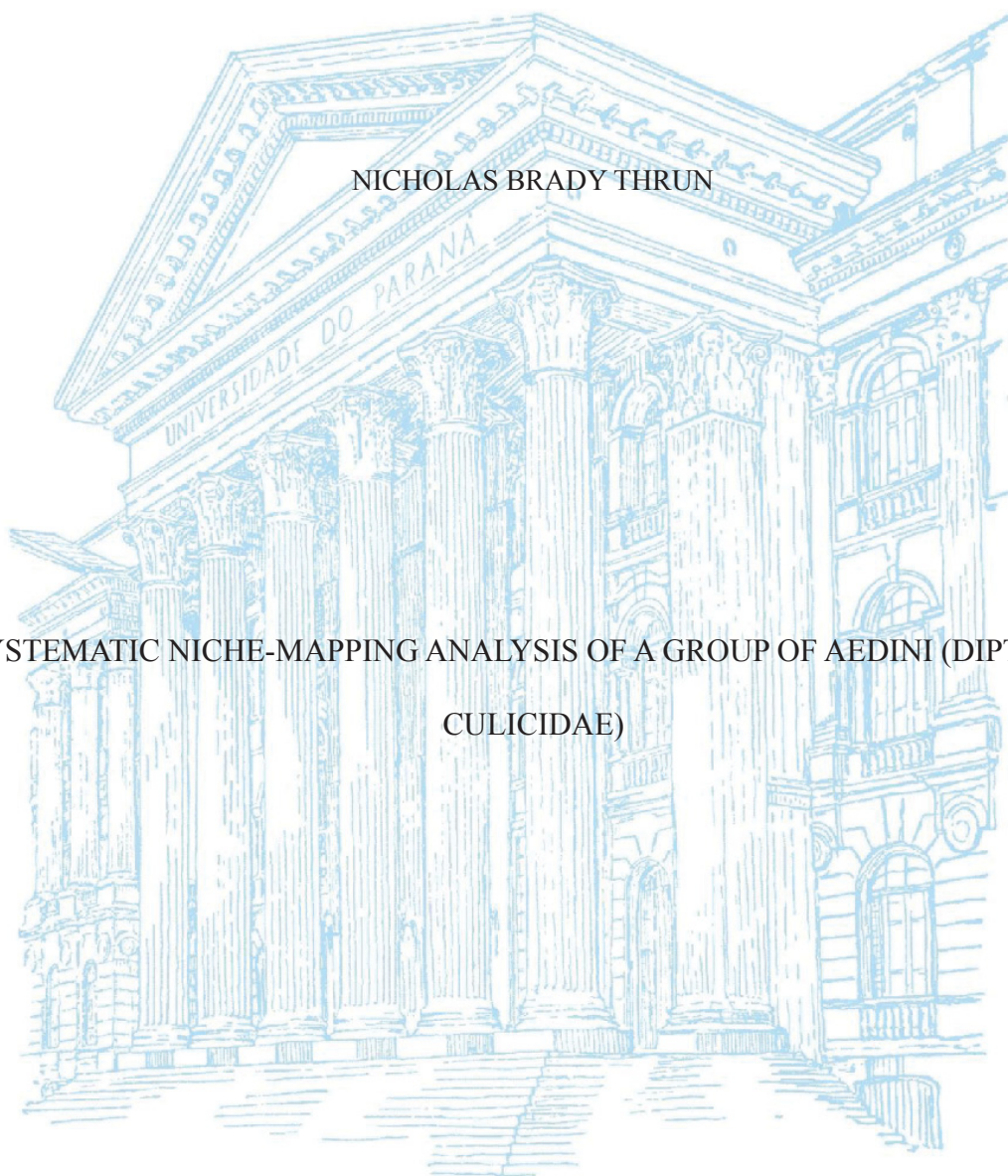


UNIVERSIDADE FEDERAL DO PARANÁ

NICHOLAS BRADY THRUN

SYSTEMATIC NICHE-MAPPING ANALYSIS OF A GROUP OF AEDINI (DIPTERA:
CULICIDAE)



CURITIBA

2022

NICHOLAS BRADY THRUN

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CULICIDAE)

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Orientador: Prof. Dr. Mário Antônio Navarro da Silva

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*Mir hilft der Geist, auf einmal seh ich Rat;
Und schreibe getrost: «Im Anfang war die Tat!»*

-Johann Wolfgang von Goethe

*System of Nature! To the wisest man, wide as is
his vision, Nature remains of quite infinite depth,
of quite infinite expansion; and all Experience
thereof limits itself to some few computed
centuries, and measured square-miles.*

-Thomas Carlyle

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RESUMO

Foi realizada uma análise de mapeamento de nicho ecológico por reconstrução de estados ancestrais, com uma filogenia de 12 espécies de Aedini (Diptera: Culicidae). Por “*nicho ecológico*” são conotados caracteres relacionados com habitat e utilização de recursos, comportamento, e outros caracteres não-estruturais. Caracteres analisados compreendem sítio de, e condição da água encontrada em (e.g., salinidade, temperatura), o habitat dos imaturos; registros de utilização de hospedeiro para alimentação de sangue e/ou autogenia; comportamento de acasalamento, de oviposição das fêmeas, e fisiologia de ovos; e sazonalidade e atividade diária de fêmeas em busca de hospedeiros. Foi achado que o último ancestral comum (LCA) deste grupo de Aedini utilizava habitats de água de chão nas estágios imaturos, particularmente os que sejam permanentes inclusive zonas úmidas do tipo pântano, junto com uma ‘predisposição’ a utilizar criadouros antropogênicos de chão bem como recipientes artificiais, com uma transição intermediária a brejos bem como habitats de chão semipermanentes e temporários, então para margens de córregos, então ocos de rocha, e finalmente ocos de decápodes. Este LCA utilizava água fresca, apresentava intolerância de temperaturas extremas junto com tolerância de ambientes ácidos e de presença de vegetação, detritos, e poluição. Subsequentemente derivada foi a tolerância de águas tanto doces como salobras e hipersalinas, e finalmente ocorreram transições a utilização obrigatória de águas salobras e ligeiramente salinas, tolerância de temperaturas extremas, e intolerância de vegetação, detritos, poluição, e ambientes ácidos. Este LCA demonstrava anaautogenia e uma disposição a se alimentar de Mammalia e Aves, particularmente *Homo sapiens*, Ungulata, Rodentia, e Phasianidae. Subsequentemente colonizados foram táxons de hospedeiro Leporidae, Carnivora, Marsupialia, e Passeriformes, então *Haliaeetus* spp., Odontophoridae, e Columbiformes, então autogenia facultativa, e finalmente uma troca para Amphibia, Gobioidi, e Anguilliformes e outros grupos de peixe, junto com uma perda secundária de autogenia. Este LCA demonstrava comportamento de acasalamento simples, subsequentemente derivando a tendência de fazer enxames. Fêmeas a ovipositar do LCA demonstrava uma restrição a habitats de água permanente e postura de ovos não resistentes à dessecação, posteriormente derivaram uma tolerância de habitats de água temporária e a postura de ovos resistentes à dessecação, e finalmente derivaram uma especialização a substratos secos e postura de ovos que exigem um estímulo de condicionamento antes de eclodir. O LCA teria, se ocorreu num clima com inverno severo, passado o inverno na etapa adulta fêmea, posteriormente ou passando o inverno no ovo ou perdendo o hábito de passar inverno se ocorreu numa área sem inverno severo. Finalmente o LCA, em sua atividade diária de busca de hospedeiros, era estritamente noturno. Foi derivada mais tarde a busca de hospedeiro no período do crepúsculo. Então ocorreu uma troca de atividade de busca de hospedeiros de noite a dia, às vezes junto com uma perda do hábito crepuscular, e finalmente derivados foram o comportamento oportunista de busca de hospedeiros em qualquer horário de dia ou noite bem como uma reversão ao hábito noturno. Este trabalho constitui uma tentativa inicial de mapeamento de nicho ecológico nos Aedini. Os resultados têm implicações para estudos futuros na sistemática, ecologia teórica, biogeografia, biologia evolutiva, e evo-devo. Estudos de sistemática futuros tal como este irão ficar cada vez melhor com a coleção de dados ecológicos mais precisos em relação ao nicho e as exigências ambientais de determinado táxon, junto com a publicação de mais estudos da sistemática filogenética, particularmente os que combinam caracteres morfológicos e moleculares.

Palavra-chaves: Aedini, Culicidae, *Aedes*, *Ochlerotatus*, *Geoskusea*, *Rhinoskusea*, *Levua*, sistemática, ecologia, comportamento.

ABSTRACT

An ecological niche-mapping analysis was performed by ancestral state reconstruction on a phylogeny of 12 species of Aedini (Diptera: Culicidae). By 'ecological niche' it is connotated characters related to habitat and resource use, behavior, and other non-structural characters. Characters analyzed include locale of, and condition of water found in (*e.g.*, salinity, temperature), immature habitat; blood-feeding host records and/or autogeny; mating and female oviposition behavior and egg physiology, and seasonality and day-night host-seeking activity of adult females. It was found that the last common ancestor (LCA) of this group of taxa, including outgroups, utilized groundwater habitats in the immature stages, particularly permanent ones including swamp-type wetlands, demonstrating as well a 'predisposition' to utilize man-made groundwaters and artificial containers; with an intermediate transition to marshes as well as semi-permanent and temporary ground habitats; then stream margins; then rock pools; then finally of decapod holes. This LCA utilized freshwater, had an intolerance of temperature extremes, and a tolerance of acidic environments and presence of vegetation, detritus, and pollution; subsequently derived were taxa which tolerate fresh as well as brackish and hypersaline habitats; and finally occurred transitions to obligate brackish and moderately saline water utilization, tolerance of temperature extremes, and intolerance of vegetation, detritus, pollution, and acidic environments. This LCA demonstrated anautogeny and would have fed on Mammalia and Aves, particularly *Homo sapiens*, Ungulata, Rodentia, and Phasianidae; subsequently colonized were host taxa Leporidae, Carnivora, Marsupalia, and Passeriformes; then came *Haliaeetus* spp., Odontophoridae, and Columbiformes; then the advent of facultative autogeny; and finally a switch to Amphibia, Gobioidae, and Anguilliformes and other fish groups, along with a secondary loss of autogeny. This LCA demonstrated simple mating behavior, subsequently deriving a propensity to swarm. Ovipositing females of the LCA demonstrated a restriction to permanent water habitats and laying of non-desiccation-resistant eggs, later deriving a tolerance for temporary water habitats and laying desiccation-resistant eggs, and finally deriving a preference for dry substrate and laying eggs which require a conditioning stimulus prior to hatching. The LCA would have, if in a climate with harsh winters, overwintered in the adult female, later deriving overwintering in the egg or losing the overwintering habit if in an area without harsh winters. And finally the LCA, in its day-night host-seeking behavioral activity, was strictly nocturnal; host-seeking at dusk was derived later; then occurred a switch from nighttime to daytime host-seeking activity, sometimes accompanied by the loss of dusk habits, and finally derived were the opportunistic behavior of host-seeking at any time of day or night as well as a reversal to nighttime host-seeking activity. This paper constitutes a groundwork attempt at ecological niche-mapping in the Aedini. The results have implications for further studies in systematics, theoretical ecology, biogeography, evolutionary biology, and evo-devo. Future systematic works such as this one will be improved by collection of more precise ecological data regarding a given taxon's niche and environmental requirements, as well as by publication of more studies of phylogenetic systematics, particularly those that combine morphological and molecular characters.

Keywords: Aedini, Culicidae, *Aedes*, *Ochlerotatus*, *Geoskusea*, *Rhinoskusea*, *Levua*, systematics, ecology, behavior.

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1. INTRODUCTION

Among the 11 tribes of subfamily Culicinae (Diptera: Culicidae), Aedini is one of the more well-studied systematically (see, *e.g.*, Reinert *et al.*, 2004, 2006, 2008, 2009; Huang *et al.*, 2010; Soghigian *et al.*, 2017). Aedini is furthermore the most speciose tribe of mosquitoes, with 1,262 species (Harbach, 2013). Some of the most charismatic (*e.g.*, *Psorophora ciliata*) and medically-important mosquito species (*e.g.*, *Stegomyia aegypti*) are contained within this tribe. As compared to other tribes of mosquitoes, Aedini appears to present a great degree of derivation, both in its *bauplan* and in its subsequent diversification, as well as variability among its species. This is evident in its member species' structural characters such as morphology and anatomy, as well as in non-structural characters such as physiology, ecological niche utilization, and behavior (Harbach, 2013, 2007; Rattananarithikul *et al.*, 2010; Reinert, 2000).

If for no other reason than by historical convention, it is typically such structural characters¹ that serve, not only as criteria for the separation of insect species into taxonomic entities, but also as criteria for grouping in phylogenetic systematics, by hypotheses of homology. It is by this methodological process that organisms see themselves at once defined by contrasts which serve to distinguish them for the purposes of taxonomic diagnosis as well as conceptually united in phylogenetic hypotheses which serve as jumping-off points for the subsequent study of evolution. There is however somewhat of a preconception against non-structural characters, such as those of behavior, which says that they are unsuitable for the purposes of taxonomy and systematics, due to their putatively high degree of evolutionary lability, a characteristic which would make them non-homologizable. This charge has been amply rejected as false by de Queiroz and Wimberger (1993): these authors found behavioral characters not to be any more homoplastic than morphology (see also Wimberger & de Queiroz, 1996; Miller & Wenzel, 1995). Furthermore, Proctor (1996) found that the reason many systematists do not use behavioral characters is not because they believe them to be too labile, but simply due to logistical and financial difficulties in facilitating the collection of such data. It is an undeniable fact that behavioral data is difficult, time-consuming, and potentially expensive to collect, as compared with regular specimen morphology review at the microscope or indeed the increasing ease and cost-effectivity of molecular sequencing, and it is probably this fact that most impedes the realization of systematic analyses of

¹ In the past, and in taxonomy in general, this is typically exclusively by morphology. Today, molecular characters are becoming more and more standard for this work, particularly in systematics.

behavior. In the context of data collection for this study, what ultimately proved most practical was a literature review, but it is important to keep in mind that this and/or future analyses could greatly benefit from the energy, time, and financial investment above alluded to. An important caveat of the literature review approach is the lack of standardization of terminology between different studies. As one example of this, many studies employ the vague phrase ‘ground pools’ to connote a wide variety of temporary, semi-permanent, and permanent accumulations of water, in the context of various types of wetlands (marsh, swamp, *etc.*), grass-lying floodwaters, ponds, and artificial ‘ground’ habitats. Other studies may offer any variety of degree of higher terminological precision.

In the present work the author’s intention is to perform an initial attempt at systematic ecological and behavioral analysis in the study of Aedini, systematically analyzing four broad category types of characters which occur in species of this tribe and their outgroups: 1) immature habitat locale and water condition found therein (*e.g.*, salinity, turbidity), 3) bloodmeal host sources and/or autogeny, 4) mating behavior, female oviposition behavior, and egg physiology, and 5) seasonality and time of day-night host-seeking activity of females.

For this study, the sixteen-species phylogenetic hypothesis (considering the twelve Aedini taxa as ingroup, and four tribal-level outgroups) produced by Huang *et al.* (2010) was chosen as a base onto which to determine ancestral states of behavioral and ecological characters of these taxa. The twelve aedine taxa are *Abraedes papago*, *Aedes cinereus*, *Aedes esoensis*, ‘*Ochlerotatus*’ (‘*Coetzeemyia*’) sensu auctorum *fryeri*, *Ochlerotatus (Juppius) caballus*, *Ochlerotatus (Empihals) vigilax*, *Ochlerotatus dufouri*, *Levua geoskusea*, *Rhinoscusea longirostris*, *Rhinoscusea wardi*, *Geoskusea baisasi*, and *Geoskusea longiforceps*, and the four non-aedine taxa are *Culiseta (Culiseta) inornata* (Culisetini), *Orthopodomyia signifera* (Orthopodomyiini), *Culex (Culex) quinquefasciatus* (Culicini), and *Mansonia (Mansonia) titillans* (Mansoniini).

By ‘niche’ it is here understood what other authors have termed ‘ecotype’²: “the species’ response to the full range of environmental variables to which it is exposed” (Whittaker *et al.*, 1973). Importantly, this species response is not restricted to single populations of a particular biological community: when it is asserted in the work of Hamon (1953), for example, that *Cx. quinquefasciatus* is known to utilize rock pools on the island of

² The present author does however avoid totally embracing the use of the term ‘ecotype’ as it has become somewhat antiquated within the science of ecology, and for this reason ‘niche’ in a general sense is retained in its place.

Réunion, this observation is not to be discounted just because workers dealing with this species in, *e.g.*, Argentina have not observed any such habitat utilization. Rather, this observation may represent a local specialization of merely one population of the cosmopolitan species: specifically, either gravid adult females' response to the stimulus of a rock pool is to oviposit in it, or otherwise the response of larvae to finding themselves in such a habitat is to feed and develop as normal for mosquito larvae, despite the seemingly strange context as compared with other populations of the same species. In evidence of this general fact, though working with another culicomorph family, Serra *et al.* (2017) compared data on niche utilization and environmental requirements of Holarctically-distributed Chironomidae, compiled for the Nearctic and Palearctic regions, respectively, and found that individual species often vary dramatically over their expansive distributions.

More generally in the context of mosquito biology, it is known in *e.g.*, oviposition site selection, that water quality, odor, light incidence, presence of other organisms, and food and vegetation availability play an important role; and in *e.g.*, host location, that various olfactory, visual, and thermal stimuli present in concert to the host-seeking mosquito a diagnostic distinctive to that host taxon (Becker *et al.*, 2020). For the purposes of this study, only those characters which can reasonably be expected to present heritability, as opposed to environmental plasticity, will finally be systematically analyzed, the analysis then being limited to solely those thought to represent the former possibility. Foucault *et al.* (2018) provided an interesting example of the disentanglement of heritable and environmentally plastic characters in *Chironomus riparius* (Diptera: Chironomidae), showing that differences in high temperature tolerance can be inherited over as little as three generations and thus such tolerances are not the product of merely individual plasticity.

One recent example of a systematic study of ecological characters is that of the evolutionary biology analysis of Soghigian *et al.* (2017). These authors broke down larval habitats utilized by aedines into five categories: 'ground pool', 'salt pool', 'rock pool', 'container', and 'crab hole'; and found a ground pool-inhabiting ancestor for Aedini, with repeated subsequent colonizations of container habitats. This result would apparently confirm Belkin's (1962a, p. 47) assertion that "ground water habits are undoubtedly the primitive breeding sites of the Culicidae." Soghigian *et al.* (2017) further endeavored to demonstrate, via a likelihood-based ancestral character state estimation analysis, that such container colonizations confer an evolutionarily adaptive advantage to the taxa which manage them and, once gained, are selectively conserved, in Aedini. Closer scrutiny of the character coding

methodology of these authors, however, reveals some problems which could challenge the tenability of this result.

It is an unfortunate artifact of long-standing traditions in mosquito ecology (particularly efforts undertaken insofar as considered necessary for vector control — not necessarily pure biological description) that some vague phrase like ‘ground pools’ should be employed in place of any more precise habitat description. Mattingly (1952, p. 271) pointed out that this common practice of classifying larval habitats into such broad categories does not reflect a natural classification of these niches, and that, insofar as possible, these categories much be further critically considered and subdivided in order to achieve a result which matches reality as closely as possible. Given the five categories of Soghigian *et al.* (2017) listed above (ground pools, salt pools, rock pools, artificial containers, and crab holes), we can immediately see that further subdivision of these character states could drastically alter the conclusion of these authors, namely the apomorphic colonization of container habitats by a plesiomorphically ground pool-inhabiting ancestor. In order to facilitate an increase in the precision and utility of future literature review-based systematic ecological studies such as that of Soghigian *et al.* (2017) and the present one, future pure ecological studies should take detailed physical, chemical, and biological parameters for the extent and nature of habitats utilized by mosquito species. Adherence to the highest degree of habitat specificity possible was attempted in the present study, given the existence of at least some more detailed studies, resulting in a distinguishing of various types of groundwater and permanent habitats.

On the other hand, at another level it is desirable to hypothesize essential features which may unite more specific habitat categories, such as the difference between groundwater and container habitats which is widely recognized to often reflect a real biological compartmentalization in the preferences and/or tolerances of each taxon. Mattingly (1969, p. 90) for example suggests, as a rationale for the separation of permanent from temporary ground pool habitats, that permanent ones exist long enough for a “specialized flora of aquatic vascular plants” to develop, while temporary ones do not necessarily. To this end, an attempt was made to reconstruct ancestral states at two or sometimes even three levels of biophysical specificity, particularly for immature habitat locale and hematophagous habits.

But there is another significant problem with the methodology that produced Soghigian *et al.* (2017)’s results, however: for the vast majority of taxa, these authors take their habitat categories to be non-overlapping. For many aedine taxa, this supposition could

not be further from the case; it ostensibly invokes a differentiation between ‘preferred’ and ‘occasional’ occurrence of immatures in a given habitat (see Mattingly, 1952, p. 270), clearly giving preference for coding purposes only to those habitats considered ‘preferred’. In reality it is quite a messy affair to assert for example that a ‘crab hole’-inhabiting species is exclusive to that habitat, as if it were totally distinguishable from the salt-water containing, coastal mangroves in which, in fact, all such crab holes may well occur — from this example alone, we find that this species lives at the ecological crossroads of swamps, tidal pools, ‘salt pools’, and ‘crab holes’, and quite an involved effort must be undertaken in order to critically decide exactly how to code it in relation to other ingroup and outgroup taxa. Interestingly, Mattingly (1952, p. 270) in contemplating this noted the high tolerance of many species for diverse habitat types and suggests that in many cases the apparent restriction of certain species’ immature stages to some ‘preferred’ habitat or other may merely reflect females’ tendencies regarding ovipositional site selection rather than any such fact about tolerance or intolerance of conditions found therein (see also Roberts, 1986). For the present study then, it is to be hoped that such critical character analysis will aid in the discovery of the real ‘character phylogenies’ (Mickevich & Weller, 1990; more on this in ‘Methods’ section below) which gave rise to the diversification of the taxa analyzed herein.

Many larval habitat classifications like that employed in the analysis of Soghigian *et al.* (2017) have been attempted for the Culicidae before. Among the first were those of Shannon (1931), Hopkins (1936, 1952), and Bates (1949); works since then have largely culminated in those of Laird (1988) and Service (1993). Importantly, almost all of these attempts are largely pragmatic in character and intention, serving first and foremost to direct field researchers as to how to find mosquito habitats and sample them, and serving only secondarily, or not at all, to represent evolutionarily-derived behavioral and ecological characteristics of mosquitoes as natural organisms. These works are thus necessarily divorced from the objective taxa and immediate observations at hand, attempting to map a human conceptual construct, *viz.*, that of ‘larval habitats’ as understood by scientists and field researchers, onto an objective natural diversity of phenomena. In this work a more open-ended approach is employed: to let the species-wise data and reports dictate the conceptual categories assumed, *a posteriori*, rather than applying any *a priori* conceptual categorization scheme.

I here follow the lead of Shannon (1931) in treating the ‘condition’ of water of immature habitats separately from its spatial manifestation; that is, the *locale* of the aquatic habitat — considered chiefly as a spatial entity (*e.g.*, a treehole versus a marsh) — is

conceptually divorced from *features* associated with the water occurring therein, such as pH and temperature. This approach stands in contrast to that adopted by, for example, Soghigian *et al.* (2017), in treating ‘salt pools’ as a category on the same footing as ‘ground pools’ and ‘rock pools’. The reasoning for this is that no justification can be found for assuming *a priori* that the presence or absence of salt (or any other attribute) in water should fundamentally alter the habitat locale. Rather, it is more conservatively assumed here only that each species simply has a certain tolerance threshold for degree of salinity which could present a relationship with virtually any type of habitat locale. Indeed, mosquito taxa representing a vast range of habitat locales present an incredible diversity of tolerance for salinity and other such factors of water condition (Roberts & Irving-Bell, 1997).

Also followed here is Shannon’s (1931) impetus to segregate those aspects of mosquito biology which are influenced by anthropogenic factors from those not. Shannon recognized that most species groupings arising from factors related to anthropogenic interactions will be highly artificial, due to the necessary modifications to mosquito habitat and behavior involved. That thread is here taken further along an evolutionary line, given the relative recency of such anthropogenic factors in evolutionary time: the advent in the past 10,000 years of a landscape categorically transformed by urban and suburban development is a recent enough phenomenon for which it is unlikely the majority of mosquito taxa will have had sufficient time to adapt and diversify in any way comparable to the way in which they have diversified in pre-anthropogenic habitats over tens of millions of years. Accordingly, immature habitats such as ‘roadside ditches’ and ‘artificial containers’ are conservatively considered totally separate from more general potential categories such as ‘ground pools’ and ‘containers’, respectively. Of course, undoubtedly some ‘ditches’ (to take the first example) will present precisely the kinds of conditions necessary to stimulate adult female ovipositional habitat selection and subsequent larval development, identical to those found in certain types of ‘ground pools’ in pre-anthropogenic time, but this cannot be assumed in advance. And equally undoubtedly some ‘artificial’ habitats represent an entirely novel stimulus for anciently-derived mosquito lineages, such as concentrated effluent from sewage treatment pipes or discarded industrial tin plates. Thus when the utilization of artificial habitats comes up during analysis, expression of the ‘evolutionary derivation’ of utilization of such habitats, in prehistoric time, is qualified using the word ‘predisposition’, which indicates uncertainty about what exactly stimulates females to oviposit and/or larvae to successfully develop there.

As for systematic analysis of ecological niche and behavioral characters beyond those of immature habitat, the author is aware of no prior work in the Culicidae. In this paper, in addition to immature habitat and the conditions found therein, also treated are records of blood-feeding and autogeny, mating behavior, female oviposition behavior and egg physiology, seasonality, and day-night host-seeking activity of females. In the history of culicidology, including prior to the Hennigian revolution, there has been a good deal of speculation about the evolutionary origins of many of these characters, but larval habitat remains by far the most hotly debated. Belkin (1962a,b) for example is widely acknowledged to have initially proposed the hypothesis that mosquitoes originated in ground pool habitats and only subsequently colonized container habitats, as noted above. Likewise Mattingly (1965, 1971) was among the first to speculate inter-generic relationships based on ecological data. Unfortunately, however, neither of these authors employed a systematic (in the sense of cladistics) framework to test their hypotheses, and thus the vast majority of their hypotheses remain completely untested. Nevertheless, the speculations advanced by these early researchers are among the most interesting hypotheses yet proposed in all the Culicidae literature which the author has encountered.

Mattingly (1965, p. 31), implicitly discussing the Culicidae or a very close ancestor of them, speculates that rock pools were the first habitats to support fully aquatic larvae. Given the probably fully-aquatic ancestor of Culicidae as well as all other basal Diptera (Wiegmann *et al.*, 2011), it readily becomes apparent that this speculation is not one to be evaluated at the level of the Culicidae, but at a much broader evolutionary clade of Diptera such as Culicomorpha, hence we may equally readily discard his hypothesis as being ‘neither here nor there’ for the purposes of this work. Of somewhat more pertinence, Mattingly (1965, p. 31; 1971, p. 45) apparently takes it rather for granted that rock pools should represent an ‘intermediate’ habitat type, at least insofar as the groups *Stegomyia* and ‘*Finlaya*’ sensu auctorum are concerned, presumably between unspecialized ground pools and these primarily treehole-utilizing groups and/or other groups which utilize specialized ground (*e.g.*, crab hole) or plant container habitats. It is not immediately clear that he means this in an evolutionary sense (as at times it seems clear that his thought is merely typological), but we may nevertheless consider the possibility in a cladistic framework.

As noted above, it bears keeping in mind that records of occurrence and absence of a species from one habitat type generally reflect the ovipositional tendencies of adult females rather than any intrinsic ecological requirement on the part of immatures (Mattingly 1969, p. 87). Therefore no attempt is made here to differentiate between ‘preferred’ and ‘occasional’

habitats by appealing to the latter putative fact. It is worth taking note of however Belkin's (1962, p. 57) suggestion that, in the case of historical invasion and evolution in the South Pacific, almost all ground pool utilizing species, upon initial colonization of an island, speciated and subsequently were either extinguished or hybridized with coexisting taxa, resulting in the present-day fauna which includes many species with a wide range of ecological tolerances. In what follows are considered in turn the remaining behavioral and ecological characters to be analyzed.

In much the same way that various bodies of water can be considered environmental resources for use as habitats by mosquito species in their immature stages, bloodmeal hosts may be considered a type of environmental resource for use by mosquito species in their adult stages. Thus, the behaviors related to host usage and preferences are here analyzed accordingly. Again, no assumptions of resource uniformity (as ready-made categories 'mammal', 'bird', *etc.*) are made *a priori*, but rather the species-wise data reports themselves are left to dictate the scope of the analysis. Put another way, it is meant that the analysis is just as precise and detailed as the behavioral characters as noted in the literature will permit. Naturally this also entails that with future accumulation of behavioral character observations, further analyses of these characters will be on the whole more complete and precise than the present analysis. Additionally, Mattingly (1969, p. 126) pointed out that host availability plays a sufficiently significant role in blood-feeding behavior and host 'choice' that the term 'host preference' is generally insufficiently studied and consequently misleading. Hence all records considered here are merely blood-feeding observations, to say nothing of 'preferences'; *e.g.*, no presumption is made of any distinction between 'fixed' and 'opportunistic' behaviors in the taxa (Edman *et al.*, 1972). The distinction above invoked in relation to immature habitat changes resulting from the transition from a pre- to a post-anthropogenic Earth also has an important impact on our understanding of blood-feeding: the effect that the colonization of the ends of the Earth by man and his associated domestic animals has had on host resource utilization by mosquitoes. It is widely known among culicidologists that mosquitoes were absent from Hawaii until just a few hundred years ago, but less commonly acknowledged in discussions of mosquito blood-feeding activity is that places such as La Réunion Island, where *Oc. dufouri* occurs endemically, were entirely devoid of man or associated domestic animals until virtually just as recently. Of similar note is the colonization of regions such as the Americas and Oceania as recently as just a dozen or so thousand years ago, a timespan again not remotely on the same scale as that of mosquito evolution at large. We should give pause to consider, then,

what exactly it means when we suggest that a given mosquito species has a ‘disposition’ to feed on man or, *e.g.*, his horses, particularly in these ‘new’ regions of the world. As before, we may certainly grant that many mosquito species, for example those which already had fed on native primates or ungulates in their ranges, may very easily adopt these new blood sources, but again this assumption must not be made in advance. Further on this latter point, and although exhaustive treatment of this problem is out of the scope of this study, it is important to acknowledge that the advent of host specificity in mosquitoes is generally related to several different biological phenomena. These include differential derivable nutrition from, and digestive energy cost of, bloodmeals; defensive behaviors by hosts; and other physiological, behavioral, and ecological factors which vary among and between different host taxa (Lyimo & Ferguson, 2009). In this, however, the possibility that mosquito taxa come into contact with only certain hosts due to other factors (*e.g.*, the interaction of locale of immature development and adult dispersal capacity effectively limit the diversity of potential host taxa thereby encountered) cannot be discounted (Lyimo & Ferguson, 2009). As a final note on hematophagy, the phenomenon of autogeny is particularly interesting within the Culicidae, almost certainly occurring secondarily, as reversals to an earlier dipteran condition, in the taxa in which it is known, *e.g.*, *Toxorhynchites* (Lorenz et al. 2021). It may in a sense be considered a type of ‘host usage’, or perhaps more aptly, the absence of any.

Mating behavior is taken to comprise fairly straightforward concepts for analysis and hence warrants no particular discussion here. Oviposition behavior simultaneously comprises the stimulus-driven comportment of females and the physico-spatial contexts in which they are thereby induced to deposit their eggs for subsequent development. One of the more interesting character categories included in the analysis is that of egg physiology, for it represents neither ‘niche,’ nor behavior, nor structure in the strict sense. Yet its importance to biological integrity in each species cannot be overstated.

As regards seasonality, the taxa here analyzed represent the full spectrum, from organisms strictly tied to an annual four-season cycle including a harsh winter, to taxa inhabiting tropical zones almost completely devoid of seasonal variation. Therefore, this component of the analysis will contain the most information for those taxa closest to the former extreme of the spectrum.

By ‘day-night host-seeking activity’ it is meant which approximate periods of the day the species is active in the adult stage. This is perhaps one of the trickiest character categories to make sense of, due to the great tendency of taxa to overlap each other inexactly (*e.g.*, taxa that are active at dusk and night such as *Ma. titillans*, as compared to taxa which are strictly

nocturnal such as *Cx. quinquefasciatus*; Reisen, 2012; Loroso *et al.*, 2010, Clark-Gil & Darsie, 1983).

It is somewhat fortuitous that the phylogenetic hypothesis (Huang *et al.*, 2010) here mapped upon, chosen on technical grounds, derives from sixteen taxa which afford a great diversity of ecological niches, behaviors, largely island distributions, and other non-structural characters for systematic analysis. It is important also to keep in mind that the character states identified as plesiomorphic in this study, based on the four outgroup taxa considered, apply only to the Aedini. Conclusions from this study do not necessarily apply to distant taxa such as Uranotaeniini or Anophelinae.

2. GOALS

2.1. GENERAL

Investigate the ecological diversification of a group of Aedini, resultant from the history of their evolution.

2.2. SPECIFIC

(1) Reconstruct ancestral states of all analyzed characters using Hennigian cladistic analysis.

(2) Characterize general patterns in the evolution of environmental resource utilization, in the senses of both bloodmeal host sources (or lack thereof, as in the case of autogeny) and larval habitat type and condition.

(3) Characterize general patterns in the evolution of behavioral diversification as regards mating, oviposition, seasonality, and day-night activity of host-seeking females.

(4) Characterize general patterns in the evolution and physiological diversification of functional features of aedine eggs.

(5) Put results found in the context of the geographical distributions of the taxa, providing a framework for a future biogeography of the species.

(6) Test long-standing untested evolutionary hypotheses of mosquito evolution in a cladistic framework.

3. MATERIALS AND METHODS

The generic classification of Aedini followed was the “Phylogenetic classification” of Harbach (2013), largely derived from the conclusions of Reinert *et al.* (2009). Subspecific classifications were ignored for the purposes of this work; any attribution to a particular subspecies was duly attributed to that entire species for the ecological analysis (*e.g.*, *Oc. vigilax vansomeranae* and *Oc. vigilax vigilax* were considered together as simply *Oc. vigilax*).

The taxa analyzed herein are the 16 taxa studied in the morphology-based successively-weighted parsimony phylogenetic analysis of Huang *et al.* (2010). There are reasons to consider this phylogenetic hypothesis the best available among published alternatives (Reinert *et al.*, 2009; Soghigian *et al.*, 2017) for the following reasons. Although the importance of the work of Reinert *et al.* (2009) for jumpstarting the systematic investigation of Aedini cannot be overstated, Huang *et al.* (2010, 2012) made the following serious criticism of the results of that work: the concept of *Levua* (as utilized for the phylogeny of Reinert *et al.*, 2009) has been incorrectly defined since the time of Danilov (1981; citation in Huang *et al.*, 2010), with putative *Levua* species ‘*Oc.*’ sensu auctorum *fryeri* (as *Le. fryeri*; abbreviations following Reinert, 2009 and Harbach, 2013) and *Oc. dufouri* (as *Le. fryeri*) showing just as many (or more) affinities to *Ochlerotatus* as they do to *Levua*. The former was therefore placed by Huang *et al.* (2010) in the novel monotypic subgenus ‘*Coetzeemyia*’ and the latter in *Ochlerotatus*, leaving *Levua* monotypic and containing only *Le. geoskusea*. Furthermore, Huang *et al.* (2010) corrected the coding of eleven of, and further modified five more of, Reinert *et al.* (2009)’s characters. Therefore for the purposes of this systematic study, the topology presented in Huang *et al.* (2010) may be considered to represent an improvement on that of Reinert *et al.* as regards these 16 taxa. This morphology-only phylogenetic hypothesis is furthermore to be preferred, for the purposes of this study, to the molecular-only hypothesis of Soghigian *et al.* (2017); many of the taxa analyzed in this latter analysis were represented by just one or two molecular characters (generally COI and/or one other molecular marker), of a total of just seven characters analyzed; just twelve of their 260 taxa were represented by all seven molecular markers, and only one outgroup, *Cx. quinquefasciatus*, was utilized for the analysis. Of course, by limiting analysis to one category of data such as morphology *or* molecules, as has so far been the norm in the Aedini, less has generally been achieved than had these two data types been combined in simultaneous analysis. Should such a work ever be realized in Aedini or Culicinae, future niche-mapping studies such as this one will no doubt benefit greatly from it.

While in the work of Huang *et al.* (2010) the nine most-derived taxa were considered the ingroup, with a seven-taxa outgroup, in the present study it was chosen to consider the twelve most-derived taxa the ingroup and the remaining four the outgroup. Evolutionary derivation of characters specific to the Aedini was of interest here, so exclusion of these three intermediately-derived aedine taxa would have been antithetical to the study. Furthermore, the four remaining outgroups, each representing a distinct tribe of Culicinae, were considered a sufficient and well-rounded outgroup for polarization of character states against the ingroup. Choosing any other outgroup furthermore would have increased the likelihood of an incorrect topology, as intergeneric and intertribal relationships among Culicinae are poorly understood (Harbach, 2007).

Examination of the literature for compilation of characters was fairly straightforward. Searches began with Google Scholar inputs such as “[specific epithet], Culicidae, bionomics”. From there, references of individual papers were consulted insofar as they were relevant, and in some cases exhaustive searches were attempted on specific databases such as the Walter Reed Biosystematics Unit website (Walter Reed Biosystematics Unit, 2015) and the Mosquito Taxonomic Inventory (Harbach, 2013), as well as in general reference books such as those of Carpenter and LaCasse (1955), Horsfall (1955), Wood *et al.* (1979), and Becker *et al.* (2020), until a substantial body of literature reports was assembled for each species (March 2022 being the cut-off date for incorporation of literature reports). Avoiding specifying the genus was generally necessary due to historical changes in taxonomic recognition and use (*e.g.*, from the classification changes brought by Reinert *et al.*, 2009), and similarly necessary at times was searching synonyms of specific epithets (*e.g.*, *Cx. ‘fatigans’* instead of *quinquefasciatus*). In some cases of by-taxa comparisons of specific characters it was necessary to search something to the effect of “[specific epithet], Culicidae, [*e.g.*, diapause]”. Data on geographic distribution was also noted.

In addition to the four broad character categories of interest here analyzed phylogenetically, several additional potential character categories, hypothetically of interest, were considered. These, however, had to be abandoned before or during the final analysis, due to either paucity of records precluding successful comparison and application of parsimony or failure to satisfactorily derive a character phylogeny for them (see below for a precise definition of this term). They included pathogens transmitted by each species, altitude, presence of sun during biting activity, oxygen content and sun exposure as conditions of water in immature habitats, ‘clearness’ of water in immature habitats, time of swarming, stenogamy, diapause, voltinism, endophily, pollination, flight range, and dispersal.

Some further comment on the nature of the characters that in the end were included in the analysis is warranted.

It was argued by Mickevich and Weller (1990), drawing on distinctions originally made by Jardine and Sibson (1971), that hypothetically heritable *characters*, typically structural but also including those behaviors intimately connected to a specific *act* (in the sense of motion) displayed by an organism, should be distinguished from *attributes*, which would be any feature of a taxon which is too environmentally labile to be heritable. This subject was in part addressed in the introduction, albeit there at the level of structural versus all behavioral and/or ecological characters. Essentially this talk of ‘attributes’ seeks to differentiate such ritualized behaviors from ecological facts which are severed from any behavioral acts which should precede them, indeed such as ecological niche utilization or parasite host usage. Although these authors allow that such attributes can yield some degree of meaningful information when analyzed on a phylogeny, they tacitly deny that it can really be systematic work, and they certainly deny that any such characters could be homologized and serve as the basis for a to-be-constructed phylogeny. This preconception appears to have its origin in Jardine and Sibson’s (1971) resistance to phylogenetic systematics, these authors rather seeking recourse to multiple positions held by numerical taxonomy and phenetics where they find cladistics to maintain putatively intenable positions. For this reason, this charge, *viz.*, that ‘ecological attributes’ cannot be *a priori* assumed to contain phylogenetic information, is here rejected, at least until the analysis has been done and these features have had a chance to stand up for themselves. Therefore no *a priori* distinction or triaging is made between act-based and act-severed behavioral characters.

For the systematic character analysis, the methodology employed was the traditional Hennigian *Methode der wechselseitigen Erhellung*, or the approach of ‘check, correct, recheck,’ as it is also known (Hennig, 1966, p. 21-22, 122; 1982, p. 28, 121), rather than any computer-aided analysis. Also followed was Hennig’s ‘auxiliary principle’ (1966, p. 121), identified with, and explored further by later authors as, the principle of parsimony (Farris 1983/1994). Although such principles are most commonly applied in cladistic analyses which seek to hypothesize a tree for a group of taxa whose relationships are as-of-then yet unknown, they are also appropriate in the tracing of ancestral states deep within existing ‘known’ phylogenetic trees (Brower & Schuh, 2021). Qualitative behavioral and ecological characters functionally manifest themselves much like qualitative morphological characters: each character is split into a series of two or more alternate possibilities, these possibilities themselves being determined essentially subjectively by the reviewer and are therefore

entirely vulnerable to subsequent reinterpretation by later authors (de Queiroz & Wimberger, 1993; Wenzel, 1992). Characters must furthermore be independent of one another and for this reason are best analyzed independently, one at a time, in relation to an outgroup, and all character state transition sequences and their polarity decisions made on that basis (Brower & Schuh, 2021; Mickevich & Weller, 1990). Some characters yield themselves to presence-absence coding, while others make evident that there should be a line of increasing complexity; the latter are thus best represented by multi-state character coding.

In the assignment of character polarities, the justifications on which coding decisions are based cannot be trivial, and neither can one approach *a priori* be assumed more parsimonious than the other: importantly, the adherence to a presence-absence coding scheme is just as much an assumption (albeit a less complex one) as that in favor of the multi-state scheme — the plausibility and applicability of one or the other can only be judged on an individual basis for each character tree (Mickevich & Weller, 1990; see more on this below). The comparison with outgroups for the purposes of character polarization is nothing more than the ‘search for the sister group’ of Brundin (1972). Outgroups in this context, somewhat differently from their more common utility in initial phylogeny discovery, serve merely to contrast to-be-mapped character states with those found in the ingroup, ideally representing different character states than the ingroup for every character of interest, although this is hardly ever strictly the case in reality (Grandcolas et al. 2004).

Although modeling-based methods are becoming more and more common in systematics in general, being particularly well-suited to the enormous datasets of molecular characters in which assignment of individual nucleotide-wise hypotheses of synapomorphy is practically impossible or even unjustifiable, they are not appropriate for a study seeking to establish baseline hypotheses of how evolution has proceeded in a given group, such as the present one. Such modeling methods are entirely dependent upon the accuracy of the evolutionary models they assume the truth of, and hence must be avoided in studies which seek initially to discover what evolution has generally been like regarding these characters in these taxa, a task which is logically and operationally quite prior to the application of evolutionary theory as such (Brower & Schuh, 2021, Brower, 2016). Hence this work presumes no specific mechanism of evolution, limiting itself to the parsimony criterion, a methodological heuristic which serves precisely the function of minimizing assumptions (Brower & Schuh, 2021). The assignment of mathematical values to nodes is here also deemed superfluous, as it has at best a highly questionable biological basis (Borkent, 2018); its ultimate goal is to improve mathematical ‘consistency’, a metric which does not aid in the

critical evaluation of hypothetical adherence to empirically-obtained biological data (Brower & Schuh, 2021).

One particularly challenging aspect (also mentioned above) particularly related to behavioral characters is the fact that character transitions are at times incomplete (*i.e.*, are polymorphic), in contrast to the general completeness of transition generally observed in the analysis of morphological or molecular characters. By this it is meant that many times the colonization of, *e.g.*, rock pools is observed in Culicidae immatures, without the total abandonment of an ancestral utilization of marsh habitat, all within the same taxon, including potentially within a species. Whereas in the analysis of morphological data, much more often it is observed, *e.g.*, that a pale-scaled patch is gained in the post-procoxal area, and no representative individuals which should lack it are left in the novel lineage; or in molecular data, *e.g.*, the full transition of one nucleotide sequence to another in a novel taxon. For this reason, in this analysis of ecological and behavioral characters it generally has to be made explicit not only when a new apomorphy is derived, but also when (if ever) an old plesiomorphy is lost.

As it turns out, the majority of the character complexes presented here were found *a posteriori* to be almost by necessity multi-state. It is inevitable that conclusions about relationships between character states and/or character trees are subjectively determined by individual workers, and it is even furthermore conceivable that some worker or other would choose to force every observation into a binary or presence-absence framework, but a thorough evaluation of the observations to be compiled reveals that in remarkably few cases would such an approach be best. As for which multi-state character dependencies (Forey & Kitching, 2000) are to be presumed, the only sensible processual approach is that they be postulated in an initial attempt at “getting it right,” and then through critical analysis of the strength of the results thus derived, they then be continually refined. This is nothing more than a special application of Hennig’s (1966, 1982) razor of ‘check, correct, recheck’ which is so central to phylogenetic systematics as such.

Discovery of the transformation series (=character state tree, of Mickevich, 1982) for each character leading to the diversity of ecological characters observed in this group of taxa was done by means of the ‘cladogram characters’ method of Mickevich (1982; Mickevich & Weller, 1990). At least insofar as ancestral state reconstruction, with a cladogram in hand, is concerned, the criticism of this method leveled by Lipscomb (1992), which says that the method “discards observed similarity evidence for homology” in its preoccupation with finding best descriptions of the cladogram under analysis, is not compelling. This author’s

criticism may hold some weight when such techniques are utilized in the discovery of a novel phylogenetic hypothesis, but not in this case of the hypothesizing of novel apomorphies for shared character states observed in a cladogram taken as provisionally ‘true’. Importantly, Mickevich and Weller (1990) further note that the method is not equivalent with the ‘optimization’ method of Farris (1970). Directionality of these transformation series was discovered by outgroup comparison, on the basis of two important assumptions: first that the four outgroup taxa constitute a representative sample of general ecological character diversity in the Culicinae as a whole, and second that the ingroup taxa are sufficiently phylogenetically close to each other as well as sufficiently phylogenetically distant from the outgroup taxa such that their consideration as a closely-allied group is coherent. This latter assumption is not strictly equivalent to the assumption of the real monophyly of the ingroup (an assumption obviously also made for tribe Aedini in general, and for which there is considerable evidence [Lorenz *et al.*, 2021; Reidenbach *et al.*, 2009]), but means that the ingroup should represent a cluster of taxa quite closely related to one another relative to the outgroup. The only nodes in which the accuracy of this assumption may be considered especially suspect are those comprised by the intermediately-derived taxa, *Abraedes* and *Aedes*, taxa which indeed in the original phylogenetic hypothesis of Huang *et al.* (2010) were considered also to represent outgroups relative to the *Ochlerotatus*- and *Levua*-allied taxa there considered the ingroup of interest.

Subsequent to the discovery of the transformation series for each character, simple optimization (again, not that of Farris, 1970, but rather in the general sense as in e.g., Carpenter, 1989) by parsimony was performed in order to find the minimal number of changes along the nodes of the tree leading to the terminal taxa. Directionality of these cladogram characters was determined by means of outgroup comparison, on the basis of the assumptions above explicated, resulting in a series of hypotheses of “character phylogeny” (Mickevich & Weller, 1990).

In some cases, paucity of data precipitated deviations from the strict methodology of cladogram characters and subsequent optimization across the nodes of the tree. Where possible, data from other sources was used to ground the assumptions as to the character states possessed by these as-yet-incompletely-sampled taxa. Unfortunately, some of these cases required inference from ‘negative data’: such as the fact that some of these taxa have been extensively offered to feed on *Homo sapiens*, and never known to accept this blood source. Such fact was taken as grounds to provisionally consider these taxa as potentially compatible with an autogenous habit, either partial or complete, as is the case with several of

their close sister taxa, and this assumption subsequently influenced both cladogram character and optimization. In what follows the details of characters analyzed are given.

During the course of the analysis, it quickly became apparent that several of the four broad characters (immature habitat locale and water condition; hematophagy and autogeny; mating, oviposition and egg physiology; and seasonality and day-night host-seeking activity), taken as single characters proved far too complex for wholesale analysis. Immature habitat locale was therefore broken down into four separate, but partially overlapping, categories: wetland type (Table 1), permanence of ground pool (Table 2), ground water type (Table 3), and ground vs. container habitats (Table 4). These four ‘mini-characters’ overlap in the following senses: ‘wetlands’, considered as comprising swamp, marsh, and mire, is taken to represent a type of permanent pool, and at that a type of ground water; likewise, ‘permanent pools’, ‘semi-permanent/temporary pools’, ‘ponds’, ‘puddles’, ‘stream margins’, ‘lake margins’, ‘rock pools’, ‘decapod holes’, and ‘buffalo footprints’ too are all taken to represent types of ground waters, for facilitation of comparison with container habitats. Immature habitat water condition was broken down into three separate ‘mini-characters’: salinity (Table 5), acidity (Table 6), and tolerance of temperature extremes and presence of vegetation, detritus, and pollution (Table 7). Hematophagy/autogeny was broken down into four ‘mini-characters’: Mammalian host usage (Table 8), Aves host usage (Table 9), general host usage (Table 10), and autogeny (Table 11). Mating behavior (Table 12) remained one integral character. Oviposition behavior (Table 13) and egg physiology (Table 14), although initially considered in combination, were ultimately separated. Seasonality (Table 15) and day-night host-seeking behavior (Table 16) each remained integral characters. Finally, distributions of the species are additionally provided for reference (Table 17).

All references consulted for character coding are given in Tables 1-16. As noted above, literature review studies often do not yield a particularly consistent terminology for cross-taxa comparisons. There is also the separate but related problem of conceptually uniting seemingly-disparate ecological characters into more general categories. It is thus necessary to note some assumptions made in the assigning of character states to taxa.

‘Swamp’ was here defined as wetlands dominated by woody-stemmed flora and includes tidal swamp, lagoon, mangrove swamp, nipa palm swamp, coconut groves, and ‘semi-permanent swamp’ (Table 1). ‘Marsh’ was defined as wetlands dominated by herbaceous flora and included ‘tidal marsh at head of mangrove creek’, ‘hilly marshes’, and ‘sedge marsh’, as well as waters in association with ‘water hyacinth’, ‘water lettuce’, ‘grass roots’ *Pistia startiotes*, *Eicchornia* spp., *Araceae* spp., *Ipomea* spp., and *Typha* spp. ‘Mire’

was defined as wetlands characterized by heightened presence of peat build-up and included ‘*Carex* spp. mats in bog lakes’. These three categories together comprised ‘wetlands’ generally.

Table 1. Character coding scheme: immature habitat locale: wetlands, considered as type of groundwater.				
Taxon	Marsh	Mire	Swamp	References
<i>Culiseta (Culiseta) inornata</i>		■	■	Ortega-Morales et al. 2015, Carpenter & LaCasse 1955, Horsfall 1955
<i>Orthopodomyia signifera</i>				
<i>Culex (Culex) quinquefasciatus</i>				
<i>Mansonia (Mansonia) titillans</i>	■		■	Almiron & Brewer 1996, Escher & Lounibos 1993, Lounibos & Escher 1985, Slaff & Haefner 1985, Belkin et al. 1970
<i>Abraedes papago</i>				
<i>Aedes cinereus</i>	■	■		Becker et al. 2020, Edsall et al. 2010, Carpenter & LaCasse 1955, Horsfall 1955, Dyar 1924
<i>Aedes esoensis</i>	■			Tanaka et al. 1979, Horsfall 1955
‘ <i>Ochlerotatus</i> ’ (‘ <i>Coetzeomyia</i> ’) <i>fryeri</i>	■			Smith & Corbet 1975, Van Someren & Furlong 1964
<i>Ochlerotatus (Juppius) caballus</i>				
<i>Ochlerotatus (Empihals) vigilax</i>	■		■	Becker et al. 2020, Knight et al. 2012, Chapman et al. 1999, Dale et al. 1986, Tanaka et al. 1979, Taylor & Maffi 1978, Lambrecht 1971, Horsfall 1955
<i>Ochlerotatus dufouri</i>				
<i>Levua geoskusea</i>				
<i>Rhinoskusea longirostris</i>			■	Reinert 1976
<i>Rhinoskusea wardi</i>			■	Rajavel et al. 2007, Rajavel & Natarajan 2006, Rajavel et al. 2006a, 2006b, Reinert 1976
<i>Geoskusea baisasi</i>				
<i>Geoskusea longiforceps</i>				

‘Permanent’ water bodies here included all types of wetlands, ponds, stream margins, and ‘permanent pools’ (Table 2). ‘Temporary / Semi-permanent pools’ are grouped together because no meaningful distinction was discovered between the two concepts, and thus here included ‘puddles’, ‘temporary ponds of estuarine water left in depressions after spring tides’, ‘rainwater lying over grass’, ‘rainwater lying over soil’, ‘edge of semi-permanent pools in floodplains’, ‘semi-permanent swamp’, ‘temporary ponds of estuarine water left in depressions after spring tides’, ‘buffalo footprints’, and ‘pools and ponds affected by salt water’.

Table 2. Character coding scheme: immature habitat locale: permanence vs. impermanence of groundwater habitats.

Taxon	Temporary/Semi-permanent pool	Permanent pool	References
<i>Culiseta (Culiseta) inornata</i>		■	Ortega-Morales et al. 2015, Carpenter & LaCasse 1955, Horsfall 1955
<i>Orthopodomyia signifera</i>			
<i>Culex (Culex) quinquefasciatus</i>	■		Becker et al. 2020, Foster & Walker 2019, David et al. 2012, Rattananarithikul et al. 2005, Service 1993, Harbach 1988, Hamon 1953
<i>Mansonia (Mansonia) titillans</i>	■	■	Almiron & Brewer 1996, Escher & Lounibos 1993, Lounibos & Escher 1985, Slaff & Haefner 1985, Belkin et al. 1970
<i>Abraedes papago</i>			
<i>Aedes cinereus</i>	■	■	Becker et al. 2020, Edsall et al. 2010, Carpenter & LaCasse 1955, Horsfall 1955, Dyar 1924
<i>Aedes esoensis</i>		■	Tanaka et al. 1979, Horsfall 1955
‘ <i>Ochlerotatus</i> ’ (‘ <i>Coetzeomyia</i> ’) <i>fryeri</i>		■	Smith & Corbet 1975, Van Someren & Furlong 1964
<i>Ochlerotatus (Juppius) caballus</i>	■	■	
<i>Ochlerotatus (Empihals) vigilax</i>	■	■	Becker et al. 2020, Knight et al. 2012, Chapman et al. 1999, Dale et al. 1986, Tanaka et al. 1979, Taylor & Maffi 1978, Lambrecht 1971, Horsfall 1955
<i>Ochlerotatus dufouri</i>			
<i>Levua geoskusea</i>			
<i>Rhinoskusea longirostris</i>	■	■	Reinert 1976
<i>Rhinoskusea wardi</i>		■	Rajavel et al. 2007, Rajavel & Natarajan 2006, Rajavel et al. 2006a, 2006b, Reinert 1976
<i>Geoskusea baisasi</i>			
<i>Geoskusea longiforceps</i>			

‘Pools’ here included all semi-permanent and temporary pools as well as ‘ground pools’, ‘woodland pools’, ‘grassy pools and edges of floodplains’, ‘shallow potholes’, and ‘ground pools in tidal flats’ (Table 3). ‘Ponds’ included ‘shallow ponds supporting an abundant *Bidens* spp. flora’ and ‘pools and ponds affected by salt water’. ‘Stream margins’ included ‘margin of mountain stream’ and ‘streams’. ‘Rock pools’ included ‘rock pools in streams’, ‘coral rock pools’, and ‘coastal rock pools’. ‘Decapod holes’ included ‘crab holes’, ‘mud lobster holes’, ‘crustacean holes’, *Thalassina anomala* mounds, Sesarmidae holes, ‘swamp pools in association with crab holes’, ‘lobster burrows’, ‘large temporary flood pools with immatures concentrated over the top of crab holes’, and ‘small rock pool on the edge of which were crab holes’.

Table 3. Character coding scheme: immature habitat locale: general type of groundwater habitats utilized.

Taxon	Pools	Wetland	Edge of lake	Stream margin	Buffalo footprints	Decapod holes	Rock pools	References
<i>Culiseta (Culiseta) inornata</i>	■	■						Ortega-Morales et al. 2015, Carpenter & LaCasse 1955, Horsfall 1955
<i>Orthopodomyia signifera</i>								
<i>Culex (Culex) quinquefasciatus</i>	■						■	Becker et al. 2020, Foster & Walker 2019, David et al. 2012, Rattanarithikul et al. 2005, Service 1993, Harbach 1988, Hamon 1953
<i>Mansonia (Mansonia) titillans</i>	■	■		■				Almiron & Brewer 1996, Escher & Lounibos 1993, Lounibos & Escher 1985, Slaff & Haefner 1985, Belkin et al. 1970
<i>Abraedes papago</i>								
<i>Aedes cinereus</i>	■	■	■	■				Becker et al. 2020, Edsall et al. 2010, Carpenter & LaCasse 1955, Horsfall 1955, Dyar 1924
<i>Aedes esoensis</i>	■	■						Tanaka et al. 1979, Horsfall 1955
<i>Ochlerotatus</i> (' <i>Coetzeomyia</i> ') <i>fryeri</i>		■					■	Smith & Corbet 1975, Van Someren & Furlong 1964
<i>Ochlerotatus (Juppis) caballus</i>	■			■			■	Hanafi-Bojd et al. 2017, Jupp 1967, Horsfall 1955, Hopkins 1952
<i>Ochlerotatus (Empihals) vigilax</i>	■	■					■	Becker et al. 2020, Knight et al. 2012, Le Goff et al. 2012, Chapman et al. 1999, Dale et al. 1986, Tanaka et al. 1979, Taylor & Maffi 1978, Lambrecht 1971, Horsfall 1955
<i>Ochlerotatus dufouri</i>							■	Boussès et al. 2013
<i>Levua geoskusea</i>						■		Harbach 2013, Belkin 1962, Amos 1944
<i>Rhinoskusea longirostris</i>	■	■			■	■	■	Rajavel et al. 2005, Reinert 1976
<i>Rhinoskusea wardi</i>		■				■	■	Rajavel et al. 2007, Rajavel & Natarajan 2006, Rajavel et al. 2006a, 2006b, Reinert 1976
<i>Geoskusea baisasi</i>						■		Toma et al. 2011, Okudo et al. 2004
<i>Geoskusea longiforceps</i>						■		Taylor & Maffi 1978, Belkin 1962

‘Man-made ground pools’ here included ditches, drainage ditches, drainage canals, irrigation channels, dikes, abandoned gold prospect holes, borrow pits, ‘burrow pits [sic]’ sewers, latrines, and septic tanks (Table 4). ‘Ground water’ included all permanent and semipermanent/temporary water bodies, rock pools, and decapod holes. ‘Plant containers’ included ‘depression in a log’, ‘tree rotholes’, ‘treeholes of *Quercus alba*, *Quercus rubra*, and

Acer rubrum’, ‘basal tree holes’, ‘tree stumps’, ‘hollow tree bases’, and ‘cut bamboo stems’. ‘Artificial containers’ included ‘cesspool’, ‘manure cesspool’, ‘discrete containers’, rubber containers, ‘tin plates’, ‘metal containers’, barrels, discarded household containers, wood containers, ‘open sewers’, waste water, pit latrines, and septic tanks.

Table 4. Character coding scheme: immature habitat locale: groundwaters vs. plant containers vs. artificial containers.					
Taxon	Man-made ground pools	Ground water	Plant containers	Various artificial containers	References
<i>Culiseta (Culiseta) inornata</i>	■	■		■	Ortega-Morales et al. 2015, Carpenter & LaCasse 1955, Horsfall 1955
<i>Orthopodomyia signifera</i>			■	■	Hartwig et al. 2018, Mitchell & Rockett 1981, Wood et al. 1979, McDonald et al. 1973, Zavortink 1968, Michener 1947
<i>Culex (Culex) quinquefasciatus</i>	■	■	■	■	Becker et al. 2020, Foster & Walker 2019, David et al. 2012, Rattanarithikul et al. 2005, Service 1993, Harbach 1988, Hamon 1953
<i>Mansonia (Mansonia) titillans</i>	■	■			Almiron & Brewer 1996, Escher & Lounibos 1993, Lounibos & Escher 1985, Slaff & Haefner 1985, Belkin et al. 1970
<i>Abraedes papago</i>			■		Zavortink 1972
<i>Aedes cinereus</i>	■	■			Becker et al. 2020, Edsall et al. 2010, Carpenter & LaCasse 1955, Horsfall 1955, Dyar 1924
<i>Aedes esoensis</i>	■	■			Tanaka et al. 1979, Horsfall 1955
‘ <i>Ochlerotatus</i> ’ (‘ <i>Coetzeomyia</i> ’) <i>fryeri</i>		■			Smith & Corbet 1975, Van Someren & Furlong 1964
<i>Ochlerotatus (Juppius) caballus</i>	■	■			Hanafi-Bojd et al. 2017, Jupp 1967, Horsfall 1955, Hopkins 1952
<i>Ochlerotatus (Empihals) vigilax</i>		■			Becker et al. 2020, Knight et al. 2012, Chapman et al. 1999, Dale et al. 1986, Tanaka et al. 1979, Taylor & Maffi 1978, Lambrecht 1971, Horsfall 1955
<i>Ochlerotatus dufouri</i>		■			Boussès et al. 2013
<i>Levua geoskusea</i>		■		■	Harbach 2013, Belkin 1962, Amos 1944
<i>Rhinoskusea longirostris</i>		■		■	Reinert 1976
<i>Rhinoskusea wardi</i>		■	■	■	Rajavel et al. 2007, Rajavel & Natarajan 2006, Rajavel et al. 2006a, 2006b, Reinert 1976
<i>Geoskusea baisasi</i>		■			Toma et al. 2011, Okudo et al. 2004
<i>Geoskusea longiforceps</i>		■			Taylor & Maffi 1978, Belkin 1962

‘Brackish’ here indicated any water saltier than freshwater (at a threshold of 0.1% salt) but less than hypersaline (>3.5% salt) (Table 5). ‘Moderately saline’ included reports of up to 1.55% (as ‘1,550 parts of chlorine per 100,000’), 2.65%, and 3.1% salt, while ‘hypersaline’ included reports of up to 4.5% salt, as well as those noted to be ‘as saline as samples of sea water’ (this being ~3.5% salt). Where reports of heightened salinity were lacking, tolerance of merely brackish water utilization was presumed. Occurrence of taxa in mangrove swamp was taken to indicate tolerance of brackish water. Taxa were considered acid-intolerant when there was no record of their occurrence in acidic habitats such as bogs (Table 6). The occurrence of active larvae in ‘snowmelt’ was taken as indication of extreme cold tolerance (Table 7). ‘Effluent’ was considered a type of polluted water, while water with mud and debris as well as ‘colored’ and turbid water were considered as having detritus.

Table 5. Character coding scheme: immature habitat condition: salinity.					
Taxon	Brackish water	Freshwater	Up to 1.55-3.1% or more salinity tolerance known	Up to 3.5% or more salinity tolerance known	References
<i>Culiseta (Culiseta) inornata</i>	■	■			Ortega-Morales et al. 2015, Moore et al. 1993, Graham & Bradley 1965, Horsfall 1955
<i>Orthopodomyia signifera</i>					
<i>Culex (Culex) quinquefasciatus</i>	■	■			Becker et al. 2020, Foster & Walker 2019, Grech et al. 2013, David et al. 2012, Service 1993, Horsfall 1955
<i>Mansonia (Mansonia) titillans</i>					
<i>Abraedes papago</i>					
<i>Aedes cinereus</i>		■			Becker et al. 2020, Edsall et al. 2010, Doran & Lewis 2003, Belton 1983, Kardatzke 1979, Wood et al. 1979, Carpenter & LaCasse 1955
<i>Aedes esoensis</i>		■			Tanaka et al. 1979, Horsfall 1955
‘ <i>Ochlerotatus</i> ’ (<i>Coetzeomyia</i>) <i>fryeri</i>	■			■	Smith & Corbet 1975
<i>Ochlerotatus (Juppius) caballus</i>	■	■			Hanafi-Bojd et al. 2017, Muspratt 1955
<i>Ochlerotatus (Empihals) vigilax</i>	■	■		■	Service 1993, Dale et al. 1986, Shinkarenko et al. 1986, Tanaka et al. 1979
<i>Ochlerotatus dufouri</i>	■	■			Boussès et al. 2013
<i>Levua geoskusea</i>	■				Harbach 2013
<i>Rhinoskusea longirostris</i>	■		■		Reinert 1976, Colless 1957, Senior-White 1925

<i>Rhinoskusea wardi</i>	■		■		Rajavel et al. 2007, Rajavel & Natarajan 2006, Rajavel et al. 2006a, 2006b, Reinert 1976
<i>Geoskusea baisasi</i>	■	■	■		Toma et al. 2011
<i>Geoskusea longiforceps</i>	■				Taylor & Maffi 1978

Table 6. Character coding scheme: immature habitat condition: acidity.				
Taxon	Acidic	pH 6.7-6.8	Alkali	References
<i>Culiseta (Culiseta) inornata</i>	■		■	Ortega-Morales et al. 2015, Moore et al. 1993, Graham & Bradley 1965, Horsfall 1955
<i>Orthopodomyia signifera</i>		■	■	Woodward et al. 1998, Hanson et al. 1995, Mitchell & Rockett 1981, Wood et al. 1979, McDonald 1973, Zavortink 1968
<i>Culex (Culex) quinquefasciatus</i>	■			Becker et al. 2020, Foster & Walker 2019, Grech et al. 2013, David et al. 2012, Service 1993, Horsfall 1955
<i>Mansonia (Mansonia) titillans</i>				
<i>Abraedes papago</i>				
<i>Aedes cinereus</i>	■			Becker et al. 2020, Edsall et al. 2010, Doran & Lewis 2003, Belton 1983, Kardatzke 1979, Wood et al. 1979, Carpenter & LaCasse 1955
<i>Aedes esoensis</i>				
<i>Ochlerotatus</i> (' <i>Coetzeomyia</i> ') <i>fryeri</i>				
<i>Ochlerotatus (Juppius) caballus</i>				
<i>Ochlerotatus (Empihals) vigilax</i>				
<i>Ochlerotatus dufouri</i>				
<i>Levua geoskusea</i>				
<i>Rhinoskusea longirostris</i>		■		Reinert 1976, Colless 1957, Senior-White 1925
<i>Rhinoskusea wardi</i>				
<i>Geoskusea baisasi</i>				
<i>Geoskusea longiforceps</i>				

Table 7. Character coding scheme: immature habitat condition: temperature, and presence of vegetation, detritus, and pollution.							
Taxon	Larvae active in snowmelt (-4C)	Larvae active in water as hot as 50C	With vegetation	Without vegetation, detritus	Detritus	Polluted water	References
<i>Culiseta (Culiseta) inornata</i>			■			■	Ortega-Morales et al. 2015, Moore et al. 1993, Graham & Bradley 1965, Horsfall 1955
<i>Orthopodomyia signifera</i>			■		■		Woodward et al. 1998,

							Hanson et al. 1995, Mitchell & Rockett 1981, Wood et al. 1979, McDonald 1973, Zavortink 1968
<i>Culex (Culex) quinquefasciatus</i>			■		■	■	Becker et al. 2020, Foster & Walker 2019, Grech et al. 2013, David et al. 2012, Service 1993, Horsfall 1955
<i>Mansonia (Mansonia) titillans</i>			■				Almiron & Brewer 1996, Lounibos & Escher 1985
<i>Abraedes papago</i>					■		Zavortink 1970
<i>Aedes cinereus</i>	■		■		■		Becker et al. 2020, Edsall et al. 2010, Doran & Lewis 2003, Belton 1983, Kardatzke 1979, Wood et al. 1979, Carpenter & LaCasse 1955
<i>Aedes esoensis</i>	■		■				Tanaka et al. 1979, Horsfall 1955
<i>‘Ochlerotatus’ (‘Coetzeomyia’) fryeri</i>							Smith & Corbet 1975
<i>Ochlerotatus (Juppius) caballus</i>			■		■	■	Hanafi-Bojd et al. 2017, Muspratt 1955
<i>Ochlerotatus (Empihals) vigilax</i>		■	■				Service 1993, Dale et al. 1986, Shinkarenko et al. 1986, Tanaka et al. 1979
<i>Ochlerotatus dufouri</i>				■			Boussès et al. 2013
<i>Levua geoskusea</i>							Harbach 2013
<i>Rhinoskusea longirostris</i>			■		■		Reinert 1976, Colless 1957, Senior-White 1925
<i>Rhinoskusea wardi</i>							
<i>Geoskusea baisasi</i>							
<i>Geoskusea longiforceps</i>							

‘Domestic animals’ was too vague a phrase for use in analysis of hematophagy. Ungulata included ‘ruminants’, Bovini, cows, *Bos taurus*, carabao, buffalo, sheep, horses, pigs, deer and *Cervus nippon yesoensis* (Table 8). Rodentia included guinea pigs, chipmunks, squirrels, white-footed mouse, New England cottontail, and *Clethrionomys rufocanus*. Carnivora included dogs, cats, *Vulpes vulpes*, and raccoons. Marsupialia included opossum, Leporidae included rabbit, and Cingulata included armadillo. Mammalia included all of the above.

Table 8. Character coding scheme: hematophagy: Mammalian hosts.								
Taxon	<i>Homo sapiens</i>	Ungulata	Carnivora	Rodentia	Leporidae	Cingulata	Marsupialia	References
<i>Culiseta (Culiseta) inornata</i>	■	■	■	■	■			Moore et al. 1993, Wright & DeFoliart 1970, Horsfall 1955
<i>Orthopodomyia signifera</i>								
<i>Culex (Culex) quinquefasciatus</i>	■	■	■	■			■	Becker et al. 2020, Boussès et al. 2018, Reeves et al. 2018, Reisen 2012, Loroso et al. 2010, Johansen et al. 2009, Horsfall 1955
<i>Mansonia (Mansonia) titillans</i>	■	■	■	■	■	■	■	Viana et al. 2014, Silva et al. 2012, Loroso et al. 2010, Daffner et al. 1987, Edman 1971
<i>Abraedes papago</i>	■							Zavortink 1972
<i>Aedes cinereus</i>	■	■	■	■	■		■	Khalin & Aibulatov 2020, Molaei et al. 2008, Apperson et al. 2002, Tempelis 1975, Wright & DeFoliart 1970, Means 1968
<i>Aedes esoensis</i>	■	■	■	■				Ejiri et al. 2011, Tanaka et al. 1979
‘ <i>Ochlerotatus</i> ’ (<i>‘Coetzeomyia’</i>) <i>fryeri</i>	■							Smith & Corbet 1975
<i>Ochlerotatus (Juppius) caballus</i>	■							McIntosh 1973
<i>Ochlerotatus (Empihals) vigilax</i>	■	■	■				■	Johansen et al. 2009
<i>Ochlerotatus dufouri</i>								
<i>Levua geoskusea</i>								
<i>Rhinoskusea longirostris</i>	■	■						Schultz 1992
<i>Rhinoskusea wardi</i>	■	■						Rajavel et al. 2006
<i>Geoskusea baisasi</i>								
<i>Geoskusea longiforceps</i>	■							Toma et al. 2011

Passeriformes included American robin, acadian flycatcher, scarlet tanager, *Serinus canaria*, brown towhee, *Passer domesticus*, and *Toxostoma rufum* (Table 9). *Haliaeetus* spp. included *Haliaeetus pelagicus* and *Haliaeetus albicilla*. Phasianidae included chicken, ‘poultry’, and pheasant. Odontophoridae included bobwhites, and Columbiformes included pigeons. Aves included all of the above.

Table 9. Character coding scheme: hematophagy: Avian hosts.								
Taxon	Passeriformes	<i>Haliaeetus</i> spp.	Columbiformes	Odontophoridae	Phasianidae	Ciconiiformes	Aves undetermined	References
<i>Culiseta</i> (<i>Culiseta</i>) <i>inornata</i>					■			Moore et al. 1993, Wright & DeFoliart 1970, Horsfall 1955
<i>Orthopodomyia</i> <i>signifera</i>	■				■			Byrd et al. 2012, Wood et al. 1979, Zavortink 1968
<i>Culex</i> (<i>Culex</i>) <i>quinquefasciatus</i>	■				■		■	Becker et al. 2020, Boussès et al. 2018, Reeves et al. 2018, Reisen 2012, Loroso et al. 2010, Johansen et al. 2009, Horsfall 1955
<i>Mansonia</i> (<i>Mansonia</i>) <i>titillans</i>					■	■	■	Viana et al. 2014, Silva et al. 2012, Loroso et al. 2010, Daffner et al. 1987, Edman 1971
<i>Abraedes</i> <i>papago</i>								
<i>Aedes cinereus</i>	■		■	■	■			Khalin & Aibulatov 2020, Molaei et al. 2008, Apperson et al. 2002, Tempelis 1975, Wright & DeFoliart 1970, Means 1968
<i>Aedes esoensis</i>		■					■	Ejiri et al. 2011, Tanaka et al. 1979
‘ <i>Ochlerotatus</i> ’ (‘ <i>Coetzeomyia</i> ’) <i>fryeri</i>								
<i>Ochlerotatus</i> (<i>Juppius</i>) <i>caballus</i>								
<i>Ochlerotatus</i> (<i>Empihals</i>) <i>vigilax</i>							■	Johansen et al. 2009
<i>Ochlerotatus</i> <i>dufouri</i>								
<i>Levua geoskusea</i>								
<i>Rhinoskusea</i> <i>longirostris</i>								
<i>Rhinoskusea</i> <i>wardi</i>								
<i>Geoskusea</i> <i>baisasi</i>								
<i>Geoskusea</i> <i>longiforceps</i>								

Amphibia included *Hyla japonica* and *Fejervarya sakishimensis* (Table 10). Serpentes included *Python bivittatus*. Crocodilia included *Caiman yacare*. Gobioidae included Oxudercidae. ‘Anguilliformes and other fish’ included eels, Blenniidae, and *Rhinecanthus verrucosus*.

Table 10. Character coding scheme: hematophagy: general.								
Taxon	Mammalia	Aves	Amphibia	Serpentes	Crocodilia	Gobioidae	Anguilliformes and other fish	References
<i>Culiseta (Culiseta) inornata</i>	■	■						Moore et al. 1993, Wright & DeFoliart 1970, Horsfall 1955
<i>Orthopodomyia signifera</i>		■						Byrd et al. 2012, Wood et al. 1979, Zavortink 1968
<i>Culex (Culex) quinquefasciatus</i>	■	■		■				Becker et al. 2020, Boussès et al. 2018, Reeves et al. 2018, Reisen 2012, Loroso et al. 2010, Johansen et al. 2009, Horsfall 1955
<i>Mansonia (Mansonia) titillans</i>	■	■			■			Viana et al. 2014, Silva et al. 2012, Loroso et al. 2010, Daffner et al. 1987, Edman 1971
<i>Abraedes papago</i>	■							Zavortink 1972
<i>Aedes cinereus</i>	■	■						Khalin & Aibulatov 2020, Molaei et al. 2008, Apperson et al. 2002, Tempelis 1975, Wright & DeFoliart 1970, Means 1968
<i>Aedes esoensis</i>	■	■	■					Ejiri et al. 2011, Tanaka et al. 1979
‘ <i>Ochlerotatus</i> ’ (<i>‘Coetzeomyia’</i>) <i>fryeri</i>	■							Smith & Corbet 1975
<i>Ochlerotatus (Juppis) caballus</i>	■							McIntosh 1973
<i>Ochlerotatus (Empihals) vigilax</i>	■	■						Johansen et al. 2009
<i>Ochlerotatus dufouri</i>								
<i>Levua geoskusea</i>								
<i>Rhinoskusea longirostris</i>	■							Schultz 1992
<i>Rhinoskusea wardi</i>	■							Rajavel et al. 2006
<i>Geoskusea baisasi</i>			■			■	■	Miyake et al. 2019, Tamashiro et al. 2011, Toma et al. 2011

<i>Geoskusea longiforceps</i>	■					■		Toma et al. 2011
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‘Facultative autogeny’ included ‘autogenous in first ovarian cycle, anautogenous in second’ and ‘cold temperature-dependent autogeny’ (Table 11). ‘Possible complete autogeny’ was assigned when no host is known and human blood has been amply offered: “*ne sont pas particulièrement anthropophiles*” and “apparently the females do not attack man”. Taxa which are extensively documented as taking bloodmeals, and for which any indication of autogeny is lacking, were presumed to be obligately anautogenous. Reports of mating biology were unambiguous (Table 12).

Table 11. Character coding scheme: hematophagy: autogeny.				
Taxon	Obligate anautogeny	Facultative autogeny	Possible complete autogeny	References
<i>Culiseta (Culiseta) inornata</i>		■		Moore et al. 1993
<i>Orthopodomyia signifera</i>				
<i>Culex (Culex) quinquefasciatus</i>	■			Clements 2012
<i>Mansonia (Mansonia) titillans</i>				
<i>Abraedes papago</i>				
<i>Aedes cinereus</i>				
<i>Aedes esoensis</i>				
‘ <i>Ochlerotatus</i> ’ (‘ <i>Coetzeomyia</i> ’) <i>fryeri</i>		■		Smith & Corbet 1975
<i>Ochlerotatus (Juppius) caballus</i>				
<i>Ochlerotatus (Empihals) vigilax</i>		■		Hugo et al. 2003
<i>Ochlerotatus dufouri</i>			■	Boussès et al. 2018
<i>Levua geoskusea</i>			■	Belkin 1962
<i>Rhinoscusea longirostris</i>				
<i>Rhinoscusea wardi</i>				
<i>Geoskusea baisasi</i>				
<i>Geoskusea longiforceps</i>				

Table 12. Character coding scheme: mating: swarming.				
Taxon	Swarm	Does not swarm	Mating immediately following female emergence	References
<i>Culiseta (Culiseta) inornata</i>		■	■	Foster & Walker 2019, Wood et al. 1979, Mattingly 1971, Owen 1942
<i>Orthopodomyia signifera</i>		■		Wood et al. 1979
<i>Culex (Culex) quinquefasciatus</i>	■			Becker et al. 2020, Reisen 2012
<i>Mansonia (Mansonia) titillans</i>				
<i>Abraedes papago</i>				
<i>Aedes cinereus</i>	■			Belton 1983
<i>Aedes esoensis</i>				
' <i>Ochlerotatus</i> ' (' <i>Coetzeomyia</i> ') <i>fryeri</i>				
<i>Ochlerotatus (Juppius) caballus</i>				
<i>Ochlerotatus (Empihals) vigilax</i>				
<i>Ochlerotatus dufouri</i>				
<i>Levua geoskusea</i>				
<i>Rhinoskusea longirostris</i>				
<i>Rhinoskusea wardi</i>	■			Rajavel & Natarajan 2006
<i>Geoskusea baisasi</i>	■			Toma et al. 2011
<i>Geoskusea longiforceps</i>				

'Oviposition at or near permanent water body' included 'avoids ovipositing into bodies which are only temporarily water-filled', 'oviposition on water surface', 'oviposition at the water's edge', and 'oviposition of egg cluster underwater on submerged plant surface' (Table 13). 'Oviposition at or near temporary water' included 'egg raft' and those species whose immatures are known from 'semi-permanent swamp'. 'Oviposition on dry substrate' included 'oviposition on soil', 'oviposition on vegetation', 'oviposition in dried up depressions', and 'oviposition on cotton pads in lab'. Reports of egg physiology and seasonality were unambiguous (Tables 14 & 15).

Table 13. Character coding scheme: female oviposition habits.				
Taxon	Oviposition at or near permanent water body	Oviposition on dry substrate	Oviposition at or near temporary water	References
<i>Culiseta (Culiseta) inornata</i>	■			Pappas & Pappas 1982

<i>Orthopodomyia signifera</i>	■			Crans 2004, Woodward et al. 1998, Wood et al. 1979
<i>Culex (Culex) quinquefasciatus</i>			■	Foster & Walker 2019, Service 1993
<i>Mansonia (Mansonia) titillans</i>			■	Day 2016, Lounibos & Linley 1987
<i>Abraedes papago</i>				
<i>Aedes cinereus</i>		■		Becker et al. 2020, Kardatzke 1979, Howard et al. 1917
<i>Aedes esoensis</i>				
' <i>Ochlerotatus</i> ' (' <i>Coetzeomyia</i> ') <i>fryeri</i>				
<i>Ochlerotatus (Juppius) caballus</i>				
<i>Ochlerotatus (Empihals) vigilax</i>		■		Dale et al. 1986
<i>Ochlerotatus dufouri</i>				
<i>Levua geoskusea</i>				
<i>Rhinoskusea longirostris</i>				
<i>Rhinoskusea wardi</i>				
<i>Geoskusea baisasi</i>		■		Toma et al. 2011
<i>Geoskusea longiforceps</i>				

Table 14. Character coding scheme: egg physiology.

Taxon	Eggs conditioned by reduction in dissolved oxygen level	Eggs conditioned by drying, then hatch after flooding	Eggs desiccation-resistant	Eggs hatch continuously in either wet or dry conditions	Eggs simply hatch upon completion of embryonic development (typically just a few days)	Eggs not desiccation-resistant	References
<i>Culiseta (Culiseta) inornata</i>							
<i>Orthopodomyia signifera</i>					■	■	Crans et al. 2004, Woodward et al. 1998, Wood et al. 1979
<i>Culex (Culex) quinquefasciatus</i>							
<i>Mansonia (Mansonia) titillans</i>							
<i>Abraedes papago</i>			■				Zavortink 1970
<i>Aedes cinereus</i>	■		■				Becker et al. 2020, Kardatzke 1979, Howard et al. 1917
<i>Aedes esoensis</i>							

<i>'Ochlerotatus'</i> (<i>'Coetzeomyia'</i>) <i>fryeri</i>			■				Girod & Le Goff 2006
<i>Ochlerotatus</i> (<i>Juppis</i>) <i>caballus</i>			■				Hopkins 1952
<i>Ochlerotatus</i> (<i>Empihals</i>) <i>vigilax</i>		■	■				Dale et al. 1986
<i>Ochlerotatus</i> <i>dufour</i>							
<i>Levua geoskusea</i>							
<i>Rhinoskusea</i> <i>longirostris</i>							
<i>Rhinoskusea</i> <i>wardi</i>							
<i>Geoskusea</i> <i>baisasi</i>			■	■			Toma et al. 2011
<i>Geoskusea</i> <i>longiforceps</i>							

Table 15. Character coding scheme: seasonality.

Taxon	Overwinters in egg stage	Overwinters as third-fourth instar	Overwinters as gravid female	Quiescent overwintering as larva	References
<i>Culiseta (Culiseta)</i> <i>inornata</i>			■		Denlinger & Armbruster 2014, Clements 2012, Vinogradova 2007, Wood et al. 1979
<i>Orthopodomyia</i> <i>signifera</i>		■			Crans 2004, Bradshaw & Holzapfel 1984
<i>Culex (Culex)</i> <i>quinquefasciatus</i>			■	■	Denlinger & Armbruster 2016, Reisen 2012, Almirón & Brewer 1996, Suleman & Reisen 1979, Jupp 1969
<i>Mansonia (Mansonia)</i> <i>titillans</i>					
<i>Abraedes papago</i>					
<i>Aedes cinereus</i>	■				Vinogradova 2007, Wood et al. 1979, Ross 1947
<i>Aedes esoensis</i>					
<i>'Ochlerotatus'</i> (<i>'Coetzeomyia'</i>) <i>fryeri</i>					
<i>Ochlerotatus (Juppis)</i> <i>caballus</i>					

<i>Ochlerotatus (Empihals) vigilax</i>	■				Dale et al. 1986
<i>Ochlerotatus dufouri</i>					
<i>Levua geoskusea</i>					
<i>Rhinoskusea longirostris</i>					
<i>Rhinoskusea wardi</i>					
<i>Geoskusea baisasi</i>					
<i>Geoskusea longiforceps</i>					

‘Daytime’ included ‘early morning’, ‘9:30-11AM, bright sunlight’, and ‘biphasic (dusk and dawn)’ (Table 16). ‘Nighttime’ included ‘not during daytime’ and ‘strongly photonegative’. ‘Dusk’ included ‘biphasic (dusk and dawn)’, ‘afternoon and dusk’, and ‘vespertine’.

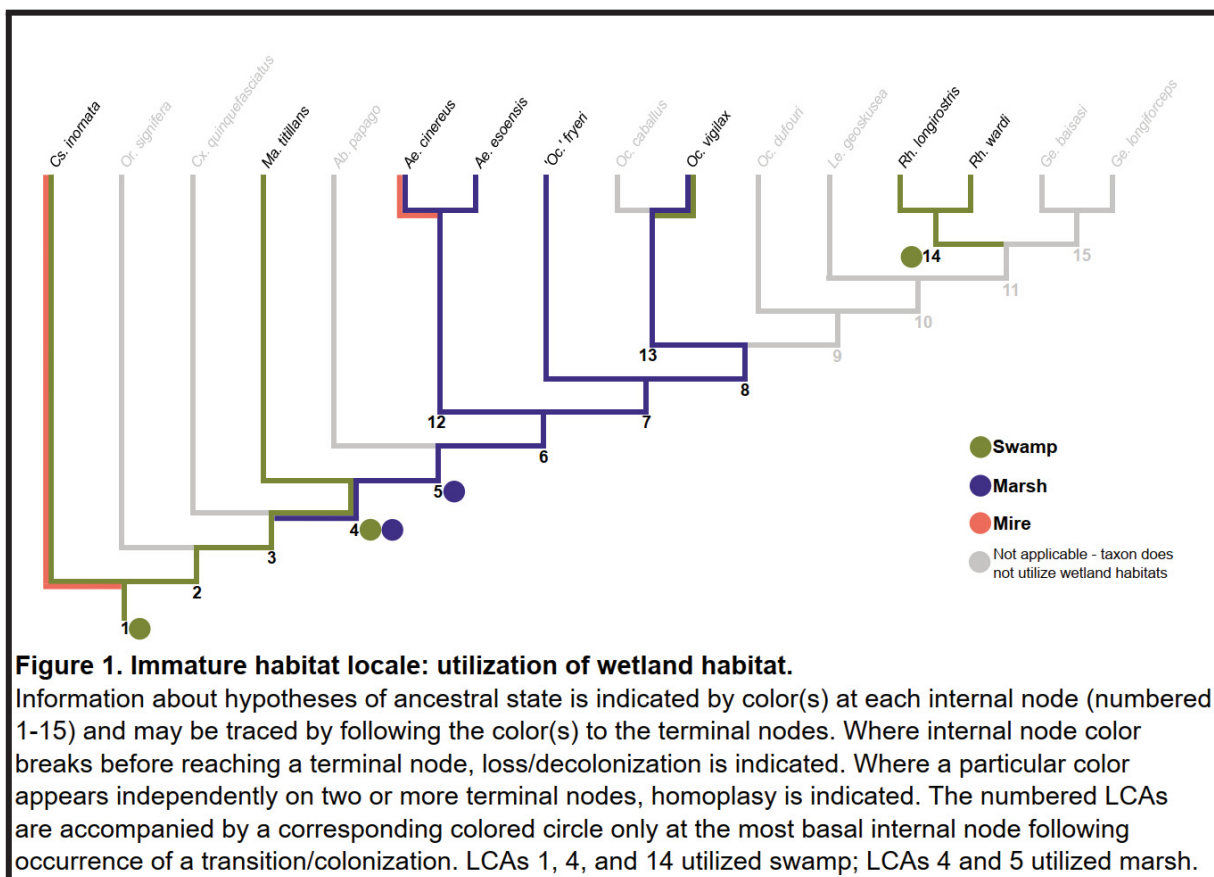
Table 16. Character coding scheme: day-night host-seeking activity of females.					
Taxon	Day	Night	Dusk	All times	References
<i>Culiseta (Culiseta) inornata</i>	■	■	■	■	Moore et al. 1993, Meyer et al. 1982
<i>Orthopodomyia signifera</i>		■			Mitchell & Rockett 1981
<i>Culex (Culex) quinquefasciatus</i>		■			Reisen 2012
<i>Mansonia (Mansonia) titillans</i>		■	■		Loroso et al. 2010, Clark-Gil & Darsie 1983
<i>Abraedes papago</i>	■				Zavortink 1972
<i>Aedes cinereus</i>	■		■		Becker et al. 2020, Belton 1983, Hocking et al. 1950
<i>Aedes esoensis</i>					
‘ <i>Ochlerotatus</i> ’ (‘ <i>Coetzeomyia</i> ’) <i>fryeri</i>	■		■	■	Huang et al. 2010, Van Someren & Furlong 1964
<i>Ochlerotatus (Juppius) caballus</i>					
<i>Ochlerotatus (Empihals) vigilax</i>			■	■	Peters & Christian 1963, Belkin 1962
<i>Ochlerotatus dufouri</i>					
<i>Levua geoskusea</i>					
<i>Rhinoskusea longirostris</i>		■			Schultz 1992
<i>Rhinoskusea wardi</i>	■				Rajavel et al. 2006a
<i>Geoskusea baisasi</i>		■	■		Okudo et al. 2004
<i>Geoskusea longiforceps</i>	■	■			Belkin 1962

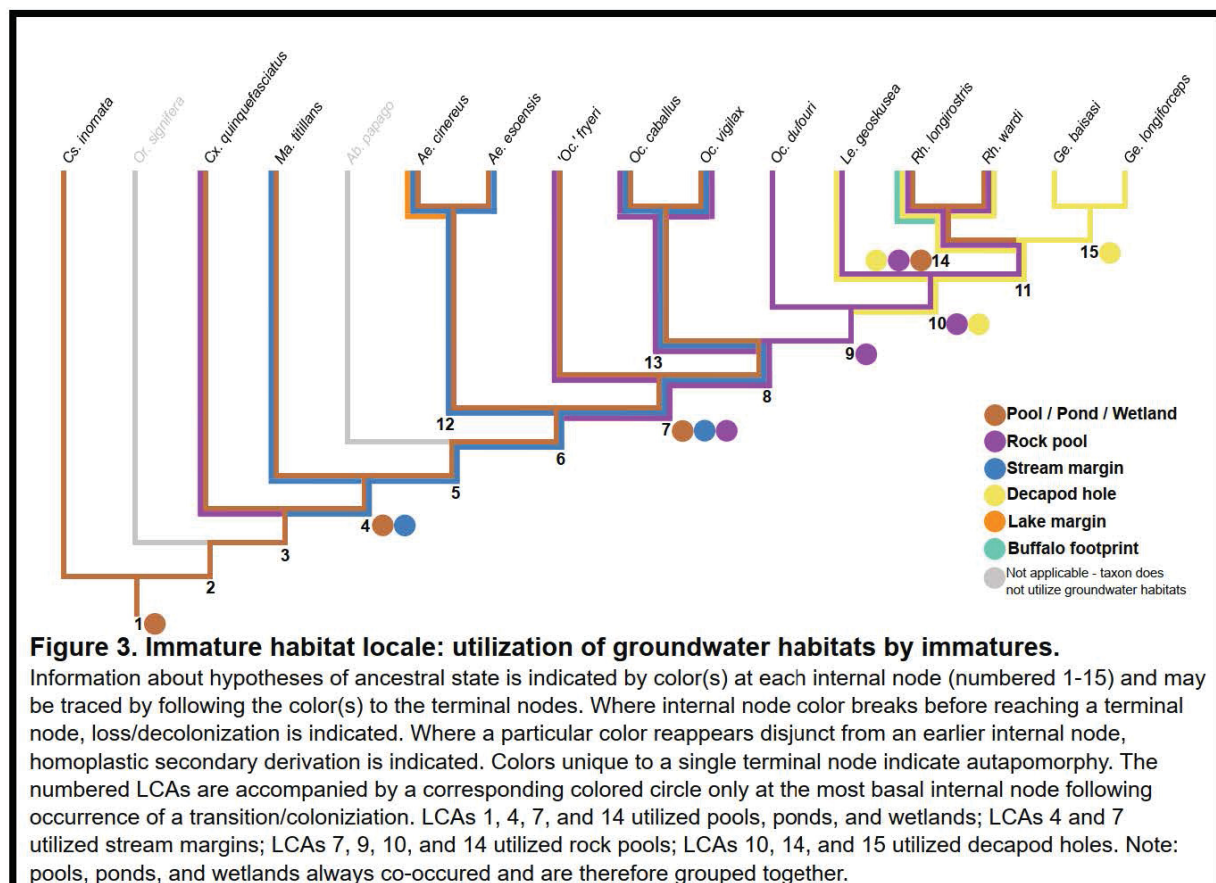
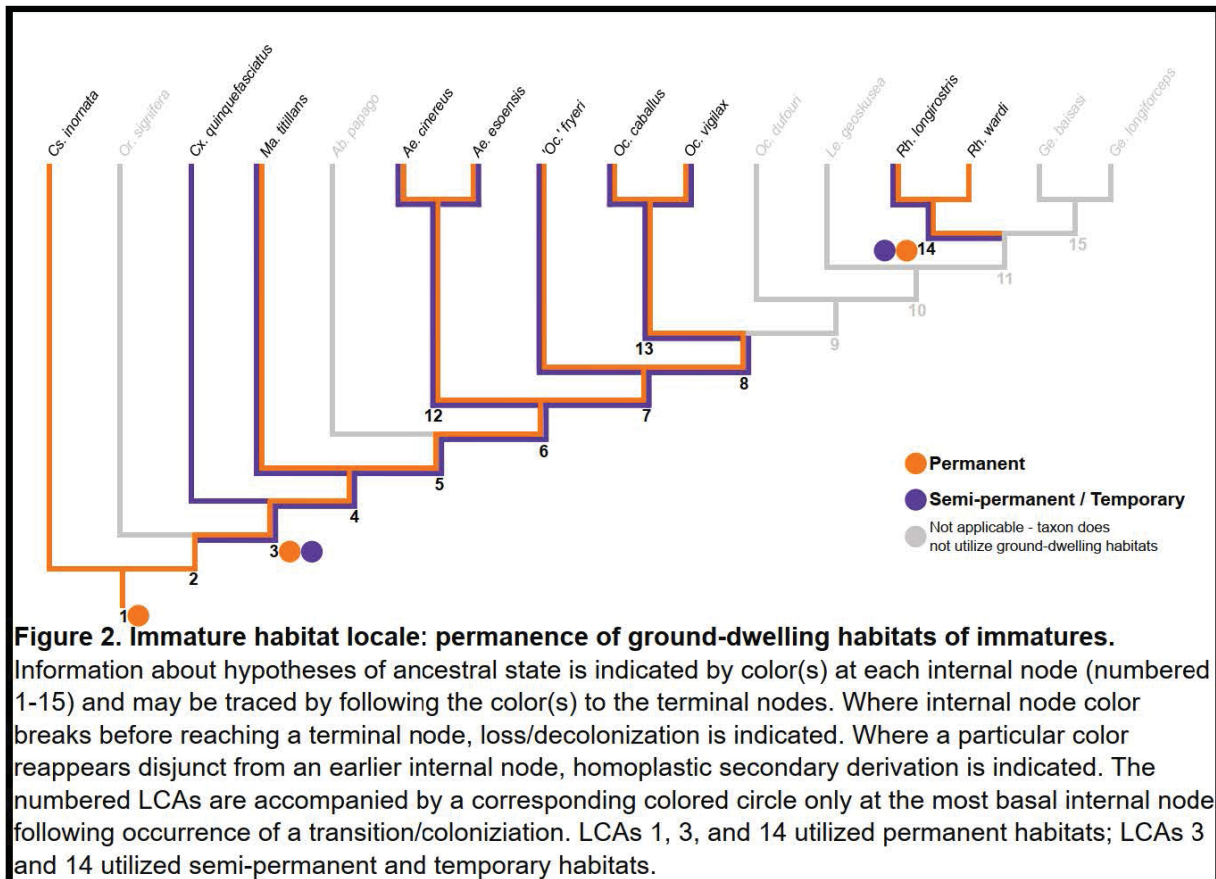
Table 17. General geographic distribution of taxa.		
Taxon	Distribution	References
<i>Culiseta (Culiseta) inornata</i>	Northwestern Canada south to northeastern Mexico	Ortega-Morales et al. 2015, Darsie & Ward 1981
<i>Orthopodomyia signifera</i>	Southern edge of central Canada south to northeastern Mexico; Caribbean	Wood et al. 1979, Shipp & Wright 1978, Zavortink 1968
<i>Culex (Culex) quinquefasciatus</i>	Cosmotropical; some extension into subtropical areas	Clements 2012
<i>Mansonia (Mansonia) titillans</i>	Southern USA south to central Argentina; Caribbean	Becker et al. 2020, Belkin et al. 1970
<i>Abraedes papago</i>	Pima Co., AZ, USA	Zavortink 1972
<i>Aedes cinereus</i>	Widespread over Holarctic region	Becker et al. 2020
<i>Aedes esoensis</i>	Widespread over northeastern Palearctic region	Халин & Горностаева 2008 [Khalin & Gornostaeva 2008]
<i>Ochlerotatus</i> ('Coetzeomyia') <i>fryeri</i>	Coasts and coastal islands of central East Africa	Boussès et al. 2018, Huang et al. 2010, Girod & Le Goff 2006
<i>Ochlerotatus (Juppius) caballus</i>	Southern Africa; Middle East	Azari-Hamidian et al. 2019, Abdoon & Ibrahim 2005, McIntosh 1973
<i>Ochlerotatus (Empihals) vigilax</i>	Seychelles; widespread over Southeast Asia and Australasia	Le Goff et al. 2012, Puslednik et al. 2012
<i>Ochlerotatus dufouri</i>	Réunion Island (French Overseas Department)	Boussès et al. 2018
<i>Levua geoskusea</i>	Viti Levu, Fiji	Belkin 1962
<i>Rhinoskusea longirostris</i>	Northern Australia north to Papua New Guinea and Southeast Asia	Lam-Phua et al. 2018, Reinert 1976
<i>Rhinoskusea wardi</i>	Philippines west to South Asia	Rajavel et al. 2007, Rajavel & Natarajan 2006, Rajavel et al. 2006a, 2006b, Reinert 1976
<i>Geoskusea baisasi</i>	Ryukyu Islands (Japan) south to Philippines	Tanaka et al. 1979
<i>Geoskusea longiforceps</i>	Solomon Islands	Steffan 1966

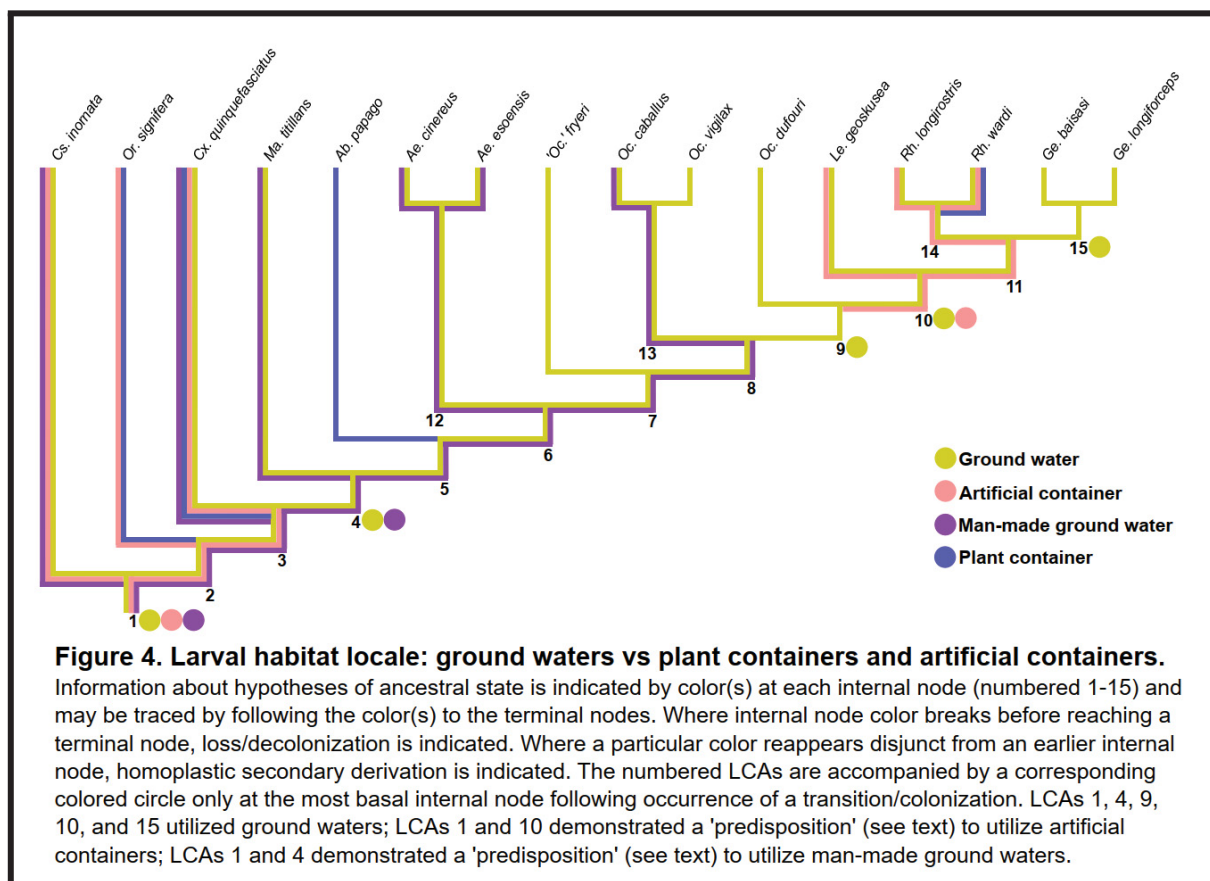
4. RESULTS

In terms of diversity of wetlands, it was here found that swamps were an ancestral immature habitat type for this group of Culicidae (Fig. 1). Subsequently invaded, and thus derived in nature, were marshes and mires. In terms of permanence of ground waters (including wetlands), it was found that permanent habitats are ancestral, while semi-permanent/temporary pools (Fig. 2) were derived. As for general type of ground waters utilized, pools, ponds, and wetlands were ancestral, stream margins and rock pools intermediately derived, and decapod holes and the most derived for this group of taxa (Fig. 3). Lake margins and buffalo footprints were autapomorphic for just one taxon each. And

finally at a third level of breadth, ground waters (including permanent habitats), as well as a ‘predisposition’ (see Introduction) to utilize man-made ground waters and artificial containers, were found to be ancestral; while plant containers were derived (Fig. 4).







The ancestral state of larval habitat utilization for all taxa here represented, including outgroups, was found to be permanent groundwaters (including wetlands and ponds), represented primarily by swamp habitat, as well as a 'predisposition' to utilize man-made groundwaters and artificial containers; this state is thus hypothesized to have occurred in the last common ancestor (LCA) of these taxa (labeled 'LCA 1' in figures; numbering system is constant across all figures) (Figs. 1, 2, 3, & 4). Subsequently invaded were semi-permanent and temporary groundwaters (such as puddles), by the last common ancestor (LCA) of *Cx. quinquefasciatus* + (*Ma. titillans* + Aedini ingroup) (LCA 3). Then, in the LCA of *Ma. titillans* + Aedini ingroup (LCA 4), the predisposition to utilize artificial containers was lost, and marshes and stream margins were colonized. Then, in the LCA of the group of Aedini here analyzed (LCA 5), swamp habitat was abandoned. *Ab. papago* then lost all groundwater habitats, and gained the plant container habitat. *Ae. cinereus* then colonized lake margins as well as mire. Then, the LCA of 'Ochlerotatus' ('Coetzeemyia') + (paraphyletic *Ochlerotatus* + (*Levua* + (*Rhinoskusea* + *Geoskusea*))) (LCA 7) colonized rock pool habitat. Then *Oc. vigilax* regained swamp habitat. Then the LCA of *Oc. dufouri* + (*Levua* + (*Rhinoskusea* + *Geoskusea*))) (LCA 9) lost all permanent and semi-permanent/temporary habitats. The LCA of

Levua + (*Rhinoskusea* + *Geoskusea*) (LCA 10) then colonized decapod holes and regained a predisposition to colonize artificial containers. *Rhinoskusea* then regained swamp and semi-permanent/temporary habitats (LCA 14), and *Rh. longirostris* colonized buffalo footprints while *Rh. wardi* colonized plant containers. *Geoskusea* then lost rock pools as well as the predisposition to utilize artificial containers, occurring exclusively in decapod-associated habitats (LCA 15).

In regards to condition of water in immature habitats, the use of freshwater (<0.1% salt), with intolerance of brackish water (>0.1% salt), was found to be ancestral; the use of brackish water and hypersaline (>3.5% salt) environments together with freshwater intermediately derived; and an intolerance of freshwater, together with tolerance of brackish and moderately saline (1.55%-3.1% salt) habitats, most derived (Fig. 5). A tolerance of acidic environments was found to be ancestral, with intolerance of acidic environments being derived (Fig. 6), likewise a tolerance for presence of vegetation, detritus, and pollution was ancestral and intolerance of such elements derived; whereas an intolerance for temperature extremes was found to be ancestral, with tolerance for such extremes (4°C and 50°C respectively) being derived (Fig. 7).

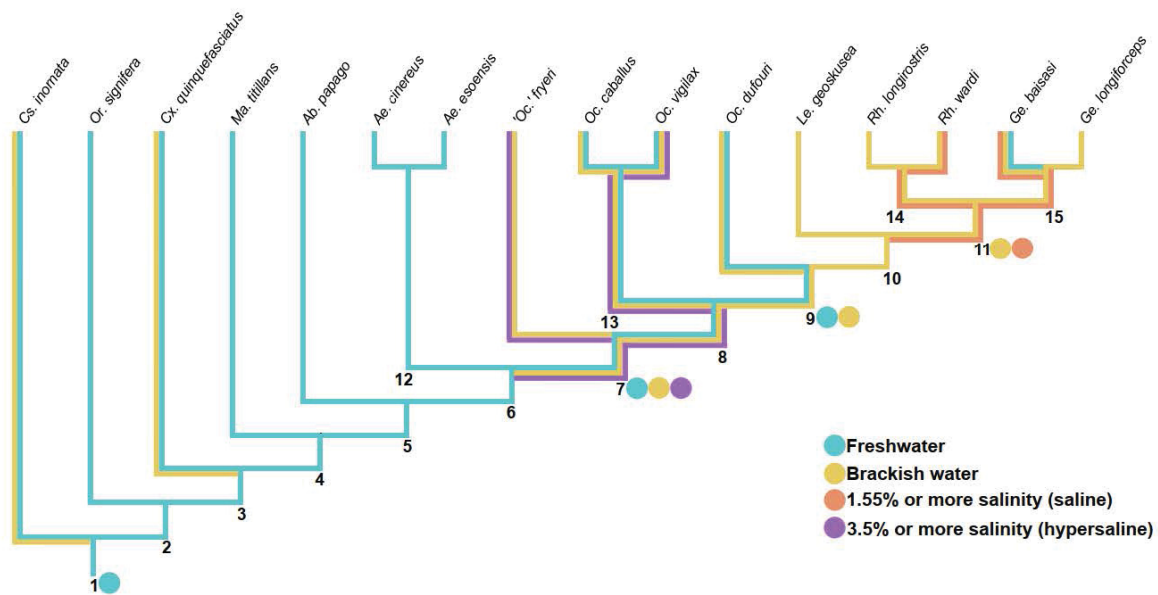


Figure 5. Immature habitat condition: freshwater, brackish water, and saline environment utilization.

Information about hypotheses of ancestral state is indicated by color(s) at each internal node (numbered 1-15) and may be traced by following the color(s) to the terminal nodes. Where internal node color breaks before reaching a terminal node, loss/decolonization is indicated. Where a particular color reappears disjunct from an earlier internal node, homoplastic secondary derivation is indicated. The numbered LCAs are accompanied by a corresponding colored circle only at the most basal internal node following occurrence of a transition/colonization. LCAs 1, 7, and 9 utilized freshwater; LCAs 7, 9, and 11 utilized brackish water; LCA 7 utilized hypersaline water; LCA 11 utilized moderately saline water.

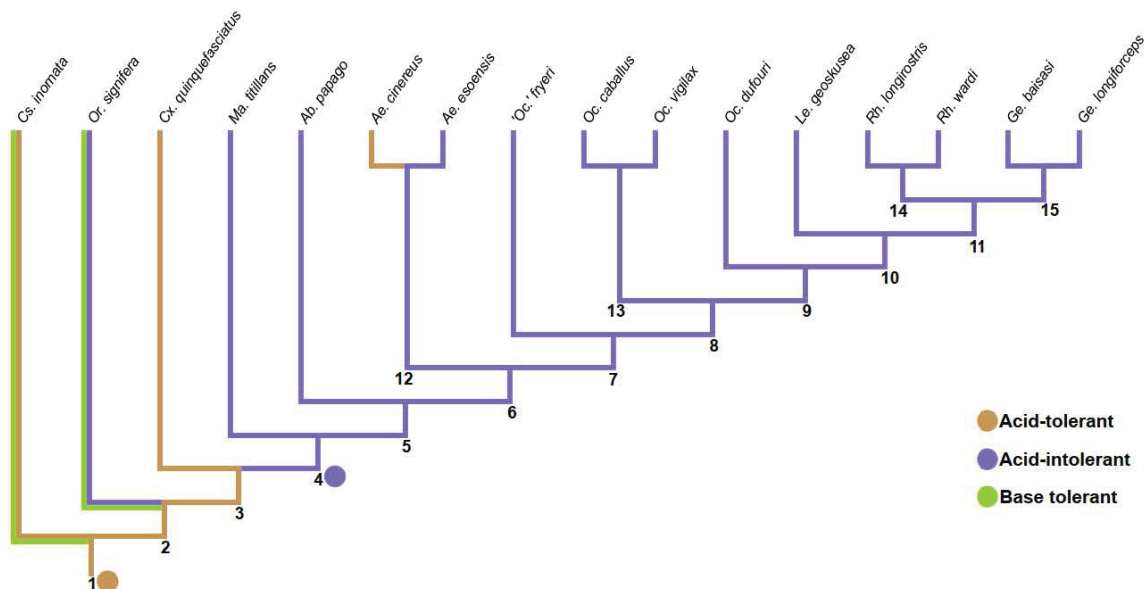
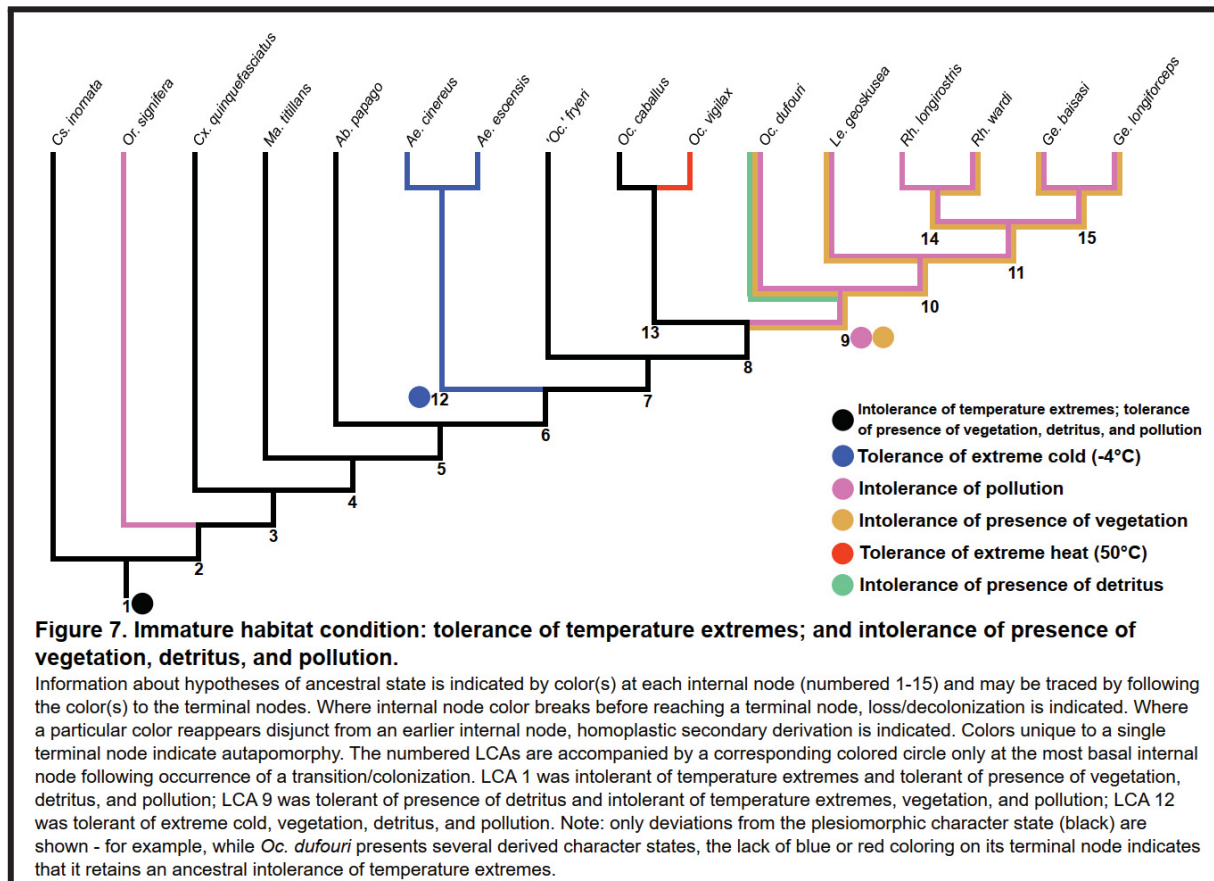


Figure 6. Immature habitat condition: tolerance of acidic water by immatures.

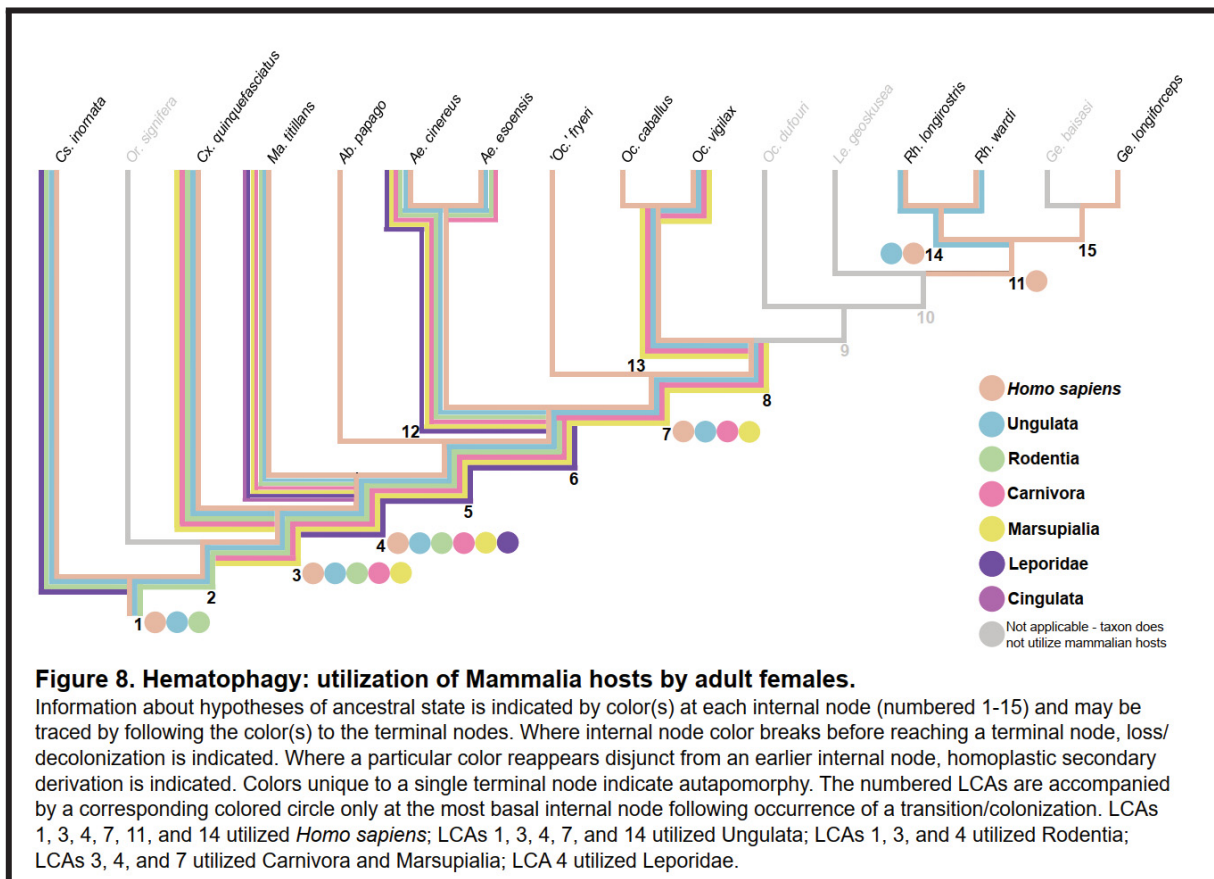
Information about hypotheses of ancestral state is indicated by color(s) at each internal node (numbered 1-16) and may be traced by following the color(s) to the terminal nodes. Where internal node color breaks before reaching a terminal node, loss/decolonization is indicated. Where a particular color reappears disjunct from an earlier internal node, homoplastic secondary derivation is indicated. The numbered LCAs are accompanied by a corresponding colored circle only at the most basal internal node following occurrence of a transition/colonization. LCA 1 was acid-tolerant; LCA 4 was acid-intolerant.

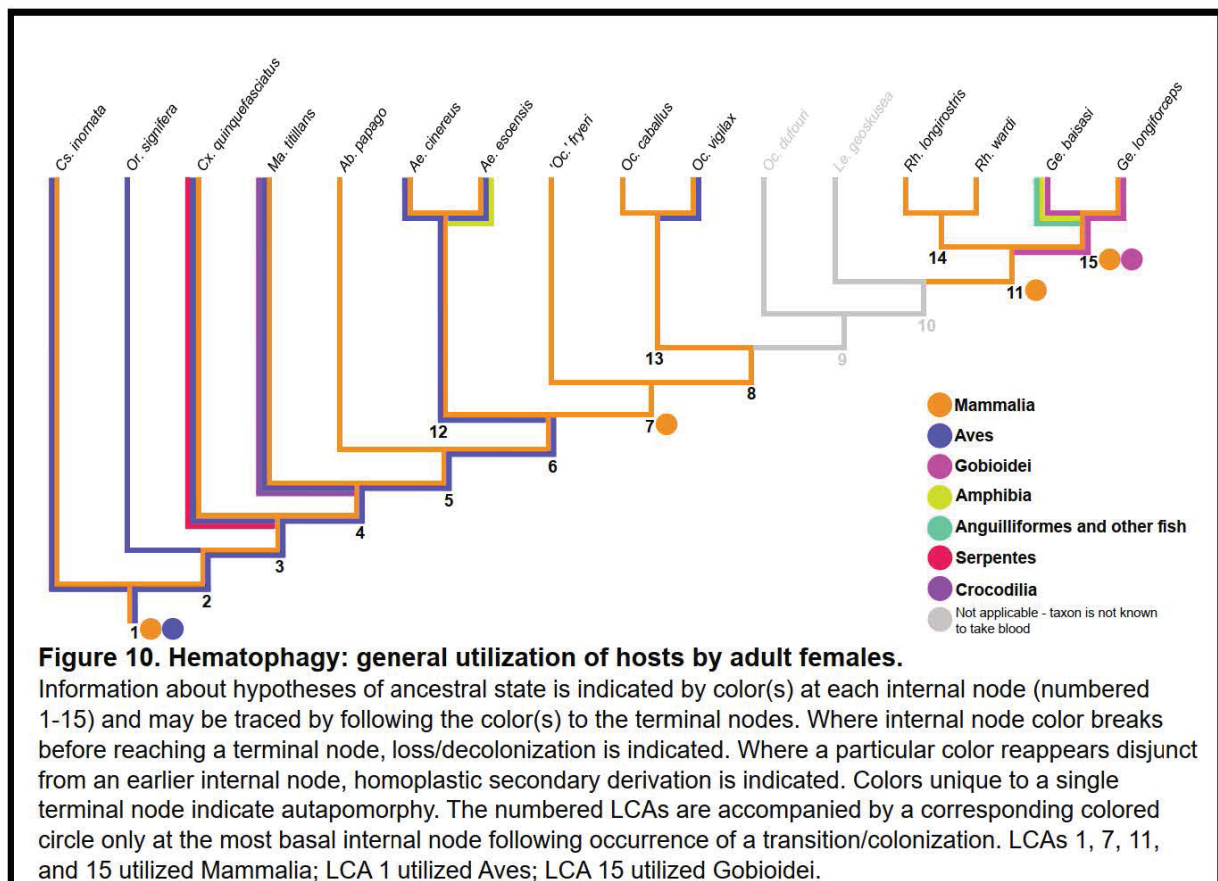
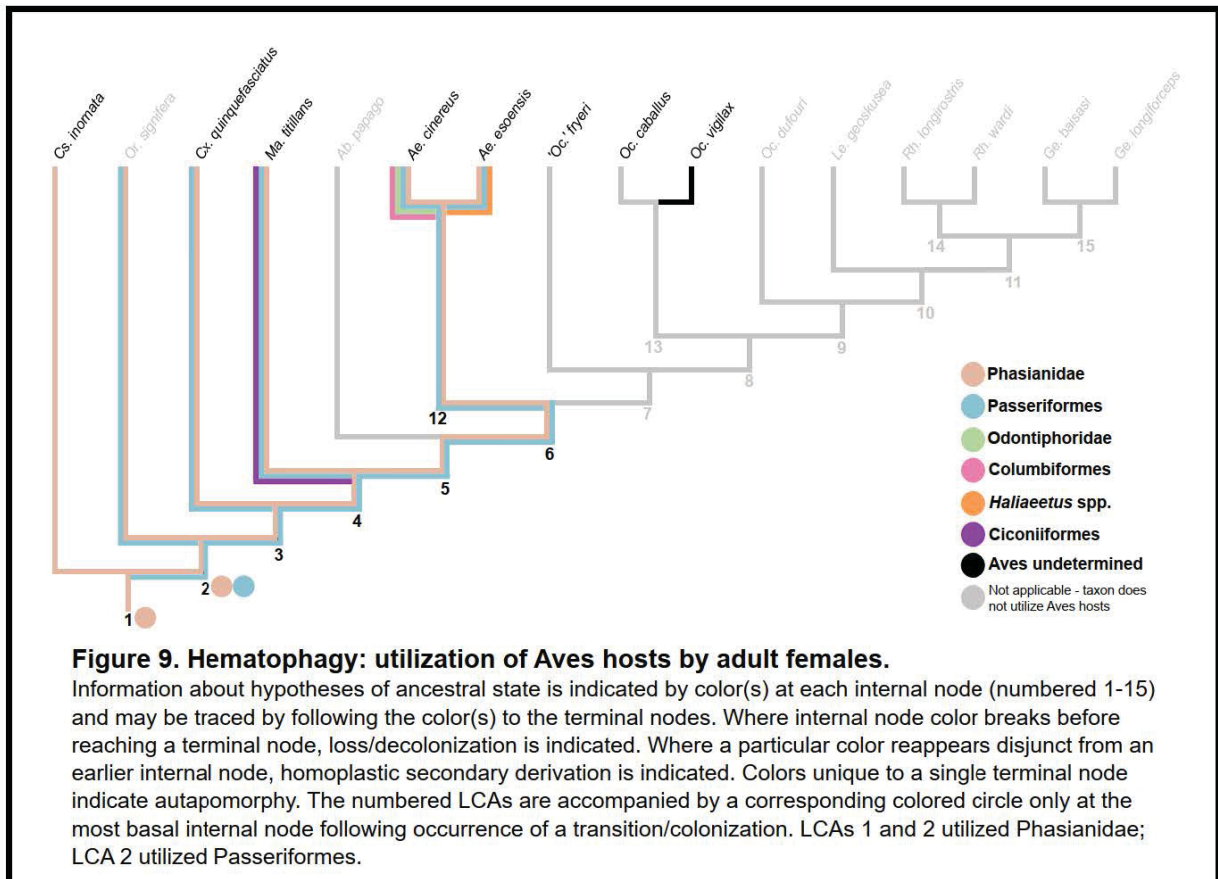


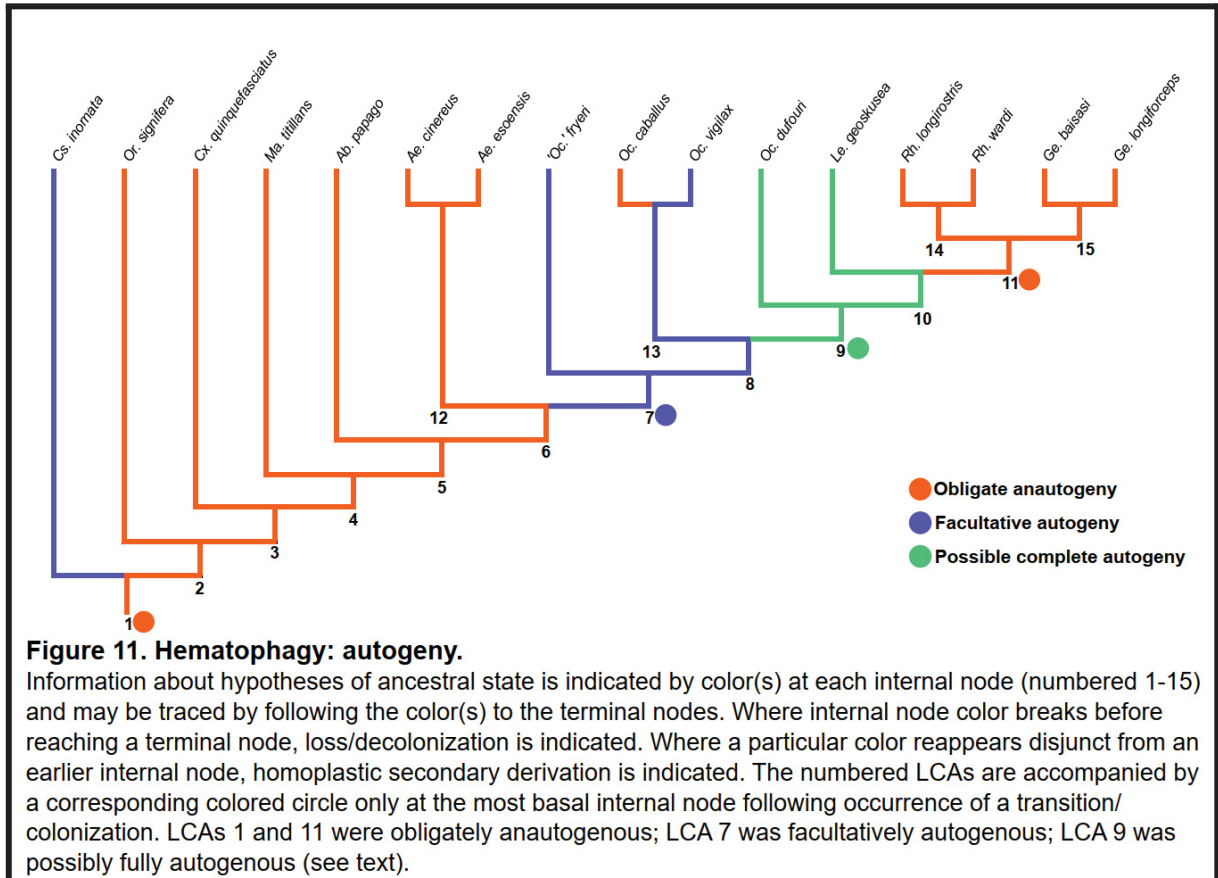
The ancestral state for condition of water utilized by immatures of all taxa here analyzed, including outgroups, was found to be freshwater, with a broad tolerance of acidic environments as well as vegetation, detritus, and pollution; and an intolerance of temperature extremes (LCA 1; Figs. 5, 6, and 7). LCA 4 then became intolerant of acidic environments. *Aedes* (LCA 12) then developed a tolerance for extreme cold. *Ae. cinereus* then regained a tolerance for acidic environments. LCA 7 then colonized brackish as well as hypersaline habitats. ‘*Ochlerotatus*’ (‘*Coetzeomyia*’) then lost a tolerance for freshwater as well as presence of vegetation, pollution, and detritus. *Oc. vigilax* gained a tolerance for extreme heat. LCA 9 then lost the tolerance of hypersaline environments. LCA 10 then also lost a tolerance for freshwater, and LCA 11 gained a tolerance of moderately saline habitats. *Ge. baisasi* subsequently regained a tolerance for freshwater.

In regards to hematophagy habits on Mammalian hosts, it was found that a predisposition to feed on *Homo sapiens*, Ungulata, and Rodentia is ancestral, while a predisposition to feed on Leporidae, Carnivora, and Marsupialia was derived (Fig. 8). On Aves hosts a predisposition to feed on Phasianidae was found to be ancestral, that on Passeriformes intermediately derived, and that on *Haliaeetus* spp., Odontophoridae, and

Columbiformes most derived (Fig. 9). Then at a broader level, a predisposition to feed on Mammalia and Aves in general was found to be ancestral, while a predisposition to feed on Amphibia, Gobioidae, and Anguilliformes and other fish was derived (Fig. 10). Finally, at a third level of breadth, the characteristic of anautogeny was ancestral, with that of facultative autogeny intermediately derived, and possibly full autogeny most derived (Fig. 11).

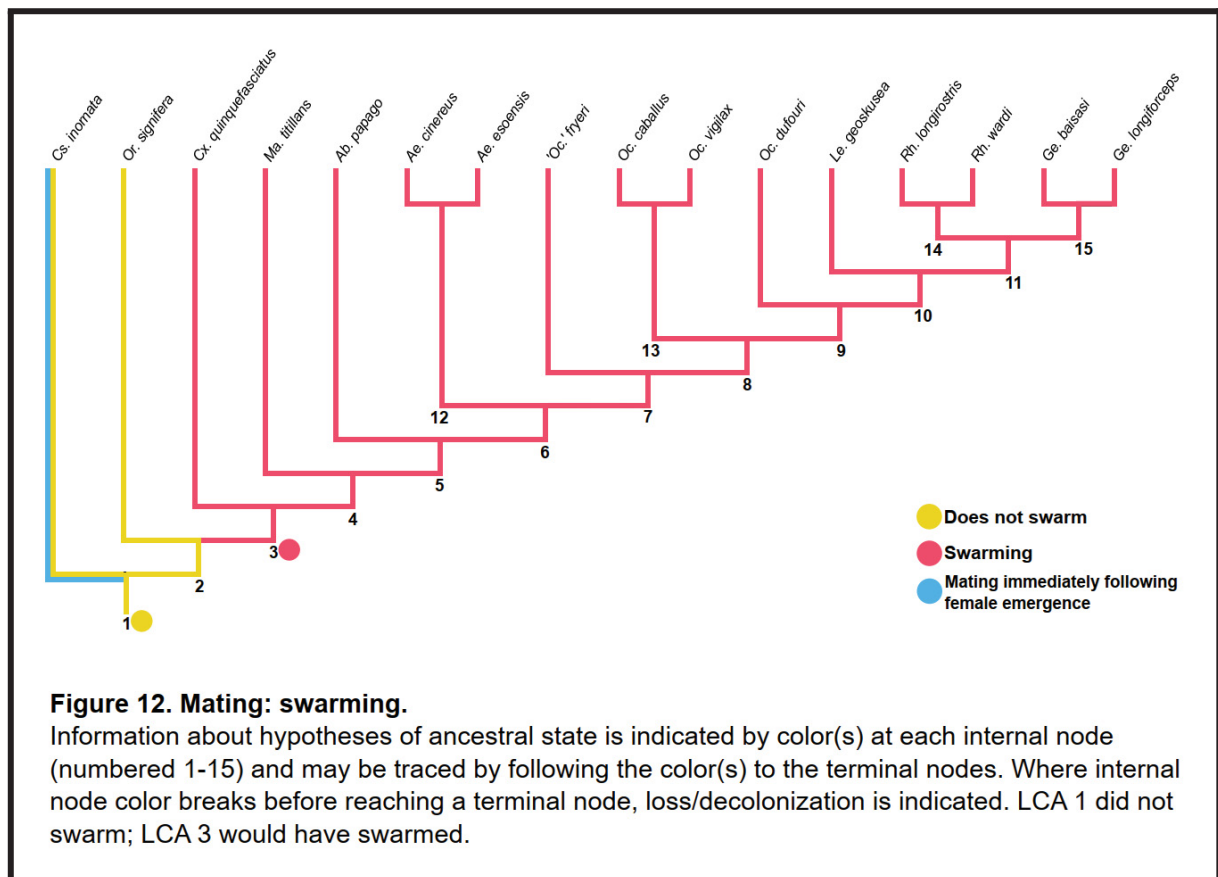


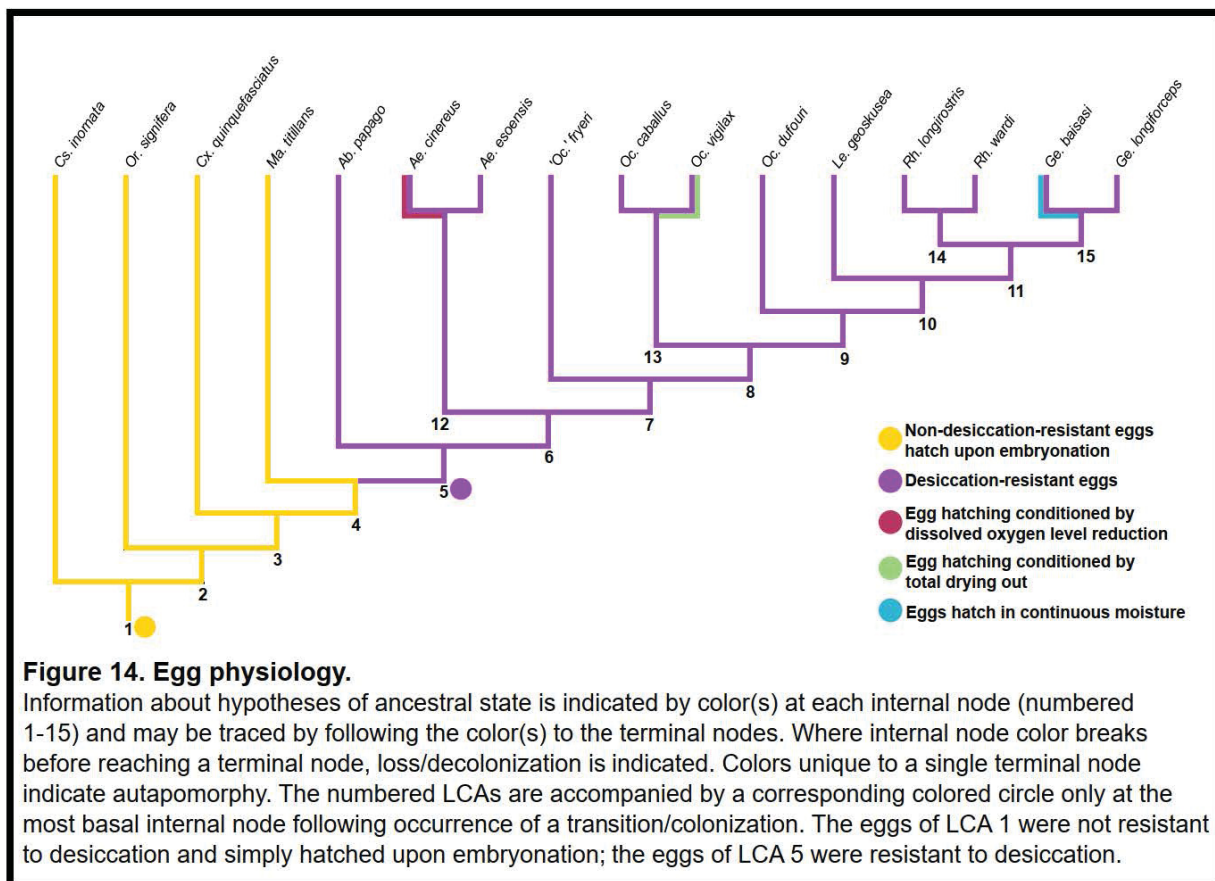
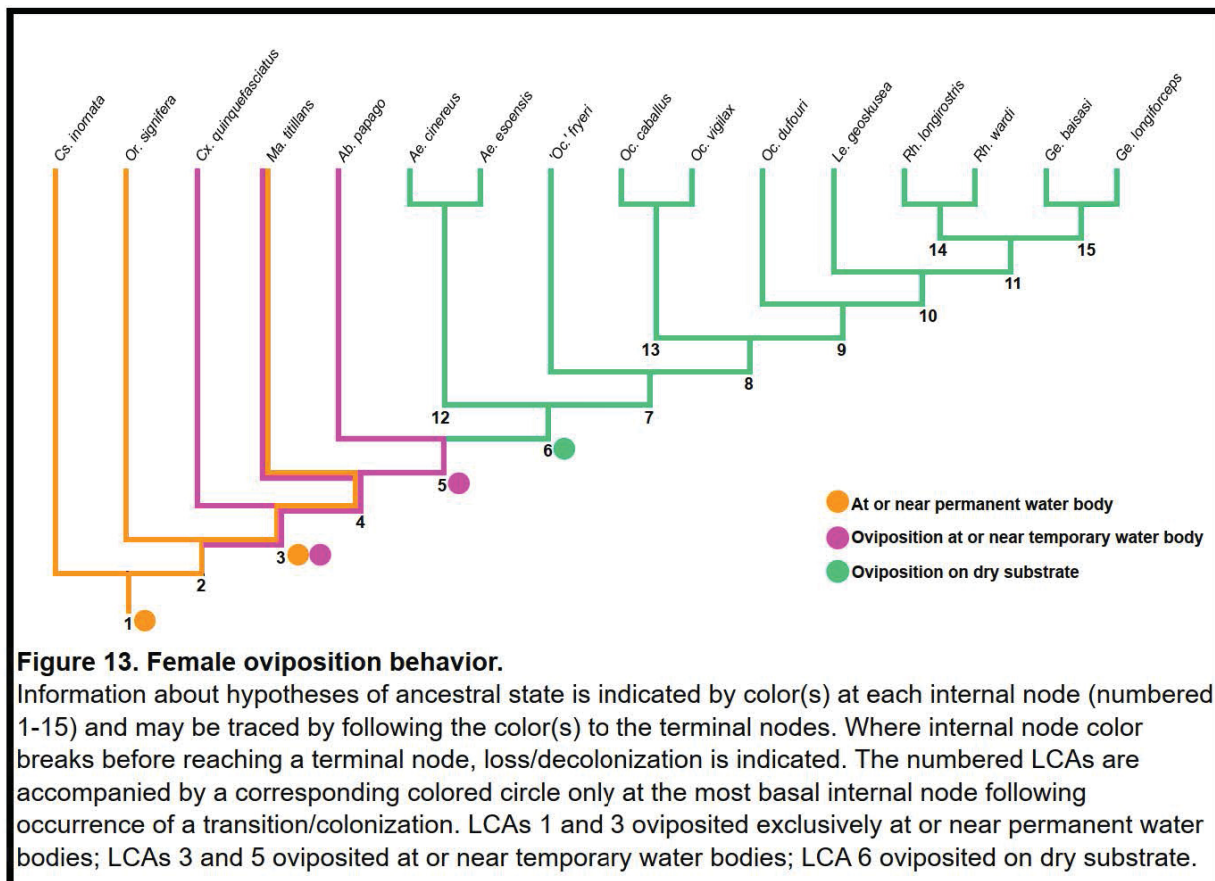




The ancestral state for hematophagy habits, including outgroups, was found to be characterized by obligatory anautogeny and specifically a predisposition to feed on *Homo sapiens*, Ungulata, Rodentia, and Phasianidae (LCA 1; Figs 8, 9, 10, & 11). LCA 2 then developed a predisposition to feed on Passeriformes. Then LCA 3 developed a predisposition to feed on Carnivora, Leporidae, and Marsupialia. *Ae. cinereus* then developed a predisposition to feed on Odontophoridae and Columbiformes, and *Ae. esoensis* a predisposition to feed on *Haliaeetus* spp. as well as Amphibia (Fig. 10). LCA 8 then lost a predisposition to feed on Rodentia, Leporidae, Phasianidae, Passeriformes, and gained the characteristic of facultative autogeny. LCA 10 then lost the predisposition to feed on all Mammalia and Aves, with the fact that neither *Oc. dufouri* nor *Levua* are known to take blood suggesting possible complete autogeny in this LCA. LCA 11 then lost the state of facultative autogeny and regained a predisposition to feed on *Homo sapiens*. *Rhinoscusea* (LCA 14) then regained a predisposition to feed on Bovini. *Geoskusea* (LCA 15) then gained a predisposition to feed on Gobioidae, with *Ge. baisasi* furthermore developing a predisposition to feed on Anguilliformes and other fish groups, Amphibia, and losing its predisposition to feed on all Mammalia.

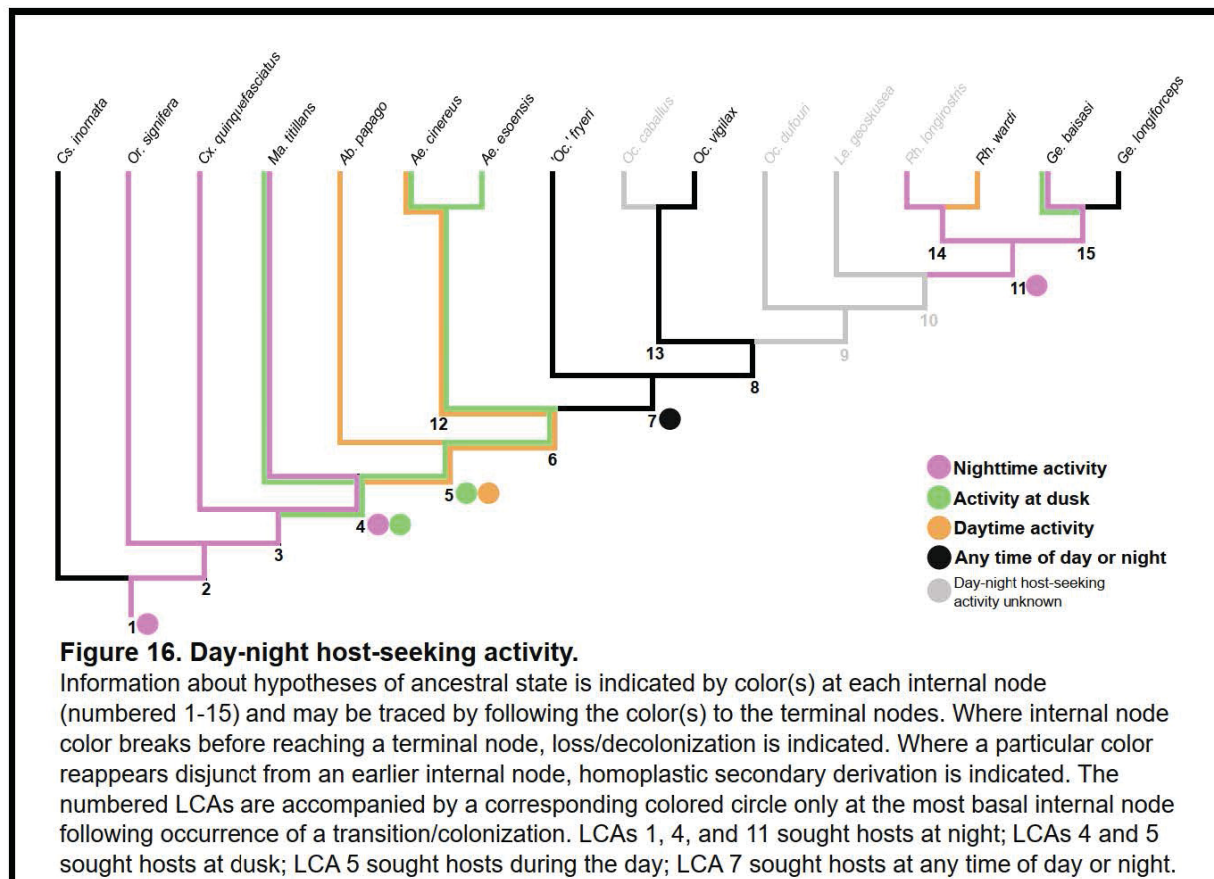
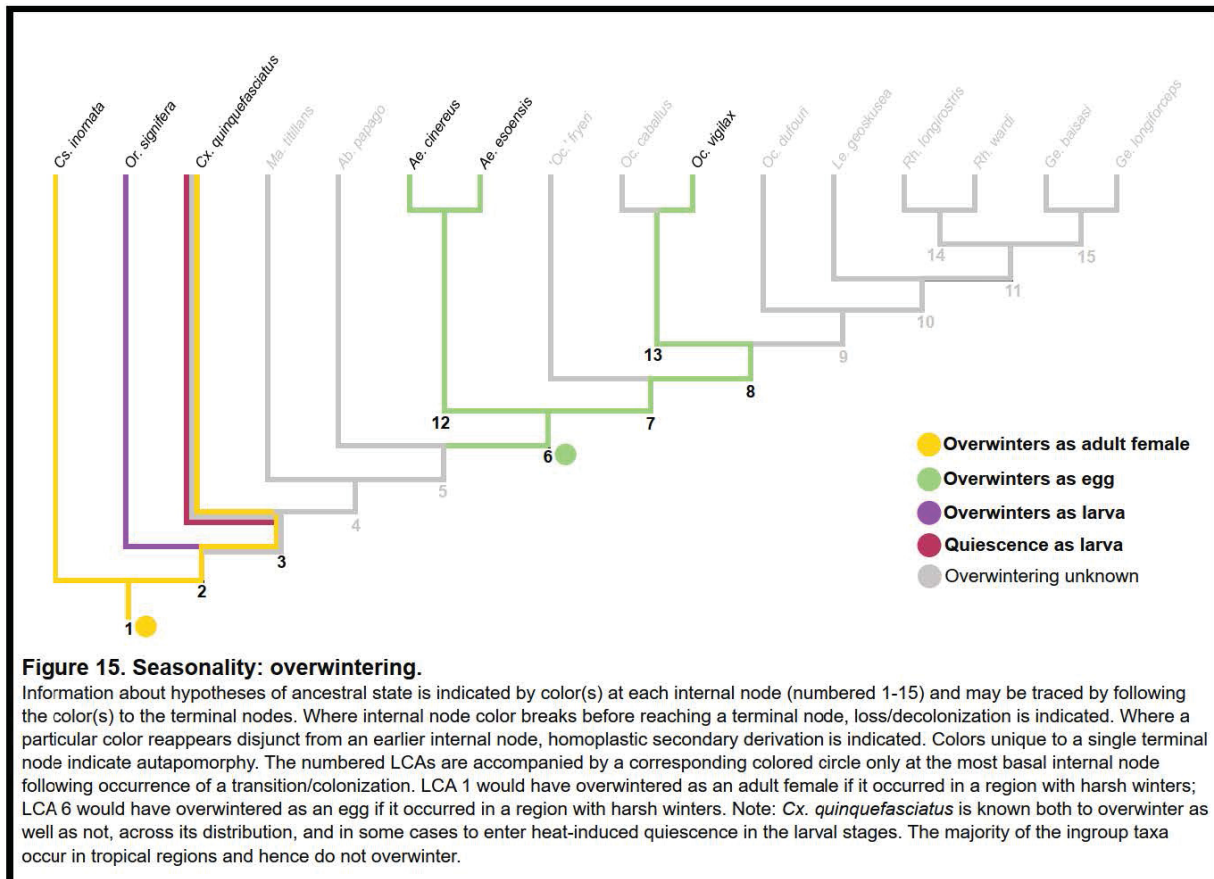
In regards to mating and egg biology, simple mating (*i.e.*, without swarming, upon encounter between a male and a female) was found to be ancestral, with swarming derived (Fig. 12). The habit of oviposition exclusively at or near a source of permanent water was found to be ancestral, while oviposition of at or near a source of temporary water (*i.e.*, habitats which may dry out are tolerated by ovipositing females) was intermediately derived, and oviposition on dry substrate was most derived (Fig. 13). The characteristic of non-desiccation-resistant eggs simply hatching upon completion of embryonic development was ancestral, while that of desiccation-resistant eggs capable of hatching long after completion of embryonic development was intermediately derived, and finally that of eggs which require a conditioning stimulus in order to hatch was most derived (Fig. 14).





The ancestral state for mating and egg biology, including outgroups, was found to be characterized by simple mating, oviposition at or near a source of permanent water, and non-desiccation-resistant eggs which hatch immediately upon completion of embryonic development (LCA 1; Figs. 12, 13, & 14). Then, LCA 3 developed the swarming habit as well as the predisposition to oviposit at or near sources of temporary water. Then, the LCA of this ingroup of Aedini (LCA 5) developed desiccation-resistant eggs. LCA 7 then developed a predisposition to oviposit on dry substrate. Subsequently, *Ae. cinereus* as well as *Oc. vigilax* came to be characterized by an egg stage which requires conditioning of some kind, in the first case by a reduction in the dissolved oxygen level of water and in the second by total drying out. Finally *Ge. baisasi* came to be characterized by an egg stage which will hatch just as well having been exposed to continuous moisture as having passed a drying-out phase.

For those taxa which overwinter, overwintering in the adult female stage was found to be ancestral, while overwintering in the egg stage is derived (Fig. 15). In regards to day-night female host-seeking activity, the nighttime habit was found to be ancestral, the dusk habit was intermediately derived, and the habit of opportunistically host-seeking at any time of day or night was most derived, with a subsequent reversion to nighttime exclusivity in LCA 11 (Fig. 16).



The ancestral state for seasonality and day-night female host-seeking activity, including outgroups, was found to be characterized by overwintering in the adult female stage (if applicable) and by taxa which are recorded as engaging in host-seeking at night (LCA 1; Figs. 15 & 16). LCA 4 then came to be characterized by host-seeking activity at dusk. The LCA of this group of Aedini (LCA 5) then lost nighttime host-seeking activity and came to do so during the day, while *Ab. papago* additionally lost host-seeking behavior at dusk. LCA 6 then came to be characterized by overwintering (if applicable) in the egg stage. LCA 7 then came to be characterized by host-seeking behavior at any time of day or night. LCA 11 secondarily reverted to nighttime host-seeking behavior, with *Rh. wardi* subsequently switching back to the daytime habit, *Ge. baisasi* additionally colonizing the dusk habit, and *Ge. longiforceps* coming to engage in host-seeking at any time of day or night.

5. DISCUSSION

Immediately apparent is that there was support for Mattingly's (1965) speculation that rock pools represent an intermediate habitat type between unspecialized ground pools and more specialized habitats associated with particular taxa, such as plant containers or decapod holes. We saw that the ancestral state of larval habitat utilization for all taxa here represented, including outgroups, was found to be groundwaters (LCA 1); somewhat later the LCA (7) of 'Ochlerotatus' ('Coetzeomyia') + (paraphyletic *Ochlerotatus* + (*Levua* + (*Rhinoskusea* + *Geoskusea*))) colonized rock pool habitat; and finally the LCA (10) of *Levua* + (*Rhinoskusea* + *Geoskusea*) colonized decapod holes.

It is interesting that three of the four outgroup taxa (*Cs. inornata*, *Cx. quinquefasciatus*, and *Ma. titillans*), together with some less derived Aedini (*Ae. cinereus*, *Ae. esoensis*, and *Oc. caballus*) are known from man-made groundwater habitats such as ditches (Fig. 1). In this study, application of strict parsimony analysis therefore hypothesized that the LCA (1) of all taxa would have then presented a 'predisposition' to utilize such anthropogenic habitats. Some conceptual disentangling followed by future study is here warranted. Given the historical recency of the advent of these environmental resources as discussed in the introduction regarding ditches for example, it could be that the stimuli/environmental cues for these niche-utilizations are simply congruent with those already faced by these mosquitoes in their ancestral habitats. Alternatively, there is of course the possibility of recent niche colonization of these resources in the majority of these terminal taxa, such eventuality then nihilating the possibility of the application of parsimony as

performed here. Future studies should therefore seek to further characterize the cues perceived and acted upon by female mosquitoes both while host-seeking and while ovipositing. Interestingly, all taxa which utilized man-made groundwaters also utilized groundwaters generally, with only *Oc. vigilax* and *Rh. longirostris* utilizing the latter but not the former.

Similarly, in consideration of the utilization of artificial containers by the three most basal outgroup taxa, as well as of the utilization of humans as hosts in nine of the ten most basal taxa, parsimony found too in the LCA (1) of all taxa ‘predispositions’ to utilize artificial containers as immature habitats and to utilize human hosts (Figs. 1 & 8). We may consider conceptual illustrations of these possibilities: *e.g.*, a tire as physically and chemically similar enough to a treehole that the ovipositional and/or larval developmental processes are absolutely equivalent, or, *e.g.*, human kairomones and body temperature as character states which are not divergent from the ancestral states of prehistorically available monkey or rodent (both ‘Suprprimates’) hosts — or even a more recent homoplastic convergence in these characters between humans and one or more prehistoric host taxon. Again, future studies which would better characterize the effects of stimuli and cues by females are necessary.

Interestingly, tolerance of hypersaline environments was found to occur in a more basal LCA (7; Fig. 5) than that which tolerated merely moderately saline environments (LCA 11), and it is particularly of note that the former occurred without associated abandonment of freshwater habitats, while the latter occurred indeed just following an abandonment of freshwater. This observation could imply that, upon initial colonization of brackish habitats by the LCA (7) of ‘*Ochlerotatus*’ (‘*Coetzeemyia*’) + (paraphyletic *Ochlerotatus* + (*Levua* + (*Rhinoskusea* + *Geoskusea*))), a tolerance of a wide range of salinities very soon followed, whereas the LCA (11) of the more derived *Rhinoskusea* + *Geoskusea* found itself tolerating a considerably narrower range of salinities. Similarly, in regard to acidity conditions, as the LCA (1; Fig. 6) of all taxa was found to tolerate acidic habitats (and the two most basal outgroup taxa even tolerate basic habitats), a restriction generally to more or less neutral pH was found in the LCA (4) of *Ma titillans* + Aedini ingroup, possibly again reflecting a derived specialization to such pH-neutral habitats. Regarding tolerance of presence of vegetation and pollution, too, a derived specialization to habitats lacking these features was observed in the LCA (9; Fig. 7) of *Oc. dufouri* + (*Levua* + (*Rhinoskusea* + *Geoskusea*)).

While feeding on Mammalia, having been lost in the LCA (9; Fig. 10) of *Oc. dufouri* + (*Levua* + (*Rhinoskusea* + *Geoskusea*)), reappeared in the LCA (11) of *Rhinoskusea* +

Geoskusea, a general switch from Aves to autogeny, then Gobioidae, was observed tracking the LCAs of ‘*Ochlerotatus*’ (‘*Coetzeomyia*’) + (paraphyletic *Ochlerotatus* + (*Levua* + (*Rhinoskusea* + *Geoskusea*))) (7), *Oc. dufouri* + (*Levua* + (*Rhinoskusea* + *Geoskusea*)) (9), and *Geoskusea* (15), respectively. In *Ge. baisasi*, in particular, which is not known to feed on Mammalia, this phenomenon is taken further, resulting in specialization to quite a variety of peculiar fish hosts available in the vicinity of its mangrove decapod holes, including eels and blennies (Miyake *et al.*, 2019; Tamashiro *et al.*, 2011; Toma *et al.*, 2011). The apparent stepwise transition from obligate anautogeny to facultative autogeny (LCA 7), then to possibly full autogeny (LCA 9) is extremely interesting; physiological studies of *Oc. dufouri* and *Le. geoskusea* are needed to confirm this prediction based on phylogeny. Also implied by this scenario would be a secondary reversal to anautogeny in LCA 11.

It is unsurprising that female oviposition behavior and egg physiology converged between LCAs 5 and 6 (Figs. 13 & 14) to oviposition on dry substrate and desiccation-resistant eggs, respectively, as these two characters are clearly very closely related. The analysis suggests that it is probable that more eggs of aedine taxa than just *Ae. cinereus* and *Oc. vigilax* require a conditioning stimulus to hatch, which would make sense ecologically, in order to have some sort of a guarantee that there is both water and food availability present in the habitat (Fig. 14); *Ge. baisasi*, however, presents a notable exception to this prediction, hatching apparently in the absence of any conditioning stimulus (Toma *et al.*, 2011).

In contrast to characters such as larval habitat locale and water condition, in which we observed a trend towards increasing specialization, that of day-night host-seeking activity indicated a general trend of decreasing specialization, at least through LCA 7: going from exclusively nighttime activity (LCA 1; Fig. 16), to nighttime and dusk (LCA 4), dusk and daytime (LCA 5), and finally to a lack of specialization to any particular time of day or night (LCA 7). A dearth of records then becomes apparent in the case of *Oc. dufouri* and *Le. geoskusea* — if these taxa and their ancestor are totally autogenous, as predicted above, then there could indeed never be a record of their ‘host-seeking’ activity. Following this potentially non-blood-feeding ancestor, there is a novel reversal to nighttime host-seeking activity in LCA 11.

Another interesting as-yet-untested evolutionary hypothesis is that of Belkin (1962, p. 55): this author speculated, based on biogeographical and morphological reflections, that the species *Le. geoskusea* is the result of hybridization between a species of *Geoskusea* and a member of the *Oc. vigilax* “section” (Marks, 1957) of *Ochlerotatus*. In today’s classification,

Oc. vigilax is the monotypic representative of subgenus *Empihals*, and the other putative members of this “section” are *Ochlerotatus* (subgenus uncertain) *antipodeus*, *Ochlerotatus* (*Buvirilia*) *edgari*, *Ochlerotatus* (subgenus uncertain) *inexpectatus*, and *Dobrowortskyius rubrithorax*. Belkin followed Marks (1957) in thinking *Oc. vigilax* to share characteristics with these four species representing several distinct genera and subgenera. Albeit noting that these are merely guesses, Belkin dated (1962, p. 57) the colonization of the South Pacific by *Oc. vigilax* to the ‘early Tertiary period’ (=Early Paleogene, ~34 to 66 mya), and the aforementioned hybridization event resulting in *Le. geoskusea* to the ‘middle Tertiary period’ (=Late Paleogene, ~23 to 34 mya). He (1962, p. 55) further speculated that *Oc. vigilax* saw itself redispersed one or more times subsequent to this initial Early Paleogene dispersal, and that, based on the hypothesized Late Paleogene hybridization event resulting in *Le. geoskusea*, that *Geoskusea* spp. would too have first dispersed just prior to this in the Late Paleogene. Elsewhere (1962, p. 332) Belkin speculated that *Geoskusea*, *Levua*, and other crab hole-inhabiting genera may represent relic lineages produced through hybridization, and, as noted in the Introduction, he also hypothesized that species with wide ecological tolerances, such as *Oc. vigilax*, were too produced through hybridization. Although it is impossible to test specifically whether hybridization was the mechanism that precipitated these speciation events, we may nevertheless test Belkin’s hypotheses of timing and location of these colonizations using what we know about the phylogeny, character evolution, ecology, and distribution of these groups.

In the absence of fossil Aedini dating back beyond the Eocene (Gilka *et al.*, 2021; Harbach, 2013), it is impossible to precisely date the occurrence of these habitat and behavior transitions. It is known that the Aedini are at least 33.9 million years old, given these Eocene fossils, and are probably as old as 123.9 million years or more, based on the molecular clock estimate of Reidenbach *et al.* (2009). Also through molecular analysis, these latter authors furthermore dated a subclade of Aedini, sister to a basal *Psorophora*, a relationship now supported by multiple studies (Lorenz *et al.*, 2021, Reidenbach *et al.*, 2009, Reinert *et al.*, 2009), to 92.05 million years ago. We may consider this latter calculation as the approximate age of the aedine ingroup of the present work.

Some conclusions may be gleaned by considering the distributions of some of the modern taxa here studied against the paleogeographical record. Of the four taxa here considered most derived (the two species each of *Rhinoskusea* and *Geoskusea*), two of these recent taxa (*Rh. wardi* and *Ge. baisasi*) have Indomalayan distributions, one has an Indomalayan-Australasian distribution (*Rh. longirostris*), and one has an Australasian

distribution (*Ge. longiforceps*). Yeates and Cassis (2017) date the initial interaction of these two biogeographical realms, following the breakup of Australasia from Gondwana, to 25 mya, via the intermediate area of Wallacea. It is difficult to say based on these taxa alone, then, whether their ancestor had dispersed initially from the south, via Australasia, or from the north, via Sundaland. The next-most derived taxon, *Le. geoskusea*, is endemic to Viti Levu, Fiji, an area of the Oceanian realm which has only had continuously exposed coastline for the past 16 million years (Anderson & Clark, 2009), so this taxon is probably not of sufficient age to help us resolve this question either. However, of help is the fact that the next four most derived taxa, *Oc. dufouri*, *Oc. vigilax*, *Oc. caballus*, and ‘*Oc.*’ *fryeri*, all present Afrotropical distributions, with just *Oc. vigilax* being represented additionally in the Indomalayan and Australasian realms. Interesting in this is that two of these taxa, *Oc. vigilax* and *Oc. dufouri*, are endemic in the Afrotropical Realm to rock pool habitats in the Seychelles and La Réunion in the Mascarenes, respectively, volcanic landmasses which split from eastern India some 93 mya (Bastia *et al.*, 2010), their seafloor having been greatly elevated from 85 to 65 mya, then the islands having served as a ‘train’ connecting Madagascar and India in the Paleocene (Ali & Aitchison, 2008). Given these facts, it would seem that the ancestor of these nine taxa was most likely of Gondwanan, not Laurasian, origin, coming through either Africa or India, then dispersing through Sundaland, the Malay realm, and finally Wallacea to Australasia and Oceania. The paleogeographical context detailed above for this conclusion is then reasonably within the time span from the estimated ancestor dating of a *Psorophora*-less Aedini by Reidenbach *et al.* (2009) to the oldest Aedini fossils (Gilka *et al.*, 2021), from 92.05 mya to 33.9 mya. This would make invasion of the Indomalayan and Australasian realms by *Oc. vigilax* secondary to an initial distribution confined to a region based in and/or around the Seychelles, following that island group’s breaking off from India. This conclusion is consistent with Belkin’s (1962) dating of the colonization of the South Pacific by *Oc. vigilax* to the Early Paleogene, as well, which likely would have occurred following an expansion by this species through the Indomalayan realm.

In summary, then, ‘*Ochlerotatus*’ (‘*Coetzeemyia*’) + ((*Ochlerotatus* (*Juppius*) + *Ochlerotatus* (*Empihals*)) + (*Oc. dufouri* + (*Levua* + (*Rhinoskusea* + *Geoskusea*)))) (LCA 7) is hypothesized to have had its origin in the intermediate area of the Seychelles-Mascarenes between Africa and India. In turn, the monotypic *Levua*, plus (*Rhinoskusea* + *Geoskusea*) (LCA 10), is hypothesized to have had an Indomalayan origin, potentially following the colonization of this area by some LCA approximately between those here designated LCAs 7 and 10. These conclusions are consistent too with Belkin’s (1962) hypothesis that *Le.*

geoskusea, which we may interpret as LCA 10 of this study, had its origin in the Late Paleogene, although notably if LCA 10 is at least 23 million years old, then *Geoskusea* (LCA 15), a derived descendant of LCA 10, could not have existed and dispersed in its present form prior to this point, let alone have hybridized with *Oc. vigilax* to produce *Le. geoskusea*, as Belkin stated. Importantly, the working concept of *Ochlerotatus* as employed here is paraphyletic — if the genus as a whole is not also polyphyletic — and so conclusions regarding this genus taken as a whole cannot be drawn from this work. Indeed, Lambrecht (1971) stated that *Ochlerotatus* (as *Aedes* subgenus *Ochlerotatus*) should be of Gondwanaland or Neotropical origin, with *Oc. vigilax* having reached the Seychelles via Australasia — quite the opposite scenario to that here postulated. As support for this speculation he notes this species' affinity for brackish seashore habitat, which could conceivably have enabled long-distance overseas migration. His scenario is here rejected in light of the phylogenetic relationships of *Oc. vigilax* and its sister taxa (a group almost entirely Afrotropical) as well as given the geological history of the Mascarenes Plateau discussed above. Refraining from speaking to genus *Ochlerotatus* taken wholesale, then, the conclusions of this work apply to the monotypic *Ochlerotatus* taxa of '*Coetzeemyia*', *Empihals*, and *Oc. dufouri*, as well as to the three taxa of *Juppius*. Further biogeographical interpretation within Aedini is here foregone, due to the probably considerable phylogenetic distance between the aforementioned group of nine species, and *Aedes* and *Abraedes* (Harbach, 2007; Reinert *et al.*, 2009).

Some important limitations of the results presented here must be made explicit. For many of the characters analyzed, it is frankly unknown if human concepts of differences between ecological entities are equivalently perceived by mosquitoes. For example, outgroup comparison led the author to conclude that swamp habitat is ancestral, and marsh and mire both derived. It is far from known to be the case that oviposition-site-seeking female mosquitoes perceive so readily such differences in floral composition among habitat as these features are conveniently delimited by man. To give one more example, it is not known with certainty what our conceptual taxonomic groupings such as 'Bovini' or 'Mammalia' represent to host-seeking female mosquitoes (barring possible apomorphies, shared by all descendants of these taxa, which would manifest as kairomones to host-seeking females), and so our tendency when recording blood-feeding events to combine host species into groups based on taxonomic classification may or may not reflect a biological fact about our parasitic organisms of interest. It is clear, then, that much work remains to be done, and the present

analysis must be considered only a point of initial entry into this complex network of biological interactions, cues, stimuli, and non-human perception as such.

Many of these characters may accurately be dubbed what Thiele (1993) called ‘overlapping characters’, a known problem in fields such as, *e.g.*, morphometrics. Although an attempt has here been made to critically make sense of the data at hand, allowing for rather than ignoring such overlapping in the analysis, the phenomenon remains problematic in the sense that the moving from one niche to another is in many cases not complete, but represents a partial expansion of niche utilization in the members of that taxon, and indeed within a single species it cannot be said for certain whether any given individual represents all of the diversity of ecology and behavior attributed to the observed sample individuals for that species in general. Indeed it is not likely, if the conclusions from the work (discussed in the introduction) of Serra *et al.* (2017) on differential niche-utilization over a distribution in the Chironomidae may be generalized. But these facts do not preclude the characters involved from being ‘cladistic’; they only make analysis that much more difficult to articulate in terms of its conclusions. But importantly, the obvious existence of this phenomenon makes it equally as important to do one’s best to coherently make sense of it.

In the cases of some potential characters, it remains a possibility that the concept of ‘niche’ colonization and decolonization is inappropriate entirely, as taxa may simply be extremely plastic and environmentally-impressionable with regard to one or more of these characters, or our human concepts of these characters are biologically unfounded or otherwise do not correspond to environmental stimulus responses of mosquitoes. One or more of these eventualities may be the case particularly for those characters noted as not analyzed for this work in the Materials and Methods section, for example oxygen content as a condition of water present in immature habitats, and stenogamy. Importantly, the testing of such eventualities stands up to the same standards of hypothesis rejection as evolutionarily systematic work itself, therefore more attention must be devoted to the disentangling of these problems.

6. FINAL CONSIDERATIONS

Systematic analyses of ecological character derivation within particular organismal groups, such as the present study, are indispensable to the biological sciences — it is through such studies that it may be possible to one day establish general principles of character evolution; moreover they are foundational for the subsequent execution of studies in

macroevolutionary biology and evolutionary-developmental (evo-devo) biology, in addition to having great relevance to biogeography and theoretical ecology. They are however limited by availability of 1) recorded character states which would facilitate cross-taxa comparisons, and 2) published phylogenetic hypotheses of the taxa in question. For further facilitation of such studies it is to be hoped, then, that in Aedini and other culicid taxa more attention is given (in relation to the first limitation) to the rigorous collection and recording of ecological data in order to more precisely define a taxon's niche and environmental requirements, and (as for the second limitation) to basic systematics efforts such as phylogenetic systematics, particularly those which would combine morphological and molecular characters.

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