Life cycle of *Poratia salvator* (Diplopoda: Polydesmida: Pyrgodesmidae)

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ABSTRACT. Even though millipedes display high plasticity in their reproductive strategies, they are poorly studied in the tropical region. The present study describes the biological cycle and reports the parthenogenetic reproduction of *Poratia salvator* Golovatch & Sierwald, 2000 under environmental conditions. Collections were carried out in Pantanal of Mato Grosso and in Várzea Grande, state of Mato Grosso, Brazil. The individuals were kept at room temperature in individual plastic pots. All 18 females used in the experiment reproduced parthenogenetically, resulting in a total of 699 young individuals. Of these, 18% survived until the adult phase. The lowest survival rate of *P. salvator* was recorded for the first developmental stage (46%). The mean duration of each developmental stage ranged between 6.8 ± 0.8 days for stage I and 14.9 ± 10.4 days for stage VI. The reproductive adult phase of females was the longest phase in the development, followed by the juvenile and pre-reproductive adult phases. The mean time of maturity was 75.1 ± 23.6 days. The mean duration of the total development was 66.1 ± 16.7 days. The number of eggs per nest varied between seven and ten, and the emergence of young individuals occurred after an average of 9.9 ± 1.4 days. Sexual proportion was 1 male:139.8 females, which indicates a tendency towards a parthenogenetic reproduction in this species.

KEY WORDS. Brazil; Life history; Mato Grosso; millipedes; parthenogenesis; reproduction.

Diplopoda, commonly known as millipedes, may contain more than 80,000 species. Based on this projected number, it has been calculated that only a small percentage of the total species richness (11-12%) has been described (HOFFMAN *et al.* 2002). The Diplopoda is the largest class of Myriapoda, probably constituting the third largest group of terrestrial Arthropoda, following Hexapoda and Arachnida (GOLOVATCH *et al.* 1995, HOFFMAN *et al.* 1996). Even though millipedes are distributed throughout the world, especially in the tropics, the North-American and European faunas are best known. The Neotropical fauna of Diplopoda corresponds to approximately 1,100-1,200 species/subspecies distributed among more than 800 genera, 47 families and 13 orders (HOFFMAN *et al.* 2002), while the whole class is currently known to comprise two subclasses, 16 orders and more than144 families, considering recent updates in millipede taxonomy, new descriptions and synonymies (SHELLEY 2003).

Some millipede populations are well-known for having a smaller number of males than females, or even a total absence of males. Such phenomenon, known as spanandry, occurs in many species in certain periods of the year or in specific habitats. Additionally, there are few confirmed cases of obligate parthenogenesis (ENGHOFF 1994). The most frequent type of parthenogenesis in this group is thelytoky, in which only females are born from unfertilized females (ENGHOFF 1976). Although common in millipedes, this reproductive strategy has only been demonstrated for a few species, some of which present obligate or opportunist parthenogenesis, and others show geographic parthenogenesis (ENGHOFF 1994, WITZEL *et al.* 2003).

It is believed that thelytoky represents a general adaptive solution to particular life history patterns of this species, such as: a) low vagility; b) distribution characterized by marked differences in the geographic and/or ecological distribution of the sexual ancestral and the thelytokous descendents; and c) distribution in habitats of low biotic diversity and more pronounced abiotic diversity, particularly in regards to temperature and seasonality (JENSEN *et al.* 2002).

Despite such limitations, the success of the parthenogenetic reproduction is warranted by the colonizing capability of these animals, which results in high reproductive rates and independence from males in environments subjected to density-independent mortality factors (JENSEN *et al.* 2002). Besides, thelytokous females are more resistant to desiccation than males from populations with bisexual reproduction. They spend less energy and are at lower risk of predation and desiccation due the copula, thus being able to more successfully transmit their genotype to the next generation (ENGHOFF 1978).

Most females of *Poratia* (Cook & Collins, 1895) reproduce parthenogenetically. Previous studies have demonstrated that parthenogenesis in individuals of this genus is determined by the presence of a flavobacteria (WITZEL *et al.* 2003).

Poratia salvator Golovatch & Sierwald, 2000, originally described from El Salvador (GOLOVATCH *et al.* 2005), is a new record for the Brazilian fauna. This species is characterized by small individuals, 3.5 mm long and 0.5 mm wide, adults with 19 body segments and yellow-brownish coloration (GOLOVATCH & SIERWALD 2000). Considering the high plasticity in millipede reproduction, still little known in the tropical region, this study describes the biological cycle and verifies the parthenogenetic reproduction of *P. salvator* contributing to the ecological and biological knowledge of Diplopoda of Mato Grosso, Brazil.

MATERIAL AND METHODS

Individuals of *P. salvator* were collected in the Retiro Novo farm, Pantanal of Mato Grosso, municipality of Nossa Senhora do Livramento, state of Mato Grosso, Brazil, and in the experimental field of Centro Universitário de Várzea Grande (UNIVAG), municipality of Várzea Grande, Mato Grosso.

The Pantanal of Mato Grosso is characterized by four well-defined seasons (HECKMANN 1998). The dry season, generally between July and September, is a period when most of the northern Pantanal is completely dry and low rainfall is recorded. The start of the rainy season, between October and December, corresponds to the flood period, when some areas may flood up temporarily as a result of intense rainfalls, but become dry again after periods of sun. During the high water period, generally between January and March or April, much of the northern Pantanal is submerged and fields and forests get flooded due to the lateral overflow of rivers and lakes. The low water is the period when the water level decreases rapidly from flooded areas, as a result of reduced rainfall in the region, and is recorded between April and June.

The experimental field is part of the UNIVAG, which is located in the urban area of Várzea Grande, Mato Grosso. The climate is tropical, hot and humid, with rainfalls concentrated between January and March, and average annual temperature of 28 °C. The experimental field, regularly used by Agronomy students for experimental research, has seven ha, mostly characterized by open spaces, strong sunlight exposure and, consequently, high temperatures and low humidity. The experimental field includes a nursery, which is shaded during all day, is relatively humid and has a large concentration of plant pots and wood that stay in direct contact with the ground. Individuals of *P. salvator* were found under these plant pots and fallen wood.

Individuals of *P. salvator* were collected by hand on a weekly basis from November, 2006 to January, 2007, in the morning. With the help of entomological tweezers, the specimens were transferred from the substrate into 500 ml transparent plastic containers containing a layer of soil previously kept in the freezer for at least 24 hours to control the proliferation of fungi and remaining arthropods that could interfere with the experiment. After being collected, the material was transported to the laboratory in a polystyrene thermal box.

Following the methodology proposed by ADIS *et al.* (2000), the biological cycle of *P. salvator* was monitored in the following manner: individuals were accommodated in plastic containers covered with lids and containing a mixture of plaster and coal (8:2) on the bottom, covered with a thin layer of soil. The plaster-coal mixture has the role of buffering the humidity inside the container, a function of the capacity these two materials have to absorb water. In the laboratory, the animals were reared in organic soil which is commercialized for gardening and contains pieces of wood, leaves, roots and chips.

All individuals received flaked dried food for carnivorous fish. This type of food is recommended for containing high levels of animal protein (tetramine), which is an important component of the exoskeleton (ADIS *et al.* 2000). The food was placed onto a piece of filter paper to avoid direct contact with the soil and to facilitate its removal and replacement due to the constant presence of fungi. The filter paper also aided in the process of separating the offspring, mainly during the first stage, as it provided better visualization of the young.

In order to assure that the females used in the experiments had not reproduced before, we used only females that had been born and carefully watched in the laboratory, thus avoiding the use of females collected directly from the field. To monitor the life cycle of *P. salvator*, 18 females (parental generation) were used: nine from the Pantanal of Mato Grosso and nine from Várzea Grande. Such females were individualized and kept at room temperature.

The parental generation was monitored three times a week with the help of a stereoscopic microscope for observation of births and determination of the development stages the young. Next, the individuals were counted, transferred to a new container, and labeled with two numbers: the number of their progenitor, and a number of their own. All young individuals in the same stage of development were grouped in one same container. Thus, the number of young individuals differed in the various rearing containers.

Offspring development was also monitored under a stereoscopic microscope three times a week. During observations, we took note of the developmental stadia of the millipedes, the development of males, and the occasional death of individuals. To identify the developmental stadia of *P. salvator*, the number of body segments with leg pairs was considered. When males were found, they were separated from the females, individualized in rearing containers and numbered according to the number on the container of their progenitors.

The duration of stadia was counted from the first day individuals in stage I were found until the first day these individuals were observed in stage II (ADIS *et al.* 2000). This procedure was repeated until they reached the seventh stage, which corre-

sponds to the adult phase. The duration of the latter stage corresponded to the first day of the adult phase until the birth of the first young. The duration of stadia included the ecdysis period.

The juvenile phase includes the first day of stage I until the first day of stage VII. The pre-reproductive adult phase is the first day of stage VII until the first birth of the young millipedes. The adult reproductive phase includes the first birth of the young until the death of the female. The age of maturity represents the time between stage I of the female until the first birth of the offspring. The longevity of females is the period from stage I to the death of the individual.

Three adult females from the offspring of each female parental generation (n = 27) were randomly selected and distributed into nine replicates to assess the fecundity of the females produced in the first generation. The number of eggs produced by *P. salvator* was not counted for all ovipositions, since visualization of the eggs was difficult through the nest chambers. We chose not to break these chambers for the purpose of egg counting to avoid affecting the development of the millipedes, once the nests act as a protection mechanism. Egg count information, therefore, was based on accidental ruptures of nests of *P. salvator*.

To evaluate the period of embryonic development, three adult females were individualized in rearing containers with a small quantity of soil so that the plaster layer was left almost totally exposed. The soil was used in amounts sufficient for building the nests. These containers were monitored daily in order to observe the exact day of egg eclosion. Three whole nests of each female (n = 9) were monitored until the birth of the young.

The life cycle of *Poratia salvator* was described based on the results obtained for the first generation of the two populations studied, without discriminating the populations.

RESULTS

All 18 females used in the experiment reproduced in the absence of males, evidencing the tendency of *P. salvator* to reproduce parthenogenetically. A total of 699 first stadium millipedes (38.8 ± 22.7 young/females) were obtained. Of these, 18% (127 individuals) survived until the adult phase.

The most pronounced decrease in the survival rate of *P. salvator* was recorded for the first stage (46% mortality), followed by the second stage (9% mortality), stabilizing around 7% for the remaining stadia until the adult phase (Tab. I). The mean duration of each development stage ranged between 6.8 ± 0.8 days for stage I and 14.9 ± 10.4 days for stage VI, which presented the highest variability among the results (Tab. I).

The reproductive adult phase showed the longest development time (80.3 ± 28.3 days), followed by the juvenile (53.1 ± 10.0 days) and pre-reproductive adult phases (13.8 ± 5.2 days). The mean time until maturity was 75.1 ± 23.6 days, and reproduction took place repeatedly until the death of the progenitors, indicating that the species has a plurivoltine cycle and may present up to five generations per year. The mean dura-

Table I. Number of individuals, percentage of the survival profile and of duration at each developmental stadia of the *P. salvator*.

Developmental stadia	Number of individuals	Percentage of the survival profile	Duration (days)
I	699	100	6.8 ± 0.8
II	378	54	7.2 ± 1.1
III	314	45	8.5 ± 1.9
IV	264	38	8.6 ± 1.9
V	223	32	9.3 ± 4.2
VI	176	25	14.9 ± 10.4
VII	127	18	10.8 ± 5.5

tion of the life cycle of *P. salvator* was 66.1 ± 16.7 days. Average female longevity was 147.2 ± 33.8 days.

The mean fecundity of adult females from the first generation was 120.8 young individuals/female (n = 34), a value much higher than the offspring produced by the females of the parental generation (38.8 young individuals/female). The number of eggs per nest ranged between seven and ten (n = 5). Also, emergence of young millipedes was observed in more than one nest at the same time, demonstrating that the between-laying period was short; however, the number of ovipositions per female was not counted.

During the experiment, eggs of *P. salvator* were laid side by side near each other. The eggs were brightly white and had a pearly aspect. Eclosion occurred after a mean of 9.9 ± 1.4 days and there were no vestiges of egg capsules in the empty nests, indicating a possible ingestion by the immature. From emergence to stage IV, young individuals were whitish and acquired their natural pinkish coloration during the subsequent stadia. Immatures of *P. salvator* are active and, in contrast with other millipedes, feed while still in the first stage, often immediately after emergence.

A total of five males individuals were obtained in this study, all of which with 19 body segments. Males could only be identified when they entered stage V, when the gonopods become evident. The sexual proportion found for *P. salvator* was highly biased towards females, 1 male:139.8 females. The mean longevity of males (106.4 ± 20.0 days) was shorter than that of females, and the adult phase had longer mean duration (60.6 ± 15.6 days) than the juvenile phase (45.8 ± 8.3 days). There were no differences between the development times of males and females of *P. salvator*.

DISCUSSION

The low survival of *P. salvator* individuals in the first stage of development observed in this study was also reported by VOHLAND & ADIS (1999) for *Pycnotropis tida* (Chamberlin, 1941), Aphelidesmidae, by SNIDER (1981) for *Polydesmus inconstans* (Latzel, 1884), Polydesmidae, and by BANO & KRISHNAMOORTHY

(1985) for *Jonespeltis splendidus* Verhoeff, 1936, Paradoxosomatidae. This high mortality can be attributed to the small body size and fragility of the exoskeleton, which are responsible for higher levels of water loss in the offspring when compared with adult individuals, making the former less resistant to desiccation or high humidity conditions (HOPKIN & READ 1992, LEWIS 1974, DAVID & VANNIER 2001). Besides, the elevated mortality rate in the initial developmental stadia is characteristic of species that reproduce through parthenogenesis (ENGHOFF 1976), as it is the case of the species studied herein.

The variability in the duration of developmental stadia observed for *P. salvator* was also demonstrated by ADIS *et al.* (2000) for two parthenogenetic populations of *Poratia obliterata* (Kraus, 1960), Pyrgodesmidae, from Germany. The stage IV and VII presented the longest duration in those two species. The highest variation occurred in the last developmental stadia which, according to HOPKIN & READ (1992), are most vulnerable to environmental factors such as temperature, which tends to affect their development time.

The plurivoltine cycle identified for *P. salvator* was observed by one of us for a population distributed in an area dominated by the "cambará", *Vochysia divergens* Pohl (Vochysiaceae), in Pantanal of Mato Grosso. In this environment, *P. salvator* is found throughout the year, and its reproductive period coincides with the flood season (October to December), extending until the high water (January to March) season.

For *P. obliterata*, a species found in flooded forests of the Amazon, BERGHOLZ (2007) reported a univoltine life cycle, with more advanced stadia also occurring during the flood period of the region. According to DAVID *et al.* (2003), the life cycle of many terrestrial arthropods is adjusted to seasonal changes, with development and reproduction alternating with dormancy periods, depending on the season. The mean duration of the life cycle *P. salvator* was different from the results obtained by ADIS *et al.* (2000), who found a longer time for parthenogenetic females of *P. obliterata*, varying from 105.3 ± 10.3 to 77.1 ± 10.1 days. Although the development times of *P. salvator* and *P. obliterata* parthenogenetic populations differed, they are amongst the shortest ever recorded for tropical millipedes, which can present a development period of up to two years (BERGHOLZ 2007, ADIS *et al.* 2000, HOPKIN & READ 1992).

The high mean fecundity of adult females from the first generation relative to the parental generation may be a reflex of the artificial conditions such as abundance of appropriate food, controlled humidity, and absence of predators. According to HOPKIN & READ (1992), the number of eggs produced by diplopods can considerably vary both intra- and interspecifically. In addition, egg number variation may be related to the conditions under which diplopods are kept, such as the diet offered (STEPHENSON 1961), the temperature (SNIDER 1981), and the intrinsic variability of the species in response to the environmental instability (BERGHOLZ 2007).

Thus, the small variation in egg number per *P. salvator*

oviposition can reflect the type and abundance of the diet offered and the stability of the environmental conditions of the experiment, mainly humidity.

ADIS *et al.* (2000) observed that in *P. obliterata* eclosion occurs between eight and 13 days after egg laying, a period longer than that found for *P. salvator*. Such difference can be explained by the different temperature regimens to which these organisms had been subjected. The rearing of *P. obliterata* included lower and controlled (15 hours at 24°C and nine hours at 29°C) temperatures, whereas *P. salvator* was kept at room temperatures (mean = 30°C, range = 25 to 34.5°C).

The influence of temperature on egg eclosion times was demonstrated for *Brachydesmus superus* Latzel, 1884, Polydesmidae, and varied according to the oviposition period, which was shorter at higher temperatures (STEPHENSON 1961). SNIDER (1981) also observed the influence of temperature on egg eclosion time for *P. inconstans*. He reported longer mean eclosion times at lower temperatures than at moderate temperatures. At high temperatures, the eggs were not viable.

The displacement of individuals after egg eclosion, as observed for *P. salvator*, is not common in millipedes. In fact, most individuals of most species remain inactive in the nest until they reach the third stage. Although HOPKIN & READ (1992) state that the egg capsule offers sufficient nutrients for the survival of individuals in the nest, our results show that this does not seem to be the case of *P. salvator*, since the juveniles search for other food sources soon after emergence.

The results obtained for males of *P. salvator* in the present study are consistent with those reported by ADIS *et al.* (2000) for non-functional males of *P. obliterata*, which also had 19 body segments, whereas functional males (bisexual population) had an additional segment. The smaller number of body segments present in males of *P. salvator* along with their low proportion indicates that this species has a strong tendency for parthenogenetic reproduction

The development synchronization between males and females of *P. salvator* is not common among millipedes. In many species, female development is delayed with respect to males. It has been hypothesized that these differences reflect reproduction optimization due to the energy investment in the formation of the ovaries (VOHLAND & ADIS 1999, HOPKIN & READ 1992), or an acceleration in the development of males to assure the insemination of recently matured females with high reproductive capacity (SNIDER 1981).

Factors such as humidity (SCHÖMANN 1956), temperature (ADIS *et al.* 2000) and contamination by bacteria (WITZEL *et al.* 2003) have been suggested to trigger the appearance of males in some parthenogenetic species of millipedes. However, none of these factors were evaluated in this study in connection with the birth of *P. salvator* males. Even though our results suggest a biased sex ratio, the high mortality of individuals in the initial developmental stadia may interfere with the estimation of the real sexual proportion in this species, once male individuals

can only be identified from stage V on. If a large number of males that hatched died before their sex could be identified, our the estimated sex ratio would not be a true estimation of the real sex ratio in these populations.

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