Parasites of the Asian Tiger Mosquito and Other Container-Inhabiting Mosquitoes (Diptera: Culicidae) in Northcentral Florida

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ABSTRACT Seven microorganisms including 4 protozoans, 2 fungi, and a bacterium infected *Aedes albopictus* (Skuse) larvae collected from 12 counties in northcentral Florida. *Ae. albopictus* and 14 other species of mosquitoes were collected from tires, flower-holding vases in cemeteries, other types of artificial containers, and treeholes. *Ascogregarina taiwanensis* (Lien & Levine) was the most common parasite of *Ae. albopictus* throughout the year. The microsporidium *Vavraia culicis* (Weiser) infected *Aedes aegypti* (L.), *Ae. albopictus, Aedes triseriatus* (Say), and *Orthopodomyia signifera* (Coquillett). A vibrio bacterium and 2 fungi (*Leptolegnia* sp. and *Smittium culisetae* Lichtwardt), infected *Ae. albopictus* larvae but were observed infrequently. *A. taiwanensis, S. culisetae,* and the vibrio bacterium previously have been reported from *Ae. albopictus*. This is the 1st report of the other 4 microorganisms parasitizing *Ae. albopictus* larvae.

KEY WORDS *Aedes aegypti*, *Aedes albopictus*, *Ascogregarina taiwanensis*, containers, parasites, Florida

Aedes albopictus (Skuse) was 1st reported in Florida in 1986 from a Jacksonville waste tire site (Peacock et al. 1988). Since then, it has spread to all 67 counties in Florida (O'Meara et al. 1995). Despite the abundance of *Ae. albopictus* in Florida, the gregarine parasite, *Ascogregarina taiwanensis* (Lien & Levine), originally described from *Ae. albopictus* larvae from Taiwan (Lien and Levine 1980), is the only parasite reported from this mosquito in Florida and the other United States (Munstermann and Wesson 1990, Garcia et al. 1994, Blackmore et al. 1995). Parasitism, seasonality, and the effect of this gregarine on *Ae. albopictus* in Florida have been discussed by Garcia et al. (1994) and Blackmore et al. (1995).

Container-inhabiting mosquitoes of Florida other than *Ae. albopictus* have been hosts to a number of parasites. Gentile et al. (1971) reported the gregarine *Lankesteria culicis* (Ross) in *Aedes aegypti* (L.), and Stapp and Casten (1971) reported the gregarine had little effect on *Ae. aegypti* populations in Florida. *Anopheles crucians* Wiedemann has been parasitized by the microsporidium *Parathelohania obesa* (Kudo) (Hazard and Weiser 1968) and the mermithid nematode *Romanomermis culicivorax* Ross & Smith (Savage and Petersen 1971). Another microsporidium, *Parathelohania anophelis* (Kudo), has been observed in *Anopheles quadrimaculatus* Say (Hazard and Weiser 1968). In the genus *Culex*, the microsporidium *Amblyospora opacita* (Kudo) has been reported from *Culex territans* Walker (Hazard and Oldacre 1975). Although these species of *Anopheles* and *Culex* do not strictly inhabit containers, they have been found in containers during times of drought.

Here, we report on *A. taiwanensis* and 6 other parasites infecting *Ae. albopictus* and parasites found in associated species of container-inhabiting mosquitoes in northcentral Florida. Our objective was to determine what parasites infected *Ae. albopictus* and associated species of container-inhabiting mosquitoes as a prelude to determining the effect of these parasites on larval mortality and the evaluation of these parasites as agents for biological control. Naturally occurring pathogens are desirable agents for control, and a continuous supply of new agents is essential for the expansion of microbial control (Burges 1981).

Materials and Methods

Larval Collection and Examination. The area sampled extended 100 km from Gainesville and included 12 counties (Fig. 1). Some cities and towns had ≥1 site where collections were made. Sites selected for sampling had definite boundaries such as a place of business, tire pile, junkyard, or cemetery, and containers within a 100-m radius were examined. The number of containers examined from a site ranged from 1 to 10 and was determined by the number that contained water.

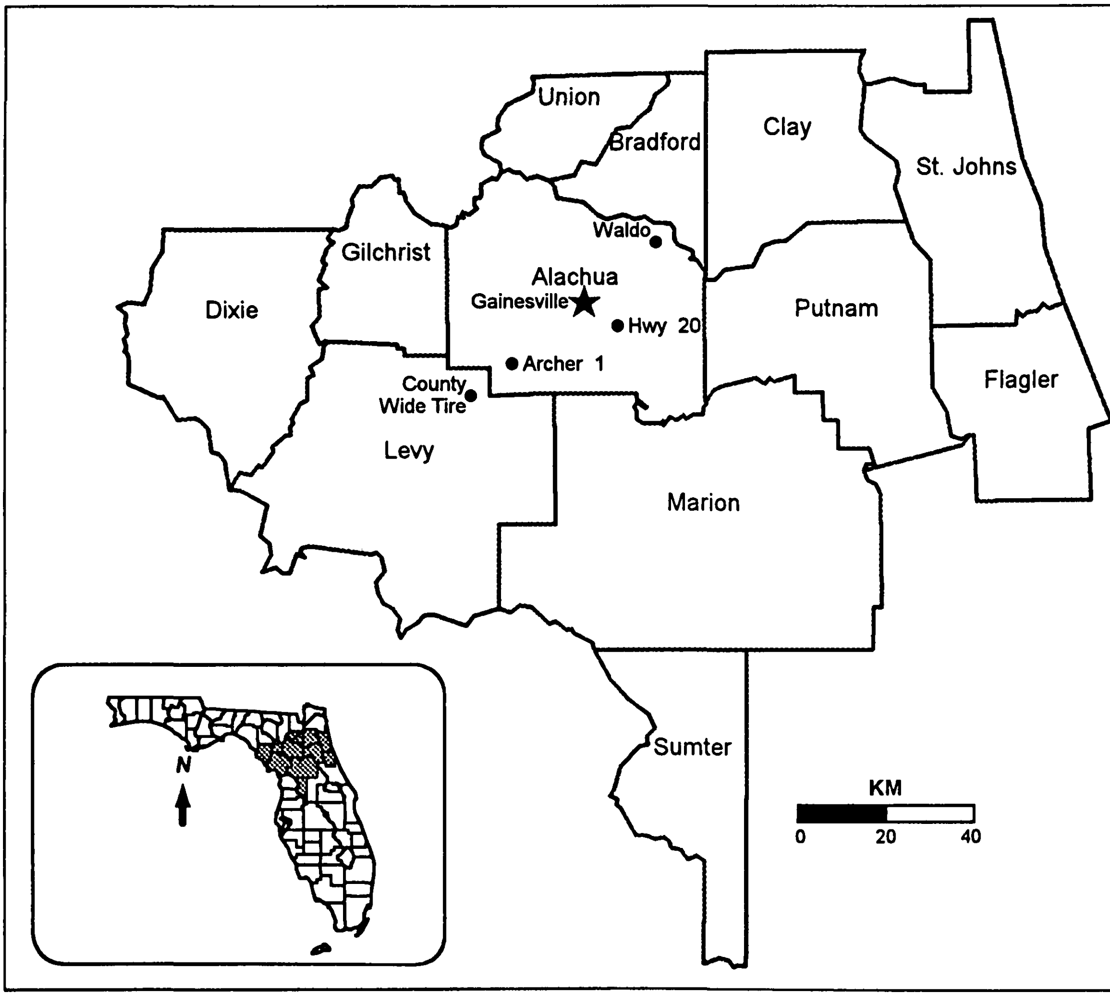


Fig. 1. The 12 northcentral Florida counties where container-inhabiting mosquitoes were collected.

Four sites (Archer 1, County Wide Tire, Highway 20, and the Waldo Salvage Yard) were monitored weekly to determine parasite seasonality. Other sites were sampled intermittently to determine parasite distribution.

Mosquito immatures were collected primarily from discarded tires and artificial containers such as steel and aluminum cans, cups, hubcaps, plasticware, and cemetery vases. Larvae also were collected from treeholes. Collections from tire piles, junkyards, and trash piles where containers were subject to change were pooled. Collections from individual treeholes and specific gravestone flower vases were kept separate by source.

Larvae and water from the habitat were transferred to bottles or plastic bags and taken to the laboratory for examination. Larvae were examined for gross abnormalities by viewing them through a dissecting microscope at 16× magnification, with top lighting and a black background.

Parasite Identification. The presence and type of microorganism(s) in apparently infected larvae was verified by light microscopy. Identification and occurrence of gut parasites was determined by examination of the guts of larvae (a sample of 20 in large collections). Other suspect tissues were examined with phase microscopy. Fresh smears of tissue infected with microsporidia were examined and photographed using a Zeiss Photo Microscope III. Fresh spores were measured with a Vicker's Image Splitting Micrometer using a phase contrast microscope at 1,000×. The ultrastructure of parasites was studied by dividing infected tissue into 0.1-mm^2 sections and fixing the tissue in 2.5% (vol:vol) glutaraldehyde buffered with 0.1 M sodium cacodylate containing calcium chloride for 2.5 h at room temperature. Tissue then was postfixed in 1% (wt:vol) osmium tetroxide for 2 h, dehydrated through a graded ethanol series into acetone, and embedded in Epon-Araldite (Mollenhauer 1964). Thin sections were poststained in methanolic uranyl acetate, followed by lead citrate (Reynolds 1963), and examined and photographed using a Hitachi H-600 electron microscope at 75 kV.

Table 1. Prevalence of parasites of container-inhabiting mosquito larvae collected from 111 sites in northcentral Florida

Host	No. sites	No. collections	No. larvae examined	Parasite	No. positive sites	No. positive collections	No. infected larvae	% infected	
								Range	Avg.
Aedes aegypti	7	24	230	*Ascorgregarina taiwanensis*	1	5	21	14.0–66.7	45.5
				Vavraia culicis	1	2	2	100	
Aedes albopictus	96	489	40,348	*Ascogregarina taiwanensis*	56	163	1,865	0.3–100	22.6
				Crithidia	1	1	1	2.4	
				Leptolegnia sp.	1	2	23	11.1–24.4	17.8
				Microsporidium 1	2	6	9	1.5–3.6	2.4
				Smittium culisetae	3	3	132	0.6–21.2	9.0
				Vavraia culicis	3	27	155	0.3–53.8	9.7
				Vibrio	1	1	1	0.2	
Aedes triseriatus	52	228	6,217	*Ascogregarina barretti*	6	17	99	2.9–100	39.0
				Smittium culisetae	2	2	5	2.4–5.6	4.0
				Vavraia culicis	1	1	3	6.3	
Culex quinquefasciatus	57	169	7,980	*Tetrahymena* sp.	1	1	1	1.8	
				Vibrio	1	1	1	7.7	
Culex restuans	25	77	4,314	*Culicospora magna*	2	2	5	1.2–1.8	1.5
Orthopodomyia signifera	32	109	1,696	Microsporidium 2	1	1	2	2.9	
				Vavraia culicis	2	2	4	2.7–4.6	3.6

Results

Overall, 510 larval collections were made from 111 sites in Alachua, Bradford, Clay, Dixie, Flagler, Gilchrist, Levy, Marion, Putnam, St. Johns, Sumter, and Union counties of northcentral Florida; most collections were near Gainesville in Alachua County. In total, 65,517 larvae were collected and examined from 356 tires, 57 cemetery vases, 48 treeholes, and 49 miscellaneous artificial containers between 12 March 1991 and 28 March 1994. Approximately 37% of the 510 collections contained 2,329 larvae parasitized with 1 or more microorganisms. Six of 15 mosquito species collected were infected with 11 different species of microorganisms (Table 1). *Aedes taeniorhynchus* (Wiedemann), *An. crucians, Anopheles perplexans* Ludlow, *An. quadrimaculatus*, *Culex nigripalpus* Theobald, *Culex salinarius* Coquillett, *Cx. territans, Toxorhynchites rutilus rutilus* (Coquillett), and *Uranotaenia lowii* Theobald were collected but were not parasitized. The most frequently observed infections in mosquito larvae were by Protozoa, which included 2 species of gregarines, 4 microsporidia, 1 ciliate, and 1 flagellate. Mosquitoes also were found to be infected with 2 species of fungi and 1 bacterium.

Gregarines. *Ascogregarina taiwanensis* was the most common parasite of *Ae. albopictus* larvae. Its distribution was widespread and was present in larvae collected from all container habitat types (Table 2). *A. taiwanensis* was found in *Ae. albopictus* larvae collected throughout the survey except for the months of January 1992 and 1994. Monthly totals of *Ae. albopictus* larvae examined and *A. taiwanensis* prevalence from the 4 sites monitored weekly are presented in Fig. 2. *A. taiwanensis* also was observed in the guts of *Ae. aegypti* larvae in 5 of 24 collections made from tires at the County Wide Tire Shop near Bronson in Levy County. There were no infections of gregarines in collections of *Ae. aegypti* larvae from 6 other sites.

Ascogregarina barretti (Vavra) was observed in the guts of *Aedes triseriatus* (Say) larvae from 17 of 228 collections. Infected larvae were found in 12 (Gainesville) of 40 treehole collections, 4 (3 in Gainesville, 1 from Wacahoota) of 138 tires, 1 (Cresent City) of 22 cemetery vases, and 0 of 14 artificial containers. *Ae. triseriatus* infected with *A. barretti* and *Ae. albopictus* infected with *A. taiwanensis* were found together in 5 of the collections from Gainesville, Wacahoota, and Cresent City; however, no cross-infections were observed. The morphology of the gamonts of the 2 gregarines remained distinct in each host type and was consistent with the original descriptions from *Ae. albopictus* and *Ae. triseriatus* (Vavra 1969, Lien and Levine 1980).

Table 2. *Ascogregarina taiwanensis* infections in *Ae. albopictus* larvae collected from four container types in northcentral Florida

Container type	No. collections	No. larvae examined	No. positive collections (%)	No. larvae infected	% infected	
					Range	Avg
Artificial containers	49	2,852	19 (40)	216	1.3–78.6	17.9
Cemetery vases	57	1,632	29 (51)	155	11.1–100	31.5
Tires	356	35,336	112 (31)	1,479	0.3–100	22.6
Treeholes	48	528	3 (6)	15	16.0–21.6	19.7

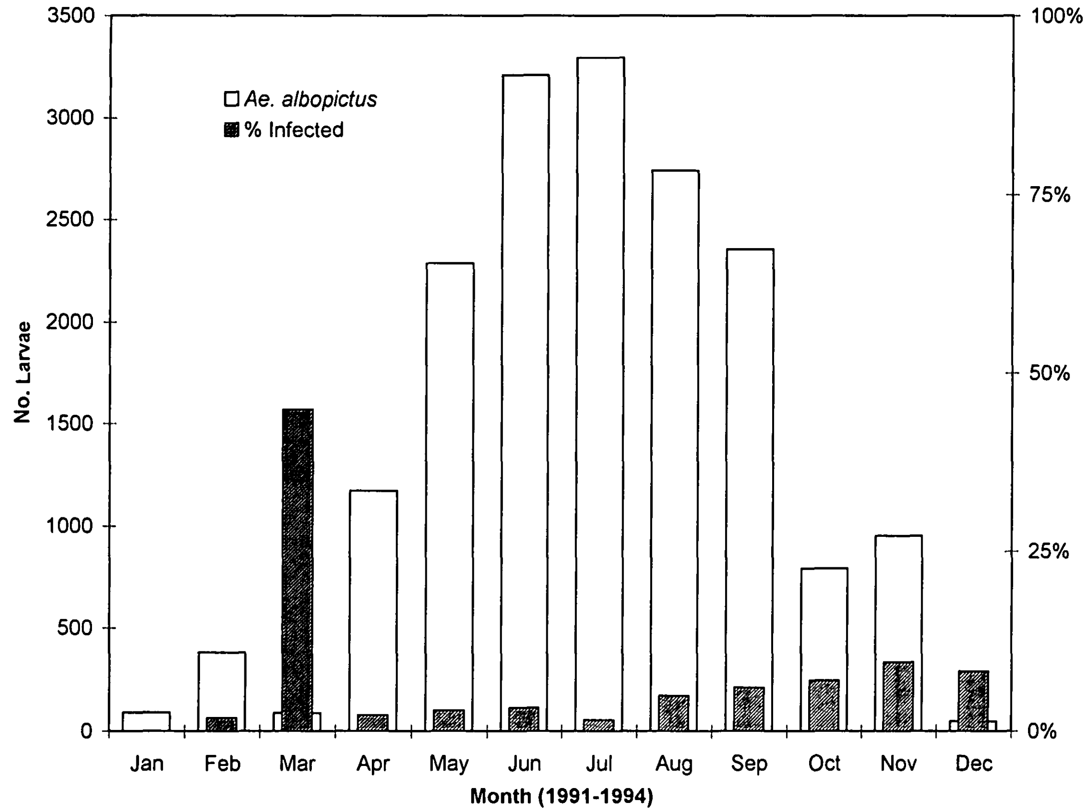
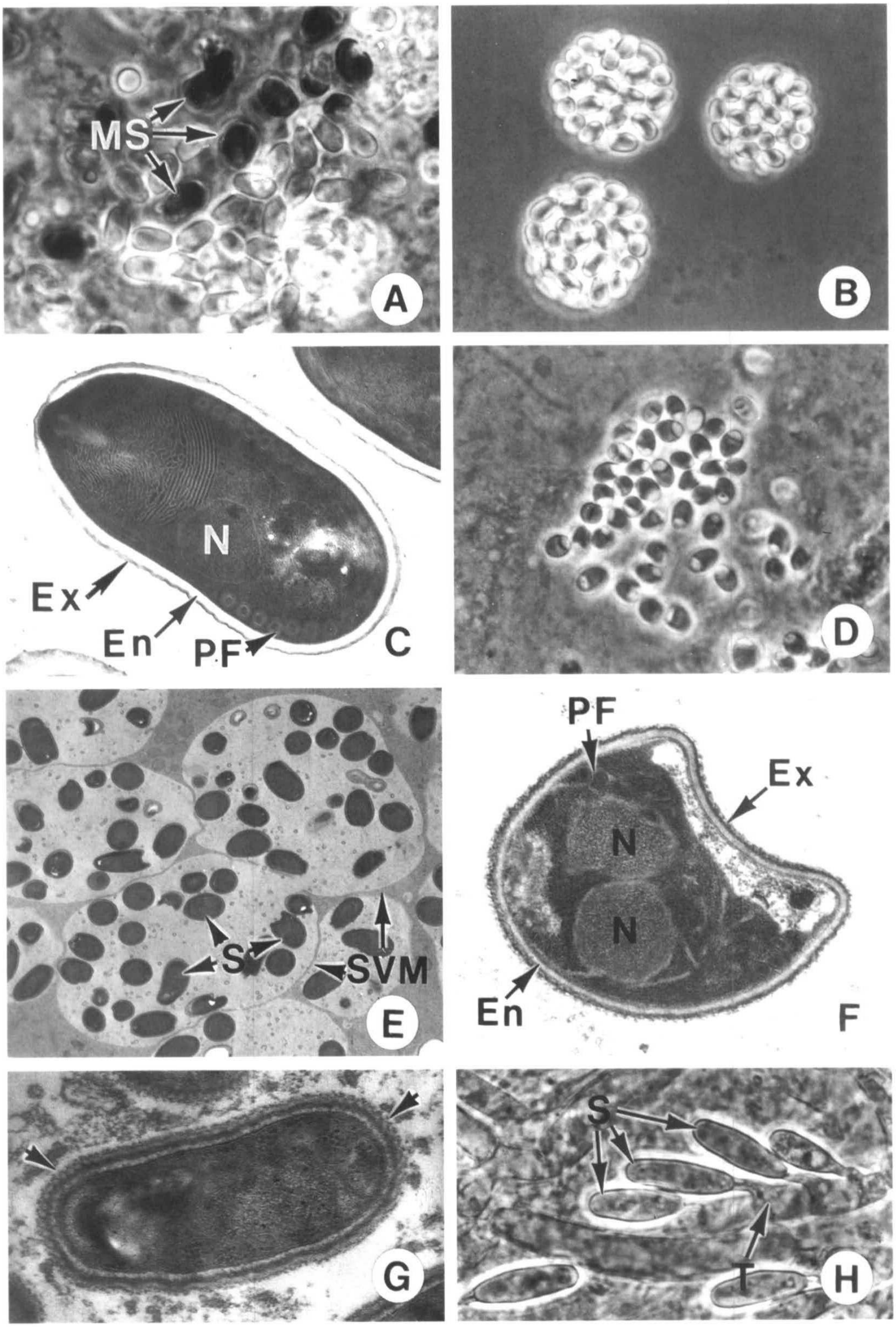


Fig. 2. Monthly occurrence of *A. taiwanensis* and *Ae. albopictus* larvae at 4 sites (Archer 1, County Wide Tire, Highway 20, and Waldo) from 1991–1994.

Microsporidia. *Vavraia culicis* (Weiser) was the most common microsporidium infecting *Ae. albopictus* larvae, with infections apparent as white opaque patches of spores in the fat body of the 4th and 5th abdominal segments. There appeared to be host reaction to the infection as melanization of spores occurred (Fig. 3A). Fresh preparations revealed large groups of oblong spores within a sporophorous vesicle (Fig. 3B). These spores measured 5.4 ± 0.4 by 3.2 ± 0.1 μm (n = 25). Ultrastructurally, spores comprised a single nucleus, a thin rugose exospore, a thick endospore, lamellate polaroplast, and an isofilar polar filament with ≈11 turns (Fig. 3C). Infections were present in collections of *Ae. albopictus* larvae from tires at Archer 1, Archer 2, and from Citra. *V. culicis* also was observed in larvae of *Ae. aegypti*, *Ae. triseriatus*, and *Orthopodomyia signifera* (Coquillett) collected from tires. The infection was located in the fat body and had the same general appearance as in *Ae. albopictus*. Ultrastructurally, the spores from the 4 hosts were identical; however, the fresh spores in *Or. signifera* were smaller and measured 4.9 ± 0.4 by 3.1 ± O.2 μm (n = 25) with no melanization. Infected *Ae. aegypti* larvae were collected at Citra, *Ae. triseriatus* at Wacahoota, and *Or. signifera* at Highway 20 and Citra.

Other microsproridia were observed less often. An unidentified microsporidium in *Ae. albopictus* larvae, designated Microsporidium 1, was manifest as white opaque patches in the fat body of the 2nd and 5th abdominal segments. Infections comprised small, slightly oblong spores in the fat body (Fig. 3D) that measured 3.3 ± 0.3 by 2.5 ± 0.3 μm (n = 25). Multisporous sporogony occurred within a sporophorous vesicle (Fig. 3E). Ultrastructurally the spores were binucleate with a thin exospore and endospore and a polar filament of 2–3 coils (Fig. 3F). Infected larvae were collected from tires located in a wooded area near Gainesville and from tires in a junkyard adjacent to woods at Highway 20. An unidentified microsporidium in *Or. signifera*, designated Microsporidium 2, was observed in a larva from a treehole on the University of Florida campus in Gainesville. The infection was apparent as white opaque areas in the fat body in the posterior abdomen. Spores were small, oblong, and measured 2.2 ± 0.1 by 1.4 ± 0.1 μm (n = 20). Ultrastructurally, rows of small projections gave the surface of the spore wall a corrugated appearance (Fig. 3G). The microsporidium *Culicospora magna* (Kudo) was observed in larvae of *Culex restuans* Theobald collected from containers at Highway 20 and in Gainesville . Spores were large, pyr-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iform, and occurred in the fat body and in the hemolymph of the larvae.

Other Microorganisms. Two additional protozoan parasites were encountered. A ciliate, *Tetrahymena* sp., was observed in the hemocoel of a *Culex quinquefasciatus* Say larva collected at Grove Park, and a crithidial flagellate was observed in the gut of an *Ae. albopictus* larva from Archer 1. *Leptolegnia* sp., a fungal parasite, was present in 2 collections of *Ae. albopictus* larvae from Bronson. A 2nd fungal parasite, *Smittium culisetae* Lichwardt, was observed in the larvae of *Ae. albopictus* collected from a tire at Archer 1 and a water-filled bucket in Waldo. *S. culisetae* also was observed in a collection of *Ae. albopictus* and *Ae. triseriatus* larvae from a tire in Gainesville and in an *Ae. triseriatus* larva collected from a treehole in Gainesville. Hyphae and fusiform spores formed at the ends of thalli were observed in the lumen of the hindgut of larvae infected with *S. culisetae* (Fig. 3H). A vibrio bacterium was found in an *Ae. albopictus* larva collected at Bronson and in a *Cx. quinquefasciatus* larva collected at Grove Park.

Discussion

The distribution of the parasites varied spatially. *A. taiwanensis* was found in 56 sites throughout the 12-county area where *Ae. albopictus* larvae were collected, *A. barretti* was found in 6 sites where *Ae. triseriatus* larvae were collected; however, the remaining parasites were restricted to only 1 or 2 sites. *A. taiwanensis* was present in populations of *Ae. albopictus* collected at remote tire disposal sites and isolated rural cemeteries throughout the 12 counties. The widespread distribution of this gregarine indicates the importance of dispersal by contaminated used tires and other containers (Munstermann and Wesson 1990, Blackmore et al. 1995). A likely mechanism of distribution would be by infected females depositing eggs contaminated with desiccation-resistant oocysts in containers and the subsequent movement of these containers. There have been no reports of transovarial transmission of gregarines; however, transovum transmission (contamination of egg surfaces with oocysts during oviposition by infected females) can occur (Vavra 1969). O'Meara et al. (1992) also implicated the practice of reusing plastic floral baskets as a means of dispersing *Ae. albopictus* from cemetery to cemetery in Florida. Eggs laid by an infected female in a basket at one cemetery may be transported to another cemetery that is at a greater distance than the normal flight range of the adults. This scenario would explain the presence of the gregarine in populations of *Ae. albopictus* at remote rural cemeteries. Although *A. taiwanensis* was observed in larvae from all the container types, the percentage of collections positive from treeholes was only 6% compared with ≥30% in artificial containers, cemetery vases, and tires. In the case of movable containers, infected populations may be transported by contaminated eggs attached to the containers. The proximity of other water holding containers would facilitate the spread of infections, whereas treehole infestation is dependent upon dispersal of infected adults.

The distribution of other protozoans, bacteria, and fungi that infect *Ae. albopictus* was limited to 1 or 2 sites. The rapid spread of the mosquito apparently has not been associated with rapid dissemination of these microorganisms. Blackmore et al. (1995) reported that ≥67% of the sites in their study contained larvae infected with gregarines. In our study, 58% of the sites contained gregarine infected larvae; however, ≤3% of the sites contained larvae infected with other parasites. This difference between the gregarine and other parasites maybe explained by the fact that the spread of a parasite by infected mosquitoes is related directly to parasite prevalence and its distribution in the source population (Blackmore et al. 1995). Nongregarine parasites were found at no more than 3 sites, and only in 1 parasite (*Leptolegnia* sp.) was the average infection level ≥10%. In contrast, *A. taiwanensis* infecting *Ae. albopictus* was found at 56 sites with an average infection of ≥20%. Nongregarine parasites also may not have a desiccation-resistant stage to persist through dry periods.

In agreement with Garcia et al. (1994), *A. taiwanensis* was present in *Ae. albopictus* populations throughout the year. However, the tires in their experimental study site were never allowed to dry because water was added to maintain a habitat for continuous *Ae. albopictus* breeding. In the 4 field sites that we monitored regularly, there were periods when no larvae were collected because of dry conditions. Therefore, *A. taiwanensis* infections were present in the months with abundant rainfall and high larval populations; at the County Wide Tire site, April to August 1991 and June to November 1993.

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Fig. 3. Parasites observed in container-inhabiting mosquitoes of northcentral Florida. (A) Fresh spores of *V. culicis* in *Ae. albopictus;* MS, melanized spores (1,200×). (B) Fresh preparation of sporophorus vesicles of *V. culicis* in *Ae. albopictus* (1,000×). (C) Electron micrograph of *V.culicis* spore; En, endospore; Ex, exospore; N, nucleus; PF, polar filament (20,000×). (D) Fresh spores of Microsporidium 1 in *Ae. albopictus* (1 ,200×). (E) Electron micrograph of Microsporidium 1 multisporous sporophorous vesicles; S, developing spores; SVM, sporophorous vesicle membrane (4,000×). (F) Electron micrograph of Microsporidium 1 spore; En, endospore; Ex, exospore; N, nuclei; PF, polar filament (30,000×). (G) Electron micrograph of Microsporidium 2 spore in *Or. signifera;* arrows, corrugated exospore (45,000×). (H) *S. culisetae* spores and hyphae in hindgut of *Ae. triseriatus* ; S, spore; T, thallus (1,200×).

Although *Ae. albopictus* in the United States probably originated from Japan (Hawley et al. 1987), the *A. taiwanensis* that is common in the United States has not been reported from Japan. However, there have been no survey of parasites of *Ae. albopictus* from Japan. The absence of *A. taiwanensis* in the United States before the discovery of *Ae. albopictus* indicates its probable introduction with the *Ae. albopictus* from mainland Japan (Munstermann and Wesson 1990). An earlier example of this type of introduction was observed by Laird (1982) in the Tokelau Islands, where a gregarine and a microsporidian became established in the local population of *Aedes polynesiensis* Marks after the accidental importation of infected *Ae. aegypti*. A thorough examination of *Ae. albopictus* in Japan most likely would reveal the presence of the gregarine. Of interest would be its prevalence, distribution, and effect on sympatric populations.

Ascogregarina culicis has been reported from *Ae. aegypti* (Gentile et al. 1971). On the basis of morphology and susceptibility studies, Garcia et al. (1994) determined that the gregarine parasitizing *Ae. aegypti* from the County Wide Tire site was a strain of *A. taiwanensis* originally reported from Taiwan (Lien and Levine 1980). However, *A. taiwanensis* did not complete development in *Ae. aegypti,* and no oocysts were produced in adults. This observation agrees with the original cross-infection studies of Lien and Levine (1980).

Spores of Microsporidium 1 found in *Ae. albopictus* are similar to spores of *Pilosporella chapmani* Hazard & Oldacre reported from *Ae. triseriatus* in Louisiana (Hazard and Oldacre 1975). Becnel et al. (1986) studied the development of *P. chapmani* and determined that octosporus sporogony occurred in larvae and that these octospores were uninucleate. Microsporidium 1, however, undergoes multisporus sporogony resulting in large sporophorous vesicles containing many binucleate spores. The taxonomic status of this microsporidium and Microsporidium 2 from *Or. signifera* has yet to be determined.

The results of our survey show that populations of *Ae. albopictus* and other container-inhabiting mosquitoes of northcentral Florida harbor numerous microorganisms as parasites. However, of the 11 parasites of *Ae. albopictus* listed by Hawley (1988), only *A. taiwanensis, S. culisetae,* and the vibrio bacterium were observed in *Ae. albopictus* of northcentral Florida. The majority of parasites reported from Asia were not observed in Florida. As *Ae. albopictus* spreads southward into tropical Central and South America, it remains to be seen if the gregarine will remain the prominent parasite, if other parasites observed in Southeast Asia will be found, or if new exotic parasites will be observed.

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