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Predicting potential transmission risk of Everglades virus in Florida using mosquito blood meal identifications

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The overlap between arbovirus host, arthropod vectors, and pathogen distributions in environmentally suitable habitats represents a nidus where risk for pathogen transmission may occur. Everglades virus (EVEV), subtype II Venezuelan equine encephalitis virus (VEEV), is endemic to southern Florida where it is transmitted by the endemic vector *Culex cedecei* between muroid rodent hosts. We developed an ecological niche model (ENM) to predict areas in Florida suitable for EVEV transmission based upon georeferenced vector-host interactions from PCR-based blood meal analysis from blood-engorged female *Cx. cedecei* females. Thirteen environmental variables were used for model calibration, including bioclimatic variables derived from Daymet 1 km daily temperature and precipitation values, and land use and land cover data representing percent land cover derived within a 2.5 km buffer from 2019 National Land Cover Database (NLCD) program. Maximum temperature of the warmest month, minimum temperature of the coldest month, and precipitation of the driest month contributed 31.6%, 28.5% and 19.9% to ENM performance. The land cover types contributing the greatest to the model performance were percent landcover of emergent herbaceous and woody wetlands which contributed 5.2% and 4.3% to model performance, respectively. Results of the model output showed high suitability for *Cx. cedecei* feeding on rodents throughout the southwestern portion of the state and pockets of high suitability along the northern east coast of Florida, while areas with low suitability included the Miami-Dade metropolitan area and most of northern Florida and the Panhandle. Comparing predicted distributions of *Cx. cedecei* feeding upon rodent hosts in the present study to historical human cases of EVEV disease, as well as antibodies in wildlife show substantial overlap with areas predicted moderate to highly suitable for these vector/host associations. As such, the findings of this study likely predict the most accurate distribution of the nidus of EVEV to date, indicating that this method allows for better inference of potential transmission areas than models which only consider the vector or vertebrate host species individually. A similar approach using host blood meals of other arboviruses can be used to predict potential areas of virus transmission for other vector-borne diseases.

KEYWORDS

Everglades virus, *Culex cedecei*, *Sigmodon hispidus*, ecological niche models, bloodmeal analysis

Introduction

The overlap between arbovirus host and vector distributions in environmentally suitable habitats can provide useful information toward understanding where risk for pathogen transmission to humans or domestic animals may occur. Landscape epidemiology is a sub-discipline of landscape ecology which studies dynamic interactions between pathogens, hosts, and vectors across heterogeneous environments at multiple spatiotemporal scales (1, 2). The formal concept outlining associations between vector-borne disease and specific landscape features or habitat types was initially proposed by Pavlovsky (3) who suggested that vector-borne pathogen transmission could only occur in environments which support the overlap of three critical biological elements, including: competent vectors, vertebrate hosts, and the pathogen. Together, these components are often referred to as a nidus, or an area where pathogen transmission takes place (2, 3).

Ecological niche modeling (ENM) is used to predict potential geographic distributions of species by correlating environmental values with georeferenced occurrence points for model calibration (4). In the study of vector-borne diseases, ENMs have been used to predict distributions of vectors (5, 6), vertebrate hosts (7) and pathogens (8, 9), based upon georeferenced detections of each independent aspect of the nidus. Ecological niche models predicting the potential distribution of vectors, hosts, or pathogens are typically generated separately. While important, these single-species ENMs provide potential distributions for just one component of the nidus of pathogen transmission, a practice that is likely to overestimate the areas where pathogen transmission occurs. Although methods are available that can test whether environmental similarities exist in predicted distributions across two species (10–12), overlap is not evidence of interactions (typically blood-feeding) between vectors and hosts. Furthermore, separate models may not use the same covariates in model calibration, introducing an added challenge to making predictions across broader geographic areas. Similarly, several factors could affect the interactions between vector, host, and pathogen, such as the presence of dilution hosts which may draw vector species away from potential reservoir hosts (13), which are not accounted for when modeling disease system components individually. Therefore, a niche model using presence data available for known interactions (actual contact through blood-feeding) of two or more components of the nidus overlap should constitute a more accurate approach to predicting areas of potential pathogen transmission than modeling just vector, host, or pathogen distributions alone.

Everglades virus (EVEV) is an enzootic subtype of Venezuelan equine encephalitis virus (VEEV) and is maintained between the enzootic vector mosquito and rodent hosts in southern Florida (14). *Culex cedecei* is the only confirmed natural vector of EVEV in Florida, and feeds to a large extent on rodents, especially the hispid cotton rat (*Sigmodon hispidus* Say and Ord) (15–18). For this reason, interactions between *Cx. cedecei* and the hispid cotton rat are generally considered to be the primary drivers of EVEV transmission (16, 19). However, several other muroid rodent species are the natural hosts of EVEV and other enzootic subtypes of VEEV distributed throughout the Americas (14, 20). This is supported by the detection of substantial virus titers for VEEV subtypes from *Rattus* spp. in Colombia (21), along with high seroprevalence for EVEV in cotton mice (*Peromyscus gossypinus* Le Conte) and hispid cotton rats in Florida (22–25). *Culex cedecei* has also been found to feed heavily on black rats (*Rattus rattus* Linnaeus), cotton mice, and hispid cotton rats in areas where EVEV has been detected from mosquitoes in nature (17), providing evidence that all three rodent species are capable of developing viremias high enough for the transmission of EVEV to *Cx. cedecei* in Florida. Everglades virus can therefore be considered a single vector system, with few host species, wherein only rodent hosts and the mosquito vector are necessary to maintain pathogen transmission (12).

In the case of EVEV, modeling only the hosts would not provide a distribution representative of the risk of EVEV, but could greatly overestimate EVEV risk due to the ability of the three primary rodent hosts to utilize a range of habitat types (26, 27) and their wide geographical distributions. Likewise, although the distribution of the vector species *Cx. cedecei* may be more limited in its utilization of habitats and known distribution, modeling only the distribution of this species may also result in over-estimation of the potential distribution of EVEV foci if *Cx. cedecei* does not feed on EVEV hosts throughout its range, or across all types of climate and land cover habitats. It is also not straightforward to model the distribution of incidence of disease in humans, as these infections may not accurately represent the locations where they contracted the pathogen. Sloyer et al. (2022) has previously modeled the potential distributions of *Cx. cedecei* in Florida using environmental variables which include temperature, precipitation, and enhanced vegetation index (EVI). Results showed that the potential distribution of *Cx. cedecei* ranges from low to high suitability throughout much of the southern half of the Florida Peninsula. This model however did not take into account landscape composition and configuration and also cannot inform on whether or not *Cx. cedecei* feeds upon rodent hosts of EVEV in all areas. Aside from direct observations, and baited traps, the most common method of determining mosquito-host interactions is by performing polymerase chain reaction (PCR)-based blood meal analysis from blood-engorged mosquitoes to determine the host species origin of the blood meal (28, 29).

The objective of this study was to use geographically referenced blood meals of *Cx. cedecei* to generate an ENM

using climate and landscape variables to predict suitable environments for interactions between *Cx. cedecei* and rodent hosts of EVEV in Florida. Resulting outputs generated by the ENM predict areas suitable for *Cx. cedecei* to feed on rodent hosts. We then overlay EVEV infections from previous studies in humans (30–33), wildlife (24, 25, 34, 35), and dogs (36) to illustrate how our model corresponds to natural infections in vertebrate animals. This method allows for the generation of a model representing the interaction between two components of the nidus of pathogen transmission rather than a single component, making it potentially more informative for inferring areas of potential transmission of EVEV.
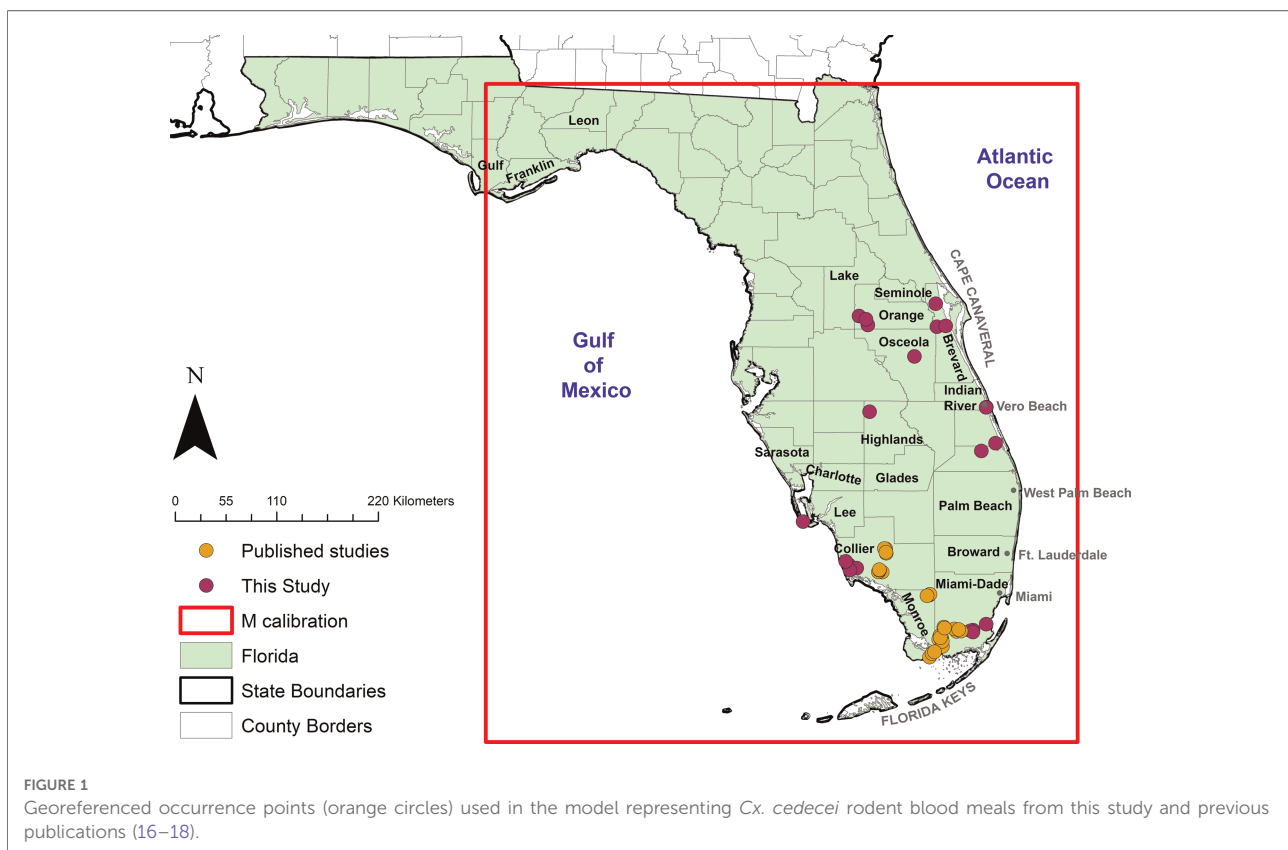
Materials and methods

Data collection and preparation

Georeferenced *Cx. cedecei* and rodent host interactions derived from blood meals were compiled from a combination of targeted field collections and previously published data (16–18). In both targeted field collections and published data, blood-engorged *Cx. cedecei* were collected using resting shelters, as previously described (37). Targeted data collection for this study occurred from the central counties of Orange and Brevard to Miami-Dade and Collier counties in the south

(Figure 1). Previously published data of blood-engorged *Cx. cedecei* were processed and collected in a similar manner to those collected for the present study and described in greater detail therein (Supplementary Table S1) (16–18).

Vertebrate host identifications were determined using PCR-based blood meal analysis of blood-engorged *Cx. cedecei*. For samples collected for this study, blood-engorged mosquitoes were stored on dry ice or in a −80 freezer until samples were processed. To process blood meals, engorged abdomens of *Cx. cedecei* were smeared onto Whatman Flinders Technology Associates (FTA) cards to preserve host DNA (38). For this, small sections of the blood samples of FTA cards were cut out using box-cutter razor blades, disposing of contaminated blades between each sample, and placed into 1.5 ml micro centrifuge tubes with five to ten glass beads for DNA extraction. DNA from blood-engorged *Cx. cedecei* was extracted using InstaGene™ Matrix (Catalog #: 732-6030; Bio-Rad Laboratories, Inc., Hercules, CA, USA) using a previously described protocol (39, 40). Host DNA was amplified from the extracted product using primers which target the 16s rRNA and cytochrome *b* genes as described in previous publications of mosquito host-use (16, 18, 41). Samples were screened using two 16s rRNA targeting primers including 16L1/H3056 for reptiles and amphibians, and L2513/H2714 for mammals (42, 43). The primer pair L0/H1, which targets the same region of the cytochrome *b* gene was used for

FIGURE 1
Georeferenced occurrence points (orange circles) used in the model representing *Cx. cedecei* rodent blood meals from this study and previous publications (16–18).

species-level identification of birds (44). Polymerase chain reaction cycling conditions were identical to those previously published (40, 41). Successful amplicon products were sent to Eurofins Genomics for Sanger sequencing and resulting sequences were identified to vertebrate host species using the National Center for Biotechnology Information (NCBI) GenBank Basic Local Alignment Search Tool (BLAST) (39).

Georeferenced occurrence points for vector-rodent interactions were defined as one of three rodent species blood meals from the primary enzootic vector *Cx. cedecei* from field sites in Florida, USA. The three rodent species chosen to build the model included hispid cotton rat, cotton mouse, and black rat based on potential to develop infectious titers of VEEV subtypes as reported in host competence studies and viral isolations (19, 21, 25, 45, 46). Sequences for *Rattus* spp. (*R. rattus* and *Rattus norvegica* Berkenhout) were highly similar (99% similarity). For this analysis, we consider *Rattus* spp. blood meals to originate from *R. rattus* (black rat) due to a small but consistently higher percent match in NCBI BLAST. After combining a total of 49 positive georeferenced rodent blood meals from this study and previous studies, the data were thinned spatially within a 0.001 degree distance (~1 km), which removed 1 record, with 48 occurrence points remaining for model calibration (Figure 1).

Model calibration area

We defined our model calibration area using the *M*-calibration region described in (47, 48). We limited the calibration area based on the known and modeled distributions of *Cx. cedecei* in Florida, including all of Peninsular Florida, the Florida Keys, and part of the Florida Panhandle, as previously described by Sloyer et al. (6). The calibration region for this model extends further north and west than the current known distributions of *Cx. cedecei* in Florida.

Environmental data

Bioclimatic variables derived from Daymet 1 km daily temperature and precipitation values from 2010 to 2020 (49) using the "bioclim" function in the "dismo" package in R served as climate variables in the model (50). National Land Cover Database (NLCD) from 2019 served as the land use and land cover data in the model (51). Percent land cover representing landscape composition, and edge density representing landscape configuration were calculated within a 2.5 km distance and aligned to the bioclimatic variables using custom R code, allowing us to combine these data in the same model and predict across new locations.

The bioclimatic and landscape variables were checked for multicollinearity to identify variable candidate sets, as

correlation between layers can contribute to difficulty in model interpretation (52). To check for multicollinearity, bioclimatic and landscape variable raster data were first masked to the M-calibration region and the variance inflation factor (VIF) values were calculated using the "vif" function in the "usdm" package in R (53). Four bioclimatic variables and percent land cover and edge density for ten NLCD habitat classes were checked for multicollinearity with a threshold value >5.

Two candidate sets of climatic and landscape variables were compiled based on their putative suitability to predict suitable habitat for *Cx. cedecei* and rodent species which include the hispid cotton rat, cotton mouse, and black rat, based on published habitat observations or recorded host-utilization by *Cx. cedecei* (6, 17, 26, 27, 54).

Model calibration and evaluation

Models were run in the ENMeval v.2.0.0 package in R using the "glmnet" algorithm (55, 56). Georeferenced occurrence data was partitioned into training and testing data internally using the "ENMevaluate" function with a spatial block method to help reduce potential bias from spatial autocorrelation. Candidate models were generated separately for the two candidate sets including either edge density or percent land cover values using all combinations of the following feature classes: "linear", "product", "quadratic", "linear + product", "quadratic + product", "linear + quadratic", and "linear + product + quadratic". Regularization multiplier values ranged sequentially between 1 and 4. Models were evaluated using an information criterion approach, ranking the models from lowest to highest Akaike's information criterion score (AIC) values and observing differences between the AUC train and test values (57). The best performing model was then run in Maxent (58) using the same parameter settings, but running with 50 bootstrapped replicates, and no extrapolation or clamping to produce variable response curves. Models were then projected to a broader area across Florida using the default "cloglog" output.

Results

Results of the blood meal analyses on mosquitoes captured with resting shelters were categorized as vector-host interactions and georeferenced to provide occurrence records for ecological niche models. These models were used to predict where suitable habitat for *Cx. cedecei* and EVEV rodent hosts (cotton rats, cotton mice, and black rats), may be located, in order to predict areas where higher potential EVEV transmission may occur in Florida. A total of 175 blood engorged *Cx. cedecei* were collected and processed as a part of the present study. Of these, 150 blood meals (85.7%) were successfully identified to vertebrate host species. There were

an additional five host species identifications which were identified from multiple host species blood meals, accounting for a total of 155 vertebrate host identifications. Vertebrate hosts included 13 species of mammal comprising 95.5% of identifications, four species of birds (3.9% of all host identifications), and one reptile host (Table 1). From the 155 host identifications, 68 (43.9%) were found to be derived from one of the three rodent hosts, with 22 of the 68 rodent host identifications from unique locations and used in the model (Table 1). Another 27 georeferenced rodent blood meals were compiled from previously published studies (16–18).

Calculations of the VIF initially showed correlation between environmental variables, especially landscape metrics, resulting in the evaluation of two separate candidate sets and the elimination of the developed medium habitat classification in the final set of variables. Two candidate variable sets were used in model runs, each set included the four bioclim variables and either the edge density habitat classes or the percent land cover habitat classes (Supplementary Table S2). All calculated VIF values were <5 for both candidate sets, indicating low correlations between layers used for model calibration.

A total of 48 models were generated using ENMeval across the two candidate sets of environmental variables. The best performing model in ENMeval was identified based on the lowest AICc value = 920.3, a high AUC of the receiver operating characteristic (ROC) (0.925) and a low difference in the AUC of the ROC of the internal training and testing data

(0.057). The best model was generated using the "percent land cover" variable set (Table 2), a combination of "linear" + "product" feature classes, and a regularization multiplier value of 3. Results from the model run in the Maxent software program using the same parameter values had an AUC of the ROC value = 0.919. Four environmental variables and nine habitat classifications were retained in the best performing model (Table 2). Three of the four environmental variables contributed to 80% of the model performance, with maximum temperature of the warmest month contributing the most (31.6%), followed by minimum temperature of the coldest month (28.5%), and precipitation of the driest month (19.9%) (Table 2). Precipitation of the wettest month contributed the least to model performance of the four environmental variables (4.6%). In contrast, landscape variables including percent land cover contributed less to model performance, with six of the nine variables contributing <1.0% (Table 2). The three habitat classifications contributing the greatest to the model performance included emergent herbaceous wetlands (5.2%), woody wetlands (4.3%), and developed open (2.7%).

For maximum temperature of the warmest month, the environmental variable with the greatest percent contribution, environmental suitability remained high across the entire range of maximum temperature of the warmest month (Figure 2). In contrast, environmental suitability generally increased as maximum temperature of the coldest month increased, with suitability tapering off at the highest

TABLE 1 Number and percent of total of host blood meals by host-class use and species use for *Cx. cedecei* in Florida.

Host class	Common name	Latin name	Blood-meals (*n*)	Percent of total (%)
Mammal			**148**	**95.5**
	Nine-banded armadillo	*Dasypus novemcinctus*	6	3.9
	Black rat	*Rattus rattus*	9	5.8
	Dog	*Canis lupus familiaris*	1	0.6
	Rabbit	*Sylvilagus* spp.	34	21.9
	Eastern woodrat	*Neotoma floridana*	10	6.5
	Hispid cotton rat	*Sigmodon hispidus*	49	31.6
	Human	*Homo sapiens*	1	0.6
	Cotton mouse	*Peromyscus gossypinus*	10	6.5
	Raccoon	*Procyon lotor*	7	4.5
	River otter	*Lontra canadensis*	1	0.6
	Virginia opossum	*Didelphis virginiana*	15	9.7
	White-tailed deer	*Odocoileus virginianus*	4	2.6
	Wild boar	*Sus scrofa*	1	0.6
Bird			**6**	**3.9**
	Chicken	*Gallus gallus*	3	1.9
	Common yellowthroat	*Geothlypis trichas*	1	0.6
	Green heron	*Butorides virescens*	1	0.6
	Loggerhead shrike	*Lanius ludovicianus*	1	0.6
Reptile			**1**	**0.6**
	Brown anole	*Anolis sagrei*	1	0.6
Total			**155**	

Blood meals determined by PCR-based assays using primers which targeted the 16s rRNA and cytochrome *b* genes for reptiles, amphibians, birds, and mammals. DNA sequences were identified to vertebrate host species using the National Center for Biotechnology Information (NCBI) GenBank Basic Local Alignment Search Tool (BLAST).

TABLE 2 Estimates of the relative contributions and permutation importance of each environmental and landscape variable used in the final model.

Variable	Percent contribution	Permutation importance
Max temperature of warmest month (bio5)	31.6	0.3
Min temperature of coldest month (bio6)	28.5	27.8
Precipitation of driest month (bio14)	19.9	3.9
Emergent herbaceous (percent land cover) (95)	5.2	6.2
Precipitation of wettest month (bio13)	4.6	4.1
Woody wetlands (percent land cover) (90)	4.3	4.8
Developed open (percent land cover) (21)	2.7	11.1
Mixed forest (percent land cover) (43)	0.9	0.3
Developed low (percent land cover) (22)	0.9	3.0
Cultivated crops (82)	0.8	0.9
Grassland/Herbaceous (percent land cover) (71)	0.5	4.4
Shrub/Scrub (percent land cover) (52)	0.1	1.0
Deciduous forest (percent land cover) (41)	0.1	0.3

temperatures (Figure 2). Response curves for precipitation of the driest month indicated a marginal decrease in suitability with increased precipitation during the driest month, whereas precipitation of the wettest month showed a slight decrease as precipitation decreased during the wettest month (Figure 2). The three habitat classifications contributing greatest to model performance included the emergent herbaceous wetlands, woody wetlands, and developed open landscapes (Table 2). Suitability values for these variables all showed slight increases as percent land cover increased for each of the classes (Figure 2).

The model predicted that substantial areas of Florida are suitable for *Cx. cedecei* feeding upon rodents, with large suitable areas in the southern half of the Florida Peninsula, and smaller areas along the Atlantic Coast, the central peninsula, and the northern Gulf Coast (Figure 3). The area of highest predicted environmental suitability was observed in southernmost Florida, consisting of the Florida Keys, and the majority of Collier, Miami-Dade and Monroe Counties. Standard deviation values in these regions were low, indicating lower variability in model outputs and higher confidence in the predicted suitability in these areas (Figure 3). Areas of high suitability were also predicted throughout Lee County, Glades County, Highlands County, the western half of Charlotte County, and the southern half of Sarasota County, with areas of moderate suitability predicted throughout much of these counties (Figure 3). Although low suitability was predicted throughout the Miami Metropolitan Area (City of Miami to West Palm Beach), there were pockets of predicted high suitability on the western margins of these urban areas, where they reach the greater Everglades area (Figure 3). Standard deviation values were higher in these transitional areas, indicating lower model confidence for these areas (Figure 3). An area of patchy high suitability was observed west of Cape Canaveral, consisting of substantial

portions of five counties (Brevard, Lake, Orange, Osceola and Seminole Counties) (Figure 3). Smaller low-to-moderate areas of suitability are also predicted for coastal portions of the entire Atlantic Coast, excluding the Miami Metropolitan area. Small areas of the Florida Panhandle were predicted to have low suitability for vector-host interactions (portions of Franklin, Gulf and Leon Counties) (Figure 3). These areas of low predicted suitability in the Panhandle had moderate standard deviation values, indicating relatively high variability in model confidence in these regions.

Discussion

The objective of this study was to model areas where EVEV virus transmission could occur using georeferenced *Cx. cedecei* blood meals derived from rodent hosts, to investigate whether this method produces accurate models to predict the distribution of the nidus of transmission. The best performing model included four climate variables summarizing parameters for precipitation and temperature, and nine landscape variables using percent land cover data. Collectively, climate variables contributed to 84.6% of the model, while landscape variables contributed only 15.4% of the model. In general, areas with the greatest predicted suitability for *Cx. cedecei* feeding upon rodents included subtropical regions of southern Florida, as well as less-developed areas in central Florida (Figure 3). Other areas with high predicted suitability included areas inland from Cape Canaveral through Lake County, and along the Atlantic Coast (Figure 3).

Precipitation and temperature bioclimatic variables contributed the greatest to model performance, suggesting that climatic factors play an important role in constraining the distribution where *Cx. cedecei* encounters and feeds upon rodents. This is likely due to the way that water levels and
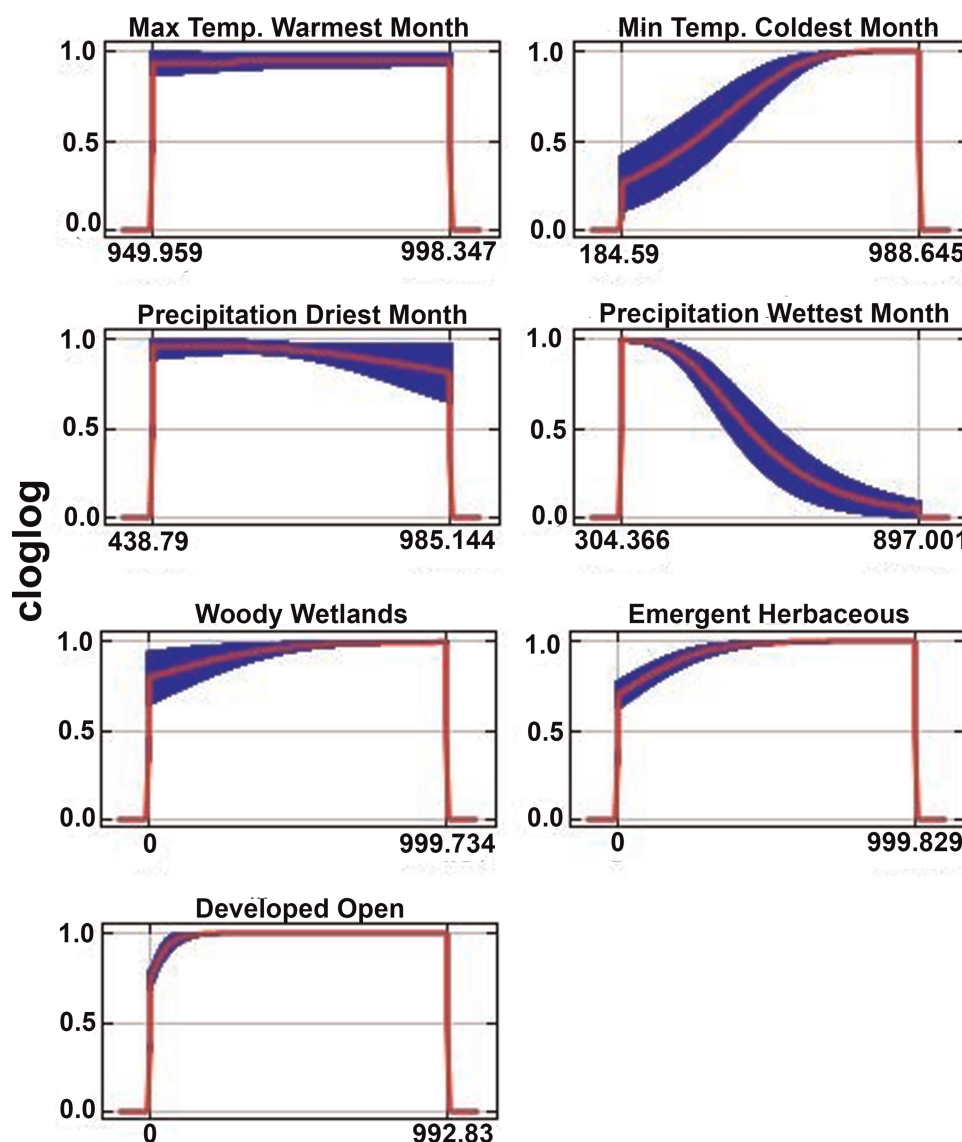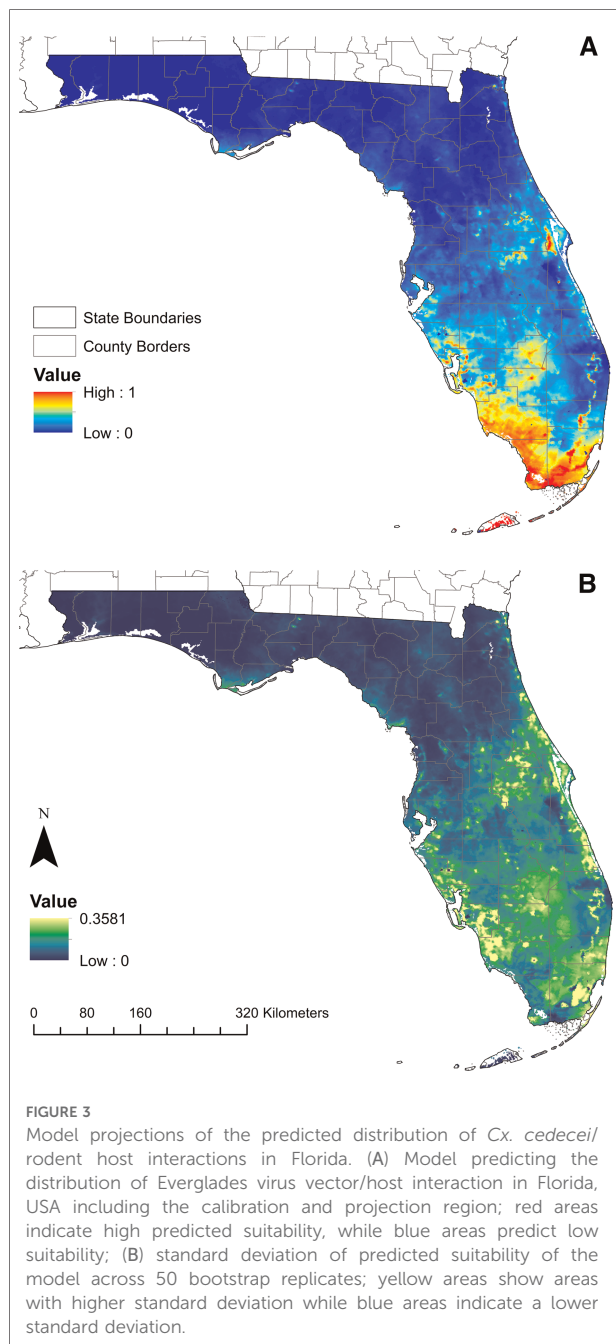
FIGURE 2
Response curves of bioclimatic and habitat classification variables used in the final model. X-axes represent standardized values of model variables. Only the seven variables contributing the most (>96%) to model performance were included in the figure.

temperature limits throughout the year influence both the subterranean larval habits of *Cx. cedecei* as well as the reproductive cycles of the vertebrate hosts of EVEV. For example, the model showed that suitability of *Cx. cedecei* feeding on rodent hosts increased with increased minimum temperatures during the coldest month, which corresponds to the subtropical portions of the state (**Figure 2**). Since the minimum temperature during the coldest month contributes a substantial amount to the performance of our model, it is understandable that much of southwestern Florida is considered suitable for *Cx. cedecei* to feed on rodent hosts, since these are where the warmest temperatures occur in

Florida (59). This interpretation is supported by the finding that suitability for *Cx. cedecei* feeding upon rodents remained high, and even increased slightly, with increasing temperatures during the warmest month (**Figure 2**), which contributed the most to model performance (31.6%), indicating that *Cx. cedecei* feeds upon rodents in tropical areas of the state. Therefore, with potentially increased annual temperatures and changes in mean daily minimum and maximum temperatures predicted in the coming years due to climate change (60, 61), prevalence of suitable areas were *Cx. cedecei* feeds upon rodent hosts is likely to increase, especially in areas with similar woody wetland coastal habitats such as areas along the

FIGURE 3
Model projections of the predicted distribution of *Cx. cedecei*/rodent host interactions in Florida. **(A)** Model predicting the distribution of Everglades virus vector/host interaction in Florida, USA including the calibration and projection region; red areas indicate high predicted suitability, while blue areas predict low suitability; **(B)** standard deviation of predicted suitability of the model across 50 bootstrap replicates; yellow areas show areas with higher standard deviation while blue areas indicate a lower standard deviation.

months of July through October (the height of the wet season) in the northern Everglades, when both hispid cotton rats and cotton mice are concentrated in hardwood hammocks of the Everglades (45). Because climate might be a primary driver of the phenology of both vector and host, it is predictable that precipitation and temperature variables had such high contributions to model performance (24, 45).

Landscape variables contributed considerably less than climate variables to the performance of the best model (Table 2). The landscape variables with the greatest contributions were percentage of emergent herbaceous wetland, which contributed 5.2% to model performance, and percentage of woody wetlands, contributing 4.6% (Table 2). Emergent herbaceous wetlands constitute marshes, which cover a large portion of the Greater Everglades Ecosystem, and during the dry season, are important reproductive habitats for the cotton rat and cotton mouse. Woody wetlands include mangrove forests, cypress swamps, and hardwood swamps, which are confirmed habitats of *Cx. cedecei*. *Culex cedecei* may encounter and feed upon muroid rodents where these habitat classes overlap, indicating that EVEV transmission may not be restricted to the hardwood hammocks of the southern Everglades ecosystem, as earlier studies indicated (15, 19, 23, 25, 45, 62). This is corroborated by Fish et al. (63) which reported EVEV isolations from mosquito pools from diverse habitats, including hardwood hammocks, mangroves, strand swamp, and pinelands. Everglades virus was detected in mosquitoes in all of these habitats in 2013 (a high-water year), but in only hardwood hammocks in 2014 (a low-water year), suggesting that hardwood hammocks may be primarily crucial for the maintenance of EVEV during dry periods, likely because hardwood hammocks provide subterranean refuge for larval *Cx. cedecei* in limestone solution holes and are the only available dry habitat accessible to rodents during periods of high water (35). Although the landscape variables used here did not contribute as much to model performance as expected, it is possible that additional habitat variables such as water table depth would have a more substantial impact on the model, considering the larval habitat of *Cx. cedecei* in many subterranean habitats such as solution-holes or crab burrows.

Areas predicted suitable for *Cx. cedecei* blood meals from rodents corresponded closely to locations of historical EVEV cases in humans and infections in wildlife, but not for a recent serosurvey of dogs (Figure 4). The seven locations where historical human cases and detection of antibody presence of EVEV recorded from 1960 to 1971 occurred in areas predicted to be moderate to highly suitable for *Cx. cedecei*/rodent interactions in Florida, supporting the notion that proximity to areas where *Cx. cedecei* actively feeds upon rodent amplifying hosts could lead to higher spillover risk to humans (30–33). In some areas, however, such as Vero Beach, Florida, where a 78-year-old man was diagnosed with EVEV in 1968, our models predict only moderately suitable
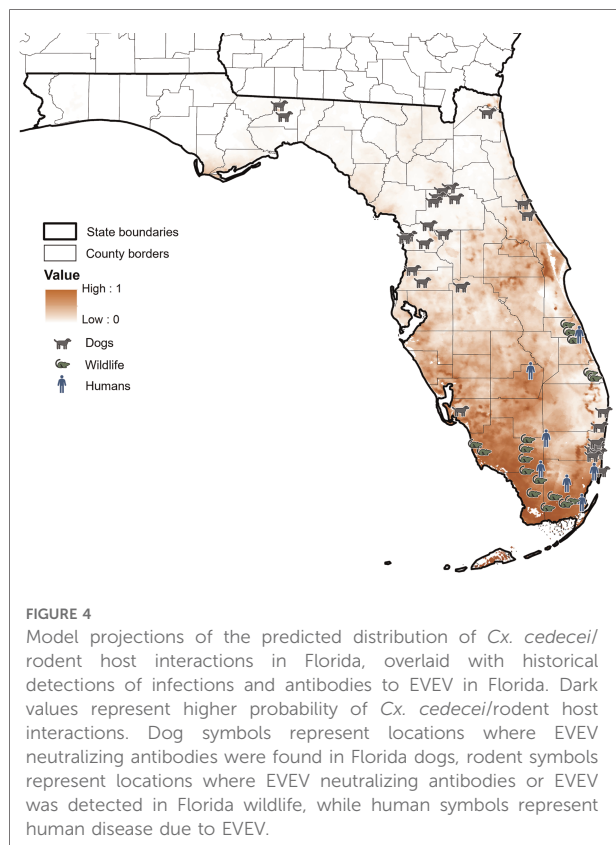
Gulf Coast including the Florida Panhandle, Louisiana, and the Texas coast. Finally, that suitability for *Cx. cedecei* to feed on rodent hosts decreased with increasing precipitation during the driest month (contributing to 19.9% of model performance) could be due to poorly defined wet and dry seasons of temperate (northern) Florida, where *Cx. cedecei* is not known to occur. However, that increased precipitation during the driest months would decrease suitability for vector/host interactions is somewhat surprising, considering that most EVEV amplification is thought to occur during the

FIGURE 4
*Model projections of the predicted distribution of Cx. cedecei/
rodent host interactions in Florida, overlaid with historical
detections of infections and antibodies to EVEV in Florida. Dark
values represent higher probability of Cx. cedecei/rodent host
interactions. Dog symbols represent locations where EVEV
neutralizing antibodies were found in Florida dogs, rodent symbols
represent locations where EVEV neutralizing antibodies or EVEV
was detected in Florida wildlife, while human symbols represent
human disease due to EVEV.*

environments for *Cx. cedecei*/rodent host interactions for EVEV
(32). Vero Beach has undergone significant development since
the 1960's, likely reducing suitable habitat for EVEV
transmission since that period (64). Georeferenced data of
antibody and virus detection in wildlife in Florida undertaken
in the 1970's also show much overlap with areas predicted
highly suitable for *Cx. cedecei* blood meals from rodent hosts
(22, 24, 34), also supporting the importance of these
organisms in transmission of EVEV. In contrast, the predicted
distribution of *Cx. cedecei* blood meals from rodent hosts in
Florida do not overlap substantially with serological evidence
of EVEV exposure in sentinel dogs (36). Coffey et al. (36)
speculate that other vector species may be involved in
transmission of EVEV, however no other mosquito species in
Florida feeds heavily upon rodents, the only recognized
vertebrate hosts of EVEV (15, 65–69). Therefore, more work
is needed to determine whether *Cx. cedecei* occurs outside of
the regions predicted to be suitable for the presence of *Cx.
cedecei* feeding on rodent hosts in this study to help to
explain antibody detections in sentinel dogs in these areas.
Although most previous studies suggest that the distribution
of *Cx. cedecei* blood meals from rodent hosts are likely to be
accurately represented by our model, future studies of
arbovirus detection in other Florida mosquito and vertebrate
populations may still be warranted to rule out the possibility

of other vector and host species involvement in the
transmission of EVEV.

In total, there were 14 locations where *Cx. cedecei* was
collected but no rodent blood meals were detected, indicating
that *Cx. cedecei* feeds readily upon nonrodents and host use of
this vector is influenced by local environment and available
mammals (16, 18). At most of these sites (13 of 14), insufficient
sampling (two or fewer blood meals), may have affected the lack
of rodent blood meals. However, given the naturally high
fraction of blood meals that Cx. cedecei takes from rodents
[>50% (15),], we expect that even with low sample sizes ($n > 3$)
rodent blood meals would be detected. At one location in Polk
County, three blood-engorged *Cx. cedecei* were analyzed, and
two females fed upon cottontail rabbit while a third fed upon
Virginia opossum, suggesting that even if *Cx. cedecei* does
encounter rodents at this site, its feeding upon rodents could be
too infrequent to support EVEV transmission.

This study has several limitations. Sampling was fairly
opportunistic, relying heavily upon properties with access
granted to mosquito control district personnel. More
sampling, across more sites distributed across all landcover
classes may have yielded additional insights into the
environmental drivers of vector-host contact. The assumption
that EVEV is maintained in nature solely by *Cx. cedecei* and
rodent amplifying hosts, while supported by many studies (15,
65–70), is not supported by evidence from EVEV-seropositive
dogs in areas of Florida that are far outside the known range
of *Cx. cedecei* (36). There are also other aspects of virus
transmission which are not considered in this model such as
pathogen presence and detection, vector competency of
varying populations of *Cx. cedecei*, and host competency of
rodent populations and subspecies (71) (vector and host
competence is known to change between populations of
mosquito species and subspecies of hosts). Pathogen presence
and detection was not undertaken as a part of this study
primarily because we wished to model the potential areas
where pathogen transmission might occur due to the
interactions between vectors and vertebrate hosts.
Furthermore, many thousands of mosquitoes (approximately
1,000 female mosquitoes per site) must be collected, identified
and pool screened by virus isolation or PCR to sample the
minimum number needed to detect EVEV in the vector,
exponentially increasing the effort and expense. We show that
with a small number of blood-engorged specimens per site,
the conditions conducive for transmission can be modelled
across the landscape. Including virus data may also limit risk
estimates, and potentially introduce spatial sampling biases
(12). Finally, in this effort, we did not compare predicted
distributions of *Cx. cedecei* rodent bloodmeals with predicted
distributions of vector or host species alone. Cotton rats have
a ubiquitous distribution occupying a broad range of habitats
in the study area and therefore model predictions may not be
widely informative. However, Sloyer et al. (2022) presents an

ecological niche model predicting the potential distribution of *Cx. cedecei* in Florida, which exhibits greater habitat constraints, and this model output provides a valuable resource for visual comparison.

In this study, we employ geographically referenced *Cx. cedecei* blood meals to investigate whether locations where vectors feed upon EVEV pathogen hosts can be used in ecological niche modeling to predict areas where the potential nidus of transmission may occur. This method should allow for better inference of potential transmission areas than models which only consider the vector or amplification host species individually. Comparing predicted distributions of *Cx. cedecei* feeding upon rodent hosts in the present study to historical human cases of EVEV disease, as well as antibodies in wildlife show substantial overlap with areas predicted moderate to high for these vector/host associations. As such, the findings of this study likely predict the most accurate distribution of the nidus of EVEV to date. A similar approach using host blood meals of other arboviruses can be used to predict potential areas of virus transmission for other vector-borne diseases such as eastern equine encephalitis virus, West Nile virus, or St. Louis encephalitis virus in the United States. Knowledge of areas where vectors feed on amplifying hosts of arboviruses can infer risk to potential human spillover of disease, drawing attention to areas where undiagnosed causes of encephalitis or acute disease in humans could be the result of arbovirus infection.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

Author contributions

KS, LC, NB-C designed the study. KS and LC completed the modelling and statistical analysis. KS, DK, and NB-C performed the fieldwork. KS and TS performed the molecular analysis. NB prepared habitat and environmental variables for the model. KS led the writing of the manuscript and edits were made by NB-C and LC. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fepid.2022.1046679/full#supplementary-material.

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