

LIFE CYCLE OF *SARRACENIOPUS NIPPONENSIS* (HISTIOSTOMATIDAE:
ASTIGMATA) FROM THE FLUID-FILLED PITCHERS OF *SARRACENIA ALATA*
(SARRACENIACEAE)

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ABSTRACT — Specimens of a population of *Sarraceniopus nipponensis* collected from *Sarracenia alata* in Louisiana, USA, slightly differ morphologically from specimens of the type location in Japan. Hence, a subspecies level for the USA-population is proposed. Ecological and behavioral data of *S. nipponensis* (Louisiana, USA) were collected. The development from larva to adult, skipping the facultative deutonymphal stage, takes about 6 days at an average temperature of 24°C (room temperature). Adults show a sex ratio of 1 : 2.6 in favor of females. Deutonymphs have a partially reduced sucker plate, apparently dispersing through ambulatory activity rather than phoresy. Precopulatory mate guarding occurs many hours before tritonymphs enter quiescence. During mate guarding, males rest motionlessly on the tritonymphal dorsum, but are not unable to move meanwhile. Legs I are conspicuously thick and serve as 'safeguard holder' while legs II are used to fight against male competitors and to hold on to the female regardless of environmental disturbances. The precopulated tritonymph is held in position directly beneath the male.

KEYWORDS — *Sarraceniopus nipponensis*; Histiotomatidae; life cycle; *Sarracenia alata*; ecology; subspecies; Kisatchie National Forest; USA-Louisiana

INTRODUCTION

Different kinds of habitats are colonized by the histiotomatids (Hunter and Hunter 1964; Nesbitt 1954), such as the fluid-filled pitchers of plants belonging to *Nepenthes* and *Sarracenia*. Mite species of *Creutzeria*, *Nepenthacarus* and *Zwickia* live in the pitchers of old world *Nepenthes*, while mite species of the genus *Sarraceniopus* are present on the New World *Sarracenia* (endemic to the South and South-East of the USA) which convergently developed similar pitchers as those observed in species of the genus *Nepenthes*. These pitcher plants pro-

duce a digestive fluid, which forms a diverse microhabitat for different kinds of animals such as the histiotomatid mite species: *Sarraceniopus nipponensis* Tagami, 2004 (Tagami 2004). Fashing and OConnor (1984) and Wirth (2004) proposed that the group of histiotomatid "pitcher-plant-mites" is monophyletic.

Although mites are commonly found in such watery habitats, the biology of most of them is poorly known (Fashing 2004a). The phenomenon of mate guarding (pre-copulation) is common in histiotomatid mites. However, within the mono-

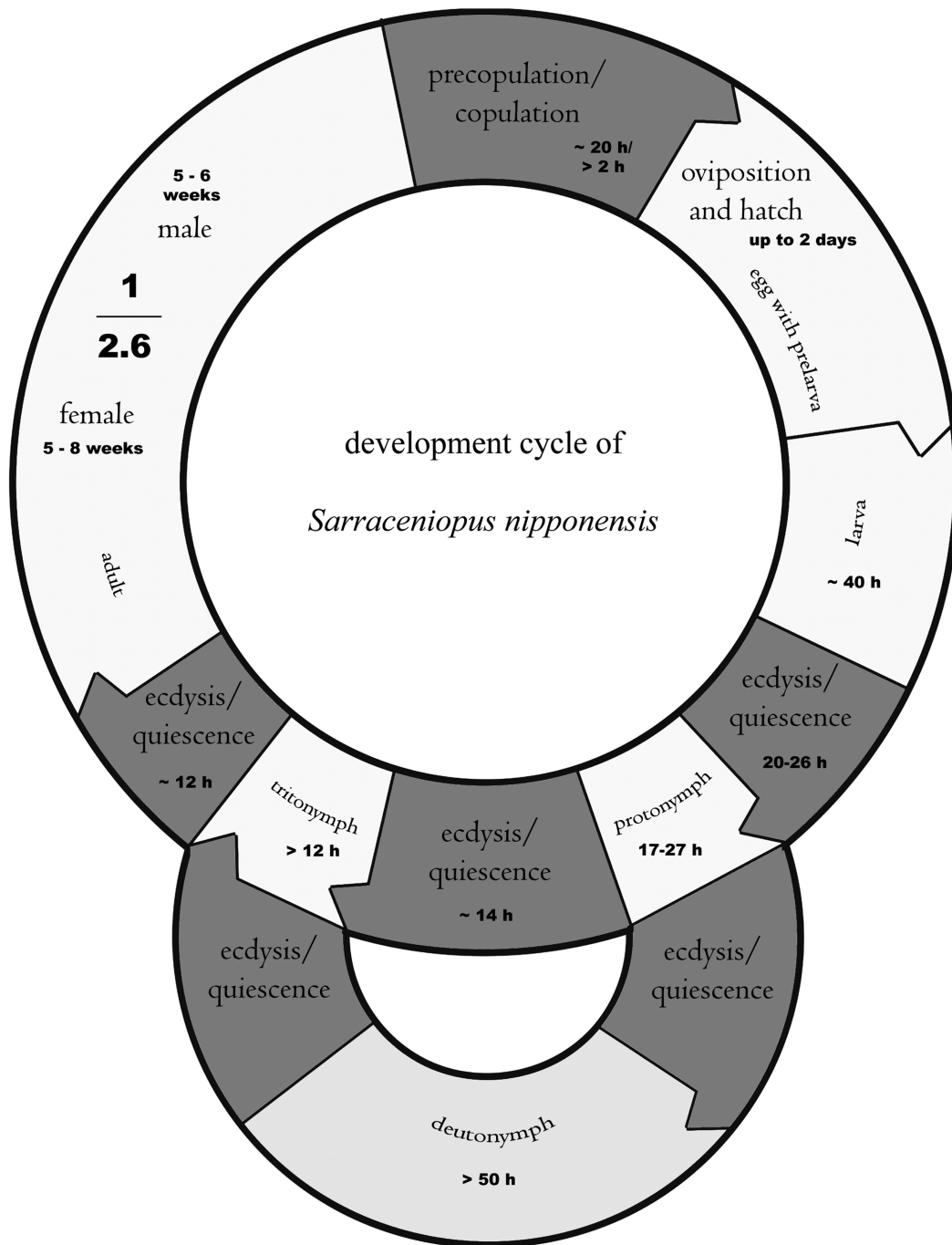


FIGURE 1: **Schematized life cycle of *Sarraceniopus nipponensis* (USA)** – big cycle: obligate cycle, small cycle: optional part with molting into deutonymphs, grey: ecdysis stages, slightly grey: active developmental stage. The first ecdysis from prelarva to larva occurs within the egg. Development from larva to adult without forming a deutonymph takes on average 6 days for both sexes. Sex ratio males : females $\approx 1 : 2.6$.

phyletic pitcher plant group, it has only been studied for *Zwickia* sp. and *Sarraceniopus darlingtoniae* Fashing and OConnor, 1984 (Fashing 2004a,b). This behavioral pattern is assumed to ensure mating success. First, pre-copulation ensures reproduction; second, it prevents other males from accessing to a virgin female. Generally, ecological and behavioral data on astigmatid mites are poorly known (Evans 1992). Life cycle studies are thus required to understand the ecological role of mites in their micro-habitats. The aim of the present paper is to present some life cycle aspects of *S. nipponensis*. These data are based on specimens collected from USA, Louisiana. Furthermore, due to morphological differences between the type material of *S. nipponensis* from Japan (Tagami 2004) and the presently studied specimens of *S. nipponensis* from Louisiana, USA, sub-species level is discussed.

MATERIALS AND METHODS

Specimens of *S. nipponensis* were sampled on *Sarracenia alata* Wood, 1863 by S. Wirth in the Kisatchie National Forest (31°1'41. 64 N; 92°53'19.90 W), Alexandria (Louisiana, USA) in August 2007. The mites were cultured on 1.4 % water agar in Petri dishes of different sizes at about 24°C ($\pm 3^\circ\text{C}$). The water agar was prepared without sterilization, and the agar solution was casted into the Petri dishes after being heated in a microwave. Two or three pieces of potatoes in form of cuboids (with a side length of about 1 cm and an altitude of 0.5 cm) were added to stimulate microorganism growth in Petri dishes with 5.5 cm in diameter, as food for the mites. The potato pieces were regularly moistened with water and once a month with beef heart solution instead of faucet water. Beef heart solution consists of faucet water, into which chunks of beef heart had been placed previously. Beefheart solution was monthly newly prepared from small pieces (2 – 3 cubes of meat with about 0.5 cm in diameter) of a frozen beef heart that were put into water (a Petri dish, 9 cm in diameter, nearly completely filled with water) and kept there for at least three days at room temperature (about 25°C).

Single larvae were isolated in small Petri dishes (5.5 cm in diameter) to determine the life cycle du-

ration of mites. A total of 85 single larvae were isolated, 38 of them developed until adulthood. Because these specimens needed a complex micro-habitat, in which the water-agar and small rotting pieces of potatoes offered hideaways, not all of these 38 individuals were continuously visible at all developmental stages. Therefore, figure 1 presents a mosaic-like pattern of data from only those individuals that were visible for a whole duration of a developmental stage including times of ecdysis. These were 1 – 3 specimens per stage, and its average duration is depicted in figure 1. The whole developmental durations of females and males of all 38 specimens are depicted in figure 2.

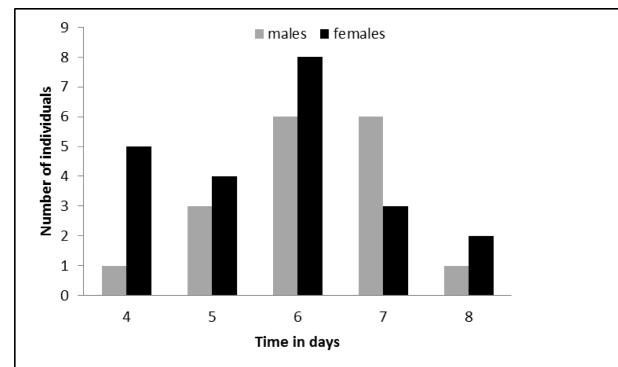


FIGURE 2: Individual whole development period of *Sarraceniopus nipponensis* (USA) males and females, which were observed from the larval stage until adulthood at a temperature of 24°C ($\pm 3^\circ\text{C}$) on daytime. On average, males need 6.2 days ($n = 16$), females need 5.7 days ($n = 22$) to complete their development.

Not all individuals moulted into deutonymphs. Data about the deutonymphal development resulted from more or less freshly molted deutonymphs, which were isolated from older cultures (9 cm in diameter), containing many mite individuals, into smaller Petri dishes (5.5 cm in diameter), in which these deutonymphs could be observed individually. Sex ratios were tested by placing mated females into Petri dishes (5.5 cm in diameter) and rearing their offspring in the same dish until they reached adulthood and also by observing entire cultures with many mite specimens. Isolated unmated females produced no offspring; hence parthenogenesis was not considered to be of importance in this

species at our rearing conditions. The sex ratio of precopulated tritonymphs was determined by separating 18 couples in precopula into 5.5 cm Petri dishes each, after molting.

For light microscopic preparations (Axioplan of Carl Zeiss, Germany), individuals of all stages (deutonymphs, other nymphs and adults) were transferred into hydroxypropionic acid (98%); after a few hours, the animal's tissues were dissolved so that only the chitinous exoskeleton remained. Then mites were mounted on slides in "Berlese solution" and after the medium solidified, the slides were sealed with glycel (a varnish).

For scanning electron microscopy (SEM, Fei Quanta 200) preparations, single individuals of deutonymphs and adults were transferred into ethanol (98%). They were then ultrasonically treated to remove dirt from their surfaces. Fixed individuals were dried in 1, 1, 1, 3, 3, 3-hexamethyldisilazane and afterwards sputtered with gold.

The holotype and the paratypes of *S. nipponensis* (Japan) were studied and compared using the light-microscopy with the mites from Louisiana, USA. At least 50 slides with specimens of each, adult and deutonymph of *S. nipponensis* (Louisiana, USA) were used for these morphological studies.

Images of the embedded objects were taken using a Nikon SC-17, an Axioplan light microscope and a Wild Heerbrugg M5A stereomicroscope. Drawings were made using a camera lucida attachment on the Axiophot light microscope, and digitized with the aid of the drawing tablet Aiptek Hyperpen 12000U, the software Adobe Illustrator 10 and Adobe Photoshop 7.0.

RESULTS

Life cycle

It took about 40 hours for the larva from hatching the egg until the next ecdysis (Figure 1). From this point until the development of tritonymphs, mites needed another 82 hours, including two quiescence periods (20 – 26 hours between larva and protonymph and 14 hours between protonymph

and tritonymph, Figure 1), Molting into deutonymphs is facultative and usually did not happen under the observed conditions. Instead protonymphs molted directly into tritonymphs.

At a temperature of 24°C ($\pm$ 3°C), males and females both developed in about 6 days (Figure 2) beginning with the free-living larval stage. Adult females lived for an average period of 46 days (38 – 55; n = 4); males only 36 days (22 – 42; n = 4).

The sex ratio was observed to be almost 1 : 3 when reared from eggs (6.2 males : 17.8 females). Sex ratio data were also determined from culture plates containing the offspring of many females. The sex-ratio observed then was of 1 : 2.5 (81 males : 204 females). These two ratios were quite similar.

A dense aggregation of about 50 deutonymph individuals was once observed; some had entered quiescence towards the tritonymphal stage, some were completing ecdysis, and others were still active (Figure 3).

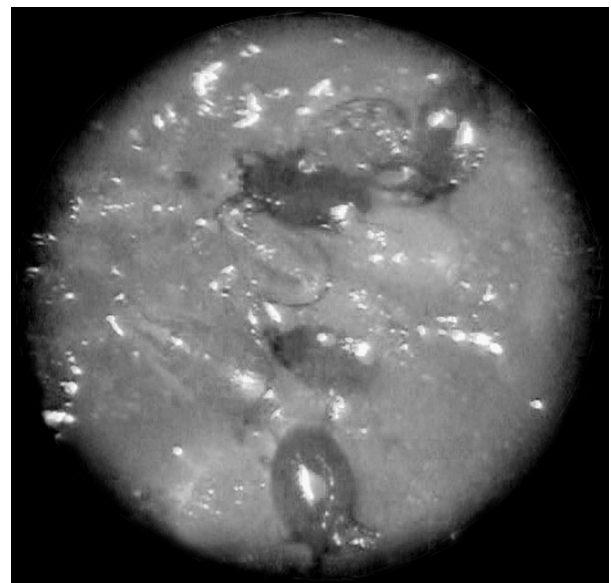


FIGURE 3: Aggregation of *Sarraceniopus nipponensis* (USA) deutonymphs during molting into tritonymphs.

Mating and reproduction

Mate guarding or precopulation (Figure 5d) to monopolize female tritonymphs was observed regularly in cultures of *S. nipponensis* from Louisiana.

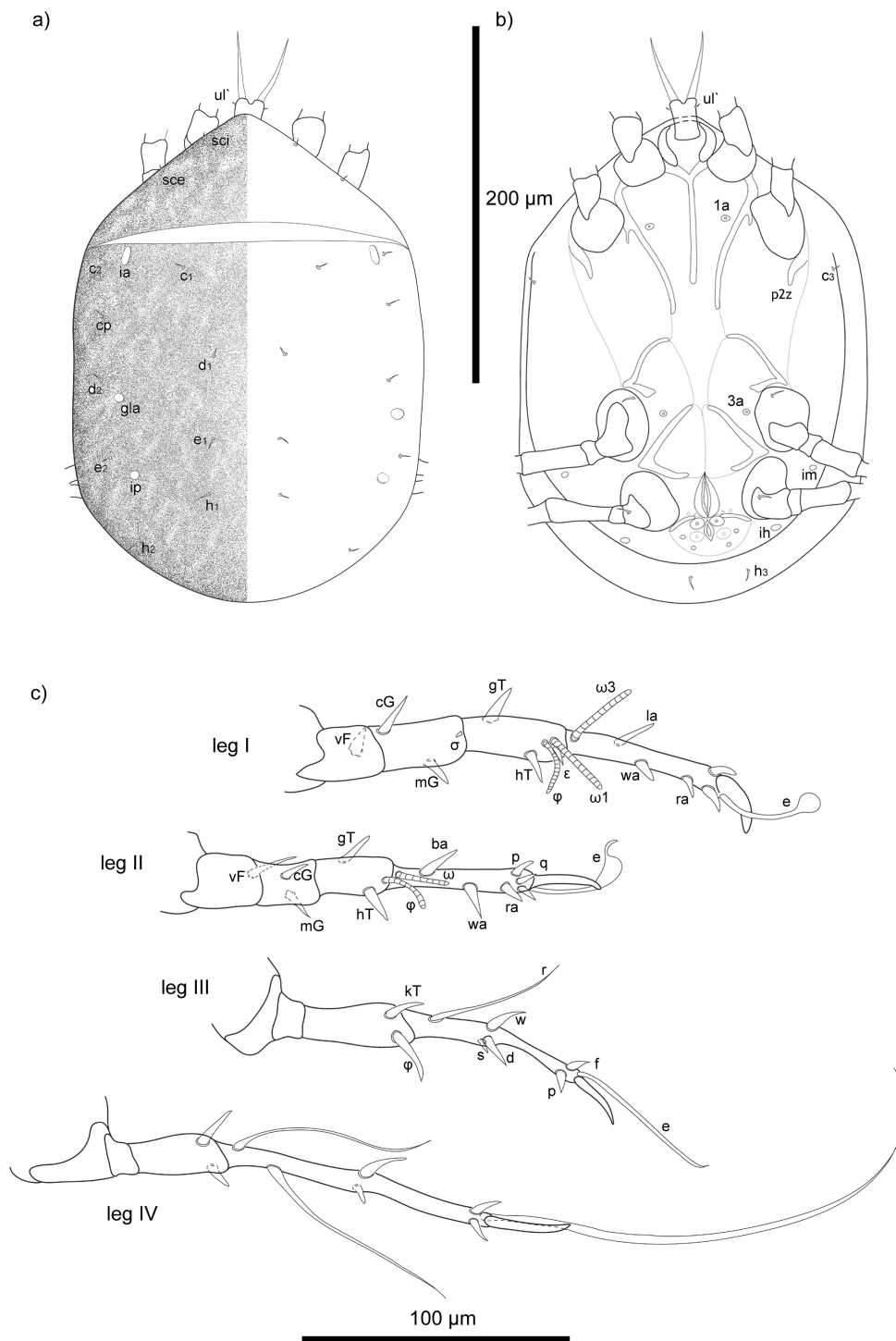


FIGURE 4: Deutonymph of *Sarraceniopus nipponensis* (USA). a – dorsal view, b – ventral view, nomenclature of dorsal and ventral setae and conoids after Fashing & OConnor (1984). Legs I-IV. φ , ω , ω_1 , ω_2 , ω_3 , σ , σ_1 , σ_2 are solenidia (setae as chemoreceptors). ih, im and ip are cupules (mechanoreceptors without hair-like structures on body surface). All other setae named in figure are mainly mechanoreceptors.

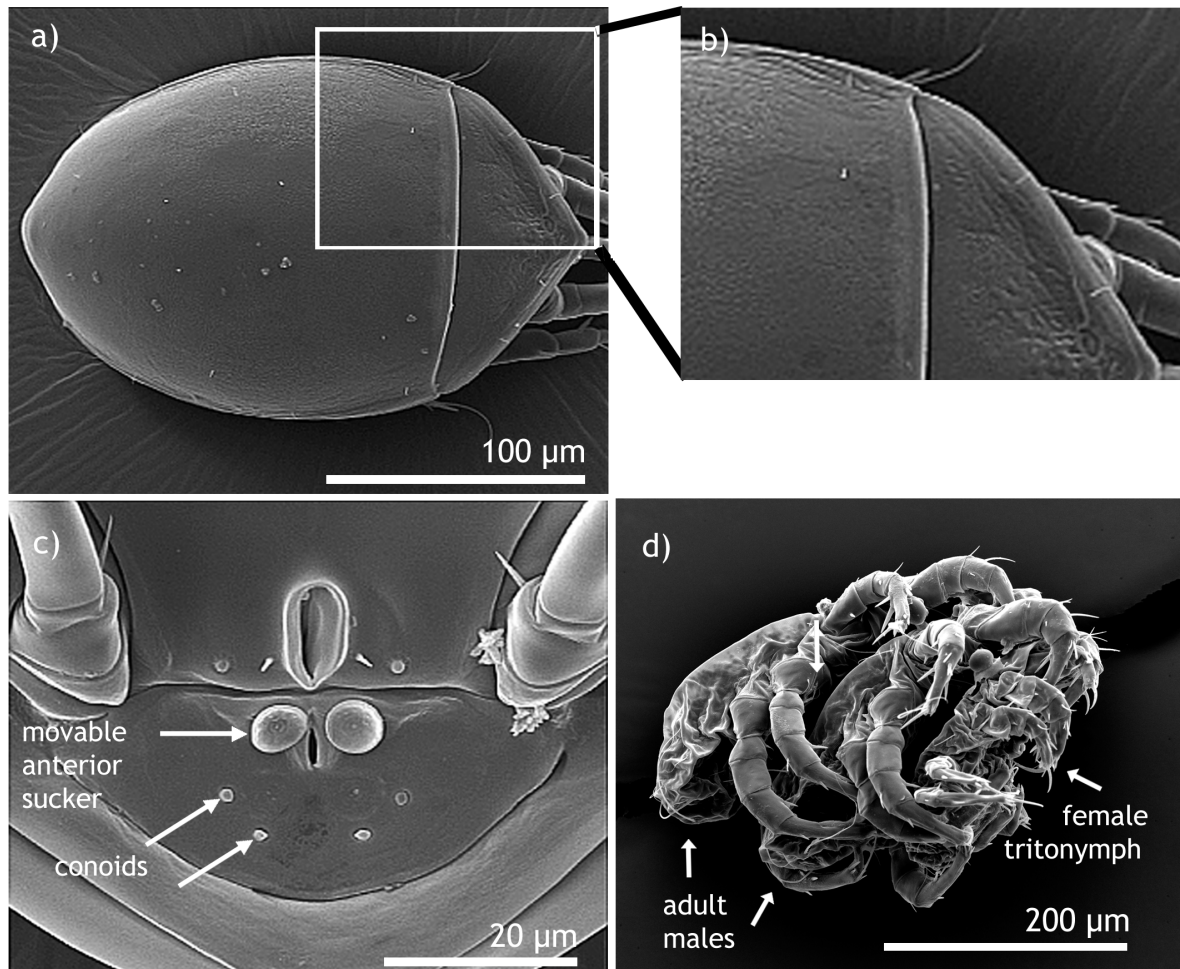


FIGURE 5: SEM pictures of *Sarraceniopus nipponensis* (USA). a – Dorsal view of the deutonymph, b – Detail of the cuticle's surface, c – Ventral suckerplate, d – lightmicroscopic picture of two adult males trying to perform a precopulation.

Mate guarding was observed 8 hours before the tritonymphs went into quiescence. Fifteen of the eighteen guarded tritonymphs that were examined after their molts were found to be females, and three were males, hence resulting in a sex ratio of 1:5 (males to females).

Precopulated tritonymphs averaged almost the same idiosomal length as males (Figure 5d): 338 µm (305 µm – 365 µm; n = 12): 346 µm (315 µm – 389 µm; n = 5). Males apparently preferred tritonymphs more or less their own size, even if further experiments would be needed to test this hypothesis. Indeed, we observed a larger variability in tritonymphal sizes, but this was not measured in

detail.

Reproduction in *S. nipponensis* was observed only to occur after copulation with males. Tests with isolated virgin females showed no evidence for parthenogenesis, as no eggs were produced. Mated females produced 30 individuals on average within about 7 days (24 – 42; n = 5).

The mate guarding behavior was observed in detail: males climbed onto the tritonymphal body from behind or from the side and finally came to rest on the tritonymph's dorsum. The whole procedure took about 15 minutes. The first legs of the males clasped over the first pair of legs of the tritonymph while legs III and IV of the male

grasped the tritonymph's body posteriorly behind its legs III and IV (Figure 5d). The second legs of males often rested between tritonymphal legs II and III, but could also be found between tritonymphal legs I and II. Legs I, III and IV of the male firmly clasped the mate's body, while the second legs lay freely against the mate's body surface and remained mobile for defense against rival males. In this way, the male guarded the tritonymph, which was restrained directly underneath. The mating position on adult females was the same. Females were observed to be copulated several times by different males. It is unknown, whether they are also inseminated several times. In detail, two out of three females observed for this specific behavior copulated and also produced offspring. After about two weeks they were put into new dishes with new males, where they copulated again.

The first legs of males are the thickest. They were of special importance for climbing and clasping their mates. They additionally act as 'safeguard holders'. In case of disturbance during the mate guarding or finally during the copulation, these legs never lost contact with the adult female or the tritonymph. During pre-copulation or the final mating, males were never observed performing any kind of active movements while riding the females; instead they rested motionless on their mates. In experiments with water droplets that were dropped on pre-copulating and copulating couples males were unable to stabilize their copulation position in an upright position and slipped to the side of the female's back.

The elongated hind legs of males were observed to envelop the posterior end of adult females firmly, but were found hanging limply on the agar surface behind the mate when guarding very small female tritonymphs (2 observations), or accidentally guarding against other males (2 observations).

Frequently, up to five males (5 males were observed once) were sitting on each other on a single tritonymph during its molting (Figure 5d).

Phylogeny

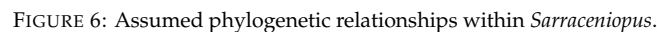
Specimens of both populations from Louisiana, USA, and from Japan share important common

morphological characters (Figures 4a, b, c), and therefore are interpreted by us to belong to the same species, *S. nipponensis*. Slides with specimens from Louisiana, USA, are deposited in the collections of S. Wirth (Berlin, Germany), of J. Moser (Pineville, Louisiana, USA) and additionally in the Museum für Naturkunde, Berlin (2 slides containing several specimens, deutonymphs and adults, ZMB 48433, ZMB 48434). Specimens from both populations could be compared using the holotype and paratypes of *S. nipponensis* (slides) from Japan and slides of mites from Louisiana, USA. They possess the following synapomorphic characters of the deutonymph: the apodemes between leg II and III are fused (Figure 4b). The sucker plate is reduced to a vestigial structure and in particular the shape of its remnants, e.g. the small conoid-rests, is similar in both species (Figures 4b, 5c). The deutonymph of specimens from Japan (smooth dorsal surface) differs from those from USA (Louisiana) in having a sculptured dorsal surface (Figures 4a, 5 a, b), being also visible with the light microscope.

Two sclerotisations anterior to the dorsal hysterosoma in males occur in *S. nipponensis* (Louisiana, LA), *S. nipponensis* (Japan) and *S. darlingtoniae*. This character is interpreted as apomorphy of the common stem species of these three forms (Figure 6). Therefore *S. darlingtoniae* is hypothesized to be the sister-species of *S. nipponensis*, which is in consistence with the phylogenetic argumentation of Fashing and OConnor (1984). An apodeme that we herewith name p2z is distinctly developed in the deutonymph; it seems to be of systematic relevance, and has never been emphasized before (Figure 4b). It is interpreted as a plesiomorphic character, which presumably evolved in the stem species of the genus *Sarraceniopus*.

DISCUSSION

The *Sarraceniopus nipponensis* population found on *S. alata* in Louisiana, USA, may be a subspecies of *S. nipponensis* from Japan (Figure 6). Further investigations are needed, whether morphological and ecological differences refer to a subspecies' level, or just due to general variability within a same species.



It is assumed that an active self-dispersal by walking over short distances could be the preferred mechanism for dispersal of deutonymphs in *S. nipponensis*. Presumably, this allowed for the reduction of the sucker plate components (Figures 4b, 5c). It is not known whether these sucker plates are still able to attach the mites to other arthropods. It is thus possible that deutonymphs of *S. nipponensis* disperse through ambulatory activity rather than phoresy, as described for *S. darlingtoniae* (Fashing 2004a).

Elongated hind legs of males were found in some histiostomatids such as *Zwickia* sp. (Fashing 2004b) belonging to the "pitcher-plant group". These were used for active movements when the mate was underneath, and fixed in this position by the forelegs. But the function of conspicuously elongated legs in males of *S. nipponensis* is still unclear, because movement and control of the whole couple by this on top sitting male using these legs to make a contact to the ground as a possible function could not be observed. Generally, the conspicuously elongated legs may just support a fixation of the male onto the female's back during precopulation and copulation.

In three of 18 pre-copulations, males inadvertently selected other males. Therefore, it can be assumed that males can hardly differentiate the own gender from those of females during ecdysis. That was what Fashing (2004a) presumed for *S. darlingtoniae*. The sex ratio of 3 : 1 in favor of the females

is conspicuous and differs from the ratio typically found for other histiostomatids, which was mostly 1:1 (Wirth 2004).

Perhaps, the ability to differentiate tritonymphal females from tritonymphal males is unnecessary within a population, since there is a threefold better chance of encountering a female.

Pre-copulation occurs many hours before tritonymphal ecdysis starts, which is different from the behavior of males in *Zwickia* species. Males of *Zwickia* sp. seek already quiescent tritonymphs for guarding against competition from other males (Fashing 2004b). The phenomenon of many males aggregating on single tritonymphs is not yet understood. There is no evidence that the male releases his female tritonymph at any time during pre-copulation in such situations, or during the subsequent copulation. Direct fighting behavior among aggregated males was not observed. Mass copulations were observed in relatively old cultures (i.e. more than three months old) with high population density, and never in younger cultures. Perhaps some sort of emergency causes this behavior at times when competition is extremely high. Competition pressure presumably rises with increasing numbers of males or decreasing number of females in the culture.

In other species of Histiostomatidae, arrhenotoky is commonly observed (unfertilized females produce males). This differs from *S. nipponensis*, as we show that fertilization is required to produce eggs. This does not mean that parthenogenesis generally does not exist. It just means that a copulation obviously is necessary for a production of eggs afterwards. However, this phenomenon is not an isolate case among the family Histiostomatidae, since it was also observed in *Histiostoma maritimum* Oudemans, 1914 (Wirth 2004).

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