minutissimus* carry their eggs in a bundle in their chelicerae until the spiderlings are fully developed and capable of moving about on their own. The number of eggs varies from 1–9; 4–6 eggs per clutch were most common. Eggs were produced by 17 of the 22 captive females with egg numbers varying from 1–9. In three cases eggs were abandoned before they developed. In three other cases females produced a second clutch of eggs, and in one case three clutches. In all, ten clutches were produced that resulted in viable spiderlings. A few females were observed to drop their clutch of eggs briefly and return to it. Four females died in the first two months. It was not until November 2001 that the remaining females were dying regularly.

All 22 females were dead by the end of December 2001.

When the eggs are extruded they are ovoid in shape and uniformly translucent. The entire clutch is extruded in a matter of hours, usually during the night. The eggs are more or less arranged in one layer to a pad of silk which the female carries in her chelicerae. For the first 9–11 days the eggs showed no clear sign of development but became more elongate and showed subtle changes in density. Over the next week the leg structure became apparent and development proceeded rapidly with the abdomen and thorax becoming distinct. The developing spiderlings were bunched together with their tarsi connected to the pad of silk. The spiderlings were arranged facing outwards (Fig. 1). Just before leaving the mother, on average three weeks after extrusion, the spiderlings engaged in an activity that we describe as doing 'pushups'. This consisted of actively flexing their legs, which tended to push their body away from the mother and appeared to be associated with the shedding of the first instar exoskeleton. This process was readily observed because at this time females typically positioned themselves upside-down in their webbing. Once they separated from their mother, the spiderlings soon assumed an upside-down orientation in the web. On average, the time between egg production and release of the spiderlings was three weeks to one month. Spiderlings remained in the webbing originally made by the female. The pad of silk containing the remnants of the first instar exuviae remained in the web as well. Within two days after the spiderlings hatched the female moved elsewhere in the dish and established a new web. These relatively dense, irregular sheet webs usually extended from the top of the dish to the side and sometimes to the bottom.

The second instar spiderlings are colorless and virtually transparent. Ten were measured averaging 0.39 mm in body length. Within 2 or 3 days, all but one spiderling produced from each clutch was separated from the mother and placed in separate petri dishes. A few spiderlings were observed attempting to capture smaller springtails. With disappointing regularity the spiderlings disappeared after about 10–14 days whether they were living alone or with the mother. It could not be determined if they had been preyed upon by

Figure 1.—Separately raised *Theotima minutissimus* with clutch of four well developed spiderlings. Spiderlings still attached by legs to a pad of silk carried by the female. The abdomens are directed inward. Space bar = 1 mm.

large springtails. We suspect they died after depleting any energy stores they inherited and/or because they lacked appropriate prey.

In April 2001, four newly hatched spiderlings were transferred into separate petri dishes with soil. Two individuals survived, one for 54 days before it died. The second survived and reached a length of 0.75 mm by January 2002. At this point the spider was judged to be penultimate. On 11 March this spider produced a clutch of four eggs which developed normally. These spiderlings hatched 4 April. The female produced a second clutch of four eggs on 14 April, which developed normally and all four spiderlings became free of the mother (hatched) on 8 May.

While not the first to suggest that some spiders might be parthenogenetic, Machado (1964) extensively examined the possibility for a maleless population of *Theotima* and concluded that the species had to be parthenogenetic. At this time it is not possible to say that the species he studied was or was not con-specific with *Theotima minutissimus*. Lake (1986) reported that a female *Isopeda insignis*, family Heteropodidae, had reproduced parthenogenetically. Gruber (1990), following up an earlier report by Deeleman-Rhinhold (1986) suggesting that *Dysdera hungarica*, family Dysderidae, might be able to reproduce parthenogenetically, kept female *Dysdera hungarica* isolated from males. The females produced egg sacs and a few of the unfertilized eggs ultimately became adults which in turn produced a further generation. Gunnarsson & Andersson (1992) studied chromosome variation in *Pityohyphantes phrygianus*, family Linyphiidae. They discovered that 1–2% of embyros were haploid which suggested development from unfertilized eggs. Shimojana & Nishihira (2000) described a trogloditic spider, *Coelotes troglocaecus*, family Amaurobiidae. No males were found and the females had degenerate copulatory organelles.

It would appear that there may be many roads to achieving parthenogenesis in spiders.

Ultimately the question of whether or not an endosymbiotic bacterium such as *Wolbachia* may be involved is worth pursuing now that it has been demonstrated to exist in *Nephila clavata* (Oh et al. 2000). Weeks et al. (2002) caution that there are other endosymbionts or nuclear effects that could be involved. Whatever the case with respect to *Theotima minutissimus,* its world wide distribution and success in its chosen habitat points up the potential evolutionary significance of adopting the parthenogenetic mode of reproduction.

Voucher specimens are deposited in the American Museum of Natural History, New York.

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