## Taxonomic works on mosquitoes (Diptera: Culicidae) of the Americas

by

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#### Abstract

mosquitoes (Diptera: Culicidae) in the Americas, with emphasis on species in the subfamily Culicinae. In Chapter 2, I provide a comprehensive review of the literature that led to the need for a taxonomic revision of the subgenus Ochlerotatus Lynch Arribálzaga, 1891, followed by the revision itself. This work is a major taxonomical reappraisal of the group and provides a definition for the subgenus, a critical step towards stabilizing the genus Ochlerotatus Lynch Arribálzaga. In total, two species are elevated out of synonymy, and two synonymized names are moved to the list of synonyms of another taxon. One new species is described, Ochlerotatus (Ochlerotatus) brisolai sp. nov., and nine species are redescribed. In Chapter 3 we review of the fauna of Culicidae of Wisconsin, USA, based on both a comprehensive review of the literature and identification of museum specimens. These data are compiled in an annotated checklist with figures, tabular data and maps to show the distribution and composition of the fauna of the state. The work presented in Chapter 3 inspired a survey of the mosquito fauna of the UW-Madison Arboretum, described in Chapter 4. This work contains information on the species composition, seasonality and habitat distribution of the Culicidae in a natural area in Madison, WI, USA. That work also provided material that led to Chapter 5, which is a description of a new species of the Stimulans Group of mosquitoes, Ochlerotatus mirabilis sp. nov. Finally, the works performed in the Arboretum were one of many inspirations for a reappraisal of characters used to distinguish two species of *Culex* that are common to this area and play important roles in the transmission of West Nile Virus (Chapter 6). This body of research highlights the importance of correctly identifying a specimen according to its designated classification, as a fundamental step in research in the biological sciences.

#### Dedication

I dedicate this work to my family, who always stood by my side, cheering my victories and helping me learn from my defeats. Especially my parents, Jairo and Sandra, who made me the person I am, and my sister Aline, who always lent me a friendly shoulder in my moments of need. I'm also very grateful to Prof. Brisola, whose work and passion for the insects inspired me, and someone who I have immense respect for.

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I also thank all of my friends and colleagues, physically present or not, that were part of my life in these last years, and provided me with the force to keep on going, even if they didn't know. To all I give my sincere thanks.

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Eu dedico este trabalho à minha família, que sempre esteve ao meu lado, celebrando minhas vitórias e me ajudando a aprender com as minhas derrotas. Em especial meus pais, Jairo e Sandra, que me fizeram quem sou hoje, e minha irmã Aline, que sempre foi um ombro amigo nos momentos de desepero. Também agradeço imensamente ao Prof. Brisola, cujo trabalho e paixão pelos insetos me inspirou, e para quem eu tenho imenso respeito.

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私達は間違えながら学ぶ。

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#### **Chapter 1. Introduction**

The ability to correctly identify a specimen according to its taxonomic designation is fundamental to many research pursuits in the organismal biological sciences. Mosquitoes in the Tribe Aedini (Diptera: Culicidae), are a prime example. There are 3,601 known species of culicids; 1,261 of these are in the Aedini, and of those, 116 (9%) are associated with transmission of pathogens of medical or veterinary significance (see Wilkerson *et al.*, 2015). Accurate identification of vector and non-vector species therefore is essential to evaluating and mitigating risk of pathogen transmission. For example, identification is essential to surveys done at the inception of a disease outbreak for the purpose of incriminating a vector, for surveying vector species to assess abundance and pathogen infection status, and for decision-making associated with implementing mosquito control.

Perhaps less appreciated, mosquitoes also provide ecosystem services by visiting particular plants to source nectar and sap, and can function as biological indicators (Dorvillé, 1996). There are indeed species of mosquito with particular habitat demands that reveal underlying features of an ecosystem (Dorvillé, 1996, Forattini, 2002). In this case, an accurate identification that confirms presence of a particular species can drive policy and decision-making in service of habitat conservation. For example, the presence of particular mosquito species impacted construction of dams for hydroelectric power plants and creation of new protected areas (Gomes *et al.* 2009; Lopes & Lozovei 1995; Marchi *et al.* 2010), and informed assessment of the environmental impact of such power plants (Müller *et al.* 2014).

Both identification of a biological unit and assessment of its evolutionary relationships are dependent on the current taxonomic state of understanding of the taxon. As it relates to the Aedini, Harbach & Kitching (1998) found the tribe Aedini to be paraphyletic in relation to Mansoniini, and provided evidence that Aedini itself is a polyphyletic assemblage. Thereafter, collective works of Reinert (1999, 2000a, b) and Reinert *et al.*, (2004, 2006, 2008, 2009) resulted in 74 new or resurrected genera from the genus *Aedes*. Wilkerson et al (2015) suggested that the phylogeny proposed in these works was "weakly supported," but did "not challenge the phylogenetic hypotheses created" and proposed simplified generic designations, in part to be more useful to the broad community of people who work with vector and nuisance species in the genus (Wilkerson et al., 2015). There is much work to be done to clearly resolve the taxonomy of the group so that it supports and is supported by the phylogeny.

#### This study

The goal of the work presented in this dissertation was revise a particular genus (*Ochlerotatus*) that was elevated from the *Aedes*, and to

provide resources for identification of mosquitoes in the Midwest, U.S.A. In Chapter 2, I present a taxonomic review of the nominotypical subgenus of the genus *Ochlerotatus* (Diptera: Culicidae) *sensu* Reinert *et al.* (2008); Chapter 3 is a review of the mosquito fauna of the state of Wisconsin, USA; Chapter 4 is a presentation of an ecological survey of the mosquito fauna of the UW-Madison Arboretum, Madison, WI, USA; Chapter 5 is a description of a new species found in Wisconsin, USA; Chapter 7 is a reassessment of morphological characters used to identify two species of *Culex* (Diptera: Culicidae). Finally, I provide a partial key for the mosquitoes of the Nearctic region.

The genus *Aedes* has long been recognized as an unnatural assemblage (Edwards 1932; Harbach & Kitching 1998). Attempts to split the genus and resolve the Tribe Aedini classification (Reinert 1999, 2000a, b; Reinert *et al.* 2004, 2006, 2008, 2009) were met with criticism by the community of medical entomologists, public health practitioners and mosquito control personnel who work regularly with *Aedes* species in particular. The controversy culminated in the publication of a provisional "utilitarian" approach to the Aedini nomenclature, the justification being that a more stable state of nomenclature could be achieved (Wilkerson *et al.* 2015). This "utilitarian" approach fails to address the underlying problem with the taxa involved. My work addresses this problem with a revision of one of the most contentious taxa involved, the genus *Ochlerotatus* Lynch

Arribálzaga, 1891, as understood by Reinert *et al.* (2008). The genus has no known synapomorphies and is potentially polyphyletic. The fundamental issue with the *Ochlerotatus* is the lack of definition for the nominotypical subgenus, which was only provisionally defined by the author as the Scapularis group of Arnell (1976). This group is itself, however, ill-defined and the descriptions of its species contain many points of contention.

Therefore, the goal of taxonomic revision of the subgenus *Ochlerotatus* is to provide the evidence needed to stabilize the genus *Ochlerotatus* Lynch Arribálzaga, 1891, as understood by Reinert *et al.* (2008). This work, presented in Chapter 2, improves the ability of future researchers to accurately place new species in the genus, and improves our understanding of the relationship of mosquitoes in this genus to others in the genus *Aedes* and Tribe Aedini. Toward this end, I completed a comprehensive assessment of *Ochlerotatus* specimens. Based on this assessment, certain types were fixed by neotype designation (as necessary), all species were redescribed, and the subgenus was redescribed and defined.

The goal of the "Mosquitoes of Wisconsin" Chapter 3 was to review the species and provide an updated checklist of species of Culicidae in the state of Wisconsin, U.S.A. The data presented were collected both from an exhaustive literature research, and review of all of the specimens in the Wisconsin Insect Research Collection in the Department of Entomology at the UW-Madison. The Chapter includes a detailed discussion of all of the species, written and tabular summaries of the data and maps of species distributions according to county records.

In that same vein, the work entitled "Survey of the mosquito fauna (Diptera: Culicidae) of the University of Wisconsin-Madison Arboretum, WI, USA" Chapter 4 was completed at surveying the mosquito fauna from a natural area in Madison, WI. Detailed bionomic data are presented for the community structure at this locale. This work contributes to the understanding of the fauna present in the UW Arboretum, in terms of species composition, distribution and bionomics.

In the process of assessing specimens associated with the previous chapter, we noted specimens that did not clearly align with morphological descriptions of the Stimulans group. In Chapter 5 "Ochlerotatus mirabilis (Diptera: Culicidae) a new species from Wisconsin, USA" we describe a new species and provide a brief introduction to the taxonomic problems surrounding the Stimulans Group of mosquitoes, particularly the Ochlerotatus stimulans complex.

The specimens collected in Chapter 4 also inspired and facilitated Chapter 6, "An evaluation of characters for the separation of two *Culex* species (Diptera: Culicidae) based on material from the Upper Midwest". Therein, we provide description of five morphological characters that are useful for identification of two *Culex* species that are commonly found in surveillance for West Nile virus, and are generally regarded as indistinguishable in the literature. We also present a taxonomic reappraisal of characters commonly used to identify six species of *Culex* mosquitoes.

Finally, all of these efforts inspired development of a partial key to the mosquitoes of the Nearctic region to supplement of the existing key (Darsie & Ward, 2005) that is used by laboratories across the U.S., but was published in 1981 and modified most recently in 2005. This key is included in the Appendix.

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Chapter 2. Taxonomy of the subgenus *Ochlerotatus* (Diptera: Culicidae) Lynch Arribálzaga, 1891

# Chapter 2.1. The taxonomic history of *Ochlerotatus* (Diptera: Culicidae) Lynch Arribálzaga, 1891

#### The description of Ochlerotatus

The genus *Ochlerotatus* was described by Lynch Arribálzaga (1891), who distinguished it from the similar valid genera at time, *Culex*, *Janthinosoma*, *Psorophora* and *Taeniorhynchus*, based on the morphology of the palps of females and the tarsal claws (of both sexes). In the newly described genus he included *Culex albifasciatus* Macquart, 1838, synonymizing under it *Culex vittatus* Philippi, 1865. He also described a new species, *Ochlerotatus confirmatus*, based on material he collected in May of 1887 near the Salado river, Buenos Aires province, Argentina, and material sent to him from Dr. Eduardo L. Holmberg from Formosa, Chaco province, Argentina. The author remarks on the similarity of this species with *Culex vittatus* Philippi, 1865, but points to the lack of an abdominal dorsal stripe on his specimens as very distinctive.

Although no indication was made by Lynch Arribálzaga on the whereabouts of his specimens in his publication, in the early 1900's he gave his entomological collections to the museum now known as the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN) (Papavero 1973). O. H. Casal designated a Lectotype from material found in the MACN; the Lectotype is a female specimen in good condition with labels by F. Lynch Arribálzaga, identified as *Ochlerotatus confirmatus* and collected in May 1887 from Navarro (a partido in close proximity to Salado river), Buenos Aires province, Argentina (Casal *in* Belkin *et al.* 1968).

Thereafter, in his publication on the type species of North American Diptera, Coquillett (1910) designated the second species of Lynch Arribálzaga's publication as the type species for *Ochlerotatus*, also adding *Protomacleaya* Theobald, 1907 and *Pseudohowardina* Theobald, 1907 to the synonym list of the taxon, which previously included several described genera from his previous designation, including: *Culicada* Felt, 1904; *Culicelsa* Felt, 1904; *Ecculex* Felt, 1904; *Protoculex* Felt, 1904; *Pseudoculex* Dyar, 1905 (Coquillett 1906a).

#### From Culex to Aedes, genera with all-encompassing definitions

Giles (1900) was the first to synonymize Lynch Arribázaga's genera (including *Heteronycha* and *Ochlerotatus*) with *Culex*. Even though he did not give a synonymy list for this genus, his treatment of species made it clear that he believed those genera belonged within *Culex* entirely. Later Theobald (1901a; b) independently utilized the same systematic treatment but gave a full synonymy list for *Culex* along with a brief explanation for his preferred schema. But it was Blanchard (1905) who first published a reinterpretation of these taxa and a full explanation for the basis of his classification schema, which relied heavily on Theobald's, focusing mostly on palpal morphology and on the shapes of scales. Moreover, Blanchard (1905) synonymized *Ochlerotatus confirmatus* Lynch Arribálzaga, 1891 (and the treatments as *Culex confirmatus* by Giles (1900, 1902) and Theobald (1901b)) under *Culex scapularis* Rondani, 1848, and provided a redescription.

Dyar & Knab (1906b) discussed both Theobald's recent classification system and Blanchard's interpretation. Dyar & Knab (1906b) expressed concern over the validity of palpal length and scale shape as reliable characters with which to define genera. They claimed that larval characters would be of much more value for the Culicidae classification, and rearranged a large portion of it, with new generic definitions based on larval characters, in one of the first large revisions of the family.

One of Dyar & Knab's (1906b) most important contributions was their redefinition of the genus *Aedes* Meigen, 1818. Meigen (1818) published the description of *Aedes* containing a single species *Aedes cinereus*; the description is very short but one character stands out in it: '*palpi brevissimi*'. The presence of short palpi both in males and females became the mark of Meigen's definition of *Aedes*. As short palpi in both sexes is a rare character among the Culicidae, this added a lot of weight to Meigen's already fairly restrictive definition of the genus. Thus, the genus historically held very few species, many of which were later removed in favor of the formation of other genera such as *Haemagogus, Uranotaenia, Aedeomyia* and *Wyeomyia*. When Theobald published his monograph on the Culicidae of the world, he included only six species under the name *Aedes*, four of which were new species.

Dyar & Knab (1906b), by strictly utilizing larval characters for taxonomic evaluation, created broadly defined generic groupings within the subfamily Culicinae. Importantly for this work, in their classification they synonymized 13 other genera within *Aedes* (namely: *Ochlerotatus*, *Haemagogus*, *Stegomyia*, *Grabhamia*, *Howardina*, *Verrallina*, *Culicelsa*, *Culicada*, *Ecculex*, *Protoculex*, *Pseudoculex*, *Gymnometopa* and *Lepidoplatys*). Their definition of *Aedes* is currently more representative of the tribe Aedini rather than a single genus, and their action had important repercussions on the systematics of the Aedini.

#### Early subgeneric divisions of Aedes

Edwards (1917) was the first to make subgeneric divisions to *Aedes* sensu Dyar & Knab, 1906b. Based on adult female characters, with supplemental information from male genitalia structures, he broke the genus into five subgenera (*Aedes, Armigeres, Ochlerotatus, Skusea* and *Stegomyia*), further dividing *Aedes* (*Ochlerotatus*) into three groups (*Diceromyia*, *Finlaya* and *Ochlerotatus*), with the nominotypical group divided into three sections (*Aedimorphus*, *Ecculex* and *Ochlerotatus*).

Dyar (1918b) reinterpreted and complemented Edward's findings with more data from New World species. The author split *Aedes* into two informal series, the Old World series and the New World series. Dyar's (1918b) groupings were based exclusively on male genitalia structures, which he claimed to contain the most valuable characters for subgeneric divisions.

Dyar (1918b) identified six groups in the Old World series, each of which he assigned a roman numeral and then tentatively named according to Edward's subgenera, as follows: Group I – *Stegomyia* Theobald, 1901; Group II - *Skusea* Theobald, 1903; Group III - *Finlaya* Theobald, 1903; Group 3IV - *Armigeres* Theobald, 1901; Group V - *Ecculex* Felt, 1904, *Aedimorphus* Theobald, 1903 and *Diceromyia* Theobald, 1911; Group VI -*Aedes* Meigen, 1818.

Within the New World series, Dyar (1918b) applied the same system, dividing the series into five groups and tentatively naming his groups according to Edward's subgenera or, when none was available, as he found appropriate: Group I - *Howardina* Theobald, 1903; Group II - *Gualteria* Lutz, 1904; Group III - *Taeniorhynchus* Lynch Arribálzaga, 1891; Group IV -*Ochlerotatus* Lynch Arribálzaga, 1891; Group V - *Culicada* Felt, 1904. Later, Dyar (1920) formalized his groups as subgenera, making some modifications to his original analysis and leaving the divisions as follows:

*Howardina* [=New World series, Group I (Howardina)];

*Heteronycha* [=New World series, Groups IV (Ochlerotatus) and V (Culicada)];

Taeniorhynchus [=New World series, Group III (Taeniorhynchus)];

*Finlaya* [=Old World series, Group III (Finlaya) and New World series, Group II (Gualteria)];

Stegomyia [=Old World series, Group I (Stegomyia)];

Aedes [=Old World series, Group VI (Aedes)];

*Ecculex* [=Old World series, Group V (Ecculex, Aedimorphus and Diceromyia)].

Most notably for the work presented herein, he decided to change the name of his New World Series, Group IV from *Ochlerotatus* to *Heteronycha*. Furthermore, Dyar (1920) created several informal groups inside the subgenus he named *Heteronycha*, namely: Group *pullatus*, Group *serratus*, Group *curriei*, Group *punctor*, Group *scapularis*, Group *impiger*, Group *stimulans*, Group *thibaulti*, Group *trichurus*, and Group *innuitius*.

It is important to note that in Lynch Arribálzaga's (1891) description of the genus *Heteronycha* he included a single (newly described) species, *Heteronycha dolosa*, which is the type by monotypy. Later, Giles (1900) clearly treated *Heteronycha* as synonymous with *Culex* (along with other genera described by Lynch Arribálzaga (1891)), even though he does not give a formal synonymy list for *Culex*, as evidenced by his use of the combination *Culex dolosus* for Lynch Arribálzaga's species. Theobald (1901b), in his "A monograph of the Culicidae" performed a major taxonomic revision of the family, also synonymizing *Heteronycha* under *Culex*, however, Theobald synonymized the name *Heteronycha dolosa* under *Culex fatigans* Wiedemann, 1828. Currently, *Culex fatigans* is a synonym of *Culex quinquefasciatus* Say, 1823, while the name *Heteronycha dolosa* is recognized under the combination *Culex dolosus*.

Howard et al. (1917) noted the morphological discrepancies between the descriptions of the male and the female in Arribálzaga's description, observing that, although the described male was possibly a *Culex*, the female clearly belonged to *Aedes*, and so they synonymyzed *Heteronycha* with *Aedes*, creating the combination *Aedes dolosa*. Later Dyar (1919) suggested that *Aedes* (*Ochlerotatus*) *lynchii* could be a synonym of *Aedes dolosa sensu* Howard et al. (1917) and, if that were true, in his own words, he believed *Heteronycha* would take precedence over *Ochlerotatus*, hence Dyar's erroneous substitution of the name *Ochlerotatus* with *Heteronycha* in his paper on the classification of American *Aedes* (Dyar, 1920). The substitution was erroneous because the name *Ochlerotatus confirmatus* appears in an earlier page in Lynch Arribálzaga's 1891 publication of both name bearing contestants for Dyar's subgenus (namely, *Ochlerotatus*  *confirmatus* and *Heteronycha dolosa*), and, as such, takes position precedence as the name for the taxon (recommendation 69A.10 of the Code, ICZN, 2000). Afterwards Dyar (1921) seems to believe the principle of priority would restrict the name *Heteronycha* to the synonymy of *Culex*, on the basis of Theobald's treatment. If that were the case, he arguably failed to recognize that the earliest treatment of the name as *Culex* was actually by Giles. Even so, he was mistaken in his assertion, because none of the actions taken by Giles (1900), Theobald (1901b), Howard et al. (1917) or Dyar (1919, 1920, 1921) would have any effect under consideration of the Code (ICZN, 2000).

Regardless, Dyar did, in the end, reverse his actions, and reinstituted *Aedes scapularis* syn. *Ochlerotatus confirmatus* as the name bearer for his subgenus (as oposed to *Aedes dolosa sensu* Howard *et al.*, 1917), resurrected *Aedes* (*Ochlerotatus*) *lynchii* and treated the name *Heteronycha dolosa* under *Culex*, albeit giving the incorrect combination *Culex dolosa* [*sic*]; the correct combination would be *Culex dolosus*, as the name *Culex* is of masculine gender and the specific epithet is a Latin adjective (*dolosa being the feminine form of dolosus*, meaning deceitful), gender agreement applies (Article 31.2 of the Code, ICZN, 2000). For completion, it should be noted that O. H. Casal (in Belkin *et al.*, 1968) designated a Lectotype for *Heteronycha dolosa* Lynch Arribálzaga, 1891, and as the specimen is a male

*Culex*, this action finally resolved the name *Heteronycha* as a synonym of *Culex*.

In the end, the type species for Dyar's subgenus *Ochlerotatus* is *Ochlerotatus confirmatus* Lynch Arribálzaga, 1891 as the subjective synonym of *Culex scapularis* Rondani, 1848. Because of that, Dyar's Group *scapularis* (later referred to as Scapularis Group (Dyar 1922)) became a very important group as a point of reference for taxonomic changes in the subgenus, which grew significantly in the following years.

#### The evolution of the definition of Ochlerotatus scapularis

Rondani (1848), in possession of material brought by Vittore Ghiliani from from Pará, Brazil, to the Museo e Instituto di Zoologia Sistemática dell'Università di Torino (TORINO), Torino, Italy, published a study on Brazilian Diptera, in which he describes *Culex scapularis*. Rondani was an independent entomologist and he did not leave his collections to any particular museum. Many museums acquired material from Braudi & Truqui and from Rondani. Some believe he might had left his collections in the TORINO, because Ghiliani worked there. Papavero (1973) stated that the Rondani material there was lost. Belkin *et al.* (1968) stated the material is "probably in Bologna", and Arnell (1976) claimed "possibly Naples". The material from the TORINO was transferred to the Museo Regionale di Scienze Naturali, Torino, Italy, in 1978. Dr. Fulvio Giachino, the curator for the entomology section of this museum, informs that, there are only three pins with labels written "Culex" present in the collection, but no specimens or any other indications (personal communication, May 11, 2016). Dr. Luca Bartolozzi, from the Florence Natural History Museum, informed me that there is no material from Rondani housed there (personal communication, November 22, 2012). Dr. Stefano Maretti, from the Pavia Natural History Museum, informed me that only Rondani's material of Italian origin is housed there (personal communication, December 6, 2018). The natural history museum in Milano was completely destroyed during the second World War. Dr. Alessandra Sforzi, who is currently compiling a catalog of the Rondani material, kindly informed me she had visited all of the Italian museums that collected Rondani's material and there is a single pinned specimen that bears Rondani's label "Culex scapularis", in the Napoli museum (MZUN) [M. Zool. Num: 10866], however the specimen is, in her words, very moldy, and in such bad condition that the sex is not determinable (personal communication, December 7, 2018). Due to the lack of a syntype in suitable conditions for a lectotype designation, and given the polymorphic nature of the current understanding of the taxon, a taxonomic revision of the species should include a neotype designation from topotypical material.

After Blanchard (1905) synonymized *Ochlerotatus confirmatus* Lynch Arribálzaga, 1891, under the name *Culex scapularis* Rondani, 1848, Dyar & Knab (1906b) described many species from specimens identified by others as *Culex confirmatus*. Those are: *Aedes infirmatus* for specimens from Baton Rouge, Louisiana, USA; *Aedes habanicus*, from Havana, Cuba (currently a subjective synonym of *Ochlerotatus tortilis*); and *Aedes hemisurus* from near Spanish Town, Saint Catherine Parish, Jamaica (currently a subjective synonym of *Ochlerotatus scapularis*). In the case of the material from Jamaica, Dyar & Knab did not believe that the insular population could be conspecific with the one described from Argentina, thus they proposed a new name.

Later Pazos (1909) added *Aedes hemisurus* Dyar & Knab, 1906 to the synonymy list of *Aedes scapularis* (Rondani, 1848). In the following year Theobald (1910) transferred *Aedes scapularis* to the genus *Leucomyia* Theobald, 1907, which is currently a synonym of the subgenus *Culex*. Other authors kept the species in the genus *Aedes*. In fact, Howard *et al*. (1917) synonymized *Aedes indolescens* Dyar & Knab, 1907 and Dyar (1920) later synonymized *Aedes (Ochlerotatus) camposanus* Dyar, 1918a under *Aedes scapularis*.

In successive publications, Dyar frequently revised his assessment of the Scapularis Group. In his first revision of his Scapularis Group, Dyar (1922), synonymized three taxa as subspecies (thus creating *Aedes scapularis euplocamus, Aedes scapularis infirmatus* and *Aedes scapularis condolescens*. Later, he suggested the name *Pseudohowardina* Theobald, 1907 for a subgeneric treatment of the Scapularis Group (Dyar 1924). Moreover, the author decided to redescribe syn. *Aedes hemisurus* as a subspecies of *Aedes scapularis*, synonymizing *Aedes indolescens* under it, and also redescribed and elevated all three taxa from his previous publication to species again (i.e. *Aedes euplocamus, Aedes infirmatus* and *Aedes condolescens*).

Dyar later abandoned a subgeneric treatment of the Scapularis Group (in favor of maintaining it inside the subgenus *Ochlerotatus*) and also moved syn. Aedes *camposanus* from synonymy with *Aedes scapularis* to the synonym list of *Aedes euplocamus* (Dyar 1925b). He also revoked the status of subspecies from *Aedes scapularis hemisurus*, treating it only as a synonym of *Aedes scapularis* (Dyar 1928).

In the 1950's, Levi-Castillo (1951, 1952) resurrected and redescribed *Aedes* (*Ochlerotatus*) *camposanus* Dyar, 1918a. Unfortunately he did not assign a type to this name, for which a type was not originally designated, which compounds the confusion surrounding this taxon. Similarly Belkin *et al.* (1970) redescribed *Aedes* (*Ochlerotatus*) *hemisurus* Dyar & Knab, 1906b (also a typeless taxon), synonymizing *Ae. indolescens* under it, in agreement with Dyar (1924).

Arnell (1976) performed the last major revision of the Scapularis Group. In his work he synonymized *Aedes camposanus, Aedes hemisurus* (together with *Aedes indolescens*) and *Aedes (Ochlerotatus) rhyacophylus*  Costa Lima, 1933 under *Aedes* (*Ochlerotatus*) *scapularis*. Not much later, *Aedes* (*Ochlerotatus*) *rhyacophylus* was resurrected and redescribed by Sallum *et al.* (1988), complete with a Lectotype designation.

No taxonomic revision of *Ochlerotatus scapularis* or the Scapularis Group has been performed since Arnell published in 1976, and there is understandably significant confusion about the morphological characters that constitute the species and define the group, making its distribution records partly unreliable, especially for *Oc. scapularis*. It is clear that the elevation of *Ochlerotatus* Lynch Arribálzaga, 1891 to genus (Reinert 2000) and the subsequent works on the phylogeny of Aedini (Reinert *et al.* 2004, 2008, 2009) suffer from the taxonomic uncertainty surrounding the type species of the group. This can be ascertained by Reinert *et al.*'s (2008) action in their treatment of the nominotypical subgenus for *Ochlerotatus*; when faced with a lack of definition with which to assign species to this taxa, they choose to simply "define" it as the Scapularis Group *sensu* Arnell (1976) as a provisional action.

# Current problems with the definition of *Oc. scapularis* and related taxa

Below I provide detailed discussions of relevant characters used to describe taxa that are or were at some point within the definition of *Ochlerotatus scapularis*. For each of these discussions, the translations of
the original text for the relevant taxa descriptions are presented first. The translated text is between double quotes (""), with translation notes provided between square brackets ([]).

Translation of the description of *Culex scapularis* Rondani, 1848, from Latin:

"Brown. First antenomere yellow. Proboscis all dark. Ommatidia dark, irregularly some silvery white (Always? Also while alive?). Occiput white scaled. Thorax dorso-anteriorly white scaled, posteriorly pilose; short setae reddish, long setae brown. Pleura reddish brown, with white spots. Scutellum and mesopostnotum reddish brown. Halteres with pedicelum light brown, capitellum darker. Abdomen dark dorsally, with a pale stripe medially extending from second segment to last; at last segments more conspicuous and wider. All segments with triangular pale spots at each side. Sternites pale scaled. Legs dark anteriorly, yellowish-white posteriorly; tarsi dark. Wing membrane transparent, fourth and seventh dorsal [veins] equidistant to base of wing".

Translation of the description of *Ochlerotatus confirmatus* Lynch Arribálzaga, 1891, from Latin:

"Brown; occiput with silky-gray scales; Mesonotum with more than half of the anterior part covered in silky-silvery-goldish, posteriorly with brown falcate scales, with dark setae; dorsal part of the abdomen dark brown, slightly purplish, base of the segments silky-white, sternites silkygray; Antennae, palpi, knees of tibiae and apex of tarsi dark. Apex of proboscis dark, base at venter lighter. Legs pale yellow. Antennae darkbrown with apexes of antenomeres with dark setae, tori and ventral base of the first antenomere scaled. Head anteriorly [clipeus?] glabrous pitch-black, posteriorly [occiput] silky-gray slightly silvery scales, brown setae, ventrally dark. Eyes in life green, after death greyish olive. Proboscis with dark scales, apex ventrally dark. Palpi dark almost black. Thorax above antral suture [scutum] densely covered in the middle with apressed sivery-grey, slightly silky-goldish, scales, posteriorly and both sides outer edges with dark falcate scales, ateriorly apparently without setae but posteriorly densely covered with long dark setae. Scutellum with dark falcate [scales?] and brown setae. Pleurae anteriorly dark, medially and posteriorly dark and silky-grey bright silvery. Wing transparent densely covered with dark scales. Halteres pale, capitellum lightly scaled. Legs pale yellow, tarsi anteriorly slightly obfuscated; knees of posterior tibial apex and tarsi diluted dark [more pale?]. Abdominal tergites dark-brown, under the light iridescent, slightly purple at margins, crosswise with a silky-white stripe, sternites with silky-gray scales.".

It is important to note here that only the patch of pale scales on the anterior part of the mesonotum and the stripe of pale scales on the tergites overlap between the original descriptions of *Culex scapularis* Rondani and Ochlerotatus confirmatus Lynch-Arribálzaga. Other currently recognized species also present a patch of pale scales on the anterior portion of the mesonotum (Oc. tortilis, Oc. condolescens, Oc. euplocamus, Oc. patersoni, Oc. infirmatus, Oc. raymondi and Oc. rhyacophilus) and at least one other (Oc. phaenotus) also presents (in addition to the mesonotal character) a stripe of pale scales on the tergites. Yet another (*Oc. comitatus*) is unknown in the female form, but assumed by Arnell (1976) to also present both characters. Additionally, it is clear that Arnell does not attribute much importance to the abdominal stripe character, and he says that the character is indistinct and occurs "often", implying that it may be absent. With this consideration, the number of species that could fit the description increases considerably.

Translation of the redescription of *Culex scapularis* and synonymy of *Ochlerotatus confirmatus* within it, from French (Blanchard, 1905):

"Head: falcate light cream scales medially, ochre more laterally and posteriorly, spatulate laterally; scales ochre in life [?]. Eyes: purple black and silver, encircled with spatulate ochre scales. Thorax divided into 2 zones: anteriorly, falcate scales in bright yellow, silky; posteriorly and laterally, brown scales, with 4 rows of golden brown bristles. Abdomen dark brown, with a mid-dorsal line of ochre scales, thicker at the base of the segments, clearer and wider at last one. Each segment with a white laterobasal spot. ungues formula: 1.1. - 1.1. - 0.0 in the female. 2.1. - 2.1. - 1.1. in the male".

The ungues formula was used by culicidologists mostly in the early 1900's, to describe the state of character regarding the ventral projections of the unguis (*i.e.*: presence and number of "teeth") for the outer and inner unguis for the front, mid and hind legs, respectively. However, little is known about the conception of this formula, and its use (in lieu of a simple description) seems to have had little to no benefit for culicidologists, as its use was phased out from the literature around midcentury (personal communication, Thomas Zavortink, Ralph Harbach, December 11, 2018).

It should be noted that, for the females of *Ochlerotatus* sensu Reinert (2008) the inner and outer ungues of each leg are equal. It is clear Blanchard (1905) thought the female had unequal front and midungues, also that they had the hindungues both untoothed (simple), but Arnell (1976) considers the species as having all ungues toothed. Blanchard worked at the Faculté de Médecine de Paris, however, I could not locate information about a voucher collection of Culicidae deposited there; thus it was impossible for me to verify what specimens he had worked with.

Blanchard simply mentions a distribution including Chile, Buenos Aires (likely meaning Argentina, in general), Brazil, Guyana and Jamaica. I have seen specimens from Argentina, Brazil and Jamaica, including the lectotype of Oc. confirmatus and topotypical material for Oc. scapularis (from Belém, Pará, Brazil). Every specimen that I have identified as Oc. aff. scapularis presents hindungues toothed, in accordance with Arnell (1976). Arnell describes simple hindungues (untoothed) for Ochlerotatus condolescens, giving its distribution as follows: Bahamas, Cuba and Cayman Islands. I observed that the holotype of Culex bracteatus (a syn. of Ochlerotatus tortilis) also presents simple hindungues, and Arnell considers the Tortilis subgroup, which includes Oc. tortilis and Oc. auratus, to have hindungues toothed or simple. Oc. tortilis is considered to be distributed as follows: southern Florida, Bahamas, Cuba, Grand Cayman Island, Antilles; and Oc. *auratus* occurs only in Jamaica. The scutal patterning of these species is not very similar to the one from the Oc. scapularis nor syn. Culex confirmatus, however the descriptions of the shape of the patterning which were available at the time are too vague. Therefore it is possible that Blanchard may have analyzed specimens of these aforementioned species (perhaps from Jamaica), and not Oc. scapularis or Oc. confirmatus. However, I think it is much more likely Blanchard mistakenly identified a species of *Culex*, in the modern sense, as an Ochlerotatus, based on Theobald's description of Culex confirmatus (Theobald, 1901b). This is likely because Theobald

mentioned "fore and mid unguis equal, toothed" with no mention of the state of the hind unguis. This description also seems to have caused confusion to others as well, because later Theobald (1910), under his treatment of *Leucomyia scapularis* [*Oc. scapularis sensu* Arnell] mentions that Ludlow wrote in a letter to him that she had identified a similar species to *Culex confirmatus* from Georgia, USA, with the only exception in character description being that the hind unguis were toothed. In response, Theobald clarifies that he never had seen any specimen that did not have the hind unguis toothed, and that he simply had not included the character earlier.

#### Aedes hemisurus Dyar & Knab, 1906b:

This species was originally described from the larva and apparently synonymized with *Oc. scapularis* by Pazos (1909). Pazos gives both *Ochlerotatus confirmatus* and *Aedes hemisurus* as synonyms of *Aedes scapularis sensu* Pazos, and states the synonymy list is given by Dyar & Knab. However, those authors did not synonymize *Aedes hemisurus* in any of their publications. Pazos provides a text formatted as a reference right beside his mention of *Aedes hemisurus*, the text is as follows: "Pazos. Rev. Med. Trop., Habana (1908). t. J. p. 99.". I was unable to locate any publication or journal archive under this name, thus the synonymy apparent in the literature is Pazos, 1909. Pazos (1909) also provides a description, in Spanish, for the name *Aedes scapularis*. Pazos states the description is from Theobald, thus giving the impression it is only a translation. He is misleading in this, as his description is different from Theobald's.

Original English text from the redescription of *Culex confirmatus* by Theobald (1901b):

"Female. Head dark brown, clothed with pale creamy curved scales in the middle and with ochraceous ones at the sides and behind, and with upright ochraceous forked ones; sides with flat scales; eyes deep purplishblack and silver, with flat ochraceous scales round them; clypeus deep purplish-brown; antennae dark brown, basal joint and the greater part of the second joint testaceous; palpi short, black scaled; proboscis covered with shiny black scales. Thorax clothed in front with pale, silky, yellowish, narrow curved scales, which gradually become pure silky white about halfway across the mesonotum, the remaining part of the mesonotum darker, covered with scattered brown scales, as also are the sides, the posterior half of the mesonotum has four rows of golden-brown bristles; scutellum deep brown when viewed in one direction, ochraceous brown in the other, with creamy scales and a border of golden-brown bristles; in some specimens pale in the middle, dark at the sides; metanotum [corr.: mesopostnotum] chestnut-brown with a dull purplish tinge; pleurae chestnut-brown, with patches of white scales. Abdomen with the segments

covered with deep blackish-brown scales, ground colour testaceous, this colour showing through the bases of the segments to a slight extent; down the middle of the abdomen runs a line of ochraceous scales, which are thickest at the bases of the segments, and which become lighter and spread out over the whole of the last segment; in some specimens these ochraceous scales are absent; each segment has a basal lateral patch of pure white; the hairs on the posterior borders pale brown; venter covered with creamyyellow scales; in some specimens the apical borders of the venter have a triangular black patch on each side. Legs covered with deep brown scales with a bronzy ochraceous reflection in some lights; femora whitish beneath nearly to the apex, which is dark, coxae testaceous; hind meta tarsi [first tarsomere of hind leg] not guite so long as the hind tibiae; fore and mid unguis equal, toothed. Wings with the first sub marginal cell longer and narrower than the second posterior cell, its stem equal to about two thirds the length of the cell; stem of the second posterior cell nearly equal to the length of the cell; posterior cross-vein about its own length distant from the mid cross-vein; costa, first long vein and third long veins blackish; halteres pale with slightly fuscous knob. Length. 4.5 to 6 mm. Male. Antennae pale brownish ochraceous, with dark brown bands and brown plume-hairs; proboscis nearly as long as the palpi, dark brown; palpi covered with steelblack scales, last joint dark, like the rest of the palpus, hairs dark brown. Abdominal segments ochraceous at their bases, dark dusky-black on the apical half, which is covered with deep, dull, purplish-black scales, bases of the segments pale, partly owing to the ochraceous ground colour and partly to pale ochraceous scales, there are also a few basal white scales; from the fourth to the seventh segments are more or less triangular patches of white scales placed laterally and at the base of the segments; the last segment is covered with pule fuscous scales; claspers steel black ; posterior border of the segments and the sides with long golden hairs. unguis unequal on the fore and mid legs, equal on the hind legs, similar to *C. serratus*".

Translation of the redescription of *Aedes scapularis* and synonymy of *Aedes hemisurus* within it, from Spanish (Pazos 1909):

"Color: dark brown; thorax silvery white. Size: usually 4.5 to 6.0 mm of width [wingspan, probably]. Head: dark brown, with pale yellow scales medially and ocher laterally and posteriorly with some forked, erect and ocher in color; laterally flat scales; eyes dark, dark purple and silver; clipeus brown, dark purple; antennae dark brown; palpi with black scales; proboscis covered in shining scales. Thorax: covered anteriorly with narrow, falcate scales, silky, pale yellow, which gradually become all silky white up to the middle of the metanotum [corr.: mesonotum]; the rest of the metanotum [corr.: mesonotum] darker, covered in brown scales, tanned the same on the sides; the scutellum brownish with cream scales and dark golden setae; pleura dark brown with patches of white scales. Abdomen: dark brown with purple shine; with median line of ocher scales, denser [wider] at the base of the segments; sternites covered by cream yellow scales. Wings: with first submarginal cell [cell  $R_2$ ] longer and narrower than the second posterior [cell  $M_1$ ]; with the pedicel [vein  $R_{2+3}$ ] the same [size] as both margins of the cell [veins  $R_2$  and  $R_3$ ]; posterior transversal vein [ $M_{3+4}$ ] its own length distant from the median transversal vein [rm], but its location variable. Legs: covered in dark brown scales and femora whitish. unguis: Female: all equal, not toothed. Male: anterior and median unequal, the smaller not toothed; posterior equal and not toothed".

The most contentious aspects of these descriptions are 1) the lack of mention of the possibility for the absence of the median line of pale scales on the tergites by Pazos, in contrast with Theobald; 2) the possible presence of triangular patches of dark scales on the sternites was omitted by Pazos, who describes all sternites are pale scaled, and 3) the unguis of the female, which Pazos states are all not toothed, where Theobald says the front and mid ones are toothed (and also clarifies later the hind one also is toothed (Theobald 1910)); similarly Pazos states the unguis of the male are all not toothed with exception of the larger front ones, while Theobald says they are similar to *Culex serratus*. Theobald (1901b) described the unguis of *Culex serratus* as follows: "fore and mid unguis unequal, larger one with two, smaller with one tooth; hind unguis equal, each with small thick tooth and basal swelling.". In my view, these aspects of Pazos' description are irreconcilable with Theobald's.

Later, Dyar (1924) recognized the taxon as a subspecies, *Aedes* scapularis hemisurus, on the basis of two male genitalia characters: 16-20 setae on basal lobe, spine [larger seta of basal lobe] stout (=*Aedes* scapularis hemisurus); 10 setae on basal lobe, spine [larger seta of basal lobe] slender (=*Aedes* scapularis scapularis). But the author later did not recognize this subspecific status for the taxon, and slightly changed his view of the characters of the male genitalia of *Aedes* scapularis, stating: "[...] basal lobe with four or five setae and other minute ones adjacent to a large spine with swollen base" (Dyar, 1928).

Belkin *et al.* (1970) recognized specific rank to *Ae. hemisurus* and provided a redescription of all life stages. These authors emphasized that the main difference of this species from *Aedes scapularis* is the lack of a retrorse process on the claspette filament, and the presence of fewer setae on the distal part of the basal lobe; this is a departure from the previous interpretation by Dyar, *i.e.* that *Aedes scapularis* has fewer setae on the basal lobe.

Arnell (1976) synonymized the taxon again within *Aedes scapularis*, together with two more species (*Aedes camposanus* and *Aedes rhyacophilus*), in addition to his keeping of the synonyms given by earlier authors. He also gives a broader description of the male genitalia of this new concept of Ae. scapularis, such that it became indistinguishable from Aedes phaenotus, a species of his own description. Aedes phaenotus is separable from Ae. scapularis only in the female based on the color of the anterior patch of pale scales of the mesonotum, which should be yellowish tan for Aedes phaenotus and white for Aedes scapularis. This is not a usable character, for the following reasons: 1) scale bleaching is a problem for identification of stored specimens, and 2) I have observed extreme variability in the coloration of the mesonotal patch of pale scales from mosquitoes from one single collection event, including freshly collected mosquitoes where the median portion of the patch was white and the edges were golden yellow. This in itself does not preclude the possibility that one species may emerge with white scaling and the other develops the character by sun exposure whilst alive. However, it would be impossible to tell which of these phenomena has happened for any given specimen. Arnell restricted the occurrence of Aedes phaenotus to Grenada, but I have observed mosquitoes from Brazil which have yellow anterior patches of mesonotal scales, which is in accordance with previous descriptions (1976). Arnell was the first to restrict the coloration of the mesonotal anterior scale patch to white (1976).

Aedes indolescens Dyar & Knab, 1907:

This species was described from the adult female and not differentiated in the description from *Aedes scapularis*, and later synonymized with it by Howard *et al*. (1917) and with *Aedes hemisurus* by Dyar (1924), with no indication of the justification for the decision.

#### Aedes camposanus Dyar 1918b

This species is described both in the female and the male, with the main difference from other species described for the male genitalia, *i.e.* the claspette filament without a retrorse expansion.

#### Aedes rhyacophilus Costa Lima, 1933

This species was described in the adult female and male, including male genitalia, and larva, with the main differences presented for the male genitalia, the most important of which was the absence of the apical lobe of the gonocoxite. Arnell (1976) synonymized the species under *Aedes scapularis* but did not mention the possibility for lack of an apical lobe. Sallum *et al.* (1988), resurrected and redescribed the species, clearly showing various differences from Arnell's description of *Aedes scapularis*, including but not limited to: the presence of posterior scutal fossal setae and achrostical setae, and the absence of a median stripe of pale scaling on the tergites in the adult female; the lack of apical lobe of the gonocoxite in the adult male genitalia.

In conclusion, this literature review reveals major disagreements with the treatments of several characters that are understood to as fundamental for the group definition and for specific differentiation, mainly the distribution of the mesonotal setae. The review makes clear the need for the fixation of a Neotype for the species *Ochlerotatus scapularis* (Rondani, 1848), as well as redescription of the species, in order to better understand variations within the group.

The presence or absence of the occipital dorsolateral spots of dark scaling, treated as a variable feature in the entirety of the group by Arnell (1976), here is proposed to be absent in the subgenus, and present elsewhere (see Chapter 2). The achrostical setae development has been treated too broadly and not consistently observed, as indicated by the treatment of *Ochlerotatus ryacophilus* by Arnell as a synonym of *Ochlerotatus scapularis*. He describes *Oc. scapularis* as not having achrostical setae, but *Oc. rhyacophilus* has them developed in a complete row (Sallum *et al.* 1988). This feature is important and it has been misrepresented in Arnell's descriptions, which served as basis for analysis of the *Ochlerotatus* and its definition by Reinert *et al.* (2008). The dorsocentral setae, similarly, were considered to be present posteriorly and absent anteriorly for the group; however, I have found that the anterior row is not absent in every species included in the group by Arnell, revealing yet

another of his oversights. Scutal fossal setae presence and distribution has been found to be of taxonomical interest, but is only mentioned as present or absent by Arnell. Even then, many of his descriptions are in disagreement with previous descriptions of species or synonyms he presented. This is clear in the example of *Oc. rhyacophilus* and *Oc. brisolai*, both of which present posterior scutal fossal setae (Sallum et al. 1988; see Chapter 2.2), which Arnell (1976) considers absent in Oc. scapularis, but both would be within this species in his work. The white scaling patterns in the tibiae and tarsi also have been found to be different than reported for the Scapularis subgroup of Arnell. The taxonomic significance of the presence of simple claws is also to be discussed, as it is not addressed by Arnell, who synonymized Oc. bracteatus within Oc. tortilis. There are also other reasons to believe this synonymy is incorrect. The scaling patterns of the abdomen have been treated by Arnell as important for species

differentiation. However, the description of *Oc. scapularis* features all ranges of variation of said character, which not only renders the character unusable but also incorrect. Arnell overemphasizes the shape of the mesonotal pattern of scales, making various concessions to several character variations considered important in other mosquito species. I hope to clarify the variation on the abdominal scaling pattern and to discuss its relative importance within the group in Chapter 2.2.

Additionally, I have found variations in male genitalia characters, especially the shape of the apical lobe and the aedeagus, between the populations of mosquitoes identified to *Ochlerotatus scapularis* from Belém, Pará, Brazil and Santa Catarina, Brazil, as well as Chaco, Argentina.

# Chapter 2.2. Taxonomic revision of the subgenus *Ochlerotatus* Lynch Arribálzaga, 1891

The types housed at the USNM and the MACN were examined on site; all other examined material in the sections discussed is housed at the USNM; the vast majority was loaned to me for analysis, and very few were from personal collection, but will be deposited at the USNM.

Standard nomenclature for mosquito morphology is observed (Harbach & Knight 1980).

A pragmatic understanding of species delimitation is followed, based on the identification of sets of morphological traits that are consistent within and between males and females that identify a taxon.

# Subgenus Ochlerotatus, taxonomy and discussion

Subgenus Ochlerotatus Lynch Arribálzaga

Type species: *Ochlerotatus confirmatus* Lynch Arribálzaga, 1891, Navarro, Buenos Aires, Argentina, May 1887 [collection of F. Lynch Arribálzaga], female, Lectotype by O. H. Casal *in* Belkin *et al.*, (1968) [MACN].

Female: Head. Occiput with paddle shaped scales dorsolaterally, all pale, and falcate pale scales medially. Erect scales of occiput generally confined to posterior region, mostly pale, sometimes those closer to cervix darkened. Vertex scales falcate, pale, short. Tori a dark shade of yellow, with a dark brown spot medially, this spot varies in size, but usually covers around 1/3 of the tori. Tori vestiture present, micro-setae not numerous but somewhat conspicuous, both bristle and paddle shaped micro-setae present after emergence). Thorax. Paratergite (easily lost scales absent. Supspiracular scale patch present. Postspiracular scale patch present or absent. Proepisternal scaling present but not extening to lower anterior. Mesokatepisternal scaling not reaching angle. Mesepimeral scaling covering about upper half of the sclerite. Mesomeron without scaling. Metameron without scaling. Postprocoxal memebrane without scaling. Scutum. Anterior scutal fossal setae present or absent, posterior present or absent. Anterior achrostical setae absent (should not be confused with setae of the anterior promontory). Posterior achrostical setae absent. Anterior dorsocentral setae absent. Posterior dorsocentral setae present. Antealar patch of pale scaling present or absent. Supraalar patch of pale scales present. Achrostical line of scales always concolorous with anterior gap (AG). Lateral preescutelar scales always concolorous with dorsocentral line, both always pale (golden yellow to silvery white). Abdomen. Tergites mostly dark scaled but usually all with white scale banding, varied in size, at least always on last segments. Sternites mostly white, sometimes with variable dark scaling. Legs. All coxae with pale scaling, or sometimes fore coxa with dark scales anteriorly. Femora mostly pale, with dark scales basolaterally. Tibiae all dark or with thin to large stripe of pale scales anteromedially. Tarsomeres all dark or with thin stripe of pales scales anteromedially on the

first tarsomeres. Foreunguis toothed, midunguis and hindunguis often toothed, but variable. Wing. Scaling all dark.

Male: Generally similar to female except for sexual differences.

Male genitalia: Gonocoxite three to four times as long as it is wide. Basal lobe present, protruding basally and tapering distally, bearing one highly differentiated seta dorsally and many small setae facing medially (*Ochlerotatus comitatus* bearing an extra differentiated setae basally). Apical lobe present or absent, when present small, rounded, and restricted to apical 1/4 of the gonocoxite. Gonostylus around same size or 3/4 of the size of gonocoxite. Gonostylar claw around 1/5 of the size of gonostylus. Claspette filament long and bearing a crest, however variable in conformation. Aedeagus often long and pyriform, sometimes small and ovoid, however rounded at tip and presenting an invagination (apical dip).

Pupa, Larva: Not treated here (see discussion below).

Taxonomic discussion: Arnell (1976) places great importance in the form and color of the patch of pale scaling of the scutum for his internal classification of the Scapularis Group, however, he fails to provide sufficient justification for his choices, which makes it difficult to compare the characters he describes to the types and other specimens available. One such example is the difference in coloration between the mesonotal patches of pale scales of *Oc. phaenotus* and *Oc. scapularis sensu* Arnell. He stated *Oc. phaenotus* should be yellow or tan and *Oc. scapularis* silvery white, often yellow to tan on the lateral borders of the patch. However, the type specimen of Oc. confirmatus has a fully golden yellow (tan) scaled scutal patch, while Oc. brisolai has a variable coloration ranging from fully golden vellow to fully silvery white, with intermediate specimens presenting central portions of the scutal patch silvery white and the borders of the patch golden yellow, this border varying greatly in thickness. Furthermore, Oc. scapularis from the type locality (Belém, Pará, Brazil) present a fully golden yellow (tan) scaled scutal patch. It should be noted that patterns of scutal coloration within this group can be usefully subdivided; the achrostical line of the scutum, the anterior and posterior gaps between the achrostical line and the dorsocentral line, the dorsocentral line, and the area laterad of the dorsocentral line. All of those regions of coloration seem to present a unicolorous display within this group, with the variation in patterns being in how these become differentially colored and how large an area they occupy. These patterns of coloration seem to coincide with scutal chaetotaxy; the mosquitoes included in this subgenus all lack achrostical setae as well as anterior dorsocentral setae. The variability of scutal fossal setae therefore seems to be of value for identification of the species, as does the number of posterior dorsocentral setae, to a lesser extent. Male genitalia characters that also coincide with all the above discussed characters of the adult (including in the male), are the small apical lobe of the gonocoxite and the aedeagus presenting an apical dip. The apical lobe can range from absent,

to vestigial, to small; it is never as large as half the length of the gonocoxite and it often presents few setae to no setation. The larval and pupal characters for the species here presented are not discussed in this work, for the most part for lack of reliable material for most species, that is, material with associated exuviae, availabe to me for analysis. Furthermore, it has become clear that the treatment given by Arnell (1976) and Dyar & Knab (1906a, 1907) might not be entirely accurate and it probably has caused many misidentifications, given the unreliability of their usage of adult characters, I cannot vouch for the accuracy of any of the larval and pupal characters there presented without having associated specimens for my own analysis.

It should be noted that this definition is rather restrictive and currently more than half of the species previously within the subgenus are to be excluded. This may seem rather extreme, however I believe this is more of a product of the extremely generalistic description of the group as proposed by Arnell, in such a way that promoted much confusion and led to many misidentifications. This was clear in the case of the resurrection of *Oc. rhyacophilus* by Sallum *et al.* (1988) and it should be clear in the indepth discussions of each species treated herein. Furthermore, the generalistic nature of the characters previously associated with the subgenus made it impossible to provide a definition that did not overlap with other subgenera nor allowed for systematic analysis of the subgenus,

as shown in the work of Reinert *et al.* (2008). A more restrictive definition of the subgenus that provides unequivocal identification will aid in future systematic analysis and promote a better understanding of the species contained within.

### Species included in the subgenus Ochlerotatus

#### Ochlerotatus (Ochlerotatus) brisolai sp. nov.

Type material: Holotype male, Baixada do Maciambu, Palhoça, Santa Catarina, Brazil, 24 August 2013 [collection of V. F. de Freitas & R. M. França], [temporarily at personal collection, repository to be decided, VFF01]; Paratypes: 1 male, 3 females, same data as holotype [temporarily at personal collection, repository to be decided].

Female: Anterior and posterior scutal fossal setae absent. Postspiracular setae ~7. Upper mesokatepisternal setae ~9. Postspiracular patch absent. Tergites mostly dark with few pale scales mediobasally, forming very small patch. Sternites all pale. Coxa all pale scaled. Tibiae and first tarsomeres with thin stripe of pale scales. All unguis toothed.

Male: Generally similar to female except for sexual differences.

Male genitalia: (see Plate 1) Differentiated dorsal seta of the basal lobe of gonocoxite around 1.5x as long as the larger width of the gonocoxite. Apical lobe of gonocoxite abstent to vestigial. Gonostylus around 0.8 the length of the gonocoxite. Claspette filaments with smooth crest (without a retrorse process). Aedeagus somewhat long and pyriform.

Systematic discussion: Similar to *Oc. patersoni* in the female, however differentiable by the chaetotaxy of the thoracic sclerites. Readily differentiable in the male genitalia by the absence of a retrorse process in the claspette filaments.

Bionomics: Collected in the *restinga* (the Brazilian sandy coastal plains), an area of sandy soil, mostly covered with low vegetation and containing many large wetlands; this species was mostly collected in semipermanent shallow water between turfs of vegetaion in the sandy soil. Apparently a diurnal species, because adults were collected mostly during daylight. Specimens were collected biting humans and, in one case, a dog.

Distribution: Brazil: Santa Catarina: Brusque, Palhoça, Florianópolis.

Additional material examined: Brazil: Santa Catarina (n=76).

Etymology: the specific epithet is given after Dr. Carlos Brisola Marcondes; Prof. Brisola was my mentor and is a reference in Psychodidae taxonomy and bionomics, Culicidae bionomics, and Medical Entomology in Brazil. Ochlerotatus (Ochlerotatus) comitatus (Arnell)

*Aedes (Ochlerotatus) comitatus* Arnell, 1976, Bosque Ocoa, east of Villavicencio, Meta, Colombia, 23 May 1944 [collection of W. H. W. Komp], female, Holotype by Arnell (1976) [USNM, KO 200-17].

Female: Unknown, however presumably similar to male, except for sexual differences.

Male: Postpronotal setae few (2-4). Postspiracular setae few (2-4). Postspiracular patch of scales absent. Scutal fossal setae all absent. Front coxa with all pale scales.

Male genitalia: (see Plate 2) Differentiated dorsal seta of the basal lobe of gonocoxite around 1.5x as long as the larger width of the gonocoxite. Basal lobe bearing a large differentiated seta basal to the main one, this seta much thinner than the dorsal counterpart, however much longer and about twice as thick as the other seate of the basal lobe. Apical lobe of gonocoxite abstent to vestigial. Gonostylus around 3/4 the length of the gonocoxite. Claspette filaments with smooth crest (without a retrorse process). Aedeagus long and pyriform.

Systematic discussion: *Ochlerotatus comitatus* is the most unusual member of the subgenus *Ochlerotatus*, however, I do not consider its differences enough to exclude it from the subgenus, as the most important combination of characters that are readily associated between adults and male genitalia seem to be present in this species, namely the small (vestigial-like) apical lobe of the gonocoxite, the format and presentation of the aedeagus and differentiated seta of the basal lobe. However the presence of a secondary differentiated seta in the basal lobe, ventral to the normal one, albeit much thinner, is unique to this species in the entire subgenus. This feature was unique to this species in relation to all specimens for which I examined the male genitalia.

Although my description given disagrees at times with the one given by Arnell (1976) it is clear to me that his decision to differentiate this species from other members of similar looking mosquitoes (Scapularis group) was a wise one, as the differences in male genitalic features as well as some adult features of the male are discrete and unique.

Bionomics: The bionomics of this species is unknown, this is possibly related to the difficulty of it's identification, which requires male genitalic confirmation, thus it is possible that this species has been identified as a variety of different species, especially the females. Very few specimens of this species have been collected with sweep nets (Hutchings *et al.* 2016) and CDC-UV traps (Hutchings *et al.* 2016, 2018) in Brazil, only male specimens were identified to this species.

Distribution: Colombia: Meta [known from type series only] (Arnell, 1976); Brazil: Amazônia (Hutchings *et al.* 2011, 2016, 2018).

Additional material examined: Colombia: Meta: KO 200-2.

Ochlerotatus (Ochlerotatus) condolescens (Dyar & Knab)

Aedes condolescens Dyar & Knab, 1907, Nassau, New Providence, Bahamas, 24 June 1903 [collection of T. H. Coffin], female, Holotype by Dyar & Knab (1907) [USNM 10248].

Female: Postpronotal setae few (4). Postspiracular setae relatively few (4-7). Postspiracular patch of scales present. Subspiracular patch of scales very small. Anterior scutal fossal setae present, few (1-2). Posterior scutal fossal setae absent. Largest width of scutal pale patch well posterior to scutal angle. All tergites with crescent-shapped bands of pale scaling. Front coxa with all pale scales. Hind tarsi all dark, but sometimes front and mid with pale markings. Mid and hindunguis simple.

Male: Generally similar to female except for sexual differences.

Male genitalia: (see Plate 3) Differentiated seta of basal lobe about the same length as largest width of the gonocoxite. Apical lobe of gonocoxite very small. Gonostylus around 3/4 of the size of gonocoxite. Claspette filaments with retrorse process on crest. Aedeagus small and ovoid.

Systematic discussion: This species is easily distinguishable both on the basis of female and male genitalic characters. It is possibly related to *Oc. euplocamus* and *Oc. patersoni* based on the male genitalic characters here presented. Bionomics: The bionomics of this species is largely unknown. It reportedly has been collected feeding on human hosts in the Cayman Islands (Arnell, 1976).

Distribution: Bahamas: Andros, Eleuthera, Long Island, New Providence, San Salvador (Arnell, 1976); Cayman Islands: Gran Cayman, Little Cayman (Arnell, 1976); Cuba: Havana, Guantánamo, other localities in the former "Oriente" region (Arnell, 1976); USA: Florida Keys (Hribar *et al.* 2011)

Additional material examined: Bahamas: THCoffin Coll. #21, #115; Cuba: JHPazos Coll. 211, 219, 365, 385, 467, 802, 803.

### Ochlerotatus (Ochlerotatus) confirmatus Lynch Arribálzaga

Ochlerotatus confirmatus Lynch Arribálzaga, 1891, Navarro, Buenos Aires, Argentina, May 1887 [collection of F. Lynch Arribálzaga], female, Lectotype by O. H. Casal *in* Belkin *et al.* (1968) [MACN].

Female: Subspiracular patch of scales large. Postspiracular patch absent. Anterior and posterior scutal fossal setae present (~1 and ~2, respectively). Supraalar setae pale, darkened at tips. Pale scaled patch of scutum widest at scutal angle. Only last two tergites with pale scaling, this scaling consisting of a pale median stripe. All tibia and tarsi with pale markings (stripes). All unguis toothed.

Male: Generally similar to female except for sexual differences.

Male genitalia: (see Plate 4) Differentiated dorsal seta of the basal lobe of gonocoxite around 1.5x as long as the larger width of the gonocoxite. Apical lobe of gonocoxite present, small but clearly protuded, with few small ventral setae. Gonostylus usually around 3/4 the length of the gonocoxite. Claspette filaments bearing retrorse process on crest. Aedeagus long and pyriform.

Systematic discussion: This species should be easily distinguishable in the female from other similar species given the characters shown in this description, both in the female and male genitalia. All the specimens from Argentina in the USNM collection that were identified as *Oc. scapularis* were either *Oc. confirmatus, Oc. crinifer* or *Oc. rhyacophilus* (although the reader might believe this is the first record of *Oc. rhyacophilus* in Argentina, Rossi & Lestani, 2014 reported the species for the first time in Missiones).

Bionomics: The bionomics of this species is unknown. Presumably this species occurs over a large range in the southern part of the South American continent, however I cannot confirm it's presence beyond Argentina at this time. However, several of the Argentine provinces for which the occurrence of this species I can confirm are in close proximity with, and sometimes border, Paraguay, Uruguay and the southern states of Brazil; due to that I strongly believe this species may be found at those places as well. Distribution: Argentina: Buenos Aires (Lynch Arribálzaga 1891).

Material analyzed: Argentina: Buenos Aires (n=16), Iguazu (n=1), Formosa (n=2), Chaco (n=2), Salta (n=8), Tucuman (n=15).

### Ochlerotatus (Ochlerotatus) euplocamus (Dyar & Knab)

Ochlerotatus (Ochlerotatus) euplocamus (Dyar & Knab 1906a), Ciruelas, Costa Rica, 14 November 1920 [collection of A. Alfaro], male, Neotype by this work [USNM].

Female: Postspiracular scale patch absent. Anterior and posterior scutal fossal setae present (~2 and ~1, respectively). Pale scaled patch of scutum widest posterior to scutal angle. Tergites mostly dark, sometimes with small patch of pale scales, more proeminent at first tergites. Sternites mostly pale, some with medial dark scales present. Coxa with dark scaling anteriorly. Tibiae and tarsi all dark. All unguis toothed.

Male: Generally similar to female except for sexual differences.

Male genitalia: (see Plate 5) Basal lobe small. Differentiated dorsal seta of the basal lobe of gonocoxite almost 1.5x as long as the larger width of the gonocoxite. Apical lobe of gonocoxite absent. Gonostylus usually around 0.8 or more the length of the gonocoxite. Claspette filaments bearing retrorse process on crest. Aedeagus long and pyriform.

Systematic discussion: This Neotype designation is necessary to fix the subgeneric definition of the *Ochlerotatus* beyond reasonable doubt, given several characteristics of the group that were misinterpreted by Arnell and later authors. Beyond that, Arnell states the male genitalia is indistinguishable from many other species in the group, which I find not to be the case. Furthermore, Syntypes are not availabe for Lectotype designation, according to Stone & Knight (1956), which I also confirmed in my visit to the USNM. It is unfortunate that the closest (in locality) specimens I could observe were from Ciruelas, Costa Rica, which is about 230km from Zent, Costa Rica, the original type locality. The two localities are low elevation near coastal areas but with slightly different vegetation types (*bosque seco* for Ciruelas and *bosque lluvoso* for Zent) but with low elevation passages between the mountain range of Costa Rica, which makes it plausible for populations of mosquitoes from the two regions to be connected even if they are restricted to low elevation, which might not necessarily be the case.

Bionomics: I consider the bionomics of this species to be largely unknown due to the previous difficulty of identification imposed by Arnell's description of the species, as well as confusion with other species by prior authors, such as Dyar (1922, 1925b). It seems to be distributed through lowland in Central America and northern South America, apparently occurring at high elevations occasionally (one collection in San José, Costa Rica); it is however difficult to judge collection effort from historical collections. Females were collected in a forest in the small town of Xmtkuil, near Mérida city, Yucatán, Mexico (Baak-Baak *et al.* 2016), as well as in Nuevo Becar, La Unión and Ejido Lázaro Cárdenas, in Quintana Roo, Mexico (Ortega-Morales *et al.* 2010); both states are part of the southeastern tip of Mexico. It is possible the species would be found in Belize in the future, as well as Cuba.

Distribution: Costa Rica: Ciruelas, El Coco, San José. Turrúcares; Colombia: Bogota; El Salvador; Honduras: Tocoa, Tela; Mexico: Oaxaca, Veracruz; Venezuela: locality unspecified (Arnell 1976), Yucatán (Baak-Baak *et al.* 2016), Quintana Roo (Ortega Morales *et al.* 2010); Panama: Bocas del Toro, Canal Zone (Arnell 1976; Blanton & Peyton 1958); Nicaragua (Woke 1947).

Material analyzed: Costa Rica: Ciruelas (n=8), El Coco, San José (CR 6), Turrúcares (CR 308-10, CR 308-105, CR 309-100, CR 309-103); Colombia: Bogota (n=4); El Salvador (n=14); Honduras: Tocoa (n=3), Tela (n=7).

# Ochlerotatus (Ochlerotatus) hemisurus (Dyar & Knab)

Aedes hemisurus Dyar & Knab, 1906, Kingston and St. Andrews, Ferry, about 1 mi along Red Hills Road from Spanish Town Road, Jamaica, 8 August 1967, [USNM JA 896], Neotype by this work. Aedes indolescens Dyar & Knab, 1907, [new synonymy] Cayamas, Cuba, date unspecified [E. A. Schwarz], female, Lectotype by Stone & Knight, 1956 [USNM, 10249]

Female: Postpronotal setae many (~7). Postspiracular setae many (12-14). Subspiracular patch of scales large. Postspiracular patch of scales absent. Anterior and posterior scutal fossal setae present (~2 each). Posterior dorsocentral setae few (~3). Pale scaled patch of scutum widest at scutal angle. All tergites with basal median pale scaling, this spot not connecting to basolateral pale scale spots, and extending posteriorly on last three segments (almost forming a continuous stripe). Sternites mostly pale, with visible dark scale spots apicolaterally. All coxae pale scaled. Tibiae with stripe of pale scales anteromedially, this stripe very large at hind leg. All first tarsi with conspicuous pale markings. All unguis toothed.

Male: Generally similar to female except for sexual differences.

Male genitalia: (see Plate 6) Differentiated dorsal seta of the basal lobe of gonocoxite only about as long as the larger width of the gonocoxite. Apical lobe of gonocoxite small. Gonostylus usually around 3/4 the length of the gonocoxite. Claspette filaments smooth on crest (not bearing retrorse process). Aedeagus somewhat long and pyriform.

Systematic discussion: Here *Oc. hemisurus* (Dyar & Knab 1906b) is resurrected from synonymy with *Oc. scapularis*, previously made by Arnell (1976). Moreover, in accordance with Belkin *et al.* (1970), I also independently found *Oc. indolescens* to be a synonym of *Oc. hemisurus*, removing it from the synonymy with *Oc. scapularis* made by Arnell (1976), following Howard *et al.* (1917). It should be noted both Belkin *et al.* (1970) and Arnell (1976) mistakenly report Kingston, Jamaica as the type locality for *Oc. hemisurus*, however the correct type locality is the Rio Cobre Canal Dam, near Spanish Town, Jamaica, as stated by Grabham (1905). The neotype was necessary as no type was fixed at publication and no syntype collection is known to exist. Moreover, the resurrection from *Oc. scapularis* and comparison to other taxa required a type to be affixed, to avoid confusion. Thankfully I was able to find USNM specimens from near the type locality. The female is closer to *Oc. confirmatus* than to *Oc. scapularis*, but it can be differentiated from the former by the tergite scaling pattern, the apicolateral dark spots at the sternites, the number of postspiracular setae, as well as the all pale scaling of the front coxae.

Bionomics: Largely unknown. It is potentially one of the most widespread mosquitoes of this subgenus in Jamaica and Cuba, and it has been observed to bite humans frequently.

Distribution: Cuba: Cayamas; Jamaica: Rio Cobre, Kingston, St. Andrews.

Material analyzed: Cuba: Cayamas (n=18); Jamaica: Kingston, St. Andrews (n=75). Ochlerotatus (Ochlerotatus) infirmatus (Dyar & Knab, 1906) Baton Rouge, Louisiana, United States of America, 7 June 1941 [collection of W. W. Wirth], female, Neotype by this work [USNM VFF Inf].

Female: Anterior and posterior scutal fossal setae present (often 1 at each position). Postspiracular patch of scales absent. Pale patch of scutal scales not significantly extending laterally of dorsocentral line, appearing more or less as a wide stripe anteriorly. Abdominal tergites all dark, except for basolateral patch. Sternites all pale. Tibiae and tarsi all dark.

Male: Generally similar to female except for sexual differences.

Male genitalia: (see Plate 7) Basal lobe relatively small. Differentiated dorsal seta of the basal lobe of gonocoxite about as long as the larger width of the gonocoxite. Apical lobe of gonocoxite small but relatively well developed. Gonostylus usually around 3/4 the length of the gonocoxite. Claspette filaments bearing retrorse process. Aedeagus somewhat long and pyriform.

Systematic discussion: This species is more or less easily differentiable in the female from other members of the group by the presence of both anterior and posterior scutal fossals and the absence of postspiracular scales and pale banding on the tergites. Bionomics: The species has been reported mostly throughout the southeast coast of the United States, but there are few reports as far inland as Illinois.

Distribution: USA: Louisiana (Dyar & Knab 1906), Florida Keys (Hribar *et al.*, 2011), Arkansas, Delaware, Mississippi, Missouri, North Carolina, South Carolina, Virginia, Illinois, Texas (Arnell 1976).

Material analyzed: USA: Louisiana: New Orleans (n=20), La Fourcha (n=1), Ibarville (n=2), Alexandria (n=1), Baton Rouge (n=4).

Ochlerotatus (Ochlerotatus) patersoni (Shannon & Del Ponte)

*Aedes (Ochlerotatus) patersoni* Shannon & Del Ponte, 1928, San Pedro de Jujuy, Jujuy, Argentina, 27 April 1926 [Paterson, Shannon & Shannon], female, Lectotype by O. H. Casal *in* Belkin *et al.* (1968) [INM].

Female: Anterior and posterior scutal fossal setae absent. Postspiracular setae ~4. Upper mesokatepisternal setae ~5. Postspiracular patch of pale scales absent. Tergites mostly dark with few pale scales mediobasally, forming very small patch. Sternites all pale. Coxa all pale scaled. Tibiae and first tarsomeres with thin stripe of pale scales. All unguis toothed.

Male: Generally similar to female except for sexual differences.

Male genitalia: (see Plate 8) Basal lobe well protuded. Differentiated dorsal seta of the basal lobe of gonocoxite almost 1.5x as long as the larger

width of the gonocoxite. Apical lobe of gonocoxite absent. Gonostylus usually around 0.8 or more the length of the gonocoxite. Claspette filaments bearing retrorse process on crest. Aedeagus long and pyriform.

Systematic discussion: It is clear that this species has been misidentified as *Oc. scapularis* by many authors, including Arnell (1976) whose collection present in the USNM contains many specimens of established species *Oc. crinifer*, *Oc. phaenotus* and *Oc. patersoni* identified as *Oc. scapularis*, not including synonymous species at the time. This coupled with the fact this species seems to be quite widespread underlines the importance of this work in aiding improved identification for a better understanding of the correct distribution of the species.

Bionomics: It is clear this species is a lot more widespread than previously thought. It was recovered in Brazil from ephemeral pools of rain water in grass, as well as semi-permanent pools in sandy soil, associated with members of *Psorophora*, which agrees with the records of collection from Argentina (Arnell, 1976).

Distribution: Argentina: Jujuy; Bolivia: Muyapampa; Brazil: Santa Catarina.

Material analyzed: Argentina: Jujuy: San Pedro de Jujuy: (n=10) ARG 755 (n=41) ARG 742 (n=7); Bolivia: Muyapampa (n=4); Brazil: Santa Catarina: Palhoça (n=15).
#### Ochlerotatus (Ochlerotatus) phaenotus (Arnell)

Type material: *Aedes* (*Ochlerotatus*) *phaenotus* Arnell, 1976, Chantimelle Village, near Sauteurs, Saint Patrick Parish, Grenada, 30 October 1963 [collection of R. Martinez], female, Holotype by Arnell (1976) [USNM, GR 104-102].

Female: Postpronotal setae usually 5. Postspiracular setae around 6-7. Subspiracular scale patch long, very thin. Postspiracular scale patch absent. Anterior and posterior scutal fossal seate present (~1 and ~2, respectively). Posterior dorsocentral setae usually 4-5. Pale scaled patch of scutum widest posterior to scutal angle. All tergites with pale scaling basomedially, not connected to basolateral patch, this scaling extending posteriorly at last two segments, almost forming a median stripe. All coxae with pale scaling only. Tibiae mostly pale scaled, dark scaling reduced to dorsal stripe. First tarsomeres mostly pale, with dark on dorsum, hind one in particular with more dark at apex. All unguis toothed.

Male: Generally similar to female except for sexual differences.

Male genitalia: (see Plate 9) Basal lobe small. Differentiated dorsal seta of the basal lobe of gonocoxite about as long as the larger width of the gonocoxite. Apical lobe of gonocoxite small, sometimes inconspicuous. Gonostylus usually around 3/4 the length of the gonocoxite. Claspette filaments smooth on crest (not bearing retrorse process). Aedeagus somewhat long and pyriform. Systematic discussion: This species is easily distinguishable from other members of the group by both female and male genitalic characters.

Bionomics: Unknown.

Distribution: Grenada: Saint Patrick Parish; Saint Andrew Parsih [only known from type series].

Material analyzed: Grenada: locality unknown (n=28), Chantimelle Village, St. Patrick Parish (n=2); Belair School, St. Andrew Parish (n=1).

#### Ochlerotatus (Ochlerotatus) scapularis (Rondani)

Type material: *Ochlerotatus (Ochlerotatus) scapularis* (Rondani 1848), APEG Forest, Belém, Pará, Brazil, 24 September 1970 [collection of T. H .C Aitken & Toda], male, Neotype by this work [USNM SCAP03].

Female: Subspiracular patch of scales small. Postspiracular patch absent. Anterior scutal fossal setae present (1-2). Posterior scutal fossal setae absent. Supraalar setae all pale. Pale scaled patch of scutum widest posterior to scutal angle. Only last two tergites with pale scaling, this scaling consisting of a pale median stripe. All tibia and tarsi with pale markings (stripes). All unguis toothed.

Male: Generally similar to female except for sexual differences.

Male genitalia: (see Plate 10) Differentiated dorsal seta of the basal lobe of gonocoxite around the same length as the larger width of the gonocoxite. Apical lobe of gonocoxite present, small but clearly protruded, with few small ventral setae. Gonostylus at least 0.8 the length of the gonocoxite, usually around the same length. Claspette filaments with smooth crest (without a retrorse process). Aedeagus long and pyriform.

Systematic discussion: This species should be considered carefully, however the combination of either the female or male characters should be enough to separate it from any other species of the group.

Bionomics: This is the most difficult species to discuss, as it has been misidentified and used as a catch-all taxon. Although I cannot be sure this species does not occur in Argentina nor in the USA, the material available to me, identified to 'Oc. scapularis', from those places contains no specimens that agree with the description here given, but with many other species, both in and out of the group; it is my experience that even members of the genus Howardina have been misidentified as 'Oc. scapularis'. Southern North America and Central America contain a variety of possibly endemic species from this group, as well as other groups, and it is difficult for me to evaluate all the literature records of this species, however from the collection records available to me it becomes increasingly difficult to believe Oc. scapularis, as described here, occurs west of the Andes, including all of the continental Central America and most Caribbean islands, excluding Cuba, for which I identified some specimens, and perhaps islands near the coast of South America, such as Trinidad & Tobago and Grenada.

Distribution: Brazil: Belém; Cuba: Marianao. Possibly distributed all over the Amazon basin, east of the Andes.

Material analyzed: Brazil: Belém (n=13); Cuba: Marianao (n=7).

# Species not included within the subgenus *Ochlerotatus*, moved to subgenus uncertain

Ochlerotatus angustivittatus (Dyar & Knab)

Type material: *Aedes angustivittatus* Dyar & Knab, 1907, Port Limon, Costa Rica, date unspecified, female, Holotype by Dyar & Knab (1907) [USNM, 10140];

Aedes cuneatus Dyar & Knab, 1908 [synonym], Cordoba, Vera Cruz, Mexico, date unspecified, female, Lectotype by Stone & Knight (1956) [USNM, 11964];

Aedes argentescens Dyar & Knab, 1908 [synonym], Cordoba, Vera Cruz, Mexico, date unspecified, male, Lectotype by Stone & Knight (1956) [USNM, 11965]

Aedes traversus Dyar, 1925a [synonym], Zulia River, Zulia, Venezuela, date unspecified [collection of L. H. Dunn], female, Lectotype by Stone & Knight (1956) [USNM, 28480].

Systematic discussion: On the female characters, the dorsocentral line is pale scaled, while the gap and achrostical line are dark scaled; furthermore, the male has the basal lobe undeveloped (unprotuded), the aedeagus without apical evagination (dip), and a gonostylus around about 1/2 the size of the gonocoxite. All of those characters fall outside of the current understanding of the subgenus *Ochlerotatus*, as described in this revision.

## Ochlerotatus atactavittatus (Arnell)

Type material: *Aedes (Ochlerotatus) atactavittatus* Arnell, 1976, eastern outskirts of Lerma, Campeche, Mexico, 5 August 1970, female, Holotype by Arnell (1976) [USNM, MEX 610-16].

Systematic discussion: This species is to be removed from the subgenus *Ochlerotatus* on the basis of similar female characters as previosly discussed for *Oc. angustivittatus*, that is, the dorsocentral line is pale scaled, while the gap and achrostical line are dark scaled; as well as on the basis of their unique male genitalic characters, the undeveloped (unprotruded) basal lobe, coupled with the large apical lobe, extending more than half the gonocoxite and bearing many long setae.

## Ochlerotatus auratus (Grabham)

Type material: *Aedes auratus* Grabham, 1906, Kingston, Jamaica, 10 July 1906, male, Lectotype by Belkin *et al.* (1970) [USNM, 680827-15]. Systematic discussion: *Ochlerotatus auratus* is hereby removed from the subgenus Ochlerotatus on the basis of male genitalic characters: 1) the development of the apical lobe into a large bulbous protrusion extending from the mid-length of the gonocoxite. 2) the development of the basal lobe into a detached structure, expanding medially at a ~45° angle and, at around 1/4 of the base of the gonocoxite, presenting a flat surface, almost at

a 90° angle with the gonocoxite; this surface covered by long thin setae, but no highly differentiated seta is present. 3) the presentation of the aedeagus, that tapers to a point distally and presents no visible dip.

# Ochlerotatus bogotanus (Arnell)

Type material: *Aedes* (*Ochlerotatus*) *bogotanus* Arnell, 1976, Ogamora, near Soacha, Cundinamarca, Colombia, 28 November 1965, female, Holotype by Arnell (1976) [USNM, COB 96-11].

Systematic discussion: This species presents many characters that conflict with the definition of the subgenus *Ochlerotatus*, in the female: The sternites with a contiguous stripe of dark scales from II to VI, otherwise pale. All paddle shapped scales immediatelly laterally of the medial line of falcate pale scales, dark; the line quite thick, but laterally all paddle shapped scales pale. The erect scales of the occiput dark medially, pale laterally. Dorsocentral line not concolorous with achrostical line. Anterior dorsocentral setae present, few. In the male genitalia: Aedeagus with no dip. Apical lobe very large, starting from just near the mid range of the gonocoxite.

#### Ochlerotatus camposanus (Dyar)

Aedes (Ochlerotatus) camposanus Dyar, 1918, Guayaquil, Ecuador, date unspecified [collection of Prof. F. Campos R.], male, Lectotype by Stone & Knight (1956) [USNM, 22916].

Systematic discussion: I believe this species should be taken out of synonymy with *Oc. scapularis*, on the basis of the male genitalia, which does not present a developed basal lobe, as well as a small aedeagus without an apical emargination, both of which also take this species out of the subgenus. Furthermore this species has tarsi speckled with white scales, differing from the subgenus *Ochlerotatus*. See Levi-Castillo (1952).

## Ochlerotatus crinifer (Theobald)

*Culex crinifer* Theobald, 1903, São Paulo, Brazil, date unspecified [collection of A. Lutz], female, Lectotype by Belkin (1968) [NHM].

*Culex lynchii* Brèthes, 1911 [synonym], Buenos Aires, Argentina, 19 January 1903 [collection of J. Brèthes], female, Lectotype by O. H. Casal *in* Belkin *et al.* (1968) [MACN] *Culex tapinops* Brèthes, 1917 [synonym], San Isidro, Buenos Aires, Argentina, 2 February 1917 [collection of J. Brèthes], male, Lectotype by O. H. Casal *in* Belkin *et al.* (1968) [MACN].

Aedes (Ochlerotatus) iguazu Shannon & DelPonte, in Dyar, 1928 [synonym], Missiones, Argentina, no type affixed.

Aedes synchytus Arnell, 1976 [new synonym], Iguazu, Misiones, Argentina, 4-10 October 1927 [collection of R. C. Shannon & E. M. Shannon], female, Holotype by Arnell (1976) [USNM].

Systematic discussion: I believe the description given by Arnell (1976) for *Ae. synchytus* is very clear on the difficulty to differentiate those species, giving a single character in the female, the coloration of the scutal scaling, and stating the male genitalia is indistinguishable. Zavortink (1991) talks extensively on how scale coloration might not be a viable character for taxonomy in mosquitoes and Dyar (1918b) argues extensively about the usefulness of male genitalic characters. Not only that, but the coloration and patterning of scales in the scutum of *Oc. crinifer* has been known to be variable for Brazilian fauna for a long time (Antunes & Lane 1934), and it is very possible this applies for the entire range of this species, including Argentina. This species is hereby removed from the subgenus *Ochlerotatus* by the presence of achrostical setae as well as anterior dorsocentrals in the females, as well as the absence of the apical invagination on the aedeagus and the great development of the apical lobe in the male genitalia.

#### Ochlerotatus deficiens (Arnell)

Aedes (Ochlerotatus) deficiens Arnell, 1976, Hacienda "Conjera", 3 km from Suba, Cundinamarca, Colombia, 7 November 1965 [collection of Morales, Ochoa & Pardo], female, Holotype by Arnell (1976) [USNM, COB 92-10].

Systematic discussion: This species is not known in the male, the female characters however are enough to remove this species from consideration with the subgenus: dorsocentral line not concolorous with achrostical line. Anterior dorsocentral setae present, few. It is clear this species in need of a revision, as it is possibily conspecific with *Oc. bogotanus*.

## Ochlerotatus incomptus (Arnell)

*Aedes* (*Ochlerotatus*) *incomptus* Arnell, 1976, 6.5 km West of summit of Cerro Mali, Serrania del Darien (approximately 29 km Northeast of Pucro), Darien, Panama, 8 June 1963 [collection of A. Quinonez], female, Holotype by Arnell (1976) [USNM, PA 379-110].

Systematic discussion: This species is removed from the subgenus on the basis of the following characters: Scutum with all bronzy brown scales. Tergites all dark. Dark spots of paddle shapped scales above eyes. Male genitalia: Gonostylus only about 0.6x the size of gonocoxite. Apical lobe large, starting from mid range of gonocoxite. Insertion of differentiated seta of basal lobe distally in comparison to members of the subgenus *Ochlerotatus*.

# Ochlerotatus obturbator (Dyar & Knab)

Aedes obturbator Dyar & Knab, 1907, Tarpon Bay, Eleuthera, Bahamas, 7 July 1903 [collection of T. H. Coffin], female, Holotype by Dyar & Knab (1907) [USNM, 10141].

Systematic discussion: This species should not belong to the subgenus *Ochlerotatus*, on the basis of both female and male characters. The female presents the achrostical line not concolorous with the dorsocentral, also they present achrostical setae. As for the male, the conformation of the basal lobe as a flat surface anteriorly is very dvergent from any of the members of the *Ochlerotatus* subgenus, not only that but the aedeagus also does not present an apical invagination.

## Ochlerotatus pectinatus (Arnell)

*Aedes (Ochlerotatus) pectinatus* Arnell, 1976, Rio Cuja, near Fusagasuga, Cundinamarca, Colombia, 29 October 1964 [collection of E. Osorno *et al.*], female, Holotype by Arnell (1976) [USNM, COB 1-10 to 1-13].

Systematic discussion: The female has tan falcate scales extending from medial line to lateral area of the occiput; all dark erect scales extending far to the front of occiput; all scutal scales concolorous, bronzy; achrostical setae present, in the front few ( $\sim$ 2), posteriorly very developed, many ( $\sim$ 6).

## Ochlerotatus rhyacophilus (Costa Lima)

Aedes (Ochlerotatus) rhyacophilus Costa Lima, 1933, Coqueto Farm, "5 de novembro" river, Canaan Valley, Espírito Santo, Brazil, 1932 [collection of J. Serafim], female, Lectotype by Sallum *et al.* (1988) [IOC].

Systematic discussion: See Sallum *et al.* (1988). This species presents achrostical setae, as well as anterior dorsocentrals in the female, and an undeveloped basal lobe in the male genitalia.

# Ochlerotatus thelcter (Dyar)

Aedes (Taeniorhynchus ?) thelcter Dyar, 1918, Brownsville, Texas, United States of America, 29 August 1916 [collection of M. M. High], female, Holotype by Dyar (1918) [USNM, 21728].

Aedes (Ochlerotatus) keyensis Buren, 1947, Key West, Florida, United States of America, 15 October 1946 [collection of E. Fernandez], female, Holotype by Buren (1947) [LU] (See systematic discussion).

Systematic discussion: *Ochlerotatus thelcter* is very aberrant in comparison to many of the other species treated here (with the exception of *Oc. crinifer*); however, taking into consideration the major characters I

interpret to be key to the subgenus, such as the setation of the scutum, as well as several of the characteristics of male genitalia, I consider it may be one of the most closely related species to the subgenus *Ochlerotatus*. It is removed from the subgenus on the basis of male genitalic characters: the presentation of the gonocoxite, the small differentiated setae of the basal lobe, the development of the basal lobe (somewhat triangular).

#### Ochlerotatus (Ochlerotatus) tortilis (Theobald)

*Culex tortilis* Theobald, 1903b, Kingston, Jamaica, 20 August 1903 [collection of M. Grabham], female, Lectotype by Belkin (1968) [NHM].

*Culex bracteatus* Coquillett, 1906 [synonym], Havana, Cuba, 1 November 1902 [collection of J. R. Taylor], female, Holotype by Coquillett (1906) [USNM, 7753]

Aedes habanicus Dyar & Knab, 1906 [synonym], Havana, Cuba, 28 October 1903 [collection of J. R. Taylor], larval fragments, Lectotype by Stone & Knight (1956) [USNM] (See discussion)

Aedes balteatus Dyar & Knab, 1907 [synonym], Santo Domingo, Dominican Republic, August 1905 [collection of A. Busck], female, Holotype by Dyar & Knab (1907) [USNM, 10142]

Aedes plutocraticus Dyar & Knab, 1907 [synonym], Nassau, Bahamas, 21 June 1903 [collection of T. H. Coffin], female, Holotype by Dyar & Knab (1907) [USNM, 10251] Aedes tortilis virginensis Dyar, 1922 [synonym], Saint Thomas, United States Virgin Islands, August 1905 [collection of A. Busck], female, Holotype by Dyar (1922) [USNM, 24898] (recommendation for further research and future removal from synonymy, after review of species, see discussion)

Systematic discussion: The description given by Arnell (1976) does not fully represent the specimens I've observed from the type locality, their scutal scaling is always fully uniform, tan brown; the tergites all dark and with dark scaling laterally at occiput. These characters of the female already disagree with my view of the subgenus Ochlerotatus, however, more confusion exists within this species, as other specimens closely resemble the types of Oc. plutocraticus (Bahamas), Oc. balteatus (St. Domingo) and Oc. bracteatus (Cuba). The type of Aedes habanicus is not analyzable, time has rendered the mount of larval fragments, already difficult to view, completely unviewable; and the type of *Aedes tortilis virginensis* seems to belong to a different species. It is sufficient to say in the specimens from the Bahamas, St. Domingo and Cuba that the pale scaling present in the scutum is not contiguous from the achrostical line to the dorsocentral, and in the Bahamas specimens as well as the St. Domingo ones the erect scales of the occiput are dark laterally and there exists a dark spot of scaling above the eyes. The specimens from the Bahamas also present simple claws. The combination of all those characters make me believe this group of potentially multiple species should not be considered to be within the subgenus *Ochlerotatus*, and furthermore is in dire need of revision.

## Ochlerotatus trivittatus (Coquillett)

*Culex trivittatus* Coquillett, 1902, Chester, New Jersey, United States of America, 14 September year unspecified, female, Holotype by Coquillett, 1902 [USNM 6702];

*Culex inconspicuus* Grossbeck, 1904 [synonym], Garret Mountain, Paterson, New Jersey, United States of America, 5 October year unspecified, male, Lectotype by Stone & Knight (1956) [USNM GR159].

Systematic discussion: This species shares the same set of characters used to remove *Oc. angustivittatus* from the subgenus.

## Incertae sedis

#### Ochlerotatus meprai (Martínez & Prosen)

*Aedes (Ochlerotatus) meprai* Martínez & Prosen, 1953, Reserva Nacional "Finca El Rey", Departamento de Anta, Salta, Argentina, November 1952, female, Holotype by Martínez & Prosen (1953) [LU] (See systematic discussion).

Systematic discussion: Martínez & Prosen (1953) in the description of the species indicate the existence of paratypes at the institute "Misión de Estudios de Patologia Regional Argentina" of the Universidad Nacional de Buenos Aires, but the institute does not exist anymore; it was closed in 1958 but there is no mention in the literature or other media of the fate of their material; the government of Argentina claims all the institute's material was lost<sup>1</sup>. Even though I have not seen the type, if descriptions are correct, this species bears resemblance to both *Oc. angustivittatus* and *Oc. trivittatus*, and should be considered out of the subgenus for the same reasons.

## Ochlerotatus raymondi (Del Ponte et al.)

Aedes (Ochlerotatus) raymondi Del Ponte et al., 1951, San Pedro, Jujuy, Argentina, 27 April 1926 [collection of Shannon & Shannon], female, Holotype by Del Ponte et al. (1951) [INM].

Systematic discussion: This species was described from two specimens from the type series of *Oc. patersoni*, and bear resemblance to it with the exception of a dark achrostical line of scales at the scutum, according to Arnell (1976). Unfortunatelly this species is only known from it's type series and I could not observe any specimens. I feel this species needs a revision before anyone can assign a subgeneric designation to it, especially if the descriptions given of it are correct, which suggest that the species may be similar to *Oc. crinifer* and other allied species.

<sup>1</sup> http://www.gob.gba.gov.ar/legislacion/legislacion/f-12527.html

## Key to species of the subgenus Ochlerotatus

# **Key to females:**

The female of Oc. comitatus is unknown.

- Not as above.....other subgenera and subgenus uncertain

- Postspiracular patch of scales absent. All unguis toothed......3

3. Abdominal tergites all dark, except for basolateral patch. Sternites all pale. Coxae all pale scaled. Tibiae and tarsi all dark......Oc. infirmatus

- Characters variable, not as above......5

5 - Posterior scutal fossal setae present......6

- Posterior scutal fossal setae absent......8

6 - Tibiae mostly dark scaled, with thin stripe of pale scales. Oc. confirmatus

- Tibiae mostly pale scaled, dark scaling reduced to dorsal stripe.....7

7. Postpronotal setae many (around 7). Postspiracular setae many (around 12-14). Scutal pale patch widest at scutal angle......Oc. hemisurus

- Postpronotal setae few (around 5). Postspiracular setae few (around 6-7). Scutal pale patch widest posterior to scutal angle......Oc. phaenotus

8 - Anterior scutal fossal setae present. Only last two tergites with pale scaling, this scaling consisting of a pale median stripe......Oc. scapularis

- Anterior scutal fossal setae absent. Tergites mostly dark with few pale scales mediobasally, forming very small patch......9

# Key to male genitalia:

1. Aedeagus with dip at apex; apical lobe of gonocoxite absent to small, never expanding half the length of the gonocoxite; basal lobe of gonocoxite expanding medially at base and tapering distally; differentiated seta of basal lobe 1 to 1.5x the width of the gonocoxite.....subgenus *Ochlerotatus* – 2

- Not as above.....other subgenera and subgenus uncertain

2. Claspette filaments with retrorse process	3
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- Claspette filaments without retrorse process......7

3. Apical lobe of gonocoxite small, but visibily developed......4

- Apical lobe of gonocoxite abstent......6

4. Basal lobe relatevely small. Differentiated dorsal seta of the basal lobe of gonocoxite about as long as the larger width of the gonocoxite. Aedeagus somewhat long and pyriform......Oc. infirmatus

- Basal lobe normally developed. Other characters variable but not in the combination above......5

5. Differentiated seta of basal lobe about the same length as largest width of the gonocoxite. Aedeagus small and ovoid......Oc. condolescens

- Differentiated dorsal seta of the basal lobe of gonocoxite around 1.5x as long as the larger width of the gonocoxite. Aedeagus long and pyriform...... .....Oc. confirmatus

6. Basal lobe small.....Oc. euplocamus

- Basal lobe well protuded......Oc. patersoni

7. Basal lobe bearing a large differentiated seta basal to the main one, this seta much thinner than the dorsal counterpart, however much longer and about twice as thick as the other seate of the basal lobe......Oc. comitatus

8. Differentiated dorsal seta of the basal lobe of gonocoxite around 1.5x as long as the larger width of the gonocoxite. Apical lobe of gonocoxite abstent to vestigial. Gonostylus around 0.8 the length of the gonocoxite. *Oc. brisolai* 

9. Apical lobe of gonocoxite present, small but clearly protuded, with few small ventral setae. Gonostylus at least 0.8 the length of the gonocoxite, usually around the same length. Aedeagus long and pyriform..*Oc. scapularis* 

10. Basal lobe small.....Oc. phaenotus

- Basal lobe normally developed......Oc. hemisurus

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Appendix A1. The transmission of the names within Ochlevatus Dynch Artiklaaga, 1891; the orignal combinations Actes infirmatus Dync & Knab,, 1906; Actes condotecents Dync & Knab,, 1906; Actes condotecents Dync & Knab,, 1906; Actes (Ochlevatus) Compounds Dync & Knab,, 1906; Actes (Ochlevatus) Componing Dync & Knab,, 1906; Actes (Ochlevatus) Componing Compounds Dync & Knab,, 1906; Actes (Ochlevatus) Componing Compounds Dync & Knab,, 1906; Actes (Ochlevatus) Componing Compounds Dync & Knab,, 1906; Actes (Ochlevatus) Componing Componing Compounds, 1881; Ochlevatus Dync & Knab,, 1906; Actes (Ochlevatus) Compounds, Dync & Knab,, 1906; Actes (Ochlevatus) Compounds, Compounds, Compound Dync & Knab,, 1906; Actes (Ochlevatus) Compounds, Compounds, Compound Dync & Knab,, 1906; Actes (Ochlevatus) Compounds, Compound Dync & Compound Dync & Knab,, 1906; Actes (Ochlevatus) Compounds, Compound Dync & Knab,, 1906; Actes (Ochlevatus) Compound Dync & Compound Dync & Compound Dync & Knab,, 1906; Actes (Ochlevatus) Compound Dync & Compound Dync & Knab,, 1906; Actes (Ochlevatus) Compound Dync & Knab,, 1906; Actes (Ochlevatus) Compound Dync & Knab,, 1906; Actes (Ochlevatus) Compound Dync & Compound



<sup>1848</sup> 

Legend: for each node, the specific and subspecific (when applicable) epithets are given in bold on top, followed by the author; afterwards follows the generic and subspecific (when applicable) treatments at the time (given by the bold) and the time (given by the bold) and the time (given by the bold) and subspecific (when applicable) treatments at the time (given by the bold) and the times represent the shifting understanding of each original combination; the x-axis gives the years for the pipelicable) are applicable.

Appendix A2. The taxonomic history of the names within Ochlerotatus (Ochlerotatus) Lynch Arribálzaga. 1891: the orignal combinations Acdes (Ochlerotatus) mervai Martínez & Prosen, 1953; Acdes (Ochlerotatus) traversus Dyar, 1925; Acdes argentescens Dyar & Knab, 1908; Acdes angustivittatus Dyar & Knab, 1907; Culex inconspicuus Grossbeck, 1904 and Culex trivittatus Coquillett, 1902.



Legend: for each node, the specific and subspecific (when applicable) epithets are given in bold on top. followed by the author; afterwards follows the generic and subgeneric (when applicable) treatments at the inter given by the position of the advoid); at the bottom the reference is given for the the inter represent the shifting understanding of each original combination; the x-axis gives the years for the publications, with the shadows (in gray) persenting the distance of that year from the most recent year in the figure (s.t. 2000).

Appendix A3. The taxonomic history of the names within Ochlevotatus (Ochlevotatus) Lynch Arribálzaga, 1891: the orignal combinations Cular lynchii Brèthes, 1911; Cular tapinops Brèthes, 1917; Acdes (Ochlervotatus) spaced shall also show and Acdes (Ochlervotatus) patersoni Shannon & Del Ponte, 1927; the taxonomic history of the original combination Hetervorded dolors draft Arrich Arribálzag, 1830n that interests the one of this group is also shown.



Legend: for each node, the specific and subspecific (when applicable) epithets are given in bold on top, followed by the author; afterwards follows the generic and subgeneric (when applicable) treatments at the time (given by the position of the nodo); at the bottom the reference is given for the treatment at the time; the lines represent the shifting understanding of each original combination (a grey line represents a name that does not pertain to this revision, the taxonomic history it shares with this group is presented in the figure, but not beyond that); the x-axis gives the years for the publications, with the shadows (in gray) representing the distance of that year from the nost recent year in the figure (i.e. 2000).

Appendix A4. The taxonomic history of the names within Ochlerotatus (Ochlerotatus) Lynch Arribálzaga, 1891: the orignal combinations Aedes (Ochlerotatus) tortilis virginensis Dyar, 1922; Aedes plutocraticus Dyar & Knab, 1907; Aedes balteatus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1906; Aedes balteatus Concertatus Dyar & Knab, 1907; Aedes balteatus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1906; Aedes balteatus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1906; Aedes balteatus Dyar & Knab, 1907; Aedes balteatus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1906; Aedes balteatus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1906; Aedes balteatus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1906; Aedes habanicus Dyar & Knab, 1906; Aedes habanicus Dyar & Knab, 1906; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1906; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1906; Aedes habanicus Dyar & Knab, 1906; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1906; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1906; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1906; Aedes habanicus Dyar & Knab, 1907; Aedes habanicus Dyar & Knab, 1908; Aedes habanicus Dyar & Knab, 1908; Aedes habanicus Dyar & Knab, 1908; Aedes habanicus Dyar &



Legend: for each node, the specific and subspecific (when applicable) epithets are given in bold on top, followed by the author; afterwards follows the generic and subgeneric (when applicable) treatments at the time; time (given by the position by the position of a the bottom the retenent at the time; the time (given by the position of a the bottom the retenent at the treatment at the time; the first of the position of the order); at the bottom the retenent at the time; the mast resent the shifting understanding of each original combination; the x-axis gives the years for the publications, with the shake of systy persensing the distance of that year from the most recent year in the figure (i.e. 2000).

Appendix A5. The taxonomic history of the names within Ochlerotatus (Ochlerotatus) Lynch Arribálzaga, 1891: the orignal combinations Aedes (Ochlerotatus) raymondi Del Ponte et al., 1951; Aedes (Ochlerotatus) beyonds them, instruction the Arcie (Termorhynchen) Reider Obra, 1918 and Aedes obturbator Dyar & Knab, 1907; the taxonomic history of the original combinations Aedes milleri Dyar, 1922 and Culex bitmative Theobald, 1903 that intersects the non of this group is also shown.



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Appendix A6. The taxonomic history of the names within Ochlerotatus (Ochlerotatus) Lynch Arribálzaga, 1891: the orignal combinations Aedes (Ochlerotatus) incomptus Arnell, 1976; Aedes (Ochlerotatus) begolarus Arnell, 1976; Aedes (Ochlerotatus) disficiens Arnell, 1976; Aedes (Ochlerotatus) attactus) synchytus Arnell, 1976; Aedes (Ochlerotatus) phaenotus) phaenotus Arnell, 1976; Aedes (Ochlerotatus) attactive) attaction of the orignal computes Arnell, 1976; Aedes (Ochlerotatus) attaction of the orignal computes Arnell, 1976; Aedes (Ochlerotatus) attactive) attactive) attactive) attactive) attactive) and Aedes (Ochlerotatus) phaenotus) phaenotus Arnell, 1976; Aedes (Ochlerotatus) attactive) attacti

incomptus Arnell Ocheratius Reinert, 2000	bogotanus Arnell Ochlerotana) Reinert, 2000	deficiens Aruell (Odderatuus) Reinert, 2000	us atactavittatus Arnell Ochérendaus Reinert, 2000	synchytus Aruell Ooklerotaus Reinert, 2000	phaenotus Arnell Ocherotaus Reinert, 2000	comitatus Arnell Ocherotaus Reinert, 2000	pectinatus Arnell Oddervatus (Oddervatus) Relnert, 2000	2000	
incomptus Arnell Aete (Ochlervlatus) Scapularls Group Arnell, 1976	bogotanus Arnell Acte (Ochlervlatus) Scapularis Group Arnell, 1976	deficiens Arnell Aete (Ochlervtatus) Scapularis Group Arnell, 1976	atactavittatı Arnell Aedes (Ochlerviatus) Scapularis Group Arnell, 1976	synchytus Arnell Acte (Ochlerotatus) Scapularis Group Arnell, 1976	phaenotus Arnell Actes (Ochlerotatus) Scapularis Group Arnell, 1976	comitatus Arnell Aeder (Ochlerviatus) Scapularis Group Arnell, 1976	pectinatus Arnell Aeda (Ocherptatus) Scopularia Group Arnell, 1976	1976	1976

Legend: for each node, the specific and subspecific (when applicable) epithets are given in bold on top, followed by the author; afterwards follows the generic, subgeneric and informal grouping (when applicable) treatments at the inter (the interment of the interments at the inter (the interments of the interments at the inter (per bub) is the position of the adol); at the bottom the reterence is given to the treatment at the inter (the inter of the interments at the inter (the intervention); which are applicable) treatments at the inter (group) is the position of the adol); at the bottom the reterence is given to the most recent year in the ligne (i.e. 2000).

**Appendix B.** Plates for species of *Ochlerotatus* (*Ochlerotatus*) Lynch Arribálzaga, 1891. For each plate the gonocoxite (including the gonostylus) is figured on the right, the claspette (including the claspette filament) is figured on the upper left, and the aedeagus is figured on the lower left. All structures are presented in the dorsal view in the pre-rotation sense.



Ochlerotatus brisolai


Ochlerotatus comitatus



Ochlerotatus condolescens



Ochlerotatus confirmatus



Ochlerotatus euplocamus



Ochlerotatus hemisurus



Ochlerotatus infirmatus



Ochlerotatus patersoni



Ochlerotatus phaenotus



Ochlerotatus scapularis

### Chapter 3. An annotated checklist of the mosquitoes (Diptera: Culicidae) of Wisconsin, USA

Ferreira-de-Freitas, Vinícios, Thrun, Nicholas B., Paskewitz, Susan, and Bartholomay, Lyric C.

#### Introduction

The landscape of the state of Wisconsin, and our understanding of the mosquitoes present in the state, have changed significantly since the last taxonomic checklist was published (Dickinson, 1944). In response, we present an updated checklist of mosquito fauna of Wisconsin that is based on a comprehensive review of museum specimens and the literature. More specifically, the personal mosquito collection of Dr. Robert J. Dicke (Professor Emeritus, Department of Entomology, UW-Madison 1912-2003), that is housed in the Wisconsin Insect Research Collection (WIRC) at the University of Wisconsin-Madison, was re-identified and the classification updated, and the data are presented herein. We report 3455 specimens from the WIRC, collected from 1930 through 1989, none of which have been the subject of a previous report. Within the collection there are two new records for the state: Anopheles perplexens Ludlow and Anopheles smaragdinus Reinert. We also add Richards et al. (2019), the first report of Stegomyia albopicta (Skuse) in the state of Wisconsin.

#### Historical records of the mosquitoes of Wisconsin

The first mosquitoes from Wisconsin were identified by van der Wulp (1867) who received material collected by Thure Kumlien in Wisconsin, most likely in Dane County. van der Wulp (1867) described two new mosquitoes, *Culex testaceus* (today a synonym of *Coquillettidia perturbans* (Walker)) and *Anopheles annulimanus* (today a synonym of *Anopheles quadrimaculatus* Say) albeit without giving a specific locality, other than North America. Belkin (1968), however, reported that van der Wulp's types bear labels that inform the locality of collection as Wisconsin. van der Wulp's types were originally housed at the State Museum of Natural History (Leiden, Netherlands), but now they are most likely located in the Rijksmuseum van Natuurlijke Historie (Naturalis Biodiversity Center).

Later Howard *et al.* (1915, 1917) and Dyar (1922), in their revisions of the mosquitoes of North America and the United States (respectively), further expand the knowledge of the mosquitoes of Wisconsin by providing detailed information on collection localities.

The first comprehensive checklist of the mosquito fauna of Wisconsin included reports of 39 taxa, with county occurrence for each (Dickinson, 1944). Dickinson's list represents 37 currently valid species and two names that have since been synonymized. Later, Ryckman (1952) published a taxonomic checklist for the mosquitoes of Lafayette County, Wisconsin, based on a study on mosquito ecology in southern Wisconsin. Although this list contains only 12 names, one of those (*Ochlerotatus sticticus* (Meigen)) represented a new report for the state at the time.

Additional insight on the mosquito fauna in the state come from studies of mosquito biology and ecology. Thompson & Dicke (1965) sampled populations of Ochlerotatus trivittatus (Coquillett), Ochlerotatus stimulans (Walker) and Aedimorphus vexans (Meigen) from Madison, providing additional information on the behavioral patterns of those species. Siverly & DeFoliart collected larvae and adults in northern Wisconsin to assess population fluctuations in culicids (1968a,b). Gojmerac & Porter collected mosquitoes in Point Beach State Forest and Wyalusing State Park in order to compare different trapping methods for collecting select species of mosquitoes (1969). Later these authors used the data from Point Beach State Forest to report a checklist of mosquito species collected in that area between 1967 and 1968, and presented two new records for the state (Anopheles barberi Coquillett and Orthopodomyia signifera (Coquillett)) (Porter & Gojmerac, 1970). Notably, Or. signifera was also independently reported by another group in the same year (Loor & DeFoliart, 1970). Amin & Hageman (1974) made an effort to catalog the mosquitoes of southeastern Wisconsin, considerably expanding knowledge of the mosquito fauna distribution within the state.

Additional studies including Loor & DeFoliart (1970), who reported some mosquitoes landing during field observations, and Wright & DeFoliart (1970), who studied the host preference of some mosquitoes in Wisconsin, cast new light on the range of a few mosquito species in the state. Additionally each of these reports also resulted in the first report of a new species occurrence for Wisconsin, *i.e.* 'Ochlerotatus' hendersoni (Cockerell) and Psorophora horrida (Dyar & Knab), respectively.

Grimstad & DeFoliart published on the ecology of Wisconsin mosquitoes (1975), and Arnell (1976) mentioned mosquitoes from Wisconsin in his revision of the Scapularis Group. Decades later (see Figure 2), Gilardi & Hilsenhoff studied the bionomics of several mosquitoes in Wisconsin, and reported 21 species, two of which were new state records, *Ochlerotatus decticus* (Howard, Dyar & Knab) and *Ochlerotatus euedes* (Howard, Dyar & Knab) (1992). Meece *et al.* (2003) provided a list of 25 species collected during West Nile virus surveillance efforts in the state and report three new species, *Anopheles crucians* s.l. (Wiedemann), *Culex erraticus* (Dyar & Knab) and *Ochlerotatus sollicitans* (Walker). Thereafter, Hughes *et al.* (2008) reported on the invasion and spread of *Hulecoteomyia japonica*  *japonica* (Theobald) in the state of Wisconsin, also as a result of active mosquito surveillance. Most recently, Richards *et al.* (2019) described invasion events for *Stegomyia albopicta* (Skuse) in two Wisconsin counties.

#### **Discussion of faunal composition**

The state of Wisconsin has 53 recognized taxa (species and subspecies) in 18 subgenera and 14 genera (only counting established populations), while the Nearctic has, to date, 181 recognized taxa in 30 subgenera and 26 genera (Harbach, 2019). Thus Wisconsin has roughly 30% of the species, and more than 50% of the subgenera and genera present in the Nearctic as a whole. It is not possible for us to make any assertions on the relative importance of this richness as not many states have a recent mosquito fauna checklist published. The only recent checklist of mosquito species from another Midwestern state is from Iowa, which reports that the state of Iowa has  $55^1$  recognized taxa in 18 subgenera and 14 genera (Dunphy *et al.*, 2014). Based on this limited analysis, Wisconsin has a similar species richness albeit with a considerably different species composition.

<sup>1</sup> The record of *Psorophora mathesoni* is almost certainly a misidentification, making the total number 54. More is discussed later in the text.

#### **Discussion of individual species**

The species of Wisconsin are presented below in the format of an annotated checklist, *i.e.*, with some discussion of the record, taxonomy and bionomics of the species, and associated County records. The nomenclature for the species presented below follows Reinert *et al.*, (2009), with special reference to the nomenclature of Wilkerson *et al.* (2015) in square brackets. The subgeneric abbreviations follow Reinert (2009). All species discussed in this section are present in Table 1 with their respective reference list. Refer to Figures 3 and 4, as well as Table 3, for additional information on the timing of each species collection or mention in a publication. Publications with an asterisk (\*) do not have precise county data, they only mention counties of collection and species present overall.

## 'Ochlerotatus' hendersoni (Cockerell) sensu auctorum [=Aedes hendersoni] and 'Ochlerotatus' triseriatus (Say) sensu auctorum [=Aedes triseriatus]

*'Ochlerotatus' hendersoni* may be considered quite rare in the state of Wisconsin, as it has been reported only twice in the literature (Loor & DeFoliart, 1970; Grimstad *et al.*, 1974), and in both cases sympatrically with

'Ochlerotatus' triseriatus. Furthermore, (Loor & DeFoliart, 1970) collected only 42 specimens of 'Oc.' hendersoni with ovitraps and one specimen in a tree hole, alongside 635 and 532 specimens of 'Oc.' triseriatus, with ovitraps and in tree holes, respectively, in the same region (Iowa County, WI).

'Oc.' hendersoni is rarely mentioned in literature involving mosquito surveillance in the states of Minnesota, Michigan and Illinois, and thus appears to be also quite rare in those states. Further strengthening this point, the mosquito has been reported as "uncommon" in the state of Iowa (Dunphy *et al.*, 2014).

Truman & Craig Jr (1968) found that, at various sites throughout the Midwest, 'Oc.' hendersoni was present in only half of their collection sites, furthermore they claim the species was found only in tree holes in which the water contained high amounts of organic matter and was very turbid. Furthermore, ecological data collected by Shipp *et al.* (1978) in Ontario, Canada, has shown that 'Oc.' hendersoni, in Ontario, has a preference for particular species of trees in which to oviposit. With those restrictions, it is clear that 'Oc.' hendersoni is most likely less abundant than 'Oc.' triseriatus, but the species might not be as rare as the literature portraits it to be, as this apparent rarity might only be a product of a lack of knowledge

of its biology.

Recently, the authors have found '*Oc.' hendersoni* in ovitraps in La Crosse, WI, during successive years (unpublished data).

**Distribution for 'Ochlerotatus' hendersoni: Iowa County:** Loor & DeFoliart (1970)\* as Aedes hendersoni; Grimstad et al. (1974) as Aedes hendersoni. **La Crosse County:** Grimstad et al. (1974) as Aedes hendersoni.

Distribution for 'Ochlerotatus' triseriatus: Brown County: WIRC (1941); Dickinson (1944) as Aedes triseriatus. Chippewa County: Dickinson (1944) as Aedes triseriatus. Columbia County: WIRC (1956). Crawford County: DeFoliart et al. (1972)\* as Aedes triseriatus. Dane County: Dickinson (1944) as Aedes triseriatus; WIRC (1949, 1950, 1951, 1952, 1954, 1955, 1978); Anslow et al. (1969) as Aedes triseriatus; Wright & DeFoliart (1970) as Aedes triseriatus; DeFoliart et al. (1972)\* as Aedes triseriatus; Meece et al. (2003)\* as Ochlerotatus triseriatus. Forest County: Dickinson (1944) as Aedes triseriatus; Siverly & DeFoliart (1968b) as Aedes triseriatus. Iowa County: DeFoliart (1970) as Aedes triseriatus; Siverly & DeFoliart (1968b) as Aedes triseriatus. Iowa County: Wright & DeFoliart (1970) as Aedes triseriatus. Iowa County: Wright & DeFoliart (1970) as Aedes triseriatus. Iowa County: Wright & DeFoliart (1970) as Aedes triseriatus. Iowa County: Dickinson (1944) as Aedes triseriatus. Iron County: Dickinson (1944) as Aedes triseriatus. Iowa County: Dickinson (1944) as Aedes triseriatus. Iron County: Dickinson (1944) as Aedes triseriatus. Iowa County: Dickinson (1944) as Aedes triseriatus. Iron County: Dickinson (1944) as Aedes triseriatus.

Kenosha County: Amin & Hageman (1974) as Aedes triseriatus; Meece et al. (2003)\* as Ochlerotatus triseriatus. La Crosse County: Dickinson (1944) as Aedes triseriatus; Grimstad et al. (1974) as Aedes triseriatus. Lafayette County: Ryckman (1952) as Aedes triseriatus. Lincoln County: Dickinson (1944) as Aedes triseriatus; Siverly & DeFoliart (1968b) as Aedes triseriatus. Manitowoc County: WIRC (1968); Porter & Gojmerac (1970) as Aedes triseriatus; Grimstad & DeFoliart (1975) as Aedes triseriatus. Marinette County: WIRC (1941); Dickinson (1944) as Aedes triseriatus. Milwaukee County: Meece et al. (2003)\* as Ochlerotatus triseriatus. Monroe County: Dickinson (1944) as Aedes triseriatus. Oneida County: Siverly & DeFoliart (1968b) as Aedes triseriatus. Outagamie County: Dickinson (1944) as Aedes triseriatus. Pepin County: Dickinson (1944) as Aedes triseriatus. Pierce County: Dickinson (1944) as Aedes triseriatus. Racine County: Meece et al. (2003)\* as Ochlerotatus triseriatus. Rock County: Dickinson (1944) as Aedes triseriatus. Rusk County: WIRC (1952). Vernon County: Grimstad et al. (1974) as Aedes triseriatus. Vilas **County:** Dickinson (1944) as *Aedes triseriatus*. Walworth County: Dickinson (1944) as Aedes triseriatus. Washburn County: WIRC (1953). Washington County: Dickinson (1944) as Aedes triseriatus. Waukesha **County:** Meece *et al.* (2003)\* as *Ochlerotatus triseriatus*.

#### Aedes cinereus Meigen

This species is broadly distributed throughout the state of Wisconsin and is a fairly common mosquito. Its larvae apparently inhabit a large variety of habitats such as rain pools, marshes, bogs and lake margins (Carpenter & LaCasse, 1974).

Distribution for Aedes cinereus: Adams County: Gilardi & Hilsenhoff (1992). Bayfield County: Gilardi & Hilsenhoff (1992). Brown County: Dickinson (1944). Buffalo County: Dickinson (1944); Gilardi & Hilsenhoff (1992). Burnett County: Gilardi & Hilsenhoff (1992). Chippewa County: WIRC (1957). Columbia County: Dickinson (1944); WIRC (1956, 1957). Crawford County: DeFoliart et al. (1972)\*. Dane County: Dyar (1922) as Aëdes cinereus; Dickinson (1944); WIRC (1952, 1954, 1978); Anslow et al. (1969); Wright & DeFoliart (1970); DeFoliart et al. (1972)\*; Meece et al. (2003)\*. Dodge County: Dickinson (1944). Douglas County: Dickinson (1944). Dunn County: Gilardi & Hilsenhoff (1992). Florence County: Gilardi & Hilsenhoff (1992). Fond du Lac County: Dickinson (1944); Gilardi & Hilsenhoff (1992). Forest County: Dickinson (1944); Siverly & DeFoliart (1968b); Siverly & DeFoliart (1968a); Gilardi & Hilsenhoff (1992). Grant County: Dickinson (1944); DeFoliart et al. (1972)\*. Iowa County: Wright & DeFoliart (1970). Jefferson County:

Dickinson (1944); Gilardi & Hilsenhoff (1992). Juneau County: Dickinson (1944). Kenosha County: Amin & Hageman (1974); Meece et al. (2003)\*. La Crosse County: Dickinson (1944). Langlade County: Dickinson (1944). Lincoln County: WIRC (1951); Siverly & DeFoliart (1968a); Siverly & DeFoliart (1968b); Gilardi & Hilsenhoff (1992). Manitowoc County: WIRC (1968); Porter & Gojmerac (1970); Grimstad & DeFoliart (1975). Marinette County: Dickinson (1944); Gilardi & Hilsenhoff (1992). Marguette County: Dickinson (1944); Gilardi & Hilsenhoff (1992). Milwaukee County: Dickinson (1944); Meece et al. (2003)\*. Oneida County: WIRC (1967); Siverly & DeFoliart (1968a); Siverly & DeFoliart (1968b). Pepin County: Dickinson (1944); Gilardi & Hilsenhoff (1992). Polk County: Dickinson (1944); Gilardi & Hilsenhoff (1992). Portage County: Dickinson (1944). Price County: Gilardi & Hilsenhoff (1992). Racine County: Dickinson (1944); Meece et al. (2003)\*. Richland County: Gilardi & Hilsenhoff (1992). Rock County: Dickinson (1944); Gilardi & Hilsenhoff (1992). Rusk County: WIRC (1952, 1957, 1968, 1969, 1986). Sheboygan County: Dickinson (1944); Gilardi & Hilsenhoff (1992). Taylor County: Dickinson (1944). Vilas County: Dickinson (1944); Siverly & DeFoliart (1968a). Walworth County: Dickinson (1944); Wright & DeFoliart (1970); Gilardi & Hilsenhoff (1992). Washburn County: Dickinson (1944); WIRC (1950, 1951, 1952). Waukesha County: Dickinson (1944); Gilardi &

Hilsenhoff (1992); Meece *et al.* (2003)\*. **Waushara County:** Howard *et al.* (1917) as *Aëdes fuscus*; Dickinson (1944). **Wood County:** Grimstad & DeFoliart (1975).

#### Aedimorphus vexans vexans (Meigen) [=Aedes vexans vexans]

Aedimorphus vexans vexans is not only widely distributed throughout the state but also extremely abundant. Part of why *Am. vexans vexans* is so common is its behavior of long-distance migration and its predisposition to invade urban areas given it is readily attracted to light and it is reportedly anthropophilic, which is generally why authors consider it a nuisance mosquito (Thompson & Dicke, 1965; Carpenter & LaCasse, 1974; Briegel *et al.*, 2001). Furthermore, the larvae of this mosquito inhabit a variety of temporary habitats, such as rain pools, flood plains and irrigation seepage (Carpenter & LaCasse, 1974), which further contributes to its abundance.

Although the presence of the subspecies *Aedimorphus vexans nipponii* in the United States is refuted by Reinert (1973), it should be noted that the mosquito was reported at least twice, but those reports were dismissed by Reinert (1973). Moreover, mosquitoes that present the characters of *Am. vexans nipponii* have been found in many states, such is the case for New Jersey (Crans & Gandek, 1968), Iowa (Dunphy and Bartholomay, unpublished) and Wisconsin (data not published). More research is needed to provide insight into the true morphological variation and taxonomical identity of the individuals currently classified as *Am. vexans vexans* in the United States.

Distribution for Aedimorphus vexans vexans: Adams County: Gilardi & Hilsenhoff (1992) as Aedes vexans. Bayfield County: Gilardi & Hilsenhoff (1992) as Aedes vexans. Brown County: Dickinson (1944) as Aedes vexans. Buffalo County: Dickinson (1944) as Aedes vexans. Burnett **County:** Gilardi & Hilsenhoff (1992) as Aedes vexans. Calumet County: Gilardi & Hilsenhoff (1992) as *Aedes vexans*. Columbia County: Dickinson (1944) as Aedes vexans; WIRC (1956, 1957). Crawford County: DeFoliart et al. (1972)\* as Aedes vexans. Dane County: Dickinson (1944) as Aedes vexans; WIRC (1952, 1954, 1955); Thompson & Dicke (1965) as Aedes vexans; Anslow et al. (1969) as Aedes vexans; Wright & DeFoliart (1970) as Aedes vexans; DeFoliart et al. (1972)\* as Aedes vexans; Grimstad & DeFoliart (1975) as Aedes vexans; Meece et al. (2003)\* as Aedes vexans. **Dodge County:** Dickinson (1944) as *Aedes vexans*. **Dunn County:** Gilardi & Hilsenhoff (1992) as Aedes vexans. Fond du Lac County: Dickinson (1944) as Aedes vexans; Gilardi & Hilsenhoff (1992) as Aedes vexans. Forest County: Siverly & DeFoliart (1968a) as Aedes vexans; Siverly &

DeFoliart (1968b) as Aedes vexans; Gilardi & Hilsenhoff (1992) as Aedes vexans. Grant County: Dickinson (1944) as Aedes vexans; Gojmerac & Porter (1969) as Aedes vexans; DeFoliart et al. (1972)\* as Aedes vexans. **Green County:** Dickinson (1944) as *Aedes vexans*. **Iowa County:** Loor & DeFoliart (1970)\* as Aedes vexans; Wright & DeFoliart (1970) as Aedes vexans. Jackson County: Anslow et al. (1969) as Aedes vexans. Jefferson **County:** Dickinson (1944) as *Aedes vexans*; Gilardi & Hilsenhoff (1992) as Aedes vexans. Kenosha County: Dickinson (1944) as Aedes vexans; Amin & Hageman (1974) as Aedes vexans; Meece et al. (2003)\* as Aedes vexans. La Crosse County: Dickinson (1944) as *Aedes vexans*. Lafayette County: Ryckman (1952) as Aedes vexans. Lincoln County: Siverly & DeFoliart (1968a) as Aedes vexans; Siverly & DeFoliart (1968b) as Aedes vexans; Gilardi & Hilsenhoff (1992) as *Aedes vexans*. Manitowoc County: Dickinson (1944) as Aedes vexans; WIRC (1967, 1968); Gojmerac & Porter (1969) as Aedes vexans; Porter & Gojmerac (1970) as Aedes vexans; Grimstad & DeFoliart (1975) as Aedes vexans. Marinette County: Dickinson (1944) as Aedes vexans; Gilardi & Hilsenhoff (1992) as Aedes vexans. Marquette County: Gilardi & Hilsenhoff (1992) as Aedes vexans. Milwaukee County: Dickinson (1944) as Aedes vexans; Meece et al. (2003)\* as Aedes vexans. Monroe County: Dickinson (1944) as Aedes vexans. **Oneida County:** Siverly & DeFoliart (1968a) as Aedes vexans;

Siverly & DeFoliart (1968b) as Aedes vexans. Outagamie County: Dickinson (1944) as Aedes vexans. Pepin County: Dickinson (1944) as Aedes vexans; Gilardi & Hilsenhoff (1992) as Aedes vexans. **Pierce County:** Dickinson (1944) as Aedes vexans. Polk County: Dickinson (1944) as Aedes vexans; Gilardi & Hilsenhoff (1992) as Aedes vexans. Price County: Gilardi & Hilsenhoff (1992) as *Aedes vexans*. Racine County: Dickinson (1944) as Aedes vexans; Meece et al. (2003)\* as Aedes vexans. Richland County: Gilardi & Hilsenhoff (1992) as Aedes vexans. Rock County: Dickinson (1944) as Aedes vexans; Gilardi & Hilsenhoff (1992) as Aedes vexans. Rusk **County:** WIRC (1952, 1957, 1966, 1968). **Sheboygan County:** Gilardi & Hilsenhoff (1992) as Aedes vexans. Taylor County: Dickinson (1944) as Aedes vexans. Vernon County: Dickinson (1944) as Aedes vexans; WIRC (1955); Anslow et al. (1969) as Aedes vexans. Vilas County: Siverly & DeFoliart (1968a) as Aedes vexans. Walworth County: Dickinson (1944) as Aedes vexans; Wright & DeFoliart (1970) as Aedes vexans; Gilardi & Hilsenhoff (1992) as Aedes vexans. Washburn County: WIRC (1952). Waukesha County: Dickinson (1944) as Aedes vexans; Meece et al. (2003)\* as Aedes vexans. Waupaca County: Dickinson (1944) as Aedes vexans. Winnebago County: Dickinson (1944) as Aedes vexans. Wood County: Dickinson (1944) as Aedes vexans; Grimstad & DeFoliart (1975) as Aedes vexans.

#### Anopheles barberi Coquillett

The report by Porter & Gojmerac (1970) of this species in Wisconsin is the only one in literature, but the species had been collected in Wisconsin in 1953 and in 1968 according to specimens in the WIRC.

Anopheles barberi is a container breeding mosquito, often associated with tree rot cavities and stumpholes, but sometimes found in artificial containers when sufficient leaf debris is present (Carpenter & LaCasse, 1974).

**Distribution for** *Anopheles barberi*: **Dane County**: WIRC (1953). **Manitowoc County**: WIRC (1968); Porter & Gojmerac (1970).

#### Anopheles crucians sensu lato Wiedemann

Meece *et al.* (2003) provided the first report of this species in Wisconsin in the literature; however, specimens exist in the WIRC from collections in 1951 and 1962 in Wisconsin. Wilkerson *et al.* (2004) showed that the *Anopheles crucians* complex is comprised of six genetically distinct entities, informally referred to as "genetic species", amongst which are three currently recognized species *An. crucians, Anopheles bradleyi* and *Anopheles georgianus*. As of now, only *An. bradleyi* is recognizable by

molecular techniques, while identification for other two named species is unknown. More research is needed in order to unveil the *An. crucians* complex; additionally, further collection and molecular analysis of the *An. crucians* s.l. from Wisconsin may provide insight into the distribution of the genetic forms.

Distribution for Anopheles crucians s.l.: Dane County: WIRC (1951); Meece et al. (2003)\* as Anopheles crucians. Kenosha County: Meece et al. (2003)\* as Anopheles crucians. Milwaukee County: Meece et al. (2003)\* as Anopheles crucians. Racine County: Meece et al. (2003)\* as Anopheles crucians. Racine County: Meece et al. (2003)\* as Anopheles crucians. Washington County: WIRC (1962). Waukesha County: Meece et al. (2003)\* as Anopheles crucians.

#### Anopheles earlei Vargas

Dickinson (1944) identified some Anopheles specimens to Anopheles maculipennis but followed trends of the time which restricted the name Anopheles maculipennis only to the European specimens (as described by Dickinson (1944) p. 346, referencing a personal correspondence with Matheson). The author thought that, from the Maculipennis Complex, the specimens he had most resembled Anopheles occidentalis. But it is clear that the specimens he had were *Anopheles earlei* Vargas, 1943. This mosquito's type locality is Jefferson County, WI (Vargas, 1943).

The larvae inhabit the shallow margins of ponds or similar semipermanent and permanent water bodies Carpenter & LaCasse (1974).

Distribution for Anopheles earlei: Barron County: Dickinson (1944) as Anopheles occidentalis. **Brown County:** WIRC (1941); Dickinson (1944) as Anopheles occidentalis. Burnett County: Dickinson (1944) as Anopheles occidentalis. Chippewa County: Dickinson (1944) as Anopheles occidentalis. Columbia **County:** Dickinson (1944)as Anopheles occidentalis. Crawford County: DeFoliart et al. (1972)\*. Dane County: Dickinson (1944) as Anopheles occidentalis; DeFoliart et al. (1972)\*. Dodge **County:** WIRC (1941). **Douglas County:** Dickinson (1944) as Anopheles occidentalis. Florence County: WIRC (1954, 1957). Fond du Lac County: Dickinson (1944) as Anopheles occidentalis. Forest County: Dickinson (1944) as Anopheles occidentalis; Siverly & DeFoliart (1968b). Grant County: DeFoliart et al. (1972)\*. Iron County: WIRC (1941); Dickinson (1944) as Anopheles occidentalis. Jackson County: WIRC (1941); Dickinson (1944) as Anopheles occidentalis. Jefferson County: Dickinson (1944) as Anopheles occidentalis. Juneau County: Dickinson (1944) as Anopheles occidentalis. La Crosse County: Dickinson (1944) as Anopheles

occidentalis; WIRC (1989). Lincoln County: Siverly & DeFoliart (1968b). Manitowoc County: WIRC (1967); Porter & Gojmerac (1970); Grimstad & DeFoliart (1975). Marinette County: Dickinson (1944) as Anopheles occidentalis. Marguette **County:** Dickinson (1944) as Anopheles occidentalis. Monroe County: Dickinson (1944) as Anopheles occidentalis. **Oneida County:** Siverly & DeFoliart (1968b). **Outagamie County:** Dickinson (1944) as Anopheles occidentalis. Pepin County: Dickinson (1944) as Anopheles occidentalis. Polk County: WIRC (1941). Rock County: Dickinson (1944) as Anopheles occidentalis. Rusk County: WIRC (1951, 1952, 1953, 1968). Sauk County: Dickinson (1944) as Anopheles occidentalis. Sawyer County: WIRC (1941); Dickinson (1944) as Anopheles occidentalis. Sheboygan County: Dickinson (1944) as Anopheles occidentalis. Taylor County: Dickinson (1944) as Anopheles occidentalis. Vernon County: Dickinson (1944) as Anopheles occidentalis. Vilas County: WIRC (1941, 1951); Dickinson (1944) as Anopheles occidentalis. Washburn County: Dickinson (1944) as Anopheles occidentalis; WIRC (1950, 1951). Waukesha County: Dickinson (1944) as Anopheles occidentalis. **County:** Dickinson (1944)Anopheles Waupaca as **County:** Dickinson (1944) occidentalis. Winnebago as Anopheles occidentalis. Wood County: WIRC (1941); Dickinson (1944) as Anopheles occidentalis; Wright & DeFoliart (1970); Grimstad & DeFoliart (1975).

#### Anopheles punctipennis (Say) and Anopheles perplexens Ludlow

Anopheles perplexens was described in 1907 by Ludlow (1907) and later synonymized with Anopheles punctipennis by Howard *et al.* (1917). In his redescription and resurrection of the species, Bellamy shows that, despite the synonymity with *An. punctipennis*, many authors made reference to the "perplexens form" in Georgia, Florida and Pennsylvania (1956). Bellamy's work also demonstrated that *An. perplexens* could be easily recognized by the morphology of the egg and proposed characters to aid the differentiation of larval and adult female stages, although he admits the character states sometimes overlap and that more studies were necessary to achieve a reliable distinction, pointing towards some promising characters the author had found.

Nothing more has been published on the morphological separation of the two species, despite Kreutzer & Kitzmiller (1972) showing the lack of genetic introgression between the two. Even though only one character is of value for separating adult females (*i.e.* ratio of the subcostal pale spot to wing length) and overlap has been shown to exist, we have found strong evidence of the presence of *An. perplexens* in Wisconsin from the material deposited at the WIRC. Most of these specimens with a ratio of 0.01 to 0.04, these are well below the overlap shown by Bellamy (1956); moreover, the two were sympatric in five counties (Dane, Florence, Rusk, Vilas and Washburn) with only three counties (Columbia, Iron and Manitowoc) presenting only An. perplexens and three (Buffalo, Dodge and Forest) presenting only An. punctipennis. Where they were sympatric, for what we considered An. perplexens the character ranged from 0.02 to 0.07, and although 0.07 is within overlapping range, only 6 out of 25 specimens had a ratio of above 0.06 (i.e. entered overlapping range). We considered a specimen to be An. punctipennis when the character ranged from 0.08 to 0.12. For the counties where only An. punctipennis was present (Buffalo, Dodge and Forest) only Buffalo County had an appreciable number of specimens (17), for which the character ranged from 0.08 to 0.12, while for the other counties there was only one specimen. For the counties where only An. perplexens was found (Columbia, Iron and Manitowoc), both Iron County and Manitowoc County had only one specimen, and the specimen from Iron County had a 0.07 ratio, while the one from Manitowoc had a ratio of 0.02. Columbia County had four specimens, and the ratio ranged from 0.01 to 0.04.

It is clear that more research is needed to better characterize these two species. However, for the specimens housed at the WIRC, we are confident that the ones presenting the identifying character further from the range of overlapping states are in fact *An. perplexens*. *An. perplexens* is therefore considered a new record for the state of Wisconsin.

Anopheles punctipennis larvae inhabit many different habitats, including temporary and permanent water bodies, as well as natural and artificial habitats. They've been reported from spring pools and pools on flowing streams to roadside puddles and rain barrels, when water was clear (Carpenter & LaCasse, 1974). Nothing is known for the larval habitats of *An. perplexens*.

Distribution for Anopheles punctipennis: Barron County: Dickinson (1944). Brown County: Dickinson (1944). Buffalo County: WIRC (1939); Dickinson (1944). Burnett County: Dickinson (1944). Chippewa County: Dickinson (1944). Clark County: Dickinson (1944). Columbia County: Dickinson (1944). Dane County: Dickinson (1944); WIRC (1950, 1951, 1952, 1954, 1955, 1956, 1966, 1978, 1989); Anslow et al. (1969); Meece et al. (2003)\*. Dodge County: WIRC (1941); Dickinson (1944). Dunn County: Dickinson (1944). Florence County: WIRC (1954). Fond du Lac County: Dickinson (1944). Forest County: WIRC (1941); Dickinson (1944). Grant County: Dickinson (1944). Iron County: Dickinson (1944). Jackson County: Dickinson (1944). Jefferson County: Dickinson (1944). Juneau County: Dickinson (1944). Kenosha County: Amin & Hageman (1974); Meece et al. (2003)\*. La Crosse County: Dickinson (1944). Lafayette County: Dickinson (1944); Ryckman (1952). Langlade County: Dickinson (1944). Manitowoc County: Dickinson (1944); Porter & Gojmerac (1970). Marinette County: Dickinson (1944). Marquette County: Dickinson (1944). Marrin County: WIRC (1941). Milwaukee County: Dickinson (1944); Meece et al. (2003)\*. Monroe County: Dickinson (1944). Pepin County: Dickinson (1944). Racine County: Meece et al. (2003)\*. Rusk County: WIRC (1952, 1953, 1980, 1981, 1982, 1983, 1984, 1985, 1988). Sheboygan County: Dickinson (1944). Taylor County: Dickinson (1944). Vernon County: Dickinson (1944). Vilas County: WIRC (1941); Dickinson (1944). Walworth County: Dickinson (1944). Washburn County: Dickinson (1944); WIRC (1951, 1952). Waukesha County: Dickinson (1944); Meece et al. (2003)\*. Waupaca County: Dickinson (1944). Waushara County: Dickinson (1944). Winnebago County: Dickinson (1944). Wood County: Dickinson (1944); Grimstad & DeFoliart (1975).

Distribution for Anopheles perplexens: Columbia County: WIRC (1947). Dane County: WIRC (1941, 1954, 1955). Florence County: WIRC (1954). Iron County: WIRC (1941). Manitowoc County: WIRC (1967).
Rusk County: WIRC (1952, 1980, 1981, 1982, 1983). Vilas County: WIRC (1941). Washburn County: WIRC (1952).

# Anopheles quadrimaculatus sensu stricto Say and Anopheles smaragdinus Reinert

The Quadrimaculatus Complex of species was described by Reinert *et* al. who restricted the name Anopheles quadrimaculatus and described four new species (Reinert et al., 1997). From these five species that comprise the complex, one is An. quadrimaculatus s.s., which was one of the first mosquito species reported for the state of Wisconsin, under the name Anopheles annulimanus (van der Wulp, 1867). Moreover, in our review of the Culicidae housed at the WIRC we identified on the basis of the female morphology Anopheles smaragdinus, we found several specimens of that species are present at the WIRC, collected from as early as 1938, as well as from samples collected by the authors in Dane County in 2016 (data not published). The literature reports of Anopheles quadrimaculatus that date prior to 1997 are regarded as An. quadrimaculatus sensu lato. The specimens in the WIRC have been identified to species utilizing the characters given by Reinert et al. (1997). It should be noted that there is only one report in the literature of this group dated after 1997, i.e. Meece et al. (2003) who simply report An. quadrimaculatus s.l.

Reinert *et al.* (1997) reported that *An. quadrimaculatus* s.s. are opportunistic in regards to habitat selection, with the larvae inhabiting

various semipermanent and permanent water bodies, mainly associated with emergent and/or floating vegetation. Reinert *et al.* (1997) also reports that *An. smaragdinus* larvae were found mainly in swamps. We do not know where the larvae might be developing in the habitats available in the state of Wisconsin, but it seems likely that bogs and marshes might provide a similar habitat to the swamps from which Reinert *et al.* (1997) reports this species.

Distribution for Anopheles quadrimaculatus s.l.: Buffalo County: Dickinson (1944) as Anopheles quadrimaculatus. Burnett County: Dickinson (1944) as Anopheles quadrimaculatus. Columbia County: Dickinson (1944) as Anopheles quadrimaculatus; WIRC (1956). Crawford County: Dickinson (1944) as Anopheles quadrimaculatus. Dane County: van der Wulp (1867) as Anopheles annulimanus; Dickinson (1944) as Anopheles quadrimaculatus; WIRC (1954); Meece et al. (2003)\*. Dodge County: Dickinson (1944) as Anopheles quadrimaculatus. Grant County: Dickinson (1944) as Anopheles quadrimaculatus. Grant County: Dickinson (1944) as Anopheles quadrimaculatus. Jefferson County: Dickinson (1944) as Anopheles quadrimaculatus. Juneau County: Dickinson (1944) as Anopheles quadrimaculatus. Juneau County: Dickinson (1944) as Anopheles quadrimaculatus. Kenosha County: Meece et al. (2003)\*. La Crosse County: Dickinson (1944) as Anopheles quadrimaculatus. Manitowoc County: Porter & Gojmerac (1970) as Anopheles quadrimaculatus. Milwaukee County: Meece et al. (2003)\*. Monroe County: Dickinson (1944) as Anopheles quadrimaculatus. Pepin County: Dickinson (1944) as Anopheles quadrimaculatus. Racine County: Dickinson (1944) as Anopheles quadrimaculatus; Meece et al. (2003)\*. Rock County: Dickinson (1944) as Anopheles quadrimaculatus. Sauk County: Dickinson (1944) as Anopheles quadrimaculatus. Vernon County: Dickinson (1944) as Anopheles quadrimaculatus. Vernon County: Dickinson (1944) as Anopheles quadrimaculatus. Walworth County: Dickinson (1944) as Anopheles quadrimaculatus. Walworth County: Dickinson (1944) as Anopheles quadrimaculatus. Walworth County: Dickinson (1944) as Anopheles quadrimaculatus. Waukesha County: Dickinson (1944) as Anopheles quadrimaculatus; Meece et al. (2003)\*. Waushara County: Howard et al. (1917) as Anopheles quadrimaculatus; Dyar (1922) as Anopheles quadrimaculatus; Dickinson (1944) as Anopheles quadrimaculatus.

Distribution for Anopheles quadrimaculatus s.s.: Buffalo County: WIRC (1939). Columbia County: WIRC (1941). Crawford County: WIRC (1961). Dane County: WIRC (1938, 1951, 1954). Grant County: WIRC (1941). Sauk County: WIRC (1941). Washburn County: WIRC (1951). Washington County: WIRC (1962). Winnebago County: WIRC (1941).

**Distribution for** *Anopheles smaragdinus*: **Buffalo County**: WIRC (1939). **Dane County**: WIRC (1938, 1954, 1966). **Grant County**: WIRC (1941). **Manitowoc County**: WIRC (1967). **Sauk County**: WIRC (1941).

Vernon County: WIRC (1941). Winnebago County: WIRC (1941).

#### Anopheles walkeri Theobald

This species has been collected consistently and all throughout the state since it was first reported by Dickinson (1944).

The larvae are reported to inhabit mostly marshes and to be closely associated with emergent vegetation (Carpenter & LaCasse, 1974).

Distribution for Anopheles walkeri: Barron County: WIRC (1941); Dickinson (1944). Buffalo County: WIRC (1939). Chippewa County: Dickinson (1944). Dane County: WIRC (1951, 1954, 1955, 1966); Meece et al. (2003)\*. Dodge County: WIRC (1941); Dickinson (1944). Forest County: Dickinson (1944); Siverly & DeFoliart (1968b). Grant County: Dickinson (1944); WIRC (1951). Jefferson County: WIRC (1941); Dickinson (1944). Kenosha County: Dickinson (1944); Amin & Hageman (1974); Meece et al. (2003)\*. La Crosse County: WIRC (1941); Dickinson (1944). Lincoln County: Siverly & DeFoliart (1968b). Manitowoc County: WIRC (1968); Porter & Gojmerac (1970). Marinette County: WIRC (1941); Dickinson (1944). Milwaukee County: Meece et al. (2003)\*. Monroe County: Dickinson (1944). Oneida County: Siverly & DeFoliart (1968b).
Polk County: Dickinson (1944). Racine County: Dickinson (1944); Meece et al. (2003)\*. Rock County: Dickinson (1944). Rusk County: WIRC (1952, 1968). Sauk County: Dickinson (1944). Sawyer County: WIRC (1941); Dickinson (1944). Sheboygan County: Dickinson (1944). Vilas County: WIRC (1941); Dickinson (1944). Walworth County: WIRC (1944); Dickinson (1944). Walworth County: WIRC (1944); Dickinson (1944). Washburn County: WIRC (1941, 1950, 1951, 1952).
Waukesha County: WIRC (1941); Dickinson (1944); Meece et al. (2003)\*.
Waupaca County: WIRC (1941); Dickinson (1944). Winnebago County: Dickinson (1944). Wood County: Dickinson (1944); Wright & DeFoliart (1970); Grimstad & DeFoliart (1975).

## Coquillettidia perturbans (Walker)

Together with *Anopheles quadrimaculatus, Coquillettidia perturbans* (under the name *Culex testaceus*) is one of the two first mosquito species reported for the state of Wisconsin (van der Wulp, 1867). This mosquito is widely distributed through the state and extremely abundant.

**Distribution for** *Coquillettidia perturbans*: Adams County: Dickinson (1944) as *Mansonia perturbans*. Brown County: Dickinson (1944) as *Mansonia perturbans*. Buffalo County: WIRC (1939); Dickinson (1944) as Mansonia perturbans. Crawford County: DeFoliart et al. (1972)\* as Mansonia perturbans. Dane County: van der Wulp (1867) as Culex testaceus; Howard et al. (1917) as Aëdes testaceus; Dickinson (1944) as Mansonia perturbans; WIRC (1950, 1951, 1952); Wright & DeFoliart (1970) as Mansonia perturbans; DeFoliart et al. (1972)\* as Mansonia perturbans; Meece et al. (2003)\*. Dodge County: Dickinson (1944) as Mansonia perturbans. **Door County:** Dickinson (1944) as Mansonia perturbans. **Douglas County:** Dickinson (1944) as *Mansonia perturbans*. Fond du Lac **County:** Dickinson (1944) as *Mansonia perturbans*. Forest County: Dickinson (1944) as *Mansonia perturbans*; Siverly & DeFoliart (1968b) as Mansonia perturbans. Grant County: Dickinson (1944) as Mansonia perturbans; DeFoliart et al. (1972)\* as Mansonia perturbans. Iowa County: Wright & DeFoliart (1970) as *Mansonia perturbans*. Iron County: Dickinson (1944) as Mansonia perturbans. Jefferson County: Dickinson (1944) as Mansonia perturbans. Juneau County: Dickinson (1944) as Mansonia perturbans. Kenosha County: Dickinson (1944) as Mansonia perturbans; Amin & Hageman (1974); Meece et al. (2003)\*. Langlade **County:** Dickinson (1944) as *Mansonia perturbans*. Lincoln County: Siverly & DeFoliart (1968b) as *Mansonia perturbans*; Anslow *et al.* (1969) as Mansonia perturbans. Manitowoc County: WIRC (1968); Porter & Gojmerac (1970) as Mansonia perturbans. Marinette County: Dickinson (1944) as Mansonia perturbans. Marquette County: Dickinson (1944) as Mansonia perturbans. Milwaukee County: Dickinson (1944) as Mansonia perturbans; Meece et al. (2003)\*. Monroe County: Dickinson (1944) as Mansonia perturbans. Oneida County: Siverly & DeFoliart (1968b) as Mansonia perturbans. Pierce County: Dickinson (1944) as Mansonia perturbans. Polk County: WIRC (1941); Dickinson (1944) as Mansonia perturbans. Racine County: Dickinson (1944) as Mansonia perturbans; Meece et al. (2003)\*. Rock County: Dickinson (1944) as Mansonia perturbans. Rusk County: WIRC (1951, 1952, 1966, 1968). Sauk County: Dickinson (1944) as Mansonia perturbans. Sawyer County: Dickinson (1944) as Mansonia perturbans. Sheboygan County: Dickinson (1944) as Mansonia perturbans. Vilas County: Dickinson (1944) as Mansonia perturbans. Walworth County: Dickinson (1944) as Mansonia perturbans; Wright & DeFoliart (1970) as *Mansonia perturbans*. Washburn County: Dickinson (1944) as Mansonia perturbans; WIRC (1950, 1951, 1952). Waukesha County: Dickinson (1944) as Mansonia perturbans; Meece et al. (2003)\*. Waupaca County: Dickinson (1944) as Mansonia perturbans. Waushara County: Howard et al. (1915) as Mansonia perturbans; Dyar (1922) as Mansonia perturbans; Dickinson (1944) as Mansonia perturbans. Winnebago County: Dickinson (1944) as Mansonia perturbans. Wood **County:** Grimstad & DeFoliart (1975).

### Culex erraticus (Dyar & Knab)

Meece *et al.* (2003) is the first literature report of this species in Wisconsin, however specimens exist in the WIRC that were collected in 1939 and 1962 in Wisconsin and are housed in the WIRC. This is the only representative of the subgenus *Melanoconion* Theobald in Wisconsin.

Distribution for *Culex erraticus*: Buffalo County: WIRC (1939). Dane County: Meece *et al.* (2003)\*. Kenosha County: Meece *et al.* (2003)\*. Milwaukee County: Meece *et al.* (2003)\*. Racine County: Meece *et al.* (2003)\*. Washington County: WIRC (1962). Waukesha County: Meece *et al.* (2003)\*. Meece *et al.* (2003)\*.

### Culex pipiens sensu lato Linnaeus

The *Culex pipiens* complex of mosquitoes is exceptionally challenging taxonomically. Fonseca *et al.* (2004) and Farajollahi *et al.* (2011) reviewed in great detail the situation of this taxon and it is clear that more research is needed in order to provide good characters for the separation of its units. It is unknown to us if *Culex quinquefasciatus* or hybrids of *Culex pipiens pipiens* and *Cx. quinquefasciatus* exist in Wisconsin, nor do we know if the state harbors hybrids of *Cx. pipiens pipiens* form pipiens *sensu* Farajollahi

et al. (2011) (=Cx. pipiens sensu Fonseca et al. (2004)) and Cx. pipiens pipiens form molestus (=Culex molestus sensu Fonseca et al. (2004)).

**Distribution for** *Culex pipiens* s.l.: Brown County: Dickinson (1944) as *Culex pipiens*. Columbia County: WIRC (1956). Crawford **County:** DeFoliart *et al.* (1972)\* as *Culex pipiens*. **Dane County:** Dickinson (1944) as Culex pipiens; WIRC (1949, 1950, 1952, 1954, 1989); Anslow et al. (1969) as Culex pipiens; Wright & DeFoliart (1970) as Culex pipiens; DeFoliart *et al.* (1972)\* as *Culex pipiens*; Grimstad & DeFoliart (1975) as *Culex pipiens*; Meece *et al.* (2003)\* as *Culex pipiens*. **Dodge County**: Dickinson (1944) as Culex pipiens. Grant County: DeFoliart et al. (1972)\* as *Culex pipiens*. **Iowa County:** Wright & DeFoliart (1970) as *Culex pipiens*. Jefferson County: Dickinson (1944) as Culex pipiens. Juneau County: Dickinson (1944) as *Culex pipiens*. Kenosha County: Dickinson (1944) as Culex pipiens; Amin & Hageman (1974) as Culex pipiens; Meece et al. (2003)\* as Culex pipiens. La Crosse County: Dickinson (1944) as Culex pipiens. Marathon County: Dickinson (1944) as Culex pipiens. Milwaukee **County:** Dickinson (1944) as *Culex pipiens*; Meece *et al.* (2003)\* as *Culex* pipiens. Monroe County: Dickinson (1944) as Culex pipiens. Racine **County:** Meece *et al.* (2003)\* as *Culex pipiens*. **Rock County:** Dickinson (1944) as *Culex pipiens*. **Rusk County:** WIRC (1952). **Vilas County:** Dickinson (1944) as Culex pipiens. Waukesha County: Meece et al. (2003)\*

as *Culex pipiens*. **Wood County:** Dickinson (1944) as *Culex pipiens*; Grimstad & DeFoliart (1975) as *Culex pipiens*.

# **Culex restuans** Theobald

The identification of *Culex restuans* has presented somewhat of a challenge because of its similarity to *Culex pipiens* s.l. in the adult female and given the poor taxonomic characterization of *Culex pipiens*. This has prevented the separation of the two taxa in the adult stage. To address this challenge, we conducted an in-depth analysis of adult female characters and found informative characters to separate these two species (see Chapter 6).

Distribution for *Culex restuans*: Barron County: Dickinson (1944). Bayfield County: Dickinson (1944). Buffalo County: Dickinson (1944). Chippewa County: Dickinson (1944). Columbia County: Dickinson (1944); WIRC (1956, 1957). Crawford County: DeFoliart *et al.* (1972)\*. Dane County: Dickinson (1944); WIRC (1949, 1950, 1951, 1952, 1954, 1956, 1989); Anslow *et al.* (1969); Wright & DeFoliart (1970); DeFoliart *et al.* (1972)\*; Grimstad & DeFoliart (1975); Meece *et al.* (2003)\*. Dodge County: Dickinson (1944). Door County: Dickinson (1944). Fond du Lac County: Dickinson (1944). Forest County: Siverly & DeFoliart

(1968b). Grant County: Dickinson (1944); DeFoliart et al. (1972)\*. Iowa County: Wright & DeFoliart (1970). Jefferson County: Dickinson (1944). Juneau County: Dickinson (1944). Kenosha County: Dickinson (1944); Meece et al. (2003)\*. La Crosse County: Dickinson (1944). Lafayette County: Dickinson (1944); Ryckman (1952). Lincoln County: Siverly & DeFoliart (1968b). Manitowoc County: WIRC (1968); Porter & Gojmerac (1970); Grimstad & DeFoliart (1975). Marinette County: Dickinson (1944). Milwaukee County: Meece et al. (2003)\*. Monroe County: Dickinson (1944). Oneida County: Siverly & DeFoliart (1968b). Pepin County: Dickinson (1944). Racine County: Dickinson (1944); Meece et al. (2003)\*. Rock County: Dickinson (1944). Rusk County: WIRC (1951, 1952, 1968). Sauk County: Dickinson (1944). Sheboygan County: Dickinson (1944). Vernon County: Dickinson (1944). Vilas County: Dickinson (1944). Walworth County: Dickinson (1944); Wright & DeFoliart (1970). Washburn County: Dickinson (1944); WIRC (1951, 1952). Waukesha County: Dickinson (1944); Meece et al. (2003)\*. Winnebago County: Dickinson (1944). Wood County: Dickinson (1944); Grimstad & DeFoliart (1975).

## **Culex salinarius Coquillett**

This species has been collected consistently in the state, but mostly in the southern counties. Although this could be a function of sampling bias given that southern Wisconsin was historically sampled more extensively than northern Wisconsin, the fact that this species does not occur in Canada lends credibility to the hypothesis that *Culex salinarius* is mostly a southern species in the state of Wisconsin.

Distribution for Culex salinarius: Buffalo County: Dickinson (1944). Chippewa County: Dickinson (1944). Columbia County: WIRC (1957). Crawford County: DeFoliart et al. (1972)\*. Dane County: Dickinson (1944); WIRC (1950, 1951, 1952, 1954, 1955, 1956, 1966, 1986); Anslow et al. (1969); Wright & DeFoliart (1970); DeFoliart et al. (1972)\*; Meece et al. (2003)\*. Dodge County: Dickinson (1944). Grant County: DeFoliart et al. (1972)\*. Green County: Dickinson (1944). Green Lake County: Dickinson (1944). Iowa County: Wright & DeFoliart (1970). Jefferson County: Dickinson (1944). Juneau County: Dickinson (1944). Kenosha County: Meece et al. (2003)\*. La Crosse County: Dickinson (1944). Lafayette County: Ryckman (1952). Marquette County: Dickinson (1944). Milwaukee County: Meece et al. (2003)\*. Monroe County: Dickinson (1944). Pepin County: Dickinson (1944). Polk County:

WIRC (1941); Dickinson (1944). Racine County: Dickinson (1944); Meece et al. (2003)\*. Rock County: WIRC (1957). Sheboygan County: Dickinson (1944). Taylor County: Dickinson (1944). Walworth County: Dickinson (1944). Washburn County: WIRC (1951). Waukesha County: Meece et al. (2003)\*. Waupaca County: Dickinson (1944). Winnebago County: Dickinson (1944). Wood County: Dickinson (1944); Grimstad & DeFoliart (1975).

### Culex tarsalis Coquillett

Collections of this species are somewhat scarce, giving the impression this is a fairly uncommon mosquito in Wisconsin, with only five literature mentions. The species is only fairly common (around 3.5% of the specimens) in the study by Meece *et al.* (2003), and it is very rare in both Siverly & DeFoliart (1968b) (<0.01%) and DeFoliart *et al.* (1972) (<0.1%) studies (Dickinson (1944) and Ryckman (1952) don't give any information on abundance); furthermore it comprises only around 2.5% of the mosquito specimens from Wisconsin at the WIRC, with around the same representation as other uncommon species such as *Ochlerotatus intrudens* and *Culiseta morsitans*, for example. It is certainly pertinent to notice however this species is supposedly very abundant in other parts of the country (Jenkins, 1950; Carpenter & LaCasse, 1974), including states very close to Wisconsin, *e.g.*: Iowa (Dunphy *et al.*, 2014).

Distribution for Culex tarsalis: Buffalo County: WIRC (1939). Columbia County: Dickinson (1944). Crawford County: DeFoliart et al. (1972)\*. Dane County: WIRC (1939, 1950, 1951, 1952, 1954); Dickinson (1944); DeFoliart et al. (1972)\*; Meece et al. (2003)\*. Dunn County: Dickinson (1944). Forest County: Siverly & DeFoliart (1968b). Grant County: DeFoliart et al. (1972)\*. Juneau County: Dickinson (1944). Kenosha County: Meece et al. (2003)\*. La Crosse County: Dickinson (1944). Lafayette County: Dickinson (1944); Ryckman (1952). Lincoln County: Siverly & DeFoliart (1968b). Milwaukee County: Dickinson (1944); Meece et al. (2003)\*. Monroe County: Dickinson (1944). Oneida County: Siverly & DeFoliart (1968b). Pepin County: Dickinson (1944). Pierce County: Dickinson (1944). Polk County: WIRC (1941); Dickinson (1944). Racine County: Meece et al. (2003)\*. Rock County: Dickinson (1944). Rusk County: WIRC (1952, 1968). Vernon County: Dickinson (1944). Washburn County: WIRC (1941, 1950); Dickinson (1944). Waukesha County: Meece et al. (2003)\*. Winnebago County: Dickinson (1944). Wood County: Dickinson (1944).

### **Culex territans Walker and Culex apicalis Adams**

Bohart revised the species previously known as *Culex apicalis* and restricted the name to forms known to him from California and Arizona (1948). He resurrected the name *Culex territans* and assigned it to forms found all over the northwest and midwest of the United States, he also described two new species, one from Arizona and another from California. Although Bohart only analyzed forms from Iowa, Michigan and Minnesota, from the Midwest, it is safe to assume that Dickinson's Cx. apicalis (Dickinson, 1944) is in fact *Cx. territans*. Later Ryckman (1952) also reports Cx. apicalis in Wisconsin; unfortunately no details are provided for the means of identification, preventing us from knowing whether the author has identified Cx. apicalis sensu Bohart (1948) or if his keys were simply outdated. Because there has been no other report of Cx. apicalis since Ryckman (1952) and there are no vouchers of this species in the WIRC, we assume that it is more likely that Ryckman had a specimen of *Cx. territans*. *Culex apicalis* is not considered as a member of the mosquito fauna of Wisconsin.

Another important note is that Dyar (1922) used the name *Culex testaceus* while referring to *Cx. territans*; this is visible by his synonymy of the name *Cx. apicalis* under the name *Cx. testaceus*. This is simply wrong,

as the name Cx. testaceus is a synonymy of Coquillettidia perturbans. Furthermore Dyar used the name Cx. territans to refer to Culex restuans (1922).

It should be noted that the larvae of Cx. territans collected in Dane County display a lack of pigmentation of the abdominal segment IV, which contrasts with the pigmentation of segments III and V, giving the larvae a banded look. This character is primarily associated with the species *Culex reevesi* and *Culex boharti*, with Cx. territans described as having the segment IV with the same pigmentation as segments III and V. Despite that, the paler abdominal segment IV character can appear in Cx. territans specimens from several different parts of the US (Bickley & Harrison, 1989). To confirm that the specimens we collected were in fact Cx. territans we slide mounted male genitalia from males emerged from reared larvae collected at the University of Wisconsin-Madison Arboretum, all of which presented the paler segment IV. It is unknown how prevalent this character is in Cx. territans populations from Wisconsin.

*Culex territans* is the only representative of the subgenus *Neoculex* Dyar in Wisconsin.

**Distribution for** *Culex territans*: **Barron County**: WIRC (1950). **Buffalo County**: WIRC (1939); Dickinson (1944) as *Culex apicalis*. **Burnett** 

**County:** Dickinson (1944) as *Culex apicalis*. **Chippewa County:** Dickinson (1944) as Culex apicalis. Columbia County: WIRC (1957). Dane County: WIRC (1939, 1948, 1949, 1950, 1951, 1952, 1954, 1955, 1956). Florence County: Dickinson (1944) as Culex apicalis. Fond du Lac County: Dickinson (1944) as *Culex apicalis*. Forest County: Dickinson (1944) as Culex apicalis; Siverly & DeFoliart (1968b). Grant County: Dickinson (1944) as Culex apicalis. Iron County: Dickinson (1944) as Culex apicalis. Jackson County: Dickinson (1944) as Culex apicalis. Jefferson County: Dickinson (1944) as Culex apicalis. Juneau County: Dickinson (1944) as Culex apicalis. Kenosha County: Dickinson (1944) as Culex apicalis. La **Crosse County:** Dickinson (1944) as *Culex apicalis*. Lafayette County: Ryckman (1952) as Culex apicalis. Lincoln County: Siverly & DeFoliart (1968b). Manitowoc County: WIRC (1968); Porter & Gojmerac (1970); Grimstad & DeFoliart (1975). Marinette County: Dickinson (1944) as Culex apicalis. Monroe County: Dickinson (1944) as Culex apicalis. **Oneida County:** Siverly & DeFoliart (1968a); Siverly & DeFoliart (1968b). **Pepin County:** Dickinson (1944) as *Culex apicalis*. **Racine County:** Dickinson (1944) as *Culex apicalis*. **Rock County:** Dickinson (1944) as Culex apicalis. Rusk County: Dickinson (1944) as Culex apicalis; WIRC (1951, 1952, 1968). Sauk County: Dickinson (1944) as Culex apicalis. Sawyer County: Dickinson (1944) as Culex apicalis. Sheboygan County:

Dickinson (1944) as *Culex apicalis*. **Vernon County:** Dickinson (1944) as *Culex apicalis*. **Vilas County:** WIRC (1941, 1949); Dickinson (1944) as *Culex apicalis*; Siverly & DeFoliart (1968a). **Walworth County:** Dickinson (1944) as *Culex apicalis*. **Washburn County:** Dickinson (1944) as *Culex apicalis*; WIRC (1951, 1952, 1953). **Waukesha County:** Dickinson (1944) as *Culex apicalis*. **Waupaca County:** Dickinson (1944) as *Culex apicalis*. **Winnebago County:** Dickinson (1944) as *Culex apicalis*. **Winnebago County:** Dickinson (1944) as *Culex apicalis*. **Wood County:** Dickinson (1944) as *Culex apicalis*. **Winnebago County:** Dickinson (1944) as *Culex apicalis*.

### Culiseta impatiens (Walker)

Although this species has only been reported once for Wisconsin (Dickinson, 1944), it has been collected in several counties in the state of Wisconsin throughout the years, based on the specimens in the WIRC.

**Distribution for** *Culiseta impatiens*: **Dane County**: Dickinson (1944) as *Culicella impatiens*; WIRC (1952, 1954). **Marinette County**: Dickinson (1944) as *Culicella impatiens*. **Monroe County**: Dickinson (1944) as *Culicella impatiens*. **Monroe County**: Dickinson (1944) as *Culicella impatiens*. **Rusk County**: WIRC (1952, 1957). **Taylor County**: Dickinson (1944) as *Culicella impatiens*. **Vilas County**: Dickinson (1944) as *Culicella impatiens*. **Vilas County**: Dickinson (1944) as *Culicella impatiens*. **Vilas County**: Dickinson (1944) as

(1944) as *Culicella impatiens*; WIRC (1955). Washburn County: WIRC (1953).

# Culiseta inornata (Williston)

Of all the *Culiseta* in the state of Wisconsin, *Cs. inornata* is the most common, and, together with *Cs. impatiens*, represents the only members of subgenus *Culiseta* in the state.

Distribution for Culiseta inornata: Barron County: Dickinson (1944) as Culicella inornata. Burnett County: Dickinson (1944) as Culicella inornata. Chippewa County: Dickinson (1944) as Culicella inornata. Columbia County: Dickinson (1944) as Culicella inornata; WIRC (1956, 1957). Dane County: Howard et al. (1915) as Culiseta inornatus; Dyar (1922) as Culiseta inornatus; Dickinson (1944) as Culicella inornata; WIRC (1948, 1949, 1950, 1951, 1952, 1953, 1954, 1955); Anslow et al. (1969); Wright & DeFoliart (1970); Meece et al. (2003)\*. Dodge County: Dickinson (1944) as Culicella inornata. Forest County: Siverly & DeFoliart (1968b). Iowa County: Wright & DeFoliart (1970). Jackson County: Anslow et al. (1969). Juneau County: Dickinson (1944) as Culicella inornata.

Amin & Hageman (1974); Meece et al. (2003)\*. La Crosse County: Dickinson (1944) as *Culicella inornata*. Lafayette County: Ryckman (1952). Lincoln County: Siverly & DeFoliart (1968b). Manitowoc County: WIRC (1968); Porter & Gojmerac (1970); Grimstad & DeFoliart (1975). Milwaukee County: Meece et al. (2003)\*. Monroe County: Dickinson (1944) as Culicella inornata. Oneida County: Siverly & DeFoliart (1968b). Outagamie County: WIRC (1954). Pepin County: Dickinson (1944) as Culicella inornata. Polk County: Dickinson (1944) as Culicella inornata. Racine County: Meece et al. (2003)\*. Rock County: Dickinson (1944) as Culicella inornata. Rusk County: WIRC (1951, 1952, 1953, 1954, 1957, 1968, 1989). Sauk County: Dickinson (1944) as Culicella inornata; WIRC (1956). Vernon County: Dickinson (1944) as Culicella inornata. Walworth County: WIRC (1955); Wright & DeFoliart (1970). Washburn County: Dickinson (1944) as Culicella inornata; WIRC (1950, 1951). Waukesha County: Meece et al. (2003)\*. Wood County: Dickinson (1944) as Culicella inornata.

### Culiseta melanura (Coquillett)

Extremely rare, the type of the subgenus *Climacura* is also the only representative of it in Wisconsin.

Distribution for *Culiseta melanura*: Dane County: Dickinson (1944) as *Culicella melanura*; WIRC (1954). Forest County: Siverly & DeFoliart (1968a); Siverly & DeFoliart (1968b). Lincoln County: Siverly & DeFoliart (1968b). Oneida County: Siverly & DeFoliart (1968b). Rusk County: WIRC (1952, 1968). Washburn County: WIRC (1951).

# Culiseta minnesotae Barr and Culiseta morsitans (Theobald)

From the two representatives of the subgenus *Culicella* in Wisconsin, *Cs. minnesotae* is the most recent name, described in 1957 by Barr and reported for the first time in the state by Siverly & DeFoliart (1968a). *Cs. morsitans* has been known to be present in the state since 1915, reported as *Culex dyari* (Howard *et al.*, 1915). It is not possible to say if any specimens reported as *Cs. morsitans* before 1957 are actually *Cs. minnesotae*, if any exist, the numbers are most likely very low, because *Cs. minnesotae* is very rare in most of Wisconsin, and the two species don't bear much resemblance to the trained eye. It may be of interest to note *Cs. minnesotae* was fairly common in Verona, Dane County, according to specimens collected for surveillance efforts between July and August of 2017 (unpublished).

Distribution for Culiseta minnesotae: Dane County: WIRC (1951, 1952, 1953, 1954, 1955). Forest County: Siverly & DeFoliart (1968b) as Culiseta silvestris minnesotae; Siverly & DeFoliart (1968a) as Culiseta silvestris minnesotae. Lincoln County: Siverly & DeFoliart (1968b) as Culiseta silvestris minnesotae. Manitowoc County: Grimstad & DeFoliart (1975) as Culiseta silvestris minnesotae. Oneida County: Siverly & DeFoliart (1968b) as Culiseta silvestris minnesotae. Rusk County: WIRC (1952, 1968). Washburn County: WIRC (1950, 1951, 1952, 1953). Wood County: Grimstad & DeFoliart (1975) as Culiseta & DeFoliart (1975) as Culiseta & Silvestris minnesotae.

Distribution for *Culiseta morsitans*: Crawford County: DeFoliart et al. (1972)\*. Dane County: WIRC (1951, 1952, 1954, 1955); Wright & DeFoliart (1970); DeFoliart et al. (1972)\*; Meece et al. (2003)\*. Forest County: Siverly & DeFoliart (1968b). Grant County: DeFoliart et al. (1972)\*. Iowa County: Wright & DeFoliart (1970). Kenosha County: Meece et al. (2003)\*. Lincoln County: Siverly & DeFoliart (1968a); Siverly & DeFoliart (1968b). Manitowoc County: WIRC (1968); Porter & Gojmerac (1970); Grimstad & DeFoliart (1975). Milwaukee County: Dickinson (1944) as *Culicella dyari*; Meece et al. (2003)\*. Oneida County: Siverly & DeFoliart (1968a); Siverly & DeFoliart (1968b). Polk County: Dickinson (1944) as *Culicella dyari*. Racine County: Meece et al. (2003)\*. Rusk County: WIRC (1951, 1952, 1968). Vilas County: Siverly & DeFoliart (1968a). Walworth County: Wright & DeFoliart (1970). Washburn
County: Dickinson (1944) as *Culicella dyari*; WIRC (1951, 1952).
Waukesha County: Meece *et al.* (2003)\*. Waushara County: Howard *et al.* (1915) as *Culex dyari*; Dyar (1922) as *Culex dyari*; Dickinson (1944) as *Culicella dyari*; Dickinson (1944) as *Culicella dyari*; Dickinson (1944) as *Culicella parodites*.

## Georgecraigius atropalpus (Coquillett) [=Aedes atropalpus]

Despite poor representation both in literature and in the WIRC material, this species seems to be widespread in the state, including urban environments, as it was captured in water from tires in Wisconsin during the summer of 2017 (data unpublished). The natural larval development habitat for this species is rock pools, usually in or near bodies of permanent water, mostly streams or rivers; but may be found in rock pools filled by rain water, relatively far from streams (Carpenter & LaCasse, 1974).

Although this mosquito species may have a predisposition to colonize artificial containers, it is clear from historical collections and descriptions that it favors rock pools. The same is true for the mosquito *Hulecoeteomyia japonica japonica* (Tanaka *et al.*, 1979). Although more research is needed to access the frequency of this phenomenon, there is evidence that the establishment of *Hl. japonica japonica* can displace *Gc. atropalpus* from its natural habitat (Armistead *et al.*, 2008); this could lead to an increase in invasion of artificial containers over time.

**Distribution for** *Georgecraigius atropalpus*: Chippewa County: WIRC (1941); Dickinson (1944) as *Aedes atropalpus*. Waushara County: Howard *et al.* (1917) as *Aëdes atropalpus*.

# Hulecoeteomyia japonica japonica (Theobald) [=Aedes japonicus japonicus]

Hughes *et al.* (2008) published the first report of this species in Dane County. More recently, Richards *et al.* regularly collected this species in ovipositional traps in fifteen additional counties (Richards *et al.* 2019 and unpublished).

Distribution for Hulecoeteomyia japonica japonica: Buffalo County: Richards et al. (2019) as Aedes japonicus japonicus. Dane County: Hughes et al. (2008) as Aedes japonicus japonicus; Richards et al. (2019) as Aedes japonicus japonicus. Eau Claire County: Richards et al. (2019) as Aedes japonicus japonicus. Grant County: Richards et al. (2019) as Aedes japonicus japonicus. Green County: Richards et al. (2019) as Aedes japonicus japonicus. Iowa County: Richards et al. (2019) as Aedes japonicus japonicus. Jefferson County: Richards et al. (2019) as Aedes japonicus japonicus. Kenosha County: Richards et al. (2019) as Aedes japonicus japonicus. La Crosse County: Richards et al. (2019) as Aedes japonicus japonicus. Lafayette County: Richards et al. (2019) as Aedes japonicus japonicus. Milwaukee County: Richards et al. (2019) as Aedes japonicus japonicus. Milwaukee County: Richards et al. (2019) as Aedes japonicus japonicus. Monroe County: Richards et al. (2008) as Aedes japonicus japonicus. Racine County: Richards et al. (2019) as Aedes japonicus japonicus. Rock County: Richards et al. (2019) as Aedes japonicus japonicus. Vernon County: Richards et al. (2019) as Aedes japonicus japonicus. Walworth County: Richards et al. (2019) as Aedes japonicus japonicus. Walworth County: Richards et al. (2019) as Aedes japonicus japonicus. Walworth County: Richards et al. (2019) as Aedes

#### **Ochlerotatus** Lynch Arribálzaga species

The Wisconsin mosquito fauna consists of 21 species of *Ochlerotatus*, representing just above 40% of its total composition. In comparison Iowa has 16 species, representing just under 30% of the state's composition. This difference is most likely due to habitat suitability. Considering there are a number of species of *Ochlerotatus* associated with snow pools, including the Punctor group, most Communis group species, many Stimulans group species and the species of the subgenus *Woodius*, which is one of the two subgenera of *Ochlerotatus* that occurs in Wisconsin but not Iowa (the other being the subgenus *Rusticoidus*). Similarly, the subgenus *Protoculex* of *Ochlerotatus* is the only of its genus that occurs in Iowa but not in Wisconsin, represented by one of the four species present in the Nearctic; this subgenus is mostly tropical and fewer species are present in subtropical areas. In the U.S.A. the distribution is mostly confined to the southeastern region.

# Ochlerotatus abserratus (Felt & Young) [=Aedes abserratus] and Ochlerotatus punctor (Kirby) [=Aedes punctor]

These two species are the only known representatives of the Punctor subgroup in Wisconsin thus far. The subgroup presents incredible difficulties in terms of morphological characterization. There are no current characters available to separate adult females of *Ochlerotatus punctodes* and, at best, contentious characters for the separation of adult females of all of the other species including, *Ochlerotatus aboriginis, Ochlerotatus abserratus, Ochlerotatus hexodontus, Ochlerotatus punctor*. The species of the group are more readily identified in the larval stage with the characters given by Carpenter & LaCasse (1974) and Gimnig (2000), with exception of *Oc. punctodes* which may present overlapping characters with *Oc. punctor*. The male genitalia is useful for separating *Oc. aboriginis* + *Oc. hexodontus* + *Oc. punctor* from *Oc. abserratus* + *Oc. punctodes* (the species within each of the two presented "clusters" are indistinguishable from each other) (Carpenter & LaCasse, 1974).

Siverly & DeFoliart (1968a), Porter & Gojmerac (1970) and Gilardi & Hilsenhoff (1992) used larval identifications and Porter & Gojmerac (1970) used male genitalia characters to confirm the presence of both Oc. abservatus and Oc. punctor in the state. Based on this, from available adult female characters presented in Carpenter & LaCasse (1974) redescriptions, we used the presence of a stripe of reddish brown scales along the median line of the mesonotum, sharply contrasting with golden scaling along the median area of the scutal fossa to identify Oc. abserratus. As for the identification of *Oc. punctor* we observed a mostly unicolorous mesonotum, or if a darker area was present near the midline, the stripe not sharply defined (the character presents itself as a less striking difference in coloration of the scales of the midline and scutal fossa). These characters are, of course, not ideal, because Zavortink (1991) showed 'bleaching' of scales due to light exposure *post mortem* can further complicate these types of characterizations. Current recognized characters for separating the other

female species are equally unreliable. Until a revision of the Punctor Subgroup is performed, and a better characterization of its species is presented, we may never truly know the true composition of the subgroup or the distribution of its species in Wisconsin and beyond.

Distribution for Ochlerotatus abserratus: Bayfield County: Gilardi & Hilsenhoff (1992) as Aedes abserratus. Buffalo County: Gilardi & Hilsenhoff (1992) as Aedes abserratus. Burnett County: Gilardi & Hilsenhoff (1992) as Aedes abserratus. Dane County: Meece et al. (2003)\*. Florence County: Gilardi & Hilsenhoff (1992) as Aedes abserratus. Fond du Lac County: Gilardi & Hilsenhoff (1992) as Aedes abserratus. Forest **County:** Siverly & DeFoliart (1968b) as *Aedes abserratus*; Siverly & DeFoliart (1968a) as Aedes abserratus; Gilardi & Hilsenhoff (1992) as Aedes abserratus. Jefferson County: Gilardi & Hilsenhoff (1992) as Aedes abserratus. Kenosha County: Meece et al. (2003)\*. Lincoln County: Siverly & DeFoliart (1968b) as Aedes abserratus; Siverly & DeFoliart (1968a) as Aedes abserratus; Gilardi & Hilsenhoff (1992) as Aedes abserratus. Manitowoc County: WIRC (1968); Porter & Gojmerac (1970) as Aedes abserratus. Marinette County: Gilardi & Hilsenhoff (1992) as Aedes abserratus. Marquette County: Gilardi & Hilsenhoff (1992) as Aedes abserratus. Milwaukee County: Meece et al. (2003)\*. Oneida **County:** Siverly & DeFoliart (1968b) as *Aedes abserratus*. **Price County:** 

Gilardi & Hilsenhoff (1992) as Aedes abserratus. Racine County: Meece et al. (2003)\*. Richland County: Gilardi & Hilsenhoff (1992) as Aedes abserratus.
Rock County: Gilardi & Hilsenhoff (1992) as Aedes abserratus.
Rusk County: WIRC (1968). Sheboygan County: Gilardi & Hilsenhoff (1992) as Aedes abserratus.
(1992) as Aedes abserratus. Waukesha County: Meece et al. (2003)\*.
Waushara County: Gilardi & Hilsenhoff (1992) as Aedes abserratus. Wood
County: Wright & DeFoliart (1970) as Aedes abserratus.

Distribution for Ochlerotatus punctor: Bayfield County: Gilardi & Hilsenhoff (1992) as Aedes punctor. Burnett County: Gilardi & Hilsenhoff (1992) as Aedes punctor. Columbia County: Dickinson (1944) as Aedes implacabilis. Dane County: WIRC (1954). Florence County: Gilardi & Hilsenhoff (1992) as Aedes punctor. Forest County: Siverly & DeFoliart (1968b) as Aedes punctor; Siverly & DeFoliart (1968a) as Aedes punctor; Gilardi & Hilsenhoff (1992) as Aedes punctor. Lincoln County: Dickinson (1944) as Aedes implacabilis; Siverly & DeFoliart (1968a) as Aedes punctor; Siverly & DeFoliart (1968b) as Aedes punctor; Gilardi & Hilsenhoff (1992) as Aedes punctor. Lincoln County: Dickinson (1944) as Aedes implacabilis; Siverly & DeFoliart (1968a) as Aedes punctor; Siverly & DeFoliart (1968b) as Aedes punctor; Gilardi & Hilsenhoff (1992) as Aedes punctor. Manitowoc County: WIRC (1968); Porter & Gojmerac (1970) as Aedes punctor. Marinette County: Gilardi & Hilsenhoff (1992) as Aedes punctor. Marinette County: Gilardi & Hilsenhoff (1992) as Aedes punctor. Siverly & DeFoliart (1968b) as Aedes punctor (1968b); Porter & Gojmerac (1970) as Aedes punctor. Marinette County: Gilardi & Hilsenhoff (1992) as Aedes punctor. Marinette County: Gilardi & Hilsenhoff (1992) as Aedes punctor. Siverly & DeFoliart (1968b) as Aedes punctor.

Pepin County: Gilardi & Hilsenhoff (1992) as Aedes punctor. Price County: Gilardi & Hilsenhoff (1992) as Aedes punctor. Richland County: Gilardi & Hilsenhoff (1992) as Aedes punctor. Rock County: WIRC (1980); Gilardi & Hilsenhoff (1992) as Aedes punctor. Rusk County: WIRC (1952, 1954, 1966, 1968, 1969, 1986). Sheboygan County: Gilardi & Hilsenhoff (1992) as Aedes punctor. Vilas County: Dickinson (1944) as Aedes implacabilis; Siverly & DeFoliart (1968a) as Aedes punctor. Waukesha County: Dickinson (1944) as Aedes implacabilis. Waushara County: Dyar (1922) as Aëdes dysanor; Dyar (1922) as Aëdes punctor; Dickinson (1944) as Aedes punctor; Dickinson (1944) as Aedes implacabilis. Wood County: Wright & DeFoliart (1970) as Aedes punctor; Grimstad & DeFoliart (1975) as Aedes punctor; Gilardi & Hilsenhoff (1992) as Aedes punctor.

# Ochlerotatus aurifer (Coquillett) [=Aedes aurifer]

This species is seemingly rare throughout the state, with scattered records and few voucher specimens in the WIRC.

**Distribution for** *Ochlerotatus aurifer*: **Bayfield County**: Gilardi & Hilsenhoff (1992) as *Aedes aurifer*. **Columbia County**: Dickinson (1944) as *Aedes aurifer*. **Dane County**: Dickinson (1944) as *Aedes aurifer*. **Fond du** 

Lac County: Dickinson (1944) as Aedes aurifer. Forest County: Siverly & DeFoliart (1968b) as Aedes aurifer. Grant County: Dickinson (1944) as Aedes aurifer. Juneau County: Dickinson (1944) as Aedes aurifer. Lincoln County: Siverly & DeFoliart (1968a) as Aedes aurifer; Siverly & DeFoliart (1968b) as Aedes aurifer. Oneida County: Siverly & DeFoliart (1968b) as Aedes aurifer. Rusk County: WIRC (1966). Sheboygan County: Dickinson (1944) as Aedes aurifer. Walworth County: Gilardi & Hilsenhoff (1992) as Aedes aurifer. Washburn County: WIRC (1952). Winnebago County: Dickinson (1944) as Aedes aurifer. Wood County: Wright & DeFoliart (1970) as Aedes aurifer; Grimstad & DeFoliart (1975) as Aedes aurifer.

# Ochlerotatus campestris (Dyar & Knab) [=Aedes campestris] and Ochlerotatus dorsalis (Meigen) [=Aedes dorsalis]

Only two records exist of *Ochlerotatus campestris* (Dickinson, 1944; Gilardi & Hilsenhoff, 1992), with no vouchers found in the WIRC. It is plausible that the authors had all of the information needed to separate it from the morphologically similar species, *Ochlerotatus dorsalis*. It is possible that this is an extremely rare mosquito in the state. In their description of the bionomics of *Oc. campestris* Carpenter & LaCasse suggest that the species is more abundant in Canada than in the United States (1974).

*Ochlerotatus dorsalis* was regularly collected in Wisconsin and it is well represented in the WIRC. It is also a flood water mosquito, as opposed to *Oc. campestris*, which is a snow pool mosquito. This likely contributes to its abundance as compared to *Oc. campestris*.

**Distribution for Ochlerotatus campestris: Bayfield County:** Gilardi & Hilsenhoff (1992) as Aedes campestris. **Dane County:** Dickinson (1944) as Aedes campestris. **Sheboygan County:** Dickinson (1944) as Aedes campestris.

Distribution for Ochlerotatus dorsalis: Crawford County: DeFoliart et al. (1972)\* as Aedes dorsalis. Dane County: Howard et al. (1917) as Aëdes curriei; Dyar (1922) as Aëdes dorsalis; WIRC (1930, 1941, 1951, 1952, 1954, 1955, 1956); Dickinson (1944) as Aedes dorsalis; DeFoliart et al. (1972)\* as Aedes dorsalis; Meece et al. (2003)\*. Grant County: DeFoliart et al. (1972)\* as Aedes dorsalis. Jefferson County: WIRC (1941); Dickinson (1944) as Aedes dorsalis. Kenosha County: Amin & Hageman (1974) as Aedes dorsalis; Meece et al. (2003)\*. Milwaukee County: Meece et al. (2003)\*. Racine County: Meece et al. (2003)\*. Rusk County: WIRC (1951, 1952, 1959). Sheboygan County: WIRC (1941). Waukesha County: Meece et al. (2003)\*.

# Ochlerotatus canadensis canadensis (Theobald) [=Aedes canadensis canadensis]

Ochlerotatus canadensis canadensis is very abundant late winter and early spring and its numbers decline significantly during the rest of the year (unpublished data), a finding that agrees with the description of the species' bionomics (Carpenter & LaCasse, 1974). This is the only representative of the subgenus *Culicada* in the state.

Distribution for Ochlerotatus canadensis canadensis: Bayfield County: Gilardi & Hilsenhoff (1992) as Aedes canadensis. Burnett County: Gilardi & Hilsenhoff (1992) as Aedes canadensis. Columbia County: Dickinson (1944) as Aedes canadensis; WIRC (1956). Crawford County: DeFoliart et al. (1972)\* as Aedes canadensis. Dane County: Dickinson (1944) as Aedes canadensis; WIRC (1949, 1950, 1951, 1952, 1954); Wright & DeFoliart (1970) as Aedes canadensis; DeFoliart et al. (1972)\* as Aedes canadensis; Grimstad & DeFoliart (1975) as Aedes canadensis; Meece et al. (2003)\* as Ochlerotatus canadensis. Dodge County: Dickinson (1944) as Aedes canadensis. Bunn County: Gilardi & Hilsenhoff (1992) as Aedes canadensis. Florence County: Gilardi & Hilsenhoff (1992) as Aedes canadensis. Forest County: Dickinson (1944) as Aedes canadensis; Siverly & DeFoliart (1968b) as Aedes canadensis; Siverly & DeFoliart (1968a) as Aedes canadensis; Gilardi & Hilsenhoff (1992) as Aedes canadensis. Grant **County:** DeFoliart *et al.* (1972)\* as Aedes canadensis. **Iowa County:** Loor & DeFoliart (1970)\* as Aedes canadensis; Wright & DeFoliart (1970) as Aedes canadensis. Jackson County: Anslow et al. (1969) as Aedes canadensis. Jefferson County: Gilardi & Hilsenhoff (1992) as Aedes canadensis. Kenosha County: Dickinson (1944) as Aedes canadensis; Meece et al. (2003)\* as Ochlerotatus canadensis. Lincoln County: WIRC (1951); Siverly & DeFoliart (1968b) as Aedes canadensis; Siverly & DeFoliart (1968a) as Aedes canadensis; Gilardi & Hilsenhoff (1992) as Aedes canadensis. Manitowoc County: WIRC (1968); Porter & Gojmerac (1970) as Aedes canadensis; Grimstad & DeFoliart (1975) as Aedes canadensis. Marinette County: Gilardi & Hilsenhoff (1992) as Aedes canadensis. Marquette County: Gilardi & Hilsenhoff (1992) as Aedes canadensis. Milwaukee County: Meece et al. (2003)\* as Ochlerotatus canadensis. Oneida County: Siverly & DeFoliart (1968a) as Aedes canadensis; Siverly & DeFoliart (1968b) as Aedes canadensis. Pepin County: Gilardi & Hilsenhoff (1992) as Aedes canadensis. Price County: Gilardi & Hilsenhoff (1992) as Aedes canadensis. Racine County: Dickinson (1944) as Aedes canadensis; Meece et al. (2003)\* as Ochlerotatus canadensis. Richland County: Gilardi & Hilsenhoff (1992) as Aedes canadensis. Rock County: Dickinson (1944) as Aedes canadensis; WIRC

(1960); Gilardi & Hilsenhoff (1992) as Aedes canadensis. Rusk County: WIRC (1951, 1952, 1966, 1968, 1969, 1973). Sauk County: Gilardi & Hilsenhoff (1992) as *Aedes canadensis*. **Sheboygan County:** WIRC (1941); Dickinson (1944) as Aedes canadensis; Gilardi & Hilsenhoff (1992) as Aedes canadensis. Taylor County: Dickinson (1944) as Aedes canadensis. Vilas **County:** Dickinson (1944) as *Aedes canadensis*; Siverly & DeFoliart (1968a) as Aedes canadensis. Walworth County: Dickinson (1944) as Aedes canadensis; Wright & DeFoliart (1970) as Aedes canadensis; Gilardi & Hilsenhoff (1992) as Aedes canadensis. Washburn County: WIRC (1950, 1951, 1952). Waukesha County: Meece et al. (2003)\* as Ochlerotatus canadensis. Waupaca County: WIRC (1941); Dickinson (1944) as Aedes canadensis. Waushara County: Dickinson (1944) as Aedes canadensis. Winnebago County: Dickinson (1944) as Aedes canadensis. Wood County: Dickinson (1944) as Aedes canadensis; Grimstad & DeFoliart (1975) as Aedes canadensis.

### Ochlerotatus communis (de Geer) [=Aedes communis]

Ochlerotatus communis is a mostly northern mosquito in the Nearctic (Carpenter & LaCasse, 1974; Darsie Jr & Ward, 2005), and the distribution of this mosquito in Wisconsin reflects this, with fewer records in the southern counties. Given the characters provided in the most recent revisions of the sibling species of the *Ochlerotatus communis* complex (Ellis & Brust, 1973; Brust & Munstermann, 1992), we are certain the specimens in the WIRC are *Ochlerotatus communis* s.s.

Distribution for Ochlerotatus communis: Bayfield County: Gilardi & Hilsenhoff (1992) as Aedes communis. Crawford County: DeFoliart et al. (1972)\* as Aedes communis. Dane County: DeFoliart et al. (1969) as Aedes communis; Anslow et al. (1969) as Aedes communis; DeFoliart et al. (1972)\* as Aedes communis; Grimstad & DeFoliart (1975) as Aedes communis. Florence County: Gilardi & Hilsenhoff (1992) as Aedes communis. Forest County: Siverly & DeFoliart (1968a) as Aedes communis; Siverly & DeFoliart (1968b) as Aedes communis; Gilardi & Hilsenhoff (1992) as Aedes communis. Grant County: Gojmerac & Porter (1969) as Aedes communis; DeFoliart et al. (1972)\* as Aedes communis. Iowa County: Loor & DeFoliart (1970)\* as Aedes communis. Jackson **County:** Anslow *et al.* (1969) as *Aedes communis*. **Kenosha County:** Amin & Hageman (1974) as Aedes communis. Lincoln County: Siverly & DeFoliart (1968a) as Aedes communis; Siverly & DeFoliart (1968b) as Aedes communis; Gilardi & Hilsenhoff (1992) as Aedes communis. Manitowoc **County:** WIRC (1968); Gojmerac & Porter (1969) as Aedes communis; Porter & Gojmerac (1970) as Aedes communis; Grimstad & DeFoliart (1975)

as Aedes communis. Marinette County: Gilardi & Hilsenhoff (1992) as Aedes communis. Oneida County: Siverly & DeFoliart (1968b) as Aedes communis. Price County: Gilardi & Hilsenhoff (1992) as Aedes communis. Rusk County: WIRC (1966). Vilas County: Dickinson (1944) as Aedes communis. Wood County: Grimstad & DeFoliart (1975) as Aedes communis.

## Ochlerotatus decticus (Howard, Dyar & Knab) [=Aedes decticus]

This species was only collected once by Gilardi & Hilsenhoff (1992) in two counties in northern Wisconsin. Although this is mainly a far northern species, it is present both in Michigan (Carpenter & LaCasse, 1974) and Minnesota (Barr & Balduf, 1965; Crane & Moon, 2010). As such the record is most likely correct, but it is probably a rare species even in the northern counties of Wisconsin.

**Distribution for Ochlerotatus decticus: Florence County:** Gilardi & Hilsenhoff (1992) as Aedes decticus. Lincoln County: Gilardi & Hilsenhoff (1992) as Aedes decticus.

### Ochlerotatus diantaeus (Howard, Dyar & Knab) [=Aedes diantaeus]

This species is one of the two belonging to the subgenus *Woodius*. *Ochlerotatus diantaeus* specimens can be found throughout the northern counties in Wisconsin. The larvae generally inhabit snowmelt pools (Carpenter & LaCasse, 1974).

**Distribution for Ochlerotatus diantaeus: Bayfield County:** Gilardi & Hilsenhoff (1992) as Aedes diantaeus. **Florence County:** Gilardi & Hilsenhoff (1992) as Aedes diantaeus. **Forest County:** Siverly & DeFoliart (1968a) as Aedes diantaeus; Siverly & DeFoliart (1968b) as Aedes diantaeus. **Lincoln County:** Siverly & DeFoliart (1968a) as Aedes diantaeus; Siverly & DeFoliart (1968b) as Aedes diantaeus; Siverly & DeFoliart (1968b) as Aedes diantaeus; Siverly & DeFoliart (1968b) as Aedes diantaeus; Gilardi & Hilsenhoff (1992) as Aedes diantaeus. **Marinette County:** Gilardi & Hilsenhoff (1992) as Aedes diantaeus. **Oneida County:** Siverly & DeFoliart (1968b) as Aedes diantaeus. **Rusk County:** WIRC (1968, 1969).

### Ochlerotatus implicatus (Vockeroth) [=Aedes implicatus]

This is a rare species with few specimens collected over the years. These mosquitoes inhabit temporary pools as larvae and may emerge as adults as soon as early spring (Carpenter & LaCasse, 1974; Gilardi & Hilsenhoff, 1992).

Distribution for Ochlerotatus implicatus: Bayfield County: Gilardi & Hilsenhoff (1992) as Aedes implicatus. Forest County: Siverly & DeFoliart (1968a) as Aedes implicatus; Siverly & DeFoliart (1968b) as Aedes implicatus. Iowa County: WIRC (1967). Lincoln County: Siverly & DeFoliart (1968b) as Aedes implicatus. Manitowoc County: WIRC (1968); Porter & Gojmerac (1970) as Aedes implicatus. Oneida County: Siverly & DeFoliart (1968b) as Aedes implicatus. Price County: Gilardi & Hilsenhoff (1992) as Aedes implicatus.

### Ochlerotatus intrudens (Dyar) [=Aedes intrudens]

This is one of the two species belonging to the subgenus *Woodius*. It is generally a northern species, but has been collected in Wisconsin as far south as Columbia County. This species inhabits a variety of aquatic habitats, from temporary to semipermanent environments (Carpenter & LaCasse, 1974).

**Distribution for** *Ochlerotatus intrudens*: Adams County: Gilardi & Hilsenhoff (1992) as *Aedes intrudens*. Bayfield County: Gilardi &

Hilsenhoff (1992) as Aedes intrudens. Burnett County: Dickinson (1944) as Aedes intrudens; Gilardi & Hilsenhoff (1992) as Aedes intrudens. Chippewa County: WIRC (1957). Columbia County: WIRC (1957). Door County: Dickinson (1944) as Aedes intrudens. Florence County: Gilardi & Hilsenhoff (1992) as Aedes intrudens. Forest County: Siverly & DeFoliart (1968b) as Aedes intrudens; Gilardi & Hilsenhoff (1992) as Aedes intrudens. Lincoln County: Dickinson (1944) as Aedes intrudens; Siverly & DeFoliart (1968b) as Aedes intrudens; Siverly & DeFoliart (1968a) as Aedes intrudens; Gilardi & Hilsenhoff (1992) as Aedes intrudens. Manitowoc County: WIRC (1968); Porter & Gojmerac (1970) as Aedes intrudens. Marinette County: WIRC (1955); Gilardi & Hilsenhoff (1992) as Aedes intrudens. Marquette **County:** Gilardi & Hilsenhoff (1992) as *Aedes intrudens*. **Oneida County:** Siverly & DeFoliart (1968b) as Aedes intrudens. Pepin County: Gilardi & Hilsenhoff (1992) as Aedes intrudens. Price County: Gilardi & Hilsenhoff (1992) as Aedes intrudens. Rusk County: WIRC (1957, 1968). Vilas County: Dickinson (1944) as Aedes intrudens. Washburn County: WIRC (1951).
#### Ochlerotatus provocans (Walker) [=Aedes provocans]

Ochlerotatus provocans is widely distributed throughout the state. This is one of the first species to emerge in late winter and early spring. It is relatively abundant in the entirety of its range and the larvae inhabit a variety of habitats including shallow grass pools (Carpenter & LaCasse, 1974; Gilardi & Hilsenhoff, 1992).

Ochlerotatus provocans is the only representative of the subgenus Rusticoidus in Wisconsin.

Distribution for Ochlerotatus provocans: Adams County: Gilardi & Hilsenhoff (1992) as Aedes provocans. Bayfield County: Gilardi & Hilsenhoff (1992) as Aedes provocans. Buffalo County: Gilardi & Hilsenhoff (1992) as Aedes provocans. Burnett County: Gilardi & Hilsenhoff (1992) as Aedes provocans. Florence County: Gilardi & Hilsenhoff (1992) as Aedes provocans. Fond du Lac County: Gilardi & Hilsenhoff (1992) as Aedes provocans. Forest County: Siverly & DeFoliart (1968b) as Aedes trichurus; Gilardi & Hilsenhoff (1992) as Aedes provocans. Jefferson County: Gilardi & Hilsenhoff (1992) as Aedes trichurus; Gilardi & Hilsenhoff (1992) as Aedes trichurus; Gilardi & Hilsenhoff (1992) as Aedes provocans. Lincoln County: Siverly & DeFoliart (1968a) as Aedes trichurus; Siverly & DeFoliart (1968b) as Aedes trichurus; Gilardi & Hilsenhoff (1992) as Aedes provocans. Lincoln County: Siverly & DeFoliart (1968a) as Aedes trichurus; Siverly & DeFoliart (1968b) as Aedes trichurus; Gilardi & Hilsenhoff (1992) as Aedes provocans. Marinette County: Gilardi & Hilsenhoff (1992) as Aedes

provocans. Marquette County: Gilardi & Hilsenhoff (1992) as Aedes
provocans. Oneida County: Siverly & DeFoliart (1968b) as Aedes
trichurus. Pepin County: Gilardi & Hilsenhoff (1992) as Aedes provocans.
Polk County: Gilardi & Hilsenhoff (1992) as Aedes provocans. Price
County: Gilardi & Hilsenhoff (1992) as Aedes provocans. Richland
County: WIRC (1955); Gilardi & Hilsenhoff (1992) as Aedes provocans.
Rusk County: WIRC (1957, 1966, 1968). Sheboygan County: Gilardi &
Hilsenhoff (1992) as Aedes provocans. Walworth County: Gilardi &
Hilsenhoff (1992) as Aedes provocans. Waushara County: Howard et al.
(1917) as Aëdes trichurus; Dyar (1922) as Aëdes cinereoborealis; Dickinson
(1944) as Aedes trichurus; Gilardi & Hilsenhoff (1992) as Aedes provocans.

## Ochlerotatus spencerii spencerii (Theobald) [=Aedes spencerii spencerii]

*Ochlerotatus spencerii spencerii* is a prairie mosquito, usually inhabiting temporary pools in open fields as larvae and flying during the day as adults (Carpenter & LaCasse).

The distribution records for this species in Winsconsin are scattered; however this is likely sampling bias, because the trap often used for mosquito surveillance is a CDC light trap is placed in tree and runs overnight, so would not typically collect this species.

Distribution for Ochlerotatus spencerii spencerii: Bayfield County: Gilardi & Hilsenhoff (1992) as Aedes spencerii. Buffalo County: Dickinson (1944) as Aedes spenceri. Burnett County: Gilardi & Hilsenhoff (1992) as Aedes spencerii. Dane County: Dickinson (1944) as Aedes spenceri. Dodge County: Dickinson (1944) as Aedes spenceri. Florence County: Gilardi & Hilsenhoff (1992) as Aedes spencerii. Fond du Lac County: Dickinson (1944) as Aedes spenceri. Marinette County: Dickinson (1944) as Aedes spenceri. Marinette County: Dickinson (1944) as Aedes spenceri. Price County: Gilardi & Hilsenhoff (1992) as Aedes spencerii. Rock County: Dickinson (1944) as Aedes spenceri. Rusk County: WIRC (1951, 1952). Sheboygan County: Dickinson (1944) as Aedes spenceri. Vilas County: Dickinson (1944) as Aedes spenceri.

#### Ochlerotatus sticticus (Meigen) [=Aedes sticticus]

This mosquito inhabits floodwater and rainwater pools, emerging very early in the spring, with numbers decreasing after May (Carpenter & LaCasse, 1974). *Ochlerotatus sticticus* is spread all throughout Wisconsin and it is fairly abundant when present.

Distribution for Ochlerotatus sticticus: Bayfield County: Gilardi & Hilsenhoff (1992) as Aedes sticticus. Buffalo County: Gilardi & Hilsenhoff (1992) as Aedes sticticus. Burnett County: Gilardi & Hilsenhoff (1992) as Aedes sticticus. Crawford County: WIRC (1955). Dane County: WIRC (1950); Wright & DeFoliart (1970) as Aedes sticticus. Forest County: Siverly & DeFoliart (1968b) as *Aedes sticticus*; Gilardi & Hilsenhoff (1992) as Aedes sticticus. Grant County: WIRC (1955, 1967). Iowa County: Wright & DeFoliart (1970) as Aedes sticticus. Juneau County: WIRC (1956). Lafayette County: Ryckman (1952) as Aedes sticticus. Lincoln **County:** Siverly & DeFoliart (1968b) as *Aedes sticticus*; Gilardi & Hilsenhoff (1992) as Aedes sticticus. Manitowoc County: WIRC (1968); Porter & Gojmerac (1970) as Aedes sticticus. Marinette County: WIRC (1955). Marquette County: Gilardi & Hilsenhoff (1992) as Aedes sticticus. Oneida **County:** Siverly & DeFoliart (1968b) as *Aedes sticticus*. **Pepin County:** Gilardi & Hilsenhoff (1992) as Aedes sticticus. Richland County: WIRC (1955); Gilardi & Hilsenhoff (1992) as Aedes sticticus. Rock County: WIRC (1980). Rusk County: WIRC (1951, 1952, 1957, 1959, 1966, 1968, 1986). Sheboygan County: Gilardi & Hilsenhoff (1992) as Aedes sticticus. Walworth County: Wright & DeFoliart (1970) as Aedes sticticus. Wood **County:** Grimstad & DeFoliart (1975) as *Aedes sticticus*.

### **Stimulans Group of Ochlerotatus**

The Stimulans group of *Ochlerotatus* is composed of several species that present a set of morphological similarities in the male genitalia thought to represent evolutionary closeness of its members (Dyar, 1920b,a).

Some species are readily separated in the adult female, such as *Ochlerotatus flavescens* and *Ochlerotatus riparius*, while others have presented challenges in identification of the adult female for many years. Although the use of the front tarsal claw for differentiation of the species within the Stimulans group has shown to be useful to many authors (Vockeroth, 1950; McDaniel & Webb, 1974; Wood, 1977; Nielsen, 2009), and came to be the default character in modern keys (*e.g.*: Darsie Jr & Ward, 2005), it should be pointed out however that these authors are in disagreement with each other in many aspects; most notably on the differentiation of the claws of *Ochlerotatus stimulans* and *Ochlerotatus fitchii*, likely due to the analysis of specimens from different regions.

Careful examination of material collected as larvae in Dane County revealed that adults identified to *Ochlerotatus stimulans* on the basis of the characters given by McDaniel & Webb (1974) are in disagreement with other characters of adult female (as described by Carpenter & LaCasse (1974)), the larva (as described by Carpenter & LaCasse (1974)), the pupa (as described by Darsie (1951)) and the male genitalia (as described by Carpenter & LaCasse (1974) and by Dyar (1920a)). These specimens are here referred simply as *Ochlerotatus stimulans* s.l., further research already showed us that a new species is amongst those specimens collected in Madison, which we describe in Chapter 5; however, other specimens are still not easily definable, and it is clear a full review of the Stimulans Group is necessary to resolve the *Oc. stimulans* complex and better define characters for each species of the Stimulans Group in general.

### Stimulans Group: Ochlerotatus euedes (Howard, Dyar & Knab) [=Aedes euedes]

Ochlerotatus euedes was collected only once in Wisconsin (Gilardi & Hilsenhoff, 1992). The collection consisted of very few larval specimens from central, east and northeast counties in the state. Even though there are no voucher specimens available from these collections, as this species is readily identified at the larval stage, there is little doubt as to the identification of the species. Furthermore, *Oc. euedes* is also present in Michigan (Wilmot *et al.*, 1987) and Minnesota (Rueger, 1958; Crane & Moon, 2010).

It is possible that this species has been collected before and after the collections of Gilardi & Hilsenhoff (1992) as adult females, and identified as *Ochlerotatus fitchii* or *Ochlerotatus stimulans*. The reason for this is that the characters used to distinguish these three species are contentious.

In the key from Carpenter & LaCasse (1974) Oc. euedes is still in synonymy with Ochlerotatus excrucians and the characters used to separate Oc. fitchii from Oc. stimulans are the presence or absence of lower mesepimeral setae, if present, the number of lower mesepimeral setae and the coloration of tori vestiture. McDaniel & Webb (1974) however showed that the mesepimeral setae were unquestionably an unreliable character for the separation of *Oc. fitchii* from *Oc. stimulans*, something that was already suggested by data presented earlier by Lunt & Nielsen (1971). This character has first appeared as a key character in the work of Matheson (1929) and later used by others, including in the keys by Wood *et al.* (1979), for Canadian fauna, and by Darsie Jr & Ward (2005), the most recent key for US fauna. It should be noted that, in the keys cited, the use of the character is supplemented by the presence/absence of upper mesomeral scales. Regardless of that they fail to acknowledge the mesepimeral setae character overlap for Oc. stimulans specifically and we believe the ease of loss of the upper mesomeral scales would be enough to yield incorrect identifications for specimens of Oc. stimulans which present absence of lower mesepimeral setae.

The tori vestiture coloration character used by Carpenter & LaCasse (1974) is the same presented by Gjullin (1946) and in both works the authors acknowledge the partial overlap of the character (indicating the presence of pale scales on the dorsum of the tori for Oc. fitchii and the presence or absence of pale scales in the region for *Oc. stimulans*). Despite that McDaniel & Webb (1974) found that in their material the character had complete overlap (i.e.: specimens with and without white scales existed within both species). Regardless of that, this character is vaguely described in the key for Canada mosquitoes by Wood et al. (1979) and in the most recent key for the United States fauna, Darsie Jr & Ward (2005), as mostly pale versus mostly dark scales on the tori (separating Oc. stimulans from *Oc. fitchii* + *Oc. euedes* in the key by Wood *et al.* (1979) and separating *Oc.* fitchii from Oc. stimulans + Oc. euedes in the key by Darsie Jr & Ward (2005)). It should be noted that the character is supplemented with the scale coloration patterning of the scutum; notably Wood *et al.* (1979) and Darsie Jr & Ward (2005) use the same wording in the couplet to separate *Oc. stimulans* differently. This is further evidence that character should be unreliable for *Oc. stimulans*, as it was already previously noted in the work of Carpenter & LaCasse (1974) describing the variability of the scutal scale patterning of the species. Furthermore, scutal scales are easily lost by

flying, by most trapping methods and by handling of the specimen.

Vockeroth (1950), based on material from Canada (Northwest Territories, Newfoundland and Labrador, and Ontario), separated Oc. excrucians from Oc. fitchii + Oc. stimulans based on the shape of the fore tarsal claws of the females, not finding differentiating features between *Oc*. fitchii and Oc. stimulans. Later McDaniel & Webb (1974) studied material from Maine, USA, and described differentiating characters in the shape of the claws of the fore tarsomeres of Oc. fitchii and Oc. stimulans females. Wood (1977) resurrected Oc. euedes from synonymy and illustrated the females' fore tarsal claws, illustrating Oc. fitchii and Oc. stimulans as well, based on mosquitoes of Ontario and Quebec. These were later used to convey a separating character in the key for the mosquitoes of Canada (Wood et al., 1979). The character used, fore claws longer and straighter in *Oc. euedes* and shorter and more strongly curved in *Oc. fitchii*, is not only difficult to portray objectively but also the material from Maine studied by McDaniel & Webb (1974) present different characteristics for Oc. fitchii, resembling Oc. aloponotum and Oc. riparius from Wood (1977), except more strongly curved. We note that the character of the shape of the fore tarsal claws is used in the key by Wood et al. (1979) as a supplement for other characters: the presence (for Oc. euedes) or absence (for Oc. fitchii) of pale scales in the cercus, proboscis and tarsomere I (beyond the basal pale ring).

However, Wood *et al.* (1979) note the partial overlap in *Oc. euedes*, in particular for "northern specimens". This couplet (presence/absence of pales scaling in various areas and the shape of tarsal claw) was later used in the key by Darsie Jr & Ward (2005), the most recent for the US fauna, but with no indication of possible partial overlap for the supplemental characters, possibly with the assumption that all US fauna would be morphologically similar to the southern Canada fauna and not the northern. This is an assumption that we do not share, as we interpret the wording on Wood *et al.* (1979)'s key to be carefully chosen as to avoid misidentifications when the overlap occurs, which shows that the authors might not be completely sure as to the geographical extent of the phenotypic variability. It is possible that populations in the US also present such variability, and as such, the character is contentious.

Distribution for Ochlerotatus euedes: Fond du Lac County: Gilardi & Hilsenhoff (1992) as Aedes euedes. Forest County: Gilardi & Hilsenhoff (1992) as Aedes euedes. Marinette County: Gilardi & Hilsenhoff (1992) as Aedes euedes. Marquette County: Gilardi & Hilsenhoff (1992) as Aedes euedes. Sheboygan County: Gilardi & Hilsenhoff (1992) as Aedes euedes.

# Stimulans Group: Ochlerotatus excrucians (Walker) [=Aedes excrucians]

This species is generally regarded as differentiable, however, there is disagreement in the presentation of the usefulness of many characters by different authors, please see the discussion under *Ochlerotatus euedes*. This species is well spread throughout the state of Wisconsin and the Midwest, occurring in Illinois, Minnesota, Michigan and in Canada (Carpenter & LaCasse, 1974). We recommend the use of the keys by Wood *et al.* (1979) as the most carefully crafted key that contains this species group at the moment.

Distribution for Ochlerotatus excrucians: Adams County: Gilardi & Hilsenhoff (1992) as Aedes excrucians. Bayfield County: Gilardi & Hilsenhoff (1992) as Aedes excrucians. Brown County: Dickinson (1944) as Aedes excrucians. Burnett County: Gilardi & Hilsenhoff (1992) as Aedes excrucians. Columbia County: WIRC (1956). Dane County: Dickinson (1944) as Aedes excrucians; WIRC (1949, 1950, 1951, 1952, 1954); Meece et al. (2003)\*. Dodge County: Dickinson (1944) as Aedes excrucians. Thoreace County: Gilardi & Hilsenhoff (1992) as Aedes excrucians. Florence County: Gilardi & Hilsenhoff (1992) as Aedes excrucians. Florence County: Dickinson (1944) as Aedes excrucians.

excrucians. Forest County: Siverly & DeFoliart (1968b) as Aedes excrucians; Siverly & DeFoliart (1968a) as Aedes excrucians; Gilardi & Hilsenhoff (1992) as Aedes excrucians. Jefferson County: Gilardi & Hilsenhoff (1992) as Aedes excrucians. Kenosha County: Meece et al. (2003)\*. La Crosse County: Dickinson (1944) as Aedes excrucians. Lafayette County: Ryckman (1952) as Aedes excruscians. Lincoln **County:** Siverly & DeFoliart (1968b) as *Aedes excrucians*; Siverly & DeFoliart (1968a) as Aedes excrucians: Gilardi & Hilsenhoff (1992) as Aedes excrucians. Manitowoc County: WIRC (1968); Porter & Gojmerac (1970) as Aedes excrucians. Marinette County: Dickinson (1944) as Aedes excrucians; Gilardi & Hilsenhoff (1992) as Aedes excrucians. Marquette County: Gilardi & Hilsenhoff (1992) as Aedes excrucians. Milwaukee **County:** Dickinson (1944) as Aedes excrucians; Meece et al. (2003)\*. Monroe County: Dickinson (1944) as Aedes excrucians. Oneida County: Siverly & DeFoliart (1968b) as Aedes excrucians. Pepin County: Gilardi & Hilsenhoff (1992) as Aedes excrucians. Polk County: Dickinson (1944) as Aedes excrucians; Gilardi & Hilsenhoff (1992) as Aedes excrucians. Price **County:** Gilardi & Hilsenhoff (1992) as *Aedes excrucians*. **Racine County:** Dickinson (1944) as Aedes excrucians; Meece et al. (2003)\*. Richland County: Gilardi & Hilsenhoff (1992) as Aedes excrucians. Rock County: Gilardi & Hilsenhoff (1992) as Aedes excrucians. Rusk County: WIRC

(1951, 1952, 1966, 1968). Sheboygan County: Dickinson (1944) as Aedes excrucians; WIRC (1950, 1952); Gilardi & Hilsenhoff (1992) as Aedes excrucians. Walworth County: Dickinson (1944) as Aedes excrucians; Gilardi & Hilsenhoff (1992) as Aedes excrucians. Washburn County: Dickinson (1944) as Aedes excrucians; WIRC (1950, 1951). Waukesha County: Gilardi & Hilsenhoff (1992) as Aedes excrucians; Meece et al. (2003)\*. Waushara County: Howard et al. (1917) as Aëdes abfitchii; Dyar (1922) as Aëdes excrucians; Dickinson (1944) as Aedes excrucians; Gilardi & Hilsenhoff (1992) as Aedes excrucians. Wood County: Grimstad & DeFoliart (1975) as Aedes excrucians.

#### Stimulans Group: Ochlerotatus fitchii (Felt & Young) [=Aedes fitchii]

This species is very difficult to differentiate from *Oc. stimulans* s.l. and *Oc. euedes*, especially in the female. See discussion under *Oc. euedes*. It is distributed all throughout the state and present in all neighboring states, and Canada (Carpenter & LaCasse, 1974). The keys by Wood *et al.* (1979) are recommended for correct identification.

**Distribution for** *Ochlerotatus fitchii*: Adams County: Gilardi & Hilsenhoff (1992) as *Aedes fitchii*. Bayfield County: Gilardi & Hilsenhoff

(1992) as Aedes fitchii. Brown County: Dickinson (1944) as Aedes fitchii. Burnett County: Gilardi & Hilsenhoff (1992) as Aedes fitchii. Columbia **County:** WIRC (1956). **Dane County:** Dickinson (1944) as Aedes fitchii; WIRC (1946, 1949, 1950, 1951, 1952, 1954, 1955); Meece et al. (2003)\*. **Dodge County:** Dickinson (1944) as *Aedes fitchii*. **Door County:** Dickinson (1944) as Aedes fitchii. Florence County: Gilardi & Hilsenhoff (1992) as Aedes fitchii. Fond du Lac County: Gilardi & Hilsenhoff (1992) as Aedes fitchii. Forest County: Siverly & DeFoliart (1968b) as Aedes fitchii; Siverly & DeFoliart (1968a) as Aedes fitchii; Gilardi & Hilsenhoff (1992) as Aedes fitchii. Iron County: Dickinson (1944) as Aedes fitchii. Jefferson County: Gilardi & Hilsenhoff (1992) as Aedes fitchii. Juneau County: Dickinson (1944) as Aedes fitchii. Kenosha County: Meece et al. (2003)\*. Lincoln County: Siverly & DeFoliart (1968b) as Aedes fitchii; Siverly & DeFoliart (1968a) as Aedes fitchii; Gilardi & Hilsenhoff (1992) as Aedes fitchii. Manitowoc County: WIRC (1968); Porter & Gojmerac (1970) as Aedes fitchii. Marinette County: Dickinson (1944) as Aedes fitchii; Gilardi & Hilsenhoff (1992) as Aedes fitchii. Marquette County: Gilardi & Hilsenhoff (1992) as Aedes fitchii. Milwaukee County: Dickinson (1944) as Aedes fitchii; Meece et al. (2003)\*. Oneida County: Siverly & DeFoliart (1968b) as Aedes fitchii. Outagamie County: Dickinson (1944) as Aedes fitchii. Pepin County: Gilardi & Hilsenhoff (1992) as Aedes fitchii. Polk County:

Gilardi & Hilsenhoff (1992) as Aedes fitchii. Price County: Gilardi & Hilsenhoff (1992) as Aedes fitchii. Racine County: Dickinson (1944) as Aedes fitchii; Meece et al. (2003)\*. Richland County: Gilardi & Hilsenhoff (1992) as Aedes fitchii. Rock County: Dickinson (1944) as Aedes fitchii; Gilardi & Hilsenhoff (1992) as Aedes fitchii. Rusk County: WIRC (1951, 1952, 1966, 1968). Sheboygan County: Dickinson (1944) as Aedes fitchii; Gilardi & Hilsenhoff (1992) as *Aedes fitchii*. Vilas County: Dickinson (1944) as Aedes fitchii. Walworth County: Dickinson (1944) as Aedes fitchii; WIRC (1948); Gilardi & Hilsenhoff (1992) as Aedes fitchii. Washburn County: Dickinson (1944) as Aedes fitchii; WIRC (1951). Waukesha County: Dickinson (1944) as Aedes fitchii; Gilardi & Hilsenhoff (1992) as Aedes fitchii; Meece et al. (2003)\*. Waupaca County: Dickinson (1944) as Aedes fitchii; WIRC (1947). Waushara County: Howard et al. (1917) as Aëdes fitchii; Dyar (1922) as Aëdes fitchii; Dickinson (1944) as Aedes fitchii; Gilardi & Hilsenhoff (1992) as Aedes fitchii. Wood County: Dickinson (1944) as Aedes fitchii; Grimstad & DeFoliart (1975) as Aedes fitchii.

# Stimulans Group: Ochlerotatus flavescens (Müller) [=Aedes flavescens]

This species is one of the most easily distinguishable from the Stimulans Group. It is rarely collected, having few reports in the state, however these are fairly widespread. Additionally, the species is present in all neighboring states and Canada (Carpenter & LaCasse, 1974).

Distribution for Ochlerotatus flavescens: Bayfield County: Dickinson (1944) as Aedes flavescens. Buffalo County: Gilardi & Hilsenhoff (1992) as Aedes flavescens. Dane County: Dickinson (1944) as Aedes flavescens. Forest County: Siverly & DeFoliart (1968b) as Aedes flavescens. Juneau County: Dickinson (1944) as Aedes flavescens. Lincoln County: Siverly & DeFoliart (1968b) as Aedes flavescens. Marquette County: Gilardi & Hilsenhoff (1992) as Aedes flavescens. Milwaukee County: Dickinson (1944) as Aedes flavescens. Oneida County: Siverly & DeFoliart (1968b) as Aedes flavescens. Oneida County: Siverly & DeFoliart (1968b) as Aedes flavescens. Oneida County: Siverly & DeFoliart (1968b) as Aedes flavescens. Oneida County: Siverly & DeFoliart (1968b) as Aedes flavescens. Oneida County: Siverly & DeFoliart (1968b) as Aedes flavescens. Oneida County: Siverly & DeFoliart (1968b) as Aedes flavescens. Oneida County: Siverly & DeFoliart (1968b) as Aedes flavescens. Oneida County: Siverly & DeFoliart (1968b) as Aedes flavescens. Oneida County: Dickinson (1944) as Aedes flavescens. Rock County: Gilardi & Hilsenhoff (1992) as Aedes flavescens. Vilas County: Dickinson (1944) as Aedes flavescens. Waukesha County: Dickinson (1944) as Aedes flavescens.

# Stimulans Group: Ochlerotatus riparius (Dyar & Knab) [Aedes riparius]

This mosquito has been reported from a variety of counties both in the southern and northern parts of Wisconsin, however it seems to be rare in collections. It occurs in Canada, as well as Iowa, Michigan and Minnesota (Carpenter & LaCasse, 1974).

Distribution for Ochlerotatus riparius: Burnett County: Gilardi & Hilsenhoff (1992) as Aedes riparius. Dane County: Dickinson (1944) as Aedes riparius; WIRC (1966). Door County: Dickinson (1944) as Aedes riparius. Florence County: Gilardi & Hilsenhoff (1992) as Aedes riparius. Fond du Lac County: Dickinson (1944) as Aedes riparius. Jefferson County: Gilardi & Hilsenhoff (1992) as Aedes riparius. Juneau County: Dickinson (1944) as Aedes riparius. Marquette County: Gilardi & Hilsenhoff (1992) as Aedes riparius. Price County: Gilardi & Hilsenhoff (1992) as Aedes riparius. Rusk County: WIRC (1966). Waukesha County: Dickinson (1944) as Aedes riparius. Waushara County: Howard et al. (1917) as Aëdes riparius; Dickinson (1944) as Aedes riparius; Gilardi & Hilsenhoff (1992) as Aedes riparius.

### Stimulans Group: Ochlerotatus stimulans sensu lato (Walker) [=Aedes stimulans sensu lato]

This species complex occurs all throughout the upper Midwest and large part of Canada (Carpenter & LaCasse, 1974). It is deserving of a taxonomic review and a new species whose type locality is Madison, WI, is in the process of description, soon to be published.

Distribution for Ochlerotatus stimulans: Adams County: Gilardi & Hilsenhoff (1992) as Aedes stimulans. Bayfield County: Gilardi & Hilsenhoff (1992) as Aedes stimulans. Brown County: Dickinson (1944) as Aedes stimulans. Buffalo County: Gilardi & Hilsenhoff (1992) as Aedes stimulans. Burnett County: Gilardi & Hilsenhoff (1992) as Aedes stimulans. Calumet County: Gilardi & Hilsenhoff (1992) as Aedes stimulans. Columbia County: WIRC (1956). Crawford County: DeFoliart et al. (1972)\* as Aedes stimulans. Dane County: Dickinson (1944) as Aedes stimulans; WIRC (1949, 1950, 1951, 1952, 1954, 1955); Thompson & Dicke (1965) as Aedes stimulans Group; DeFoliart et al. (1969) as Aedes stimulans; Anslow et al. (1969) as Aedes stimulans; Wright & DeFoliart (1970) as Aedes stimulans; DeFoliart et al. (1972)\* as Aedes stimulans; Meece et al. (2003)\*. Dodge County: Dickinson (1944) as Aedes stimulans. Door County: Dickinson (1944) as Aedes stimulans. Dunn County: Gilardi

& Hilsenhoff (1992) as Aedes stimulans. Florence County: Gilardi & Hilsenhoff (1992) as Aedes stimulans. Fond du Lac County: Gilardi & Hilsenhoff (1992) as *Aedes stimulans*. Forest County: Dickinson (1944) as Aedes stimulans; Gilardi & Hilsenhoff (1992) as Aedes stimulans. Grant **County:** WIRC (1966); Gojmerac & Porter (1969) as Aedes stimulans; DeFoliart et al. (1972)\* as Aedes stimulans. Iowa County: WIRC (1966); Wright & DeFoliart (1970) as Aedes stimulans; Loor & DeFoliart (1970)\* as Aedes stimulans. Iron County: Dickinson (1944) as Aedes stimulans. Jefferson County: Dickinson (1944) as Aedes stimulans; Gilardi & Hilsenhoff (1992) as Aedes stimulans. Kenosha County: Amin & Hageman (1974) as Aedes stimulans; Meece et al. (2003)\*. Lincoln County: Siverly & DeFoliart (1968a) as Aedes stimulans; Gilardi & Hilsenhoff (1992) as Aedes stimulans. Manitowoc County: WIRC (1966, 1968); Gojmerac & Porter (1969) as Aedes stimulans; Porter & Gojmerac (1970) as Aedes stimulans; Grimstad & DeFoliart (1975) as Aedes stimulans. Marinette County: Dickinson (1944) as Aedes stimulans; Gilardi & Hilsenhoff (1992) as Aedes stimulans. Marquette County: Gilardi & Hilsenhoff (1992) as Aedes stimulans. Milwaukee County: Dickinson (1944) as Aedes stimulans; Meece et al. (2003)\*. Outagamie County: WIRC (1954). Pepin County: Gilardi & Hilsenhoff (1992) as Aedes stimulans. Polk County: Dickinson (1944) as Aedes stimulans; Gilardi & Hilsenhoff (1992) as Aedes stimulans.

Racine County: Meece et al. (2003)\*. Richland County: Gilardi & Hilsenhoff (1992) as Aedes stimulans. Rock County: Dickinson (1944) as Aedes stimulans; Gilardi & Hilsenhoff (1992) as Aedes stimulans. Rusk County: WIRC (1952, 1957, 1968). Sheboygan County: WIRC (1941); Dickinson (1944) as Aedes stimulans; Gilardi & Hilsenhoff (1992) as Aedes stimulans. Taylor County: WIRC (1966). Vernon County: Gilardi & Hilsenhoff (1992) as Aedes stimulans. Vilas County: Dickinson (1944) as Aedes stimulans. Walworth County: Dickinson (1944) as Aedes stimulans; WIRC (1948); Wright & DeFoliart (1970) as Aedes stimulans; Gilardi & Hilsenhoff (1992) as Aedes stimulans. Washburn County: WIRC (1951). Washington County: Dickinson (1944) as Aedes stimulans. Waukesha **County:** Dickinson (1944) as *Aedes stimulans*; Gilardi & Hilsenhoff (1992) as Aedes stimulans; Meece et al. (2003)\*. Waushara County: Dickinson (1944) as Aedes stimulans; Gilardi & Hilsenhoff (1992) as Aedes stimulans. Wood County: Grimstad & DeFoliart (1975) as Aedes stimulans.

#### Ochlerotatus trivittatus (Coquillett) [=Aedes trivittatus]

This species seems to be more pervasive in the southern parts of Wisconsin, but it does occur in the northern counties. It constitutes one of the largest portions of the community structure of mosquitoes in many counties. This species also occurs in southern parts of Canada, and in Minnesota, Iowa and Illinois (Carpenter & LaCasse, 1974).

Distribution for *Ochlerotatus trivittatus*: Columbia County: WIRC (1957). Crawford County: DeFoliart et al. (1972)\* as Aedes trivittatus. Dane County: Dickinson (1944) as Aedes trivittatus; WIRC (1950, 1951, 1952, 1954, 1955, 1966); Thompson & Dicke (1965) as Aedes trivittatus; Anslow et al. (1969) as Aedes trivittatus; Wright & DeFoliart (1970) as Aedes trivittatus; DeFoliart et al. (1972)\* as Aedes trivittatus; Arnell (1976) as Aedes trivittatus; Meece et al. (2003)\*. Forest County: Dickinson (1944) as Aedes trivittatus. Grant County: Dickinson (1944) as Aedes trivittatus; Gojmerac & Porter (1969) as Aedes trivittatus; Anslow et al. (1969) as Aedes trivittatus; DeFoliart et al. (1972)\* as Aedes trivittatus. Green Lake County: Dickinson (1944) as Aedes trivittatus. Iowa County: Wright & DeFoliart (1970) as *Aedes trivittatus*. Jefferson County: Dickinson (1944) as Aedes trivittatus. Juneau County: Dickinson (1944) as Aedes trivittatus. Kenosha County: Amin & Hageman (1974) as Aedes trivittatus; Meece et al. (2003)\*. Lafayette County: Ryckman (1952) as Aedes trivittatus; Arnell (1976) as Aedes trivittatus. Lincoln County: Dickinson (1944) as *Aedes trivittatus*. Manitowoc County: WIRC (1968); Gojmerac & Porter (1969) as *Aedes trivittatus*; Porter & Gojmerac1970 as Aedes trivittatus. **Marguette County:** Dickinson (1944) as *Aedes* 

trivittatus. Mazomanie County: WIRC (1965). Milwaukee County: Dickinson (1944) as Aedes trivittatus; Meece et al. (2003)\*. Pepin County: Dickinson (1944) as Aedes trivittatus. Polk County: WIRC (1941); Dickinson (1944) as Aedes trivittatus. Racine County: Meece et al. (2003)\*. Rock County: Dickinson (1944) as Aedes trivittatus. Rusk County: WIRC (1952, 1959). Sauk County: Dickinson (1944) as Aedes trivittatus. Sheboygan County: Dickinson (1944) as Aedes trivittatus. Taylor County: Dickinson (1944) as Aedes trivittatus. Vilas County: Dickinson (1944) as Aedes trivittatus. Waukesha County: Dickinson (1944) as Aedes trivittatus; Meece et al. (2003)\*. Waupaca County: Dickinson (1944) as Aedes trivittatus. Wood County: Dickinson (1944) as Aedes trivittatus; Grimstad & DeFoliart (1975) as Aedes trivittatus.

### Orthopodomyia signifera (Coquillett)

This specimen has only been mentioned in two reports from literature associated with mosquitoes from Wisconsin, both from 1970, but several specimens are present at the WIRC, the first ones collected in 1949.

**Distribution for** *Orthopodomyia signifera*: Columbia County: WIRC (1956). Dane County: WIRC (1949, 1950, 1955). Iowa County: Loor & DeFoliart (1970)\*. Manitowoc County: WIRC (1967); Porter & Gojmerac (1970). Washburn County: WIRC (1953).

#### **Psorophora Robineau-Desvoidy species**

Iowa has 10 species of *Psorophora* in three subgenera while Wisconsin has only three species in two subgenera; this is something that also reflects the fact that the genus *Psorophora* is less specious outside of the tropics and subtropics. Although no species of the subgenus *Grabhamia* of *Psorophora* was ever collected, as its habitat consists mainly of open fields with shallow water, its niche limits in Wisconsin probably consists of the yearly temperature fluctuation. As the temperatures increase globally it may be possible that species of this subgenus could be collected in Wisconsin in the future, along with other species with similar restrictions.

#### **Psorophora ciliata (Fabricius)**

Strangely this species is only mentioned in literature by Dickinson (1944), perhaps because of its habits of flying on open fields or perhaps because of its ability to "outfly" a common CDC-type trap fan. This species

may not be particularly common but it is attracted to humans and abundant enough in open fields that it has been captured in at least two occasions in 2015 (in Dane County) (unpublished).

Distribution for *Psorophora ciliata*: Dane County: Dickinson (1944). Jefferson County: Dickinson (1944). Kenosha County: Dickinson (1944). Racine County: Dickinson (1944). Waukesha County: Dickinson (1944). Waushara County: Dickinson (1944). Winnebago County: Dickinson (1944).

#### **Psorophora ferox (von Humboldt)**

This species has been collected consistently since 1917 in Wisconsin, but it is a southern species in the state. It should be noted that the eggs of this species differ significantly from two sampled populations in a study by Linley & Chadee (1990), from Florida, U.S.A., and Trinidad, Trinidad and Tobago. This may present a taxonomical challenge for a future revision of this species, which is collected from Argentina to Canada. This is a fairly abundant species in the end of the summer in Dane County (data unpublished).

Distribution for Psorophora ferox: Brown County: Dickinson

(1944). Crawford County: DeFoliart et al. (1972)\*. Dane County: Wright & DeFoliart (1970); DeFoliart et al. (1972)\*; Meece et al. (2003)\*. Grant County: DeFoliart et al. (1972)\*. Iowa County: Wright & DeFoliart (1970). Kenosha County: Dickinson (1944); Meece et al. (2003)\*. Manitowoc County: Porter & Gojmerac (1970). Milwaukee County: Meece et al. (2003)\*. Racine County: Meece et al. (2003)\*. Sheboygan County: Dickinson (1944). Walworth County: Dickinson (1944). Waukesha County: Meece et al. (2003)\*. Waupaca County: Dickinson (1944).
Waushara County: Howard et al. (1917) as Psorophora sayi; Dyar (1922) as Psorophora sayi; Dickinson (1944).

### Psorophora horrida (Dyar & Knab)

Many specimens of this taxon were captured by Wright & DeFoliart (1970); This strengthens the view that the identification of a single specimen of *Psorophora mathesoni* by Thompson & DeFoliart (1966) was most likely the first finding of *Psorophora horrida*. The lack of collection of this species since 1970 may seem troubling, but the consistent (yearly) collection of this mosquito in Iowa (Dunphy *et al.*, 2014) lends more credibility for the presence of the mosquito in Wisconsin; the species however is most likely not as common or as well-distributed as in Iowa.

**Distribution for** *Psorophora horrida*: **Dane County**: Wright & DeFoliart (1970). **Iowa County**: Wright & DeFoliart (1970).

#### Uranotaenia sapphirina (Osten Sacken)

This is a rare species in the state; evidence of this were collections made by the authors on the summer of 2016 of a few specimens of *Uranotaenia sapphirina*, while in the summer of 2017 no specimens could be found at the same locale or in nearby locations (data unpublished).

Distribution for Uranotaenia sapphirina: Buffalo County: Dickinson (1944). Burnett County: Dickinson (1944). Chippewa County: Dickinson (1944). Columbia County: Dickinson (1944). Dane County: Dickinson (1944); Meece et al. (2003)\*. Dodge County: Dickinson (1944). Dunn County: Dickinson (1944). Forest County: Dickinson (1944). Iron County: Dickinson (1944). Jefferson County: Dickinson (1944). Kenosha County: Dickinson (1944); Meece et al. (2003)\*. La Crosse County: Dickinson (1944). Lafayette County: Ryckman (1952). Manitowoc County: Porter & Gojmerac (1970). Milwaukee County: Meece et al. (2003)\*. Pepin County: Dickinson (1944). Polk County: Dickinson (1944). Racine County: Dickinson (1944); Meece et al. (2003)\*. Sawyer County: Dickinson (1944). Vernon County: Dickinson (1944). Walworth County:
Dickinson (1944). Washburn County: Dickinson (1944). Waukesha
County: Dickinson (1944); Meece et al. (2003)\*. Waupaca County:
Dickinson (1944).

#### Wyeomyia Theobald species

The genus *Wyeomyia* is the only that occurs in Wisconsin but not in Iowa. The genus is represented in Wisconsin by one of the three species present in the Nearctic, namely *Wyeomyia smithii*; Carpenter & LaCasse (1974) informs that the species is known to breed only in the pitcher plant *Sarracenia purpurea* Linnaeus; interestingly, no species of the genus *Sarracenia* is present in Iowa (USDA, 2018), and the two other species in the Nearctic (*Wyeomyia mitchellii* and *Wyeomyia vanduzeei*) are associated with epiphytic Bromeliaceae, and as such, in the United States, are restricted to the southeastern states (Carpenter & LaCasse, 1974).

#### Wyeomyia smithii (Coquillett)

The lack of literature reports of this species after 1944 is most likely due to the scope of most publications being either studies of mosquitoes of medical and veterinary importance or studies of specific mosquito groups. Moreover specimens of *Wyeomyia* were observed during a trip to northern Wisconsin (Vilas County) (unpublished).

**Distribution for Wyeomyia smithii: Iron County:** Dickinson (1944). **Jefferson County:** Dickinson (1944). **Ozaukee County:** Dickinson (1944). **Rock County:** Dickinson (1944). **Vilas County:** Howard *et al.* (1915); Dyar (1922); Dickinson (1944).

#### Invasive mosquitoes, doubtful and incorrect records:

### Invasive: Stegomyia albopicta (Skuse) [=Aedes albopictus]

Stegomyia albopicta, also known as "Asian tiger mosquito" is an invasive species that is widely distributed throughout the southeastern United States. Although there are several reports of this species in northern parts of the Midwest, such as Iowa, Michigan and Minnesota, they are mostly very localized, do not occur every year, and are often associated with tire trade (Hahn et al., 2016).

Future research will provide insight into the nature of the colonization by *St. albopicta* of the state, but it is possible that the species may appear every summer due to repeated introduction via tire trade. Moreover, climate change may enable establishment of the species in Wisconsin in the future.

**Reported distribution for** *Stegomyia albopicta*: Dane County: Richards *et al.* (2019) as *Aedes albopictus*. Waukesha County: Richards *et al.* (2019) as *Aedes albopictus*.

# Doubtful record: Ochlerotatus sollicitans (Walker) [=Aedes sollicitans]

The report of *Ochlerotatus sollicitans* for Wisconsin is very recent (*i.e.*: Meece *et al.*, 2003). This is the only species of the subgenus *Culicelsa* reported for the state of Wisconsin. The larvae of *Oc. sollicitans* inhabit salt water environments and the adults are known to migrate in large numbers, as far as 160 km from their developing site in occasion (Carpenter & LaCasse, 1974).

In Illinois Ross (1947) reported Oc. sollicitans in the southern parts of

the state at oil drilling or mining facilities. Although he does not elaborate on the findings, it is clear that these mosquitoes can inhabit spills of and/or human-made containers with saline groundwater pumped out of oil wells and mining tunnels. Oil drilling is non-existent in Wisconsin (U.S. Energy Information Administration, 2018) and although mining exists, saline groundwater is practically non-existent in the state (Stanton *et al.*, 2017).

Accumulation of road salt in fresh water bodies has been a concern in the past few years as a source of salinification. Although theoretically it would be possible to turn fresh water bodies into saline ones by application of road salt, the concentration of chlorides found at stream and lakes in the upper midwest in recent studies (Blasius & Merritt, 2002; Dugan *et al.*, 2017) would not be sufficient to trigger osmoconformation mechanisms in mosquitoes (Wigglesworth, 1933; Garrett & Bradley, 1987) and thereby not support the development of *Oc. sollicitans* larvae. It is possible that smaller water bodies than those studied in the cited literature may become significantly saltier with evaporation, and thus may be where these mosquitoes are breeding. It is possible that the collection of *Oc. sollicitans* by Meece *et al.* (2003) consisted of specimens from such pools, but it is far more likely that this was a misidentification.

#### **Reported distribution for Ochlerotatus sollicitans: Dane**

County: Meece *et al.* (2003)\*. Kenosha County: Meece *et al.* (2003)\*. Milwaukee County: Meece *et al.* (2003)\*. Racine County: Meece *et al.* (2003)\*. Waukesha County: Meece *et al.* (2003)\*.

### Incorrect records: Ochlerotatus grossbecki (Dyar & Knab) [=Aedes grossbecki] and Psorophora mathesoni Belkin & Heinemann

Thompson & DeFoliart (1966) performed mosquito collections for arbovirus isolation and reported two new mosquito records for the state, collected in Dane County, *Ochlerotatus grossbecki* (as *Aedes grossbecki*) and *Psorophora mathesoni* (as *Psorophora varipes*).

Psorophora mathesoni has a mostly southeastern distribution in the United States. No records exist for surrounding Midwestern states including Michigan and Minnesota. Venard & Mead (1953) report *Psorophora mathesoni* as very rare in the southern counties of Ohio and that it had been collected only once in a northern county. Hart (1944) reports the species in a county in southern Indiana, whilst Nasci *et al.* (1983) reports it in a county in northern Indiana. Ross (1947) remarks that *Psorophora mathesoni* (as *Psorophora varipes*) is a southern species in the state of Illinois, occurring mainly in the southern eighth or fourth of Illinois. Rowe (1942) reports *Psorophora mathesoni* (as *Psorophora varipes*) for the state of Iowa based on a single specimen captured in May 25, 1941. The author states that although he is aware the species is very similar to *Psorophora horrida*, the markings on the hind tarsi were sufficiently different to assert the identity of the specimen. It is highly unlikely that Rowe (1942) could distinguish the two species at that time, as *Psorophora horrida* have been shown to present a range of tarsal marking variations, and other characters (such as scutal patterns and subspiracular vestiture) have been proven to be most useful to help distinguish these species (Harrison & Whitt, 1996; Harrison *et al.*, 2008). Similarly, the report by Thompson & DeFoliart (1966) of *Psorophora mathesoni* (as *Psorophora varipes*) is most likely a result of misidentification. The fact that no other researcher has ever collected this species since strengthens this view.

Ochlerotatus grossbecki has a slightly confusing distribution pattern, as Ross (1947) cites that Ochlerotatus grossbecki (as Aedes grossbecki) has only been collected in the extreme south of the state of Illinois, while Venard & Mead (1953) report its occurrence mostly in the northern counties of Ohio. There are no reports of this species for Michigan, Minnesota or Iowa, however, and the lack of collection of this species after Thompson & DeFoliart (1966)'s in 1966 implies that it is more likely to have been a misidentification. For the reasons above cited both these species are considered to not occur in Wisconsin.

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## Figures





publication (sorted by year)

Figure 2: The absolute number of publications per year, in regard to mosquito presence and/or abundance in the state of Wisconsin.



Figure 3: Light grey squares represent the species is not known to occur in Wisconsin at the time of publication; dark grey squares represent a species is known at the time of publication, but has not been featured in that specific publication; black squares represent a species has been featured in the specific publication.



Figure 4: Light grey squares represent the species does not have any vouchers for that year in the WIRC; black squares represent a species has one or more vouchers for that year in the WIRC.



Appendix A. Maps of records of species of mosquitoes in the state of Wisconsin. Left: WIRC records + Literature records. Upper right corner: WIRC records only. Lower right corner: Literature records only. Each county is white, if no record exists, grey if a record exists, and ranging from grey to black depending on the abundance of specimens deposited at the WIRC, grey if few specimens, black if many. WIRC records are given a number of specimens. Literature records are given a letter corresponding to the publication: A) van der Wulp, 1867; B) Howard et al., 1915;C) Howard et al., 1917; D) Dvar, 1922; E) Dickinson, 1944; F) Ryckman, 1952; G) Thompson & Dicke, 1965; H) Siverly & DeFoliart, 1968b; I) Siverly & DeFoliart, 1968a; J) Anslow et al., 1969; K) Gojmerac & Porter, 1969; L) DeFoliart et al., 1969; M) Porter & Gojmerac, 1970; N) Wright & DeFoliart, 1970; O) Loor & DeFoliart, 1970; P) DeFoliart et al., 1972; Q) Amin & Hageman, 1974; R) Grimstad et al., 1974; S) Grimstad & DeFoliart, 1975; T) Arnell, 1976; U) Gilardi & Hilsenhoff, 1992; V) Meece et al., 2003; W) Hughes et al., 2008.





















































































































# Chapter 4. Survey of the mosquito fauna (Diptera: Culicidae) of the University of Wisconsin-Madison Arboretum, WI, USA

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# Introduction

The mosquitoes (Diptera: Culicidae) are a group of insects best known for the blood-feeding behavior of the adult females and the associated potential to transmit etiologic agents of disease to humans and other animals. They are abundant both in the flying adult stage and in aquatic habitats as larvae and pupae. They play important roles in the ecosystems they inhabit, serving as food for other animals (Bay, 1974; Naeem, 1988) and providing important pollination roles in the adults (Brantjes & Leemans, 1976; Thien, 1969, Gorham, 1976), and can be used as bioindicators of environmental quality (Dorvillé, 1996).

The University of Wisconsin-Madison Arboretum is a 1,200 acre piece of land surrounded by the City of Madison, Wisconsin, U.S.A. The land was dedicated in 1934 by then director Aldo Leopold, with the goal of reestablishing the landscape and plant communities that predated European settlement, thereby pioneering the concept of ecological restoration (UW-MA, 2019). Although there have been intermittent reports of mosquito

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species from the Arboretum since 1965, there has not been a comprehensive survey of the fauna of this area as a whole.

The first published account of mosquito fauna in the UW-Madison Arboretum was by Thompson & Dicke (1965), reporting the presence of Ochlerotatus Aedimorphus vexans vexans (Meigen), (Ochlerotatus) trivittatus (Coquillett), and the Ochlerotatus stimulans group (Walker). Four years later, DeFoliart et al. (1969) reported what we now know was Ochlerotatus communis (de Geer) (as "Aedes communis group", denoting that certainty about the differentiation between the species *Oc. communis*, Oc. punctor (Kirby), Oc. abserratus (Felt & Young), Oc. sticticus (Meigen), and Oc. provocans (Walker) was not possible at the time). In that same year Anslow et al. (1969) reported Aedes cinereus Meigen, Culex pipiens s.l. Linnaeus, Culex restuans Theobald, Culex salianarius Coquillett, 'Ochlerotatus' ('Protomacleaya') triseriatus s.l. sensu auctorum (Say), and Culex Linnaeus spp.. Wright & DeFoliart (1970) reported Psorophora (Janthinosoma) ferox (von Humboldt). Grimstad & DeFoliart (1974, 1975) reported Oc. communis s.s, and later Ochlerotatus (Ochlerotatus) canadensis canadensis (Theobald). Some reports of mosquitoes collected in Dane County include mention of the Arboretum, but it is not possible to ascertain which species were collected there specifically, (e.g.: Wright & DeFoliart, 1970; DeFoliart et al., 1972; Meece et al., 2003).

The Arboretum presents a unique opportunity to collect mosquitoes in distinct ecosystems that have long been carefully preserved and to develop a baseline for future long term research. Therefore, we conducted surveys of the mosquitoes of the UW-Madison Arboretum in 2016, 2017 and 2018.

#### **Materials and methods**

# Adults:

We performed 15 collections events of adult mosquitoes in 2016. All events took place with the use of three traps in different locations, Wingra Woods [43°02'49"N, 89°25'36"W], Teal Pond [43°02'27"N, 89°25'29"W] and Lost City Forest [43°02'32"N, 89°24'58"W], with each trap running from approximately 14:30 to 9:30. The first collection event utilized two CDC-like incandescent light traps baited with  $CO_2$ , and a Mosquito Magnet<sup>®</sup>. Thereafter, we substituted the Mosquito Magnet<sup>®</sup> for a CDC-like UV-light trap baited with  $CO_2$  for subsequent events. The collection event dates are as follows: 1-2.vi.2016, 13-14.vi.2016, 15-16.vi.2016, 20-21.vi.2016, 21-22.vii.2016, 27-28.vii.2016, 21-22.vii.2016, 22-23.vii.2016, 26-27.vii.2016, 27-28.vii.2016, 21-22.vii.2016, 22-23.vii.2016, 26-27.vii.2016, 27-28.vii.2016, 21-22.vii.2016, 22-23.vii.2016, 26-27.vii.2016, 27-28.vii.2016.

All Anopheles Meigen spp. and Culex spp. specimens were removed from the sample in the trap upon retrieval from the field and stored in a -80°C freezer for a separate study other than this mosquito survey conducted by our collaborators. Similarly, a subset of Ochlerotatus canadensis canadensis and Coquillettidia perturbans (Walker) was also removed and stored at a -80°C freezer for other purposes. We were able to identify all the Anopheles to species during retrieval. The Culex were not identified to species; however some vouchers were kept and identified to species. The remaining material from the collection events, from 1-2.vi.2016 to 27-28.vi.2016, were dry-mounted and deposited in the Culicidae collection of the School of Veterinary Medicine, Department of Pathobiological Sciences, University of Wisconsin-Madison, voucher material was also deposited at the Wisconsin Insect Research Collection (WIRC), at the University of Wisconsin-Madison.

We collected adults sporadically in 2017 by manual collection, and in one trapping event utilizing a CDC-like incandescent light trap baited with CO<sub>2</sub> [14 April 2017, Gallistel Woods, 43°02′35″N, 89°25′28″W]. These were performed only for confirmation of faunistic composition. Similarly, in 2018 we performed sporadic trapping events utilizing CDC-like incandescent light and UV-light traps baited with CO<sub>2</sub> [Wingra Woods, 43°02′49″N, 89°25'36"W]. These were also dry-mounted and are deposited at the collections reported above.

#### **Immatures**:

We report identifications for 14 collection events for immatures between 2016, 2017 and 2018, utilizing manual collection, with larval dippers and pans. Each collection event consisted in the inspection of a set of known sites, numbered from 1 to 6: 1) Curtis Prairie [43°02′26″N, 89°25′43″], 2) Gallistel Woods (flooded forest) [43°02′35″N, 89°25′28″W], 3) Gallistel boardwalk [43°02′33″N, 89°25′25″W], 4) Teal Pond [43°02′27″N, 89°25′27″W], 5) Teal Pond Wetlands [43°02′24.4″N 89°25′29.6″W], and 6) Gardner Marsh [43°03′21″N, 89°24′22″W]. The dates for the collection events are as follows: 27.vi.2016, 28.vi.2016, 6.vii.2016, 7.vii.2016, 14.iv.2017, 2.v.2017, 10.v.2017, 5.vi.2017, 6.vi.2017, 13.vi.2017, 20.vi.2017, 22.viii.2017, 5.xi.2017, and 31.v.2018. Dates for which we did not find any immatures in our sampling areas are not reported.

Larvae collected in the field were brought to the laboratory and reared individually in plastic cups with tap water and fed dry yeast. Pupae collected in the field and developed in the lab were placed in small cups inside closed mesh containers for collection of the adult. Larval exuviae, pupal exuviae and adult association was recorded. Adults were drymounted, and immature exuviae were slide mounted in Canada balsam. Male genitalia were slide mounted in Canada balsam when available. All specimens are deposited at the collections previously mentioned.

Identifications of specimens were made using keys in Carpenter & LaCasse (1974), Darsie & Ward (2005), Stojanovich (1997), Wood *et al.* (1979), and Darsie (1951). Species descriptions were consulted for confirmation of identifications.

The majority of *Anopheles* and *Culex* mosquitoes collected in 2016 were removed immediately upon trap collection and stored in a -80°C freezer for virus detection. *Anopheles* were identified to species at this time, but *Culex* were not. Only the voucher *Culex* were identified to species, at a later time; most of the total *Culex* catch are represented by the identification "*Culex* spp." It is the former voucher specimens upon which we report species richness of *Culex*.

# Larval habitats:

The trail markers referenced consist of wooden poles affixed to the ends of trails within the UW-Madison Arboretum; a map with the positions and names of the markers can be found at the UW-Madison Arboretum website1. The sites in our standard sampling are labeled 1 to 5 and are as follows:

Curtis Prairie (1) – Exposed, tall grass brush prone to occasional inundation resulting from rain. Just southeast of trail marker post A4.

Gallistel Woods flooded forest (2) – Fairly open oak forest about 5 meters away from Gallistel Boardwalk. Just past G3. Sampling site almost always muddy.

Gallistel Boardwalk (3) – Samples made over the sides of this boardwalk. Most samples made amidst aquatic vegetation, but some sampled portions were on the border with the forest and hence contained more woody plants and dead plant material; never dry, but water level varying considerably at the points of collection.

Teal Pond (4) – Fairly large pond surrounded by forest. Just east of F3. Perennial body of water but points of collection (near the boardwalk) sometimes with lowered water level, to the point of becoming mostly mud.

Teal Pond Wetlands (5) – About halfway between F2 and L3, this area was similar to the Curtis Prairie location (1), also consisting of grass, but with a few more trees in the surrounding area.

<sup>1</sup> https://arboretum.wisc.edu/content/uploads/2018/07/Map-Rev-6\_7-winter-2017\_web.pdf

An additional site was sampled once [5.xi.2017] for confirmation of a species presence, this is labeled site 6. This site was only sampled once, on 5 November 2017, and so its fauna is not directly comparable with the over five sites:

Gardner Marsh (6) – Sampling site defined by its perimeter which was a large tree root, effectively separating a small body from the main body of Gardner Marsh. Site about ~25 m west of the boardwalk across from the Mills Street parking lot. Very stagnant, turbid water.

# Results

We collected at least 21 mosquito species, representing ten genera, in the UW-Madison Arboretum. Twenty of these species were present in the adult traps, and 13 of these were collected in immature stages.

For the first year, 3739 adult mosquitoes were trapped from 1 June 2016 to 28 June 2016. Along with a gradual increase in raw numbers (Table 1), we also observed changes in community structure (Figure 2). *Ochlerotatus* aff. *stimulans* s.l. decreased in relative abundance from 0.233 to 0.004. Likewise, *Culex* spp. decreased from 0.023 to 0.009. *Ochlerotatus* 

(*Culicada*) *canadensis canadensis* decreased from 0.442 to 0.044, and in our samples this species dropped in absolute abundance to zero by 7-8 July 2016. *Aedes cinereus* decreased from 0.023 to 0.001. *Anopheles* (*Anopheles*) *punctipennis* decreased slightly, from 0.047 to 0.031, but seemed to remain relatively constant throughout the month, averaging in abundance at 0.03 (SD 0.01). *Aedimorphus vexans vexans* also stayed relatively constant, averaging 0.183 (SD 0.056).

The most abundant mosquito trapped was *Coquillettidia* (*Coquillettidia*) perturbans (Figures 1 and 2), comprising 0.605 of all mosquitoes caught. We saw an increase in relative abundance of this species from zero to 0.707, then a slight decrease to 0.598. *Ochlerotatus* (*Ochlerotatus*) trivittatus (Coquillett) increased in abundance from 0.023 to 0.12.

Of the five larval sites that were regularly sampled during the spring and summer of 2017 (Sites 1-5), *Am. vexans* was found at every site (Table 2). *Ochlerotatus trivittatus* was found at all but one site. *Ae. cinereus* was found at all but one site. *An. punctipennis* (Say) and *An. perplexens* Ludlow were present at two sites.

Curtis Prairie (Site 1) had two species in two genera. Gallistel Woods flooded forest (Site 2) had 5 species in 3 genera. Gallistel boardwalk (Site 3) had 7 species in 6 genera, Teal Pond (Site 4) had 7 species in 5 genera, and Teal Pond Wetlands (Site 5) had two species in two genera.

# Discussion

The nomenclature for the taxa presented below follows Reinert *et al.*, (2009), with special reference to the nomenclature of Wilkerson *et al.* (2015) in square brackets. The subgeneric abbreviations follow Reinert (2009).

# Aedes cinereus Meigen [=Aedes (Aedes) cinereus]

This species was uncommon in adult traps (only ten total specimens collected), but, interestingly, present in most of the larval habitats sampled (sites 2, 3, 4, and 5).

Aedimorphus vexans vexans (Meigen) [=Aedes (Aedimorphus) vexans vexans]

We collected *Aedimorphus vexans vexans* in all larval habitats we sampled during spring and summer 2017, and additionally during every

trap night in June 2017. This species was the second most abundant during most trap nights (0.198 of total mosquitoes collected), and the most common species in larval collections.

#### Anopheles (Anopheles) perplexens Ludlow

This is a first record of this species in Wisconsin. Adult females appear superficially similar to *Anopheles (Anopheles) punctipennis*. There is only one well-established character for the separation between the two species: the ratio of the subcostal pale spot to wing length is 0.06 or less in *Anopheles (Anopheles) perplexens* and usually 0.8 or more in *Anopheles (Anopheles) punctipennis* (Bellamy, 1956). All of our specimens that conform to the 0.06 or less diagnosis of *An. perplexens* are here identified to said species. *T*his mosquito was relatively uncommon in adult catches (only 8 specimens), only appearing in late June, and few larvae were collected.

### Anopheles (Anopheles) punctipennis (Say)

Individuals of this species complex that presented the subcostal pale spot to wing length ratio above 0.09 were identified as *An. punctipennis*, in accordance with Bellamy (1956). Adults were moderately common in trap catches (0.029 of total mosquitoes), especially in late June, and few larvae were collected.

# Anopheles (Anopheles) quadrimaculatus s.l. Say

Specimens that fall within the Anopheles (Anopheles) quadrimaculatus complex of species that could not be further identified to species due to poor specimen condition are reported here. This includes only one trap-caught adult, and two specimens collected as larvae and reared to and identified as adults. See discussion of Anopheles (Anopheles) smaragdinus for information about that particular species, which is also in this complex.

#### Anopheles (Anopheles) smaragdinus Reinert

Only two specimens were caught in traps, and one specimen was collected as a larva and reared to and identified as an adult. Separation from *Anopheles* (*Anopheles*) *quadrimaculatus* s.s. in the adult involves the use of three characters: the number of interocular setae, the number of scutal fossal setae, and the number of pre-alar setae (Reinert *et al.,* 1997). This is the first record of this species for Wisconsin.

#### Coquillettidia (Coquillettidia) perturbans (Walker)

This mosquito was by far the most commonly collected species in adult traps, and it was also the most common species every trap night from mid to late June. We attempted to locate and collect larvae, but were unsuccessful. Larvae live in direct contact with aquatic plants, and Lewis & Bennett (1980) note that larvae have been collected from *Carex lasiocarpa*, *Typha glauca*, *Typha latifola*, and *Sparganium eurycarpum* (among other aquatic plant species); all of these species have been reported in the UW-Madison Arboretum (Hall & Zedler, 2010).

# Culex (Culex) pipiens s.l. Linnaeus

This species complex was collected in the larval and pupal stages in November 2017, from a small pocket of water separated from the main body of Gardner Marsh by a very large tree root. As the species in this complex overwinter in the adult female stage (Carpenter & LaCasse, 1974), it may be that these late-stage larvae and pupae could have emerged just before the winter season, to then overwinter as adults. This suggestion is in line with the observation of Spielman (1971) that breeding stopped in October in Boston, Massachussetts for *Culex* (*Culex*) *pipiens* s.s. We noted that larvae presented a siphon index which was at odds with that usually reported for this species complex in North America; typically, the siphon index was closer to that usually reported for *Culex* (*Culex*) *salinarius* and key out to such in the keys by Darsie & Wards (2005). However, the identification of larvae were confirmed by careful comparison with available descriptions of each species (Harbach *et al.*, 1985, Carpenter & LaCasse, 1974). As almost all *Culex* caught in adult traps that could be immediately identified to *Culex* spp. were saved for virus detection, we lack adultcollected vouchers for this species. Nevertheless, because the number of *Culex* kept as vouchers was quite low, we believe it is still very likely that *Culex pipiens* s.l. was indeed also collected in the adult stage.

#### Culex (Culex) restuans Theobald

This species was collected in the adult stage only. Due to its morphological similarity to *Culex* (*Culex*) *pipiens*, male genitalia of kept *Culex* voucher specimens were identified to confirm its presence in our samples.

#### Culex (Culex) salinarius Coquillett

This species was collected only in the adult stage. While abundance isn't known (see discussion of level of identifications of *Culex*, under *Culex* (*Culex*) *pipiens* s.l.), its presence was confirmed from voucher specimens. The morphological characters used for identification were as discussed in Apperson *et al.* (2002) and two specimens were additionally confirmed by PCR-based species identification using the ITS primers and protocol of Crabtree *et al.* (1995).

#### Culex (Neoculex) territans Walker

This species was collected both in immature stages and in adult traps. Larvae presented characters that resulted in the incorrect identification to *Culex (Neoculex) boharti* and *Culex (Neoculex) reevesi* by utilization of the keys of Darsie & Ward (2005). However, larval characters from Bickley & Harrison (1989) and characters from male genitalia showed beyond reasonable doubt only *Cx. (Neoculex) territans* is present at the collection site.

#### Culiseta (Culiseta) inornata (Williston)

This species was collected in adult traps only. In April 2017, adult trap collections were made at Gallistel Woods flooded forest, which contained almost entirely *Cs. inornata*. This species is well-documented as overwintering in the adult stage in the northern parts of North America (Carpenter & LaCasse, 1974), so these may have been females exiting their overwintering cycle.

Hulecoeteomyia japonica japonica (Theobald) [=Aedes (Hulecoeteomyia) japonicus japonicus]

Only one specimen of this invasive species was collected during our adult trapping efforts in June 2016.

Ochlerotatus (Culicada) canadensis canadensis (Theobald) [=Aedes (Ochlerotatus) canadensis canadensis]

During our first trap night, 1-2 June 2016, this was among the most abundant of species. Its abundance declined throughout the season however, and by 16 July 2016 and thereafter, it was completely absent. Although this species overwinters in the egg stage and hatches in early spring, which is suggestive of a univoltine life cycle, there are reports of continuous breeding throughout the warm season as well (Carpenter & LaCasse, 1974; Rudolfs & Lackey, 1929).

# Ochlerotatus trivittatus (Coquillett) [=Aedes (Ochlerotatus) trivittatus]

This species was common in both larval collections and adult traps. Larval comb scales did not fit the key presented by Darsie & Ward (2005); this key says that the median spine of the comb scale is to be at least twice as long as the subapical ones, while our specimens often had the median one only 1.5 times as long as the submedian ones. However, we confirmed our identification in the female and in the male genitalia of reared specimens.

# Ochlerotatus punctor s.l. (Kirby) [=Aedes (Ochlerotatus) punctor s.l.]

Representatives of this species complex were fairly rare, only appearing in early June 2016 collections. This could be reflective of primarily early season activity, as it is typically associated with pools of melting snow (Carpenter & LaCasse, 1974). Just one larva was observed, from Gallistel Woods flooded forest, on 20 June 2017, but failed to develop in the laboratory.

#### Ochlerotatus aff. sticticus (Meigen) [=Aedes aff. sticticus]

This species was rare in traps, with only three individuals observed, during mid-June 2016. Other individuals were collected manually, however, in 2017 and 2018. Although these individuals keyed out to Oc. sticticus in the keys used, the specimens differed from currently published descriptions in many aspects (Carpenter & LaCasse, 1974, Gutsevich et al., 1974, Wood et al., 1979, Harbach et al., 2017). Namely, Oc. sticticus is reported as having narrow abdominal bands, even absent on some tergites; in our collections the bands on some specimens were quite broad and usually present on every tergite from II-VII. Further, Oc. sticticus is reported as either having a few pale scales on the costa, or having the wing entirely dark; however most of these specimens had basal pale scaling on the costa, 'streak' just underneath the costa near the humeral region, and a sometimes further pale scaling on the base of the subcosta and radius. Additionally, Oc. sticticus is sometimes reported as having the mesokatepisternal scales sparse along the anterior edge; these specimens had a complete line of scales extending to the anterior angle. Oc. sticticus is

reported as having the lower 0.25 to 0.33 of the mesepimeron bare, whereas these specimens sometimes have scales extending past this mark. None of the specimens fit descriptions to any currently recognized species, and *Oc. sticticus* is the most superficially similar species to our collections, even if only for its broad description.

Ochlerotatus aff. stimulans s.l. (Walker) [=Aedes aff. stimulans s.l.]

Adult females were fairly common in light trap catches throughout the trapping period. Larvae were collected in Gallistel Woods flooded forest on 14 April 2017 and 2 May 2017. This group is notorious for the difficulty of identifying certain life stages, including the adult female. However, larvae reared to adult males revealed that this is in fact a new species of the Stimulans group, based on morphology of the male genitalia and pupal exuviae (manuscript in preparation).

'Ochlerotatus' ('Protomacleaya') triseriatus s.l. [=Aedes (Protomacleaya) triseriatus s.l.]

Only two adult individuals were collected in traps, during the trap night of 28 June 2016.

#### Psorophora (Janthinosoma) ferox (von Humboldt)

We collected *Psorophora (Janthinosoma) ferox* in the larval stage only once, on 20 June 2017. This mosquito is reported to be present in the larval stages in temporary pools or stream overflow pools from May to September in the north of North America (Carpenter & LaCasse, 1974). However, as we found a single specimen in a semi-permanent water body (marsh area underneath Gallistel Woods boardwalk), it may be that we did not actually find *Ps. ferox* habitat, but that this sample represents only an incidental occurrence of the species. The eggs may have been laid spuriously by a female, outside of normal larval habitat, or rain may have washed eggs from nearby floodplains to this marsh area.

# Uranotaenia (Uranotaenia) sapphirina (Osten Sacken)

This species was caught just once in an adult trap, and larvae were found only in Teal Pond. Although this is the first report of this species for the Arboretum specifically, it has been reported twice for Dane County (Dickinson, 1944; Meece *et al.*, 2003).

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# **Tables and Figures**

Table 1. Total numbers of adults trapped at the UW-Madison Arboretum from 1 June to 28 June 2016 utilizing three traps in three different locations.

	TOTAL
1-2.Jun.2016	43
13-14.Jun.2016	260
15-16.Jun.2016	388
20-21.Jun.2016	625
21-22.Jun.2016	895
27-28.Jun.2016	1528
TOTAL	3739

Table 2. Collection report for adults trapping and larval sites (labeled 1 through 6), as well as previous records for the UW-Madison Arboretum.

	Adult	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Previous records
Aedes cinereus	Х		Х	Х	Х	Х		а
Aedimorphus vexans vexans	Х	Х	Х	Х	Х	Х		b
Anopheles (Anopheles) perplexens	х			Х	Х			
Anopheles (Anopheles) punctipennis	Х			Х	Х			
Anopheles (Anopheles) quadrimaculatus s.l.	Х				Х			
Anopheles (Anopheles) smaragdinus	Х				Х			
Coquillettidia (Coquillettidia) perturbans	Х							
Culex (Culex) pipiens s.l.	?						Х	а
Culex (Culex) restuans	Х							а
Culex (Culex) salinarius	Х							а
Culex (Neoculex) territans	Х			Х				
Culiseta (Culiseta) inornata	Х							а
Hulecoeteomyia japonica	Х							
Ochlerotatus (Culicada) canadensis canadensis	Х							С
Ochlerotatus trivittatus	Х	Х	Х	Х	Х			b
Ochlerotatus punctor	Х		Х					
Ochlerotatus aff. sticticus	Х							
Ochlerotatus aff. stimulans s.l.	Х		Х					
'Ochlerotatus' ('Protomacleaya') triseriatus s.l.	Х							а
Psorophora (Janthinosoma) ferox	Х			Х				d
Uranotaenia (Uranotaenia) sapphirina	Х				Х			

Legend: Site 1: Curtis Prairie; Site 2: Gallistel Woods flooded forest; Site 3: Gallistel Woods Boardwalk; Site 4: Teal Pond; Site 5: Teal Pond Wetlands; Site 6: Gardner Marsh. a) Anslow *et al.*, 1969; b) Thompson & Dicke, 1965; c) Grimstad & DeFoliart, 1975; d) Wright & Defoliart, 1970. Figure 1. Total species composition of adult mosquitoes trapped at the UW-Madison Arboretum from 1 June to 28 June 2016 utilizing three traps in three different locations.



Figure 2. Seasonality of community structure of adult mosquitoes trapped at the UW-Madison Arboretum from 1 June to 28 June 2016 utilizing three traps in three different locations.



# Chapter 5. *Ochlerotatus mirabilis* sp. nov., a new mosquito (Diptera: Culicidae) from Wisconsin, USA

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#### Abstract

We describe a new species of mosquito (Diptera: Culicidae) collected in the University of Wisconsin-Madison Arboretum, a natural area within the municipality of Madison, Dane County, WI. *Ochlerotatus mirabilis* sp. nov. is a member of the Stimulans Group, however it has distinctive characters in the male genitalia and pupa that do not fit any described species or possible phenotypic variation. Additionally we describe at least two female adult characters that may aid in the identification of this life stage. Given the necessity of a full revision of the Stimulans Group, the adult female characters described are tentative and male genitalia should be used to confirm the identification of this species.

Keywords: Ochlerotatus mirabilis, Culicidae, Aedini, Taxonomy

# Introduction

During the spring of 2017, several mosquito larvae (Diptera: Culicidae) were obtained from a flooded marsh area in the UW-Madison Arboretum, Madison, Wisconsin, USA, for which identification was challenging. Observation of adult male genitalia characters revealed that the species in question belongs to the Stimulans group as understood by Dyar (1918, 1920) and that it does not fit the description of any currently recognized species or synonym of that group. The adult female is generally similar to *Ochlerotatus stimulans* (Walker), *Ochlerotatus euedes* (Howard, Dyar & Knab), *Ochlerotatus fitchii* (Felt & Young) and, to a lesser extent, *Ochlerotatus excrucians* (Walker).

The new species is described herein. The morphological terminology follows Harbach & Knight (1980) and generic abbreviations are given by Reinert (2009).

#### **Materials and Methods**

#### Mosquitoes:

The specimens used for description were collected as larvae at the University of Wisconsin-Madison Arboretum, Madison, Wisconsin, USA, as part of the effort described in Chapter 3 of this dissertation. Additional specimens were collected as adults in CDC-like light traps at the same location. Larvae were reared individually in tap water in plastic cups, with dry yeast as a food source. Larval and pupal exuviae were collected from the water and mounted in Canada Balsam (SERVA Electrophoresis GmbH, Heidelberg, Germany). Adults were dry mounted post emergence. Genitalia from male specimens were removed and also mounted in Canada Balsam. All specimens are associated by the code AR#, where mounts with the same number belong to a single specimen. All specimens here referenced will be deposited at the National Museum of Natural History; Smithsonian Institution, also known as the United States National Museum (USNM).

# Molecular methods:

The holotype (AR4, male) and type series specimens AR1 [male], AR2 [female], AR7 [male], AR8 [male], AR27 [female], AR43 [female] and AR54 [female] had a portion of the midleg removed for DNA extraction, as described by Johnson et al. (2015). Specifically, each mosquito leg was placed in a microcentrifuge tube with 50  $\mu$ L of 25 mM NaOH + 0.2 mM EDTA pH 12. The tube was heated to 95°C for an hour, then cooled down to 4°C, and 50  $\mu$ L of 40 mM EDTA pH 5 was added. The "universal" primers for cytochrome *c* oxidase subunit I of invertebrates, LCO1490 and HCO2198, were used for PCR amplification of ~700 base pairs of the gene (Folmer *et al.* 1994). PCR products were sent to the University of Wisconsin-

Madison Biotechnology Center for sequencing. Sequences were manually cleaned to remove 40bp at the 5' end from each sequence, in order to remove low quality base calls, and the trailing end of the sequencing product, which presented no discernible peak in the chromatogram (beyond 650bp in our case). The sequences will be deposited in GenBank and the Barcode of Life Database.

#### Ochlerotatus mirabilis sp. nov.

Type data: *Holotype* male, with genitalia slide and associated larval and pupal exuviae slides (AR4), "Gallistel Woods", UW-Madison Arboretum, Madison, Wisconsin, USA (43°02′35″N, 89°25′28″W), 14 April 2017, coll. V. Ferreira-de-Freitas & N. Thrun, collected as larva from a marsh. Paratopotypes, 1 male, with genitalia slide and associated larval and pupal exuviae slides (AR1), 19 males, with genitalia slide and associated pupal exuviae slide (AR5, AR6, AR7, AR8, AR11, AR16, AR18, AR21, AR24, AR26, AR31-33, AR38, AR39, AR41, AR44, AR45, AR47), 2 females, with associated larval and pupal exuviae slides (AR2, AR3), 23 females, with associated pupal exuviae slide (AR12-15, AR17, AR19, AR20, AR22, AR23, AR25, AR27-30, AR34-37, AR40, AR42, AR43, AR46, AR48), same data as holotype. Primary type material and most secondary types will be deposited at the Wisconsin Insect Research Collection at the University of WisconsinMadison, Madison, WI, USA; a few secondary types will be deposited at the United States National Museum, Smithsonian Institution, Washington, D.C. (material yet not deposited).

Female: Head: Many erect forked setae, all dark, extending all over occiput, up to near the vertex. Medial area of occiput scaled differently than lateral area; the medial area here describes is very broad, with its lateral edge aligned with the lateral end of the tori. The medial area posteriorly with pale falcate scales, somewhat disheveled. The medial area anteriorly with slightly smaller falcate scales, all tan brown, appearing orderly. Lateral area with very broad appressed paddle shaped scales, almost all pale, except for a spot of dark scales at the most lateral position (postgena), this spot large, anterior in position (closer to eye), touching vertex. Base of second and third palpal segment with pale scales, sometimes one or two scattered further apically, otherwise dark scaled. Proboscis dark, with very few pale scales basoventrally. Thorax: Large patch of pale scales present at postprocoxal membrane; pale scales covering metameron; mesomeron with small patch of pale scales at upper posterior corner. Lower  $\sim 0.25$  of mesanepimeron bare (devoid of scales). One mesepimeral setae present. Scaling of the mesokatepisternum reaching anterior angle. Paratergite scales thin and falcate anteriorly, moderately broad and paddle shaped

posteriorly. Subspiracular patch of scales large and "double", that is, consisting of two distinct regions, one anterior and small, the other posterior and elongated along edge of mesokatepisternum; the anterior portion with thin and falcate scales, the posterior portion with moderately and paddle shaped scales. Postpronotal setae many ( $\sim$ 7). broad Postpiracular setae very thin, few ( $\sim 6$ ). Postspiracular patch of pale scales covering much of the sclerite, densely scaled, these scales small but generally paddle-shaped and somewhat elongated. Scutum: Scutal scales slightly broader and falcate at edges of scutum, at dorsocentral line, and below "scutal V" (along the prescutellar area), also appearing slightly paler. Remainder of scutum with small hairlike tan brown scales. Achrostical setae few, small, inconspicuous, more close together posteriorly. Dorsocentral setae present both anteriorly ad posteriorly, few anteriorly, not very conspicuous, somewhat small, gradually extending posteriorly, the ones near scutellar area very long and conspicuous. Antealar setae dark, supraalar setae pale (white gold). Anterior and posterior scutal fossal setae present, few, but somewhat long. Abdomen: Abdominal tergites II-VI, at least, with very discernible band of pale scales, merged with basolateral patch of pale scales, this band with a straight edge. Sternites with all pale scales. Legs: Tarsomeres  $I_1$ -III<sub>1</sub> with white scales scattered, mostly basally, darker apically. White markings present basally at remaining tarsomeres,
extending more than 1/3 apically, frequently almost covering half the segment, except for Tarsomere I<sub>5</sub>-III<sub>5</sub>, where the with scaling usually covers slightly less than 1/3 basally. Fore tarsal unguis somewhat bent after mid lenght of large tooth; small tooth very blunt; distance between base and apex of small tooth clearly shorter than distance from apex of large tooth to apex of small tooth (Figure 3). Wing: CuA with some scattered pale scales at base (very few). M with some pale scales, mostly near rm, same for M1+2. Rs and R2+3 same. Many pale scales at C, Sc, basal R and R1.

**Male:** Genitalia: (Figure 1) Gonocoxite (Gc) long, about four to five times longer than the base width. Basal lobe of gonocoxite protruded dorsally and sunken ventrally, apex somewhat rounded and bearing a strongly developed seta which is distally recurved on its distal end; the lobe bears many setae ventrally from the apical one, including within the sunken medial area of the gonocoxite directly behind the dorsal protrusion of the basal lobe, these setae arise from papillae, which may be quite tall in some specimens, but relatively small in others; the papillose area usually does not extend apically in the gonocoxite much beyond the base line of the dorsal protrusion of the basal lobe. Apical lobe of gonocoxite thin and with strong edges; bearing many small setae, those with distal ends recurved dorsally. Gonostylus (Gs) slightly smaller than 2/3 of the gonocoxite; gonostylar claw (GC) around 1/4 of the size of the gonostylus. Aedeagus long, around three

times as tall as basal width, expanded basally, 1.5 times as wide as basal width at lower third and 1 at upper third. Lobes of the IX tergite rounded, generally as tall as they are wide, with some variation, bearing 3 to 5 long setae.

**Pupa:** (Figure 2; Table 1) Ct4 2 branched; Ct5 3 branched; Ct6 single; Ct7 3 branched; Ct8 4-5 branched; Ct9 2 branched; MT10 long, 6 branched; MT11 long, 2 branched; MT12 not as long as 11, 2 branched; I1 multi branched, general number uncountable; I2 small, 2-3 branched; I3 medium 3 branched; I4 medium, 5 branched; I5 medium, 4-5 branched; I6 very long, reaching upper third of next segment, single; I7 very long, reaching middle of next segment, 2 branched; I9 very small, single; II0 minute, single; II1 medium to long, 6 branched; II2 short, single; II3 medium, 2-3 branched; II4 medium, 3 branched; II5 long, reaching upper third of next segment, 3 branched; II6 very long, reaching beyond middle of next segment, 2-3 branched; II7 long, single; II9 minute, single; III0 minute, single; III1 long, reaching near middle of next segment, 4 branched; III2 very small, single; III3 long, reaching upper third of next segment, 2 branched; III4 medium 3 branched; III5 medium, 6 branched; III6 very long, reaching near middle of next segment, 2 branched; III7 medium 2-3 branched; III8 small, 3 branched; III9 minute, single; III10 very long, reaching middle of next segment, 2-3 branched; III11 small, single; IIIp laterad of III4; IV0 minute,

single; IV1 long, reaching near middle of next segment, 2-3 branched; IV2 small, single; IV3 medium, 1-5 branched; IV4 medium, 2-3 branched; IV5 extremely long, reaching beyond end of next segment, 2 branched; IV6 extremely long, reaching upper third of next segment, 1-2 branched; IV7 medium to long, 2 branched; IV8 medium, 2 branched; IV9 very small, single; IV10 very long, reaching middle of next segment, 1-2 branched; IV11 small, single; IV14 minute, single; IVp medially from IV4; V0 minute, single; V1 very long, reaching lower third of next segment, 1-2 branched; V2 small, single; V3 long, reaching middle of next segment, 2 branched; V4 medium, 5 branched; V5 extremely long, reaching beyond next segment, up to around one third of segment VII, 2 branched; V6 very long, reaching near lower third of next segment, single; V7, long, 5 branched; V8 small, 2-3 branched; V9 very small, single; V10 very long, reaching end of next segment, single; V11 very small, single; V14, minute, single; Vp laterad of V4; VI0 very small, single; VI1 long, reaching middle of next segment, 2 branched; VI2 small, single; VI3 very long, reaching middle of next segment, 2 branched; VI4 medium to long, 4-5 branched; VI5 extremely long, reaching beyond end of next segment, around upper one third of segment VIII, 1-4 branched; VI6 very long, reaching middle of next segment, single; VI7 long, reaching upper one third of next segment, single; VI8 medium, 3 branched; VI9 very small, single; VI10 very long, reaching lower third of next segment, single;

VI11 small, single; VI14 minute, single; VII0 very small, single; VII1 long, reaching middle of next segment, single; VII2 small, single; VII3 long, reaching middle of next segment, single; VII4 long, reaching lower third of next segment, 1-2 branched; VII5 extremely long, reaching well beyond end of next segment, single; VII6 small, 4-5 branched; VII7 very long, reaching beyond end of next segment, single; VII8 medium to long, 3 branched; VII9 medium, 2 branched; VII10 long, reaching end of next segment, single; VII11 small, single; VII14 very small, single; VII10 very small, single; VII14 very small, single; VII10 very small, single; VII14 very long, about 1.5 times its segment length, 2 branched; VII19 long, about same length of segment to slightly longer, 4-6 branched; VII14 small, single; Pa1 single.

**Larva:** Indistinguishable from *Ochlerotatus stimulans* complex. Matches the description given by Wood *et al.* (1979) for the species *Ochlerotatus stimulans*.

Etymology: The specific epithet chosen, *mirabilis*, refers to the remarkable finding of a new species, in the author's view, from perhaps one of the most thoroughly studied countries in regards to mosquito fauna.

Distribution: Only reported for the type locality.

Bionomics: We found these mosquitoes to breed in shallow flood marshes in a deciduous forest, appearing in abundance mid-spring (April), and persisting up to the beginning of the summer (June). Blood feeding habits are unknown, but females were attracted to  $CO_2$  baited CDC traps operated from 16:00 to 10:00 (overnight) during the month of June.

Taxonomic discussion: Ochlerotatus mirabilis is clearly a member of the Stimulans group as described by Dyar (1918, 1920), and it is possible it has been observed before in several localities in the female and classified as Oc. stimulans. The adult female appears indistinguishable from Oc. stimulans based on the literature, however it is probable, as discussed earlier, that those descriptions reflect an amalgamation of many "forms" that likely comprise a complex of species. A redescription of Oc. stimulans based on the type (female, at the Natural History Museum in London, NHM) and topotypical material (Nova Scotia, Canada) would inform the true distribution of both species in the USA, and perhaps reveal more species within the complex. The adult male is apparently easily distinguished from Oc. stimulans from literature (which I call here Oc. stimulans s.l.) (i.e.: Carpenter & LaCasse 1974; Dyar 1920, 1928; Howard et al. 1917; Wood et al. 1979) on the basis of the following two male genitalia characters: 1) Gonocoxite (Gc) long, about four to five times longer than the base width; 2) gonostylar claw (GC) around 1/4 of the size of the gonostylus. There are other characters that are divergent from each specific interpretation of authors, since their descriptions are already divergent, however it is

impossible to know which, if any, of those refer to Oc. stimulans s.s. and these two characters should suffice to distinguish this species from any of those interpretations. The pupa of *Oc. stimulans* was only described by Darsie (1951) from material from New York, USA, and Saskatchewan, Canada. Oc. mirabilis can be differentiated from it by the combination of the following characters: II-1 6 branched, III-4 3 branched, IV-3 1-5 branched, VI-4 4-5 branched, VI-5 1-4 branched. Darsie only describes the dorsal setae of the abdominal segments, so more differences may exist in the ventral setae. Additionally, given the perceived complexity of the Stimulans group, it is possible that populations from Darsie's collection are not conspecific, which would render his description an amalgamation of characters. The larva seems indistinguishable from other Ochlerotatus stimulans s.l., as it matches the description given by Wood et al. (1979) for Ochlerotatus stimulans. This is possibly a reflection of the description of that species, which agglomerates characters from many populations and possibly different species.

Discussion of COI sequencing: ClustalW alignment (performed by Clustal Omega software) shows (using MView) a minimum of 88.9% identity (max. 94.3%) between each of the sequences generated by sequencing from the forward primer (LCO1490). They show a minimum of 83.1% (max. 88.1%) between each other in the sequences generated by sequencing from

the reverse primer (HCO2198). This low identity could have arisen from low fidelity PCR or problems with the sequencing. However, it is clear that "barcoding", or the usage of the COI region for identification of species, does not work well for this group (see Table 2), as has been shown for other groups of mosquitoes (e.g.: Laurito et al. 2013). Using blastn, we compared all sequenced strands with the database for each of the species of the Stimulans group mosquitoes currently deposited at the GenBank (Oc. stimulans, Oc. excrucians, Oc. fitchii, and Oc. euedes), for both light and heavy strands (LCO and HCO); we noted the lowest and highest matches in % identity, and checked that those sequences were obtained utilizing the same primers (see Table 2). We found variation from  $\sim$ 79% identity to  $\sim$ 96% identity with the four species available. The highest identities were with Oc. stimulans from Canada and Oc. excrucians from Japan. We also used blastn to check the consistency of COI based identification across the four species of the Stimulans group for which sequences are available; for this we chose one sample sequence for each species (GU907957.1, GU907891.1, GU907899.1 and GU907883.1), and performed blastn as described before. We found that Oc. stimulans shows more than 99% identity to Oc. excrucians; both Oc. excrucians and Oc. euedes show more than 99% identity to Oc. fitchii; and furthermore, Oc. euedes had higher identity to *Oc. fitchii* than the sample of *Oc. fitchii* chosen (Table 2). It is possible these

results can arise due to misidentification, however, given the variability in % identity (shown in Table 2), associated with the variability of our own samples, we believe it is likely that this group cannot be distinguished to species based on high ancestral polymorphism in COI sequence. Although our own samples have scored higher % identities with *Oc. stimulans*, the variability falls well within all other species for most specimens, also they all fall below the variability shown by each sample species to match to itself, except for *Oc. fitchii*.

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### **Tables and Figures**

Figure 1. Male genitalia of Ochlerotatus mirabilis.



**Figure 2.** Pupal chaetotaxy of *Ochlerotatus mirabilis*, general aspect, dorsal and ventral.



Figure 3. Fore tarsal unguis of *Ochlerotatus mirabilis*.



**Table 1.** Setal branching range for pupal chaetotaxy of Ochlerotatusmirabilis.

	Seta	
	10	6
MT	11	2
	12	2
	2	2-3
	2	20
	1	5
т	4± E	J 4 E
1	С С	4-3
	6	1
	/	2
	9	1
	0	1
	1	6
	2	1
	3	2-3
II	4	3
	5	3
	6	2-3
	7	1
	9	1
	0	1
	1	4
	2	1
	3	2
	4	2
	5	6
III	6	0
	07	2
	/ 0	∠-J 2
	0	ں 1
	9	
	10	2-3
	11	1
	0	1
	1	2-3
IV	2	1
ŢĀ	3	1-5
	4	2-3
	5	2

	6	1-2
	/ 8	2 2
	Q Q	ے 1
	10	1-2
	11	1
	14	1
	0	1
	1	1-2
	2	1
	3	2
	4	5
<b>N</b> 7	5	2
V	6 7	I F
	/ 8	ט ז_2
	Q Q	2-J 1
	10	1
	11	1
	14	1
	0	1
	1	2
	2	1
	3	2
	4	4-5
VI	5 6	1-4
V I	0 7	1
	8	3
	9	1
	10	1
	11	1
	14	1
VII	0	1
	1	1
	2	1
	ろ 1	
	4 5	1-Z 1
	5	1 1_5
	7	
	8	3

325

	9	2
	10	1
	11	1
	14	1
	0	1
VIII	4	2
	9	6-4
	14	1

**Table 2.** Results from balstn analysis for COI sequences from *Oc. mirabilis* and relates species. Each cell shows lowest and highest % identity matches to the sequences produces from specimens described herein for *Oc. mirabilis* (AR2, AR4, AR7, AR8, AR27, AR43 and AR54).

	Oc. stimulans	Oc. excrucians	Oc. fitchii	Oc. euedes
AR4* LCO	89.80% - 92.52%	90.88% - 91.71%	81.86% - 91.54%	88.85% - 89.37%
AR4* HCO	92.07% - 93.25%	90.77% - 92.28%	82.18% - 91.93%	89.77% - 90.84%
AR1 LCO	92.86% - 94.74%	93.37% - 94.20%	84.19% - 94.03%	91.51% - 92.35%
AR1 HCO	89.66% - 90.41%	88.12% - 89.33%	80.10% - 89.33%	87.17% - 87.87%
AR2 LCO	94.90% - 95.82%	94.06% - 95.05%	84.49% - 94.72%	91.91% - 93.28%
AR2 HCO	93.91% - 95.30%	92.30% - 93.81%	83.42% - 93.77%	91.79% - 92.72%
AR7 LCO	89.29% - 91.32%	89.68% - 90.55%	80.37% - 90.22%	87.52% - 88.43%
AR7 HCO	90.62% - 91.50%	88.98% - 90.52%	80.56% - 90.13%	87.97% - 89.16%
AR8 LCO	86.48% - 88.95%	87.13% - 88.23%	79.04% - 87.95%	85.52% - 86.38%
AR8 HCO	81.12% - 82.65%	80.43% - 81.34%	73.19% - 81.27%	79.26% - 80.49%
AR27 LCO	94.13% - 95.03%	93.68% - 94.53%	84.53% - 94.20%	91.51% - 92.72%
AR27 HCO	92.86% - 94.16%	91.60% - 92.82%	83.03% - 92.42%	90.82% - 91.78%
AR43 LCO	84.95% - 88.36%	86.80% - 87.89%	78.55% - 87.46%	85.02% - 85.82%
AR43 HCO	87.97% - 88.67%	87.09% - 87.91%	78.78% - 87.85%	85.69% - 86.92%
AR54 LCO	90.31% - 92.50%	91.09% - 92.04%	81.85% - 91.42%	89.02% - 90.11%
AR54 HCO	92.05% - 92.72%	90.98% - 92.14%	81.94% - 91.80%	89.66% - 91.03%
<i>Oc. stimulans</i> GU907957.1	99.22% - 100.00%	97.81% - 99.06%	88.73% - 98.59%	95.77% - 97.76%
Oc. excrucians GU907891.1	98.28% - 98.90%	98.44% - 100.00%	90.86% - 99.06%	96.40% - 98.13%
Oc. fitchii GU907899.1	89.74% - 91.25%	90.44% - 91.68%	90.75% - 99.69%	91.04% - 91.69%
<i>Oc. euedes</i> GU907883.1	95.93% - 97.59%	96.24% - 97.34%	89.83% - 99.84%	97.01% - 99.84%

Legend: For each of the sample species' sequences (GU907957.1, GU907891.1, GU907899.1 and GU907883.1) its own sequence was left out this comparison.

### Chapter 6. An evaluation of characters for the separation of two *Culex* species (Diptera: Culicidae) based on material from the Upper Midwest

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#### Abstract

Mosquitoes (Diptera: Culicidae) in the *Culex pipiens* complex play a key role in the transmission and therefore epidemiology of a number of human and animal pathogens globally. These mosquitoes, and sympatric *Culex* species that are not within the Cx. *pipiens* complex, are often considered impossible to distinguish by morphology in the adult female stage. In the U.S.A., this is particularly true for *Culex pipiens* s.l. and *Culex restuans* Theobald, both of which are competent vectors of West Nile virus, but likely play different roles in the transmission cycle. Toward this end, we undertook an in-depth morphological evaluation of matched larval exuviae and adult specimens that revealed five useful morphological characters that are informative to distinguish Cx. *pipiens* s.l. from Cx. *restuans*. Herein, we provide a comprehensive review of the literature on these two, and four additional, morphologically similar *Culex* species, and a proposed key to adult female specimens. Keywords: *Culex pipiens* s.l., *Culex restuans*, morphology, character reappraisal, occipital erect scales.

#### Introduction

Culex pipiens Linnaeus is the name bearing taxon for the family Culicidae (order Diptera) and is a member of one of the most taxonomically challenging complexes of species of mosquitoes. In the United States, the Culex pipiens complex is represented by Culex pipiens Linnaeus and Culex quinquefasciatus Say, as well as hybrid forms of those two (Farajollahi *et al.* 2011; Smith & Fonseca 2004). These mosquitoes, and sympatric Culex species that are not within the Cx. pipiens complex, are often considered impossible to distinguish by morphology in the adult female stage. In the U.S.A., this is particularly true for Culex pipiens s.l. and Culex restuans Theobald.

*Culex pipiens* and other *Culex* species transmit a number of neuroinvasive arthropod-borne viruses including Western equine encephalitis virus, St Louis encephalitis virus, and West Nile virus (WNV). In particular, in Eastern and Midwestern states, members of the *Cx. pipiens* complex and *Cx. restuans* are key players in the transmission of WNV (Johnson *et al.* 2015). Because of their vector status, adult female *Cx. pipiens* complex and *Cx. restuans* are regularly targeted in arbovirus

surveillance efforts. Unfortunately, the morphological traits used to separate adult female members of the *Culex pipiens* complex and *Culex restuans* Theobald present in current keys are fraught with ambiguity. Indeed, many authors have chosen to not differentiate between these taxa in their publications, as most collections consist of adult females (e.g.: Kilpatrick et al., 2005, Kinsley et al., 2016, Meece et al., 2003, Michener, 1947).

This course of action is understandable given the morphological similarity of females of these species, but is problematic for the study of the particular contribution of each of these species to West Nile virus enzootic and endemic or epidemic transmission cycles. Although both species prefer to feed on avian hosts, Cx. pipiens s.l. may feed on mammals more than previously thought (Farajollahi *et al.* 2011) and possibly more than Cx. restuans (Apperson *et al.* 2002). As such, Cx. restuans may be less likely to transmit the virus to humans, but may be critical for early season amplification of the virus in the enzootic cycle. It is important then to be able to differentiate between these taxa in order to further evaluate the relative importance of each species to West Nile virus transmission to humans in the United States.

Although it is possible to distinguish these species using PCR-based diagnostics, morphological identification would facilitate rapid processing of

field-collected mosquitoes. Therefore, we set the goal to develop accurate morphological diagnostic criteria for these two *Culex* species. Towards this goal, we reviewed characters used in North American keys and all characters described for both taxa in the literature. We provide comprehensive discussion of the use and utility of characters represented in keys and faunistic reviews for the mosquitoes of North America. Importantly we also report that four other *Culex* species were poorly characterized in certain keys and other reference type literature, and could confuse the utility of the characters found, so we included those in our analysis. We also performed an in-depth analysis of distinct character states between *Culex* pipiens s.l. and Culex restuans, and report five characters that may be useful in the differentiation of these two species. The utility of these characters for separating Culex pipiens s.l. and Culex restuans was validated using the PCR diagnostic for *Culex pipiens*, *Cx. restuans* and *Cx.* salinarius as described by Crabtree et al. (1995). The molecular diagnostic confirmed the morphological identification for every specimen (N=113) tested collected from Wisconsin, Illinois and Minnesota, U.S.A. Finally, we provide a provisional key for six morphologically superficially similar *Culex* species, including both *Cx pipiens* s.l. and *Cx. restuans*.

#### Literature review

The characters that are used in key couplets to separate adult females *Cx. restuans* and *Cx. pipiens* s.l. from one another and other morphologically similar species are presented here for each of the published keys to mosquitoes of North America, to include Ross (1947), Carpenter & LaCasse (1974); Means (1987); Wood *et al.* (1979), Darsie & Ward (2005) and Andreadis *et al.* (2005).

# Characters in common use to separate *Cx. pipiens* s.l and *Cx. restuans*:

The most commonly used character to separate Cx. restuans from Cx. pipiens s.l. is the mid-dorsocentral patches of pale scales on the scutum (Andreadis *et al.* 2005; Carpenter & LaCasse 1974; Means 1987; Ross 1947; Wood *et al.* 1979). Unfortunately, it is also clear from literature that this character is unreliable because a portion of Cx. restuans in any given sample will simply not present the phenotype. This is even noted by authors of these works; for example, Wood et al. (1979) use the qualifier "usually" to warn the reader that the spots might not be present. The proportions vary greatly from place to place and possibly seasonally, making it impossible to generalize and/or predict the occurrence of variation.

The second most used character (and perhaps the one portrayed by

keys as the most reliable choice) is the narrowing basal "pale banding" towards the edges of the abdominal terga, which Ross used to separate Cx. *quinquefasciatus* from Cx. *pipiens* + Cx. *restuans* (1947). And readis, Carpenter & LaCasse, Means and Darsie and Ward present this as a character that separates Cx. *pipiens* from Cx. *restuans*.

#### Characters used to distinguish morphologically similar species:

Morphological similarities similarly confound the identification of Cx. erythrothorax, Cx. interrogator, Cx. chidesteri, Cx. salinarius, and Cx. pipiens s.l. and Cx. restuans. In the analysis below, we present key characters used to distinguish these species in the context of the body regions for which diagnostic characters have been reported.

#### **Characters on the scutum:**

Ross separated Cx. restuans from Cx. pipiens by the middorsocentral pale scaling on the scutum (1947). Carpenter & LaCasse (1974) separated Cx. interrogator from Cx. restuans by the lack or usually presence of middorsocentral spots of pale scaling on the scutum, respectively. Means and Andreadis *et al.* also mention the existence of the patch of middorsocentral pale scales on the scutum of Cx. restuans as diagnostic for the species (1987, 2005).

Carpenter & LaCasse (1974) considered the scaling on the scutum to be "somewhat coarse" and "golden" for Cx. pipiens s.l., and "fine" and "golden brown" for Cx. interrogator + Cx. restuans. Means also separated Cx. restuans from Cx. pipiens based on the character of "fine scaling" vesus "coarse scaling" respectively (1987). Apperson *et al.* (2002) explained the character in more detail stating the scutal scales are typically longer, falcate and lighter brown on Cx. pipiens, and shorter, not falcate and darker brown for Cx. restuans. The result is an appearance that the scaling is "lighter, coarse and scruffy" in Cx. pipiens, and "darker, smooth and well arranged" in Cx. restuans.

#### **Characters on the scutellum:**

Andreadis *et al.* used the character of the scutellum of Cx. salinarius as having short brown scales, while Cx. pipiens + Cx. restuans have long pale scales (2005).

#### **Characters on the lateral thorax:**

Carpenter & LaCasse (1974) separated Cx. *nigripalpus* from Cx. salinarius + Culex chidesteri Dyar by the pleural scaling pattern. The authors do not provide a clear explanation of this character but Belkin *et al.* (1970) state that the pleural pale scaling of Cx. *nigripalpus* is restricted to a few broad pale scales on the upper and lower mesokatepisternum and a few narrow pale scales on the upper mesepimeron. They treat the pleural scale patches of Cx. chidesteri as equal to that of Cx. quinquefasciatus, other than presenting a few post-spiracular scales, *i.e.* a small patch of broad pale scales on upper proepisternum, broad pale scales on the upper and lower mesokatepisternum, a large patch of elongate broad pale scales on the mid mesepimeron and many elongate pale scales on the upper mesepimeron. To the best of our knowledge the distribution pattern of pleural scaling of Cx. salinarius is not described by any author to date.

#### Characters on the tibia and tarsi:

Carpenter & LaCasse (1974) differentiate *Cx. chidesteri* from *Cx. nigripalpus* with the description that all of the tibiae in *Cx. chidesteri* have more or less conspicuous "knee spots" of pale scales as well as extremely narrow rings of pale scaling at the tarsal joints whereas *Cx. nigripalpus* has inconspicuous "knee spots", except sometimes on hind leg, and all tarsi dark. Means (1987) also considers the tibial "knee spots" of *Cx. restuans* as "prominent" while the ones on *Cx. pipiens* "moderate".

#### **Characters on the abdominal tergites:**

Ross stated that *Culex salinarius* Coquillett can be distinguished from

Cx. restuans + Cx. pipiens sl. presentation very narrow "pale banding" on all or most of the otherwise brown scaled tergites. He further separates *Cx*. quinquefasciatus from Cx. restuans + Cx. pipiens based on the same character forming narrowing "pale banding" towards the edges of the segments. Carpenter & LaCasse (1974) likewise separated these Culex species according to those that have broader "basal banding" on the abdominal tergites (Cx. pipiens s.l. + Cx. interrogator + Cx. restuans) from the species that have narrow to nonexistent basal "pale banding" (Culex nigripalpus Theobald + Cx. salinarius + Cx. chidesteri). Means also separated *Cx. salinarius* from *Cx. restuans* + *Cx. pipiens* by the presence of the very narrow "pale banding", often absent, adding that segments VII and VIII are mostly covered by pale yellow scales and uses the character of narrowing basal "pale banding" of the tergites to separate *Cx. pipiens* from Cx. restuans (1987). Andreadis et al. also used the character of the tergite basal "pale bands" narrowing towards the edges of the segment in Cx. *pipiens* to separate it from *Cx. restuans* in their key (2005).

Carpenter & LaCasse also separated Cx. salinarius from Cx. chidesteri by contrasting abdominal tergite scaling. They reported pale yellow scaling scattered near the apex of each segment and tergite VII mainly or entirely covered by pale yellow scales for Cx. salinarius. For Cx. chidesteri, the tergites have only dark scaling at apex, with tergite VII mainly covered by dark scaling (1974).

#### **Critical evaluation of characters using field-caught specimens**

Apperson *et al.* used the following characters to differentiate Cx. salinarius, Cx. pipiens s.l. and Cx. restuans specimens collected in n the Borough of Queens, New York City: 1) the presence of short brown scales on the midlobe of the scutellum of Cx. salinarius, in contrast to these scales long and pale (cream or white, in their words) in Cx. pipiens s.l. and Cx. restuans; 2) the sternites entirely covered by "copper" scales in Cx. salinarius; 3) the "dingy yellow to copper colored" scales forming the basal "bands" of the tergites, these very narrow to absent on II and III, in contrast with the same character as yellow or "cream" colored and appearing "convex" for *Cx. pipiens* s.l. (narrowing towards edges), while the ones from *Cx. restuans* white colored and appearing broad and connected with the basolateral patch. The author did note that some character overlap exists with Cx. pipiens s.l. and Cx. restuans; 4) the "dingy yellow to copper colored" scales covering tergites VII and VIII, presumably in contrast with the scaling pattern of tergites VII and VIII not different from other tergites in Cx. pipiens s.l. and Cx. restuans; 5) "reddish-brown" coloring of the thoracic integument in Cx. restuans, in contrast with the "tan to brown" coloring in *Cx. pipiens* s.l.; the scales of the scutum "dark brown", short and not falcate in *Cx. restuans*, in contrast to "light brown", long and falcate in *Cx. pipiens* s.l. The authors report a success rate of identification in utilizing this combination of characters of 100% for *Cx. pipiens*, 100% of *Cx. salinarius* and 80% for *Cx. restuans*, for which the remaining (20%) of misidentified specimens were *Cx. pipiens* (Apperson *et al.* 2002). Their morphological identifications were confirmed using the technique described by Crabtree *et al.* (1995).

Harrington & Poulson re-evaluated the characters presented by Apperson *et al.* to differentiate *Cx. pipiens* s.l. from *Cx.* central New York (2008). They report the combination of characters would correctly identify only ~30% of *Cx. pipiens* s.l. and ~60% of *Cx. restuans,* based on results from PCR diagnostics designed by Crabtree *et al.* (1995) They conclude that these mosquito species cannot be conclusively identified by morphological characters used by Apperson et al. (2002).

McKinnish *et al.* (2013) evaluated specimens from Pennsylvania and Virginia collected with gravid traps and confirmed morphological identification using the PCR diagnostic designed by Crabtree *et al.* (1995). Only 31% of their *Cx. restuans* specimens had middorsocentral pale scaling spots on the scutum. They also found the difference in abdominal banding pattern from "straight" bands in *Cx. restuans* to "curved" bands in *Cx. pipiens* s.l. could be used to correctly identify 87% of *Cx. restuans* and 84% of Cx. *pipiens* s.l. Interestingly, they also evaluated the presence of pale erect scales on the head for Cx. *pipiens* s.l. in contrast with the presence of only dark erect scales for Cx. *restuans* and report the character correctly identified 92% of Cx. *pipiens* s.l. and 93% of Cx. *restuans*. They concluded the combination of these characters should be diagnostic for these species

#### **Materials and methods**

#### **Molecular methods:**

Genomic DNA was extracted from a single mosquito leg utilizing the protocol described by Johnston *et al.* (2015), where the mosquito leg is placed on a tube with 50  $\mu$ L of 25 mM NaOH + 0.2 mM EDTA at pH 12, which is heated to 95°C for an hour then cooled down to 4°C and 50  $\mu$ L of 40 mM EDTA at pH 5 is added.

In accordance with the previous papers, the primers used for molecular identification were the ones designed by Crabtree *et al.* (1995) for the ITS regions of the rDNA gene group, with the following sequences (shown 5' to 3'): reverse primer, (CP16) GCGGGTACCATGCTTAAATTTAGGGGGGTA; forward primer for *Cx. pipiens* s.l. (PQ10) CCTATGTCCGCGTATACTA; forward primer for *Cx. restuans* (R6) CCAAACACCGGTACCCAA; forward primer for *Cx. salinarius* (S20) TGAGAATACATACCACTGCT. The PCR mixture for each tube consisted of 1

 $\mu$ L of extraction product, 1.5 μL of each primer (CP16, PQ10, R6, S20) at a concentration of 10 μM, 5 μL of GoTaq Flexi Buffer (5x), 2.5 μL of MgCl<sub>2</sub>, 0.5 μL of dNTPs, 0.125 μL of GoTaq DNA Polymerase, Promega, Madison, WI, USA, in a total reaction volume of 25 μL. The thermocycler program was set to one cycle at 96°C for 4 minutes, 40 cycles at 96°C for 30 seconds, then 51°C for 30 seconds, then 72°C for 90 seconds, and one cycle at 72°C for 4 minutes. The PCR products were subject to electrophoresis in 2% agarose gel with ethidium bromide, a PCR product without DNA template was used as negative control. *Culex pipiens* s.l., *Culex restuans* and *Culex salinarius* were identified according to the amplicon size (698 bp, 506 bp and 175 bp respectively) as visualized on the gel, as described by Crabtree *et al.* (1995).

#### Mosquitoes specimens and morphological analysis:

The mosquitoes (*Culex pipiens* s.l. and *Culex restuans*) used for analysis of separation characters were individually reared from eggs collected in tire standing water in Monona [21.VI.2017, 12.VI.2017, 14.VI.2017], DeForest [21.VI.2017] and Janesville [15.VI.2017], Wisconsin, USA. Larvae were reared in tap water in individual plastic cups and fed dry yeast. The 4<sup>th</sup> instar exuviae were collected and slide mounted in Canada Balsam (SERVA Electrophoresis GmbH, Heidelberg, Germany) for confirmation of taxonomic identity, while the adults were point mounted and stored dry.

Characters of interest for identification of Cx. *pipiens* s.l. and Cx. *restuans* were determined utilizing these specimens (n=14). The same specimens were used to confirm the mosquitoes of our region were correctly identified by the primers from Crabtree *et al.* (1995).

Additional adult female mosquitoes from field collections were used to confirm the utility of the characters found by the previously discussed morphological analysis (n=99). For each specimen, the taxonomic identity was determined using the morphological characters here proposed, then a leg sample was taken and encoded to be subjected to a blinded molecular identification utilizing the molecular methods described. These additional mosquitoes were collected from All Saints Cemetery, Des Plaines, Illinois, USA [42°03'54.7"N, 87°53'45.8"W; gravid trap; 19.VI.2018, 31.VII.2018, 14.VIII.2018] and from Blaine [45°07'42.96"N, 93°15'02.52"W; CDC trap CO<sub>2</sub> baited; 10.VII.2018], Anoka [45°12'51.12"N, 93°22'09.12"W; gravid trap; 18.VII.2018], Waconia [44°51'15.12"N, 93°47'46.68"W; CDC trap CO<sub>2</sub> baited; 7.VIII.2018], Mendota Heights [44°52'14.16", 93°11'49.56W"; CDC trap CO<sub>2</sub> baited; 31.VII.2018], Minnetrista [44°56'47.4"N, 93°42'39.96"W; CDC Lauderdale  $CO_2$ baited; 24.VII.2018], [44°59'22.2"N, trap 93°11'54.6"W; gravid trap; 15.VIII.2018], St. Paul [44°53'48.84"N,

93°10'08.4"W; CDC trap CO<sub>2</sub> baited; 24.VII.2018], Shakopee [44°45'45.72"N, 93°31'49.8"W; CDC trap CO<sub>2</sub> baited; 31.VII.2018], and Cedar Lake [44°34'16.32"N, 93°25'45.12"W; gravid trap; 25.VII.2018], Minnesota, USA.

All specimens were deposited in the Culicidae collection in the School of Veterinary Medicine, Department of Pathobiological Sciences at the University of Wisconsin-Madison.

The nomenclature of characters generally follows Harbach & Knight (1980), except where indicated otherwise. Generic abbreviations follow Reinert (2009).

#### Results

## Characters for morphologically distinguishing Cx. pipiens s.l. from Cx. restuans:

We analyzed and uncovered five adult female characters that may be of use for the distinction of *Cx. pipiens* s.l. and *Cx. restuans*.

We observed that the erect scales on the dorsal area of the head (ESc), distributed from occiput to vertex, were all dark colored in *Culex restuans* (Fig. 1b); these same scales were, at least medially, pale in *Culex pipiens* s.l. (Fig. 1a). This is generally consistent with the literature; however, Carpenter & LaCasse (1974) present this character as "usually"

the case for *Cx. pipiens* s.l. We found no evidence to say this is not always the case in our samples. Furthermore, in our samples, these scales (ESc) were more numerous in *Cx. restuans* than in *Cx. pipiens* s.l. (Fig. 1a,b).

The number of setae present in the upper proepisternum (PeSU) on the thorax, as viewed laterally, was useful for separation of Cx. *pipiens* s.l. (six to twelve setae) (Fig. 1c), from Cx. *restuans* (four to seven setae) (Fig. 1d). These characters have not been described in the literature for Cx. *restuans*. Harbach *et al.* (1985) state that upper proepisternal setation varies from eight to thirteen setae for the neotype series of Cx. *pipiens*, and Sirivanakarn & White (1978) report a total of ten to twelve proepisternal setae present in the neotype series of Cx. *quinquefasciatus*.

The coloration of the setae of the postpronotum (PpS) and of the mid lobe of the scutellum (MSS) proved to be useful in our samples as well. For this character, some or all (usually at least one) of the setae of the postpronotum golden/pale in Cx. pipiens s.l. (Fig. 1c), as well as some or all (usually at least one) of the setae of the mid-lobe of the scutellum golden/pale in Cx. pipiens s.l. (Fig. 1e); in contrast all of the setae of the postpronotum and the mid-lobe of the scutellum were dark in Cx. restuans (Fig. 1d, f). These characters have not been reported in literature.

We also found the wing remigial setae (ReS) may be of some use in the separation of those species, as they appear to be lighter colored and thinner in appearance in Cx. *pipiens* s.l. (Fig. 1g) and very dark and thicker in appearance in Cx. *restuans* (Fig. 1h). Furthermore, we observed three to four remigial setae generally present in Cx. *restuans*, while only two to three setae generally present in Cx. *pipiens* s.l., but overlap proved to occur too frequently to make the seta count useful. To the best of our knowledge, the only account of remigial setae in a description of these species is in the description of the neotype of Cx. *pipiens*, in which Harbach *et al.* (1985) reports the specimen as having two remigial setae.

### Paired morphological and molecular identification of *Culex pipiens* s.l. and *Culex restuans*:

To confirm the molecular identification of lab-reared adults, for which each specimen's morphological identification was confirmed based on its larval exuvia, DNA was extracted from a single leg and subjected to PCR for the ITS segment using methods and primers from Crabtree *et al.* (1995). To then validate these characters in specimens from elsewhere, we acquired and identified 73 adult female specimens to *Cx. pipiens* s.l. and 26 adult female specimens to *Cx. restuans*, from material collected from Des Plaines, Illinois, USA (50 *Cx. pipiens* s.l., 13 *Cx. restuans*), and from Blaine, Anoka, Waconia, Mendota Heights, Minnetrista, Lauderdale, St. Paul, Shakopee, and Cedar Lake, Minnesota, USA (23 *Cx. pipiens* s.l., 13 *Cx. restuans*). Each specimen was morphologically identified using the combination of characters described above, then subjected to PCR identification again using the Crabtree *et al.* method (1995). There was 100% agreement between morphological and molecular identification for both species for every specimen tested.

### Paired morphological and molecular identification of *Culex* salinarius:

In addition to these findings, we tested and found support for the utility of the presence of short brown scales on the midlobe of the scutellum on Cx. salinarius, as compared to long and pale scales in Cx. pipiens s.l. and Cx. restuans to distinguish these species (Apperson *et al.* 2002). We observed the only 2 specimens of Cx. salinarius in our collection (UW-Madison Arboretum, Madison, WI, USA) and confirmed the presence of the character, and PCR for the ITS sequence yielded a product with the correct band size using the primers and methods described by Crabtree *et al.* (1995). We note that in our limited experience with Cx. salinarius, the character described by Apperson *et al.* (2002) presents itself more as bright in color than brown *per se*, so we refer to it as "copper-colored" scaling.

#### Discussion

In the process of assessing these specimens and character states, we also had the opportunity to assess the characters previously used to distinguish these species using existing dichotomous keys. Our experience with characters related to the coloration and shape of the abdominal tergite basal "pale bands" corroborates that of Apperson *et al.* and Harrington & Poulson, who noted that the character state overlaps in specimens and is of little use for separation of said species (2002, 2008). Based on specimens observed from WI, IL and MN, we likewise observed that abdominal tergite scaling pattern is not useful for identification of these two species because 1) there is significant overlap of the character between the species such that some specimens of both species present the character as reduced in some or most tergites, thereby forming a very thin "band" or no "band," and 2) because of potential confusion with other *Culex* species if this is the character on which the observer is focused.

Furthermore, we did not find that thoracic integument coloring was useful for identification of these mosquitoes from this region. Also, the use of this character is not consistent in the literature, with most modern Nearctic keys attributing the "reddish brown" character as exclusive to *Culex erythrothorax* Dyar (e.g.: Carpenter & LaCasse 1974; Darsie Jr & Ward 1981, 2005). The same can be said of the character of scutal scaling.
We did find that the scutal scales are dark brown and short in *Cx. restuans*, as compared to bright yellow and rather long (giving the impression the scutum scaling to be quite "shaggy") in *Cx. pipiens* s.l.; however, the shape of the scales is not well described as "not falcate" in *Cx. restuans*, as reported by Apperson *et al.* (2002). The scutal scales of *Cx. restuans* in our sample seem to be falcate, but not dorso-ventrally expanded by a significant amount, which gives them a more "hair-like" appearance at a glance, rather than the more archetypal falcate scales present in *Cx. pipiens* s.l. This character may furthermore be misinterpreted as the "hair-like" scales of *Cx. erythrothorax* as presented in the keys of Carpenter & LaCasse (1974) and Darsie Jr & Ward (1981, 2005), resulting in a misidentification. Additionally the *Cx. salinarius* of Wisconsin also present the character of short brown scutal scales, with lack of significant dorsovental expansion, giving a more "hair-like" appearance than the ones of *Cx. pipiens* s.l.

In sum, it is clear that these two species are morphologically differentiable, but that existing keys do not sufficiently delineate morphological differences between *Cx. pipiens* s.l. and *Cx. restuans* to provide reliable identification. The combination of the characters described herein may be of use to ascertain correct identification of a few *Culex* species that currently have equivocal characters in available keys. Should the user identify *Cx. erythrothorax*, *Cx. interrogator*, *Cx. chidesteri*, *Cx.* 

salinarius, Cx. pipiens s.l. or Cx. restuans using existing keys, the characters below should provide a degree of certainty to the identification. This key is derived from characters presented in this paper, and from the literature for species other than Cx. pipiens s.l. and Cx. restuans. The key should be taken as provisional until these characters have been thoroughly vetted for specimens of these species from different regions of the Nearctic and the American continent. It is clear that more research is needed to further increase our knowledge of the morphological traits of all these species, and also to improve and update reference material (both keys and descriptions) for the Nearctic mosquito fauna.

## Key to adult female Culex (Culex) (in part)

The purpose of this key is to provide a way to confirm the identities of certain *Culex* (*Culex*) species that have been mischaracterized in reference material or have not been adequately separated in previous keys. More specifically, if by utilizing current keys to Nearctic *Culex* species, the reader arrives at the identification of Cx. erythrothorax, Cx. interrogator, Cx. chidesteri, Cx. salinarius, Cx. pipiens s.l. or Cx. restuans, we recommend the consultation of the following key, and believe it should provide a more accurate identification to the best of our knowledge, at least within Midwestern states in the U.S.A., with the limit of its usage confined to the Nearctic region because Neotropical Cx. restuans almost certainly present variation.

2. base of mid lobe of scutellum with long pale scales......*Cx. erythrothorax*base of mid lobe of scutellum with short "copper colored" scales; tergites usually with a few pale scales scattered at apex.....*Cx. salinarius*

3. Postspiracular patch of scales usually present; very narrow pale rings at joint of tasomeres usually present; erect scales of dorsum of head dark...*Cx. chidesteri* 

- Postspiracular patch of scales absent; joints of tarsomeres usually without pale markings; erect scales of dorsum of head dark or pale ones medially...4

5. middorsocentral patches of pale scaling present or absent; erect scales of dorsum of head dark (Fig. 1b); upper proepisternum with four to seven setae (Fig. 1d); postpronotum with all setae dark (Fig. 1d); mid lobe of scutellum with all setae dark (Fig. 1f); remigial setae thick, dark, three to four (usually three) (Fig. 1h).....*Cx. restuans* 

- middorsocentral patches of pale scaling absent; erect scales of dorsum of head pale medially, others dark (Fig. 1a); upper proepisternum with six to 12 setae (Fig. 1c); postpronotum usually with at least one seta pale/golden (Fig. 1c); mid lobe of scutellum with at least one seta pale/golden (Fig. 1e); remigial setae thin, pale, two to three (usually three) (Fig. 1g) *Cx. pipiens* s.l.

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Figure 1. General characters of *Culex pipiens* s.l. and *Culex restuans*.

Legend: General characters of *Culex pipiens* s.l. (a, c, e, g) and *Culex restuans* (b, d, f, h); a, b: erect scales (ESc) on the dorsal portion of head, dark laterally and pale medially (a) or all dark (b); c, d: lateral view of the thorax, postpronotal setae (PpS) pale, at least one, (c) or all dark (d), upper proepisternal setae (PeSU) six to 12 (c) or four to seven (d); e, f: dorsal view of scutellum, median scutellar setae (MSS) pale, at least one, (e) or all dark (f); g, h: dorsal view of the wing, remigial setae (ReS) thin and pale (g) or thick and dark (h).

#### **Chapter 7. Summary**

The revision of the Ochlerotatus (Ochlerotatus) Lynch Arribálzaga presented herein (Chapter 2) is essential to the future re-evaluation of the genus and its constituents. This is particularly important because the genus contains a number of significant vector and nuisance mosquito species, and this particular taxon has been in need of revision to define the subgenus. Prior to this analysis, there were a total of 40 names regarding 23 valid species within the subgenus. After revision, I confirm 11 names regarding ten valid species, and reject 30 names regarding 16 valid species from the subgenus Ochlerotatus, totaling 41 names within 26 valid species discussed in the revision. These ten valid species and the subgenus definition constitute a critical stepping stone for the redefinition of other groups within the genus *Ochlerotatus* and ultimately the redefinition of the genus itself. The new distribution records given, e.g. for Oc. patersoni, and resurrection and description of new species changes our understanding of the distribution of mosquitoes in this group. Because the group was mischaracterized previously, the impression was that Oc. scapularis was ubiguitous in all corners of the Americas, and in very diverse habitats. With more detailed understanding of the group, we can shift our attention to the many species that were misidentified as a result of the description provided by Arnell (1976). The improvement of our level of understanding of the

identity of these organisms is essential for future endeavors in systematics, ecology and epidemiology of these species.

The annotated checklist of the mosquitoes of Wisconsin (Chapter 3) is useful for entomologists and other scientists interacting with mosquitoes in the state, and complements checklists and similar works for other states of the Midwest. This work can be used to inform entomological and epidemiological surveillance for new and invading mosquito and pathogen species in the state of Wisconsin. Furthermore, this work is in line with efforts to map and catalog biodiversity in the world, and provides a baseline understanding of mosquitoes in the state currently for future and comparative work in ecology and epidemiology of the Culicidae.

The efforts associated with the annotated checklist of the state of Wisconsin also prompted a survey of the University of Wisconsin-Madison Arboretum, Madison, Wisconsin (Chapter 4), which resulted in the description of a new species, *Ochlerotatus mirabilis* sp. nov. (Chapter 5). The survey also revealed ten species never before reported for that specific locality and directly contributed to the confirmation of two new species records for the state of Wisconsin. Beyond that, the seasonality and community structure presented are a useful guide for designing future studies on specific species. For example *Oc. canadensis canadensis* was present in high numbers early in the season and subsequently disappeared

from the community, coinciding with the steady rise in numbers of *Coquillettidia perturbans* towards the end of the season.

Finally, ongoing mosquito and mosquito-borne disease surveillance in the Midwest Center of Excellence for Vector-Borne Disease inspired us to take on a persistent issue with the identification of two *Culex* species (Chapter 6). Namely, *Cx pipiens* and *Cx. restuans*, which are sympatric and important vector species associated with West Nile virus transmission; specimens of these species are challenging to identify in the adult female stage. An in-depth review of specimens from Wisconsin and of the literature and keys available for mosquitoes of the U.S.A, resulted in five new characters we proposed for their differentiation, and brought to light problems with character usage for other similar species. In Chapter 6, we provide both a key to six morphologically similar *Culex* species, and descriptions and drawings of the five characters that proved informative for differentiating *Cx. pipiens* from *Cx. restuans*.

### Future studies:

Additional collections and identification of *Ochlerotatus* (*Ochlerotatus*) species from localities across Central and South America are necessary to truly understand the distribution the species of this group. Reidentification of material deposited in institutional collections, such as what I was able to perform in the USNM, MACN and in the WIRC, might prove useful for this purpose as well. Furthermore, collection of new material to obtain male specimens for assessment of male genitalia characters may show new species that were previously misclassified as *Oc.* (*Och.*) *scapularis*. Genetic analysis, especially SNP analysis, may prove useful to delineate the evolutionary relationships and species boundaries within the subgenus.

Many levels of "domesticity" were described for the species previously known as *Oc. scapularis* (Forattini 1961, 2002, Forattini *et al.* 1989, 1995, 1997, 2000; Sant'Ana & L. 2001; da Silva & de Menezes 1996). However, these studies probably refer to many different species, of main concern amongst those are *Oc. confirmatus, Oc. patersoni, Oc. brisolai*, and *Oc. scapularis*. A reassessment of the bionomics of each of these species is in order to characterize their preferred habitats and behaviors, and aid future research in ecology and epidemiology. It is exciting to imagine that this kind of work could untangle the old mystery of the vector species associated with the outbreaks of the Rocio Virus in Brazil in the 1970's (see Iversson *et al.* 1989; Straatmann *et al.* 1997).

A reevaluation of the phylogeny of the genus *Ochlerotatus* overall is also necessary. However, for that purpose, taxonomic reviews of its other members should prove useful in this regard, in particular the species within subgenus uncertain. Amongst those, the holarctic Stimulans group is perhaps one of the least resolved groups of *Ochlerotatus* and is in dire need of review. Members of this group are spread through multiple continents and, yet, have never received a full treatment of the group; there exists a single review of the American species (Dyar 1920) and reviews of species in the former U.S.S.R. and Japan (Gutsevich *et al.* 1974; Tanaka *et al.* 1979). Although not discussed here at length the Serratus group of *Ochlerotatus*, most specious in the neotropics, is also deserving of revision and such will certainly aid the pursuit of an increasingly better understanding of the genus. From this dissertation, it is clear that *Oc. sticticus* is in need of taxonomic reevaluation.

As it relates to the work presented in Chapter 6, future studies should be done to test the utility of the characters for distinguishing *Culex pipiens* from *Culex restuans* on specimens from a broader geographic area. Furthermore *Culex restuans* is in need of a taxonomic revision for elucidation of additional characters and the nature of its phenotypic variations (see Chapter 6).

Because I will return to and intend to work in Brazil, I hope to be able to take on more of this work and further review fauna from states of Brazil. At the same time, I intend to continue pursuing the improvement of the classification of the Culicidae and hope to continue aiding in the resolution of taxonomic challenges related to the Culicidae beyond my own country.

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Appendix Chapter. Key to Nearctic Culicidae (in part).

#### Introduction

Basic mosquito anatomy

Key to genera of Nearctic female mosquitoes

Key to species of Nearctic Georgecraigius

Key to species of Nearctic Jarnellius

Key to subgenera of Nearctic female *Ochlerotatus* and *'Ochlerotatus' sensu* auctorum

Key to species of Nearctic female 'Ochlerotatus' ('Protomaclaya') sensu auctorum

Key to species of Nearctic female Ochlerotatus (Chrysoconops)
Key to species of Nearctic female Ochlerotatus (Culicada)
Key to species of Nearctic female Ochlerotatus (Culicelsa)
Key to species of Nearctic female Ochlerotatus (Ochlerotatus)
Key to species of Nearctic female Ochlerotatus (Protoculex)
Key to species of Nearctic female Ochlerotatus (Rusticoidus)
Key to species of Nearctic female Ochlerotatus (Woodius)
Key to species of Nearctic female Ochlerotatus (Woodius)
Key to species of Nearctic female Ochlerotatus (Woodius)

# Introduction

This key follows the genera classification of Reinert *et al.* (2009) for the tribe Aedini. The nomenclature of morphological characters follows Harbach & Knight (1980) and the mosquito taxonomic inventory (Harbach, 2013) as well as Reinert *et al.* (2009) were heavily consulted for the characters used, additional references are Berlin (1969), Belkin *et al.* (1970), Zavortink (1972), Reinert (1973), Harbach & Kitching (1998) and Reinert *et al.* (2004, 2006, 2008). The majority of the drawings are from Darsie Jr & Ward (2005), some are modified drawings from that publication, a few are modified from Doane (1910), Thielman & Hunter (2007) and Medlock & Vaux (2010), and some are original drawings.

# Basic mosquito anatomy

Males mosquitoes should be recognizable by their genitalic structure at the tip of the abdomen, the gonocoxites, which are very distinguishable from the structure present on the females, the cerci. Moreover, males often possess plumose antennae and long palpi, whislt female mosquitoes have pilose anntenae and varied palpal length. Exceptions include the Sabethini, in which some species males have a somewhat pilose antennae, and *Aedes*, in which both sexes have short palpi.



Figure 1: Male mosquito

Figure 2: Female mosquito

# Key to genera of Nearctic female mosquitoes

Last revised on August 27, 2019

1. Maxillary palpi as long as proboscis (Fig. 3) ..... Subfamily Anophelini

In the Nearctic, only one genus: Anopheles



Figure 3: Anopheles quadrimaculatus



Figure 4: Aedimorphus vexans

3 (2). Wing with anal vein (1A) sharply curved distally, ending before fork between cubital vein (CuA) and mediocubital crossvein (mcu) (Fig. 7); scales of the veins usually all broad and small, truncate or rounded apically, never forked at tip ..... Uranotaenia



Figure 5: Toxorhynchites rutilus





Figure 7: Uranotaenia sapphirina





4 (3). First tarsomere of the fore and midlegs longer than the other four distal tarsomeres combined, tarsomeres 4 and 5 very short, 4 shorter than 5 (Fig. 9) ...... Orthopodomyia





Figure 10: Culex pipiens

In the Nearctic, only one genus: Wyeomyia Recommended reference: Lane (1953)



Figure 11: Wyeomyia smithii



Figure 13: Wyeomyia smithii



Figure 12: Aedimorphus vexans



Figure 14: Aedimorphus vexans



7 (6). Abdomen tapering at apex 17); tarsal claws usually toothed (always both claws), especially at front tarsi, rarely simple but if so, then postspiracular setae present; postspiracular setae usualy present, rarely absent but if so, front tarsal claws toothed (always both claws)

Exceptions: if all tarsal claws simple and postspiracular setae absent, but upper mesokatepisternal setae absent and/or hind coxae more or less at same level as dorsal margin of mesomeron, then non-nearctic Aedini genera and species.



Figure 17: Kompia purpureipes

Figure 18: Culex pipiens

8 (7). Prespiracular setae present (Fig. 19) ..... Psorophora



Figure 19: Psorophora ciliata



Figure 20: Aedimorphus vexans

9 (8). Scutal scales broad and metallic; antepronotum large (Fig. 21) ...... Haemagogus In the Nearctic, only one species: Haemagogus equinus

Scutal scales narrow; antepronotum small (Fig. 22) ... Aedes sensu Wilkerson ... 10



Figure 21: Haemagogus equinus





Monobasic: Kompia purpureipes

Postspiracular setae present (Fig. 24) .....11



Figure 23: Kompia purpureipes



Figure 24: Aedimorphus vexans

Monobasic: Abraedes papago



Tergites VI and VII fully scaled (Fig. 26) .....12



Figure 26: Aedimorphus vexans

12 (11). Lateral and mid surface of tori covered with white scales (Fig. 27); lower proepisternal scales present; hindtarsi pale scaled basally; paratergite usually with scales; clipeus bare or scaled

In the Nearctic, only two species: Stegomyia aegypti and Stegomyia albopicta

If scales present on tori, only on mid surface (Fig. 28); lower proepisternal scales present or absent; hindtarsi varied; paratergite bare or with scales; clipeus bare or scaled 13



Figure 27: Stegomyia albopicta



Figure 28: Ochlerotatus infirmatus

13 (12). Scutum ornamented with pale scales forming a long median line, a long submedian line interrupted at scutal angle, a line or diffused area over wing base, a small patch just

In the Nearctic, only one species: Hulecoeteomyia japonica





 $`Ochlerotatus'\ triseriatus$ 

Figure 29: Hulecoeteomyia japonica

Figure 30: Different scutal patterns

In the Nearctic, only one species: Tanakius togoi

Scutum with different ornamentation (Fig. 32); other characters varied ......15



Figure 31: Tanakius togoi



Figure 32: Different scutal patterns

Monobasic: Lewnielsenius muelleri



Figure 33: Lewnielsenius muelleri



 $Ochlerotatus\ canadensis$ 

Figure 34: Different scutal patterns

In the Nearctic, only one species: Howardina bahamensis

Scutum and scutellum with different ornamentation or concolourous, if scutum



Figure 35: Howardina bahamensis



Figure 36: Ochlerotatus atlanticus

17 (16). Scutellum with broad scales on lateral lobes (Fig. 37); hypostigmal scales (HySc) absent; metameral scales (MeSc) usually present; maxillary palpus of females dark-scaled with pale-scaled areas; subspiracular area (SA) with or without setae (Figs. 39, 41) Jarnellius Recommended reference: Arnell & Nielsen (1972), as Aedes (Ochlerotatus) Varipalpus Group



Stm

Figure 37: Jarnellius sierrensis

Figure 38: Georgecraigius atropalpus



Figure 39: Jarnellius sierrensis



Figure 40: Ochlerotatus provocans



Figure 41: Jarnellius varipalpus



Figure 42: 'Ochlerotatus' triseriatus

In the Nearctic, only one species: Aedes cinereus

Never all three pleurite areas lacking scales (Fig. 44); other characters varied ...19



Figure 43: Aedes cinereus



Figure 44: Ochlerotatus stimulans

In the Nearctic, only one subgenus: Georgecraigius (Georgecraigius) Georgecraigius (Georgecraigius) can be recognized by the combination of the following characters: posterior dorsocentral setae well developed; postspiracular area usually with scales; paratergite, postprocoxal membrane and laterotergite of abdominal segment I bare; outer surface of hindcoxae with large patch of scales; pale tarsal markings present both at base and apex, at least in some segments; hindtarsal segment 5 usually entirely light scaled dorsally.



Figure 45: Georgecraigius atropalpus

Figure 46: Ochlerotatus canadensis

> In the Nearctic, only one species: Aedimorphus vexans Aedimorphus vexans can be recognized by its very distinct abdominal tergites bearing medially bifid basal white markings (Fig. 47) and by the pattern of wing scaling, which is mainly dark with a streak of white scales on the posterior margin of dorsal base of costa.



Figure 47: Aedimorphus vexans



Figure 48: Ochlerotatus increpitus





Figure 50: Culex pipiens

PsA

22 (21). Pulvili present (Fig. 51); postspiracular setae absent ...... Tribe Culicini ......23



Figure 51: Culex pipiens



Figure 52: Coquillettidia perturbans



Figure 53: Deinocerites pseudes

Figure 54: Culex pipiens

Wing veins never with asymmetrical scales dorsally, often all scales dark, sometimes dark and pale scales intermixed (Fig. 56); postspiracular setae absent (Subgenera Coquillettidia and Austromansonia) or present (Subgenus Rhynchotaenia) ..... Coquillettidia

In the Nearctic, only one species: Coquillettidia (Coquillettidia) perturbans

*Coquillettidia perturbans* can be recognized by the combination of the following characters: wing veins with moderately broad scales dorsally, with dark and pale scales intermixed, usually more dark then pale; proboscis bearing a broad median ring of pale scales; hindtarsomere I with a narrow ring of pale scales basally and a broader one just beyond middle.




Figure 55: Mansonia titilans

Figure 56: Coquillettidia perturbans

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### Key to species of Nearctic Georgecraigius

Key adapted from Zavortink (1972) and Reinert et al. (2006)

One or more posterior scutal fossal setae present; hindfemur mostly dark; interocular space at most 2.0 facets; paratergite and postprocoxal membrane bare ..... *Georgecraigius epaticus* 

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# Key to species of Nearctic Jarnellius

Key adapted from Arnell & Nielsen (1972)

1	. Postcoxal area	with patch of	white scales	 	 	2
	Postcoxal are	a without scale	2S			3

3 (1). Subspiracular area with light setae in between scale patch ..... Jarnellius varipalpus

# References

Arnell, J. H. & L. Nielsen, 1972. Mosquito studies (Diptera, Culicidae) XXVII: The varipalpus group of Aedes (Ochlerotatus). Contributions of the American Entomological Institute 8:1–48.

# Key to subgenera of Nearctic female Ochlerotatus and 'Ochlerotatus' sensu auctorum

Last revised on August 27, 2019

1. Paratergal scales (PaSc) present; hypostigmal scales (HySc) absent; lower mesepimeral setae absent; hind claws simple 'Ochlerotatus' ('Protomacleaya') sensu auctorum (page 405)

Recommended reference: Zavortink (1972), as Aedes (Protomacleaya)

Integument variously colored, scutum integument lacking pair of dark postlateral spots

3(2).	Hindtarsi pale banded		4
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Hindta	rsi p	oale	bande	ed	basal	ly	onl	у	••	••	• •	••	•••	••	•	• •	•••	•••	• •	•	•••	• •	• •	•	• •	• •	·	• •	• •	•	• •	•	•••	•••	• •	• •	6
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Variable, but never with both lower proepisternal and hypostigmal areas scaled ... 9

9 (8). Metameron bare; scutum with narrow falcate scales, with various patterns of dark and pale scales; scutellum with narrow scales on mid-lobe, scales sparse or absent on lateral lobes; paratergite bare; hypostigmal scales absent; wing entirely dark-scaled or with patch of white scales at base of costa; hindfemur mostly pale-scaled with dorsal line of dark scales extending and broadening from mid-length to apex ...... Ochlerotatus (Ochlerotatus) (page 410)

Not as above ...... Ochlerotatus subgenus uncertain (in part) (page 415)

# Key to species of Nearctic female 'Ochlerotatus' ('Protomaclaya') sensu auctorum

Adapted from Zavortink (1972)

White markings	on hindtarsal	segments	restricted	to first	tarsomere;	hindfemur	never
with dark basal band			Kompi (	Group			5

#### Key to species of Nearctic female Ochlerotatus (Chrysoconops)

Adapted from Darsie Jr & Ward (2005)

1. Dark integumental spot anteriorly to postspiracular area; abdominal tergites mostly yellow scaled, with dark scales apically from II to VI ..... Ochlerotatus fulvus pallens

#### Key to species of Nearctic female Ochlerotatus (Culicada)

Adapted from Darsie Jr & Ward (2005)

1. Broad basal and apical rings on hindtarsomeres 1 to 4; hindtarsomere 5 entirely pale scaled; scutum unicolorous, golden brown ...... Ochlerotatus canadensis canadensis

#### Key to species of Nearctic female Ochlerotatus (Culicelsa)

Adapted from Darsie Jr & Ward (2005)

1. Abdominal terga with transverse pale bands, lacking median longitudinal stripe of pale scales; wing dark scaled ...... Ochlerotatus taeniorhynchus

2 (1). Wing dark scaled; hypostigmal scales absent ..... Ochlerotatus mitchellae

Hindtarsomere 1 usually without median band of pale scales, if present, those scales whiteish; abdominal basolateral patches of scales yellow ...... Ochlerotatus nigromaculis

#### Key to species of Nearctic female Ochlerotatus (Ochlerotatus)

Adapted from Arnell (1976)

Scutal scales not as above, scutal fossa never completely covered with pale scales .....2

4 (3). Scutal pale scales spreading laterally posteriorly to scutal angle (on supraalar area), reaching area above paratergite ...... Ochlerotatus tortilis

5(4).	Abdomen	with mediar	ı basal	white	patche	s or b	ands of	n mos	t tergites	•••••	• • •
						••••		0	Ochlerotatus	condolesc	ens
	Abdomina	l tergites all	dark						Ochlerotate	us infirma	ntus

#### Key to species of Nearctic female Ochlerotatus (Protoculex)

Adapted from Roberts & Scanlon (1979) and Shroyer et al. (2015)

1. Occiput usually without or with few dark scales laterally ..... Ochlerotatus dupreei

2 (1). Scutal stripe of pale scales very narrow, broken or even absent anteriorly, but always conspicuous towards prescutelar space, where it forks and surrounds the area (occasionally the stripe may manifest as a small spot before the prescutellar area) *Ochlerotatus pertinax* 

Scutal stripe wider, complete ..... Ochlerotatus atlanticus-tormentor complex ..... 3

3 (2). Scutal stripe tapering posteriorly, forking at the beginning of the prescutellar area, bordering the space; abdominal pale scales usually distinctly white *Ochlerotatus tormentor*\*

Scutal stripe more or less continuous in width from the anterior promontory to the prescutellar space, not forking noticeably but rather encompassing the space, populating the interior borders with scales; abdominal pale scales usually cream to bronze colored .....

.....Ochlerotatus atlanticus\*

\*Ochlerotatus atlanticus and Ochlerotatus tormentor form a complex of species that is generally regarded as almost indistinguishable in the female. The characters here presented were proposed by Roberts & Scanlon (1979) and no major review on those was performed afterwards. Such characters should be interpreted carefully or, if available, characters from male genitalia or immatures should be used instead.

#### Key to species of Nearctic female Ochlerotatus (Rusticoidus)

Adapted from Carpenter & LaCasse (1974)

Palpi and proboscis all dark scaled; wing dark, with small patch of pale scales restricted to base of costa; tergites with narrow basal bands of pale scales, broadening laterally; sternites mostly pale scaled, with apical dark patches; tarsi dark ...... Ochlerotatus provocans

# Key to species of Nearctic female Ochlerotatus (Woodius)

Adapted from Carpenter & LaCasse (1974) and Reinert et al. (2009)

1. Mesokatepisternal scaling reaching angle; hypostigmal scales absent; sternites with dark apical bands; tergites usually without basal bands of pale scales, or very narrow ones; lower mesepimeral setae usually absent, rarely 1 or 2; palpi dark ..... Ochlerotatus diantaeus

# Key to species of Nearctic female *Ochlerotatus* subgenus uncertain

Adapted from Knight (1951), Beckel (1954), Vockeroth (1954), Ellis & Brust (1973), Carpenter & LaCasse (1974), McDaniel & Webb (1974), Wood (1977), Brust & Munstermann (1992), Lanzaro & Eldridge (1992), Gimnig (2000), Darsie Jr & Ward (2005) and Nielsen (2009)

Hindtarsi not pale banded, completely or mostly dark (pale streaks might be present) 17

Wing vein costa mostly pale scaled; tergum VII with more pale than dark scales ... 4

4 (3). Wing vein  $R_{4+5}$  with more dark scales than veins  $R_2$  and  $R_3$ ; foreclaw almost straight in the middle ..... Ochlerotatus dorsalis

Wing vein  $R_{4+5}$  with as many dark scales as  $R_2$  and  $R_3$ ; foreclaw abruptly curving near attachment of tooth ...... Ochlerotatus campestris

At least some dorsal scales narrow, dark and pale scales unevenly distributed ......8

7 (6). Proboscis with many dark and pale scales intermixed; scutum with mixed brown and pale scales laterally ...... Ochlerotatus squamiger

Proboscis with few scattered pale scales on basal 0.5; scutum with mostly pale scales laterally ...... Ochlerotatus grossbecki

8 (6). Abdominal terga entirely clothed with yellow scales ..... Ochlerotatus flavescens

> It is strongly advised to use larval characters to confirm the identities of the mosquitoes within the Stimulans Group.

9 (8). Front tarsal claw sharply bent and sinuous; tooth long, extending over halfway from insertion to apex of main claw, and somewhat parallel to main claw; proboscis largely dark

scaled, with few pale scales scattered ...... Ochlerotatus excrucians

> Ochlerotatus increpitus Ochlerotatus clivis Ochlerotatus washinoi

Proboscis with pale scales intermixed, more numerous towards middle; hypostigmal scale patch present; sternites with dark and pale scales intermixed ... Ochlerotatus riparius

13 (12). Palpi dark, sparsely speckled with pale scales, with irregular pale rings at bases of segments 3 and 4, terminal segment yellowish white at apex; proboscis dark, sprinkled with few pale scales (sometimes appearing all dark); tergites with basal pale bands and some pale

scales scattered throughout each segment, but with apices mostly dark scaled *Ochlerotatus fitchii* 

14 (13). Proboscis usually clothed with many pale scales, rarely appearing all dark; frontal tarsal claw distinctly bent just distad of insertion of tooth ...... Ochlerotatus stimulans

Proboscis all dark scaled; frontal tarsal claw smoothly curved along its length .....

Mesepimeron with at least ventral 0.25 devoid of scales	31
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20 (19). Abdominal terga with at least longitudinal stripe of pale scales, or almost entirely pale scaled; scales on dorsal 0.5 of postpronotum brown ... Ochlerotatus spencerii spencerii

22 (21). Hypostigmal scale patch present ...... Ochlerotatus pullatus

Hypostigmal scale patch absent ...... Ochlerotatus communis Complex ......23

> Ochlerotatus tahoensis Ochlerotatus nevadensis Ochlerotatus

#### churchillensis

The members of the Punctor Subgroup are almost indistinguishable in the female, especially Ochlerotatus punctodes, which shares many characters with both "tundra" varieties of Ochlerotatus punctor and Ochlerotatus hexodontus (Carpenter & LaCasse, 1974; Gimnig, 2000). Larval characters should be used to avoid assuming identities based on distribution alone. Moreover, morphological studies of these species are greatly needed, as Gimnig (2000) points out, since the known "type" and "tundra" varieties of the before mentioned species are thought by many to represent cryptic species, as it was found with other members of the Communis Group. Useful characters for the separation of Ochlerotatus punctodes females are unknown, for the remaining species a tentative key follows.

Sternites mostly pale scaled, some with a median patch of black scales apically 30

All femora dark with scattered pale scales dorsally; tarsi dark, the proximal segments sprinkled with pale scales; proepisternum fully scaled ...... Ochlerotatus hexodontus\*

30 (28). Tarsomere I with pale markings on posterior surface ..... Ochlerotatus abserratus\*

Tarsi all dark ..... Ochlerotatus punctor\*

\*The morphological characters here presented are based on the works of Carpenter & LaCasse (1974) and Gimnig (2000), these should be used carefully and preferably confirmed using larval characters.

31 (17). Scutum with many long setae, appearing hirsute; postpronotum with many setae scattered over posterior 0.5 ..... Ochlerotatus nigripes

32 (31). Hypostigmal scales present, but few; wing dark scaled with numerous white scales scattered over anterior veins; legs with a mixture of dark and pale scales on all segments, giving them a grayish appearance; abdominal tergites with broad basal pale bands, sometimes with pale scales forming medial stripe; sternites all white scaled . Ochlerotatus niphadopsis

35 (34). Femora and tibiae speckled with pale scales anteriorly, tarsomere I with streak of pale scales ...... Ochlerotatus ventrovittis

Fore and hind femora speckled with pale scales anteriorly, mid femora all dark anteriorly; tibiae with streak of pale scales, continuous with the one on the first tarsomere

	Ochlerotatus	sticticus
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Postpronotum with narrow brown scales dorsally, and broader, white scales ventrally; scutum mostly with pale golden scales, with brown scaling forming two submedian longitudinal stripes often divided by a narrow stripe of golden scales, sometimes not evident, thus brown scaling appearing to form a single broad median stripe, also brown scaling is often present at the middle of supraalar area, surrounded by pale scaling ..... Ochlerotatus decticus

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