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ARTÍCULO:

A critical review of reports of parthenogenesis in Scorpions (Arachnida)

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Abstract:

Parthenogenesis has been reported in thirteen species of scorpion, and is suspected or implied in another one. The criteria for considering a species to be parthenogenetic are reviewed. Birth by an unmated, virgin female is irrefutable evidence for this phenomenon, whereas iteroparity and female-biased sex ratios are not necessarily so and should be thoroughly investigated. Thelytokous parthenogenesis is accepted for the following seven taxa: *Centruroides, gracilis Tityus columbianus, Tityus metuendus, Tityus serrulatus, Tityus trivittatus, Tityus uruguayensis* and *Liocheles australasiae.* Thelytokous and/or facultative parthenogenesis are rejected for *Ananteris coineaui, Cazierius asper, Hottentotta hottentotta, Tityopsis inexpectatus, Tityus stigmurus* and *Vaejovis spinigerus*; and arrhenotokous parthenogenesis is rejected for *Tityus metuendus*. Further studies are needed in *Pseudolychas ochraceus*.

Key words: Virgin birth, thelytoky, arrhenotoky, captive breeding, biased sex-ratios.

Revisión crítica de los informes sobre partenogénesis en Escorpiones (Arachnida)

Resumen:

La partenogénesis ha sido señalada en trece especies de escorpiones, y se sospecha que está implicada en otra. En esta nota se revisan los criterios para considerar que una especie es partenogenética. El parto de una hembra virgen es una evidencia irrefutable para este fenómeno, mientras que la iteroparidad y la proporción de sexos sesgado hacia las hembras no son necesariamente pruevas suficientes y deben de ser investigados en profundidad. La partenogénesis telitoka es aceptada para los siete siguientes taxones: *Centruroides gracilis*, *Tityus columbianus*, *Tityus metuendus*, *Tityus serrulatus*, *Tityus trivittatus*, *Tityus uruguayensis* y *Liocheles australasiae*. Telitoquia y/o partenogénesis facultativa son rechazadas para *Ananteris coineaui*, *Cazierius asper*, *Hottentotta hottentotta*, *Tityopsis inexpectatus*, *Tityus stigmurus* y *Vaejovis spinigerus*, y la partenogénesis arrenotoca es rechazada para *Tityus metuendus*. Se requiere un mayor número de estudios en *Pseudolychas ochraceus*.

Palabras clave: Nacimiento virginal, telitoquia, arrenotoquia, cria en cautividad, sesgo en la proporcion de sexos.

Introduction

Asexual reproduction seems to be the norm among prokaryotic organisms, whereas sexual reproduction is prevalent among eukaryotes. The evolution of sexual reproduction in diploid organisms usually implies; (a) in a population of organisms of the same species, the presence of two or more discrete classes of individuals belonging to different sexes; *i. e.*, producing different kinds of gametes (=anisogamy); (b) some kind of reductional cell division (*i. e.*, meiosis); and (c) independent assortment and recombination. Parthenogenesis in animals (mostly known as apomixis in plants) literally means "virgin birth", and in a stricter biological sense it is understood as "reproduction by development of an unfertilized gamete" (Mayr, 1963:409).

Parthenogenesis is a relatively rare phenomenon in the animal kingdom and has received considerable attention from zoologists for its broad implications in cytogenetics (*e.g.*, White, 1978), dispersal biogeography (*e. g.,* Cuellar, 1994), and both the genetic (Williams, 1975; Maynard Smith, 1978) and developmental (*e.g.*, West-Eberhard, 2003) aspects of the evolution of sexual reproduction. The various forms of parthenogenesis in insects (*e.g.*, obligatory, facultative, cyclical, etc.) are summarized by Thornhill & Alcock (1983). Thelytoky is the more widespread form of parthenogenesis, where a virgin female produces an all-female clutch or litter; arrhenotoky is a form of parthenogenesis mostly confined to hymenopteran insects (ants, bees and wasps) where non-virgin females can lay diploid, fertilized eggs that develop into daughters, or lay haploid, unfertilized eggs that develop into sons.

Among arachnids parthenogenesis has been reported in mites (Acari), harvestmen (Opiliones), and scorpions (Scorpiones) (Lourenço & Cuellar, 1994); as well as spiders (e.g., see Edwards *et al.*, 2003). Parthenogenesis has been associated, in one form or another, with 14 species of scorpion, belonging to 3 families, 8 genera and 3 subgenera which should make scorpions an ideal group to analyze numerous aspects (*e.g.,* cytogenetics, ecology, biogeography and evolution) of this phenomenon. Nonetheless, some of those reports have been controversial and others are fraught with shortcomings and/or factual inaccuracies. It is the aim of this review to analyze critically each one of those claims/reports, in chronological order, exposing their strengths and weaknesses, and identifying areas of opportunity for future research.

Broad overview of the problems associated with claims of parthenogenesis in scorpions

If parthenogenesis literally means "virgin birth," then the most obvious and direct way to determine its presence is by observing an unmated (*i.e.*, virgin) female deliver a litter of young. Iteroparity (successive parturitions by one female even without intervening insemination) is widespread among scorpions (*e.g.,* Polis & Sissom, 1990), and thus sperm storage and/or delayed implantation, or both, are implied in scorpion reproductive biology (*e.g.,* Kovoor *et al.*, 1987). Therefore, one or multiple parturitions in captivity by an adult, field-caught female cannot be construed as, and does not constitute incontrovertible evidence of parthenogenesis. Sexually immature female scorpions (subadult or younger) are as a general rule both unreceptive and unattractive to sexually mature males. Thus, parturition in captivity by a female which was caught as an immature, and which molted (once or more) to attain sexual maturity while in captivity, can be construed as, and in my opinion does constitute, indisputable evidence of parthenogenesis. Parturition in captivity by a female born in captivity and raised in isolation until sexually mature is incontrovertible evidence for parthenogenesis. The single report by Toscano-Gadea (2001) of a post-parturition molt by a field-caught female scorpion needs to be verified, for it could have serious implications with respect to claims of parthenogenesis involving field-caught "subadult" females which molted to "maturity" in captivity (*i.e.*, the females could have been sexually mature and mated before an additional molt). However, literally thousands of female scorpions of many different taxa have been raised and bred in captivity, and to my knowledge no other post-parturition molts have been reported.

Does the presence of an "all-female" population constitute evidence of parthenogenesis? Special situations aside, such as gynogenesis where sperm from males of a different species are used to stimulate cell division but the male chromosomes are not incorporated into the egg nucleus, "all-female" populations are strong candidates for thelytokous parthenogenesis and should be studied further (*e.g.*, dissections to establish clearly the presence of only one kind of reproductive system, cytogenetic studies to establish whether the population exhibits ameiotic or meiotic parthenogenesis, rearing at least two consecutive generations under isolation conditions in the laboratory) in order to establish "beyond reasonable doubt" that virgin births do occur. Why are these extra steps necessary? Because there are several alternatives to parthenogenesis to account for female-biased sex ratios.

First, as Charnov & Bull (1977) pointed out, "There are several sex determining mechanisms which produce two sexes in a population (dioecy or gonochorism). In many of these, the offspring´s sex is determined at or before conception, as in male or female heterogamety. In several organisms, however, the offspring´s sex is determined later than conception, by some environmental influence upon the offspring" (p. 828)

Environmental sex determination (ESD) is known in several plants and animals, among the latter are mermithid nematodes, echiurid marine worms, parasitic isopods, monstrillid crustaceans (Charnov & Bull, 1977), and a species of shrimp (Taylor *et al.,* 1988). Temperature-dependent sex determination in reptiles and amphibians (Bull 1980) is responsible for enigmatic sex ratios (Bull & Charnov, 1989), *i.e.,* those that deviate from the 1:1 expected from genotypic sex determination (GSD) mechanisms. In those reptiles where the sex of the clutch is strongly correlated with the incubation temperature, a female-biased sex-ratio is no more evidence for thelytokous parthenogenesis than a male-biased sex-ratio is for arrhenotoky.

Second, sex ratios in nature can be distorted by sexual differences in age at maturity and by differential survival of males and females, independently of the underlying mechanism(s) of sex determination (Girondot & Pieau, 1993), and scorpions are no exception (Polis & Sissom, 1990).

Third, female-biased sex ratios in field samples can result from seasonal differences in surface activity between females and juveniles (earlier in the season) and males (later in the season) (Polis, 1980).

Fourth, at least in the case of scorpions, is the matter of sample size: how many field-caught females,

without any males, constitute indisputable evidence of an "all-female" population? Does a reported sample size of "several" females and no males really support a claim for thelytoky? Does the fact that only three males survived to maturity in captivity out of a litter of 22 young scorpions constitute evidence for arrhenotoky?

Finally, intracellular infection with the alpha-proteobacteria *Wolbachia* can severely disrupt the reproductive process in arthropods, inducing parthenogenesis, selectively killing male embryos and inducing the feminization of genetic males (Werren *et al.*, 1995, Stouthamer *et al.*, 1999). *Wolbachia* has been recently reported in scorpions (Baldo *et al.*, 2007, Suesdek-Rocha *et al.*, 2007), raising the possibility that female-biased sex-ratios are due to infection of the observed females, and not due to natural parthenogenesis throughout the population.

Sex in scorpions

Two challenging issues are discussed in this section: the mechanism(s) for sex determination, and the problems associated with sexual identification or recognition by investigators. Sex determination in diploid animals usually has a strong genetic component and is often manifested in the karyotype, *i.e.*, there are chromosomal differences between the two sexes. Some familiar examples are the XX/XY, the XX/XO and the X_1X_1/X_2X_2 sex chromosomes. More information concerning parthenogenesis in particular can be found in White (1978) and Cuellar (1977, 1987). As indicated above, arrhenotoky involves a special case of parthenogenesis and sex-determination in hymenopterans, where males develop from unfertilized eggs and hence are haploid, and females develop from fertilized eggs and hence are diploid. How can a claim for arrhenotoky in scorpions (Lourenço & Cuellar, 1999) be supported without any karyotyping?

In scorpions we know that females bear live young (Francke, 1982), and that males transfer their gametes indirectly by means of a spermatophore (Francke, 1979). However, we do not have the slightest evidence of the mechanisms underlying sex determination, and this is an open opportunity for research. This being the present state of knowledge about scorpion sex determination mechanisms: how can a claim for arrhenotoky (Lourenço & Cuellar, 1999) be sustained? The karyotype has never been reported in either sex of the species in question; thus, are the males really haploid as an hypothesis of arrhenotoky would predict? Could ESD account for the biased sex ratios reported by some investigators?

One of the serious challenges faced by scorpionologists is that of establishing the age and sex of field-caught samples based on external morphology alone. Sexual identification in scorpions is often a complex problem, created to some extent by scorpion diversity. Secondary sexual characteristics of scorpions in general are reviewed by Polis & Sissom (1990), and will not be repeated here. Some taxa exhibit strong sexual dimorphism between adult males and females, and on those the problem might be simply that of sexing juveniles. Among other taxa, however, establishing the age of field-caught specimens is a tenuous approximation at best (see *Tityopsis inexpectatus* below); sexual maturity can usually be confirmed by dissection of the gonads, but among immatures (especially when only a few specimens are available) clues such as "relative" degree of pigmentation and sclerotization permit tentative groupings. Identifying the sex depends on the taxon in question and the age of the individuals involved, and usually revolves around the genital opening and the pectines. In numerous taxa the males have genital papillae which the females lack; however, these soft structures are usually retracted and /or hidden under the genital operculi, especially in juveniles, complicating matters. Regarding the pectines, two somewhat variable characters are occasionally available. The first, ontogenetically fixed, is the number of teeth on each pectinal comb; on some taxa the male and female counts do not overlap and can be reliably used to sex individuals of any age (*e. g.,* Francke & Jones, 1982); whereas on other taxa they overlap partially or completely, rendering this character useless to sort the sexes. The second, ontogenetically variable, is the relative size of the teeth: at birth males and females are indistinguishable, but with each molt the male teeth become progressively longer relative to those of the females, rendering it possible to sort an adequate sample of middle-sized individuals (however, it is often impossible to sex with absolute certainty a single individual without the relative reference as to what constitutes "longer" versus "shorter").

These issues of sex determination and sex-and-age identification play a prominent role in some of the reports on parthenogenesis in scorpions.

Chronological review

Tityus serrulatus Lutz & Mello, 1922 (Buthidae)

The first report of parthenogenesis in scorpions is that of Matthiesen (1962), who successfully raised three females born in captivity (from a "brood" of 20) to sexual maturity, and which in turn produced litters of 16, 19 and 20 young, respectively. As Matthiesen indicated, Toledo-Piza (1940) had not found any males in a sample of 36 individuals of this species (I assume they were adult; no data were provided by Toledo-Piza as to the dates or season(s) of collection).

Bücherl (1956), discussing the possibility of iteroparity in *Tityus* C. L. Koch, 1836, rather casually indicated that over several decades he had never seen a male of *T. serrulatus* at the Instituto Butantan, in Sao Paulo, Brazil, where numerous specimens were kept in captivity for the production of antivenin. He also indicated that 425 specimens had been dissected in an attempt to find and study the male reproductive system, to no avail. However, he noted that the females gave birth in captivity each year, raising the possibility of iteroparity if they had mated before capture. Bücherl does not mention the word "parthenogenesis" in his work; Mat-

thiesen, however, following up on Bücherl's report, dissected more than 500 adult specimens in the Instituto Butantan and found no males, raising the possibility that this species exhibits thelytokous parthenogenesis. This was subsequently confirmed by Matthiesen (1962).

A few years later, San Martin & Gambardella (1966) corroborated Matthiesen's observations. They obtained adult females from Dr. Bücherl and the Instituto Butantan in July, 1963, and transported them to Montevideo, Uruguay. There, two females gave birth to litters of 7 and 12 young, respectively. The young were isolated as second instars, and two individuals (F_1) from each of those litters survived in captivity until mature at the fifth instar, and produced one F_2 litter, respectively. In addition, one additional female from each F_1 litter died as a pregnant, adult fifth-instar female with well-developed embryos inside (p. 83, Tables 1 and 2 "muere el ejemplar adulto en avanzado estado de gravidez").

Matthiesen (1971) following up on his earlier observations, reported the successful birth of an F_3 generation of parthenogens in captivity, as well as iteroparity (with up to four consecutive births by a virgin female) in this species.

Lourenço & Cuellar (1995) reported on the medical importance of *T. serrulatus*, indicating that its geographic range in Brazil was expanding and that the successful colonization of disturbed habitats could be attributed to its parthenogenetic means of reproduction.

Finally, Lourenço & Cloudsley-Thompson (1999), in a somewhat confusing and inconclusive contribution, merely managed to create some taxonomic confusion. They proposed that *T. serrulatus* and *Tityus lamottei* Lourenço, 1981 are nothing but color morphs of *Tityus stigmurus* (Thorell, 1877) and should be synonymized under the latter. Nonetheless, Lourenço *et al.* (2000:267) continue to refer to "the *Tityus serrulatus* morph Lutz & Mello, 1922"—a totally unacceptable nomenclatural arrangement. Nomenclatural problems aside, on the issue of parthenogenesis they state (Lourenço & Cloudsley-Thompson, 1999): "However, recent unpublished field observations by Lourenço show that the morphs *T. serrulatus* (=confluenciata) and *T. stigmurus* (=unifasciata) reproduce by parthenogenesis. Moreover, the sexual individuals of *T. stigmurus* occur in an undisturbed region...., Two other sexual morphs (confluenciata/maculata and trisfaciata [sic!] occur in undisturbed regions." (p. 155)

These authors then proceed to give a "formal" taxonomic diagnosis of the sexual *confluenciata* form (*i. e., Tityus serrulatus*, an hypothesized obligatory thelytokous parthenogen!). The quote above is remarkable because out of nowhere and without any evidence whatsoever, a second taxon (species? morph?) has been "shown" to be parthenogenetic. Further, Lourenço (2002) states: "Since then, T. serrulatus has been transferred to Tityus stigmurus (Thorell) (Lourenço & Cloudsley-Thompson 1996), a parthenogenetic species consisting of at least three distinct all-female morphs (Lourenço & Cloudsley-Thompson 1999) of which the original *T. serrulatus* represents one." (p. 77)

Now there are three all-female morphs, one of which is nominally unidentified, and for two of which no evidence whatsoever is provided! Furthermore, after having "diagnosed" the sexual form of the *confluenciata* morph a year earlier, it now turns out that we are back to an all-female population.

I propose the following temporary solution for this taxonomic mess: (1) pending a thorough taxonomic revision of this species complex, the mostly all-female parthenogenetic taxon of medical importance should continue to be referred to as *Tityus serrulatus* Lutz & Mello (as the various morphs designated by Lourenço & Cloudsley-Thompson have no validity according to the International Code of Zoological Nomenclature, 1999); (2) pending the publication of such revision, the so-called "sexual *confluenciata* form" has not taxonomic standing whatsoever, and its phylogenetic/evolutionary relationships with the all-female *T. serrulatus* populations remain unknown; and (3) pending the publication of evidence in support thereof, *T. stigmurus* (Thorell, 1876) (*i. e.,* the *unifasciata* morph) and the third, unnamed morph are not to be considered parthenogenetic.

The final chapter on these problems has yet to be written in light of very recent developments. First, *Wolbachia* has been detected in *T. serrulatus,* and now its possible influence in inducing parthenogenesis and in selectively killing males must be studied in full (Suesdek-Rocha *et al.*, 2007). Second, the taxonomic picture is still undergoing changes, as the supposed male of *T. serrulatus* described by Lourenço & Cloudsley-Thompson (1999) apparently belongs to a different species and the true male has been discovered elsewhere in Brazil (Souza, *et al.*, 2007).

Tityopsis inexpectatus (Moreno, 1940) (Buthidae)

Armas (1980), under a primary heading "Partenogénesis" wrote as follows: "Aunque no se ha demostrado experimentalmente, *Tityus i. inexpectatus* parece ser una especie partenogenética, pues en más de 40 ejemplares examinados, ninguno ha resultado ser macho. Armas (1974) describió dos machos de esta subespecie; pero consistió en un error, pues recientemente, al disecar dichos ejemplares, comprobé que los mismos eran hembras (una de ellos con huevos)." (p. 22)
{Although not demonstrated experimentally, *Tityus i. inexpectatus* appears to be a parthenogenetic species, because in over 40 specimens examined, none is a male. Armas (1974) described two males of this subspecies; but that was an error, because recently, upon dissecting those specimens, I found that they are females (one of them with eggs).} (my translation).

Thus far, this example is important because it shows: (a) the difficulties that can be encountered when relying exclusively on external morphology to sex specimens; and (b) the tendency to hypothesize parthenogenesis based on female-biased sex ratios that are not statistically analyzed. I will say more on that below, because Armas (1984) described the males of *T. inexpectatus* again; it turns out that they had been present in that sample of over 40 specimens, but were misidentified as subadult females! Once again, we have a prime example of the difficulties often encountered in aging and sexing scorpions and of the dangers of hypothesizing parthenogenesis on indirect evidence.

Tityus trivittatus **Kraepelin, 1898** (Buthidae).

Maury (1970) studied this taxon in Argentina and indicated that: "It seems to be parthenogenetic in this area, as only one male was found among 146 Argentine specimens… (in Brazil Bücherl (1956) records a ratio of one male to three females."(p. 405) .
"...pero la rareza de los machos de *T. t. trivittatus* nos inclinaría a suponer (sin tener ningún argumento para probarlo) que esta subespecie en nuestro país puede ser ocasionalmente partenogenética." (p. 418).
{…but the scarcity of males of *T. t. trivittatus* would lead us to suppose (without solid arguments to support it) that this subspecies in our country can be occasionally parthenogenetic.} (my translation).

Maury's caution regarding claims about parthenogenesis is understandable and his paper is useful to illustrate some of the problems associated with forthcoming taxa. Maury does not indicate the sex or the age of those 137 specimens, so it is not really as if he had a 1♂ : 136♀♀ sex ratio. In this species the number of pectinal teeth in males and females overlap considerably (Lourenço, 1978, p. 43: "Nombre de dents du peigne....les différences d'ordre sexuel étant minimes."), so sex detection in juveniles is problematic. Thus, there could have been several undetected juvenile males among the Argentinian material.

Another problem is probabilistic. For the sake of argument let's assume that indeed Maury observed 1 male and 136 females, and that in normal bisexual populations of this taxon the sex ratio is 3♀♀ : 1♂ (p=¾, q=¼) as reported by Bücherl; then, then probability of sampling 1 male and 136 females from such population P= 0.75^{136} (=1.02 x 10^{-17}) and highly significant. We would have to reject the null hypothesis that the sample came from a "normal bisexual" population, and accept an alternative explanation such as thelytokous parthenogenesis or an environmentally dominated sex-determining mechanism. However, Maury's records come from 99 independent samples, 83 of which consist of a single individual (P=3/4 or 75%), seven with two individuals (P=9/16 or 56% of the time), four with three (P=27/64 or 42% of the time), one with four individuals (P=81/254 or 31%), two with five (P=243/1024 or 24%), one with six (P=729/4096 or 18%) and one with seven individuals (P=2187/16,384 or 13%); none are statistically significant and they conform to the predicted 3♀♀ : 1♂ sex ratios found elsewhere in the geographic range of the subspecies. Thus, there would be no need to search for alternative hypotheses, such as thelytoky, to account for the "biased" sex-ratio observations.

Subsequently, Maury (1997) published on the subject again. This time, however, whereas the still "biased" sex-ratio he reported of 2♂♂ : 234♀♀ can probably be dismissed again by probability analysis of independent collection events, he offered indisputable proof of parthenogenesis in *T. trivittatus*, 17 years after his original suspicions. Two immature field-caught females, raised in isolation, and which molted to maturity in captivity, gave birth to litters of 8 and 13 young, respectively. Recently, Toscano-Gadea (2004) questioned Maury's evidence, by stating that he observed a

post-parturition molt in *Tityus uruguayensis* Borelli, 1901(see below) and that therefore, "the progeny obtained by Maury would not necessarily be an [*sic*?] evidence of parthenogenesis." (p. 866). Whereas no one else has ever reported a post-maturation or a post-parturition molt in scorpions before and thus Toscano-Gadea's report is definitely questionable and in need of verification, it is also a fact that no one has ever reported mating and sperm storage by an immature female— definitely not the same as sperm storage and molting (even if it exists) by an already mated, mature female. Thus, I consider that Maury should be given proper credit for demonstrating that *T. trivittatus* is a facultative parthenogen. Subsequently, Toscano-Gadea (2004) succeeded in raising four F_2 generation litters from progeny of a litter (F_1) born and raised in captivity, corroborating Maury's (1997) findings.

Liocheles australasiae **(Fabricius, 1775)** (Liochelidae)

Parthenogenesis in this species was originally reported by Makioka & Koike (1984), from a population on Iriomoto Island, Ruykyu Islands, Japan. It is strongly supported by sex-ratio probability analysis, dissections of the reproductive system, and numerous records of virgin births in captivity.

During eight separate collecting events they obtained 156 specimens, as follows: 103 adult, pregnant females; 3 adult, non-pregnant females; 41 nymphal females (sex confirmed by dissection, revealing "numerous oocytes in the ovarian tubes (Fig. 3)" (p. 374); 9 small young nymphs "with thread-like rudimental gonads carrying a large number of germ cells (Fig. 4), sexes were not distinguishable."; and 0 adult or nymphal males. Their collections were made in July 1977, July 1978, Nov. 1979, March 1982, June 1982, Nov. 1982, May 1983 and October 1983, clearly spanning the calendar and ruling out any possible seasonal effect on the presence/absence of adult males.

Engaging again in probability analysis, we find the following. First, Koch, (1997:167) reported examining 164 males and 266 females of *Liocheles waigiensis* (Gervais, 1844), and (p. 172) eight males and 12 females of *Liocheles karschii* (Keyserling, 1885); and another related species, *Liocheles penta* Francke & Lourenço, 1991, from Rennell Island, British Solomon Islands, was described on the basis of 4 males and 3 females (Francke & Lourenço, 1991). Thus, a 1:1 sex ratio can be hypothesized as the norm for bisexual populations in the genus. The May 1983 collection of *L. australasiae* by Makioka & Koike (1984) produced one pregnant female (P=0.5), one nymphal female (P=0.5) and one young nymph; the probability of getting two females in an otherwise normal, bisexual populations would be 0.5 X 0.5 =0.25—not statistically significant. On the other extreme, on November 1979 they collected 52 specimens, of which 48 were confirmed females; the probability of such a sample from a normal population is P=0.5^{48} =3.55 X 10^{-15}, which is statistically highly significant and supporting the alternative hypothesis that

the Iriomote Island population does not have the expected 1:1 sex ratio of a bisexual population.

Furthermore, as Makioka & Koike (1984) stated: "Most of the pregnant females gave birth to nymphs during the rearing. About a week after the parturition, all of the females became pregnant again. In six females, the next parturition took place about one year or more after the first and this was followed by pregnancy." (p. 374).

Thus far there was strong evidence for iteroparity, but the possibility of sperm storage and/or delayed implantation had not been completely ruled out. Tissue staining of reproductive organs of numerous individuals was performed, and "no spermatozoa could be detected in any region of the female reproductive systems." (p.374), thus ruling out completely the matter of sperm storage in this species. Finally, "three subadult females molted into adults, and about a month after the molting, all of them were found to be pregnant. In these females pregnancy undoubtedly occurred under the isolated conditions, without any participation of males. No spermatozoa could be found in any part of the reproductive system of these females." (p. 376).

I have elaborated somewhat on the research efforts by Makioka & Koike (1984) to stress how a thorough and methodical corroboration of parthenogenesis in scorpions should be done. Research on this species has continued over the years (Makioka, 1992a, 1992b, 1993; Makioka & Koike, 1985; Yamazaki & Makioka, 2001), culminating in the report of a fifth generation of thelytokous parthenogens born in captivity (Yamazaki & Makioka, 2004).

It is probable that other populations of *L. australasiae* show facultative parthenogenesis, although this can only be verified by observing deliveries by virgin females. Koch (1977:164) reported examining 3 males and 87 females of this species from various islands in the Australo-Papuan region; and Francke & Lourenço (1991) examined 37 specimens from Rennell Island, British Solomon Islands, and they were all females.

Tityus uruguayensis Borelli, 1901 (Buthidae)

The subject of parthenogenesis in this taxon has been somewhat controversial since the beginning. Zolessi (1985a) in a one-page communication (actually an abstract of a paper presented at a scientific meeting in her native Uruguay), under the nomenclatural combination of *Tityus bolivianus uruguayensis*, indicated: (a) the absence of adult males among an undisclosed number of specimens from Uruguay she studied; (b) her failure to find paraxial organs (*i.e.*, male reproductive structures) in an undisclosed number of adult specimens dissected; and (c) the successful rearing in captivity of three generations of individuals derived from field-caught females, all reared in isolation since the second instar, and without any males being produced in the laboratory.

In a second publication on the same year, Zolessi (1985b) confirms her earlier results and provides detailed records; (a) on the date of capture of five females, their dates of parturition, and their litter (F_1) sizes; (b) on the birth and post-embryonic development of seven young from four of those five original litters of the F_1 generation; (c) the dates of sexual maturation of four F_1 females, the dates of their respective parthenogenetic parturitions (and those F_2 litter sizes); and (d) finally, she indicates that an F_3 generation followed.

Zolessi (1985a, b) emphatically stated that no males were found in Uruguay, and questioned Mello-Leitão's (1945) description of adult males of *T. uruguayensis*, suggesting instead the possibility of "geographical parthenogenesis" (1985b:30.) in this species. In the same year, however, Lourenço & Maury (1985) proved her wrong. They examined six specimens (2 males, 4 females) from Argentina, nine (1 male, 8 females) from Brazil, and 19 (1 male, 18 females) from Uruguay—ruling out the idea of geographical parthenogenesis and suggesting instead that *T. uruguayensis* is a facultative parthenogen. Toscano-Gadea (1999) confirmed the presence of males in Uruguay, likewise dismissed the hypothesis of geographical parthenogenesis and suggested instead facultative parthenogenesis in *T. uruguayensis*.

Subsequently, Toscano-Gadea (2001), although failing to dismiss the occurrence of parthenogenesis altogether, suggested "that this species has only biparental sexual reproduction." Although not directly relevant to the topic of parthenogenesis, Toscano-Gadea initially compared the sex ratio from a population in northern Uruguay (hand-capture, winter months), against pooled data from three southern populations (pit-fall trapping, summer months), making it impossible to ascertain if the differences might be due to the sampling methods, seasonality, or an interaction of the two. He did find males, which apparently was one of the primary objectives. Second, he presented a Table with data for nine presumed pregnant females (seven of them winter-caught), from southern populations, presumably hand-captured. Yet, he failed to provide complementary data on other specimens (males, non-pregnant females, juveniles) captured during those collection events, data which would have provided more meaningful "ecological" comparisons between northern and southern, hand-captured winter samples. Toscano-Gadea used the data on those nine females to argue against parthenogenesis in the species, because after an initial parturition in the laboratory those females "failed" to give birth again. It must be pointed out that: (a) one of those field-caught females never gave birth in the laboratory even though it was considered to be gravid when captured; and (b) another female from the same location presumably molted 11 months after giving birth in captivity—the first and only post-parturition molt ever reported in scorpions. Could this have been the result of a clerical error, and the molt actually involved the "non-gravid" subadult female mentioned above? Zolessi (1985b) reported a gestation period of an average of 480 days between the date of the molt to sexual maturity and parturition among the various parthenogenetic events she recorded, and five of the eight females which Toscano-Gadea had did not live long enough in captivity for a second parturition. Only three of Toscano-Gadea's field-captured females gave birth in the laboratory and

then survived long enough to complete a second pregnancy—yet failed to deliver a second litter. Toscano-Gadea interpreted this as evidence that parthenogenesis does not occur in the species. However, a failure to exhibit iteroparity by field-caught "gravid" females does not in itself constitute proof of the absence of parthenogenesis, and Zolessi (1985b) never reported iteroparity for the species either. The crucial question is: if those field-caught females had produced a second and a third litter in captivity (as expected by Toscano-Gadea), would that have constituted solid, irrefutable evidence for parthenogenesis? Knowing about sperm storage and delayed implantation, the answer would obviously be no. Consequently, based on the information available it is premature to dismiss facultative parthenogenesis in *T. uruguayensis* as reported by Zolessi (1985a, b) and supported by the rearing of three successive generations in captivity.

Tityus columbianus (Thorell, 1876) (Buthidae)

The first report for parthenogenesis in this taxon belongs to Lourenço (1991), based on both sex-ratio analysis and laboratory births. Near the village of Mosquera, Cundinamarca Province, Colombia, a one-day collecting event yielded 423 specimens of *T. columbianus* –all females and no males. Whether we hypothesize a 1♂ : 1♀ "normal "bisexual sex-ratio, or a 1♂ : 20♀♀ sex-ratio, the odds against such a "female-biased" sample are astronomical and probability analysis only reinforces the need for alternative explanations. Amazingly, 221 out of 250 field-collected adult females produced litters within six months of being captured, and "Twenty-seven subadults collected in the field were raised separately, and after one or two months they reached adulthood. In the following two or three months, 11 of these individuals produced broods, without fertilization." (p. 275).

There is little doubt that this population indeed exhibits thelytokous parthenogenesis. Interestingly, on the same trip (Lourenço, 1991) near Villa de Leiva, Boyaca Province, Colombia, "41 specimens were collected: 40 females and 1 male," indicating that a certain degree of bisexuality persists in this taxon. Additional comparative data between bisexual and parthenogenetic populations of this species can be found in Lourenço *et al.* (1996).

Hottentotta hottentotta (Fabricius, 1793) (Buthidae)

The first mention of parthenogenesis for this species appears in a review paper (Lourenço & Cuellar, 1994), as follows: "A recent analysis of several living individuals of this species collected from the delta of the Niger River in Nigeria revealed the absence of males, suggesting that this particular population may be parthenogenetic (Lourenço, unpublished). *H. hottentotta* is otherwise bisexual throughout its distribution." (p. 22).

Exactly how many is "several" individuals? Enough to show statistical significance in a simple probability analysis as done above? What is the usual or "normal" sex ratio in the "otherwise bisexual" populations of the species? Lourenço & Cuellar (1999), however, unhesitant state: "Among the almost 1500 species of scorpions throughout the world, only five are known to be parthenogenetic (Lourenço & Cuellar 1994). ... the original *T. serrulatus* is one. The other four parthenogenetic species are *Tityus uruguayensis* Borelli from Uruguay and Brazil, *Tityus columbianus* (Thorell) from Colombia, *Hottentota hot-tentota* (sic) (Fabricius) from West Africa, and *Liochelis* [sic] *australasiae* (Fabricius) from the South Pacific (Lourenço & Cuellar 1994)." (p. 149).

The assertion that *H. hottentotta* is parthenogenetic is repeated, unquestioned and unchallenged, by Lourenço *et al.* (2000), Toscano-Gadea (2001, 2004), Lourenço (2002), and Yamasaki & Makioka (2004). However, there is absolutely no published evidence to support the claim that any population of the species is parthenogenetic; not even a weak probabilistic statement that can be scrutinized!
Lourenço & Cuellar (1999) further state: "*Tityus trivittatus* Kraepelin from Argentina is also suspected of parthenogenesis (Peretti 1994, Maury 1997)." (p. 149).

The authors never stated what is "suspicious" about the two virgin births reported by Maury, yet they do not even bother to provide any evidence for their claims regarding *H. hottentotta.*

Tityus stigmurus (Thorell, 1876) (Buthidae)

Setting aside the taxonomic/nomenclatural controversy surrounding *T. stigmurus* and *T. serrulatus* (discussed above; Lourenço & Cloudsley-Thompson, 1999; Lourenço *et al.*, 2000, Lourenço 2002), here I briefly review again the subject of parthenogenesis in this taxon (the so-called *unifasciata* morph of *T. stigmurus*). Whereas Lourenço & Cloudsley-Thompson (1999) claim that "...recent unpublished field observations by Lourenço show that... both the morphs of *T. serrulatus* (=confluenciata) and *T. stigmurus* (=unifasciata) reproduce by parthenogenesis." (p. 155) no evidence whatsoever is provided to support such a claim. It is hard to imagine how anyone could observe and document "virgin birth" in the field, so we must assume the claim is based on undocumented, sex-ratio field observations. Although this species is cited as being parthenogenetic in two subsequent publications (Lourenço *et al.,* 2000; Lourenço, 2002), no additional evidence has been provided to date. This claim is therefore disregarded as unsubstantiated and invalid.

Vaejovis spinigerus (Wood, 1863) (Vaejovidae)

Concerning this species from the southwestern U.S., Warburg & Rosenberg (1996) wrote in connection with an anatomical study: "Since no males were found in the populations studied here, this species can be assumed to be parthenogenetic. However, this point needs more concrete proof." (p.751).

This statement seems a bit premature: (a) because they only collected 15 specimens, and (b) males of this species are abundant throughout its geographic range (pers. observ.). Further, Warburg (2001) stated: "As we did not encounter any males in *Vaejovis spinigerus* populations, perhaps this species is parthenogenetic....However, it could also be a seasonal phenomenon or due to different be-

haviour al pattern of activity in the males that although they were not captured could still be present in the population." (p. 355).

It is unfortunate that these authors did not consult with any North American scorpion taxonomist about the presence of males in this species, because this is yet another unsubstantiated claim of parthenogenesis in a scorpion.

Ananteris coineaui **Lourenço, 1982** (Buthidae)

Lourenço & Cuellar (1999) stated: "*Ananteris coineaui* was described from a rainforest near the Arataye River in French Guyana [sic], based on three adult females collected in a palm tree.... Since then, only one additional specimen [female] was collected from Saul (close to the original locality), also in a palm tree....Males are rare....female-biased sex-ratios may be taken as evidence of parthenogenesis. (pp. 149-151).

In this case the probabilistic arguments are clearly against the hypothesis of parthenogenesis. In a closely related species, *Ananteris balzanii* Thorell, 1891 a sex ratio of 1 ♂ : 2 ♀♀ has been reported (Lourenço & Cuellar, 1999); and if we assume this to be the norm for the genus, finding three females ($P=0.67^3=0.29$) of *A. coineaui* is not statistically significant, nor is finding a single female ($P=2/3$ or 67%). Those authors continue: "Within about two weeks, the female molted, by March 30, she gave birth to 16 young, which remained on her back until April 16 when they all died before molting. An examination of the size and sexual dimorphism of the pectines revealed that the entire brood consisted of females, suggesting parthenogenesis." (p. 150).

If only four females were known, how could they know about sexual dimorphism in the species?

In the genus *Ananteris* Thorell, 1891 the pectinal tooth counts of males and females overlap, so that criterion can not be used to sex the specimens. I have repeatedly tried to determine the sex of first-instar specimens on many broods of buthid scorpions (Francke & Perez-Sotelo, 2007), and have been unsuccessful. Sixteen first-instar young of *Ananteris* (about 5mm in total length) could be reliably sexed? I will accept that all young looked alike, but that is merely due to lack of sexual differentiation at such an early ontogenetic stage, and not necessarily that they were all of the same sex! Thus, I can not comprehend how they determined that the entire brood consisted of females. The authors insisted on the topic of parthenogenesis as follows: "The occupancy of isolated palm trees within vast areas of rain forest or savanna conforms with the concept of insular parthenogenesis proposed by Cuellar (1977, 1994)" (p. 151). Adding that: "Most parthenogens are also characterized by small size, low mobility, and low population density (Cuellar 1994). The rarity of *A. coineaui*, its occurrence in isolated palm trees, and the absence of males all suggest a parthenogenetic mode of reproduction. This may hold as a rule for other species of this genus." (p. 149).

This is all unsubstantiated speculation: (1) how many palm trees have been searched for scorpions in French Guiana? Are the scorpions really that rare? (2) Is the "rarity of species" the same as the "low population density"? What has been the collecting effort to find and collect this species? If the three original specimens were collected in the same palm tree, does not that indicate a higher density?(3) With a hypothetical sex ratio of 1♂: 2♀♀, like its congener *A. balzanii*, Are males really rare? In this case are "female-biased" sex-ratios really evidence for parthenogenesis? (4) Who said the palm trees (in the rain forest or in the savanna) were isolated? Or is this mere inference to "fit the model" of "insular biogeography" that the authors preferred? (5) "parthenogenesis....characterized by low population density"? What about the 435 female *Tityus columbianus* collected in five hours by Lourenço (1991:275)? (10) "the absence of males **suggest** a parthenogenetic mode of reproduction"; however, by the following year (Lourenço *et al,.* 2000, and Lourenço, 2002) the same species is listed as a corroborated parthenogen!
They finally concluded that: "This [parthenogenesis] may hold as a rule for other species in this genus" (p. 151).

This is more speculation based on inadequate sampling. Prendini (2001) reported finding 8 males and 2 females of *Ananteris cussinii* Borelli, 1910 in Trinidad and Tobago. Recent field collections of *Ananteris balzanii* in Brazil yielded 6 males, four females and two juveniles on one night, and 6 males and four females on the next night; and of *Ananteris mauryi* Lourenço, 1982, 3 males, 2 females and 2 juveniles (Mattoni, pers. comm.). Thus, males are not rare and there is no evidence to support such statements. One can only wonder what happened to the fine examples of scientific thoroughness set forth by Matthiesen (1962, 1971), Makioka & Koike (1984, 1985) and Maury (1970, 1997) to corroborate parthenogenesis "beyond a reasonable doubt"?

Tityus metuendus **Pocock 1898** (Buthidae)

In the same contribution, Lourenço & Cuellar (1999) report on a slightly more credible instance of parthenogenesis by a female scorpion from near Iquitos, Peru. That female, which molted to maturity in captivity, produced three consecutive litters (=iteroparity) of 21, 32, and an unreported number of young. What is less credible, however, is their claim for arrhenotoky in this particular instance. Other populations have a 1♂ : 1 ♀ sex ratio ($p=1/2$, $q=1/2$). From the first brood born in captivity, only three specimens (all male), reached sexual maturity after an undisclosed number of molts ($P=0.5^3=0.125$, not statistically significant), and the other 18 died as "immatures" (specific instars not revealed), and "examination of the pectines ...revealed that the entire brood was male." (pp). In this species, sexual dimorphism in pectinal tooth counts is non-existent (males 22-27, females 22-26) (Lourenço, 1983), and dimorphism in pectinal tooth size is minimal and only appears after the final molt. Therefore, the fact that the pectines in those 18 dead juveniles looked alike is totally expected, and the "detection" that they were all males is an unsupported inference based on the surviving three individuals. The 32 specimens of the second brood all died as second instars, an early ontogenetic stage in the life cycle where sexual identification in this genus, in the absence of discrete pectinal tooth counts, is not possible. Thus, once again it is unsurprising that all

young looked similar, but there is no evidence to support the contention that they were all males! No data were provided on the third litter (e.g., number of young, instars of death), and the assertion that it was "an all-male brood" is also inference.

As indicated earlier, arrhenotoky is the production of haploid sons by diploid mothers. In the case of *T. metuendus* not only is there no evidence to support the "all-male" claim for the three litters born in captivity, but there is also no evidence to support the hypothesis of arrhenotoky. The fact that three males from the first litter where "presumably" the only ones to reach sexual maturity is possibly due to chance alone, and the rest is inferential speculation.

A subsequent paper dealing with this species (Lourenço *et al.,* 2000) sheds additional light on the problem. *Tityus metuendus* is one of those species in which the proximal (or basal) middle lamella of the pectines is dilated on adult females, whereas it is undilated in immatures and in adult males. "The males obtained from the parthenogenetic population were also much smaller that those from the sexual populations, undoubtedly because they reached adult-hood in only four molts instead of the usual five."

"Also, adult parthenogenetic males lacked certain male secondary sex characters observed in sexual populations of *T. metuendus*." (p. 274).

Apparently, the three specimens reared by Lourenço died as fourth instars, one molt short of the final molt to maturity! For all we know, they could have been three subadult females, or an assortment of subadult males and females. The morphometric data provided by Lourenço *et al.* (2000, Fig. 14) clearly support the interpretation that those three specimens were one molt short of adulthood. The lack of distinctive secondary sexual characters (dilated proximal pectinal middle lamella in females; pedipalp chela finger scalloping and elongate metasoma in males) further supports this contention. Finally, the authors published two SEM photographs of the pectines, showing an undilated pectinal proximal middle lamella, to support their claim that the specimen was indeed a male; whereas in reality that only shows that it is not an adult female (it could be a subadult of either sex). In conclusion: (1) the observation of virgin birth in *T. metuendus* is irrefutable evidence of facultative parthenogenesis in this taxon; and (2) there is no evidence to support the hypothesis of arrhenotoky.

Centruroides gracilis (Latreille, 1804) (Buthidae)

Chronologically, the next report of parthenogenesis in scorpions belongs to this species (Teruel, 2004). Two subadult females, from different bisexual populations, molted to maturity in captivity and subsequently gave birth. From one of those litters two females reached sexual maturity, but unfortunately died shortly thereafter without producing an F_2 parthenogenetic generation. The second litter died in the second instar. Teruel correctly pointed out the difficulties with sex distinction in early instars and would not categorically

state that thelytoky occurs in the species. However, since sexually mature males and females of *C. gracilis* have been raised from litters born in captivity (Francke & Jones, 1982), we must consider this an example of facultative parthenogenesis for this taxon.

Pseudolychas ochraceus (Hirst, 1911) (Buthidae)

Concerning this scorpion from southern Africa, Prendini (2004) indicated: "Although specimens of this species are abundant in Southern African museum collections, adult males are very rare (the material examined for this study includes 5 male and 110 female specimens of *P. ochraceus*, compared with 41 male and 60 female specimens of *P. pegleri*). No males have been collected in the major cities where most of the specimens originate. It seems probable that *P. ochraceus* is parthenogenetic, as is the case in several other synanthropic buthid scorpions (Matthiesen 1962, San Martin and Gambardella 1966, Lourenço and Cuellar 1994, 1999, Peretti 1994, Maury 1997) and that this attribute may have contributed to its successful invasion of urban habitats" (p. 53).

It should be noted that the author had examined all material of this species that could be found in collections, and that in *P. ochraceus* females are unequivocally identifiable at all stages based on the enlargement of the first pectinal tooth (an ontogenetically invariant character), so the paucity of males does appear to be real. Obviously, Prendini was pointing to the need for further investigation, rather than making a categorical assertion concerning parthenogenesis in this taxon.

Cazierius asper Teruel 2006 (Diplocentridae)

An unusual sex ratio was reported in the original description of this Cuban species (Teruel, 2006). The author reported collecting 41 adult females, 11 juvenile males and 22 juvenile females. The sex ratio observed in juveniles (1♂ : 2♀♀) is similar to that reported for adults in two other species in the genus: *Cazierius gundlachii* (Karsch 1880) and *Cazierius parvus* Armas 1984 (Teruel & Cala, 2006). However, the biased sex ratio and the absence of adult males led that author to three alternative hypotheses: (1) that this species reproduces by "mixed" parthenogenesis, and even though males are born, they die before reaching sexual maturity; (2) males reach adulthood, but are short-lived (actually have a short life span or are cannibalized by females shortly after mating), or (3) males occupy a different habitat. Teruel favored the first of these hypotheses: some kind of facultative or "mixed" parthenogenesis.

Considering the total sample size (n=74, 11 males) the binomial test (c=3.37, p<0.001) leads us to reject the null hypothesis that *C. asper* has a 1♂ : 2♀♀ sex ratio as in *C. gundlachii* and *C. parvus.* However, a biased sex ratio due to ESD factors during early development, or due to differential life history parameters in nature have not been eliminated. No virgin females have been observed having young and thus the hypothesis of parthenogenesis is considered premature.

Conclusions

Parthenogenesis, *i.e.,* virgin birth, in scorpions is considered to occur when an unmated female produces a litter of young. If a female that was born in captivity, and raised in total isolation until mature, produces progeny, the evidence is irrefutable. Mating behavior has never been reported for subadult female scorpions; thus, if such a female (or younger) is captured, raised in isolation until mature (*i.e.,* undergoes one or more molts in captivity and in isolation), and then produces progeny, an equally undisputable case for parthenogenesis is provided. However, if mating behavior is ever reported for a non-adult (=non-mature) female scorpion then this alternative would no longer be so readily accepted as the maturation molt would not be a guarantee of virginity. Likewise, if the controversial topic of post-maturation (and post-parturition) molts in scorpions is convincingly demonstrated, then one would and should question whether a field-caught female which molted in captivity was actually a virgin or not. The presence or absence of iteroparity (*i.e.,* repeated parturition without intervening inseminations) does not provide evidence regarding the presence or absence of parthenogenesis.

The use of sex-ratio analysis of field-caught adult scorpions to support hypotheses of parthenogenesis has to be statistically supported, as shown in this contribution. Ideally, such an analysis should be followed up by rearing experiments to firmly demonstrate "virgin births." Sex-ratio analysis of juveniles, whether field-caught or laboratory-reared, is fraught with inaccuracies and should be avoided in all instances where clear-cut sexual identification can not be made.

At present, the evidence available supports the hypotheses of parthenogenesis for the following scorpion taxa:

Buthidae:
  Centruroides gracilis (facultative)
  Tityus columbianus (facultative)
  Tityus metuendus (facultative)
  Tityus serrulatus (obligatory?)
  Tityus trivittatus (facultative)
  Tityus uruguayensis (facultative)
Liochelidae:
  Liocheles australasiae (obligatory)

Evidence is absent or incomplete to support the hypotheses of parthenogenesis for:
Buthidae:
  Ananteris coineaui
  Hottentotta hottentotta
  Pseudolychas ochraceus
  Tityopsis inexpectatus
  Tityus stigmurus
Diplocentridae:
  Cazierius asper
Vaejovidae:
  Vaejovis spinigerus

The hypothesis of arrhenotoky in *Tityus metuendus* is also rejected for lack of credible and irrefutable evidence.

Acknowledgements

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