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RESEARCH ARTICLE

# The dacetine ant *Strumigenys arizonica*, an apparent obligate commensal of the fungus-growing ant *Trachymyrmex arizonensis* in southwestern North America

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## Abstract

Over 40 years ago, the dacetine ant *Strumigenys arizonica* was discovered in a nest of the fungus-growing ant *Trachymyrmex arizonensis* at Madera Canyon in the Santa Rita Mountains of the southwestern United States. This discovery suggested that the two species form compound nests, but this hypothesis has not been investigated. Here, we characterize this symbiosis through an analysis of collection records supplemented by recent field and laboratory observations. Our observations show that *S. arizonica* and *T. arizonensis* form compound nests that are a type of commensalistic symbiosis. Individuals of *S. arizonica* forage in galleries and tunnels of *T. arizonensis* nests but do not steal fungus or brood. Instead, individuals of *S. arizonica* hunt collembolans in the internal refuse piles of *T. arizonensis* nests. Interestingly, *S. arizonica* was never found independent of its host *T. arizonensis* over a significant portion of the geographic range of *T. arizonensis*. These results suggest a tight but asymmetric association where compound nesting is obligate for *S. arizonica* and facultative for *T. arizonensis*.

**Keywords** Attini · Commensalism · Formicidae · Mutualism · Social parasitism · Symbiosis

## Introduction

Ants (Hymenoptera: Formicidae) are involved in every conceivable form of symbiosis with their biotic environment spanning the spectrum from mutualism to parasitism (Hölldobler and Wilson 1990). Examples of mutualisms, interactions where both partners benefit, are well documented between ants and plants as well as between ants and fungi (Hölldobler and Wilson 1990, 2011; Heil and McKey 2003; Mehdiabadi and Schultz 2010; Mueller et al. 2017). Ants are also frequently involved in symbiotic interactions with other ant species (Hölldobler and Wilson 1990). These myrmecosymbioses (in reference to Stumper's 1950 "myrmecobioses") are usually socially parasitic or commensalistic (Hölldobler and Wilson 1990; Buschinger 2009) but may include rare mutualistic interactions (Vantaux et al. 2007; Menzel and Blüthgen 2010). Myrmecosymbioses occur in mixed colonies and compound nests (Huber 1810; Forel 1874; Wasmann 1891; Wheeler 1901). In mixed colonies, two ant species share a nest space, interact frequently, and store brood in a common area where they are cared for by one species, which is usually referred to as the "host" (Wheeler 1910; Hölldobler and Wilson 1990). All forms of true social parasitism in ants are examples of mixed colonies including the temporary, dulotic (or "slave-making"), and inquiline social parasites (Hölldobler and Wilson 1990; Buschinger 2009). In contrast, in compound nests two ant species share a nest space or nest close to one another and occasionally interact, but they keep and rear their brood separately (Wasmann 1891; Wheeler 1901; Hölldobler and Wilson 1990). Interactions in compound nests range from commensalism, where one species benefits from the nest environment of a host species and occasionally feeds on food scraps of the host species, to parasitic interactions, where the parasite feeds on host brood or is fed by the host via trophallaxis (Table 1)

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**Table 1** Different types of myrmecosymbioses in compound nests according to Wasmann (1891), Forel (1898), Wheeler (1901, 1910), and Hölldobler and Wilson (1990). Definitions were modified from Hölldobler and Wilson (1990), and Kaufmann et al. (2003)

| Symbiosis | Species interaction | Definition | Original references |
|---|---|---|---|
| Plesiobiosis | Neutralism | A rudimentary association in which different ant species nest close to each other but engage in little or no direct communication and are not biologically interdependent | Wheeler (1901) |
| Cleptobiosis | Commensalism or parasitism | The relation in which one species builds a nest near another species and scavenges in the refuse piles or robs its food stores | Forel (1901) |
| Lestobiosis | Parasitism or predation | The relation in which colonies of a small ant species live in the walls of a larger ant species' nest and steals food or preys on brood | Forel (1901) |
| Parabiosis | Commensalism or mutualism | The relation in which two or more species use the same nest and sometimes the same odor trails but keep their brood separate | Forel (1898) |
| Xenobiosis | Commensalism or parasitism | The relation in which one species lives in the nest of another species, but keeps the brood separate, and moves freely inside the nest of the other species to obtain food usually via trophallaxis | Wheeler (1901) |

(Hölldobler and Wilson 1990; Breed et al. 2012; Kanizsai et al. 2013; Gallego-Ropero and Feitosa 2014; McGlynn et al. 2015). Traditionally, interactions between ant species living in compound nests were categorized with a set of Hellenistic terms (Forel 1898, 1901; Wheeler 1901, 1910) (Table 1). However, these terms are not congruent with terminology and definitions used to describe species interactions nowadays, nor do they represent mutually exclusive definitions. Therefore, we apply species interactions terminology and concepts for myrmecosymbioses in this paper.

*Strumigenys* (Myrmicinae: Attini) is a hyper-diverse group of specialized hunters that prey upon Collembola and other small leaf litter dwelling arthropods (Brown and Wilson 1959; Bolton 2000). Interestingly, this genus contains multiple species that form compound nests with other ants (Wesson 1935; Kaufmann et al. 2003; Yéo et al. 2006). A well-known instance is *Strumigenys pergandei* Emery in northeastern North America, which lives as a commensal of *Aphaenogaster fulva* Roger, *Aphaenogaster rudis* Enzmann, and several *Camponotus* and *Formica* species (Wesson 1935; Brown 1964). *Strumigenys pergandei* nests adjacent to or inside the nest of its host. Workers walk around freely with few instances of direct communication or aggression with host workers and hunt collembolans near or inside the nest (Wesson 1935). Additional cases of apparently commensalistic *Strumigenys* species in compound nests include: (i) *S. biolleyi* Forel, a presumed commensal of *Cyphomyrmex cornutus* Kempf in Costa Rica (Adams and Longino 2007), (ii) an undescribed species of *Strumigenys* in Indonesia that lives as a commensal with species of *Diacamma, Ectomomyrmex* (previously *Pachycondyla*), and *Pseudolasius* (Kaufmann et al. 2003), and (iii) *S. maynei* Forel, a commensal of *Platythyrea conradti* Emery in the Afrotropics (Yéo et al. 2006). Recently, Parmentier et al. (2017) suggested that the relationship between *S. maynei* and *P. conradti* could be mutualistic. *Strumigenys maynei* provides intranidal defense against myrmecophiles and other ant species, whereas *P. conradti* constructs the communally inhabited nest (Parmentier et al. 2017). Currently, all known *Strumigenys* commensals form compound nests with other ants but can also be found independent of their hosts.

In 1975 at Madera Canyon in the Santa Rita Mountains of the southwestern United States (Arizona: Santa Cruz County), *Strumigenys arizonica* (Ward) was discovered in a nest of the fungus-growing ant *Trachymyrmex arizonensis* (Wheeler) (Ward 1988) (Fig. 1). *Trachymyrmex arizonensis* is a common ant found at mid-elevations (ca. 1000–2000 m) in various habitats throughout mountains of the southwestern United States and the Mexican states of Chihuahua and Sonora (Rabeling et al. 2007a). *Trachymyrmex arizonensis* is widespread throughout the Madrean Sky Islands, which are isolated mountains surrounded by desert that occupy the Cordilleran gap connecting montane and tropical biota of the Rocky Mountains of the United States and the Sierra Madre Occidental of Mexico (Moore et al. 2013). Nests of *T. arizonensis* are found in exposed or shaded areas under stones

**Fig. 1** A rarely documented encounter between two workers of *Trachymyrmex arizonensis* and a single worker of *Strumigenys arizonica*. Photograph courtesy of Alex Wild

**Fig. 2** Characteristic external refuse pile of a *Trachymyrmex arizonensis* nest containing exhausted fungal substrate of primarily oak catkins (red arrow)

or logs, or in open soil. Often their presence is indicated by external refuse piles consisting of exhausted fungal substrate situated near the nest entrance, which is unique among North American species of *Trachymyrmex* (Wheeler 1907, 1911; Rabeling et al. 2007a) (Fig. 2). The discovery of *S. arizonica* in a nest of *T. arizonensis* suggested that these two species form compound nests, and since this discovery, colonies of *S. arizonica* have been collected only from within nests of *T. arizonensis* (Rabeling et al. 2007a). In contrast, sympatric *Strumigenys* species, such as *S. chiricahua* (Ward) and two undescribed species in Arizona (S.P. Cover & G.D. Alpert, pers. obs.), have only been found free-livingly.

Here, we present data from field and laboratory observations to characterize the symbiosis between the dacetine ant *Strumigenys arizonica* and the fungus-growing ant *Trachymyrmex arizonensis*. We provide novel natural history information and behavioral observations for both species, describe interspecific interactions, and evaluate the geographic extent of this myrmecosymbiosis.

## Materials and methods

### Locating compound nests of *S. arizonica* and *T. arizonensis*

Field research was conducted between 1986 and 2017 across Madrean Sky Islands in Arizona and northern Mexico, where the fungus-growing ant *T. arizonensis* is common (Fig. 3). Collections were made between June and October in habitats that included oak woodlands, oak-pine-juniper woodlands, riparian chaparral, and mesquite riparian woodland (Table 2).

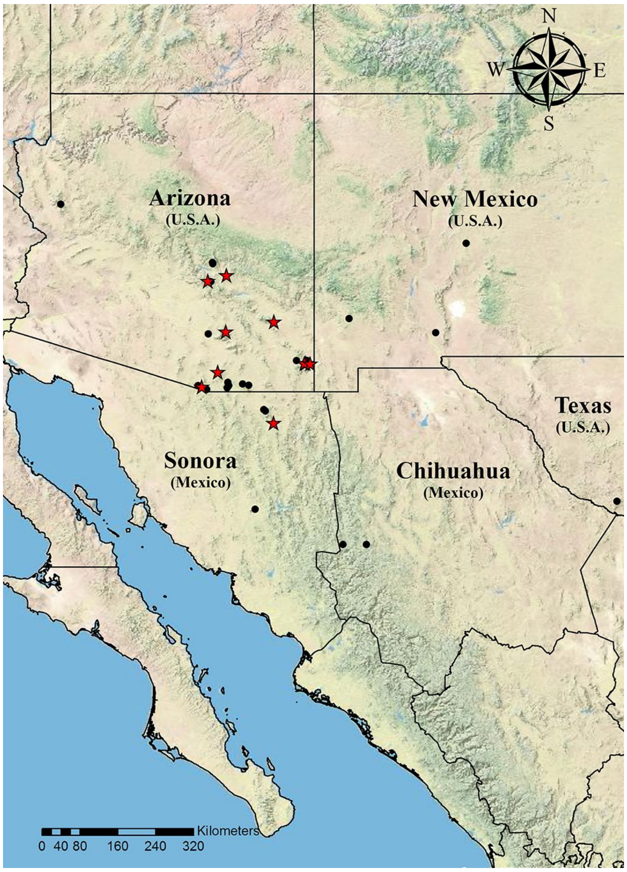

**Fig. 3** Geographic distribution of *Strumigenys arizonica* and *Trachymyrmex arizonensis* in southwestern North America. Compound nests are indicated by red stars and independent nests of *T. arizonensis* are indicated by black circles

In 2017, two localities were sampled intensively for compound nests of *S. arizonica* and *T. arizonensis*: one locality in the Superstition Mountains (USA: Arizona, Pinal County, USFS Road 287A at Pinto Creek; hereafter Pinto Creek), and two proximate localities in the Chiricahua Mountains (USA: Arizona, Cochise County, Junction West Cave Creek and West Herb Martyr Roads, and West Cave Creek Road at 1.8 km NW West Herb Martyr Road; hereafter, collectively as SWRS) (Table 2). The external refuse piles of *T. arizonensis* provided landmarks for locating nests that potentially harbored *S. arizonica* (Fig. 2). To test for presence of *S. arizonica*, nests of *T. arizonensis* were opened by turning over stones or logs to reveal the uppermost chamber or stratum which was then observed for at least two minutes. During the Arizona monsoon season (June–September), *S. arizonica* is readily visible, when present, because foragers move to upper chambers of the nest. When conditions were dry, one gallon of water was poured onto the area surrounding the nest entrance of *T. arizonensis* and then revisited 24 h later. If *S. arizonica* was not apparent, a soil sample from the entire upper chamber was collected and manually

**Table 2** Collection records for compound nests of *Strumigenys arizonica* and *Trachymyrmex arizonensis*

| Country/state | Locality | Collector(s) & code | Collection date | Latitude | Longitude | Elevation (m) |
|---|---|---|---|---|---|---|
| USA/Arizona | Cochise County: Chiricahua Mountains, Southwestern Research Station, 1.8 km NW SWRS along FSR 42. Oak-pine-juniper woodland; rocky soil | R.A. Johnson, K.W. Gray, & C. Rabeling (CR170713-01) | 12 July 2017 | 31.899 | – 109.226 | 1713 |
| USA/Arizona | Cochise County: Chiricahua Mountains, Southwestern Research Station, 0.32 km SW SWRS. Oak-pine-juniper woodland; rocky soil | K.W. Gray & C. Rabeling (KWG170715-02) | 15 July 2017 | 31.882 | – 109.209 | 1664 |
| USA/Arizona | Cochise County: Chiricahua Mountains, Southwestern Research Station, 3.38 km W SWRS. Oak-pine woodland | R.A. Johnson (RAJ2531) | 04 Aug 2001 | 31.883 | – 109.242 | 1914 |
| USA/Arizona | Cochise County: Chiricahua Mountains, Southwestern Research Station. Juniper-dominated slope | S.P. Cover (SPC807 & 808; MCZC Ent. 540667) | 05 June 1986 | 31.884 | – 109.206 | 1659 |
| USA/Arizona | Cochise County: Chiricahua Mountains, Southwestern Research Station. Oak-pine-juniper forest just south of station | B.L. Fisher (BLF12428; CASENT0066103) | 05 Aug 2005 | 31.883 | – 109.207 | 1648 |
| USA/Arizona | Cochise County: Chiricahua Mountains, Sulphur Spring Canyon, 6 km S Portal. Oak scrub along wash | G.D. Alpert (GDA256; MCZC Ent. 676863) | 14 Aug 2002 | 31.866 | – 109.123 | 1546 |
| USA/Arizona | Gila County: Superstition Mountains, Corral Creek, 19.5 km NE Globe. Mixed riparian-chaparral-pinyon-juniper | R.A. Johnson (RAJ3600) | 16 Dec 2005 | 33.558 | – 110.719 | 1624 |
| USA/Arizona | Pima County: Santa Catalina Mountains, Stratton Canyon. Oak-juniper woodland | R.A. Johnson (RAJ2109) | 31 Sept 2000 | 32.486 | – 110.736 | 1900 |
| USA/Arizona | Pinal County: Superstition Mountains, jct. FSR 287 & 287A, 11.75 km NW jct. Rt. 60 on FSR 287, at jct. FSR 287A. Open mesquite and riparian woodland with scattered sycamore, cottonwood and juniper and ash near stream | S.P. Cover (SPC7140; MCZC Ent. 655038) | 16 Aug 2005 | 33.429 | – 111.005 | 956 |
| USA/Arizona | Pinal County: Superstition Mountains, Pinto Creek, 5.15 km W. jct. FSR 287 on FSR 287A. Dense Emory oak, *Quercus turbinella*, manzanita, acacia thicket 6 m tall | S.P. Cover (SPC7414; MCZC Ent. 655193) | 12 Dec 2006 | 33.428 | – 111.056 | 1247 |
| USA/Arizona | Pinal County: Superstition Mountains, Pinto Creek, USFS Rd 287A. Oak-manzanita-acacia woodland | K.W. Gray (KWG170809-03) | 09 Aug 2017 | 33.439 | – 111.065 | 1037 |
| USA/Arizona | Pinal County: Superstition Mountains, Pinto Creek, USFS Rd 287A. Oak-manzanita-acacia woodland | K.W. Gray (KWG170809-05) | 09 Aug 2017 | 33.438 | – 111.065 | 1037 |
| USA/Arizona | Pinal County: Superstition Mountains, Pinto Creek, USFS Rd 287A. Oak-manzanita-acacia woodland | K.W. Gray (KWG170812-02) | 12 Aug 2017 | 33.439 | – 111.065 | 1037 |
| USA/Arizona | Pinal County: Superstition Mountains, Pinto Creek, USFS Rd 287A. Oak-manzanita-acacia woodland | K.W. Gray (KWG170812-07) | 12 Aug. 2017 | 33.438 | – 111.064 | 1045 |
| USA/Arizona | Pinal County: Superstition Mountains, Pinto Creek, USFS Rd 287A. Oak-manzanita-acacia woodland | K.W. Gray (KWG170813-03) | 13 Aug 2017 | 33.438 | – 111.064 | 1045 |
| USA/Arizona | Pinal County: Superstition Mountains, Pinto Creek, USFS Rd 287A. Oak-manzanita-acacia woodland | K.W. Gray (KWG170827-06) | 27 Aug 2017 | 33.437 | – 111.062 | 1081 |
| USA/Arizona | Pinal County: Superstition Mountains, Pinto Creek, USFS Rd 287A. Riparian corridor with pinyon-juniper-oak woodland | R.A. Johnson (RAJ3474) | 29 Aug 2004 | 33.439 | – 111.069 | 1046 |

**Table 2** (continued)

| Country/state | Locality | Collector(s) & code | Collection date | Latitude | Longitude | Elevation (m) |
|---|---|---|---|---|---|---|
| USA/Arizona | Santa Cruz County: Atascosa Mountains, Yanks Canyon. Oak woodland | R.A. Johnson (RAJ2088) | 23 Aug 2000 | 31.439 | – 111.192 | 1279 |
| USA/Arizona | Santa Cruz County: Patagonia Mountains, 6.12 km SE jct. FSR 139 (N. end) on FSR 58, SE of Patagonia. Grazed open Emory oak forest with up to 9 m tall canopy on NE-facing slope, grassy understory; coarse gravel | S.P. Cover (SPC5682; MCZC Ent. 585431) | 30 Aug 1999 | 31.46[a] | – 110.67[a] | 1091 |
| USA/Arizona | Santa Cruz County: Patagonia Mountains, 8.69 km SE jct. FSR 139 (N. end) on FSR 58, SE of Patagonia. Gray oak, Emory oak forest with 11 m tall canopy on slopes of large wash; sand with clay | S.P. Cover (SPC5688; MCZC Ent. 584712) | 30 Aug 1999 | 31.45[a] | – 110.66[a] | 997[a] |
| USA/Arizona | Santa Cruz County: Pajarito Mountains, Sycamore Canyon, 0.16 km W. jct. FSR 4181 (turnoff to Sycamore Canyon) on FSR 39 at jct. FSR 682. Emory oak woodland with 9 m tall canopy on gentle slope next to large creek bed | S.P. Cover (SPC6018; MCZC Ent. 654478) | 24 Aug 2000 | 31.435 | – 111.187 | 1231 |
| USA/Arizona | Santa Cruz County: Pajarito Mountains, Sycamore Canyon, 13.04 km WNW jct. Rt. 289 on FSR 39 (2.25 km ESE jct. FSR 218). Extensive Emory oak forest with 11 m tall canopy on flat with grassy groundcover; moderate grassy-herb vegetation; coarse sand | S.P. Cover (SPC5094; MCZC Ent. 584693) | 12 Aug 1998 | 31.42[a] | – 111.16[a] | 1225[a] |
| USA/Arizona | Santa Cruz County: Pajarito Mountains, Sycamore Canyon, 13.8 km SW jct. Rt. 289 on FSR 39. Steep slopes of dry wash. Emory oak woodland with up to 9 m tall canopy with grassy understory; sandy soil with rocks | S.P. Cover (SPC5678; MCZC Ent. 584708) | 29 Aug 1999 | 31.43 | – 111.179 | 1256 |
| USA/Arizona | Santa Cruz County: Pajarito Mountains, Sycamore Canyon, 13 km WNW jct. Rt. 289 on FSR 39. (2.25 km ESE jct. FSR 218). Emory oak forest, 11 m tall canopy with grassy groundcover on flat | S.P. Cover (SPC5093; MCZC Ent. 584692) | 12 Aug 1998 | 31.43[a] | – 111.19[a] | 1225[a] |
| USA/Arizona | Santa Cruz County: Santa Rita Mountains, Madera Canyon | A. Mintzer (MCZC Ent. 34836) | 02 Aug 1975 | 31.725 | – 110.88 | 1497 |
| Mexico/Sonora | Sierra Buenos Aires, Ajos- Bavispe Reserve, 2.25 km N base camp. Oak woodland | R.A. Johnson (RAJ5706) | 15 Aug 2016 | 30.75 | – 109.822 | 1493 |

[a] GPS coordinates inferred from Google Earth

sifted using a sifting tray (sieve size 1.27 cm) to remove large pieces of organic matter and rocks and then observed for 5 min to detect individuals of *S. arizonica*.

To determine whether *S. arizonica* nests and/or forages directly outside of *T. arizonensis* nests, additional soil (Berlese) and leaf litter (Winkler) samples were collected within a 1-m radius around *T. arizonensis* nests. Thirteen colonies were sampled at SWRS and eight colonies were sampled at Pinto Creek. At SWRS, *T. arizonensis* nests were commonly surrounded by leaf litter, whereas at Pinto Creek *T. arizonensis* nests were common near creek beds without surrounding leaf litter. Both Winkler and Berlese sampling were performed on *T. arizonensis* nests observed to harbor *S. arizonica* (SWRS: $n=2$, Pinto Creek: $n=6$) and independent *T. arizonensis* nests (SWRS: $n=11$, Pinto Creek: $n=2$). Leaf litter was manually sifted and arthropods were extracted with Winkler extractors for 48 h. Berlese funnel extractions were used for soil samples for 24 h. Additional hand collecting was performed at both sites to search for independent nests of *S. arizonica*. Frequency of compound nests was estimated at SWRS (July 2017) and Pinto Creek (August 2017) by comparing the number of *T. arizonensis* nests with and without *S. arizonica*.

In addition to observing and collecting compound nests, specimens and collection records from the following entomological collections in the United States were used in this study: (i) Bohart Museum of Entomology, University of California Davis, Davis, CA (UCDC), (ii) Museum of Comparative Zoology Collection, Harvard University, Cambridge, MA (MCZC), (iii) R.A. Johnson Collection, Tempe, AZ (RAJC), and the (iv) Social Insect Biodiversity Repository, Arizona State University, Tempe, AZ (SIBR).

### Behavioral interactions between *S. arizonica* and *T. arizonensis*

Field and laboratory nest observations were conducted to characterize interactions between *S. arizonica* and *T. arizonensis*. The objectives were to: (i) observe interactions between workers of *S. arizonica* and *T. arizonensis*, i.e., antennation, grasping, biting, or lunging, (ii) determine whether *S. arizonica* consumes brood and/or fungus garden of *T. arizonensis*, and (iii) document feeding habits of *S. arizonica*.

At Pinto Creek, six compound nests were observed in situ for 30 min each when *S. arizonica* was found directly under a stone with *T. arizonensis*. Workers of both species were observed, and the location of the nest chamber for both species was recorded. After in situ observations, all six compound nests were excavated following procedures described in Rabeling et al. (2007b). When a fungus-garden chamber was encountered, the fungus garden was removed using spoon and forceps, then the chamber dimensions (height × width × depth) were recorded in cm. Presence or absence of *S. arizonica* workers inside chambers of *T. arizonensis* was recorded as well as whether *S. arizonica* hunted collembolans from external or internal refuse piles. Colonies of both species and the mutualistic fungi were maintained alive for laboratory observations by placing them in 7.5 × 7.5 cm plastic nest boxes with moistened plaster-lined floors.

Six compound nests were placed in laboratory nests using the nest setup described in Sosa-Calvo et al. (2015) (Supplementary Fig. 1). Nests were maintained at 25 °C and supplied with water for humidity and cornmeal and dry, coarsely ground rice cereal as fungal substrate for *T. arizonensis*. One additional 7.5 × 7.5 cm plaster-lined box was attached to each laboratory nest consisting of a 2.5 × 2.5 × 1 cm excavated chamber with a red-tint glass cover to supply a potential nesting space for *S. arizonica*. Each nest was observed twice per day for 30-min intervals between 8:00–10:00 h and 16:00–19:00 h for the first week. Collembolans (Entomobryomorpha: Entomobryidae and Isotomidae), isopods, mites, and myriapods (Symphyla: Scutigerellidae) were introduced to one isolated *S. arizonica* colony and one compound nest to observe hunting behavior of *S. arizonica*. Additional observations were made at irregular intervals until *S. arizonica* colonies had died, after which series of workers for both species, alate gynes, and/or males, portions of the fungus garden, were preserved in 95% ethanol. Laboratory observations were conducted under a Leica MZ 9.5 stereomicroscope. Laboratory nest images were captured using a Leica MZ 12.5 stereomicroscope with SPOT 5.1 microscope imaging software. The geographic distribution map was created with the ArcMaps (version 10.5) software package. Behavioral observations were recorded using a Dino-Lite Digital Microscope (USB2.0).

## Results

### Biogeography and frequency of compound nests of *S. arizonica* and *T. arizonensis*

Since its discovery in 1975, at least 26 compound nests of *S. arizonica* and *T. arizonensis* have been discovered at ten localities across seven Madrean Sky Islands at elevations from 956 to 1914 m in the southwestern United States and the Mexican state of Sonora (Fig. 3; Table 2). All colonies of *S. arizonica* were found within *T. arizonensis* nests. No individuals of *S. arizonica* were found outside *T. arizonensis* nests in Berlese or Winkler samples or during hand collections. All records of *S. arizonica* were from central and southeastern Arizona in the United States and the northernmost portion of Sonora in Mexico, which constitutes much of the central and western portions of the geographic range of *T. arizonensis* (Fig. 3).

Of the 35 nests of *T. arizonensis* investigated at Pinto Creek and SWRS in 2017, eight harbored *S. arizonica* resulting in an overall frequency of 23% (8/35). However, the frequency of compound nests varied between the two sites. At SWRS, 2 out of 25 nests of *T. arizonensis* harbored *S. arizonica* (8%) (2/25), whereas at Pinto Creek six out of ten nests of *T. arizonensis* harbored *S. arizonica* (60%) (6/10).

## Architecture of compound nests of *S. arizonica* and *T. arizonensis*

Compound nests of *S. arizonica* and *T. arizonensis* were found under stones in both shaded and exposed areas (Fig. 4). Nests of *T. arizonensis* contained one to three chambers of refuse (exhausted fungal substrate) or active fungus gardens, which often contained brood. Nest chambers ranged from 2×2×2 cm (8 $cm^3$) to 7×9×8 cm (504 $cm^3$). Small and large chambers contained fungus gardens and brood. The uppermost nest chambers were 2–35 cm below the surface and contained refuse but occasionally housed an active fungus garden. Refuse piles were both external and internal. Internal refuse piles were in upper chambers or close to nest entrances within galleries and tunnels. Collembolans (Entomobryomorpha: Entomobryidae) were present in tunnels as well as in both internal and external refuse piles. Collembolans were not found inside fungus-garden chambers of any independent *T. arizonensis* nest or compound nest. Fungus gardens were attached to the underside of embedded stones in the soil and often appeared confined on three sides. Chambers were connected via small tunnels between the stones. Wheeler (1907, 1911) and Rabeling et al. (2007a) provide additional information on the nest architecture of North American *Trachymyrmex*. Fungal substrate primarily consisted of oak catkins. Other fungal substrates can include insect frass and withered vegetable debris (Wheeler 1911).

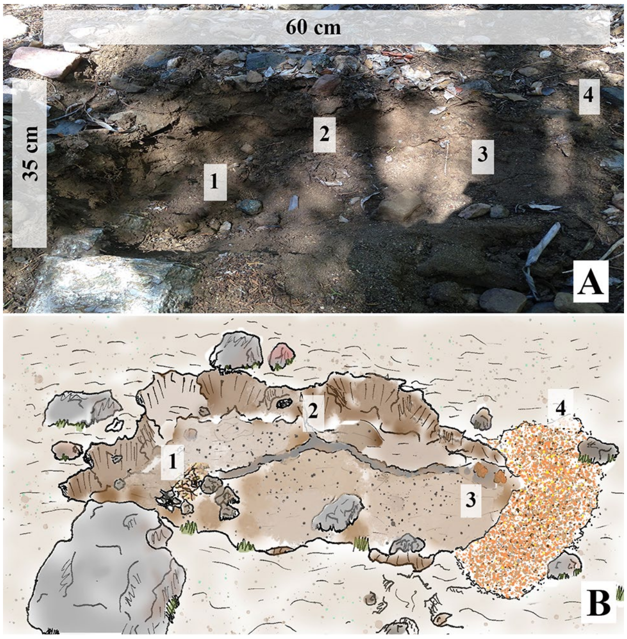

**Fig. 4** Photograph of a *Strumigenys arizonica* and *Trachymyrmex arizonensis* compound nest after removing the large stone covering the nest (**a**). Illustration of the same compound nest (**b**). Nest chamber of *T. arizonensis* (1), nest chamber of *S. arizonica* (2), two internal refuse piles of *T. arizonensis* where *S. arizonica* workers were observed to collect collembolans (3), and the external refuse pile of the *T. arizonensis* nest (4)

*Strumigenys arizonica* workers were observed in tunnels of *T. arizonensis* nests. Each compound nest contained one *S. arizonica* nest chamber and colony. Nest chambers of *S. arizonica* were 2–15 cm below the surface and were located no more than 20 cm from the nearest *T. arizonensis* chamber. One *S. arizonica* nest chamber whose structure was not destroyed by the excavation process measured 3×3×3 cm (27 $cm^3$).

## Colony composition of *S. arizonica* and *T. arizonensis*

Colonies of *T. arizonensis* contained up to 1000 or more workers and were monogynous except for one colony which had two dealate queens; the reproductive status of these two queens was not determined. *Strumigenys arizonica* colonies consisted of one or two dealate queens with 30–40 workers. *Strumigenys arizonica* males and alate queens were present from July through September, but both sexes were never found in the same nest.

## Behavioral interactions between *S. arizonica* and *T. arizonensis*

During field observations, no direct behavioral interactions, i.e., antennation, aggression, or trophallaxis, were observed between workers of *S. arizonica* and *T. arizonensis*. The fungus garden and brood of *T. arizonensis* were not observed to be attacked by workers of *S. arizonica*.

Laboratory nest observations lasted from 4 to 44 days depending on the survival of the *S. arizonica* individuals. Brief interactions between workers of *S. arizonica* and *T. arizonensis* occurred on three instances in one compound nest within 48 h of laboratory nest establishment. These interactions involved antennation and aggression, i.e., grasping or lunging. Antennation and aggression occurred at or adjacent to the food resource of either species. Antennation intervals between species lasted about 4 s and each time antennation was followed by a form of aggression. Two of the three aggressive interactions involved a *T. arizonensis* worker grasping the head of a *S. arizonica* worker and lifting her for about 5 s. The *S. arizonica* worker responded by tucking antennae into the antennal scrobes and remained completely motionless. Afterwards, the *T. arizonensis* worker placed the *S. arizonica* worker back on the ground without further aggression. The single instance of a *S. arizonica* worker showing aggression towards *T. arizonensis* occurred when the *T. arizonensis* worker approached the stockpile of collembolan prey. The *S. arizonica* worker lunged at the *T. arizonensis* worker with agape mandibles but did not make contact. This behavior was observed only once and elicited no response from the *T. arizonensis* worker. After 48 h in the laboratory nest, no form of direct interaction was observed between *S. arizonica* and *T. arizonensis*. *Strumigenys arizonica* was never observed to consume fungus garden or to rob brood of *T. arizonensis*.

## Hunting behavior of *S. arizonica*

Field observations of foraging and hunting behavior by *S. arizonica* were limited to one incident in which workers carried unidentified collembolans from the internal refuse pile near the nest entrance to their chamber. No workers of *S. arizonica* were observed to forage in the external refuse piles or in fungus gardens of *T. arizonensis*. In the laboratory, both workers and queens of *S. arizonica* were observed to hunt introduced collembolans (Entomobryomorpha: Entomobryidae and Isotomidae) as follows: (i) individuals wandered around the nest chamber until they became aware of a collembolan, (ii) the head was lowered and mandibles slowly opened while reaching toward an appendage of the collembolan, (iii) upon grasping an appendage, both the collembolan and individual were catapulted 1–2 cm via the furcula of the collembolan, (iv) the collembolan was immediately stung and paralyzed (see Supplementary Video 1), (v) subsequently, the individual started feeding on its prey (Fig. 5). The threat response (jumping) of collembolans and the stinging behavior of *S. arizonica* occurred almost simultaneously. Collembolans (Entomobryomorpha: Entomobryidae and Isotomidae) were readily accepted as food sources by *S. arizonica*, whereas isopods, mites, and myriapods (Symphyla: Scutigerellidae) were rejected.

## Discussion

Field and laboratory observations show that *S. arizonica* and *T. arizonensis* form compound nests and live in a commensalistic symbiosis. Workers of *S. arizonica* hunt collembolans in tunnels and internal refuse piles of *T. arizonensis* nests without stealing and consuming fungus garden or host brood. The feeding behavior of *S. arizonica* is consistent with observations of other short-mandibulate *Strumigenys* species described by Wesson (1935), Wilson (1953), and Masuko (1984). Interactions between the two species are rare but do include brief periods of antennation and aggression around food resources. *Strumigenys arizonica* was never found independent of its host *T. arizonensis* and the geographic distribution of *S. arizonica* covers a significant portion of the geographic range of *T. arizonensis*. These results suggest a tight but asymmetric association between the two species, where compound nesting is seemingly obligate for *S. arizonica* and facultative for *T. arizonensis*.

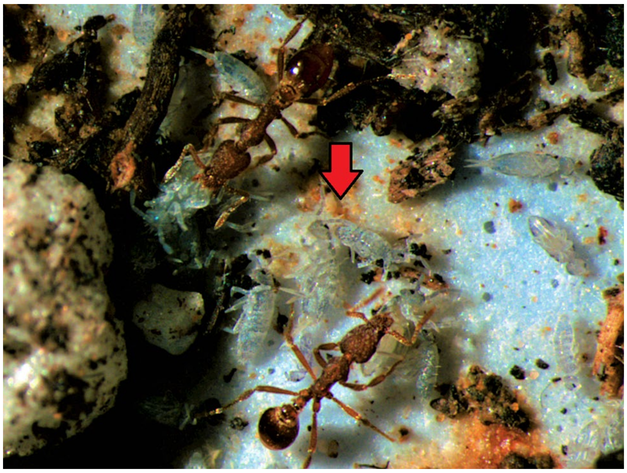

**Fig. 5** *Strumigenys arizonica* workers with a stockpile of collembolan prey (red arrow) in a laboratory nest

Nests of the fungus-growing ant *T. arizonensis* provide a suitable nesting and hunting environment for *S. arizonica*. Fungus-growing ants in general maintain a moist microclimate for their fungal symbiont by keeping their subterranean chambers at high humidity (Roces and Kleineidam 2000; Hölldobler and Wilson 2011). In general, *Strumigenys* species are well adapted to moist microclimates, which is indicated in the United States by the relatively high diversity and abundance of *Strumigenys* species in the humid southeastern states and the comparatively lower diversity and abundance in the xeric southwestern states (Ward 1988; Bolton 2000; Deyrup et al. 2000). In addition, *T. arizonensis* creates internal and external refuse piles that attract detritivores such as collembolans. These conditions are well-suited for *S. arizonica* because they provide a protected hunting ground to capture collembolans and offer environmental buffering against regularly occurring extensive droughts in southwestern North America. Three sympatric free-living *Strumigenys* species are comparatively uncommon and nest exclusively in relatively mesic habitats under rotten oak stumps that provide substantial protection against desiccation.

Despite the fact that *T. arizonensis* provides a suitable environment for *S. arizonica*, we never detected other *Strumigenys* species in nests of *T. arizonensis*. Moreover, we did not detect *S. arizonica* in nests of other sympatric

*Trachymyrmex* species such as *T. carinatus* MacKay & Mackay and *T. pomonae* Rabeling & Cover.

One question that remains unresolved is whether the apparent commensalistic symbiosis between *S. arizonica* and *T. arizonensis* is mutualistic. Mutualistic interactions have been suggested for compound nests of *Strumigenys maynei* and *Platythyrea conradti* in Ivory Coast (Parmentier et al. 2017). Under such a scenario, *T. arizonensis* would provide a stable microclimate for *S. arizonica*, whereas *S. arizonica* would provide a "pest control" service by protecting the nest environment from collembolans. Collembolans are frequently associated with fungus-growing ants (Castaño-Meneses et al. 2017), and Weber (1957), for example, regarded collembolans as significant fungus-garden pests that infest both natural and laboratory colonies. The presence of collembolans in nests of the fungus-growing ant *Cyphomyrmex costatus* Mann elicits an interesting behavioral response: workers perform a "jigging" behavior, which was interpreted as a type of colony-stress signal (Kweskin 2004). However, in both independent *T. arizonensis* nests and in compound nests, collembolans were only detected in empty tunnels, refuse chambers, and refuse piles, but never in fungus-garden chambers. This observation suggests an unlikely negative impact of collembolans on healthy *T. arizonensis* colonies. To test whether a mutualistic relationship between *S. arizonica* and *T. arizonensis* exists, fitness experiments would need to demonstrate a negative effect of collembolans on *T. arizonensis* colonies as well as establish a protective, pest control behavior of *S. arizonica*.

Our characterization of the symbiosis between *S. arizonica* and *T. arizonensis* adds to the diversity of myrmecosymbioses that involve fungus-growing ants (Hölldobler and Wilson 1990; Mehdiabadi and Schultz 2010). In general, fungus-growing ants are more frequently involved in socially parasitic rather than commensalistic interactions. Some *Megalomyrmex* species are trophic parasites and predators that usurp colonies of distantly related fungus-growing ant species (Adams and Longino 2007; Adams et al. 2012; Boudinot et al. 2013), whereas six known species of inquiline social parasites exploit closely related species in the leaf-cutting ant genus *Acromyrmex* and the lower attine genus *Mycocepurus* (Rabeling and Bacci 2010; Rabeling et al. 2014, 2015). In contrast, Sanhudo et al. (2008) observed a compound nest of *Apterostigma urichii* Forel and *Mycetophylax faunulus* (Wheeler) sharing a nest and tending a single fungus-garden, but it remains unknown whether their interactions are commensalistic or mutualistic. Additional documented but unstudied symbioses involve the arboreal fungus-growing ant *Cyphomyrmex cornutus*, which hosts a suite of ant species including the dacetine ant *Strumigenys biolleyi* in Costa Rica (Adams and Longino 2007).

Future studies will investigate the co-evolutionary history of *S. arizonica* and *T. arizonensis* across the Madrean Sky Islands of southwestern North America, as well as target poorly sampled areas in eastern portions of the geographic range of *T. arizonensis* in New Mexico and northern Mexico.

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