KARYOTYPE AND NOTES ON THE MALE REPRODUCTIVE SYSTEM AND NATURAL HISTORY OF THE HARVESTMAN *VONONES SAYI* (SIMON) (OPILIONES, COSMETIDAE)

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Abstract.—The Texas Platycynorta transversalis is regarded as a junior synonym of *V. sayi*. Karyotypes of male and female *V. sayi* from central Texas reveal 2n = 78. This number is almost twice that previously reported for any other harvestman. The male reproductive system of *V. sayi* is described and illustrated. Natural history data are provided for egg laying and developmental times. The supercooling points ranged from -2.5 to -7.2°C, with poor recovery and death following thawing.

Key Words: Karyotype, reproductive system, natural history, supercooling, overwintering

The identification of the Cosmetidae of the U.S.A. presents a difficult problem. How much variation in a character constitutes a different species? As presented in the literature, there is a maximum of five species in the U.S.A.: *Vonones sayi* (Simon) in central and eastern U.S.A. (type from Texas), *Vonones bimaculata* (Banks) from California (correct locality?), *Vonones ornata* (Say) from Florida and Georgia, *Vonones dentica* (Walker) from Ohio, and *Platycynorta transversalis* Roewer from Texas. Based on the original description, Roewer’s species is a synonym of *V. sayi*. Goodnight and Goodnight (1953, 1973) treated all the eastern U.S.A. populations as one species. From our examination, we find that the abdominal spines of *V. ornata* are always present and consistently larger than the similarly placed low tubercles in *V. sayi*. Even so, doubt remains about the range and extent of variation in the U.S.A. cosmetids. To help resolve this problem we attempted to karyotype *Vonones* from numerous localities in the U.S.A.

In addition to the karyological study, we obtained biological data from captive specimens. Little natural history data have been published on the members of the exclusively American family Cosmetidae. Only a single note has been published on biology of *Vonones* (Goodnight 1958): *V. "ornata"* in Indiana. Three other reports have appeared on biology of cosmetids: Juberthie (1972) on *Cynorta cubana* (Banks) from Cuba, Canals (1936) on *Gyne orensis* (Sörensen) and *Metalibitia paraguayensis* (Sörensen) from Argentina, and Goodnight and Goodnight (1976) on *Erginulus clavotibialis* (Pickard-Cambridge) from Belize.

RESULTS AND DISCUSSION

Karyology.—Parthasarathy and Goodnight (1958) reported observations on the genital morphology and karyotype of "*Vonones ornata* (Wood)" from Indiana. Obtaining countable spreads of *V. sayi* chromosomes proved quite difficult, a point not noted by Parthasarathy and Goodnight. Because juveniles are very rarely seen, adults...
were the sole source of material for karyotyping.

During 1982 and 1983, V. ornata were obtained from Florida whereas V. sayi were collected from numerous localities in Texas, Oklahoma, Nebraska (Jefferson Co.), and Tennessee (Davidson Co.). Ovaries and testes were prepared for chromosomal observation during January-March, May, July, October, and November. From these, only seven cells from one male collected during May resulted in observable (but not countable) individual chromosomes. Not until 1984 were we able to obtain living material collected in the proper season, from which we successfully karyotyped two males and one female from central Texas, collected in April.

Karyotypes using the gonads as the source of cells are best obtained from subadult harvestmen. In our case, active gonadial cells in adults were sought. From a series of animals collected 6.4 km E of Kerrville, Kerr Co., Texas, we prepared (following the air-drying method of Cokendolpher and Brown 1985) five males and 11 females on 5 April. A second series of animals from the same locality was karyotyped on 17 May. All animals had been dipped or injected with a 0.005% colchicine solution 24-40 hours prior to karyotyping. Although several cells from many slides were dividing, clear, countable spreads were only obtained from two males and a female from 5 April with a 24 hour colchicine treatment. Good spreads were only counted for one cell in the female (2n = 78) and four cells for males (n = 39, 2n = 78). Chromosomes were often overlapping in spreads with only slightly condensed chromosomes. Despite these difficulties, these spreads were used to verify the chromosome morphologies observed in more highly condensed plates. Altogether, there were 38 pairs of submeta- and meta-centric chromosomes and one pair (smallest) in which the centromere could not be detected (Fig. 1). No obvious sex chromosome could be determined by either morphology or activity.

Our results are totally unlike those of Parthasarathy and Goodnight (1958). They reported finding 2n = 24 + X in males. Because they used squash and sectioning techniques, we assume they are in error. Not
Fig. 2. Male genital system (minus penis) of *Vonones savi*, preserved in 80% ethanol, critical point dried, gold coated, and then examined with a Scanning Electron Microscope (Hitachi model S-570). See Fig. 3 for abbreviations.

only is their count much lower than ours, but an XO-XX (male heterogametic) sex determining system has never been verified in any opilionid (Tsurusaki and Cokendolpher in press). Karyotyping additional males from Indiana with an air-drying method will be required to be certain.

The highest chromosome number previously recorded for Opiliones is 2n = 40 in *Pseudobiantes japonicus* Hirst (Tsurusaki 1986, Tsurusaki and Cokendolpher in press). Although in different families, *Vonones* and *Pseudobiantes* (Phalangodidae) are the only members of the suborder Laniatores that have been karyotyped.

Although we could not obtain chromosome counts for numerous populations of

![Diagram](image.png)

Fig. 3. Diagrammatic view of *Vonones savi* male genital system. T = testis, VE = vasa efferentia, VD = vas deferens, SR = seminal reservoir, PO = propulsion organ, P = penis, DE = ductus ejaculatorius, TR = truncus, G = glans, AT = apophysis of truncus, AG = apophysis of glans. *V. savi*, we did obtain what is probably the first accurate count.

Male genital morphology.—While dissecting numerous males for this study and that of Jones and Cokendolpher (1985), we found the description and illustration of the male reproductive system of *Vonones* presented by Parthasarathy and Goodnight (1958) to be incorrect. They reported finding a single testicle connected at one end by a sperm duct to the vas deferens. As their drawing has served as the standard for the Cosmetidae (Suzuki 1966), we feel it desirable to re-illustrate the system in greater detail. After careful dissection of living animals, we find the reproductive system to be similar to other harvestmen. Although the vasa efferentia are small and difficult to see, they are attached to both ends of the testis (F).
testis (Fig. 2). Other details are as in Fig. 3 (morphological nomenclature as in Juberthie 1965, Martens 1976, and Silhavý 1966).

Natural history.—We obtained data on natural history while maintaining animals in culture over several years. Based on our studies, V. sayi spermatogenesis is most active in April and May in central Texas, and is the time period during which specimens were collected for our previous paper on spermatogenesis (Jones and Cokendolpher 1985). Similarly, peak egg laying occurs during April. Based on cytological studies, Goodnight (1958) reported V. "ornata" in Indiana must breed and lay eggs throughout the summer months. Egg laying in Tropical species is different with C. cubana laying eggs year-round (Juberthie 1972), and E. clavotibialis (Goodnight and Goodnight 1976) laying eggs during the rainy seasons (May and December).

The juvenile stages of C. cubana last 4.5-7 months at 20-25°C and E. clavotibialis requires 121 days post-hatch (temperature not reported, presumably 20-26°C as used for eggs). The lengths of the juvenile stages in V. sayi are unknown. Individual V. sayi collected as adults have been held in captivity for over three years on a diet of dead insects, live fruit flies, and fruits. Cynorta cubana is recorded (Juberthie 1972) to live 2.5 years as an adult.

Because so little is known about juveniles of the Cosmetidae, we here present our data. Females of V. sayi, in captivity, lay eggs during the winter. Several females collected in Wichita Falls, north-central Texas, produced the following numbers of eggs: December (1 egg), February (2), March (32), and April (50). These females were collected during May of the previous year. Eggs are laid singularly (usually only one per day) and packed into crevices of moist wood. The eggs are covered with small pieces of wood and soil, presumably to hide them. Covering eggs with debris is also reported in C. cubana by Juberthie (1972) and assorted cosmetids and gongyleptids by Canals (1936). The actual egg laying process was never observed. Egg development took 20-38 days (n = 17). This range is obviously influenced by temperature, which varied greatly over the egg-laying period in an unheated room (about 5-20°C). Cynorta cubana egg development lasts 16-27.5 days at 28-20°C, whereas E. clavotibialis lasts 13 and 23-27 days at 26 and 20°C. Because the V. sayi eggs were camouflaged, some shorter incubation times may be due to the fact that they went undetected for some time. Of the 17 individuals which hatched only two presumably molted to the third instar (2nd nymphal stage) (16 and 25 days) and the 16 day 3rd instar presumably molted to the 4th instar (25 days later). As these individuals died while still juveniles, the number of molts to adulthood is unknown.

Although never observed in V. sayi, it is assumed that the first instar (emerging larva) molts immediately after leaving the egg shell, as do C. cubana and E. clavotibialis. No exuviae were recovered and instars were determined by uneven jumps in body size. Molting and hatching were not observed, so the chewing (‘eating’) of the exuviae could not be verified. Such behavior is known for several cosmetids and gongyleptids (Canals 1936, Juberthie 1972) and postulated for E. clavotibialis by Goodnight and Goodnight (1976). The first instar or larva does not have a tooth-like structure to aid in hatching. The absence of this egg-tooth is also reported in the Gonyleptidae (Muñoz Cuevas 1971). Such a tooth is also missing in a drawing of the larva of E. clavotibialis (Goodnight and Goodnight 1976). Supercooling.—Cold-hardiness in arthropods can be enhanced by: (1) cold-acclimation, (2) supercooling, and (3) freezing tolerance. Preparation to avoid injury at temperatures too low for continued growth is referred to as cold-acclimation. Supercooling is the adaptation in which the freezing point of body tissues is lowered. If an animal can withstand freezing of body tissues, it is considered to be freeze-tolerant.
Because the supercooling point is the freezing point, it is the lowest temperature survived by a freeze-intolerant species. *Vonones sayi* is a member of the Cosmetidae, a primarily Tropical American family. Besides being the cosmetid with the most northern distribution (reported as *V. ornata* from Indiana by Goodnight 1958), it has the distinction of having the largest range (Indiana south to Florida, west to Nebraska and Texas in the U.S.A., and northern Mexico). Because adults are collected throughout the year and juveniles are present during winter in Texas, overwintering for this species poses several problems. To overwinter, these animals must either avoid freezing (by cold-acclimation or supercooling) or be freeze-tolerant.

Several animals from Kerr and Wichita counties (central and northern), Texas, were supercooled during 1981 (methods follow Francke et al. 1986, except probe was attached to abdomen with Vaseline on juveniles and taped to adults). Field observations revealed that these animals gather under wood and logs helping to insulate the animals from the winter cold. Two males tested during early May had supercooling points of −3.4 and −5.5°C. Immediate thawing resulted in poor recovery, a lack of ambulatory ability, and death by the third day. Two second instars and one third instar reared at room temperature were supercooled three days post-feeding. The second instars supercooled to −7.0 and −7.2°C, whereas the 3rd instar supercooled at −3.5°C. Like the adults, poor recovery was noted with death by the third day. Although the sample sizes are small and our warming periods were not timed (see Baust and Rojas 1985), the values obtained suggest that this species supercools to avoid bodily freezing.

In February 1990, winter-collected adults from Shelby County, eastern Texas, were allowed to live in the laboratory at 24–26°C for a week prior to further experiments. One group of six adults (males and females) was cooled (average rate 0.1°C/min) to −3.5°C and a second group of six adults was cooled (average rate 1.25°C) to −26°C for 36 hours. Warming the animals (at approximately the same rate as cooling) revealed: (1) none survived freezing to −26°C, (2) two (male and female) survived cooling to −3.5°C. The survivors moved and fed normally for one week, at which time their supercooling points were determined to be −2.5 and −5.0°C. Two females which had not been previously frozen were also tested. Their supercooling points were −3.2 and −3.8°C.

As a large sample of animals was not available, the animals could not have been tested at a variety of cooling and warming rates to determine if there was an optimal rate (see Baust and Rojas 1985). While the supercooling points were sufficient to protect animals in Texas, another means would be required for overwintering populations in the northern U.S.A. Goodnight (1958) reported that this species aggregates under piles of logs or brush heaps with the coming of fall and apparently disappears into the ground to avoid winter cold. It is unknown whether these animals remain relatively active or diapause and to what depth they retreat. If animals retreat deep into earth cracks and holes, their supercooling points would not have to be lower than the southern populations because of the warmer temperatures.

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LITERATURE CITED


