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SCIENTIFIC NOTE

AEDES JAPONICUS: A TENANT INVADER IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK, USA[1]

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ABSTRACT. Invasive organisms may cause ecologic, economic, and public health harm. *Aedes japonicus* is an invasive mosquito species of known ecologic and public health importance that has widely spread throughout the eastern USA since initially being recognized in Connecticut in 1998. Here, we report the known distributions of *Ae. japonicus* within the Great Smoky Mountains National Park (GSMNP) since its initial recognition in the park in 2004. From 2006 to 2022, we sampled eggs, larvae, and adult life stages through targeted, haphazard, and convenience collections. Through these efforts, we surveyed 23 (54.7%) of the 42 watersheds within the GSMNP. *Aedes japonicus* was present in 19 (82.6%) of the sampled watersheds, and the species was confirmed, in some instances, at the same location over multiple years, suggesting it remains entrenched. This species was observed in 45.2% of the GSMNP watersheds at elevations ranging from 347 to 1,478 m. Naturally occurring containers (i.e., riverine rock pools) were common collection sites in this study. The results of our findings are presented in the context of the species distribution within the park, the public health relevance given the GSMNP's public visitation rate (>12 million annually), potential species interactions, and the persistence of this species over the multiyear study.

KEY WORDS *Aedes japonicus*, invasive species, rock pools, southern Appalachia

Aedes japonicus (Theobald) was initially recognized in New York and New Jersey, USA, in 1998 (Peyton et al. 1999). During the past 25 years, this species has spread to 7 Canadian provinces and 38 states, including North Carolina and Tennessee (Peach et al. 2019, Sames et al. 2022, Cawthon et al. 2023, Monath 2023). There is molecular evidence of multiple introductions since the initial recognition (Kaufman and Fonseca 2014, Monath 2023). This invasive mosquito is known to have complex interactions with other container-inhabiting species and is commonly found in riverine rock pools in the southern Appalachian Region (Byrd et al. 2019). From a public health perspective, it is considered a secondary vector of La Crosse virus (LACV), and there are multiple reports detecting West Nile virus in field collected samples (Kaufman and Fonseca 2014, Westby et al. 2015).

Located along the border of North Carolina and Tennessee, the Great Smoky Mountains National Park (GSMNP) contains more than 522,000 acres and receives >12 million visitors annually, more than any other US national park. The GSMNP also contains over 2,100 mi (3,360 km) of riverine ecosystems and is located within a known focus of endemic LACV transmission (National Park Service [NPS] 2015, Davis et al. 2024). Indeed, there are reports of LACV exposure likely occurring in GSMNP visitors, with some historical reports dating back to 1965 (Kelsey and Smith 1978). Therefore, the invasion and entrenchment of *Ae. japonicus* within the GSMNP has direct public health relevance.

Here, we document the known distributions of *Ae. japonicus* within the GSMNP since its incidental recognition in a study of trichomycete insect symbionts in 2004 (White et al. 2006). We sampled *Ae. japonicus* eggs, larvae, and adult life stages within the GSMNP through a series of targeted surveys and haphazard or convenience collections from 2006 to 2022 (Table 1). Mosquitoes were identified using a stereomicroscope (total magnification 90X, Olympus SZ61; Olympus, Waltham, MA) and a dichotomous key (Darsie and Ward 2005). Samples were primarily taken as larvae from riverine rock pools within the GSMNP during the months of May–October. Additional collections from ovitraps, CO_2-baited Centers for Disease Control and Prevention light traps, and incidental (landing mouth aspiration) encounters with host-seeking adults also yielded *Ae. japonicus*. Other mosquito species found in rock pools with *Ae. japonicus* included *Ae. atropalpus* (Coquillett), *Culex territans* (Walker), and *Anopheles punctipennis* (Say); ovitraps that contained *Ae. japonicus* frequently yielded both *Ae. albopictus* (Skuse) and *Ae. triseriatus* (Say) (Table 1). These observations are consistent with other studies in the region (Byrd et al. 2019, Tamini et al. 2021).

Riverine rock pools are well established as suitable habitat for *Ae. japonicus* in the southern Appalachian

[1] The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the National Park Service or the Centers for Disease Control and Prevention.

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Table 1. *Aedes japonicus* collections in the Great Smoky Mountains National Park (2006–22).

Watershed (map identification)	Location	Collection method	Year	Compatriots	Elevation (m)	Coordinates
		Tennessee				
Lower Abrams Creek (2)	Campground	Larval	2019	*Anopheles punctipennis*	354	35.61056, −83.93583
Upper Abrams Creek (5)	Cades Cove	Larval	2006	—	522	35.59420, −83.84213
Cosby Creek (34)	Dump Station	Larval	2015	—	522	35.75639, −83.20920
Dudley Creek (22)	Horse Trail	Larval	2006	—	567	35.71985, −83.43683
Goshen Prong, Little River (12)	The Sinks	Larval	2022	*Ae. atropalpus*	998	35.66950, −83.66278
	Curry Mountain Trailhead	Larval	2006	—	512	35.67538, −83.64590
Le Conte Creek (17)	Twin Creeks Trail	Ovitrap	2016	*Ae. albopictus* *Ae. triseriatus*	687	35.70077, −83.51126
	Cherokee Orchard Road	Larval Adult	2006 2007	—	717	35.68004, −83.48857
Upper Little Pigeon River (23)	Greenbrier Road	Larval	2015	*Ae. atropalpus* *Culex territans*	423	35.73318, −83.41082
Rudd Branch, Little River (8)	Chestnut Top Trailhead	Larval	2006	—	347	35.66032, −83.70867
Upper West Prong, Little Pigeon River (16)	Sugarlands Headquarters	Ovitrap	2016	*Ae. albopictus*	443	35.68783, −83.53655
	Sugarlands Headquarters	Larval	2006	—	443	35.68630, −83.53770
	Mile Post 9.39 Parking	Larval	2006	—	1,146	35.63887, −83.46632
West Prong, Little River (9)	Lead Cove Trail	Larval	2006	—	625	35.60274, −83.74217
	Tremont	Ovitrap	2006 2007	—	445	35.65245, −83.70239
	Tremont	Ovitrap	2006 2007	—	457	35.63772, −83.68884
	Tremont	Larval	2006 2007	—	466	35.63944, −83.69140
	Tremont Friendship Circle	Ovitrap	2006 2007	—	448	35.63959, −83.68851
		North Carolina				
Big Creek (36)	Big Creek Trail	Larval	2014	*Cx. territans*	521–753	35.75042, −83.10975 35.74900, −83.11398 35.74738, −83.11613 35.73878, −83.12575 35.73943, −83.12653
Cataloochee Creek (40)	Cataloochee Creek Road	Larval	2022	—	786	35.64041, −83.07868
Deep Creek (20)	Deep Creek Campground	Larval	2018	—	552	35.46170, −83.43630
	Deep Creek Trail	Larval	2014	—	577	35.47184, −83.42807
Hazel Creek (10)	Hazel Creek Trail	Larval	2015	—	517	35.47279, −83.72906
Lower Jonathan Creek (42)	Purchase Knob	Adult; Ovitrap; Larval	2006 2007	—	1,478	35.58623, −83.06121 35.58403, −83.07352
Noland Creek (14)	Noland Creek Trail	Larval	2018	—	1,481	35.45835, −83.52652 35.45706, −83.52754

Table 1. Continued.

Watershed (map identification)	Location	Collection method	Year	Compatriots	Elevation (m)	Coordinates
Lower Oconaluftee River (27)	Visitor Center	Ovitrap	2016	*Ae. albopictus* *Ae. triseriatus*	552	35.51335, −83.30605
	Mingus Creek Trail	Larval	2016	—	576	35.52070, −83.30912
Upper Oconaluftee River (25)	Collins Creek Comfort Station	Adult	2016	—	752	35.56934, −83.33763
Palmer Creek (39)	Boogerman Trail	Larval	2006	—	619	35.60286, −83.10038
	Hemphill Bald Trail	Larval	2006	—	926	35.55266, −83.15348

Region, and some authors have postulated movement through riverine corridors (Gray et al. 2005, Byrd et al. 2019). Thus, we organized our collections based on watershed systems with the GSMNP. In total, 23 of the 43 total watersheds in the GSMNP were surveyed, and *Ae. japonicus* was present in 19 watersheds, representing 82.6% of watersheds surveyed and 45.2% of the total watersheds in the park (Fig. 1). *Aedes japonicus* was also observed in the same watersheds, including the same collection location, over multiple years, demonstrating that this species is likely well established in the park.

Notably, *Ae. japonicus* was collected across a wide range of elevations from 347 to 1,478 m (Table 1), which supports prior observations that elevation may not grossly constrain the distribution of the mosquito in the southern Appalachians (Bevins 2007). *Aedes atropalpus*, an autogenous native rock pool species, was occasionally observed with *Ae. japonicus*; however, most of the rock pools that contained mosquito larvae did not contain the native species. Prior studies in the region suggest that riverine rock pools that are less exposed (i.e., shaded) and maintain cooler temperatures are more likely to contain *Ae. japonicus* (Byrd et al. 2019). Most rivers and creeks within the GSMNP are narrow and have well-established tree canopies at the riverbanks; some primary creeks are completely covered by canopies. Taken together, most of the riverine rock pools within the GSMNP are likely highly suitable habitat for the invasive species. Unfortunately, little is known about the historical distribution of *Ae. atropalpus* within the GSMNP, and the impacts of competition or other interactions between these species remain unresolved; however, cooler water temperatures may limit *Ae. atropalpus* population growth in the southern Appalachian Region (Armistead et al. 2008, Byrd et al. 2019, Day et al. 2021).

The extensive distribution of *Ae. japonicus* within the park has potential public health implications. Although the relative importance of *Ae. japonicus* in endemic arbovirus transmission remains unclear, it is a competent vector of LACV and has been found naturally infected with the virus in eastern Tennessee (Sardelis et al. 2002, Westby et al. 2015). From 2000 to 2020, North Carolina reported an average of 17 neuroinvasive LACV infections annually, with most (>90%) occurring in western North Carolina counties, including counties containing or bordering the GSMNP (Davis et al. 2024). The GSMNP is the most visited US national park, with more than 12 million annual visitors; many visitors will camp, hike, or otherwise recreate within the park boundaries and therefore risk direct exposure to *Ae. japonicus*. Given the high volume of annual visitors, public health messaging to park visitors emphasizing personal protection measures (i.e., effective repellents registered with the US Environmental Protection Agency, protective clothing to minimize exposed skin, and the use of screens or nets when camping) to prevent

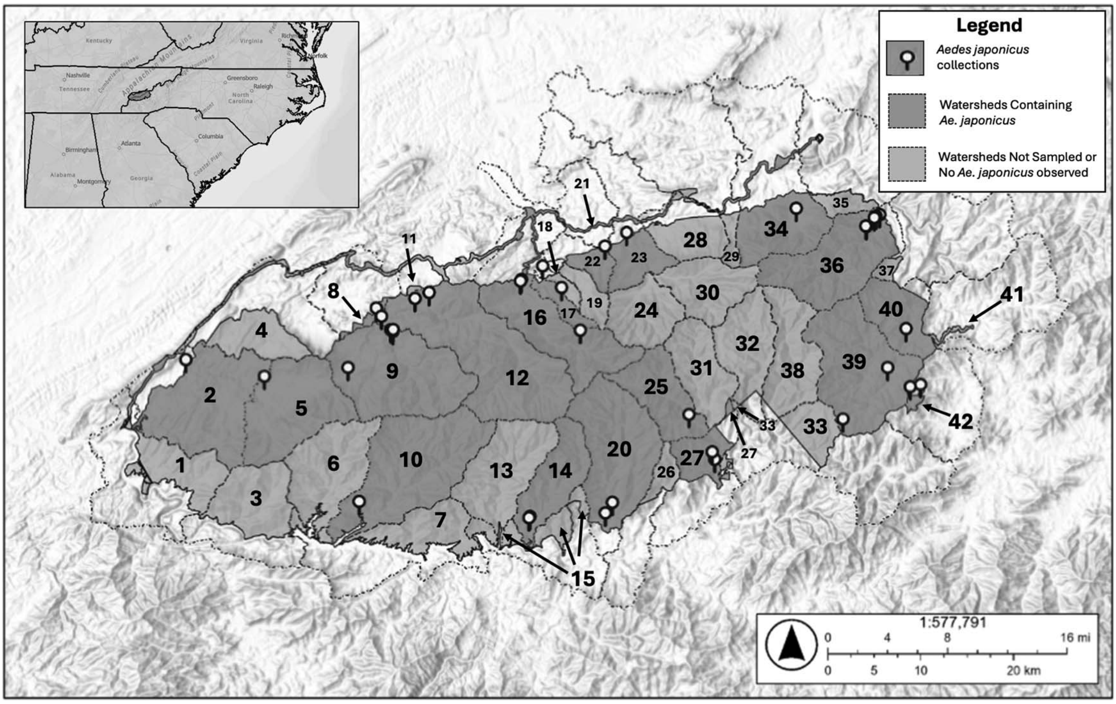

Fig. 1. Survey for *Aedes japonicus* in the Great Smoky Mountains National Park (NPS 2015); *Ae. japonicus* collected from the dark grey watersheds ("*" denotes watershed with *Ae. japonicus*): 1) Chilhowee Lake, Little Tennessee River; 2) Lower Abrams Creek*; 3) Lake Cheoah, Little Tennessee River; 4) Hesse Creek, Little River; 5) Upper Abrams Creek*; 6) Eagle Creek; 7) Lower Fontana Lake, Little Tennessee River; 8) Rudd Branch, Little River*; 9) West Prong, Little River*; 10) Hazel Creek*; 11) Walden's Creek; 12) Goshen Prong, Little River*; 13) Forney Creek; 14) Nolan Creek*; 15) Fontana Lake, Tuckasegee River; 16) Upper West Prong Little Pigeon River*; 17) Le Conte Creek*; 18) Baskin's Creek; 19) Roaring Fork; 20) Deep Creek*; 21) Bird Creek; 22) Dudley Creek*; 23) Upper Little Pigeon River*; 24) Porter's Creek; 25) Upper Oconaluftee River*; 26) Kirkland Creek, Tuckasegee River; 27) Lower Oconaluftee River*; 28) Webb Creek; 29) East Fork Little Pigeon River; 30) Middle Prong Little Pigeon River; 31) Bradley Fork; 32) Upper Raven Fork; 33) Lower Raven Fork; 34) Cosby Creek*; 35) Cripple Creek, Pigeon River; 36) Big Creek*; 37) Cold Springs Creek, Pigeon River; 38) Straight Fork Raven Fork; 39) Palmer Creek*; 40) Cataloochee Creek*; 41) Walter's Lake, Pigeon River; and 42) Lower Jonathan Creek.*

exposure is appropriate during the summer and early fall (i.e., May–September) when LACV disease is most prevalent in the region.

The NPS is tasked, as are all federal agencies (Executive Order 13112), to prevent, detect, respond to, and monitor invasive species through practical programmatic and research efforts. Here, we document the presence of *Ae. japonicus,* an invasive mosquito, in >40% of the GSMNP watersheds and repeated observations at the same collection location over multiple years. Taken together, these observations suggest the entrenchment of this invasive species within the GSMNP. However, this scientific note is limited, and our observations likely do not completely define the distribution of *Ae. japonicus* within the GSMNP. Similarly, the observations reported here were conducted over more than 15 years and do not address the seasonality or relative abundance of *Ae. japonicus* in the GSMNP. Additional longitudinal surveys within the park are warranted.

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