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Spiders (Aranei) and their association with microorganisms

Пауки (Aranei) и их связи с микроорганизмами

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КЛЮЧЕВЫЕ СЛОВА: Gnaphosidae, Lycosidae, Pisauridae, Salticidae, бактерии, грибы.

ABSTRACT. The paper discusses what has been published in the scientific literature about the microbial carriage by spiders and then presents the results of our own limited study. Spiders for our pilot study were collected in wooded and grassy areas near lakes and streams located in two counties of New York, just upstate of New York City. A vertical sampling of soil, deep and top leaf litter, and tall grasses was undertaken. Eight species of spiders were studied: *Micaria pulicaria* (Sundevall, 1831), *Gnaphosa muscorum* (L. Koch, 1866), *Pardosa moesta* (Banks, 1892), *Trochosa terricola* (Thorell, 1856), *Pisaurina mira* (Walckenaer, 1837), *Phidippus audax* (Hentz, 1845), *Salticus scenicus* (Clerck, 1757), and *Sitticus fasciger* (Simon, 1880). *Gnaphosa muscorum* inhabit soil and leaf litter and this spider carried mainly *Bacillus*- and *Streptomyces*-type organisms. Three of our spiders, which inhabit the upper surface of soil and the top of leaf litter, *P. moesta*, *T. terricola* and *P. mira,* carried a large number of microorganisms but of varying diversity. *Pisaurina mira* carried predominantly *Bacillus* species, with an occasional pseudomonad and others. *Pardosa moesta* carried very predominantly a heavily encapsulated microorganism as well as a second – with both possibly being pseudomonads. *Trochosa terricola* had the most bacterial diversity – with some *Bacillus* species as well as pseudomonad-type microorganisms. Interestingly, none of these spiders carried fungi. The ground spider species, *Micaria pulicaria,* like two of the three Salticidae family members, *P. audax* and *S. fasciger*, carried molds and did not carry any bacteria. *Salticus scenicus* lacked fungi and bacteria.

РЕЗЮМЕ. В статье обсуждаются результаты ранее опубликованных данных по взаимоотношениям микроорганизмов с пауками, а также представлены результаты предварительных исследований авторов по этой теме. Пауки для предварительных исследований были собраны авторами в раздичных биотопах: лес и луга по берегу озера и ручья, в двух графствах штата Нью-Йорк, расположенных на север от Нью-Йорка. Пауки собирались в четырех вертикальных ярусах: почва, подстилка, поверхность подстилки, луговой травостой. Всего было изучено 8 видов пауков: *Micaria pulicaria* (Sundevall, 1831), *Gnaphosa muscorum* (L. Koch, 1866), *Pardosa moesta* (Banks, 1892), *Trochosa terricola* (Thorell, 1856), *Pisaurina mira* (Walckenaer, 1837), *Phidippus audax* (Hentz, 1845), *Salticus scenicus* (Clerck, 1757) и *Sitticus fasciger* (Simon, 1880). Обитающая на почве и в подстилке *Gnaphosa muscorum* в основном переносит бактерии из родов *Bacillus* и *Streptomyces.* Трое из исследованных пауков, *P. moesta*, *T. terricola* and *P. mira,* обитающих на почве и на поверхности подстилки, переносят большее количество микроорганизмов и с большим их разнообразием. *Pisaurina mira* переносит в основном виды рода *Bacillus*, а также иногда псевдомонад и другие бактерии. *Pardosa moesta* переносит в основном сильно инкапсулированные микроорганизмы, которые скорее всего являются псевдомонадами. У *Trochosa terricola* микроорганизмы наиболее разнообразны, они представленны как видами рода *Bacillus,* так и *Pseudomonas.* Отмечено также, что эти три вида не переносят споры грибов. Вид *Micaria pulicaria,* обитающий на почве, а также два вида из трех исследованных видов семейства Salticidae, *P. audax* and *S. fasciger*, являются переносчиками спор плесени (грибов) и не переносят никаких других микроорганизмов (бактерий). Вид *Salticus scenicus* не переносит ни грибов, ни бактерий.

Introduction

Spiders (Aranei) are a relatively ancient order of organisms, probably first coming into existence almost 400 millions years ago in the Devonian period [Selden et al., 1991; Foelix, 1996]. Therefore, spiders have had the chance to establish extremely complex interrelationships with the even more ancient bacterial microorganisms that came into existence billions of years ago [Madigan and Martinko, 2006]. Given this likely long interaction extending over millions of years, surprising, very little is known about possible spider — microorganism relationships. Most of the literature that does exist is devoted to these associations in terms of spiders serving as possible bacterial sources or reservoirs for human infection even though spiders have not been associated as vectors of human diseases at this time [Murray et al., 2005]. What is known about bacteria-spider associations will be discussed below.

Most spiders are predatory, eating invertebrates in the ecosystem using their venom to immobilize and kill their prey. Only a few spiders such as the black widow spider (genus *Latrodectus*) and the brown recluse spider (genus *Loxosceles*) appear to produce toxic enough venoms that have ill and occasionally lethal effects on human beings. In other cases, bite wounds lead to a phenomenon known as necrotic arachnidism or araneism [Diaz, 2004; Swanson & Vetter, 2005]. Necrotic arachnidism has been associated with the brown recluse spider (*Loxosceles reclusa*) in the Americas [Swanson & Vetter, 2005]. Several papers have addressed the idea that the tissue necrosis is not just due to the spider venom but might also be a result of microbes introduced into the bite wound at the time of the spider bite. Some researchers have found certain microorganisms like *Staphylococcus* and *Streptococcus* Group G at the bite site but these could easily have stemmed from the skin of the infected person [Banks et al. 2004].

A more convincing association of bacteria with the phenomenon of necrotic arachnidism was obtained by Monteiro et al. [2002]. They demonstrated that the fangs and the venom passing through the fangs of the brown recluse spider, *Loxoceles intermedia*, sometimes carry microorganisms that could potentiate the venom effects. Approximately 25% of the spider fangs carried *Clostridium perfringens* and approximately 37% carried microorganisms such as *Staphylococcus* species, *Bacillus* species and *Acinetobacter*, which are all bacteria that can be found in the environment and most of these organisms are routinely found in the soil. They focused on the anaerobic, spore-former *Clostridium perfringens* because of its association with gas gangrene. In a more recent study, Silvestre et al. [2005] looked for the presence of Clostridium species in in the spider species, *Loxoceles similis* (a recluse spider). They determined that *L. similis* can be a carrier of *C. septicum* in its fangs. Like *C. perfringens*, this clostridium is associated with the production of several exotoxins, such as alpha toxin that can promote tissue necrosis [Silvestre et al., 2005].

It has been suggested that the brown recluse spider might be more prone to having bacteria associated with it due to how it acquires food [Crawford, 2003]. Sandidge [2003] showed that the brown recluse spider, *L. reclusa*, is unusual in terms of feeding habits. They are scavengers actually preferring dead over living prey. Thus, they are more likely to contaminate their mouthparts with microorganisms. Additionally, it had been reported by Dr. Bleakley et al. [2003] that the brown recluse spider, *L. reclusa,* carries *Bacillus sphaericus*; however, this bacterium has not been associated with human disease at this time.

At one point, necrotic arachnidism had also been associated with the white-tailed spiders, *Lampona cylindrata* (L. Koch, 1866) and *L. murina* L. Koch, 1873 of Australia and a number of researchers had looked at the possible association of *Mycobacterium ulcerans* with the bites of these particular white-tailed spiders, *L. cylindrata* and *L. murina.* Many including Banks et al. [2004] have concluded that the evidence does not support a role for *M. ulcerans* in necrotic arachnidism and have even discounted the association of white-tailed spider bites with necrotic arachnidism.

Overall, a direct association of bacterial infectious disease arising after a spider bite has not been easy to establish. Isbister and Gray [2002] performed a large study in Australia. They analyzed 761 definitive spider bites from 18 families with dominant families being Sparassidae, Araneidae, Lamponidae and Theridiidae. They attributed these spider bites to seven possible secondary bacterial infections. They based this on the appearance of the bite wound, which showed "chemical features consistent with local infection (redness, swelling and pain, often delayed)."

Similarly, another Australian study suggested that two individuals might have infections of the nematode-associated *Photorhabdus luminescens* (Family: Enterobacteriaciae) in association with spider bites [Peel et al., 1999]. In a related publication, Weissfield et al. [2005] described an individual with an alleged spider bite on the left breast whose wound and blood showed infection with *Photorhabdus asymbiotica*.

Fagan et al. [2003] claim there is a correlation of severe human soft tissue infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) with spider bite wounds. It is unclear from their work as to whether they believe that the spiders are serving as a vector or allowing MRSA into the wound by providing a portal of entry to MRSA that might already be on the skin. Baxtrom et al. [2006] tried to show whether spiders could be vectors of MRSA. They collected 100 common household spiders, with 85 identified to the family level. The seven spider families collected were: Pholcidae, Theridiidae, Lycosidae, Salticidae, Anyphaenidae, Agelenidae and Gnaphosidae. Two were identified to species level and they were: *Pholcus phalangioides* (Fuesslin, 1775) and *Steatoda triangulosa* (Walckenaer, 1802). Microbiota associated with the spiders' exterior and interior surfaces were isolated on blood agar plates. Many of the spiders had on their exterior body surfaces *Bacillus* species (~50%) and *Staphylococcus epidermidis* (~35%). About 30% did not show any bacterial growth at all while only a small number had a variety of *Streptococcus*, *Micrococcus* species, *Actinomycetes* and fungi. Internally, *Bacillus* species and *S. epidermidis* were still among the more prevalent bacterial types. Among the microbes found in the interior was one potential pathogen, bacteria of the *Aeromonas* genus (found on only one specimen). Notably, Baxtrom et al. [2006] did not find any association between the spiders and MRSA strains.

Recently, Tugmon and Judge [2007] reported two garden spiders of the genus *Argiope*, *A. argentata* (Fabricius, 1775) and *A. aurantia* Lucas, 1833, carry bacterial organisms in and on them, with the intention of demonstrating that spiders could serve as vectors of human disease. The authors performed polymerase chain reaction (PCR) assays to identify the 83 bacteria they found. Among the species found were: *Enterococcus faecalis*, *Serratia marcescens* and Bacillus species. These can all be found in the environment. Interestingly, they found two medically important microorganisms on a single spider: *Klebsiella oxytoca* and *Acinetobacter baumanii / haemolyticus*.

A few recent findings shed some light on microbial associations with spiders from a non-human perspective. It has been recently determined that some spiders, like many arthropods, carry intracellular symbionts such as *Wolbachia*, *Spiroplasma*, *Rickettsia* [Rowley et al., 2004; Goodacre et al., 2006] and the recently identified endosymbiont, *Cardinium* [Duron et al., 2008]. Certain families of spiders — Linyphiidae, Agelenidae, Araneidae, Tetragnathidae — were shown by Goodacre et al. [2006] to carry all three of these intracellular microorganisms, whereas Theridiidae was shown to carry two of the three endosymbionts. Future studies will establish whether these endosymbionts have effects on the reproduction of spiders such as skewed sex ratios or parthenogenesis as has been observed for infected arthropod hosts.

It is known that some spiders such as the drumming spider, *Hygrolycosa rubrofasciata* (Ohlert, 1865), have effective immune systems which can protect the spiders from microbial infectious disease [Ahtiainen et al., 2005] and several spider species were recently shown to have antimicrobial substances in their venom [Curzo & Escoubas, 2003]. These include: *Lycosa carolinensis* (Walckenaer, 1805), *L. singoriensis* (Laxmann, 1770), *Cupiennius salei* (Keyserling, 1877), and the tarantula spider, *Acanthoscurria gomesiana* Mello-Leitao, 1923. *Acanthoscurria gomesiana* has also been shown to have the antimicrobial peptides (defensin-type molecules), gomesin and acanthoscurrin, present in its hemocytes. It is believed that the release of these defensin-type molecules into hemolymph contributes significantly to tarantula's innate immune response against both Gram positive and Gram negative bacteria [Silva et al., 2000; Fukuzawa et al., 2008]. Additionally, Fukuzawa et al. [2008] reports that hemocytes release coagulation cascade components into the hemolymph. These coagulation cascade components appear to play an important role in the innate immunity of the tarantula spider towards microbial infection.

Very little information regarding spider death due to microbial infectious disease has appeared in the literature. Nentwig [1985] reported that he had found on occasional dead spiders in the field in Panama, which appeared to have been infected with a member of the fungus *Nomuraea* species or *Gibellula* species. Greenstone et al. [1987] were able to show that certain spiders were susceptible to *Nomuraea atypicola*, when deliberately infected in the laboratory setting. Nentwig and Prillinger [1990] more recently reported that two of their laboratory stocks of spiders, *Cupiennius salei* Keyserling and *Ischnothele guyanensis* Walckenaer were infected with and dying from the fungus *Mucor hiemalis f. heimalis*. It had been reported that spiders such as *Argyrodes gibbosus* Simon, 1873 and *Pisaura mirabilis* (Clerck, 1757), have been found to be subjected to infectious disease caused by *Rickettsiella* microorganisms [Morel, 1978]. Tietjen [1986] reports that the social spider species, *Mallos gregalis* (Simon, 1909), can be subjected to contagious microbial infections, which can lead to death of many spiders in the colony.

Is infectious disease in spiders due to the presence of microorganisms such as bacteria more common than has been reported in the literature? Or does the presence of defensins such as gomesin prevent widespread bacterial infection in spiders? The presence of these defensin-type molecules might even decrease the carriage rate of bacteria by spiders on their exterior. Our study begins to address this issue in the manner that Baxtrom et al. [2006] and Tugmon and Judge [2007] do by more systematically looking at the carriage of bacteria on the exterior surfaces of spiders. We collected spiders from different ecological habitats in rural areas to look for bacterial presence without concern for the bacteria's pathogenicity toward human beings. The ecological habits represent vertical strata samplings (soil, deep leaf litter, top leaf litter, and grassy vegetation) in relatively close proximity to each other.

Materials and Methods

Spiders for our research were collected either in the Black Rock Forest (Orange County, New York; 41.430944?N, 74.053877?W) or in wooded areas near lakes and streams located in northern Westchester County of New York (41.27218?N, 73.602027?W). We chose representatives of four families of spiders: family Gnaphosidae with two species: *Micaria pulicaria* (Sundevall, 1831) and *Gnaphosa muscorum* (L. Koch, 1866); family Lycosidae also with two species: *Pardosa moesta* (Banks, 1892) and *Trochosa terricola* (Thorell, 1856); one species of family Pisauridae: *Pisaurina mira* (Walckenaer, 1837), and three species of the family Salticidae: *Phidippus audax* (Hentz, 1845), *Salticus scenicus* (Clerck, 1757), and *Sitticus fasciger* (Simon, 1880). All of our spiders are wandering spiders and do not produce catching webs and easily migrate in vertical strata. All studied specimens were mature spiders and were collected by using traditional collecting techniques. The ground dwelling spiders in the families Gnaphosidae, Lycosidae, and Pisauridae, were collected by hand. The Salticidae were collected by sweeping pasture vegetation (with the grass having a maximum height of one meter). The spiders were collected either in April 2006 (*G. muscorum*) or June 2007 (*P. moesta, P. mira, T. terricola, M. pulicaria, P. audax, S. scenicus, S. fasciger*). The live specimens were brought to the laboratory in separate, clean glass vials. After making sure the spiders were free of dirt or leaf litter debris, each spider was transferred to a sterile Petri dish containing BBL™ trypticase™ soy agar (Becton, Dickinson and Company). The plating of the microorganisms associated with the spider exteriors was accomplished by allowing the spiders to walk on the surfaces of the agar plates for approximately 24 hours as per Baxtrom et al. (2006). The spiders during this time were kept at 25?C. The spiders were removed carefully from the Petri dishes and transferred to 75% ethanol for taxonomic identification. The plates were further incubated at 25?C for a week to allow for the growth of slow growing microorganisms such as members of the bacterial family *Streptomycetaceae*. Following the incubation period, colonies on the plates were evaluated for the different types of microbial growth. Wherever possible, well-isolated distinctive bacterial colonies were selected and handled in the following manner. Using sterile technique, a portion of a selected colony was used to inoculate a small portion of the surface of a new Petri dish containing BBL™ trypticase™ soy agar. The bacterial inoculum was then streak plated to obtain a pure culture of the bacterial microorganism. Partial identification of the bacterial microorganisms was performed using traditional microbiology methodology such as Gram staining [Holt, 1977; Benson, 2002].

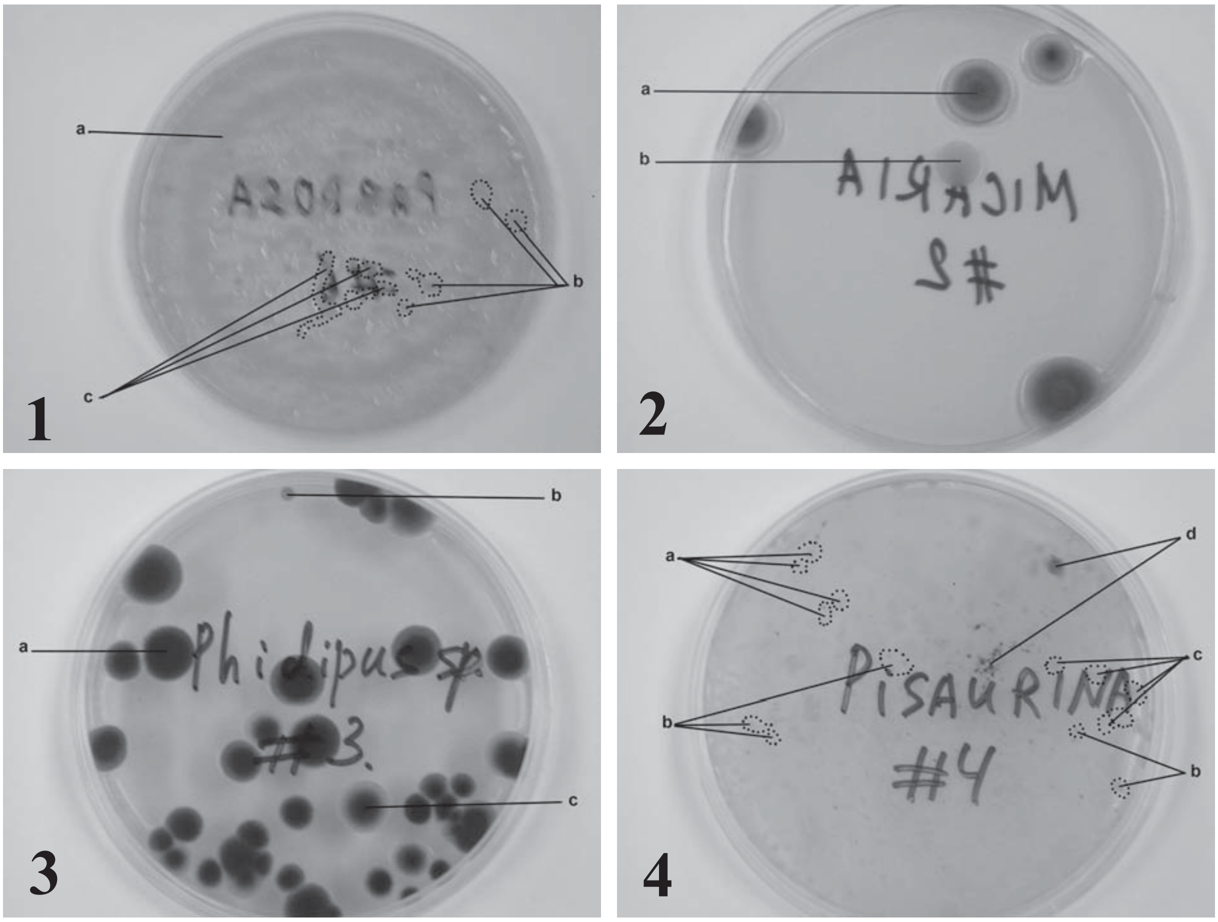
Results and Discussion

For our research, we chose spiders collected in different layers of soil, deep and top leaf litter, as well as grassy vegetation. The ground spiders are likely to have close association with soil microorganisms. However, even the Salticidae collected from the tall grasses are expected to have some contact with soil microorganisms – possibly at night or during rainy weather when they typically retreat to the leaf litter at the base of the tall grasses. As mentioned above, two ground spiders of family Gnaphosidae were represented by the species *Gnaphosa muscorum* and *Micaria pulicaria*. *Gnaphosa muscorum* is normally a nocturnal predator, inhabiting mostly the surface of soil or in deep layers of leaf litter. These spiders hunt mostly in and on the upper surface of leaf litter. *Gnaphosa muscorum* can make silken retreats for molting and laying eggs in egg sacs in the soil or the leaf litter [Platnick, Dondale, 1992]. Therefore, this species has regular and stable contact with soil and microorganisms living in the soil. The second representative is *Micaria pulicaria*. This species is most often found associated with ants. *Micaria pulicaria* are very commonly found near or even inside of ant nests. *Micaria pulicaria* prey on the ants, and they also leave their cocoons in the ant nests. *Micaria pulicaria* occur on the top of soil or on top of leaf litter, usually near ants [Cushing, 1997]. The wolf spiders are represented by two species: *Pardosa moesta* and *Trochosa terricola*. Both species have diurnal activities. *Pardosa moesta* are most often found hunting on the surface of soil, or leaf litter. They do not make retreats and the females carry cocoons at all times [Dondale, Redner, 1990]. *Pardosa moesta* can move easily on the water surface of small ponds and streams. *Trochosa terricola* spiders behave similarly to *P. moesta*. *Trochosa terricola* occupy the same habitat as *P. moesta*, also hunting on the surface of soil or leaf litter; however, unlike *P. moesta*, *T. terricola* spiders do construct retreats by digging into soil. Therefore, spiders of this species have very close contact with soil and could be contaminated by microorganisms occurring in the soil.

For our study, a nursery-web spider (Pisauridae) was also collected. *Pisaurina mira* (Walckenaer, 1837) is a large spider occurring primarily on the vegetation of swampy areas, with these swampy areas often near lakes or ponds [Dondale, Redner, 1990]. The *Pisaurina* spiders have complicated mating behaviors, which involved the male repeatedly copulating with the female while repeatedly presenting her with a fly “wrapped” in silk. While going through this mating ritual, the male is constantly moving back and forth coming into contact with soil and litter surfaces that are rife with bacteria. The female attaches the egg sacs on the vegetation and the spiders can actively move across all types of grassy vegetations. These spiders have regular contact with soil and water. We chose this species of *Pisaurina* since it occurs in all levels of vegetation, on the upper surface of soil or leaf litter, and on the water surface. Thus, the spider should eventually contact with microflora occurring in all levels of vegetation as well as soil.

Lastly, to provide contrast in terms of having less frequent contact with soil and leaf litter, several jumping spiders of the family Salticidae were collected. Jumping spiders are active diurnal predators, hunting mostly on the top of vegetation or grasses. They prefer blooming plants and hunt on insects and small spiders occurring on or near blooming flowers. At night, Salticidae are not active and as a rule, spend their nights in silken retreats amongst the low levels of vegetation or even in leaf litter. Females make and leave cocoons in a variety of places. It could be on the vegetation, in the leaf litter, under logs, under rocks or under tree bark [Kaston, 1981]. For our study, we chose jumping spiders that spend their time mostly on the upper levels of vegetation. Three species were studied: *Phidippus audax*, *Salticus scenicus*, and *Sitticus fasciger*. All three species have been observed to have very similar behavior and occupy almost the same ecological space — the tops of blooming vegetation.

Four of our five ground spiders, *G. muscorum, P. moesta*, *P. mira, and T. terricola,* had bacteria on their



Figs 1–4. Description of the colonies formed by the microorganisms isolated from the exterior of the following spiders on Petri dishes containing BBL™ trypticase™ soy agar as well as other relevant feature. The colony descriptions and features for: 1 — *Pardosa moesta* (Banks, 1892): a) The "track" of the spider is observed causing confluent bacterial growth, consisting of a mixture of the two types of bacterial colonies on the plate, with a predominance of the translucent, yellowish colony type; b) Several colonies are selected of the translucent, yellowish colony type; c) Several colonies are selected of the encapsulated, somewhat opaque, cream colored colony type. 2 — *Micaria pulicaria* (Sundevall, 1831): a) Sample of one of two mold colony types observed. The obverse is olive green with a cream colored margin and the reverse is cream colored; b) Sample of the yeast colony observed. The colony is opaque, cream colored with a buttery texture. 3 — *Phidippus audax* (Hentz, 1845): a) Sample of one of two mold colony types observed. The obverse is dark blackish-gray with a light gray margin and the reverse is light gray colored; b) The dark spot is believed to be a result of guanine hydrochloride released by the spider; c) Sample of second of two mold colony types observed. The obverse is dark gray with a cream colored margin and the reverse is cream colored. 4 — *Pisaurina mira* (Walckenaer, 1837): a) Several colonies are selected of the translucent, yellowish colony type; b) Several colonies are selected of the opaque, golden yellow colony type; c) Several colonies are selected that are opaque, whitish with a "ground glass" texture; d) The dark spots are believed to be a result of guanine hydrochloride released by the spider.

Рис. 1–4. Описания колоний микроорганизмов выращенных на чашках Петри, содержащих BBL™ trypticase™ с соевым агаром и при определенных условиях. Микроорганизмы собраны с наружных покровов четырех видов пауков. Описание колоний и их характеристики: 1 — *Pardosa moesta* (Banks, 1892): a) "Дорожка" проделанная пауком и состоящая из разросшихся и слившихся колоний бактерий двух типов присутствующих в чашке Петри, с преобладанием прозрачной, желтоватой колонии; b) Несколько отобранных колоний бактерий прозрачного и желтоватого типа; c) Несколько отобранных колоний бактерий типа: инкапсулированные, непрозрачные, кремового цвета колонии. 2 — *Micaria pulicaria* (Sundevall, 1831): a) Пример одного из двух видов колоний плесени обнаруженных в чашке Петри. Колония оливково-зеленая с кремовой окантовкой сверху и кремового цвета снизу; b) Пример обнаруженной колонии дрожжей. Колония кремового цвета, непрозрачная, с маслянистой текстурой. 3 — *Phidippus audax* (Hentz, 1845): a) Пример одного из двух видов колоний плесени обнаруженных в чашке Петри. Сверху колония темно-серая, черная, со светло-серой окантовкой, снизу колония светло-серого цвета; b) Темные пятна, вероятно гуанин гидрохлорид выделенный пауком; c) Пример второго вида колоний плесени обнаруженных в чашке Петри. Сверху колония темно-серая, с кремовой окантовкой, снизу колония кремового цвета. 4 — *Pisaurina mira* (Walckenaer, 1837): a) Несколько отобранных колоний бактерий полупрозрачного, желтоватого типа; b) Несколько отобранных колоний бактерий непрозрачного, золотисто-желтого типа; c) Несколько отобранных колоний бактерий непрозрачного, беловато-матового типа; d) Темные пятна, вероятно гуанин гидрохлорид выделенный пауком.

exteriors and no fungi. The fifth, *M. pulicaria* (Fig. 2), as well as two of the salticids, *P. audax* (Fig. 3) and *S. fasciger*, yielded fungal colonies — primarily mold-like growths. For an unknown reason, *S. scenicus*, did not manifest fungal or bacterial growth. Likewise, the reason for the dearth of bacteria on the exterior of *M. pulicaria* is unknown at this point. It is also interesting to note that the four ground spiders that carried bacteria on their surfaces had somewhat different bacterial populations — albeit many of them having characteristics typical of soil microorganisms.

For example, the *Gnaphosa* species representative, *G. muscorum,* had predominately bacteria of the genus Bacillus, which are Gram positive and endospore-forming. In addition, the plate manifested a few tough, leathery, raised, pigmented colonies typical of bacteria in the family *Streptomycetaceae*. Consistent with the presence of bacteria of the family *Streptomycetaceae*, the plate produced a characteristic geosmin odor [Ovtcharenko, Trachman, 2007].

The *Pardosa* species representative, *P. moesta*, whose constant activity was evidenced by circular track marks visible on the agar surface (Fig. 1), carried predominantly 2 types of microorganisms. The most predominant bacterial type was a heavily encapsulated Gram negative short bacillus that yields a cream colored, somewhat opaque colony. This capsule allows the microorganism to adhere to the plastic of the Petri dish. Capsules can also serve to protect the microorganism against desiccation and are also known to be a nutrient source for starved bacteria [Madigan, Martinko, 2006]. These two traits can help a soil associated microorganism. The second isolate, also a Gram negative short bacillus, produces a translucent, yellow pigmented colony. Many environmental isolates are pigmented to protect against the adverse effects of sunlight by the quenching of photo-oxidation products [Madigan, Martinko, 2006]. Both microorganisms are obligate aerobes. In addition, these bacteria were oxidase positive and catalase positive as well as motile. They both might be bacteria in the family *Pseudomonadaceae*, which are common environmental microorganisms [Ovtcharenko, Trachman, 2007; Trachman, Ovtcharenko, 2007].

The *Pisaurina* spider representative, *P. mira*, yielded mainly colonies of the Gram positive, endospore-forming bacteria of the genus *Bacillus*. Several colonies appeared to typical of *Bacillus cereus var. mycoides*. There were also several colonies of opaque, golden yellow pigmented bacteria. Gram staining showed Gram positive cocci primarily arranged as tetrads. These microorganisms were catalase positive, were obligate aerobes and were not able to grow in the presence of 7.5% sodium chloride. We believe them to be members of the *Micrococcus* genus. We also found colonies that were translucent producing yellowish green pigmentation. They were Gram negative short bacilli, which were catalase positive, oxidase positive, motile and again found to be obligate aerobes. Again, this could indicate that they are bacteria of the family *Pseudomonadaceae* (Fig. 4) [Ovtcharenko, Trachman, 2007; Trachman, Ovtcharenko, 2007].

The *Trochosa* spider representative, *T. terricola*, typically digs into soil and leaf litter and also dug into the agar surface. *Trochosa terricola* yielded a wider variety of colony types than our other spiders. Members of the *Bacillus* genus were represented but not as dominantly as we observed with the microorganisms yielded by *P. mira*. Many colonies were yellow-pigmented and translucent. Gram staining showed many to be Gram negative short bacilli, which were catalase positive, oxidase positive, motile and found to be obligate aerobes. We again suspect these to be members of the family *Pseudomonadaceae*. We also observed some colonies to be more opaque and cream-colored. These were Gram positive, non-spore forming, short bacilli with the bacteria being catalase positive, oxidase negative, and again found to be obligate aerobes [Ovtcharenko, Trachman, 2007; Trachman, Ovtcharenko, 2007].

Concluding Remarks

As part of our understanding how spiders exist in their respective habitats, we investigated the possible presence of microorganisms on the exteriors of several different spiders, five ground-dwelling spiders, *Gnaphosa muscorum*, *Pardosa moesta*, *Pisaurina mira*, *Trochosa terricola* and *Micaria pulicaria*, and three jumping spiders of the family Salticidae. Four of the five ground-dwelling spiders, *G. muscorum*, *P. moesta*, *P. mira*, and *T. terricola*, carried a variety of bacterial microorganisms and no fungi. The remaining ground spider, *M. pulicaria* as well as the two of the Salticidae, *P. audax*, and *S. fasciger,* only carried fungi — primarily mold colonies. *Salticus scenicus* did not carry fungus or bacteria.

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