Identification of *Uranotaenia sapphirina* as a specialist of annelids broadens known mosquito host use patterns

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Feeding upon vertebrate blood by mosquitoes permits transmission of diverse pathogens, including viruses, protozoa, and nematodes. Despite over a century of intensive study, no mosquito species is known to specialize on non-vertebrate hosts. Using molecular analyses and field observations, we provide the first evidence, to our knowledge, that a mosquito, *Uranotaenia sapphirina*, specializes on annelid hosts (earthworms and leeches) while its sympatric congener, *Uranotaenia lowii*, feeds only on anurans (frogs and toads). Our results demonstrate that *Ur. sapphirina* feeds on annelid hosts (100% of identified blood meals; *n* = 72; collected throughout Florida), findings that are supported by field observations of these mosquitoes feeding on *Sparganophilus* worms and freshwater leeches. These findings indicate that adult mosquitoes utilize a much broader range of host taxa than previously recognized, with implications for epidemiology and the evolution of host use patterns in mosquitoes.
Mosquitoes (Diptera: Culicidae) are the vectors of disease-causing human and wildlife pathogens\(^1\)–\(^8\), and as a result, they have received greater scientific and public attention than any other insect taxon. The evolutionary innovation of blood feeding in the nematoceran flies preceded our species by hundreds of millions of years\(^9\), but has had persistent consequences throughout human history. Because blood feeding enables the transmission of pathogens between vertebrate hosts\(^10\), it is the primary reason behind the intense study of mosquitoes and their interactions with vertebrates. Despite this, the origin of blood feeding and the evolution of host use patterns in mosquitoes or other dipteran vectors of pathogens remains poorly understood. Blood feeding presents an evolutionary challenge that requires meticulous adaptations that are specific for various host groups. To effectively utilize blood, a mosquito must not only possess olfactory, visual, or thermal machinery to locate hosts and pierce the epidermis, but overcome cellular and molecular barriers to blood feeding\(^11\) that vary between host orders\(^12\). Most vertebrate animals have evolved complex cellular mechanisms that rapidly respond to blood vessel injuries with a series of immune and hemostatic reactions. Mosquitoes that feed on endothermic hosts (birds and mammals) must also possess thermoregulatory strategies to avoid overheating\(^13\). The evolution of mechanisms around these barriers in mosquitoes, including biochemical salivary cocktails that circumvent immune and hemostatic responses of hosts, and thermoregulation and highly specialized mouthparts and sensory organs, have had tremendous implications for human health, throughout history and today.

Feeding on vertebrate blood is characteristic of mosquitoes of all genera, with few exceptions. All species of Toxorhynchites (89 species) and Malay (12 species), and possibly others (e.g., Topomyia, Maaorigoeldia) do not require a blood meal to complete egg development\(^14\)–\(^18\). Adults of species of these genera feed exclusively on plant-derived sugars, either directly\(^15\) or from the carbohydrate-rich solution regurgitated by ants\(^18\). Autogenous, or partially autogenous, mosquito species are also found in a number of genera which include species that are otherwise blood-feeders\(^19\)–\(^23\). Many blood-feeding mosquito species feed on plant-derived sugars to support metabolism\(^10\), but only females feed on blood. Female mosquitoes of hematophagous species feed on diverse vertebrate lineages, including mammals, birds, reptiles, amphibians, and fishes\(^24\). Most specialize to varying degrees on certain ranges of vertebrate classes or orders, and these patterns of host use mediate the transmission dynamics of mosquito-vectored pathogens\(^25\). No mosquito species studied to date has been found to specialize on the blood of a non-vertebrate animal.

Mitigating the impact of vector-borne pathogens is one of the greatest challenges in epidemiology and medicine. Because the transmission networks of mosquito-vectored pathogens are structured by mosquito host use patterns, understanding mosquito–host interactions is a critical element in confronting this challenge. Blood meal analysis is a collection of techniques that takes advantage of immunological or genetic specificity of host blood in adult mosquito gut contents in order to identify hosts\(^24\). Using PCR-based blood meal analyses, we investigated the host use patterns of the two Uranotaenia species that occur in eastern North America: *U. sapphirina* and *U. lowii*.

**Uranotaenia** is a taxonomically diverse genus, consisting of 270 currently recognized, primarily tropical species\(^13\), \(^26\). Although few species have been extensively studied, those investigated feed primarily on anuran hosts\(^27\), \(^28\) and at least one feeds on amphibious fishes\(^29\). *U. lowii* feeds predominantly on frogs, and host-seeking females are attracted to their songs\(^30\). Until now, the host use patterns of *U. sapphirina* were, as far as we are aware, unknown, and despite deficient evidence, assumed to parallel those of *U. lowii*. Previous research using both serological and DNA-based blood meal analyses have attempted to identify the hosts of *U. sapphirina*, but most blood meal assays failed. For example, Irby and Apperson\(^30\) identified only two (1.7%) of 120 *U. sapphirina* blood meals (both as an unknown species of snake) and Cupp et al.\(^31\) identified 2 (5.7%) of 35 (both as the ranid frog *Lithobates catesbeianus*), compared with identification rates of 85.8% and 61.4%, respectively, for all mosquito species screened, excluding *U. sapphirina*.

We determined that *U. sapphirina* is a specialist of invertebrate hosts, worms, and leeches of the phylum Annelida, while the sympatric *U. lowii* specializes on the amphibian order Anura (frogs). We demonstrate that adult *U. sapphirina* feed on diverse annelid hosts, and report the first documentation, to our knowledge, of a mosquito specializing on invertebrate hosts. We collected blood-fed *Uranotaenia* mosquitoes (*n* = 132; 88 *U. sapphirina*; 44 *U. lowii*) from multiple locations on the Florida Peninsula. We used two diagnostic PCR assays to screen *Uranotaenia* blood meals for annelid and vertebrate DNA. Annelid DNA was targeted because field observations made at River Styx, Alachua Co., Florida, USA, suggested that this mosquito was feeding on oligochaete earthworm hosts. For each blood meal, we used extracted DNA as amplification templates in two separate reactions: one that targeted the annelid 28S ribosomal RNA and one that targeted the vertebrate cytochrome c oxidase subunit I gene (COI). Amplification reactions and primers were designed to produce an amplicon only in the presence of their respective template. Products of all successful reactions were sequenced to confirm the presence of annelid or vertebrate DNA.

**Results**

**Mosquito collections.** In total, 132 blood-fed adult female *Uranotaenia* mosquitoes were collected in four counties in Florida, representing 88 *U. sapphirina* and 44 *U. lowii*. *Uranotaenia sapphirina* was collected in Columbia Co. (*n* = 18), Alachua Co. (*n* = 14), and Indian River Co. (*n* = 56). *Uranotaenia lowii* was collected in Alachua Co. (*n* = 26), Indian River Co. (*n* = 14), and Miami-Dade Co. (*n* = 4).

**Host identification.** The results of PCR assays indicated that *U. sapphirina* and *U. lowii* had distinct and disparate host specialization patterns (Fig. 1). There was a significant difference in the use of annelid and vertebrate hosts between *U. sapphirina* and *U. lowii* (two-sided Fisher’s exact test; *P* < 0.001). We found that 100% of identified *U. sapphirina* blood meal DNA was derived from annelid hosts, while 100% of identified *U. lowii* blood meal DNA was derived from anuran hosts. Templates from 80 of 88 *U. sapphirina* blood meals screened positive for annelid DNA, and of these, 72 (81%) were confirmed by Sanger sequencing. All *U. sapphirina* blood meals were negative for vertebrate DNA. Identical screens of *U. lowii* blood meals indicated that 43 of 44 were positive for vertebrate DNA, with 38 (86%) confirmed by Sanger sequencing and attributed to anuran species known to occur in Florida. All *U. lowii* blood meals were negative for annelid DNA. Recovered host DNA sequences were compared against a reference database (GenBank, National Center for Biotechnology Information), or sequences obtained from morphologically identified annelid specimens. The majority (93%) of identified *U. sapphirina* blood meals were attributed to oligochaete earthworms. *Sparganophilus tennessensis*, a spargano- philid worm, was the most frequently identified host, detected in 43 of 72 (60%) of *U. sapphirina* blood meals. Two species of freshwater leeches (*Macrobdella dietra*, *Philodella floridana*) together represented 7% of identified *U. sapphirina* blood meals. In comparison, the hosts of *U. lowii* were exclusively identified as anurans.
Female *Ur. sapphirina* at the interface between terrestrial and aquatic ecosystems. Feeding on both oligochaete worms and freshwater leeches (Fig. 2)

Unlike any previously studied mosquito species, *Ur. sapphirina* feeding on both oligochaete worms and freshwater leeches (Fig. 2) in our sample fed exclusively on annelid hosts. This finding explains the inability of previous investigations to identify *Ur. sapphirina* blood meals, as these studies were performed under the assumption, and corresponding laboratory methodology, that female mosquitoes take blood meals only from vertebrate animals. Annelids and vertebrates share enclosed circulatory systems and either extracellular (annelids) or intracellular (vertebrates) hemoglobin, which in both groups causes the characteristic red coloration of the blood. The presence of red blood in the guts of *Ur. sapphirina* females likely contributed to the confusion related to host use of this mosquito by other researchers. Future studies may need to consider the possibility that mosquitoes fed on other types of invertebrates may not display the red gut normally used to classify a mosquito as blood engorged.

The recognition of *Ur. sapphirina* as a specialist of annelid, not vertebrate, host animals has important implications in mosquito ecology and evolution, and in the epidemiology of mosquito-vectored pathogens. This finding demonstrates that the range of potential mosquito hosts is considerably broader than previously indicated. *Uranotaenia sapphirina* is a common species throughout eastern North America, where mosquitoes have been under extensive study since their involvement in pathogen transmission was recognized in 1881 (ref. 1). The presumption that adult female mosquitoes blood feed only from vertebrate hosts, is a source of bias in the methodological framework used to study mosquito ecology. Mosquito blood meals are identified primarily through methods that, by design, selectively react with only vertebrate antigens or DNA. For this reason, estimating the extent to which invertebrate hosts are utilized by female mosquitoes is not possible given previously available methods. In the laboratory, caged mosquitoes, including mammalophilic *Aedes* and *Anopheles* species have been documented locating and feeding on lepidopteran larvae in no-choice experiments, and subsequently, in some cases, to produce viable eggs (refs. 32–35). Anecdotal records of mosquitoes feeding on cicada nymphs, mantids, chironomid midges, and lepidopteran pupae reported in the early 1900s by Howard (refs. 36, 37) and occasionally referenced in the literature (refs. 56, 38) have been disputed by Downes (ref. 10) as mistaken identifications and include few substantive details. Beyond these laboratory experiments and historic records, there is no previous evidence that such interactions occur in nature, although any instance of invertebrate host use would not be detected by the traditional methods of blood meal analysis.

Field observations. To further confirm these results, we made field observations of *Ur. sapphirina* and *Ur. lowii* in Alachua Co., Florida. We observed and documented female *Ur. sapphirina* feeding on both oligochaete worms and freshwater leeches (Fig. 2) at the interface between terrestrial and aquatic ecosystems. Female *Ur. sapphirina* were observed probing the substrate with their proboscises, presumably in attempts to locate hosts (Supplementary Movie 1). While no *Ur. lowii* were observed feeding on annelids, we documented females feeding on both hyliid and ranid frogs (Fig. 2).

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Interestingly, for some mosquito and Corethrellidae (the culicomorph sister taxon to mosquitoes + Chaoboridae) species, blood meal analyses that are effective in other species have failed, which may indicate that these species also feed upon hosts that cannot be detected using the vertebrate-based methodology. As new sequencing technologies are applied to blood meal analysis, the ability to detect unexpected hosts should improve, particularly with the potential of the invertebrate feeding in mosquitoes.

Understanding the extent to which mosquitoes, particularly pathogen vectors, interact with invertebrate hosts has epidemiological implications. *Uranotaenia sapphirina* has been implicated as a potential vector for several arboviruses. Field-collected *U. sapphirina* have tested positive for Eastern equine encephalitis virus, and West Nile virus. Our results suggest that *U. sapphirina* is unlikely to be infected with these viruses through feeding on vertebrate hosts. It is possible that by feeding on hematophagous leeches, which themselves often parasitize competent arbovirus hosts, *U. sapphirina* could act as kleptoparasites, acquiring virus-infected vertebrate blood meals from their leech hosts. Similarly, interactions between *U. sapphirina* and leeches may affect the transmission of pathogens vectored by leeches. In previous studies, a small proportion of examined *U. sapphirina* blood meals were derived from snakes and the ranid frog *Lithobates catesbeianus*. Some snake species (*e.g.*, *A高铁ron piscivorus*, *Nerodia spp.*) and ranid frogs are common at the margins of vegetated waterways at night, a microhabitat where *U. sapphirina* females were observed feeding on annelid hosts. In these microhabitats, snakes and frogs would be available to host-seeking *U. sapphirina* females, and may be fed on incidentally. An alternative possibility for the detection of arboviruses in *U. sapphirina* is that this mosquito occasionally feeds on vertebrate hosts that may be competent for some arboviruses. The identification of two snake-derived *U. sapphirina* blood meals is particularly noteworthy, as the role of snakes in arbovirus persistence and transmission has been increasingly supported. Future studies should elucidate how *U. sapphirina* comes into contact with these viruses, and investigate the possibility that *U. sapphirina* could represent a complex of cryptic species with varying host use patterns.

Annelid host specialization by *U. sapphirina* raises important questions about the origin and evolution of blood feeding in mosquitoes. Foremost is whether feeding on invertebrates is an ancestral or derived trait. Divergence and radiation time estimates of Culicomorpha and vertebrate host lineages suggest that mosquitoes, or their culicomorph ancestors, adapted to vertebrate host groups after their diversification. The relationship between host and mosquito/Culicomorpha phylogenies has yet to be assessed, but other hematophagous insects have undergone stepwise transitions, with diversification of hematophagous insect lineages paralleling their host phylogenies. Understanding how invertebrates factor into the evolution of host use by mosquitoes and other Culicomorpha will ultimately depend on a more complete accounting of mosquito host use patterns and the extent of invertebrate host use, and a well-resolved mosquito phylogeny. Until more information becomes available, understanding the origins and evolution of blood feeding in mosquitoes will remain speculative. However, the evolutionary history of Culicomorpha and host animals, and the host use patterns of basilar mosquitoes may provide clues.

Birds and mammals are the major hosts of many modern mosquitoes, particularly among the more derived lineages (Fig. 3). However, it is unlikely that birds or mammals were the initial hosts of ancestral mosquitoes, as the earliest known fossil mosquito, *Burmaculex antiquus*, precedes the diversification of birds and mammals by 30–40 million years. Modern frog-biting midges (Corethrellidae), sister to the mosquitoes + phaneromorph midges (Chaoboridae), are known to feed only on anuran hosts (Fig. 3), and this association dates to the Lower Cretaceous, pre-dating *Burmaculex* by 75 million years. The antiquity of anuran host use by *Corethrella* and the use of endothermic hosts by modern mosquitoes suggests a relationship between the vertebrate and mosquito phylogenies. However, this hypothesis is not supported by the basal placement of *Anopheles*, the human malaria vectors, that are generally considered specialists of mammalian hosts (Fig. 3). The split between Anophelinae and Culicinae is estimated at 45–126 million years before *Burmaculex*, suggesting either that mammal specialization in Anophelinae is not the ancestral trait, or that the basal placement of Anophelinae is incorrect. While phylogenetic analyses based on molecular data have not yet fully resolved deeper (genus-level) divisions within Culicidae, they have estimated the time of divergence of *Uranotaenia* from other genera at >150 mya. This event would have been concurrent with the diversification of major anuran groups and the first actual fossils of frog-biting midgets, and 50 million years older than *Burmaculex*. *Uranotaenia*, with its sister group *Aedoeomyia*, is placed in a basal position within Culicinae, a diverse clade containing the majority of mosquito genera, implying an ancient origin for *Uranotaenia*. This might suggest that, if invertebrate feeding is
the pleisiomorphic state, host affinities within Uranaotaenia are indicative of early patterns of host use within Culicidae that were lost in other basal lineages (e.g., Anophelineae).

The limited evidence available indicates that the earliest lineages of Culicomorpha fed on the body fluids (hemolymph) of the open circulatory systems of insects. This trait is retained in multiple culicomorph families, including some extant chironomids and ceratopogonids, and mammalophilic Anopheles and Aedes mosquitoes are able to locate insect hosts and utilize hemolymph to mature eggs. The closed circulatory systems of annelids, by contrast, are analogous to those of vertebrates, utilizing hemolymph to mature eggs. The closed circulatory systems of annelids, by contrast, are analogous to those of vertebrates, utilizing hemolymph to mature eggs. Annelid feeding may represent an evolutionary link between an ancestral culicomorph feeding on free hemolymph in the body cavity and modern mosquitoes feeding on vertebrates with closed circulatory systems. While annelid circulatory systems are analogous to those of vertebrates, annelid immune systems and hemostatic responses are less sophisticated, suggesting that annelid feeding mosquitoes may encounter fewer defenses. In that context, annelid feeding may have primed the development of physiological, biochemical, behavioral, and morphological adaptations that would enable mosquitoes to eventually circumvent barriers to blood feeding from diverse vertebrate hosts, ultimately leading to the tremendous human health impacts caused by pathogen transmission in modern mosquitoes.

The use of annelid hosts in Ur. sapphirina alternatively could be a trait derived from frog feeding ancestors switching to worms and leeches that were encountered in habitats similar to anuran hosts. The phylogenetic position (Fig. 3) of the frog-biting midges, and their presumed ancient association with frogs may suggest that amphibians were the hosts of the common ancestor of Corethrellidae and Culicidae + Chaoboridae. However, a recent study of the hosts of Corethrella found that PCR assays successfully identified only <30% of blood meals, despite the use of primers that amplify a broad range of vertebrate hosts. The authors of that study compared their low success rate with that of Ur. sapphirina from other published works, leading them to conclude that female Corethrella may feed on additional, unidentified hosts.

The function of annelid host use by Ur. sapphirina is not yet established, and the molecular analyses and field observations we report cannot discount the possibility that annelid host use by Ur. sapphirina could be a derived trait that evolved to serve a non-reproductive function, such as preventing dehydration or supplementing energetic reserves. Blood feeding by mosquitoes serves a function that is primarily reproductive: the females of most mosquito species require nutrients, particularly proteins, from host blood to provision developing eggs, although blood-derived resources can also be diverted to meet metabolic needs or in response to dehydration. Dehydration can alter the behavior of mosquitoes by prompting them to increase host seeking and blood feeding. Carbohydrates, obtained directly or indirectly from plants, serve primarily as a metabolic resource to both male and female mosquitoes, but can also enhance the reproductive potential of a female mosquito. Our findings do not exclude the potential that female Ur. sapphirina utilize annelid blood as an energetic resource or a means of maintaining hydration; however, circumstantial evidence supports the idea that annelid feeding plays a reproductive role for this mosquito. For example, despite the collection of numerous male Ur. sapphirina, blood meals were only found in females in our samples. In addition, both males and females have been observed nectaring at flowers at field locations (L.E.R., personal observation), and collected engorged with nectar. The details of egg production following annelid feeding, and the potential for annelid blood to serve a function other than egg maturation, needs to be explored by subsequent research to better understand the evolutionary
context of these findings, as our data are limited to the identification of annelids as the hosts of *Ur. sapphirina*.

Specialization of *Ur. sapphirina* on annelid hosts demonstrates that the host breadth of mosquitoes is substantially broader than previously understood. Prior research on the host interactions of mosquitoes has centered around a minor subset of mosquito species, particularly the Aedes, Anopheles, and Culex pathogen vectors. For many genera, particularly those restricted to tropical regions, host use patterns have not been investigated, leaving substantial gaps in the understanding of mosquito–host relations. Combined with the large diversity of mosquitoes, there is potential that invertebrate host specialization extends beyond *Ur. sapphirina*. The fact that a common North American mosquito specializing on annelid hosts has gone undocumented as far as we are aware for more than a century suggests that invertebrate host use by mosquitoes is easily overlooked. Future work towards a more complete understanding of mosquito host use patterns should consider this possibility and, ideally, make use of novel molecular technologies that are compatible with the detection of invertebrate hosts.

**Methods**

**Mosquito collections.** Blood engorged *Uranotaenia* mosquitoes were collected between 28 September 2015 and 10 May 2017 at sites throughout the Florida Peninsula: Osceola National Forest (Columbia County), River Styx (Alachua County), Newnan’s Lake (Alachua County), the University of Florida Natural Area Teaching Lab (Alachua County), two sites at Blue Cypress Lake Conservation Area (Indian River County), and Everglades National Park (Miami-Dade County; Permit Number EVER-2017-SCI-0011). Mosquitoes were collected from natural resting sites (tree trunks, cypress knees, vegetation, exposed tree roots) using a battery-powered aspirator. Mosquitoes from Osceola National Forest, River Styx, Natural Area Teaching Lab, and Everglades National Park were collected in a field, promptly after collection, by exposure to ethyl-acetate-soaked plaster for approximately 10 min. Host DNA was immediately preserved in the field on Whatman Finders Technology Associates (FTA) blood cards72 and, taken to the laboratory (Florida Medical Entomology Laboratory, Vero Beach, Florida) inside polypropylene collecting cups (BioQuip), chilled on ice inside a cooler. Upon arrival, collecting cups were placed into a −20°C freezer for at least 20 min to kill mosquitoes. Mosquitoes were identified through morphological characters73. Blood-fed individuals were separated from others by visual inspection of the abdomen. DNA was extracted using the hot sodium hydroxide and tris (HotSHOT) method74 or DNEasy blood and tissue kit (Qiagen), following inspection of the abdomen. DNA was extracted using the hot sodium hydroxide method75. DNA sequencing and taxonomic identification. To confirm that the PCRs correctly detected annelid or vertebrate DNA, any PCR product that showed a band at the expected fragment size was submitted to the University of Florida Interdisciplinary Center for Biotechnology Research or Eurofins for Sanger sequencing on an ABI 3130 automated sequencer. Resulting sequence chromatograms were examined and edited for quality in the program Geneious® Version R10 (ref.76). Unambiguous sequences were searched on the National Center for Biotechnology Information, GenBank database using the Basic Local Alignment Search Tool (BLAST). For COI sequences, species-level taxonomic identities were assigned to blood meals when a host sequence was >97% similar to a sequence referenced in the GenBank database. When some unambiguous sequences did not meet this threshold. In these cases, we used the BLAST function to align and compare blood meal sequences with reference sequences obtained from tissue samples of morphologically identified species and used the same >97% homologous criterion to identify host species. For 28S ribosomal RNA sequences, low taxonomic coverage for Annelida in the GenBank database prohibited the application of the 97% similarity criterion used for COI. We attributed 28S ribosomal RNA sequences to Annelida if the most similar reference sequence to an unambiguous high-quality host sequence was derived from an annelid species. We subsequently compared 28S ribosomal RNA blood meal sequences to reference sequences obtained from annelids collected at two sites (River Styx, Alachua County; Blue Cypress Lake, Indian River Co.). Worm specimens were captured by hand from muddy substrates where *Ur. sapphirina* mosquitoes had previously been observed. Worms were immediately placed in 95% ethanol and subsequently identified to species by M. Siddall using morphological and molecular markers.

**Statistics.** The proportion of blood meals derived from vertebrate hosts and annelid hosts (detected by PCR and confirmed by sequencing) for *Ur. sapphirina* and *U. lowni* was compared using Fisher’s Exact Test. This analysis was performed in the software R® Version 3.2.0 using the stats package. Results were considered significant if *P* < 0.05.

**Data availability.** Sequence data generated by this study have been deposited in the National Center for Biotechnology Information GenBank database (Accession Numbers MH384533-MH384601 for vertebrate hosts and accession Numbers MH384497-MH384532 for annelid hosts). All other relevant data supporting the findings of this study are within the paper and its Supplementary Files. Any further data or information are available from the corresponding author upon reasonable request.

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**Table 1 Primer sequences used to screen *Uranotaenia* blood meals for vertebrate and annelid DNA**

| Primer name | Target          | Sequence                     | Amplicon size (bp) | Reference          |
|-------------|-----------------|------------------------------|--------------------|--------------------|
| RepCOI-F    | Vertebrate COI   | 5'-TNT TMT CAA ACC ACG AAG A-3' | 664                | This study         |
| RepCOI-R    | Vertebrate COI   | 5'-ACT TCT TGG TCA AAR CAC C-3' | 75                 |                    |
| SPARG_C2_416_F | annelid 28S ribosomal RNA | 5'-ATC GGT CGG CAA CCT GTA CG-3' | 416                |                    |
| 2BS_C4_R    | annelid 28S ribosomal RNA | 5'-TCC GAT TRG TCT TAC GCC CCT-3' | 416                |                    |

References

1. Finlay, C. The mosquito hypothetically considered as an agent in the transmission of yellow fever poison. *New Orleans Med. Surg. J.* 9, 601–616 (1881).
2. Cupp, E. W., Klinger, K., Hassan, H. K., Viguers, L. M. & Unnasch, T. R. Transmission of Eastern equine encephalomyelitis virus in central Alabama. *Am. J. Trop. Med. Hyg.* 68, 495–500 (2003).
3. Andreadis, T. G., Anderson, J. F., Vossbrinck, C. R. & Main, A. J. Epidemiology of West Nile virus in Connecticut: a five-year analysis of mosquito data 1999–2003. *Vector Borne Zoonotic Dis.* 4, 360–378 (2004).
4. Armstrong, P. M. & Andreadis, T. G. Eastern equine encephalitis virus in mosquitoes and their role as bridge vectors. *Emerg. Infect. Dis.* 16, 1869–1874 (2010).
5. Wilkerson, R. C. et al. Making mosquito taxonomy useful: a stable classification of tribe Aedini that balances utility with current knowledge of evolution and systematics. PLoS ONE 10, e0133602 (2015).

6. Farajollahi, A., Fonseca, D. M., Kramer, L. D. & Kilpatrick, A. M. “Bird biting” mosquitoes and human disease: a review of the role of Culex pipiens complex mosquitoes in epidemiology. Infect. Genet. Evol. 11, 1577–1585 (2011).

7. Clements, A. N. The Biology of Mosquitoes. Volume 3, Transmission of Viruses, and Interactions with Bacterial Symbionts (CAB International, Wallingford, 2012).

8. Dobson, A. & Fuopoupo, J. Emerging infectious pathogens of wildlife. Philos. Trans. R. Soc. Lond. B Biol. Sci. 356, 1001–1012 (2011).

9. Mains, B. J. Evolution of vertebrate hemostatic and inflammatory control mechanisms in blood-feeding arthropods. J. Insect. Immunol. 3, 41–55 (2011).

10. Downes, J. A. The feeding habits of biting flies and their significance in classification. Annu. Rev. Entomol. 3, 249–266 (1958).

11. Ribiero, J. M. C., Mains, B. J. & Arcá, B. An insight into the sialome of blood-feeding arthropods. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365, 259–266 (2010).

12. Didisheim, P., Hattori, K. & Lewis, J. H. Hematologic and coagulation studies in various animal species. J. Lab. Clin. Med. 53, 866–875 (1959).

13. Lahorce, C., Chaudron, B. R. M. Mosquitoes cool down during feeding to avoid overheating. Curr. Biol. 22, 40–45 (2012).

14. Harbach, R. E. Mosquito Taxonomic Inventory. http://mosquito-taxonomic-inventory.info/ (2013).

15. Steffen, W. A. & Evenhuis, N. L. Biology of Toxorhynchites. Ann. Rev. Entomol. 26, 159–190 (2001).

16. Rattanarithikul, R., Harbach, R. E., Harrison, B. A., Panthusiri, P. & Coleman, R. E. Illustrated keys to the mosquitoes of Thailand V. Genera Orthopodomyia, Kimia, Malaya, Topomyia, Tripteroides, and Toxorhynchites. Southeast Asian J. Trop. Med. Public Health 38, 1–65 (2007).

17. Snell, A. E., Derraik, J. G. B. & McIntyre, M. Maoriogelida argeraris Walker (Diptera: Culicidae): is this another threatened endemic species? NZ Entomol. 28, 95–99 (2005).

18. Foster, W. A. & Walker, E. D. in Medical and Veterinary Entomology, 2nd edn (eds Mullen, G. R. & Durden, L. A.) 207–259 (Academic Press, San Diego, 2009).

19. Tate, P. & Vincent, M. The biology of autogenous and anautogenous races of Culex pipiens L. (Diptera: Culicidae). Parasitology 28, 115–145 (1936).

20. Speilman, A. Bionomics of autogenous mosquitoes. Ann. Rev. Entomol. 16, 231–248 (1971).

21. O’Meara, G. F. & Edman, J. D. Autogenous egg production in the salt marsh mosquito, Aedes taeniorhynchus. Biol. Bull. 149, 384–396 (1975).

22. O’Meara, G. F. Variable expression of autogenic in three mosquito species. Int. J. Inve. Rep. Dev. 3, 253–261 (1979).

23. Lounibos, L. P., Van Dover, C. & O’Meara, G. F. Fecundity, autogeny, and the larval environment of the pitcher-plant mosquito, Wyeomyia smithii. Oecologia 55, 160–164 (1982).

24. Tempelis, C. H. Host-feeding patterns of mosquitoes, with a review of advances in analysis of blood meals by serology. J. Med. Entomol. 11, 635–653 (1975).

25. Kilpatrick, A. M., Kramer, L. D., Jones, M. J., Marra, P. P. & Daszak, P. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biol. 4, e82 (2006).

26. Harbach, R. E. The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. Zootaxa 1688, 591–638 (2007).

27. Christensen, H. A., de Vasquez, A. M. & Boreham, M. H. Mosquito feeding patterns of mosquitoes (Diptera: Culicidae) from central Panama. Am. J. Trop. Med. Hyg. 55, 202–206 (1996).

28. Toma, T., Miyagi, M. & Tashimori, M. Blood meal identification and feeding habits of Uranotaenia species collected in the Ryukyu Archipelago. J. Am. Mosq. Control Assoc. 30, 215–218 (2014).

29. Borkent, A. & Belton, P. Attraction of female Orthopodomyia nigripalpus (Diptera: Culicidae) to frog calls in Costa Rica. Can. Entomol. 138, 91–94 (2006).

30. Irby, W. S. & Apperson, C. S. Hosts of mosquitoes in the coastal plain of North Carolina. J. Med. Entomol. 25, 85–93 (1988).

31. Irby, W. S., Krzywinski, J., Grushko, O. G. & Besansky, N. J. Analysis of the complete mitochondrial DNA from Anopheles funestus: an improved dipteran mitochondrial genome analysis and a temporal dimension of mosquito evolution. Mol. Phylogenet. Evol. 39, 417–423 (2009).

32. Biju, S. D. & Bossuyt, F. New frog family from India reveals an ancient radiation of frogs (Anura) as the hosts of Corethrellidae (Diptera) in the southeastern United States. J. Insect Sci. 17, 95 (2017).

33. Krzywinski, J., Grushko, O. G. & Besansky, N. J. Analysis of the complete mitochondrial DNA from Anopheles funestus: an improved dipteran mitochondrial genome analysis and a temporal dimension of mosquito evolution. Mol. Phylogenet. Evol. 39, 417–423 (2009).

34. Biju, S. D. & Bossuyt, F. New frog family from India reveals an ancient radiation of frogs (Anura) as the hosts of Corethrellidae (Diptera) in the southeastern United States. J. Insect Sci. 17, 95 (2017).

35. Martin, J., Eilert, N. & Durrant, B. R. M. Mosquitoes feeding on caterpillars of the Common Buckeye butterfly, Junonia coenia (Lepidoptera: Nymphalidae). J. Res. Lepid. 47, 45–48 (2014).

36. Howard, L. O. Mosquitoes. How They Live; How They Carry Disease; How They May Be Destroyed (McClure, Phillips and Co., 1901).

37. Howard, L. O., Dyar, H. G. & Knab, F. The Mosquitoes of North and Central America and the West Indies (Carnegie Institution of Washington, The Lord Baltimore Press, Baltimore, 1912).

38. Borkent, A. Mosquitoes. How They Live; How They Carry Disease; How They May Be Destroyed (McClure, Phillips and Co., 1901).

39. Krzywinski, J., Grushko, O. G. & Besansky, N. J. Analysis of the complete mitochondrial DNA from Anopheles funestus: an improved dipteran mitochondrial genome analysis and a temporal dimension of mosquito evolution. Mol. Phylogenet. Evol. 39, 417–423 (2009).

40. Biju, S. D. & Bossuyt, F. New frog family from India reveals an ancient radiation of frogs (Anura) as the hosts of Corethrellidae (Diptera) in the southeastern United States. J. Insect Sci. 17, 95 (2017).

41. Krzywinski, J., Grushko, O. G. & Besansky, N. J. Analysis of the complete mitochondrial DNA from Anopheles funestus: an improved dipteran mitochondrial genome analysis and a temporal dimension of mosquito evolution. Mol. Phylogenet. Evol. 39, 417–423 (2009).

42. Biju, S. D. & Bossuyt, F. New frog family from India reveals an ancient radiation of frogs (Anura) as the hosts of Corethrellidae (Diptera) in the southeastern United States. J. Insect Sci. 17, 95 (2017).

43. Krzywinski, J., Grushko, O. G. & Besansky, N. J. Analysis of the complete mitochondrial DNA from Anopheles funestus: an improved dipteran mitochondrial genome analysis and a temporal dimension of mosquito evolution. Mol. Phylogenet. Evol. 39, 417–423 (2009).
84. Aitken, T. H. The canopy frequenting mosquitoes of Bush Forest, Trinidad.
83. Navia-Gine, W. G., Loaiza, J. R. & Miller, M. J. Mosquito-host interactions
82. Moreno, M. et al. Intensive trapping of blood-fed
8
COMMUNICATIONS BIOLOGY | DOI: 10.1038/s42003-018-0096-5
www.nature.com/commsbio
ARTICLE COMMUNICATIONS BIOLOGY | DOI: 10.1038/s42003-018-0096-5
www.nature.com/commsbio
85. Toma, T. et al. Bionomics of the mud lobster-hole mosquito
76. Lang, S. A., Saglam, N., Kawash, J. & Shain, D. H. Punctuated invasion of
75. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
78. FGDL (Florida Geographic Data Library). University of Florida.https://www.
72. Reeves, L. E., Holderman, C. J., Gillett-Kaufman, J. L., Kawahara, A. Y. &
71. Bidlingmayer, W. L. & Hem, D. G. Sugar feeding by Florida mosquitoes.
70. Foster, W. A. Mosquito sugar feeding and reproductive energetics. Annu. Rev.
69. Hagan, R. W. et al. Dehydration prompts increased activity and blood feeding
86. Cooper, E. L., Kauschke, E. & Cossarizza, A. Digging for innate immunity
67. Clements, A. N. Bionomics of the mud lobster-hole mosquito
66. Cooper, E. L., Kauschke, E. & Cossarizza, A. Digging for innate immunity
63. Lang, S. A., Saglam, N., Kawash, J. & Shain, D. H. Punctuated invasion of
53. Borkent, A. World catalog of extant and fossil Corethrellidae (Diptera).
50. Navia-Gine, W. G., Loaiza, J. R. & Miller, M. J. Mosquito-host interactions
47. Reyes, L. E., Holderman, C. J., Gillett-Kaufman, J. L., Kawahara, A. Y. &
46. Cooper, E. L., Kauschke, E. & Cossarizza, A. Digging for innate immunity
45. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
44. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
43. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
42. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
41. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
40. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
39. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
38. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
37. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
36. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
35. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
34. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
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32. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
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30. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
29. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
28. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
27. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
26. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
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24. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
23. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
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17. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
16. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
15. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
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12. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
11. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
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9. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
8. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
7. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
6. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
5. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
4. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
3. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
2. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).
1. Toma, T. et al. World catalog of extant and fossil Corethrellidae (Diptera).

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Author Contributions
N.D.B.C., C.J.H., P.E.K., J.L.G.K., A.Y.K., and L.E.R. conceived and designed the study. C.J.H., E.M.B., N.D.B.C., and L.E.R. collected mosquitoes from field sites throughout Florida, N.D.B.C. and L.E.R. obtained permits where necessary. A.Y.K., N. D.B.C., and L.E.R. designed laboratory protocols. P.E.K., and N.D.B.C. provided laboratory space, equipment, and supplies. C.J.H., L.E.R., and N.D.B.C. performed molecular analyses. N.D.B.C. photographed interactions between mosquitoes and frogs. L.E.R. designed primers for amplifying ameliod DNA, photographed, and filmed interactions between mosquitoes and annelids, and drafted the initial version of the manuscript. All authors provided input and edits on the manuscript, and approved the final version.

Additional information
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