Comparative water relations, temperature sensitivity, and breeding potential of *Pycnoscelus* cockroaches (Blattaria: Blaberidae)

by

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Abstract

Pycnoscelus surinamensis (L.) is a pest cockroach found worldwide, while its relatives are restricted to tropical regions. Few comparative studies have investigated physiological differences between *P. surinamensis* and its sexually reproducing sister species, *Pycnoscelus indicus* (F.), but no comparison has been made between different *Pycnoscelus* species other than the two species mentioned here. To examine potential factors contributing to the widespread range of *P. surinamensis* we compared water relations, thermal tolerance, and reproductive potential between six *Pycnoscelus* species. No significant differences were observed in water relations, thermal tolerance, and reproductive potential between *P. surinamensis* and other *Pycnoscelus* species. While we could not explain why *P. surinamensis* are more widespread than its congeners, our study opens a great opportunity to explore the physiological and biological aspects of *Pycnoscelus* species.

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CHAPTER 1

Introduction and literature review

Cockroaches and their impact on humans

Insects are probably the most successful animals on the planet. They make up about 70% of all organisms (Samways 2018). Of all the insects, one can argue that cockroaches are truly the champions of survival. They have been around before even dinosaurs and survived several mass extinction events (Copeland 2003). They also occupy diverse habitats in the ecosystem, from deserts to forests and caves to canopies and are found worldwide except in the arctic regions (Roth and Willis 1960, Schal et al. 1984). Cockroaches are also diverse in size and form. The heaviest cockroach in the world, *Macropanesthia rhinoceros* Sassure, can weigh up to 30 grams and grow to 8 cm in length (Brown et al. 2000). These gentle giants are native to Australia and spend most of their lives in underground burrows feeding on rotting leaves they have dragged down from the surface. The smallest cockroach species, *Attaphila fungicola* Wheeler, measures only 3 mm in length. This unique species lives with *Atta* species, the leafcutter ant, and forages on fungus that the ant's farm (Bell et al. 2007).

Cockroaches are perhaps the most misunderstood group of insects in the world. Of about 4600 species of cockroaches known to science, less than 1% are considered pests due to their association with humans (Rehn 1945). Of the 30 or so species of cockroaches that are commonly associated with humans, only about three are well-known as pests which include the American cockroach, *Periplaneta americana* (L.), Oriental cockroach, *Blatta orientalis* L., and the German cockroach, *Blattella germanica* (L.) (Goddard 2003). They adapt readily to various environments though they mostly prefer warm conditions found within buildings (Ifeanyi and

Odunayo 2015). These species indirectly harm us by transmitting disease organisms to the general public through mechanical means and fecal contamination (Ifeanyi and Odunayo 2015). They can also have a health impact on humans as an intermediate host of various parasites and other microorganisms (Ifeanyi and Odunayo 2015). Microbiological investigation of common pest cockroaches showed an array of 32 different bacterial isolates, 17 different parasites, 7 different fungi spp., some exotic viruses including hepatitis, and they have been implicated in the transmission of *Salmonella* spp. and *Shigella* spp., which are of severe clinical importance (Ifeanyi and Odunayo 2015). Their diet is a primary factor in their capability as a vector. As an omnivore, they can feed on a variety of food sources (Schal et al. 1984). Although they mostly prefer starchy foods, they can consume dried blood, fingernails, toenails, eyelashes, and the skin of sleeping and sick persons (Royal 1979). They also feed on mouse fecal matter amongst other animals (Ifeanyi and Odunayo 2015). The cockroaches also defecate and regurgitate as they feed, often on human food, exposing humans to their crop content and fecal matter (Royal 1979). To paint a complete picture of how strong a vector particular pest cockroaches can be, *P. americana* can be used as an example. In a survey of 322 cockroaches, 58.6% were infected with gastrointestinal parasites, including: *Balantidium coli*, Hookworms, *Entameba coli*, *Enterobius vermicularis*, *Entameba histolytica*, *Trichuris trichuira* and *Ascaris lumbricoides*. These parasites were collected from the external or the internal surfaces of the gastrointestinal tract of the cockroaches, a shocking 30.6% of the cockroach individuals surveyed harbored all the above parasites (Etim et al. 2013).

Pest cockroaches are also significant transmitters of allergens, as cockroach allergy has been shown to be strongly associated with asthma, particularly among children and young adults living in inner-city environments where significant numbers of pest cockroaches are present

(Mueller et al. 2017). Their feces, saliva, or even the cockroach themselves can be an allergen to humans. There are 12 groups of allergens associated with cockroaches, present mostly with *P. americana* and *B. germanica*. Research in the past 50 years has shown that sensitization to cockroach allergens is one of the predominant causes of asthma in lower-income urban people worldwide (Mueller et al. 2017). A recent review documents that cockroach allergy ranges from 17 to 41% in the US, with cockroach allergens detected in 85% of inner-city homes in the US. Based on the skin prick testing performed in the review, 60–80% of inner-city children with asthma are sensitized to cockroaches. (Do et al. 2016)

However, pest cockroach species are not just vectors; they also impact greenhouses and native ecosystems. One such invasive pest species is the Surinam cockroach, *Pycnoscelus surinamensis* (L.), a common greenhouse pest around the world and a widespread exotic species across the tropics and subtropics (Zangl et al. 2018, Roth 1967, 1974). Commercially, it is significant in its impact on many plant species, as it can cause damage to rose, orchid and lily plantations. It also feeds on the roots of many commercially significant plant species, such as pineapples, potato tubers, cucumbers, palms, tomatoes, papayas, figs, sweet potatoes and other plants (Bell et al. 2007). Outside of moist tropical climates where it can move around freely, it relies on humans for its transportation and spread, especially within the transportation of soil, mulch, or plants to colonize new areas (Bell et al. 2007, Zangl et al. 2018). Since it has a synanthropic or peridomestic lifestyle, it can survive in temperate regions by being transported to suitable greenhouses and households (Grandcolas et al. 1996, Zangl et al. 2018). *Pycnoscelus surinamensis* might also have an impact on native fauna through competition. When the author was traveling in tropical and subtropical climates in the southern United States (Texas, Alabama, and Florida) to observe detritivores, *P. surinamensis* was the dominant species in the leaf litter,

even in habitats devoid of or with minimal human habitation (Indian Shell Mound Park, Everglades National Park, Big Pine Key Preserve, Ocala National Forest to name a few). In some areas their density was so great that the first inch of soil surface was mainly composed of *P. surinamensis* and their frass. While the impact of *P. surinamensis* on other litter-residing invertebrates and vertebrates is not well investigated, we suspect some native soil-dwelling invertebrates such as sand cockroaches *Arenivaga* spp. and isopods face heavy competition from the *P. surinamensis*.

Anatomy and Behavior

What we currently recognize as the standard cockroach body is flattened and broadly oval, with a relatively large and shield-shaped pronotum covering the cockroach's head and neck. They always have chewing mouthparts and long, highly segmented antennae. With regards to the winged species of cockroaches, the forewings or tegmina are typically thicker, with a leathery feel to the touch, while the hindwings are usually more delicate and hyaline. The coxae of the cockroaches are flattened and modified to house the femur, so that when the legs are tucked in close to the body, the combined thickness of the two segments is reduced, making it easier for the cockroach to fold its legs inward (Bell et al. 2007). Most cockroaches are yellowish-brown in color (Ifeanyi and Odunayo 2015).

Most cockroach species, especially those that serve as pests in human habitations, are nocturnal, often inhabiting latrines and other moist areas below sinks and other water sources. Many pest species, such as *Periplaneta*, breed outdoors, with the nymphs and adults wandering inside to feed, especially in response to changing weather conditions (Appel and Rust 1985, Ifeanyi and Odunayo 2015).

Part of what allows cockroaches to succeed in many environments is their legs. Their legs are covered in tactile spines and have a complex network of microtubules that respond in adaptive pathways, allowing them to have a good sense of the surrounding areas for quick responses to any stimuli (Lund 2019). In some species, legs are fossorial or modified for digging. They can be stout and paddle-shaped, allowing cockroaches to burrow easily into the soil (Roth 1977). Others have modified pads on their feet called arolia, which allows cockroaches to climb smooth surfaces (Arnold 1974).

Cerci, a pair of appendages located on the last segment of the cockroaches, also play an important role in cockroach survival as they aid in escape response. Cerci can detect air movement in the surroundings, allowing cockroaches to detect predators that are approaching from behind (Fraser 1977, Camhi et al. 1978, Ritzmann 1984). This is achieved by the stimulation of hairs on the ventral surface of the cerci, which signals giant axons connected to mesothoracic ganglia to activate, making legs attached to mesothorax to run (Farley and Milburn 1969, Zilber-Gachelin and Chartier 1973).

Cockroaches possess two types of eyes: compound eyes and simple eyes called ocelli. Compound eyes are believed to perceive ultraviolet wavelength while the ocelli perceive light intensity below the UV wavelength (Goldsmith and Ruck 1957). This could be because cockroaches are primarily nocturnal; thus, perception of UV wavelength will allow cockroaches to see at night, and perceiving light intensity can enable cockroaches to follow day/night cycle.

However, not all cockroaches possess eyes; though most species have functioning eyes, many cave-dwelling species have lost their eyes. For example, some epigean *Nocticola* species lack eyes (Roth 1988). Eye structure can also be sexually dimorphic within cockroaches, with males

having large, bulging, nearly contiguous eyes while those of the more sedentary females exhibit flattened and farther apart eye structures (Bell et al. 2007).

One interesting note regarding their anatomy is that cockroaches tend to have a high level of fluctuating asymmetry, with slight and random differences in bilateral characters. For example, cockroach tarsus usually comprises five segments, but occasionally a single leg may have just four segments (Bell et al. 2007). Spines on the femora are another characteristic that may vary from time to time. In this case, the number between the right and left sides of the same cockroach may vary (Bell et al. 2007). In both cases, a reduction more often on the specimen's left. Wing veins may also be an example of bilateral asymmetry, with them being simple on one side and bifurcated on the other (Bell et al. 2007). An extreme example of asymmetry in cockroaches is gynandromorphy. While it occurs rarely, gynandromorphs have been reported from at least three species: *B. germanica, Byrsotria fumigata,* (Guérin-Méneville) and *Gromphadorhina portentosa* (Schaum) (Barth and Bell 1971, Ross and Cochran 1967, Graves et al. 1986). These gynandromorphic individuals often are split bilaterally in appearance, with one side exhibiting female characters and the other side exhibiting male characters.

The genus *Pycnoscelus*

The genus *Pycnoscelus* consists of a group of cockroaches comprising sixteen species (Beccaloni 2023). The sixteen species are *P. aurantia* (Hanitsch), *P. conferta* (Walker), *P. femapterus* (Roth), *P. gorochovi* (Anisyutkin), *P. indicus* (Fabricius), *P. janetscheki* (Bey-Bienko), *P. micropterus* (Hanitsch), *P. nigra* (Brunner von Wattenwyl), *P. rothi* (Anisyutkin,), *P. rufus* (Bey-Bienko), *P. schwendingeri* (Anisyutkin), *P. semivitreus* (Princis), *P. striatus* (Kirby), *P. surinamensis* (Linnaeus), *P. tenebrigera* (Walker), and *P. vietnamensis* (Anisyutkin,).

Six of the species are in the US pet trade and were obtained for this study: *P. femapterus*, *P. indicus*, *P. nigra*, *P. striatus*, *P. surinamensis*, and *P. tenebrigera*. These six species are primarily distinguished by structural differences in male genitalia, presence and absence of wings, coloration of adults, and wing venation (Roth 1998). For example, *P. femapterus* and *P. tenebrigera* have apterous (wingless) females, whereas the females of other *Pycnoscelus* species have wings. While most species in this genus are restricted to particular countries in the tropics, one species, *P. surinamensis*, can be found across tropical and subtropical regions across the globe (Roth 1967). While *P. surinamensis* is not considered a serious pest, it is often considered a greenhouse pest that damages crops and ornamental plants and can serve as an intermediate host for a chicken eye worm *Oxyspirura mansoni* (Schwabe 1948). This species likely spread through ornamental plant trade as they often hitchhike on or in flowerpots, hence why they are commonly encountered in the greenhouse.

Not much is known about the biology and ecology of *Pycnoscelus* species in nature, but we can infer their lifestyle from captive specimens. All species currently available in culture are fossorial, spending most of their time buried in the substrate and only coming out to the surface to forage. Males of some species with macropterous form tend to be more surface active than nymphs and adult females and will frequently try to fly out of the enclosure, suggesting they likely disperse through flight. In species with macropterous females, females are the ones capable of flight and likely play a role in dispersal. For example, *P. striatus* are commonly found in caves, but occasionally adult females are seen outside caves. In macropterous females of *P. nigra* and *P. surinamensis*, specimens have been observed making short-distance flights in captivity (pers. obs.).

Of the diverse array of *Pycnoscelus* species, only *P. indicus* and *P. surinamensis* are present in various areas worldwide, with *P. surinamensis* having a much broader distribution (Roth 1998, Parker et al. 1977). Two previous hypotheses have been proposed to explain the wide distribution of *P. surinamensis*; its ability to reproduce parthenogenetically allowed the species to colonize different regions, and transportation through anthropogenic activities was responsible for its widespread distribution (Gade and Parker 1977, Parker et al. 1977, Grandcolas et al. 1996). The former hypothesis would explain why *P. surinamensis* is widespread compared to its sexually reproducing sister species, *P. indicus*, but it does not explain why another parthenogenetic species, *P. nigra* is not as widespread as *P. surinamensis*. The latter would not explain why *P. indicus* and *P. nigra* have limited distribution since they are found near human habitation. With regards to the latter hypothesis, there is no indication of high invading abilities in *P. surinamensis* since it was never found far from human dwellings (Grandcolas et al. 1996). However, we have observed *P. surinamensis* in areas with limited anthropogenic influence. In our field observations, we have located large populations of *P. surinamensis* in areas such as the Hidden Lake Environmental Center (Miami-Dade County, Florida, USA - 25.3901, -80.6212) Big Pine Key - Key Deer Preserve (Monroe County, Florida, USA - 24.7058, -81.3802) and Ocala National Forest (Marion County, Florida, USA - 29.4314, -81.7660), which are all less trafficked areas far from human habitation. All these observations beg the question "are other factors involved in explaining the success of *P. surinamensis*?" One possible explanation is that *P. surinamensis* have physical or physiological adaptations that allow them to colonize a wide array of environments unsuitable for other *Pycnoscelus* species. This could be higher tolerance to drier conditions or higher tolerance to lower temperatures. Thus, we decided to investigate the potential physiological advantages that *P. surinamensis* may have through desiccation studies

and thermal sensitivity tests. We hypothesize that the distribution of the cockroaches in this genus is restricted by their desiccation tolerance and ability to tolerate extreme temperatures.

Cockroaches and Water Relations

All organisms, including cockroaches, need water to survive as it is essential for biochemical reactions that are critical for survival (Edney 1977). However, too much or too little water can be detrimental to the survival of the organism. As a result, water availability in the environment and the organism's ability to maintain a balance in its internal water content can influence the distribution of the organisms (Addo-Bediako et al. 2001, Chown et al. 2011). Insects deploy several strategies to overcome water gain or loss. They can control water intake behaviorally by seeking moist areas, moving away from wet areas, or closing their spiracles to reduce water loss from respiration (Benoit et al. 2007, Hetz and Bradley 2005). They can also have physiological adaptations to limit water loss such as having thicker cuticles with waxy (i.e., higher molecular weight cuticular hydrocarbons) layers (Gibbs et al. 1997, Yoder et al. 1997, Bazinet et al. 2010).

There are many ways to measure the water relations between different species. Cuticular permeability has been widely studied in cockroaches to determine the potential physical adaptation to a dry environment (Appel et al. 1983, Appel and Sponsler 1989, Appel 1990). Since the main path of water loss in insects is through the cuticle, cuticular permeability can give insight into how resistant an insect is to desiccation (Appel 1991). In general, insects with lower cuticular permeability live in drier environments when compared to insects in wetter environments (Cohen and Cohen 1981, Appel et al. 1986, Sponsler and Appel 1990). For example, *Arenivaga investigata* from the desert in Southern California has a CP of 12, whereas smokybrown cockroach, *Periplaneta fuliginosa* (Serville), which inhabits subtropical regions,

has a CP of 75 (Cohen and Cohen 1981, Appel et al. 1986, Sponsler and Appel 1991). Therefore, cuticular permeability is a valuable tool when comparing adaptiveness to a dryer environment between different species of cockroaches.

Percent total body water content and water loss rate are also frequently used for determining the desiccation tolerance of cockroaches. In general, insects with a higher percent total body water content tend to live in more hydric environments (Schilman et al. 2005, Zukowski and Su 2019), and insects with a higher water loss rate also tend to live in a wetter environment than those with lower water loss rate (Collins and Richards 1966, Zukowski and Su 2019). However, the percent total body water content might not be a good variable for comparing desiccation tolerance between different species of cockroaches as it can vary depending on the life stage, lifestyle, fat content, and body structure (Edney 1977, Zukowski and Su 2019).

Cockroaches and Thermal Sensitivity

The study of thermal sensitivity in insects has high implications for understanding the ecological and physiological context limiting the distribution of the insects (Appel 1991, Bale et al. 2002, Terblanche et al. 2011). Insects with a wide range of thermal tolerance are likely able to expand into a broader environment (Bale et al. 2002). Therefore, examining the insect's thermal thresholds can give insight into their distribution.

Critical thermal maxima/minima (CT max/min), and upper/lower lethal limit (U/LLL) are important for comparing the thermal tolerance between different species of insects, including cockroaches (Appel 1991, Appel et al. 1983, Hu and Appel 2004, Lighton and Turner 2004). Higher CT max values seem to indicate that the insect is more resilient to high temperatures and

can occupy a broader range of warmer habitats (Castillo-Perez et al. 2022, Roeder et al. 2021). Lower CT min value indicates that the insect can only tolerate and live in cooler environments (Addo-Bediako et al. 2000). Upper and lower lethal limits will likely play the same role as CT max/min.

Animals have a thermal neutral zone; a range of temperature in which the metabolism of the animals stays stable and the cells in their body function normally. Outside of this range, animals will exhibit certain behaviors that are generally not observed. We use these behaviors to record critical thermal temperatures. In the case of *Pycnoscelus,* when exposed to high temperature, they initially show signs of agitation, such as increased leg movements and twitching of their legs. As temperatures increase, the cockroaches will flip on their back or "faint." The temperature at which the cockroaches fainted is recorded as critical thermal maxima (Appel 1991). Once the ambient temperature exceeds the critical thermal temperature, the upper lethal limit may be reached, which will cause the cockroaches' death as the heat denatures the proteins in their cells (Appel 1991). The critical thermal maximum serves as an important measure for heat adaptation because it allows us to measure the temperature at which the insect loses its coordination. In the wild, loss of coordination would lead to the death of the individual as it can no longer escape from the predators or perform biological functions normally; thus, the upper thermal limit restricts its range.

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CHAPTER 2

Comparative water relations of nymphs and adults of seven *Pycnoscelus* spp. (Blattodea: Blaberidae)

Introduction

Water is essential for all organisms as it plays a vital role in biochemical reactions within the organisms (Edney 1977, Denny 2016). However, water can be detrimental to the organisms if too much or too little water is present within the organism. Therefore, water availability in the environment and the organisms' ability to balance their inner water content can influence the distribution of the organisms and the potential spread of insects to various habitats (Addo-Bediako et al. 2001, Chown and Nicolson 2004, Chown et al. 2011).

Insects use several strategies to balance the water content in them. Such strategies include behavioral adaptation such as burrowing deeper into the soil to avoid dry conditions or consuming large quantities of water-rich food, or physiological adaptations such as building up more fat content in the body or having thicker cuticles with waxy layers (Hadley 1994, Gibbs et al. 1997, Yoder et al. 1997, Benoit et al. 2007, Bazinet et al. 2010). Understanding these adaptations can give insight into the distribution patterns of insects.

There are several ways to study desiccation resistance. Measuring cuticular permeability is a widely used method for examining insects' potential physical adaptation to a dry environment (Appel et al. 1983, Appel and Sponsler 1989, Appel 1990). The main pathway of water efflux of cockroaches and most other insects is their cuticle, due to their relatively small size and the large surface area to volume ratio. Some level of resistance to water loss is achieved within certain cockroaches with a waterproofing layer of epicuticular lipids (Appel 1991). The

research results by Appel et al. (1983) and Appel and Sponsler (1989) concluded that there were correlations between cockroach cuticular permeability and cockroach habitat. They showed that cockroaches found in more tropical habitats generally had higher cuticular permeability than those found in more temperate areas (Appel 1991). Other studies support the idea that in general, insects with lower cuticular permeability live in drier environments when compared to insects in wetter environments (Cohen and Cohen 1981, Sponsler and Appel 1990, Appel et al. 1986). For example, *Arenivaga investigata* from the desert in Southern California has a CP of 12, whereas smokybrown cockroach, *Periplaneta fuliginosa* (Serville), which inhabits subtropical regions, has a CP of 75 (Cohen and Cohen 1981, Sponsler and Appel 1991, Appel et al. 1986).

Besides cuticular permeability (CP), percent total body water content (pTBW) and water loss rate have also been shown to help predict the habitat preference of cockroaches (Appel et al. 1983, Appel and Sponsler 1989, Appel 1991). Insects with higher pTBW and higher water loss rates typically live in wetter environments (Collins and Richards 1966, Schilman et al. 2005, Zukowski and Su 2019). However, pTBW might not be a good variable for comparing desiccation tolerance between different species of cockroaches as it can vary depending on the life stage, lifestyle, fat content, and body structure (Edney 1977, Zukowski and Su 2019).

The *Pycnoscelus* is a genus of cockroaches comprised of sixteen species (Beccaloni 2023). While most *Pycnoscelus* spp. are limited to moist tropical environments, the Surinam cockroach, *Pycnoscelus surinamensis* (L.) has been known to spread to drier areas with a more fluctuating environment, and can be found worldwide (Roth 1967, Grandcolas et al. 1996). While *P. surinamensis* is not considered a major agricultural pest, it is known to be a minor pest in greenhouses and plantations as it damages crops and ornamental plants (Roth 1967, 1974,

Zangl et al. 2018), and it is a vector of chicken eye worm, *Oxyspirura mansoni*, which is a serious pest of poultry (Schwabe 1948).

Two previous hypotheses have been proposed to explain the wide distribution of *P. surinamensis*; its ability to reproduce parthenogenetically allowed the species to colonize different regions, and transportation through anthropogenic activities was responsible for its widespread distribution (Gade and Parker 1977, Parker et al. 1977, Grandcolas et al. 1996). The former hypothesis would explain why *P. surinamensis* is widespread compared to its sexually reproducing sister species, *P. indicus*, but it does not explain why another parthenogenetic species, *P. nigra* is not as widespread as *P. surinamensis*. The latter would not explain why *P. indicus* and *P. nigra* have limited distribution since they are found near human habitation.

Since insects' ability to maintain water balance can play critical role in their distribution, we decided to investigate the desiccation resistance of *Pycnoscelus* species. Studying the rate of water loss of *Pycnoscelus* spp. can give a better understanding of why *P. surinamensis* has been the sole species to be able to spread into this large array of habitats, especially the drier environments that specific populations of *P. surinamensis* seem to be able to occupy. Thus, we hypothesize that *P. surinamensis* will have a significantly lower CP, lower pTBW, and lower water loss rate compared to other *Pycnoscelus* species.

Materials and Methods

Study Organisms

Six *Pycnoscelus* species and one unidentified *Pycnoscelus* species were obtained from two sources (Tydyeexotic.com and Roachcrossing.com) (Fig. 1). Unfortunately, the origins of these stocks are unclear for some of the species, but some information was obtainable (Table 1).

Unidentified *Pycnoscelus* species is labeled with its original locality "Thailand" in our study to distinguish it from the other populations. The "Thailand" population produces three variations that share traits of *P. nigra* and *P. surinamensis* and may represent a naturally occurring hybrid between the two species.

Three separate colonies were maintained per species to ensure we have enough specimens for the study. All cultures were maintained inside 1470 cm^3 plastic storage (McCormick Food Storage Round 12 Cup, Dollar Tree, Auburn, Alabama) with moist coconut coir (Burpee Eco-Friendly Natural Organic Garden Coir, Walmart, Auburn, Alabama), provisioned with dry dog chow (Purina, Auburn, Alabama) and kept at (26.7 ± 0.2 °C) with irregular photoperiod.

Water Relations Experiments

To compare the desiccation rates among *Pycnoscelus* species, we used both dead and live specimens for the trials. We compared both live and dead specimens because live specimens can regulate their cuticular permeability and reduce respiratory water loss by holding their breath (Noble-Nesbitt and Al-Shakur 1988, Zukowski and Su 2019). Dead specimens remove all these mechanisms of controlling water loss and just leave comparisons of the waterproofing ability of the cuticle.

For the desiccation of dead specimens, eight specimens of three similar-sized groups (small, medium, and large) were selected from each of the *Pycnoscelus* species. They were placed in 230 cm^3 plastic deli cups with a moist paper towel for 12 hours at 24 ± 2°C, 70 ± 5% RH to remove excessive debris. Specimens were then transferred to 150 cm^3 plastic vials, placed in a glass chamber, and exposed to cyanide gas for 20 minutes before being transferred to the

weighting station. The specimens were weighed individually in aluminum trays using an electronic balance (Model GH-200; A&D Company, LTD, Japan), then they were transferred to numbered aluminum trays that were divided into seven sections, and each species was placed into each section. Once the weighting was complete the trays were placed in a desiccation chamber where the air humidity was maintained close to 0% using Drierite (W.A. Hammond Drierite Co. LTD, Xenia, OH) and the temperature set at 30 °C. Specimens were removed from the chamber every two hours for weighting for 12 consecutive hours; another weight was taken 24 hours after the initial weight. Specimens were then placed into drying oven set at 50 °C and were left there overnight. Two consecutive weights were then taken between 24 and 72 hours after the specimens were placed in the drying oven to get the final weight. This procedure was repeated for different age groups of each species: small, medium, and large nymphs and adult females and males, if present.

Specimens were selected based on their size or their developmental stage. For small nymphs, specimens between 3.0 to 5.5 mm in length were chosen. For medium nymphs, specimens between 7.0 to 8.5 mm were chosen. For large nymphs, specimens between 10.0 to 17.0 mm were chosen. Adults were determined based on the presence of wings for winged species and the sclerotization of subgenital plates for wingless species.

For desiccation of live specimens, we allowed specimens to remain in 0.23L deli cups with moist paper towels for 12 hours at 24 ± 2°C, 70 ± 5% RH to remove dirt before placing them in 0.15L plastic vials with aluminum mesh netting to prevent escape. A battery-powered fan (ELUTENG 40 mm USB Fan) was placed at the lid of the desiccation chamber to allow air to circulate through the mesh-covered vials. Specimens were transferred to separate vials with a

plastic cap for weighing and returned to vials with mesh netting after weighting. Desiccation time and steps followed the same procedure as dead specimens.

Measurement of water loss

Total body water content was calculated as the difference between initial and dry mass multiplied by 100 for each age group and adult gender. The rate of percent total body water loss (%TBW loss) was calculated using this formula:

$\%TBW\ loss = [(M_{initial} - M_{dry}/M_{initial})]/[(M_{initial} - M_{dry})] \times 100$; M = mass in g

Mass loss was calculated following a formula from Benk et al. (2020); Mass loss = mg of H_2O lost (T0 − T2)/gram body weight (T0), where T0 is initial mass and T2 is mass after 2 h of desiccation. It is defined as the mg of water loss between the initial and 2-h masses per initial mass (g) per unit time (h)

CP was calculated using Edney's (1977) formula; $(M_{initial} - M_{2hour})$ per unit surface area (cm^2) per unit time (h) per unit saturation deficit (mmHg); M = mass in μg. Surface area (SA) was estimated using Meeh's formula: SA (cm^2) = 12 initial mass $(g)^{2/3}$. Meeh's formula was used rather than the empirically derived model by Haagsma et al. (1996) because it is more accurate for insects with sphere-like body shapes like *Pycnoscelus* spp. Saturation deficit (SD) was calculated as 31.824 mmHg (30°C, 0% RH).

Desiccation sensitivity

We recorded desiccation sensitivity by recording the time and weight at which the live specimen was unresponsive to a stimulus during the weighting session. Specimens were deemed unresponsive if they showed no leg and antennae movement during the weighting session.

Data analysis

For our experiment, we used a randomized complete block design. For data analysis, we used SAS version 9.3 (SAS Institute Inc. 2013) and SigmaPlot (v13.0, Systat Software, Inc., San Jose, CA). We used univariate procedure in SAS to check for normality ($\alpha = 0.05$) for Body mass, CP value, and %TBW. We used Analyses of variance (ANOVA) to test differences in initial body mass, pTBW, mass loss, and CP values among different species and life stages followed by the Waller-Duncan K-ratio test to determine if the differences among different species and life stages were statistically significant. Linear regression was used to estimate the rate of percentage of initial mass lost and %TBW lost over time. Analysis of covariance (ANCOVA) was used test differences in the rate of %TBW lost over time.

Results

Nymphs

The mean initial body mass of dead specimens ranged from 4.18 to 5.87 mg for small nymphs, 43.21 to 60.28 mg for medium nymphs, and 200.90 to 0.445.05 mg for large nymphs (Table 1). Small *Pycnoscelus femapterus* nymphs weighed significantly less than other species, and small *P. nigra* and *P. surinamensis* nymphs weighed significantly more than other species. Medium *P. tenebrigera* nymphs weighted significantly less than other species, while other species had statistically similar weights. Large *P. femapterus* nymphs weighted significantly less than other species, and large *P.* sp. "Thailand" nymphs weighed significantly more than other species.

For live specimens, the mean initial body mass ranged from 4.19 to 5.50 mg for small nymphs, 63.56 to 79.30 mg for medium nymphs, and 161.69 to 465.61mg for large nymphs (Table 2). Small *Pycnoscelus nigra* and *P. surinamensis* nymphs weighed significantly more

than other *Pycnoscelus* species while others had statistically similar weights. Medium *P. nigra* nymphs weighted significantly less than other species, and medium *P. striatus* and *P. surinamensis* nymphs weighted significantly more than other *Pycnoscelus* species. Large *P. femapterus* nymphs weighted significantly less than other *Pycnoscelus* species and large *P. nigra*, *P. striatus*, and *P.* sp. "Thailand" weighted significantly more than other Pycnoscelus species.

The mean percentage of total body water content (pTBW) of dead specimens ranged from 66.80 to 74.60 % for small nymphs, 59.96 to 72.24% for medium nymphs, and 58.48 to 66.71% for large nymphs (Table 1). Small *P. tenebrigera* nymphs had significantly lower pTBW than other species while small *P. femapterus* and *P. surinamensis* nymphs had significantly higher pTBW than other species. Medium *P. striatus* nymphs had significantly lower pTBW than other species while medium *P. nigra* nymphs had statistically higher pTBW than other species. Large *P. tenebrigera* nymphs had significantly higher pTBW than other *Pycnoscelus* species followed by *P. femapterus* while other species had statistically similar pTBW.

For live specimens pTBW ranged from 71.80 to 81.88 % for small nymphs, 58.60 to 71.91 % for medium nymphs, and 56.37 to 70.11 % for large nymphs (Table 2). Small *Pycnoscelus femapterus* and *P. striatus* nymphs had significantly lower pTBW than other *Pycnoscelus* species while small *P. surinamensis* and *P. nigra* nymphs had significantly higher pTBW than other species. Medium P. striatus had significantly lower pTBW than other species while medium *P. tenebrigera* nymphs had significantly higher pTBW than other species. Large *P. nigra* and *P. striatus* nymphs had significantly lower pTBW than other species while large *P. femapterus* nymphs had significantly higher pTBW than other species. In dead specimens, all but *P. tenebrigera* had pTBW decrease as nymph size increased. In dead *P. tenebrigera*, medium

nymphs had higher pTBW than small nymphs. In live specimens, all species had pTBW decrease as nymph size increased except for *P*. sp. "Thailand". The pTBW of medium nymphs of *P*. sp. "Thailand" was lower than pTBW of large nymph. *P. striatus* consistently showed the lowest pTBW in all nymphal stages for both live and dead specimens except for small dead nymphs. *P. tenebrigera* and *P. femapterus* showed the highest pTBW for all nymphal stages except for dead and live small nymphs and dead medium nymphs. *P. surinamensis*, our species of interest, was always close to the middle range for all stages regardless of condition (dead or live).

The mean cuticular permeability (CP) for dead specimens ranged from 15.25 to 40.25, 19.86 to 37.14, and 39.18 to 26.74 μg cm−1 h−1 mmHg−1 for small, medium, and large nymphs, respectively (Table 1). Small *P. tenebrigera* nymphs had significantly lower CP than other species while small *P. striatus* and *P. surinamensis* nymphs had significantly higher CP than other species. Medium and large *P. striatus* nymphs had significantly higher CP than other species while all the other species had statistically similar CP.

Mean CP for live specimens ranged from 23.96 to 39.52, 18.99 to 31.05, and 18.46 to 33.35 for small nymphs, medium nymphs, and large nymphs, respectively (Table 2). Small *P. striatus* and *P. femapterus* nymphs had significantly lower CP than other species while small *P*. sp. "Thailand" had significantly higher CP than other species. Medium *P. indicus* had significantly higher CP than other species while other species had statistically similar CP. Large *P*. sp. "Thailand" nymphs had significantly higher CP than other species while other species had statistically similar CP. In dead specimens, *P. striatus* consistently showed the significantly highest CP of all species throughout different nymph size. However, in live specimens, species with the highest CP values varied between different nymph sizes. For the lowest CP values of

both live and dead specimens, *P. tenebrigera* and *P. femapterus* mostly had lower values than other species, but the differences were often statistically insignificant.

The mean mass loss for dead specimens ranged from 66.59 to 181.10, 40.53 to 77.83, and 24.40 to 40.43 mg/g for small, medium, and large nymphs (Table 1). Small *P. tenebrigera* nymphs had significantly lower mass loss than other species while small *P. striatus* and *P. surinamensis* nymphs had significantly higher mass loss than other species. Medium *P. striatus* nymphs had significantly higher mass loss than other species while other species had statistically similar mass loss. Large *P. striatus* nymphs had significantly higher mass loss than other species followed by large *P. fempaterus* nymphs while other species had statistically similar mass loss.

For live specimens, the mean mass loss ranged from 109.22 to 173.48, 34.16 to 57.87, and 18.38 to 33.24 mg/g for small, medium, and large nymphs (Table 2). Small *P*. sp. "Thailand" had significantly higher mass loss than other species followed by *P. surinamensis* while *P. femapterus* had significantly lower mass loss than other species. Medium *P. indicus* had significantly higher mass loss than other species while other species had statistically similar mass loss. Large *P. striatus* had significantly lower mass loss while *P*. sp. "Thailand" had significantly higher mass loss than other species. *P. striatus* had the most mass loss in all nymph sizes for dead specimens, *P. tenebrigera* had the least mass loss in all nymph sizes except for large nymphs, in which *P. surinamensis* showed the least mass loss. Live specimens showed too much variability throughout different nymph sizes to compare to dead specimens. However, both live and dead nymphs had a negative correlation between mass loss and body size for each species.

Mean water loss rates were not significantly different between species for each nymph size (Tables 3 and 4). The water loss rate increased as nymph size decreased for each species.

Mass loss and %TBW loss of all nymphal sizes of all species tested increased linearly with desiccation time (Figs. 2-5).

Adults

The mean initial body mass of dead specimens ranged from 98.35 to 163.36 mg for adult males, and 238.02 to 382.95 mg for adult females (Table 1). Male *P. fempterus* had significantly lower mass than other species while male *P. striatus and P. indicus* had significantly higher mass. Female *P. fempterus* had significantly lower mass than other species while female *P. nigra* and *P.* sp. "Thailand" had significantly higher mass than other species.

For live specimens, the mean initial body mass ranged from 82.51 to 160.63 mg for adult males, and 182.01 to 330.28 mg for adult females (Table 2). Male *P. fempterus* had significantly lower mass than other species while male *P. striatus* and *P. indicus* had significantly higher mass than other species. Female *P. fempterus* had significantly lower mass than other species while other species had statistically similar mass.

The mean percentage total body water (pTBW) content for dead specimens ranged from 65.58 to 69.65 % for adult males, and 61.89 to 67.04 % for adult females (Table 1). Male *P. indicus* had significantly higher pTBW followed by *P. tenebrigera* while others had statistically similar pTBW. Female *P.* sp. "Thailand" had significantly lower pTBW than other species while *P. indicus* and *P. striatus* had significantly higher pTBW than other species.

In live specimens, pTBW ranged from 62.71 to 70.51% for adult males, and 68.90 to 71.17 % for adult females. Male *P. striatus* had significantly lower pTBW than other *Pycnoscelus* species while male *P. fempterus* had significantly higher pTBW than other species. All female *Pycnoscelus* species had statistically similar pTBW.

The mean cuticular permeability (CP) for dead specimens ranged from 25.51 to 91.98 and 14.52 to 50.83 μg cm^{-1} h^{-1} $mmHg^{-1}$ for adult males and adult females, respectively (Table 1). Male *P. tenebrigera* had significantly lower CP than other species, while male *P. striatus* had significantly higher CP than other species. Female *P. tenebrigera* had significantly lower CP than other species, while female *P. striatus* had significantly higher CP than other species.

For live specimens, the CP ranged from 17.78 to 30.02 and 15.49 to 46.27 μg cm^{-1} h^{-1} $mmHg^{-1}$ for adult males and adult females, respectively (Table 2). Male *P. tenebrigera* had significantly lower CP than other species while male *P. striatus* had significantly higher CP than other species. Female *P. tenebrigera*, *P. femapterus*, and *P. indicus* had significantly lower CP than other species while female *P. striatus* had significantly higher CP than other species.

The mean mass loss for dead specimens ranged from 38.71 to 129.88 mg/g for adult males and 16.48 to 59.72 mg/g for adult females. Male *P. tenebrigera* had significantly lower mass loss than other species, while male P. striatus had significantly higher mass loss than other species. Female *P. tenebrigera* had significantly lower mass loss than other species, while P. striatus females had significantly higher mass loss than other species.

For live specimens, the mean mass loss ranged from 26.24 to 44.00 mg/g for adult males and 17.57 to 53.16 for adult females. Male *P. tenebrigera* had significantly lower mass loss than other species, while male *P. femapterus* had significantly higher mass loss than other species. Female *P. tenebrigera* had significantly lower mass loss than other species, while female *P. striatus* had significantly higher mass loss than other species.

The mean water loss rates were not significantly different between species for adult males and females (Table 3,4). Mass loss and %TBW loss of all adults of all species tested increased linearly with desiccation time (Figs. 2-5).

We could not get accurate desiccation tolerance data for adults and most nymph sizes as only small nymphs died within the 12-hour period to give us accurate death time. Of the small nymphs, five species showed mortality within a 12-hour period: *P. femapterus*, *P. indicus*, *P. striatus*, *P. tenebrigera*, and *P.* sp. "Thailand".

Discussion

Pycnoscelus surinamensis is a highly successful colonizer found in almost every continent. Unlike its relatives restricted to tropical environments, *P. surinamensis* have been found in subtropical and relatively dry environments. Since this species is found in drier environment we hypothesized it to be better adapted to drier condition than other species in this genus. To compare the desiccation tolerance of this species with its relatives we measured CP, water loss rate, and pTBW. CP and water loss rate are commonly used for measuring desiccation tolerance since studies show that both are often correlated to the environment that the insect lives in; insects with higher CP and lower water loss rate often live in mesic environments while insects with lower CP and higher water loss rate live in xeric environment (Edney 1977, Hadley 1994).

Unlike what we predicted, *P. surinamensis* did not show a significant difference in CP, water loss rate, and pTBW compared to most species of *Pycnoscelus* we used in the study. Our result indicates that *P. surinamensis* has no apparent significant advantage in desiccation tolerance compared to most species of *Pycnoscelus* we used in the study. *P. striatus* had a significantly greater CP at all life stages for dead specimens (Table 1). However, *P. striatus* showed the highest CP for most stages of live specimens (Table 2). Other species showed similar but varied CP levels across the life stage. The %TBW varied across some species throughout the life stage, but most species showed no significant differences in %TBW compared to *P.*

surinamensis except in small nymphs. The water loss rate was similar across the taxa except for *P. striatus*, which had a significantly higher water loss rate than other species throughout the life stage for dead specimens.

CP data show that *P. surinamensis* has similar CP to most of the tested species with small nymphs having the highest CP. While most species had similar CP value for dead specimens, *P. striatus* showed significantly higher CP and *P. tenebrigera* showed significantly lower CP than other species through most stages. Edney (1977) lists CP of several arthropod species showing that insects in xeric environments usually have CP below 40 μg cm^{-2} h^{-1} $mmHg^{-1}$ and insects in mesic environment have CP above 50 μg cm^{-2} h^{-1} $mmHg^{-1}$. Most of the *Pycnoscelus* species used in the study fall under the xeric category despite being found in tropical regions, with *P. striatus* falling in the mesic category. It is also important to note that *P. tenebrigera* has the lowest CP value of 14.52 as adult female, which is comparable to a desert sand cockroach, *Arenivaga investigata* (CP = 12.1, Edney 1977). This species was collected from Pakistan, a country with warm and dry climate throughout most of its region (World Koppen-Geiger Climate classification). Therefore, we can predict that *P. tenebrigera* are found in drier habitat than other *Pycnoscelus* species.

Pycnoscelus striatus demonstrated significantly greater CP than other species for dead specimens. This species can be found inside Batu cave, where they live in layers of fruit bat guanos in the wild (Lucañas and Lit 2016). Batu cave has multiple seepages that allow water to flow into the cave, so it stays wet during the rainy season (McClure et al. 1967). Perhaps *P. striatus* has higher CP values than other species because it lives in a damp environment.

One possible explanation for the CP and water loss rate being similar between different *Pycnoscelus* species is their lifestyle. All life stages of *Pycnoscelus* species used in this study spend most of their lives buried in substrate unless they are foraging at night when it is more humid and cooler than the daytime (pers. obs.). Burrowing invertebrates often avoid desiccation through vertical migration in the soil to seek a moist spot to stay in (Cloudsley-Thompson 1975, Crawford and Cloudsley Thompson 1971, Holsinger and Dickson 1977). Therefore, they might not be subjected to environmental pressure by the dry environments, instead, they have a behavioral mechanism for dealing with desiccating conditions. A variety of other invertebrates have developed this behavioral adaptation with a response to a more hostile environment, such as various desert wood louse, scorpions, entomopathogenic nematodes and amphipods (Salame and Glazer 2015, Somme 1995, Holsinger and Dickson 1977). With this specific behavior selected for a wide array of invertebrates, it is reasonable to believe that it will also be selected for soil-dwelling cockroach species.

pTBW for *Pycnoscelus* showed significant differences between species that were inconsistent between different life stages. The range of pTBW was from 56 to 82%. Edney's table of pTBW between various arthropods shows that most arthropods have pTBW value from low 50s to high 80s (Edney 1977), which agrees with our data. High variation and inconsistency in % pTBW might be explained by the nature of the pTBW. While it is sometimes correlated with the environment the organism lives in, multiple studies show that pTBW is not a reliable trait to use for estimating the desiccation tolerance of the organisms as it can vary depending on the life stage, lifestyle, fat content, and body structure of the organism (Edney 1977, Zukowski and Su 2019).

While we cannot conclude that *P. surinamensis* is more desiccation tolerant than other *Pycnoscelus* species, our results open an array of questions that can be tested further; perhaps *P. surinamensis* are more successful because they can tolerate a broader range of temperatures than other *Pycnoscelus* species, or maybe this species has higher fecundity than other species. We plan to pursue these questions further through a series of experiments.

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Table 1. Profiles of *Pycnoscelus* cultures used in this study. X indicates the absence of males.

Species	**Origin**	**Habitat**	**Reproduction**	**Adult size (mm) (Female)**	**Adult size (mm) (Male)**
P. femapterus	Thailand	Under leaf litter	Sexual	11 – 17	10 – 13
P. indicus	Thailand	N/A	Sexual	18 – 25	18 – 23
P. nigra	Thailand	Under debris near heavily populated area	Parthenogenetic	18 – 28	X
P. surinamensis	Montgomery, AL, USA	In flower pot	Parthenogenetic	18 – 25	X
P. striatus	Borneo	In cave	Sexual	20 – 28	18 – 23
P. tenebrigera	Pakistan	In cave	Sexual	15 – 22	15 – 20
P. sp. "Thailand"	Thailand	Under debris near heavily populated area	Parthenogenetic	18 – 25	X

Table 2. Mean (±SE) initial fresh body mass, % total body water (%TBW), cuticular permeability (CP; μg cm^{-2} h^{-1} $mmHg^{-1}$and mass loss (mg/g) of seven dead *Pycnoscelus* species.

Species	Stages	Initial mass (mg)	%TBW	CP value	Mass loss (mg/g)
P. femapterus	Adult female	238.02 ± 15.88 c	64.64 ± 0.95 abc	32.10 ± 2.28 b	40.13 ± 3.26 b
P. indicus	Adult female	301.18 ± 10.00 b	67.00 ± 0.93 a	32.65 ± 4.16 b	37.51 ± 1.44 bc
P. nigra	Adult female	365.67 ± 7.16 a	65.39 ± 0.43 ab	26.36 ± 1.26 b	28.21 ± 1.44 bcd
P. striatus	Adult female	308.46 ± 22.24 b	67.04 ± 1.08 a	50.83 ± 7.12 a	59.72 ± 10.10 a
P. surinamensis	Adult female	276.15 ± 16.97 bc	63.78 ± 1.22 bc	28.70 ± 2.41 b	34.09 ± 3.07 bc
P. tenebrigera	Adult female	315.82 ± 20.42 b	66.74 ± 1.02 ab	14.52 ± 1.00 c	16.48 ± 1.26 d
P. sp. *"Thailand"*	Adult female	382.95 ± 15.36 a	61.89 ± 1.14 c	25.44 ± 1.34 b	26.86 ± 1.49 cd
P. femapterus	Adult male	98.35 ± 3.82 c	66.62 ± 0.42 b	43.02 ± 5.99 b	71.19 ± 9.57 b
P. indicus	Adult male	163.36 ± 8.10 a	69.65 ± 1.43 a	36.81 ± 1.99 bc	51.59 ± 2.75 bc
P. striatus	Adult male	158.46 ± 4.37 a	65.58 ± 0.60 b	91.98 ± 8.58 a	129.88 ± 11.95 a
P. tenebrigera	Adult male	130.99 ± 6.09 b	67.32 ± 0.44 ab	25.51 ± 1.90 c	38.71 ± 3.17 c
P. femapterus	Large nymph	200.90 ± 10.80 c	64.03 ± 0.81 b	26.43 ± 2.19 b	35.13 ± 3.67 ab
P. indicus	Large nymph	371.71 ± 15.96 b	58.48 ± 0.99 c	26.74 ± 1.80 b	28.49 ± 1.94 bc
P. nigra	Large nymph	387.87 ± 20.03 b	59.10 ± 0.43 c	25.39 ± 1.91 b	26.82 ± 2.29 c
P. striatus	Large nymph	412.40 ± 20.91 ab	58.39 ± 0.64 c	39.18 ± 3.75 a	40.43 ± 4.03 a
P. surinamensis	Large nymph	402.38 ± 22.19 ab	59.26 ± 1.02 c	23.41 ± 0.80 b	24.40 ± 1.06 c
P. tenebrigera	Large nymph	226.35 ± 10.67 c	66.71 ± 1.17 a	23.13 ± 1.70 b	29.134 ± 2.23 bc
P. sp. *"Thailand"*	Large nymph	445.05 ± 15.39 a	59.32 ± 0.58 c	26.25 ± 2.37 b	26.44 ± 2.59 c
P. femapterus	Medium nymph	54.02 ± 2.78 a	70.59 ± 1.02 ab	19.86 ± 1.00 b	40.53 ± 2.49 b
P. indicus	Medium nymph	51.55 ± 4.16 ab	68.06 ± 0.75 b	22.51 ± 1.61 b	46.80 ± 3.71 b
P. nigra	Medium nymph	56.56 ± 3.36 a	72.24 ± 1.51 a	23.71 ± 2.28 b	47.48 ± 4.61 b
P. striatus	Medium nymph	49.94 ± 2.04 ab	59.96 ± 1.66 d	37.14 ± 5.80 a	77.83 ± 12.80 a
P. surinamensis	Medium nymph	55.31 ± 4.87 a	69.44 ± 0.88 ab	23.28 ± 1.18 b	47.09 ± 2.33 b
P. tenebrigera	Medium nymph	43.21 ± 2.81 b	70.68 ± 1.13 ab	20.23 ± 1.85 b	44.55 ± 4.32 b
P. sp. *"Thailand"*	Medium nymph	60.28 ± 2.21 a	64.30 ± 0.96 c	24.67 ± 1.04 b	48.23 ± 2.25 b
P. femapterus	Small nymph	4.23 ± 0.12 c	74.27 ± 1.19 a	24.04 ± 1.10 b	113.54 ± 5.04 b
P. indicus	Small nymph	4.18 ± 0.19 c	70.46 ± 0.62 c	24.06 ± 1.38 b	114.40 ± 6.70 b
P. nigra	Small nymph	5.87 ± 0.24 a	73.33 ± 0.77 ab	27.60 ± 2.52 b	118.36 ± 12.23 b

P. striatus	Small nymph	4.99 ± 0.26 b	69.61 ± 1.36 c	40.25 ± 6.37 a	181.10 ± 28.63 a
P. surinamensis	Small nymph	5.69 ± 0.20 a	74.60 ± 1.17 a	39.01 ± 0.87 a	167.49 ± 4.69 a
P. tenebrigera	Small nymph	5.39 ± 0.15 ab	66.80 ± 0.47 d	15.25 ± 1.63 c	66.59 ± 7.18 c
P. sp. *"Thailand"*	Small nymph	5.11 ± 0.19 b	71.44 ± 0.61 bc	25.25 ± 1.71 b	112.80 ± 8.44 b

*Mean values within each column followed by the same letter are not significantly different according to the Waller–Duncan K-ratio mean separation test (P > 0.05).

Table 3. Mean (±SE) initial fresh body mass, % total body water (%TBW), cuticular permeability (CP; μg cm^{-2} h^{-1} $mmHg^{-1}$), and mass loss (mg/g) of seven live *Pycnoscelus* species.

Species	Stages	Initial mass (mg)	%TBW	CP value	Mass loss (mg/g)
P. femapterus	Adult female	182.01 ± 6.36 b	69.30 ± 1.20 a	17.07 ± 2.06 c	23.10 ± 2.85 cd
P. indicus	Adult female	330.28 ± 25.48 a	68.90 ± 1.20 a	17.41 ± 2.21 c	19.46 ± 2.61 d
P. nigra	Adult female	294.38 ± 21.47 a	68.90 ± 1.03 a	26.92 ± 1.03 b	31.32 ± 3.94 bc
P. striatus	Adult female	293.88 ± 16.12 a	71.17 ± 1.08 a	46.27 ± 3.15 a	53.16 ± 3.23 a
P. surinamensis	Adult female	276.00 ± 23.69 a	69.56 ± 0.39 a	27.41 ± 4.97 b	32.70 ± 6.29 bc
P. tenebrigera	Adult female	314.15 ± 20.26 a	70.63 ± 2.02 a	15.49 ± 1.12 c	17.57 ± 1.42 d
P. sp. *"Thailand"*	Adult female	311.70 ± 21.83 a	68.58 ± 1.52 a	33.36 ± 4.80 b	37.87 ± 5.48 b
P. femapterus	Adult male	82.51 ± 2.99 c	70.51 ± 0.39 a	25.21 ± 5.00 ab	44.00 ± 8.26 a
P. indicus	Adult male	153.88 ± 4.51 a	66.90 ± 0.63 b	23.03 ± 3.09 ab	32.78 ± 4.27 ab
P. striatus	Adult male	160.63 ± 2.41 a	62.71 ± 1.64 c	30.02 ± 3.45 a	41.98 ± 4.53 ab
P. tenebrigera	Adult male	140.66 ± 1.94 b	67.73 ± 0.51 b	17.78 ± 1.10 b	26.24 ± 1.81 b
P. femapterus	Large nymph	161.69 ± 6.50 d	70.11 ± 1.12 a	18.58 ± 1.89 b	26.11 ± 2.60 ab
P. indicus	Large nymph	316.88 ± 5.97 b	62.02 ± 1.42 b	21.86 ± 2.87 b	24.52 ± 3.26 ab
P. nigra	Large nymph	439.51 ± 25.49 a	58.10 ± 0.97 c	24.78 ± 2.04 b	24.98 ± 2.04 ab
P. striatus	Large nymph	460.89 ± 19.29 a	56.37 ± 0.60 c	18.54 ± 2.01 b	18.38 ± 2.00 b
P. surinamensis	Large nymph	336.23 ± 9.10 b	63.38 ± 1.30 b	22.70 ± 3.38 b	25.11 ± 3.88 ab
P. tenebrigera	Large nymph	219.75 ± 7.91 c	64.72 ± 1.61 b	18.46 ± 0.59 b	23.42 ± 0.81 ab
P. sp. *"Thailand"*	Large nymph	465.61 ± 23.25 a	62.16 ± 1.34 b	33.35 ± 4.25 a	33.24 ± 4.31a
P. femapterus	Medium nymph	65.11 ± 2.65 bc	70.93 ± 1.24 ab	21.26 ± 1.90 b	40.63 ± 3.92 b
P. indicus	Medium nymph	72.08 ± 3.93 abc	67.53 ± 1.13 c	31.05 ± 4.08 a	57.87 ± 8.18 a
P. nigra	Medium nymph	63.56 ± 1.71 c	69.01 ± 0.94 bc	22.78 ± 1.63 b	43.61 ± 3.05 ab
P. striatus	Medium nymph	79.30 ± 4.21 a	58.60 ± 1.45 d	21.93 ± 1.16 b	39.05 ± 1.97 b
P. surinamensis	Medium nymph	77.65 ± 4.61 a	69.38 ± 0.85 abc	25.36 ± 2.30 ab	46.06 ± 4.88 ab
P. tenebrigera	Medium nymph	67.51 ± 5.47 abc	71.91 ± 0.69 a	25.33 ± 2.25 ab	48.20 ± 4.51 ab
P. sp. *"Thailand"*	Medium nymph	76.56 ± 3.15 ab	60.82 ± 0.62 d	18.99 ± 1.11 b	34.16 ± 1.86 b
P. femapterus	Small nymph	4.74 ± 0.15 bc	72.43 ± 1.33 d	23.96 ± 2.53 c	109.22 ± 11.59 c
P. indicus	Small nymph	4.27 ± 0.47 c	74.54 ± 1.72 cd	27.95 ± 2.14 bc	132.05 ± 6.91 bc

P. nigra	Small nymph	5.50 ± 0.14 a	76.39 ± 1.14 a	27.96 ± 2.69 bc	120.80 ± 11.04 bc
P. striatus	Small nymph	4.62 ± 0.23 c	71.80 ± 1.42 d	25.54 ± 2.49 c	117.31 ± 11.08 bc
P. surinamensis	Small nymph	5.50 ± 0.18 a	81.88 ± 0.59 a	33.38 ± 1.83 ab	144.79 ± 8.42 ab
P. tenebrigera	Small nymph	4.19 ± 0.17 c	78.77 ± 0.84 ab	28.27 ± 2.80 bc	134.65 ± 13.96 bc
P. sp. *"Thailand"*	Small nymph	5.30 ± 0.14 ab	77.06 ± 0.60 bc	39.52 ± 2.73 a	173.48 ± 12.37 a

*Mean values within each column followed by the same letter are not significantly different according to the Waller–Duncan K-ratio mean separation test ($P > 0.05$).

Table 4. Regression statistics of % mass loss of seven dead *Pycnoscelu*s species at 30°C ± 0–2% RH (means ± SE), n = 8.

Species	Stages	Slope	Intercept	R^2	F	p
P. femapterus	Small nymph	2.62 ± 0.30	8.25 ± 3.20	0.94	76.17	0.0003
	Medium nymph	1.38 ± 0.03	0.89 ± 0.28	1.00	2852.73	<0.0001
	Large nymph	1.04 ± 0.03	0.91 ± 0.29	1.00	1493.22	<0.0001
	Adult male	1.49 ± 0.13	4.19 ± 1.36	0.97	137.38	<0.0001
	Adult female	1.11 ± 0.03	1.06 ± 0.35	0.996	1182.57	<0.0001
P. indicus	Small nymph	2.47 ± 0.29	8.30 ± 3.03	0.94	75.21	0.0003
	Medium nymph	1.48 ± 0.05	1.56 ± 0.49	1.00	1022.63	<0.0001
	Large nymph	0.79 ± 0.03	1.04 ± 0.31	0.99	750.96	<0.0001
	Adult male	1.27 ± 0.08	2.46 ± 0.80	0.98	291.85	<0.0001
	Adult female	0.82 ± 0.04	1.57 ± 0.46	0.99	359.18	<0.0001
P. nigra	Small nymph	2.56 ± 0.29	8.31 ± 3.05	0.94	80.53	0. 0003
	Medium nymph	1.57 ± 0.04	1.33 ± 0.41	1.00	1692.66	<0.0001
	Large nymph	0.83 ± 0.02	0.68 ± 0.21	1.00	1723.30	<0.0001
	Adult female	0.70 ± 0.03	0.99 ± 0.29	0.99	662.23	<0.0001
P. striatus	Small nymph	2.54 ± 0.47	14.56 ± 5.01	0.85	29.14	0.0029
	Medium nymph	1.92 ± 0.12	3.89 ± 1.29	0.98	253.25	<0.0001
	Large nymph	1.05 ± 0.04	1.50 ± 0.44	0.99	646.53	<0.0001
	Adult male	1.78 ± 0.28	9.42 ± 3.00	0.89	39.70	0.0015
	Adult female	1.34 ± 0.10	3.22 ± 1.03	0.98	190.89	<0.0001
P. surinamensis	Small nymph	2.74 ± 0.51	14.30 ± 5.39	0.85	29.33	0.0029
	Medium nymph	1.55 ± 0.03	1.20 ± 0.35	1.00	2185.92	<0.0001
	Large nymph	0.77 ± 0.02	0.61 ± 0.18	1.00	2032.70	<0.0001
	Adult female	0.90 ± 0.03	1.07 ± 0.32	0.99	893.88	<0.0001
P. tenebrigera	Small nymph	2.09 ± 0.12	3.19 ± 1.24	0.99	322.08	<0.0001
	Medium nymph	1.58 ± 0.03	1.02 ± 0.31	1.00	3022.97	<0.0001
	Large nymph	1.02 ± 0.02	0.60 ± 0.20	1.00	2822.97	<0.0001
	Adult male	1.23 ± 0.04	1.30 ± 0.41	1.00	1023.87	<0.0001

	Adult female	0.60 ± 0.01	0.35 ± 0.11	1.00	3230.76	<0.0001
P. sp. "Thailand"	Small nymph	2.41 ± 0.26	7.75 ± 2.74	0.95	88.23	0.0002
	Medium nymph	1.54 ± 0.04	1.29 ± 0.38	1.00	1876.54	<0.0001
	Large nymph	0.76 ± 0.02	0.80 ± 0.24	1.00	1186.65	<0.0001
	Adult female	0.74 ± 0.02	0.79 ± 0.24	1.00	1135.40	<0.0001

*Mean values within each column followed by the same letter are not significantly different according to the Waller–Duncan K-ratio mean separation test ($P > 0.05$).

Table 5. Regression statistics of % mass loss of seven live *Pycnoscelu*s species at 30°C ± 0–2% RH (means ± SE), n = 8.

Species	Stages	Slope	Intercept	R^2	F	p
P. femapterus	Small nymph	2.83 ± 0.25	6.97 ± 2.67	0.962	127.86	<0.0001
	Medium nymph	1.35 ± 0.04	1.34 ± 0.43	0.996	1136.70	<0.0001
	Large nymph	0.87 ± 0.02	0.60 ± 0.18	0.998	2632.52	<0.0001
	Adult male	1.64 ± 0.03	0.76 ± 0.32	0.998	3071.12	<0.0001
	Adult female	0.74 ± 0.02	0.65 ± 0.19	0.997	1705.57	<0.0001
P. indicus	Small nymph	2.89 ± 0.31	8.89 ± 3.30	0.946	86.82	<0.0001
	Medium nymph	1.38 ± 0.11	3.34 ± 1.13	0.972	171.28	<0.0001
	Large nymph	0.80 ± 0.04	1.06 ± 0.41	0.989	432.47	<0.0001
	Adult male	1.02 ± 0.04	1.17 ± 0.38	0.994	833.92	<0.0001
	Adult female	0.51 ± 0.03	0.92 ± 0.30	0.986	341.70	<0.0001
P. nigra	Small nymph	2.80 ± 0.21	6.75 ± 2.26	0.972	174.17	<0.0001
	Medium nymph	1.14 ± 0.08	2.57 ± 0.90	0.974	183.34	<0.0001
	Large nymph	0.67 ± 0.03	1.00 ± 0.30	0.991	570.67	<0.0001
	Adult female	0.79 ± 0.04	1.31 ± 0.39	0.989	455.36	<0.0001
P. striatus	Small nymph	2.80 ± 0.24	6.95 ± 2.53	0.965	138.65	<0.0001
	Medium nymph	1.30 ± 0.05	1.51 ± 0.52	0.993	724.35	<0.0001
	Large nymph	0.57 ± 0.01	0.47 ± 0.14	0.997	1900.83	<0.0001
	Adult male	1.53 ± 0.08	1.76 ± 0.88	0.986	339.37	<0.0001
	Adult female	1.29 ± 0.05	1.56 ± 0.53	0.993	683.83	<0.0001
P. surinamensis	Small nymph	3.16 ± 0.30	9.21 ± 3.21	0.957	110.31	<0.0001
	Medium nymph	1.11 ± 0.07	2.35 ± 0.74	0.981	251.30	<0.0001
	Large nymph	0.86 ± 0.03	0.89 ± 0.31	0.994	888.41	<0.0001
	Adult female	0.81 ± 0.03	1.09 ± 0.34	0.992	639.12	<0.0001
P. tenebrigera	Small nymph	3.09 ± 0.35	9.39 ± 3.71	0.940	78.73	0.0003
	Medium nymph	1.61 ± 0.04	1.28 ± 0.39	0.997	1949.91	<0.0001
	Large nymph	0.73 ± 0.02	0.79 ± 0.25	0.995	1000.37	<0.0001
	Adult male	1.20 ± 0.03	-0.15 ± 0.27	0.998	2317.03	<0.0001

	Adult female	0.53 ± 0.02	0.51 ± 0.16	0.996	1292.61	<0.0001
P. sp. "Thailand"	Small nymph	2.89 ± 0.42	12.80 ± 4.49	0.904	47.11	<0.0001
	Medium nymph	1.25 ± 0.02	0.63 ± 0.19	1.000	4705.21	<0.0001
	Large nymph	0.82 ± 0.05	1.71 ± 0.57	0.979	230.40	<0.0001
	Adult female	0.75 ± 0.06	2.18 ± 0.69	0.965	135.98	<0.0001

*Mean values within each column followed by the same letter are not significantly different according to the Waller–Duncan K-ratio mean separation test ($P > 0.05$).

Fig. 1. Seven *Pycnoscelus* species used in the desiccation trial. All the specimens represented in the figure are adults. For species with males, males are placed on the left, and females are placed on the right.

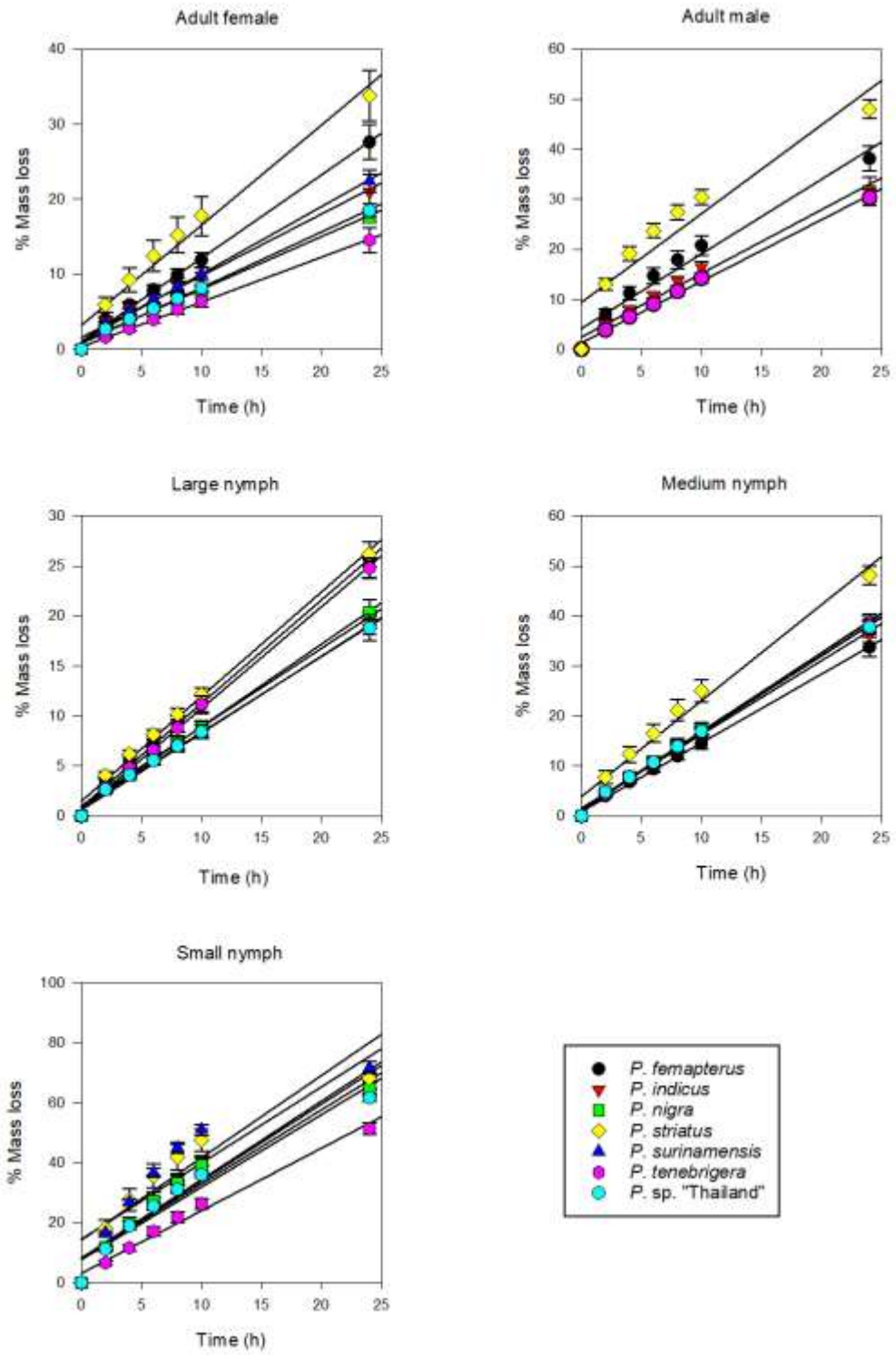

Fig. 2. Percentage of body mass loss over time of seven dead *Pycnoscelus* species.

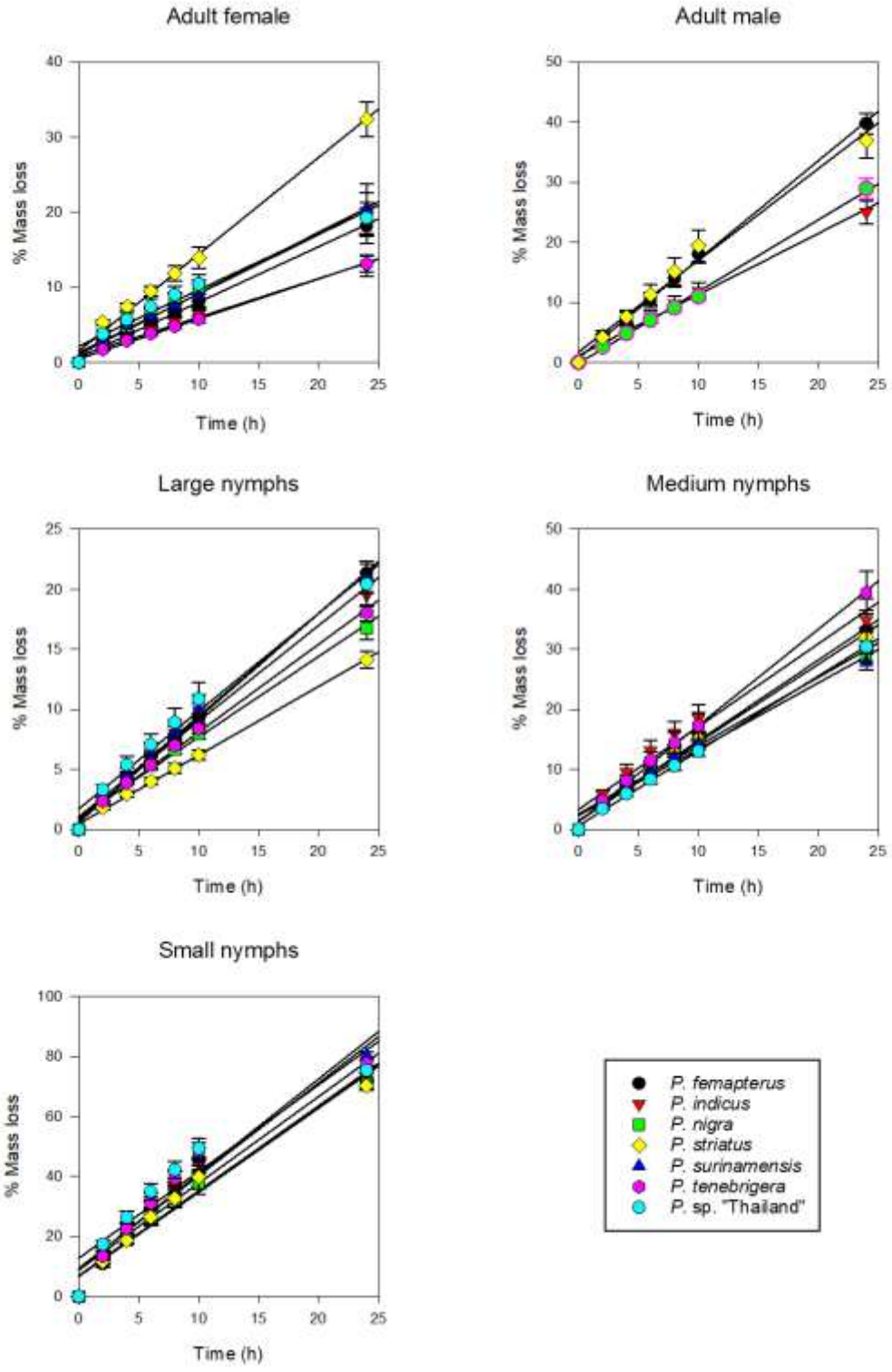

Fig. 3. Percentage of body mass loss over time for seven live *Pycnoscelus* species.

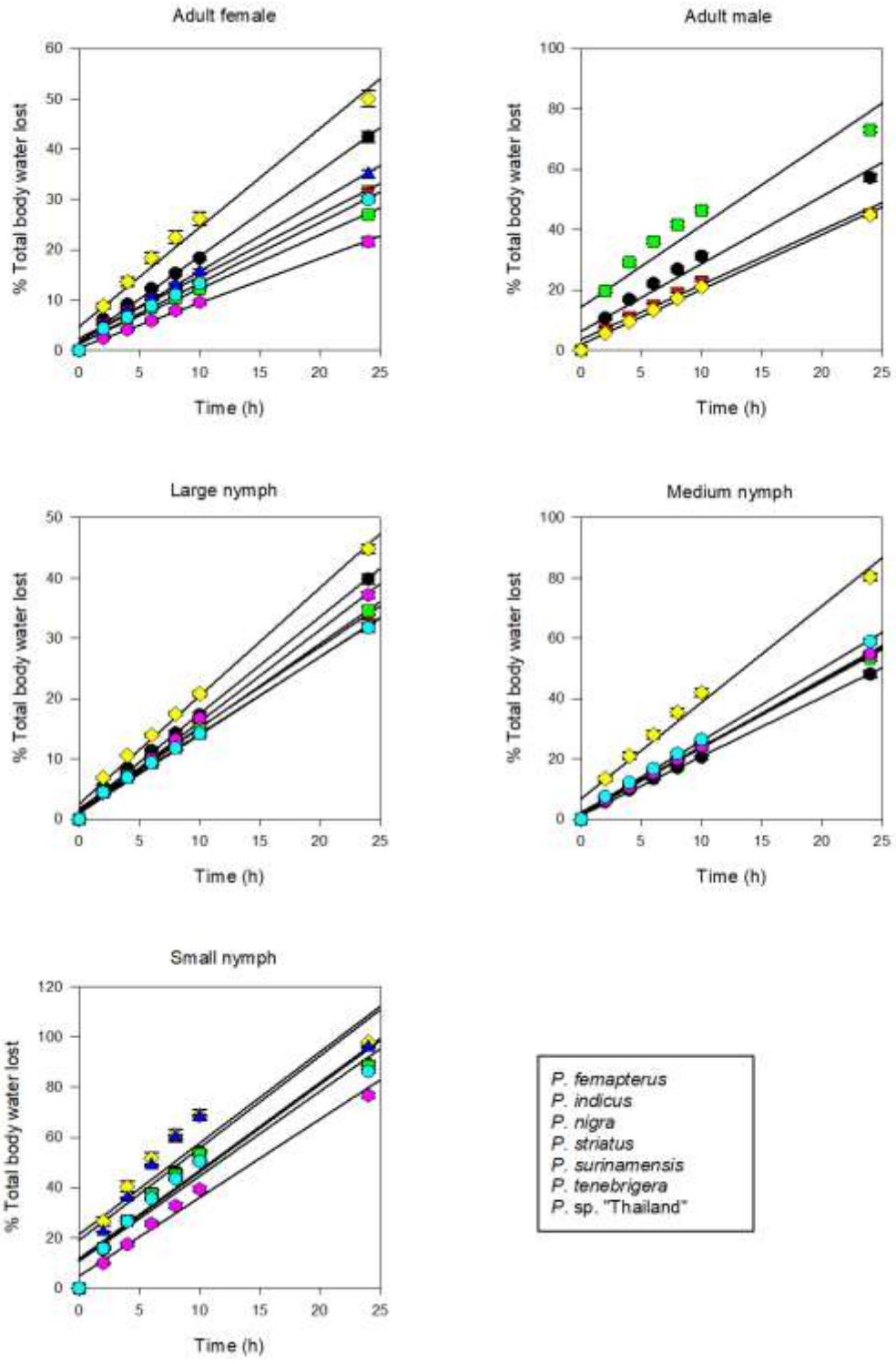

Fig. 4. Percentage total body water (%TBW) lost over time for seven dead *Pycnoscelus* species.

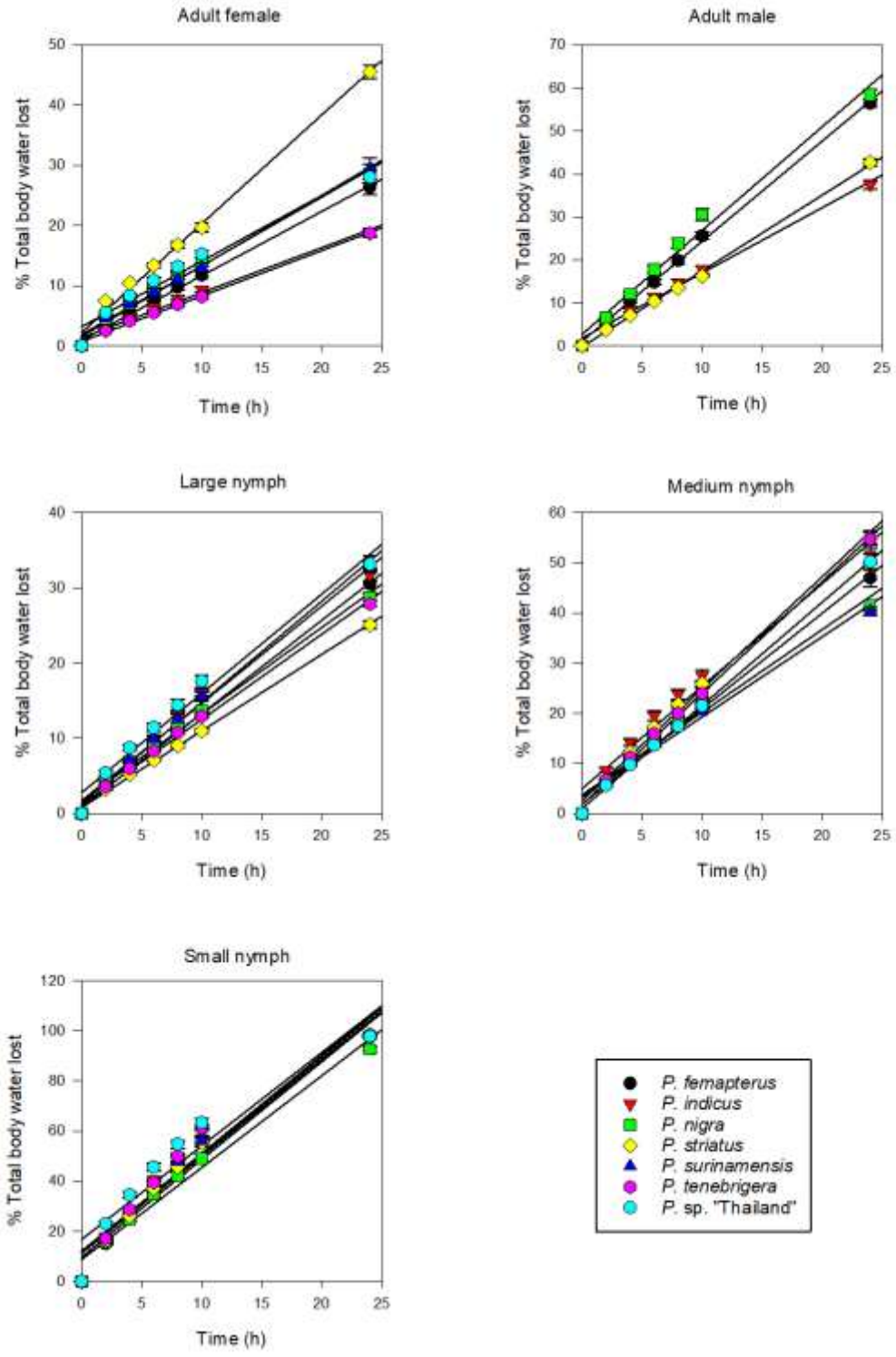

Fig. 5. Percentage total body water (%TBW) lost over time for seven live *Pycnoscelus* species.

CHAPTER 3

Thermal sensitivity of nymphs and adults of seven *Pycnoscelus* spp. (Blattodea: Blaberidae)

Introduction

Insects occupy a diverse niche in our ecosystem, even in the arctic (Strathdee and Bale 1998). But oftentimes, they are affected drastically by temperature changes (Hallman and Delinger 1991). Significant declines in insect populations in the tropics are primarily attributed to the increase in global temperature. For example, there has been a 70% insect population decline in Puerto Rico's national forests, in areas with very limited human activity, with only a 2-degree increase in the temperature since the 1970s (Lister & Garcia 2018). Since insects are poikilotherms, their capability to regulate temperatures is very limited.

Cockroaches, like all arthropods, are ectotherms. The ambient temperature they inhabit heavily influences their growth rate and reproduction (Tsuji and Mizuno 1973, Wu et al. 2017). Peterson (2019) found that across six constant temperatures ranging from 10°C to 35°C, there were significant differences in the nymphal development periods, instars count, and survivorship in *B. germanica* and *B. asahinai*, with reduced survivorship and reproduction on both the lower and higher end of the temperature ranges. For example, at 10°C and 15°C, no nymphs of *B. germanica* molted but all died in the first instar, while at 30°C, 86.78% of nymphs survived to adulthood, and at 35°C 66.09% of nymphs survived to adulthood.

Regarding thermal sensitivity, species that inhabit hot areas such as tropical and subtropical scrublands or deserts must be able to tolerate heat or have adaptations to escape the hot daylight. Animals have a thermal neutral zone, which is a range of temperatures in which the animal's metabolism doesn't change and cells in its body function normally. But as the ambient

temperature goes out of this range, causing the ectotherm's body temperature to be outside their neutral zone, the animal will exhibit certain behaviors that are not normally observed. We use these behaviors to record critical thermal temperatures.

In the case of *Pycnoscelus*, as the ambient temperature is increased, the cockroach may exhibit behaviors from agitation, heat exhaustion and even fainting. At the point the cockroach is incapacitated, the temperature recorded is the critical thermal maxima. (Appel 1991) Once the ambient temperature exceeds the critical thermal maxima, the upper lethal limit may be reached, in which case it will cause the death of the cockroach, as the heat may denature the proteins in the invertebrate's cells. (Appel 1991) The critical thermal maximum, rather than the upper lethal limit, is a relevant measure in heat adaptation due to the fact it is the temperature that said invertebrates lose coordination. In the wild, the loss of coordination would often mean death of the individual, as it can no longer escape from predators or function normally. Thus, it demonstrates the upper temperature limit an invertebrate can tolerate, and thus restricting its range.

Invertebrates are also heavily affected when the ambient temperature is lowered below the thermal neutral zone. In extremely cold temperatures, depending on how many osmotically active ions are present in an invertebrate, the water molecules in the cells may freeze. Once ice crystals are formed, the organism dies. Some insects have adaptations that can counter the effects of the ice particle formation by being able to "supercool" , having antifreeze present in their cells, like in the case of the spruce budworm *Choristoneura fumiferana*, in which case a highly active antifreeze protein helps to prevent ice crystals forming in their cells. (Kuiper et al. 2015). There have been examples of other insects dealing with low temperatures by removing ice nucleators from the gut and hemolymph. Larvae of *Ceruchus piceus* are capable of depressing

their supercooling points from approximately −7°C in the summer to −25°C in the winter through the help of lipoproteins which remove the nucleators. (Neven et al. 1986). Another example, *Celatoblatta quinquemaculata* in New Zealand have evolved to tolerate ice nucleating agents in their hemolymph and guts to survive extreme temperatures. (Wharton 2011) Many insects with low temperature tolerance also have desiccation resistance, as water is not available at low temperatures due to its quality of freezing quickly and damaging the organism's cells. In many cases, when insects are preparing for hibernation or molting, they reduce their water content and increase their sugar alcohol content to combat the potential crystallization of water.

Our study group, *Pycnoscelus* species have females generally considered flightless, and certain species have winged males capable of flight (pers. obs). They live in fragmented habitats in the tropics, preferring moist habitats with detritus, and are often found in various greenhouses around the world (Roth 1988, Parker et al. 1977, Grandcolas et al. 1996, Gade and Parker 1997, Moretti et al. 2011). The effects of temperature in the tropics are not completely cut and dry either. More specifically, wetter habitat is relatively cooler than drier habitats due to the thermal buffering properties of water. But due to the tendency of *Pycnoscelus* to inhabit fragmented habitats surrounded by harsher environments, and their inability to fly(at least within females), they cannot readily relocate when the conditions become unfavorable. Thus, we can hypothesize that thermally sensitive occupants may have a lesser range globally, especially in exposed locations prone to extreme temperatures.

The goal of this study was to measure the upper and lower thermal tolerance limits of the various life stages of six species and one variety in the genus *Pynoscleus*: *Pycnoscelus femapterus, Pycnoscelus indicus, Pycnoscelus surinamensis, Pycnoscelus striatus, Pycnoscelus tenebrigera, Pycnoscelus surinamensis, Pycnoscelus surinamensis* "Thailand". We chose these

species because they are present in the US cockroach hobby. They also have different ranges in the wild, from Kenya, the United States, Borneo to Pakistan, and so on, and thus inhabit various climate zones and habitats. We address one general question about their thermal tolerance: do *P. surinamensis* have a higher CTmax than all the other species or variety of *Pycnoscelus* tested? We predicted that *P. surinamensis* will have higher CTmax values than other species tested based on their extensive distribution worldwide, to a wide variety of ecosystems and temperature ranges. We tested these predictions with laboratory data outlined below in our materials and methods.

Materials and Methods

Six *Pycnoscelus* species and one unidentified *Pycnoscelus* species were obtained from two sources (Tydyeexotic.com and Roachcrossing.com) (Fig. 1). Unfortunately, the origins of these stocks are unclear for some species, but some information was obtainable (Table 1).

Unidentified *Pycnoscelus* species is labeled with its original locality "Thailand" in our study to distinguish it from the other populations. It produces three variations that share traits between *P. nigra* and *P. surinamensis* and may represent a naturally occurring hybrid between the two species.

All cultures were maintained inside 1470 cm^3 plastic storage with moist coconut coir (Burpee Eco-Friendly Natural Organic Garden Coir, Auburn, Alabama), provisioned with dry dog chow (Purina, Auburn, Alabama) and kept at (26.7 ± 0.2 °C) with an irregular photoperiod.

Thermal Sensitivity Experiments

As described by Appel (1990) we defined critical thermal maxima (CTMax) as the temperature that caused reversible knock-down when the temperatures were rapidly increased. This phenomenon was determined by observing specimens in regulated chambers and recording the temperature at which they started showing disorientation in movement - discoordination in the specimen's leg movement and the specimen flipping on its back. The upper lethal limit (ULL) was defined as the temperature that caused specimens to cease all movements. CTMax, CTMin, and ULL were measured using a precision temperature control unit as described in Hu and Appel (2004).

Seven *Pycnoscelus* spp. of similar size were weighted individually and placed into individual 10 mL beakers. These beakers were then placed onto the Peltier temperature-controlled plate controlled by a Pelt-5 temperature controller (Sable Systems International, Henderson, NV, U.S.A.—hereafter termed Sable Systems) inside a temperature ramping device set at 25 °C (Fig. 1). A 5 mL beaker filled approximately to ¾ was placed inside the temperature control device to maintain >50% RH. Temperatures were increased or decreased at a rate of 1 °C/min until knockdown was observed. The temperature was measured independently via a copper constantan bead thermocouple placed directly on the hot plate and connected to a TC-2000 Type-T thermocouple meter (Sable Systems). Specimens that were knocked down were moved to an incubator held at 30 °C with saturated humidity (>80% RH) for 30 minutes. Specimens were removed from the incubator and probed with a brush to observe recovery. If the specimen moved one body length after being probed we determined that knockdown was reversible.

Data analysis

For our experiment we used a randomized complete block design. For data analysis we used SAS version 9.3 (SAS Institute Inc. 2013). We used the univariate procedure in SAS to check for normality ($\alpha = 0.05$) for CT min, CT max, ULL, and mass loss. We used Analyses of variance (ANOVA) to test differences in CT min, CT max, ULL, and mass loss among different species and life stages followed by Waller-Duncan K-ratio test to determine if the differences among different species and life stages were statistically significant.

Results

The critical thermal maxima (CT max) ranged from 47.30 to 51.54 °C for small, 48.90 to 52.90 °C for medium, 49.54 to 52.93 °C for large nymphs, and 50.53 to 52.27 °C and 50.37 to 52.86 °C for adult males and females, respectively (Table 1). Small *P. striatus* nymphs had significantly lower CT max than other species while other species had statistically similar CT max. Medium *P. striatus* nymphs had significantly lower CT max than other species while medium *P. femapterus*, *P. indicus*, and *P. nigra* nymphs had significantly higher CT max than other species. Large *P. striatus* nymphs had significantly lower CT max than other species while large *P. femapterus* and *P. nigra* had significantly higher CT max than other species. Adult male *P. striatus* had significantly lower CT max than other species while other species had statistically similar CT max. Adult female *P. striatus* had significantly lower CT max than other species while adult female *P. femapterus* had significantly higher CT max than other species. *P. striatus* consistently had lowest CT max for all stages, and *P. femapterus* showed highest CT max values for most stages except small nymphs and adult males.

The upper lethal limit (ULL) ranged from 51.73 to 54.91 °C, 54.19 to 56.66 °C, 57.90 to 57.43 °C, 53.59 to 54.71 °C, and 55.19 to 56.76 °C for small nymphs, medium nymphs, large nymphs, adult males and adult females, respectively (Table 2). Small *P. striatus* nymphs had

significantly lower ULL than other species, while small *P. nigra* nymphs had significantly higher ULL than other species. Medium *P. striatus* nymphs had significantly lower ULL than other species, while medium *P. femapterus*, *P. nigra*, and *P. surinamensis* nymphs had significantly higher ULL than other species. Large *P. striatus* nymphs had significantly lower ULL than other species, while large *P. femapterus* nymphs had significantly higher ULL than other species. Adult males of all *Pycnoscelus* species used in the study had statistically similar ULL. Adult female *P. striatus* had significantly lower ULL than other species, while adult female *P. femapterus* had significantly higher ULL than other species. *P. striatus* consistently had lowest ULL throughout different stages, and *P. femapterus* showed the highest ULL for most stages except for small nymphs and adult males.

The critical thermal minima (CT min) ranged from 3.44 to 5.87 °C, 4.21 to 5.86 °C, 2.00 to 4.81°C, 3.44 to 5.29 °C, and 1.93 to 5.03 °C for small nymphs, medium nymphs, large nymphs, adult males and adult females, respectively (Table 3). Small *P. tenebrigera* nymphs had significantly lower CT min than other species, while *P. femapterus* and *P. striatus* had significantly higher CT min than other species. Medium *P. tenebrigera* nymphs had significantly lower CT min than other species while medium *P. femapterus* nymphs had significantly higher CT min than other species. Large *P. tenebrigera* nymphs had significantly lower CT min than other species while large *P. femapterus* nymphs had significantly higher CT min than other species. Adult male *P. indicus* and *P. tenebrigera* had significantly lower CT min than adult male *P. femapterus* and *P. striatus*. Adult female *P. nigra* had significantly lower CT min than other species while adult female *P. femapterus* had significantly higher CT min than other species. *P. tenebrigera* consistently had the lowest CT min throughout different stages except in

adult female, and *P. fempaterus* had the highest CT min throughout different stages except in adult male.

Discussion

Our results indicate that *P. surinamensis* does not appear to have an advantage in thermal tolerance to hot or cold temperatures compared to most other *Pycnoscelus* species used in this study. It is interesting to note that *P. striatus* consistently had the lowest CT max and ULL while *P. femapterus* showed the highest CT max and ULL for most stages. *P. striatus* are primarily found in caves (Roth 1998, Lucañas and Lit 2016), an environment often characterized as cool and moist. Therefore, it would be natural for *P. striatus* to have lower heat tolerance than other *Pycnoscelus* species, which may limit its dispersal and colonization into areas outside cave environments. It is also important to note that while some of the temperature differences are considered statistically significant according to our analysis, these differences are within 3 °C which might not be a significant difference in the biological context.

The similarity in CT max and min, and ULL among the species might be due to the specific lifestyle of the *Pycnoscelus* species. All *Pycnoscelus* species used in the study spend most of their lives buried in the substrate. Burrowing invertebrates can avoid temperature extremes through vertical migration in soil (Cloudsley-Thompson 1975, Crawford and Cloudsley Thompson 1971, Holsinger and Dickson 1977). Therefore, they might not require a physical or physiological adaptations to tolerate extreme temperatures in the wild. Also, many *Pycnoscelus* species are sympatric in tropical regions. For example, wildlife photographer and cockroach collector, Martin Ho, mentioned that he found *P. femapterus* together with *P. surinamensis* in the wild on occasion. Another collector, Djari Sabutaro, noted that he has found a group of *P.* sp.

"Thailand" living together with *P. nigra* in Thailand. Since these species occupy the same area, it would be natural for them to have similar temperature tolerance.

In our study, all the specimens used were reared in the same laboratory condition under a constant temperature and humidity. The regulation of physiological functions in insects, including respiration, immunity, metabolism, growth, and reproduction, is significantly influenced by the surrounding temperature. Consequently, these aspects impact various biological characteristics such as behavior, locomotion, dispersal, lifespan, and survival. Insects have evolved physiological strategies that enable them to adapt to seasonal changes in temperatures. For example, adaptations to warming include temporal shifts from periods of activity to quiescence via diapause and/or aestivation (Harvey et al. 2020). It is known that insects can have long-term acclimation to the environment (Harvey et al. 2020) so rearing conditions might have had an influence on the temperature tolerance of the cockroaches in this experiment. For a future study, wild-collected individuals might be ideal, as it will be a more accurate reflection of the acclimation capabilities of wild individuals. It would also be interesting to test the temperature tolerances of individuals reared in high temperature or low temperatures, such as 35°C versus 16°C, the average high and low temperatures of Alabama (National Weather Service Forecast Office 2018), which would test the different temperature acclimation ability by Surinam cockroaches, and see if *P. surinamensis* has an advantage breeding in temperature extremes, which would help explain their large ranges.

Our results show that the CT max and min ranged from 2.15 to 57.43°C for the genus *Pycnoscelus* and between 2.57 and 56.17°C for *P. surinamensis*. The ranges are within the range of other insects; for example, *Onymacris plana* have a very high CT max of 51°C, and a CT min of 12°C. CT max can be very low for certain animals, such as the salamander species

Diemictylus viridescens which only tolerate 39°C for a short period before death and acclimates to temperature changes very slowly (Hutchison 1961). Appel (1991) found the CT max of 43.2°C for *D. punctata* , 44.3°C for *P. surinamensis*, 40.9°C for *Supella longipalpa* (F.), and 51.4°C for *Cryptocercus punctulatus* Scudder. In our study, the CT max of *P. surinamensis* was higher, but it could be due to the faster temperature change rate as we measured. Overall, *Pycnoscelus* have a high CT max and a large range of temperatures they can tolerate and remain mobile and functional, allowing them to acclimate to various habitats or escape dangerously high temperatures. Although CT max does not vary drastically among the species, this removes high temperature-sensitivity as the limiting factor for the distribution of this genus. Much of the range limitations of other members of the genus must not be due to the temperature tolerance, but to other factors.

Different rates of temperature change affects CT rates. In our experiments, we used 1C per minute increments, since other studies often used this rate of temperature change. However, it is possible that we should have decreased the rate of temperature change for a more accurate result as it will allow more time for the cockroaches to acclimate internally to the new temperature.

It is also interesting to note that different life stages had similar CT max, ULL, and CT min values, suggesting size and mass of the cockroaches did not influence their temperature tolerance, which differs from other insects such as *Lucanus elaphus,* which had a positive relationship between larval mass and thermal threshold (Lawhorn and Yanoviak 2022). Similarity in CT max, ULL, and CT min might be because all life stages of *Pycnoscelus* spp. live together in the same area in the wild. Therefore, they face the same environmental pressure regardless of their life stage.

In the case of CT min, *P. tenebrigera* consistently had the lowest CT min throughout its life stages. This species originates from Pakistan and lies in the temperate zone with some areas dropping below-freezing points during winter. Therefore, this species could be more cold adapted than other species of *Pycnoscelus*. However, despite its cold tolerance, it has yet to be found outside its native range. Their dispersal might be limited by several factors: females of this species are apterous, and they are troglobitic (Roth 1998).

From these results we speculate that the success of *P. surinamensis* to colonize a broad range is not due to temperature tolerance since they are not dramatically different from the other *Pycnoscelus* species. No species with higher CT max or lower CT min have a broader distribution than *P. surinamensis*. In fact, the more tolerant species in this genus have a drastically smaller range in comparison.

This is not to say that the temperature tolerance of *P. surinamensis* did not help its dispersal. The CT max for *P. surinamensis* is around 56°C, and the CT min is around 2.5°C; this allows for a very large range of temperature where the cockroach can remain active and functional in, effectively allowing it to survive in all environments that are not exposed to desert heat or below-freezing. Having a wide range of temperatures must be a fundamental reason that allows *P. surinamensis* to survive in relatively hostile, and often human-altered environments. It might also benefit them in surviving hostile conditions during which the substrate they live in is processed or transported. But due to the similar CT max and CT min within this genus, their advantage over their relatives must lie within other characteristics such as their dispersal capability.

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Table 1. Critical thermal maxima and mass loss for seven different *Pycnoscelus* species.

Species	Stages	CTMax ± SE (°C)	Mass loss (mg/g)
P. femapterus	Small nymph	50.23 ± 1.02ab	10.17 ± 1.39a
P. indicus	Small nymph	51.49 ± 0.45a	10.32 ± 1.52a
P. nigra	Small nymph	51.43 ± 0.54a	9.89 ± 1.36a
P. striatus	Small nymph	47.30 ± 0.46c	5.01 ± 0.76b
P. surinamensis	Small nymph	51.54 ± 0.40a	12.10 ± 0.86a
P. tenebrigera	Small nymph	49.61 ± 0.41b	8.71 ± 0.76a
P. sp. "Thai"	Small nymph	51.43 ± 0.44a	11.32 ± 1.30a
P. femapterus	Medium nymph	52.90 ± 0.23a	7.44 ± 0.98bcd
P. indicus	Medium nymph	52.97 ± 0.28a	8.78 ± 1.54ab
P. nigra	Medium nymph	53.53 ± 0.15a	8.75 ± 0.66abc
P. striatus	Medium nymph	48.90 ± 0.22c	6.34 ± 0.50cd
P. surinamensis	Medium nymph	52.14 ± 0.27b	6.01 ± 0.45d
P. tenebrigera	Medium nymph	51.71 ± 0.25b	7.28 ± 0.53bcd
P. sp. "Thai"	Medium nymph	51.66 ± 0.40b	10.91 ± 0.50a
P. femapterus	Large nymph	52.93 ± 0.48a	6.44 ± 0.73ab
P. indicus	Large nymph	52.41 ± 0.30ab	7.11 ± 0.91ab
P. nigra	Large nymph	52.83 ± 0.17a	6.29 ± 0.48ab
P. striatus	Large nymph	49.54 ± 0.24c	3.45 ± 0.31c
P. surinamensis	Large nymph	52.41 ± 0.26ab	7.29 ± 0.50ab
P. tenebrigera	Large nymph	51.90 ± 0.34b	8.01 ± 1.11a
P. sp. "Thai"	Large nymph	51.73 ± 0.36b	5.80 ± 0.33b
P. femapterus	Adult male	52.06 ± 0.27a	7.03 ± 0.49a
P. indicus	Adult male	51.61 ± 0.32a	5.56 ± 0.54a
P. striatus	Adult male	50.53 ± 0.25b	5.58 ± 0.50a
P. tenebrigera	Adult male	52.27 ± 0.23a	5.35 ± 0.79a
P. femapterus	Adult female	52.86 ± 0.22a	5.49 ± 0.97bc

P. indicus	Adult female	52.64 ± 0.14ab	5.59 ± 0.62abc
P. nigra	Adult female	52.03 ± 0.44b	5.45 ± 0.88bc
P. striatus	Adult female	50.37 ± 0.35c	3.40 ± 0.42c
P. surinamensis	Adult female	51.94 ± 0.28b	4.93 ± 0.33bc
P. tenebrigera	Adult female	52.06 ± 0.25b	6.84 ± 0.70ab
P. sp. "Thai"	Adult female	52.57 ± 0.24ab	8.00 ± 1.29a

*Mean values within each column followed by the same letter are not significantly different according to the Waller–Duncan K-ratio mean separation test (P > 0.05).

Table 2. Upper lethal limit and mass loss for seven different *Pycnoscelus* species.

Species	Stages	ULL ± SE (°C)	Mass loss (mg/g)
P. femapterus	Small nymph	54.74 ± 0.19ab	12.68 ± 0.79a
P. indicus	Small nymph	54.00 ± 0.31bc	10.65 ± 0.67ab
P. nigra	Small nymph	54.91 ± 0.21a	10.93 ± 0.73ab
P. striatus	Small nymph	51.73 ± 0.54e	6.39 ± 0.39c
P. surinamensis	Small nymph	53.66 ± 0.33cd	10.98 ± 0.87ab
P. tenebrigera	Small nymph	52.79 ± 0.36d	9.89 ± 1.10b
P. sp. "Thai"	Small nymph	53.93 ± 0.27bc	11.51 ± 0.81ab
P. femapterus	Medium nymph	56.66 ± 0.29a	5.38 ± 0.53cd
P. indicus	Medium nymph	55.54 ± 0.26bc	6.71 ± 0.53bc
P. nigra	Medium nymph	56.66 ± 0.34a	8.13 ± 0.86ab
P. striatus	Medium nymph	54.19 ± 0.24d	4.84 ± 0.72d
P. surinamensis	Medium nymph	56.71 ± 0.30a	6.46 ± 0.28bcd
P. tenebrigera	Medium nymph	55.30 ± 0.30c	6.80 ± 0.60bc
P. sp. "Thai"	Medium nymph	56.27 ± 0.18ab	8.88 ± 0.73a
P. femapterus	Large nymph	57.43 ± 0.20a	7.04 ± 0.69ab
P. indicus	Large nymph	56.53 ± 0.30ab	5.75 ± 1.00ab
P. nigra	Large nymph	56.40 ± 0.52b	5.00 ± 0.73ab
P. striatus	Large nymph	54.90 ± 0.19c	4.10 ± 0.71b
P. surinamensis	Large nymph	56.17 ± 0.34b	7.41 ± 0.93a
P. tenebrigera	Large nymph	55.81 ± 0.36bc	7.93 ± 0.93a
P. sp. "Thai"	Large nymph	56.29 ± 0.37b	6.97 ± 1.10ab
P. femapterus	Adult male	53.99 ± 0.87a	7.45 ± 0.56a
P. indicus	Adult male	54.71 ± 0.18a	7.34 ± 0.86a
P. striatus	Adult male	53.59 ± 0.31a	6.69 ± 0.81a
P. tenebrigera	Adult male	54.24 ± 0.22a	7.95 ± 1.08a
P. femapterus	Adult female	56.76 ± 0.10a	4.85 ± 0.49ab

P. indicus	Adult female	56.33 ± 0.14abc	6.55 ± 0.94a
P. nigra	Adult female	56.27 ± 0.24abc	3.85 ± 0.35b
P. striatus	Adult female	55.19 ± 0.23d	3.68 ± 0.25b
P. surinamensis	Adult female	56.10 ± 0.23bc	4.06 ± 0.65b
P. tenebrigera	Adult female	55.84 ± 0.27c	6.41 ± 1.29a
P. sp. "Thai"	Adult female	56.49 ± 0.14ab	6.94 ± 0.70a

*Mean values within each column followed by the same letter are not significantly different according to the Waller–Duncan K-ratio mean separation test (P > 0.05).

Table 3. Critical thermal minima and mass loss for seven different *Pycnoscelus* species.

Species	Stages	CTMin ± SE (°C)	Mass loss (mg/g)
P. femapterus	Small nymph	5.87 ± 0.16a	1.06 ± 0.16a
P. indicus	Small nymph	4.34 ± 0.26b	0.67 ± 0.36a
P. nigra	Small nymph	3.99 ± 0.34bc	0.59 ± 0.15a
P. striatus	Small nymph	5.69 ± 0.12a	1.29 ± 0.17a
P. surinamensis	Small nymph	4.47 ± 0.17b	0.57 ± 0.22a
P. tenebrigera	Small nymph	3.44 ± 0.28c	0.67 ± 0.28a
P. sp. *"*Thai*"*	Small nymph	4.40 ± 0.15b	0.68 ± 0.20a
P. femapterus	Medium nymph	5.86 ± 0.17a	0.37 ± 0.13a
P. indicus	Medium nymph	4.90 ± 0.23b	0.65 ± 0.25a
P. nigra	Medium nymph	4.89 ± 0.24b	0.24 ± 0.09a
P. striatus	Medium nymph	5.40 ± 0.11ab	0.73 ± 0.21a
P. surinamensis	Medium nymph	5.03 ± 0.14b	0.27 ± 0.09a
P. tenebrigera	Medium nymph	4.21 ± 0.33c	0.65 ± 0.42a
P. sp.. *"*Thai*"*	Medium nymph	4.84 ± 0.16b	0.17 ± 0.10a
P. femapterus	Large nymph	4.81 ± 0.13a	0.22 ± 0.09 b
P. indicus	Large nymph	2.95 ± 0.16d	0.11 ± 0.06b
P. nigra	Large nymph	3.16 ± 0.26cd	0.17 ± 0.02b
P. striatus	Large nymph	4.20 ± 0.25b	0.81 ± 0.31a
P. surinamensis	Large nymph	3.56 ± 0.26c	0.20 ± 0.13b
P. tenebrigera	Large nymph	2.00 ± 0.23e	0.25 ± 0.10b
P. sp. *"*Thai*"*	Large nymph	3.34 ± 0.24cd	0.11 ± 0.06b
P. femapterus	Adult male	4.73 ± 0.18a	0.18 ± 0.05b
P. indicus	Adult male	4.01 ± 0.21b	0.06 ± 0.02b
P. striatus	Adult male	5.29 ± 0.17a	0.58 ± 0.18a
P. tenebrigera	Adult male	3.44 ± 0.37b	0.09 ± 0.05b
P. femapterus	Adult female	5.03 ± 0.16a	0.59 ± 0.35 a

P. indicus	Adult female	1.93 ± 0.22d	0.14 ± 0.06a
P. nigra	Adult female	2.44 ± 0.24cd	0.17 ± 0.06a
P. striatus	Adult female	3.39 ± 0.41b	0.64 ± 0.19a
P. surinamensis	Adult female	2.57 ± 0.24cd	0.31 ± 0.05a
P. tenebrigera	Adult female	3.13 ± 0.38bc	0.23 ± 0.08 a
P. sp. "Thai"	Adult female	3.07 ± 0.35bc	0.30 ± 0.07a

*Mean values within each column followed by the same letter are not significantly different according to the Waller–Duncan K-ratio mean separation test (P > 0.05).

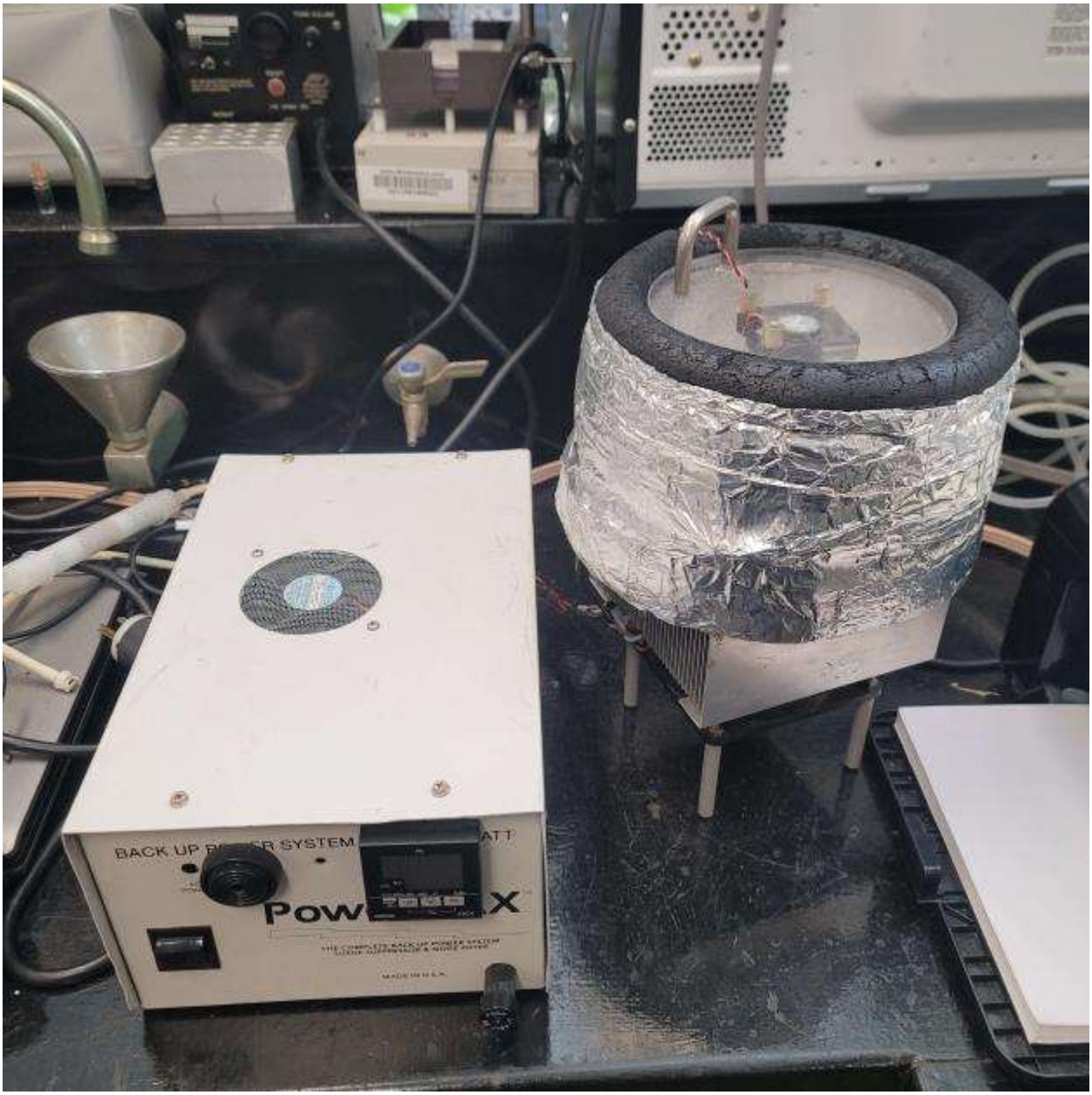

Fig. 1. Temperature control apparatus used for thermal sensitivity study. The device was custom-made by Auburn Research Support Team.

CHAPTER 4

Reproductive potential of seven *Pycnoscelus* spp. (Blattodea: Blaberidae)

Introduction

The genus *Pycnoscelus* contains sixteen species (Beccaloni 2023), with most of the members restricted to the tropics. However, one of its species, *P. surinamensis*, is found across tropical and subtropical regions worldwide, and is also considered a greenhouse pest (Roth 1967, 1974, Zangl et al. 2018).

Species in the genus consist of: *P. aurantia* (Hanitsch), *P. conferta* (Walker), *P. femapterus* (Roth), *P. gorochovi* (Anisyutkin), *P. indicus* (Fabricius), *P. janetscheki* (Bey-Bienko), *P. micropterus* (Hanitsch), *P. nigra* (Brunner von Wattenwyl), *P. rothi* (Anisyutkin,), *P. rufus* (Bey-Bienko), *P. schwendingeri* (Anisyutkin), *P. semivitreus* (Princis), *P. striatus* (Kirby), *P. surinamensis* (Linnaeus), *P. tenebrigera* (Walker), and *P. vietnamensis* (Anisyutkin,).

Little is known about the natural habitats of most species in this genus; with the limited information known, *P. striatus* can be found in bat caves, *P. femapterus* found in forest floor habitats under leaf litter and logs, *P. nigra* and *P. surinamensis* can be found near human habitation under trash and various debris (Martin GM Ho pers. comm., Lucañas and Lit 2016).

Parthenogenesis is a rare phenomenon observed in some cockroach taxon. Most Blattodea capable of parthenogenesis has facultative parthenogenesis; females generally reproduce sexually, but are capable of producing viable oothecae in the absence of males (Roth and Willis 1956, Corley et al. 2001). In most of these species, while they are capable of facultative parthenogenesis, the frequency of it is low (Griffiths and Tauber 1942, Roth and Willis 1956, Corley et al. 2001). Parthenogenesis is reported from following families and

species; Blaberidae- *Nauphoeta cinerea* (Olivier), *Pycnoscelus nigra* (Brunner von Wattenwyl), *Pycnoscelus surinamensis* (L.), Blattidae – *Periplaneta americana* (L.), *Periplaneta brunnea* Burmeister, *Periplaneta fuliginosa* Serville, and Corydiidae – *Polyphaga obscura* Chopard (Roth and Willis 1956, Cochran 1999, Corley et al. 2001). Additionally, an unidentified species of *Nocticola* species (Nocticolidae) from Malaysia was observed to reproduce parthenogenetically (pers. obs.).

In *Pycnoscelus* spp., only *P. nigra* and *P. surinamensis* are reported to breed parthenogenetically. Interestingly, *P. nigra* is capable of sexual reproduction when males are present, but *P. surinamensis* does not have fertile males present in any known populations and is obligatorily parthenogenetic (Roth 1967, 1998, Martin GM Ho pers. comm.).

Parthenogenesis is not rare across various taxa of invertebrates and fungi, and has many advantages over sexual reproduction, albeit many disadvantages. Parthenogenesis's main advantages are its energy efficiency, lack of risk of organelle conflicts, and preservation of well-adapted genomic configurations (Sun and Heitman 2011). Individuals do not need to look for mates or invest energy into sexual displays, thus making reproduction more efficient. Asexual organisms might also be better at colonizing marginal habitats as only one individual is needed to start a population in the new habitat. (Baker 1955, Gerritsen 1980, Cueller 1994). Sexual organisms in marginal habitats may also be maladapted because of the low gene flow from the main population, but well adapted asexual forms will retain the adaptation and have a competitive edge (Antonovics 1968, Peck et al. 1998, Hagg and Ebert 2004). In some cases, high fitness asexual populations out-compete inbred sexual populations and drive sexual the sexual populations local extinction (Hagg and Ebert 2004). There is also the argument that sexual reproduction may be advantageous in habitats that involve many biotic interactions, including

co-evolutionary arms races and gives an advantage to the larger genetic diversity provided by sexual reproduction (Jaenike 1978, Hamilton 1980, Lively et al. 1990, Hagg and Ebert 2004), while asexuals reproduction would have an advantage in more sparsely inhabited areas, where biotic interactions are rare and environmental factors dominating (Levin 1975, Glesener and Tilman 1978, Hamilton et al. 1990, Hagg and Ebert 2004). However, parthenogenesis does come with many drawbacks, as parthenogenesis is considered an evolutionary dead end, with a clonal population and lower genetic diversity, is less adaptive in a fluctuating environment, and less efficient in purging deleterious mutations (Sun and Heitman 2011).

Roth (1974) reported that certain strains of *P. surinamensis* have different fecundity compared to other populations, and both diploid and triploid *P. surinamensis* are present, possibly evolving multiple times from *P. indicus*. *P. surinamensis* are reproductively isolated from *P. indicus*; Roth (1974) tried to cross the two species to no avail. The males produced by the parthenogenetic strain did not mate with the females of *P. indicus*, and the female *P. indicus* never produced any viable offspring; they often did not retract the ootheca, and males of *P .indicus* successfully mated with *P. surinamensis* females, but all the offspring produced were females. This result demonstrated that *P. surinamensis* is the only known cockroach to be obligatorily parthenogenetic (Roth 1967).

As previously discussed, *P. surinamensis* have a significantly wider distribution compared to all the other members of their genus. Within investigating the reason for this large range, one would be remiss if one were not to investigate the fecundity of the species. In this study we examined the reproductive potential of seven *Pycnoscelus* species.

Materials and Method

Study organisms

Six *Pycnoscelus* species and one unidentified *Pycnoscelus* species were obtained from two sources (Tydyeexotic.com and Roachcrossing.com) (Fig. 1). Unfortunately, the origins of these stocks are unclear for some of the species, but some information was obtainable (Table 1). Unidentified *Pycnoscelus* species is labeled with its original locality "Thailand" in our study to distinguish them from the other population. It produces three different variations that share traits between *P. nigra* and *P. surinamensis* and may represent a naturally occurring hybrid between the two species.

All cultures were maintained inside 1470 cm^3 plastic storage with moist coconut coir (Burpee Eco-Friendly Natural Organic Garden Coir, Auburn, Alabama), provisioned with dry dog chow (Purina, Auburn, Alabama) and were kept at (26.7 ± 0.2 °C) with an irregular photoperiod. Soil moisture was measured and maintained at average wet level (meter reading of 6 to 8) using soil moisture tester (Economy Soil Moisture Tester, Spectrum Technologies, inc., Aurora, Illinois).

Method

Ten adult females from six *Pycnoscelus* species were placed individually into 150 cm^3 deli cups and kept at 26.7 C with food and Expert Gardener Potting Mix at an average wet soil moisture level filled to ¾ of the enclosure. Specimens were examined bi-weekly for signs of birth and were given two koi pellets (Wardley Pond Pellets, Auburn, Alabama) after each measuring session. Uneaten food was removed to prevent molding formation.

For egg counts, fifteen females from each species were selected randomly from each breeding colony that was maintained in 147 cm^3 plastic storage with moist coconut coir (Burpee Eco-Friendly Natural Organic Garden Coir, Auburn, Alabama) and maintained on dry dog chow (Purina, Auburn, Alabama) at (26.7 ± 0.2 °C) and an irregular photoperiod. The abdomen of each female was gently pressed with fingers to extrude oothecae out of their body cavities, and these eggs were placed in 70% ethanol for future egg count. The number of eggs was counted under a dissecting scope.

Data analysis

For our experiment, we used a completely randomized design because we could not control the timing of availability of female *Pycnoscelus* species used in this study. For data analysis, we used SAS version 9.3 (SAS Institute Inc. 2013). We used the univariate procedure in SAS to check for normality ($\alpha = 0.05$) for egg count and nymph count. We used Analyses of variance (ANOVA) to test differences in egg count and nymph count among different species, followed by the Waller-Duncan K-ratio test to determine if the differences among different species were statistically significant. The percent hatch rate was calculated by using the following formula:

Hatch rate = (Number of nymphs/Number of eggs) X 100%

Results

The mean egg count ranged from 21.9 to 43.6, and the mean number of nymphs ranged from 18.5 to 33.0 (Table 1).

Within sexually reproducing species, *P. indicus* produced a significantly greater number of eggs and nymphs compared to other species, and *P. femapterus* produced the lowest number of eggs and nymphs.

Within parthenogenetic species, *Pycnoscelus* sp. "Thailand" produced the most eggs, but the result was not significantly different from other species. For nymphs, *P. surinamensis* produced a significantly greater number of nymphs than the other species.

While *P. surinamensis* did show a significant difference in egg count from most of the *Pycnoscelus* species used in this study, it had the second lowest value of all species. The mean egg count was lowest in *P. femapterus* and greatest in *P. indicus*. However, *P. surinamensis* produced the second-highest number of nymphs compared to other species. The mean nymph count was greatest in *P. indicus* and lowest in *P. femapterus*.

The hatch rate ranged from 63.0 to 84.4 %. *P. femapterus* had highest hatch rate of all species while *P*. sp. "Thailand" had the lowest. *P. surinamensis* had the second-highest hatch rate of all species.

Discussion

Of 16 *Pycnoscelus* species, only *P. surinamensis* is present worldwide (Roth 1998, Parker et al. 1977). There have been two previous hypotheses on explaining the wide distribution of *P. surinamensis*; its ability to reproduce parthenogenetically, allowing the species to colonize different regions, and transportation through anthropogenic activities that could be responsible for its widespread distribution (Parker et al. 1977, Gade and Parker 1977, Grandcolas et al. 1996). The former hypothesis would explain why *P. surinamensis* is widespread compared to sexually reproducing *Pycnoscelus* species, but it does not explain why another parthenogenetic

species, *P. nigra* is not as widespread as *P. surinamensis*. Although *P. surinamensis* is the only obligate parthenogenetic member of the *Pycnoscelus* genus, *P. nigra* can also reproduce with parthenogenesis, and thus their wide distribution could possibly be attributed to the fecundity of the species. The latter hypotheses would also not explain why species like *P. nigra* and *P. indicus* are equally widespread, as both species are frequently found near anthropogenic environments (pers. comm.). Since these two hypotheses do not explain why *P. surinamensis* is more widespread than other species, we investigated the reproductive potential to see if *P. surinamensis* produces significantly more offspring than other *Pycnoscelus* species. However, we found that *P. surinamensis* produced significantly fewer eggs compared to other members of the genus, except for *P. femapterus*. However, it produced a significantly greater number of nymphs compared to other parthenogenetic species.

As discussed earlier, asexual reproduction would have an advantage in more sparsely inhabited habitats, where biotic interactions are rare and environmental factors are dominating (Levin 1975, Glesener and Tilman 1978, Hamilton et al. 1990, Hagg and Ebert 2004). The areas these *P. surinamensis* are introduced to are oftentimes greenhouses and near human habitation; therefore, they face little competition with other species occupying the same niche, thus allowing the advantage of asexual reproduction.

Being near human habitation also increases their chance of being transported through plant exports or the movement of soil and mulch. Parthenogenetic reproduction would mean a single transported individual can establish a new population, which drastically increases the chance of a quick establishment of new populations. With the sexually reproducing *Pycnoscelus*, a pair of approximately the same age would be required, at the very least, and in close proximity, for a new population to be established. Such an event is statistically less likely. Therefore, the

parthenogenetic nature of *P. surinamensis* would allow them to quickly establish themselves at large levels in new sites through transportation events and expand their range in the human-altered landscapes through the plants or debris they are transported with. Multiple authors have pointed this out in the literature (Roth and Willis 1960, White 1964, Parker and Niklasson 1995).

Some species, like *P. femapterus* produce fewer offspring compared to *P. surinamensis*. *Pycnoscelus femapterus* unsurprisingly, have a relatively small range compared to *P. surinamensis* that are found only in Malaysia and Thailand. This species has been collected together with *P. surinamensis* in Thailand, so it likely has an equal chance of being transported (pers. comm). Therefore, producing fewer offspring per ootheca is unlikely to be an advantageous trait that allowed *P. surinamensis* to be more widespread.

Furthermore, even though *P. surinamensis* have similar egg and nymph count to other members of the genus, there could be differences in development time and the survivorship of the nymphs at certain temperatures. We know that *P. surinamensis* is present in a more diverse and hostile array of habitats, and thus differences in nymph survival at these extremes could benefit them. We also do not know how many nymphs each female *Pycnoscelus* can produce throughout its adult lifespan. Within our studies, we only sampled one ootheca per female, but females can produce multiple ootheca throughout their adult lifespan, and there could be dramatic variations in the total number of nymphs produced per female due to this factor.

However, we cannot disregard the effects of fecundity on the range of a species. The second most widespread species in the genus *Pycnoscelus* is *P. indicus* (Roth 1996), which had the highest egg and nymph production in our study, and the greatest number of offspring from sexually reproducing species. Perhaps this reflects the capability of producing more offspring

does offer a competitive advantage and allow the species to spread more quickly compared to their relatives.

Looking at the hatch percentage of other species', *Blattella lituricollis* (Walker) have a 90% hatch percentage, *Blattella biligata* (Walker) with a 81% hatch percentage, *Balta longicercata* (Bolivar) with a 96% hatch percentage, *Scalida latiusvittata* (Brunner) with a 79% hatch percentage, *Margattea nimbata nimbata* (Shelford) with a 91% hatch percentage, *Lobopterella dimidiatipes* (Bolivar) with a 82% hatch percentage and *P. surinamensis* (L.) with a 70% hatch percentage (Boyer and Rivault 2004). In our experiment, *P. surinamensis* had a hatching percentage of 82.1 percent, possibly due to the difference in the conditions the cockroaches were raised. We can see through, that *P. surinamensis*, or *Pycnoscelus* spp. in general, have a lower hatch percentage than various other cockroaches.

We would also like to note that *P. surinamensis* has multiple lineages that seem to have different reproductive potentials and the strain we used (Montgomery, Alabama) are likely is the same lineage as two Florida lineages (USF a and b) that Roth used in his 1974 study. His study showed that Florida *P. surinamensis* produced significantly fewer ovarioles (eggs) and nymphs compare to most *P. surinamensis* strains used in his study, with average egg count of 30 (USFa) and 22 (USFb), and an average nymph count of 26.8 (USFa) and 17.2 (USFb). His values for USFa strain are very similar to ours, suggesting that the differences in the number of offspring produced by different strains is somewhat consistent between different lineages. With that being said, we could improve our study by adding different lineages of *P. surinamensis* to our study in the future.

It is unclear why *Pycnoscelus nigra* and *P*. sp. “Thailand” are not more widespread, as they are capable of parthenogenesis like *P. surinamensis*. It could be due to a complex combination of previously mentioned factors or simply luck.

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Table 1. Mean egg count and nymph count of different *Pycnoscelus* species (n=10 per species).

Species	Number of eggs (Mean ± SE)	Number of nymphs (Mean ± SE)	% Hatch Rate
P. femapterus	21.9 ± 0.8 d	18.5 ± 1.0 c	84.4
P. indicus	43.6 ± 1.0 a	33.0 ± 3.3 a	75.7
P. nigra	37.9 ± 0.6 b	24.6 ± 1.8 bc	64.9
P. striatus	38.9 ± 1.3 b	29.7 ± 1.6 ab	76.3
P. surinamensis	34.1 ± 1.0 c	28.0 ± 1.5 ab	82.1
P. tenebrigera	36.6 ± 0.5 b	24.7 ± 2.3 bc	67.5
P. sp. "Thailand"	38.1 ± 1.1 b	24.0 ± 3.0 bc	63.0

*Mean values within each column followed by the same letter are not significantly different according to the Waller–Duncan K-ratio mean separation test ($P > 0.05$).

CHAPTER 5

Conclusion and reflections

While we could not definitely explain why *Pycnoscelus surinamensis* is more widespread than other *Pycnoscelus* species, we can reflect on what we could do differently for future studies.

During our desiccation study, we could not get accurate desiccation tolerance data as only small nymphs died within a 12-hour period to give us an accurate death time. Of the small nymphs, only five species showed mortality within a 12-hour period; *P. femapterus, P. indicus, P. striatus, P. tenebrigera, and P.* sp. "Thailand". In further studies, we should extend the amount of time we run the desiccation study to over 72 hours. Under those conditions, it might be possible for us to observe the amount of time needed for all life stages to die, and give accurate data on the effect of desiccation on adults and large nymphs.

In our studies, all the specimens used were raised in controlled laboratory conditions with constant temperature and humidity. The temperature of the surrounding environment significantly affects the regulation of various physiological functions in insects, such as respiration, immunity, metabolism, growth, and reproduction (Appel 1991, Bale et al. 2002, Terblanche et al. 2011). Consequently, these factors have an impact on different biological characteristics including behavior, movement, dispersal, lifespan, and survival. It is worth noting that insects can adapt to their environment over a long period of time, as observed in a study by Harvey et al. (2020). Therefore, the rearing conditions in our experiment may have influenced the cockroaches' tolerance to temperature. For future studies, it would be ideal to compare individuals collected from the wild, as this would provide a more accurate understanding of their natural acclimation capabilities.

Additionally, it would be interesting to investigate the temperature tolerances of cockroaches reared under high or low-temperature conditions, such as 36°C compared to 16°C similar to the developmental study conducted by Wu et al. (2017). This would allow us to assess the cockroaches' ability to adapt to different temperature extremes, providing insights into whether *P. surinamensis* has an advantage in breeding under such conditions, which could help explain their wide distribution range.

CT max of *P. surinamensis* was higher in our study than that found in previous studies, and this might be due to the faster rate of change in temperature as we measured. Different rates of temperature change affect CT values. In our experimental setup, we used temperature increments of 1°C per minute, as done in many previous studies. However, it is worth considering that a slower rate of temperature change might be more appropriate for obtaining more precise results. By decreasing the rate of temperature change, the cockroaches would have had additional time to acclimate to the new temperature, potentially leading to a more accurate understanding of their responses.

Moreover, although *P. surinamensis* exhibits similar egg and nymph counts compared to other members of the same genus, there may be variations in their development time and the survival rate of nymphs under specific temperature conditions. Considering that *P. surinamensis* inhabits a wider range of diverse and challenging habitats, these differences in nymphal survival under extreme temperatures could potentially offer them an advantage. Additionally, our knowledge regarding the total number of nymphs produced by each female *Pycnoscelus* throughout its adult lifespan is limited. In our research, we only examined one ootheca per female, but it is important to note that each female produces multiple ootheca during its adult lifespan. Therefore, there might be significant variations in the overall number of nymphs

produced per female due to this factor. This variation might have effects on the success of *P .surinamensis* and should be investigated.

With all the data on the physiology of *P. surinamensis*, we can make educated projections on the extensive range of *P. surinamensis*. One of the heaviest contributing factors of this species must be its ability to reproduce parthenogenetically. The ability of *P. surinamensis* to reproduce parthenogenetically has played a crucial role in its colonization of different regions, while human-mediated activities have contributed to its extensive distribution (Parker et al. 1977, Gade and Parker 1977, Grandcolas et al. 1996). Previous discussions have highlighted the advantages of asexual reproduction in sparsely inhabited habitats, where biotic interactions are infrequent, and environmental factors dominate (Levin 1975, Glesener and Tilman 1978, Hamilton et al. 1990, Hagg and Ebert 2004). *P. surinamensis* is often introduced to locations such as greenhouses and areas near human settlements, where it faces limited competition from other species occupying the same ecological niche. This circumstance aligns with the benefits of asexual reproduction.

Living in proximity to human habitation also increases the likelihood of transportation through activities such as plant exports or the movement of soil and mulch. The parthenogenetic reproduction of *P. surinamensis* allows a single transported individual to establish a new population, significantly enhancing the probability of rapid population establishment. In contrast, sexually reproducing *Pycnoscelus* would require at least a pair of individuals of approximately the same age in close proximity for a new population to establish, making such an event statistically less likely. Therefore, the parthenogenetic nature of *P. surinamensis* enables quick establishment in numerous new locations through transportation events and facilitates range expansion within human-altered systems via plants or debris that accompany their transport.

Several authors have highlighted this phenomenon in the literature (Roth and Willis 1960, White 1964, Parker and Niklasson 1995).

However, *P. surinamensis* possesses advantages beyond parthenogenesis. They have developed a range of adaptations that enable their survival in harsh environments, including their ability to tolerate drought. Edney (1977) provides a list of cuticular permeability (CP) for various arthropod species, which indicates that insects in xeric environments typically have CP values below 40 μg cm^{-1} h^{-1} $mmHg^{-1}$, while those in mesic environments have CP values above 50 μg cm^{-1} h^{-1} $mmHg^{-1}$. Most of the *Pycnoscelus* species examined in the study fall into the xeric category, despite being found in tropical regions, with only *P. striatus* falling into the mesic category. This suggests that although *P. surinamensis* does not possess a distinct advantage over other members of its genus in terms of drought tolerance, it is capable of withstanding xeric conditions for extended periods. This adaptability allows them to thrive in a diverse range of environments once they are transported there, as well as in arid conditions during transportation where moisture may be scarce.

Furthermore, *P. surinamensis* exhibits a remarkable tolerance for high temperatures. In the case of insects, as the ambient temperature rises, they may display behaviors such as agitation, heat exhaustion, and even fainting, which signifies their critical thermal maxima (Appel 1991). The critical thermal maxima is a significant measure in assessing heat adaptation because it represents the temperature at which these invertebrates lose coordination. In the wild, the loss of coordination often leads to the death of the individual, as it becomes unable to escape predators or function normally. Consequently, the critical thermal maxima indicate the upper-temperature limit that an invertebrate can endure, thereby restricting its distribution. Although *P. surinamensis* does not possess a specific advantage over other members of its genus regarding

temperature tolerance, it does possess an exceptionally high critical thermal maxima, enabling it to tolerate temperatures exceeding 55°C. This capacity allows the species to survive adverse transportation conditions, where temperatures can reach extremes due to the greenhouse effect. Additionally, it facilitates the colonization of various environments inhabited by *P. surinamensis*, many of which experience high ground temperatures during the day. While our research indicates that *P. surinamensis* does not have a distinct advantage over other members of its genus in terms of temperature tolerance, it still serves as a crucial factor in the successful establishment of their population following transportation to a new location and enables their survival during transport.

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