ORIGINAL ARTICLE

A morphological identification key to the mosquito disease vectors of the Pacific

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Abstract

An identification guide is provided for female adults of the mosquito groups, complexes or species that can be morphologically differentiated and that are likely to transmit arboviruses (e.g., dengue, Zika, chikungunya, Ross River and Japanese encephalitis) or parasites (e.g., Plasmodium spp. and Wuchereria bancrofti) in the Pacific Islands, countries and territories. This dichotomous key is adapted, with permissions, from a variety of text and image sources to facilitate the identification of disease vectors by individuals with limited taxonomic training including Pacific island country Vector Surveillance and Environmental Health officers, other public health officials and students.

KEYWORDS

Aedes, Anopheles, Culex, identification, morphology, mosquitoes, Pacific Islands, taxonomy

INTRODUCTION

The Pacific is home to over 400 mosquito species. A small number of mosquitoes in the Aedes (Ae.), Anopheles (An.) and Culex (Cx.) genera (Table 1) are responsible for transmitting pathogens causing diseases in humans including malaria, lymphatic filariasis, dengue, Zika, chikungunya, Ross River and Japanese encephalitis (Matthews et al. 2022). The mosquito species that transmit these pathogens differ in their behaviours, distributions and competency to transmit pathogens with the distributions of many species overlapping (Figure 1). It is therefore essential that mosquitoes collected as a part of vector surveillance programmes are correctly identified so that their distributions, abundance, ecology and susceptibility to insecticides may be understood. This information is critical for predicting the risk of pathogen transmission and to ensure the relevance of vector control operations. For example, mosquito species that bite indoors at night can be targeted by insecticide treated nets, while mosquitoes that rest outdoors might be better controlled by harborage spraying of insecticides on vegetation (Sinka et al. 2016).

Medically important mosquitoes in the Pacific Island countries and territories

Malaria remains an important vector-borne disease with 10 000 000 people considered at risk in Papua New Guinea, the Solomon Islands and Vanuatu (WHO 2022). The malaria vectors in the region are composed of groups and complexes of closely related, morphologically similar, cryptic or sibling Anopheline species. The primary vectors are members of the Cellia sub-genus, Punctulatus group (Beebe et al. 2013). Aedes vectors, principally Aedes aegypti and Aedes albopictus, are responsible for transmitting dengue and the emerging arboviral diseases—Zika and chikungunya. The burden of these viruses has dramatically increased in recent years in the Pacific island countries (PICs) (Matthews et al. 2022). Some Aedes species also vector other public health pathogens such as Ross River virus, Japanese encephalitis virus and the helminth, Wuchereria bancrofti, responsible for lymphatic filariasis in the Polynesian countries and Fiji (WHO 2020). Medically important Aedes vectors are in the sub-genera Finlaya, Ochlerotatus, Rampamyia and Stegomyia. In the Pacific region, 113 species of the genus Culex have been

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TABLE 1	List of Anophe	eles, Aedes and	Culex species	and complexes identifiable with this morphological key.
Genus	Sub-genus	Group	Complex	Mosquito name
Anopheles	Anopheles	bancroftii	bancroftii	Anopheles (Anopheles) bancroftii s.l. Giles 1902 (genotype A, B, C, D)
		barbirostris	barbirostris	Anopheles (Anopheles) barbirostris van der Wulp 1884
	c #			Anopheles (Anopheles) campestris Reid 1962
	Cellia		longirostris	Anopheles (Cellia) longirostris s.l. Brug 1928
			subpictus 	Anopheles (Cellia) subpictus Grassi 1899
		annulipes ,	annulipes ,	Anopheles (Cellia) annulipes Walker 1856
		lungae	lungae	Anopheles (Cellia) lungae Belkin & Schlosser 1944
		punctulatus	farauti	Anopheles (Cellia) punctulatus Donitz 1901 Anopheles (Cellia) farauti s.l. Laveran 1902 (farauti s.s., farauti 4, farauti 5, farauti 8, hinesorum,
			kolionsis	Anonholos (Collia) koliansis s L (construm 1, 2)
			KOIIEIISIS	Anopheles (Cellia) kollensis s.r. (genotype 1, 5)
				Anopheles (Cellia) literalis Vina 1022
				Anopheles (Cellia) Intolaiis King 1952
Andre	Finlaya	kachi		Anopheles (Centa) karwan (James 1905)
Aedes	Finiaya	коспі		Aedes (Finlaya) Injensis Marks 1947
				Aedes (Finlaya) kochi (Donitz 1901)
	Ochlevetetus			Aedes (Ontherettus) normanansis (Teular) 1915
	Ochlerotatus	anan ikala		Aedes (Ochlerotatus) normanensis (Taylor) 1915
	D	empinais		Aedes (Ochierotatus) vigilax (Skuse) 1889
	Rampamyia Charana	notoscriptus		Aedes (Rampamyla) notoscriptus (Skuse) 1889
	Stegomyla	aegypti		Aedes (Stegomyid) degypti (Linnaeus) 1762
		scutellaris		Aedes (Stegomyid) aloopictus (Macquart) 1903
				Aedes (Stegomyia) cooki Belkin 1962
				Aedes (Stegomyia) neoriaeus Edwards 1962
				Aedes (Stegomyid) nensilii Farner 1945
				Aedes (Stegomyld) kessell Huang & HitchCock 1980
				Aedes (Stegomyid) marshallensis (Stone & Bonart) 1944
				Aedes (Stegomyid) polynesiensis Marks 1951
				Aedes (Stegomyid) pseudoscutellaris (Theobaid) 1901
				Aedes (Stegomyia) rotumae Belkin 1962
				Aedes (Stegomyia) scutellaris (Walker) 1858
				Aedes (Stegomyia) upolensis Marks 1957
	<i>c</i> /		tongae	Aedes (Stegomyia) tongae s.s. Edwards 1926
Culex	Culex	pipiens		Culex (Culex) pacificus Edwards 1916
				Culex (Culex) pervigilans (Bergroth) 1889
			pipiens	Culex (Culex) australicus Dobrotworsky & Drummond 1953
		sitiens		Culex (Culex) quinquetasciatus Say 1823
				Culex (Culex) annulirostris Skuse 1889
				Culex (Culex) gelidus Theobald 1901
				Culex (Culex) palplis Taylor 1912
				Culex (Culex) sitiens Wiedemann 1828
				Culex (Culex) tritaeniorhynchus Giles 1901
				°Culex (Culex) whitmorei (Giles), 1904
	Oculeomyia	bitaeniorhynchus		Culex (Oculeomvia) bitaeniorhynchus, Giles 1901

Note: s.l. (sensu lato, used to denote complex); s.s. (sensu stricto, used to denote species). Source: Adapted from Russell and Burkot (2023).

^aNon-vectors.



FIGURE 1 Distribution of medically important mosquito species by country (reproduced from Russell & Burkot 2023). Note that Culex pervigilans and Culex whitmorei are non-vector mosquitoes, but often collected in traps.

reported (Russell & Burkot 2023), and some of them also transmit arboviral diseases such as Ross River and Japanese encephalitis, as well as W. bancrofti in Micronesia (Wilkerson et al. 2021). The mosquitoes that transmit human pathogens in the Pacific or that can be easily confused with those disease vectors and their distributions by PICs are shown in Figure 1.

The geographic focus and purpose of the guide

This taxonomic key complements 'A guide to mosquitoes in the Pacific 2023' (Russell & Burkot 2023) and has been developed by the PacMOSSI (Pacific Mosquito Surveillance for Impact) program (https://pacmossi.org), which is supported and funded by the Australian Government Department of Foreign Affairs and Trade (DFAT), the Agence Francaise de Developpement (AFD) and the European Union (EU). The project is implemented in partnership with the Pacific Community (https://www.spc. int), which represents 22 Pacific Island Countries and Territories (PICTs), from the Northern Mariana Islands in the North, to Palau in the West and the Pitcairn Islands in the South East (see Figure 1). PacMOSSI and the Pacific Community are committed to supporting those 22 PICTs to strengthen vector surveillance and control, and prevent, contain and control mosquito-borne diseases. This guide is therefore specific to those PICTs and is intended for use by Vector surveillance and Environmental Health workers with a focus on disease vectors.

The guide is intended as a basic training resource for students, teachers and researchers and targeted at public health and environmental health officers who may be required to conduct vector surveillance across the PICTs. The geographic reach has excluded the Easter Islands, the Galapagos Islands and Hawaii. The major purpose of this guide is to cover medically important mosquito vectors.

MOSQUITO IDENTIFICATION

Taxonomically, mosquitoes belong to the family Culicidae, sub-family Culicinae, which differ from other insects by having a proboscis that is several times longer than the head. Scales are present on the veins of the wings, forming a fringe along the wing's hind edge (visible only with magnification).

Identifying mosquitoes to genus, species or complex requires specimens in good condition (e.g., with minimal

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damage). While both larvae and adult mosquitoes can be identified using their physical characteristics (their morphology), this key focuses on adult female mosquitoes using variations in physical characteristics including size, colour, wing vein patterns and the arrangement of scales and hairs on the wings and body. Scales and hairs often occur in unique designs that may interact with light to create different colours. A stereomicroscope or dissecting microscope ($10-40 \times$ magnification) is required to see many of these key features. Mosquito specimens may be manipulated with forceps or pinned into position in petri dishes to focus on specific features. Good reference specimens preserved as pinned adults or larvae mounted in resins are useful to compare with the specimens being identified.

Morphological characteristics coupled with known geographic distributions can be used to accurately identify many mosquito species, but others can be extremely difficult or even impossible to differentiate by morphology. Closely related species, with morphological characteristics that are very similar to other closely affiliated taxa, are often referred to as species complexes or sibling species. The assemblage of these very similar species is called a group. Mosquito complexes require molecular analysis to confirm their identification to species. Even when species are amenable to identification, their classification can be compromised or hesitated if they are outside their usual geographic distribution, or if they have been damaged during collection. It should be recognised therefore,



FIGURE 2 General anatomy of an adult mosquito (Source: https://freesvg.org/mosquito-parts).

that not all specimens will be identifiable and individuals attempting to identify mosquitoes should resist the temptation to force specimen identifications to species. When verification of species is required, advice from recognised experts should be sought. The user is further cautioned that mosquito species not included in this key may be found in the same habitats as the vector species listed, so not all specimens in local collections can be keyed out using this guide. Additionally, mosquito distributions are dynamic with constant movement and establishment into areas where they were not previously found. The distributions of mosquitoes shown in Figure 1, while reflecting current knowledge at the time of this publication, may not be



FIGURE 3 Head and thorax (dorsal view) of an adult mosquito (Source: modified from Disa Eklöf and Anders Lindstrom, National Veterinary Institute, Sweden).

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accurate. Table 1 lists the key species of PICTs by genus, sub-genus, group, complex and species. The taxonomic authorities are also included.

Morphology of an adult mosquito

An adult mosquito is small, generally 0.4–0.9 cm long with wings that do not extend beyond the abdomen. The proboscis is elongated and wings are covered with scales, creating a fringe-like border on the hind edge. Morphological identification is based on features of the mosquito body parts (head, thorax, abdomen, wings and legs) shown in Figure 2.

Head and thorax

The major structures of the head include the maxillary palps, antennae and proboscis. Behind the head is the thorax to which the legs, abdomen and a single pair



FIGURE 5 Mosquito wing and its venation (Becker et al. 2010).





Post spiracular area Mesothoracic spiracle Pre spiracular area

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of wings are attached. The dorsal part of the thorax (Figure 3) includes the scutum and scutellum. Scale patterns (e.g., medial or sub-medial stripes) on the scutum and scutellum are important for morphological characteristics as is the postspiracular area and mesepimeron on the lateral side (Figure 4) of the thorax.

Wings

The wings of mosquitoes (Figure 5) are long and slender, with wing veins covered with scales. The principal longitudinal veins of mosquitoes (from anterior to posterior) are the costa, subcosta, radius, media, cubitus and anal vein. The branches of these veins are differentiated by numbers; for example, the anterior branch of the media is named media-one (M_1) and the posterior branch is media-two (M_2).

Abdomen

The abdomen (Figure 6) is posterior to the thorax. The dorsal sclerite is called the 'tergum' (plural terga), and the ventral sclerite is called the 'sternum' (plural sterna). Each tergum or sternum is divided into plates called tergites and sternites, respectively. There are 10 segments in a mosquito's abdomen. Segments 1 to 8 are easily identified under a stereomicroscope. Each segment may have basal, apical or lateral patches of pale scales, which are useful taxonomic markers. Segments 9 and 10 are very small and modified as external genitalia. The paired cerci projecting beyond the last segment are part of the female terminalia and more noticeable in genera with pointed abdomens.



FIGURE 6 Abdomen of adult mosquito (a) lateral view showing dorsal side (tergum) and ventral side (sternum). (b) Dorsal view showing abdominal segments of an adult mosquito (Source: Narayan/QIMRB).

Legs

Adult mosquitoes have three pairs of jointed legs, composed of a femur, tibia and five tarsomeres (Figure 7). Identification keys often refer to 'Ta-III4' or Ta-III5'. 'Ta' refers to the tarsus, 'III' refers to the third set of legs (i.e., the hind legs) and 4 and 5 refer to tarsal segments (i.e., tarsomere 4 or tarsomere 5). The banding patterns of these different leg segments are often used to differentiate mosquito species.

THE PICTORIAL KEY AND MOSQUITO IDENTIFICATION

The resources for the preparation of this mosquito identification key were obtained from Belkin (1962), Cooper et al. (2010), Huang (1977), Huang and Hitchcock (1980), Lee et al. (1982), the Department of Health Western Australia (2020) and Wilkerson et al. (2021). The images used in the identification keys were captured by the authors, and by Chen Wu (Mosquito Control Laboratory QIMRB), Disa Eklöf and Anders Lindstrom (National Veterinary Institute, Sweden),



FIGURE 7 Hind leg of an adult mosquito (Source: Rueda 2004).

Huang (1977), Huang and Hitchcock (1980), Walter Reed Biosystematics Unit (http://www.wrbu.org/VecID_ MQ.html), Rattanarithikul et al. (2006), Rueda (2004), Robert D. Cooper (personal communication), Stephen Doggett (NSW Health Pathology) and the World Health Organisation (WHO 2020).

This pictorial guide to mosquito identification takes the form of a dichotomous key. Dichotomous keys offer a straightforward, step-by-step approach to morphological identification, presenting two choices at each step. These direct the user to the next step and, ultimately, the identification of a species. This binary choice simplifies decision making for non-experts, making it easier for users to navigate through the key. A list of terminologies and definitions for the traits mentioned in the key is given in Table 2. When cross-referenced to Figures 1–7, these will help the user understand and follow the guide's decisionmaking process.

This guide only identifies adult, female mosquitoes in the genera *Anopheles*, *Aedes* and *Culex*. To identify a specimen, follow the steps in Part A to ensure that the insect is a female mosquito (Figure 8) and to determine the genus to which it belongs (Figure 9). To differentiate whether the mosquito is male or female, look at the density of hairs on the antennae. Hairs of male antennae are numerous and feather-like (plumose). Hairs of female antennae are few and short (pilose).

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Having established the genus as *Anopheles, Aedes* or *Culex,* use the relevant figures in the rest of the key (Part B: *Anopheles* [Figures 10–14]; Part C: *Aedes* [Figures 15–21]; and Part D: *Culex* [Figures 22–26]) to examine the morphological characteristics that differentiate the sub-genera. Finally, based on all of the morphological features, identify the mosquito to complex or species level.

The key was reviewed by subject matter experts Arnaud Cannet (Directorate of Health and Social Affairs, New Caledonia), Robert D. Cooper (retired entomologist, Australian Defence Force) and Mark Schmaedick (Community College, American Samoa). The key was also tested and validated during PacMOSSI training workshops held in Brisbane (Australia) by experienced entomologists Brian Johnson (QIMRB), Andrew van den Hurk (Queensland Health) and Michael Onn (Brisbane City Council) and in Fiji by Vineshwaran Rama (Ministry of Health and Medical Services, Fiji).

TABLE 2 A glossary for understanding the pictorial key of mosquito identification.

Words	Definitions		
Anterior	Situated before or towards the front		
Apical	The tip or apex of a structure or a body part		
Basal	The base of a structure or a body part		
Clypeus	A broad plate on the front of mosquito head in front of the antennal pedicels and behind the maxillary palpi		
Integument	The outer layers of the body comprised of membranes and sclerites		
Maxillary palps	Paired sensory structures originated from the head, directly in front of the clypeus		
Median	The middle of a structure or a body part		
Posterior	Situated at, or towards the back or hindmost		
Prescutellar area	The median posterior area of the scutum, in front of the scutellum		
Scales	Extrusions of insect cuticle, made of chitin and evident as hairs or small plates. These cover large parts of the mosquito's body		
Sensu lato (s.l.)	In the broad sense. Used to indicate all members of a species complex		
Sensu stricto (s.s.)	In the strictest sense. Used to indicate a specific species within a complex		
Setae	Hair or bristle		
Sternite	The ventral sclerotized part of the exoskeleton, usually describing an abdominal segment		
Sub-median	To the side of the median or midline		
Supra-alar	A small lateral area of the scutum just above and in front of the scutellum		
Tergite	The dorsal sclerotized part of the exoskeleton, usually describing an abdominal segment		

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Part A: Identify specimen as a mosquito, and identify sex and genus







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FIGURE 10 Identifying Anopheles mosquito: An. bancroftii s.l., An. barbirostris s.s., An. campestris, An. annulipes, An. longirostris s.l., An. lungae s.l., An. punctulatus, An. farauti s.l., An. koliensis s.l., An. karwari, An. litoralis, An. subpictus s.l. and An. vagus.



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FIGURE 12 Identifying An. annulipes, An. longirostris s.l., An. lungae s.l., An. punctulatus, An. farauti s.l., An. koliensis s.l., An. litoralis, An. karwari, An. subpictus s.l. and An. vagus.





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List of Aedes species and complexes identifiable with this morphological key

Aedes (Finlaya) fijiensis Marks 1947 Aedes (Finlaya) kochi (Donitz 1901) Aedes (Finlaya) samoanus (Grünberg) 1913 Aedes (Ochlerotatus) normanensis (Taylor) 1915 Aedes (Ochlerotatus) vigilax (Skuse) 1889 Aedes (Rampamvia) notoscriptus (Skuse) 1889 Aedes (Stegomyia) aegypti (Linnaeus) 1762 Aedes (Stegomvia) albopictus (Macquart) 1903 Aedes (Stegomvia) cooki Belkin 1962 Aedes (Stegomyia) hebrideus Edwards 1962 Aedes (Stegomyia) hensilli Farner 1945 Aedes (Stegomyia) kesseli Huang & Hitchcock 1980 Aedes (Stegomvia) marshallensis (Stone & Bohart) 1944 Aedes (Stegomyia) polynesiensis Marks 1951 Aedes (Stegomyia) pseudoscutellaris (Theobald) 1901 Aedes (Stegomyia) rotumae Belkin 1962 Aedes (Stegomyia) scutellaris (Walker) 1858 Aedes (Stegomyia) upolensis Marks 1957 Aedes (Stegomyia) tongae s.s. Edwards 1926

Part C: Aedes



FIGURE 15 Identifying Ae. fijiensis, Ae. kochi, Ae. samoanus, Ae. normanensis, Ae. vigilax, Ae. notoscriptus, Ae. albopictus, Ae. hebrideus, Ae. hensilli, Ae. marshallensis, Ae. polynesiensis, Ae. pseudoscutellaris, Ae. rotumae, Ae. scutellaris, Ae. upolensis, Ae. cooki, Ae. kesseli and Ae. tongae s.l.



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The clypeus of female *aegypti* has two silvery white dots, which are lacked in mosquitoes of Scutellaris group.

FIGURE 17 Identifying Ae. normanensis, Ae. vigilax, Ae. aegypti, Ae. albopictus, Ae. hebrideus, Ae. hensilli, Ae. marshallensis, Ae. polynesiensis, Ae. pseudoscutellaris, Ae. rotumae, Ae. scutellaris, Ae. upolensis, Ae. cooki, Ae. kesseli and Ae. tongae s.l.



FIGURE 18 Identifying Ae. albopictus, Ae. hebrideus, Ae. hensilli, Ae. marshallensis, Ae. polynesiensis, Ae. pseudoscutellaris, Ae. rotumae, Ae. scutellaris, Ae. upolensis, Ae. cooki, Ae. kesseli and Ae. tongae s.l.

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FIGURE 19 Identifying Ae. hebrideus, Ae. hensilli, Ae. marshallensis, Ae. polynesiensis, Ae. pseudoscutellaris, Ae. rotumae, Ae. scutellaris, Ae. upolensis, Ae. cooki, Ae. kesseli and Ae. tongae s.l.

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b. Identifying Ae. hebrideus, Ae. marshallensis, Ae. scutellaris



Note: The following members of the *Scutellaris* group are non-overlapping in distribution. Please note the geographic location when making an identification.

Only in Tonga
Only in Rotuma island, Fiji
Confirmed in Samoan Islands, possibly present in Tonga
Only in Marshall Island and Federated States of Micronesia

FIGURE 20 Identifying (A) Ae. rotumae and Ae. upolensis and (B) Ae. hebrideus, Ae. marshallensis and Ae. scutellaris.

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FIGURE 21 Identifying Ae. cooki, Ae. kesseli and Ae. hensilli.





FIGURE 22 Identifying Cx. annulirostris, Cx. bitaeniorhynchus, Cx. gelidus, Cx. palpalis, Cx. sitiens, Cx. tritaeniorhynchus, Cx. whitmorei, Cx. quinquefasciatus, Cx. pacificus, Cx. pervigilans and Cx. australicus.





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Part D: Culex

Absent

Cx. sitiens

IDENTIFY

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Cx. annulirostris

same width in middle and edges



Cx. palpalis, Cx. sitiens

Additional cues: Cx. annulirostris's fore tibia has usually a line of pale spots on anterior surface, but Cx. palpalis and Cx. sitiens have no pale spots on anterior surface



Cx. annulirostris



FIGURE 24 Identifying Cx. annulirostris, Cx. palpalis, Cx. sitiens and Cx. tritaeniorhynchus.

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FIGURE 25 Identifying Cx. quinquefasciatus, Cx. australicus, Cx. pervigilans and Cx. pacificus.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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