Variability of larval identification characters of exotic *Aedes albopictus* (Skuse) intercepted in Darwin, Northern Territory

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Abstract

There is no morphological identification key to satisfactorily distinguish between the larvae of *Aedes (Stegomyia)* species with special consideration to endemic and exotic species in Australia. Difficulty in differentiation between exotic *Aedes (Stegomyia) albopictus* (Skuse) and *Aedes (Stegomyia) scutellaris* Walker has been described previously.[1] Aedes (Stegomyia) larvae were collected during an interception from an overseas vessel in Darwin, Northern Territory, and link bred. The adults were identified as *Ae. albopictus*. The larval skins and larvae were used to describe the variation in hair features of larval segment VII that are used to identify *Ae. albopictus*. The median hair number of hair 1–VII was three, whereas the description in Huang's identification key[2] states four. The median of hair 2–VII was one, confirming Huang.[2] However, the variability was higher than described by Huang[2] and nearly half of the specimens showed different hair numbers on both sides. Individual specimens are therefore not clearly distinguished from other members of the *Aedes (Stegomyia) scutellaris* group. This paper also describes the detection, elimination and surveillance procedures following the interception. These were successful in preventing the establishment of exotic *Ae. albopictus* in the Northern Territory following the interception. *Commun Dis Intell* 2003;27:105–109.

Keywords: Aedes albopictus, mosquitoes, surveillance

Introduction

The larvae of some species of the *Aedes (Stegomyia) scutellaris* group are difficult to identify morphologically.[3] The collection location is often essential in assisting species identification because many species are allopatric.[4] The species *Aedes (Stegomyia) katherinensis* Woodhill, for example, is endemic to the Northern Territory of Australia and the species *Aedes (Stegomyia) scutellaris* Walker is distributed locally in the Torres Strait, Queensland and also in Papua New Guinea.[4,5,6] At present it is impossible to distinguish these two species at the larval stage.[1,7]

Identification keys generally encompass all species of a zoogeographic region. However, importation of exotic species occurs with increased transport and traffic due to globalization and new species can be introduced into a region.[8,9]

Correct identification of larval mosquitoes is essential to prevent the establishment of exotic species of the *Aedes (Stegomyia) scutellaris* group in Australia. *Aedes albopictus* is a vector of dengue fever, and the prevention of its establishment is a public health priority in both tropical and temperate Australia. *Aedes albopictus* has recently spread globally and is now established in many countries.[8,10] The species is present in countries to Australia's immediate north such as East Timor and Papua New Guinea, posing a continual threat to entering Australia.[2,11,12,13] Exotic larvae recently collected in Darwin, Northern Territory, were identified as either *Aedes (Stegomyia) albopictus* (Skuse) or *Ae. scutellaris*, but with the material available no conclusive identification was possible.[1] The differentiation of *Ae. albopictus* and *Ae. scutellaris* is usually carried out using a key for the South-East Asian region by Huang.[2] However, identification can be difficult when one of the key characteristics of intercepted larvae differs from the description. There is also the possibility of the interception of an undescribed species or a species such as *Aedes (Stegomyia) alorensis* Bonne-Wepster of which larvae have not been described.[1,12]

A routine quarantine inspection of unloaded overseas cargo at an international shipping company premise in Darwin Port, Northern Territory, found mosquito larvae in water pooling on mining equipment. The larvae were collected live. Some were identified at this stage while some were link bred to adults to confirm the larval identification. The reared adults

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were identified as *Ae. albopictus*. The larvae of a bulk sample and the larval skins of the link bred specimens provided material for the investigation of morphological features used to identify exotic and endemic *Aedes(Stegomyia)* larvae. A sample of four mosquito larvae sent from a survey in East Timor was also included in this examination.

This paper describes the investigation on the larval identification characters, as well as the interception and the elimination measures taken.

Identification

Twenty-four mosquito larvae were submitted by the Australian Quarantine Inspection Services (AQIS) to the Medical Entomology Branch in Darwin (MEB). Five females and five males were link bred successfully. All were identified as *Ae. albopictus* when using the identification keys by Huang.[2,12] Pupal skins were identified as *Ae. albopictus* according to Huang.[2] Fourteen larvae were not link bred and were preserved in 70 per cent ethanol for further examination.

The larval skins and larvae of the interception sample were analysed to distinguish between *Ae. albopictus* and *Ae. scutellaris* as in Huang.[2] Hair numbers on hairs 1–VII and 2–VII were counted on the left and the right side of each specimen (referred to as sides A and B), as differences on the two sides were observed. Therefore, 48 hair number counts were made for the 24 specimens (i.e., the larval skins of the five females and five males as well as the 14 larvae).

The hair length of hairs 1–VII was assessed in two classes, as 2 times the length or 2.5 times the length of hairs 5–VII. Other classes were not observed. Not all of the specimens were preserved well enough to clearly investigate this hair length parameter in all specimens and there was no difference between sides A and B of the individuals examined. There were 22 measurements of the hair length parameter from 22 specimens.

The variability of the hair characteristics is summarised in Table 1. The median number for hairs 1–VII was three and the median number for hairs 2–VII was one. The standard deviation revealed variability for both of these hair branches (Table 1). The sides A and B of individuals were observed to show different hair numbers in 11 out of 24 specimens examined.

A sample of four larvae sent from East Timor were identified as *Ae. albopictus*, although variability of the key identification characters was observed.[2] The larvae were investigated as described above and the results are also summarised in Table 1.

Detection and eradication measures

AQIS found the mosquito larvae during a routine inspection of a vessel on 2 January 2002 at premises of a shipping company at Darwin Port. The larvae were discovered in pooling of what was probably rainwater on oil exploration machinery. The machinery had been unloaded on 31 December 2001 from a cargo vessel which had arrived on 30 December

Table 1. Larval hair characteristics of *Aedes albopictus* from interception and East Timor sample and data provided by Huang[2]

	Hair 1–VII branches	Hair 2–VII branches	Length of hair 1–VII to hair 5–VII
Interception			
n	48	48	22
Minimum	2	1	2
Maximum	4	3	2.5
Average ± standard deviation	3.25 ± 0.70	1.19 ± 0.45	2.05 ± 0.15
Median	3	1	2
East Timor specimens			
n	8	8	4
Minimum	3	1	2
Maximum	4	2	2
Average ± standard deviation	3.25 ± 0.46	1.25 ± 0.46	2.0 ± 0.0
Median	3	1	2
Huang[2]			
Ae. albopictus	4 (3–4)	1 (1–2)	2
Alternative couplet	2 (2–3)	3–4	2.5

N Number of observations

* Key couplet leads to the *Aedes* (Stegomyia) *scutellaris* group members *Ae. downsi*, *Ae. patriciae*, *Ae. riversi*, *Ae. malayensis*, *Ae. alcasidi*, or *Ae. scutellaris*

2001. The inspecting officer removed the live mosquito larvae with their original water, which were taken to MEB and reared out as described above. Parts of the equipment were sprayed with the residual insecticide deltamethrin. The consignment was fumigated at 1.30pm on 2 January 2002.

The recent history of the exploration equipment was obtained from AQIS. In March 2001 it was used offshore in the Philippines. On 19 December 2001 it was transferred to Singapore by vessel, which then departed with the equipment on 20 December 2001. The equipment arrived in Darwin via Dili, East Timor, on 30 December 2001 and was discharged in the late afternoon on 31 December 2001.

The larval sample taken to MEB also contained 5 pupal skins and one unidentifiable dead adult. This indicated that live adults could have emerged and hence the interception was treated as a risk interception. MEB conducted a fogging operation using a Leco HD aerosol fogger applying bioresmethrin on the evening of 2 January 2002 from 6.00pm to 7.00pm within the premises of the shipping company and neighbouring port areas.

Container breeding survey

A container (receptacle) breeding survey was conducted as a joint operation between MEB and AQIS within the 400 m exclusion zone of the shipping company. Most premises were inspected on 22 January 2002, one week after substantial rain. Two remaining premises were inspected on 11 February 2002, 5 days after substantial rainfall.

No exotic mosquitoes were found (Table 2). All containers were sprayed with the residual insecticide deltamethrin. The container index (the percentage of water holding containers examined that contain mosquito larvae) was calculated according to World Health Organization guidelines (Table 2).[14]

Ovitrap surveillance

MEB set two ovitraps in vegetated areas within the neighbourhood of the shipping company premise for a time period of two months. These were in addition to five routine ovitraps set in the port area. The ovitraps were collected fortnightly, commencing on 7 January 2002 and ending on 4 March 2002 with the trapping results presented in Table 3. Following the interception, exotic *Aedes* species were not recorded from any of the additional traps or from the five routine ovitraps of the MEB, or the six AQIS ovitraps in the area.

Table 2. Summary of container breeding survey conducted around the port area of Darwin

Survey parameter	Number/index
Premises inspected	6
Containers found	258
Containers holding water	103
Containers breeding mosquitoes	17
Container index	16.5
Species found†	
Ochlerotatus notoscriptus	16.5
Culex quinquefasciatus	4.9
Ochlerotatus tremulus	2.9
Culex annulirostris	1.0

* Container index = percentage of water holding containers examined that contain mosquito larvae.[14]

† The index value calculated for each species is derived as: Species container index = percentage of water holding containers that contain mosquito larvae of this species.

Table 3. Ovitrap results of two additional traps set for eight weeks

Site description	Sampled n	Positive n	Positive %	Times species recorded (total number of larvae/pupae)				
				Ochlerotatus notoscriptus pupae		*Ochlerotatus notoscriptus* larvae		Nil mosquitoes
				Count of species*	Sum of pupae	Count of species*	Sum of larvae	Count of occurrence
Near drain along Frances Bay Drive	4	4	100.0	1	4	4	304	0
Mavie Street, under bush left hand side of driveway	4	2	50.0	0	0	2	104	2
Totals	8	6		1	4	6	408	2

* The count of species details the number of times this species was found in a trap.

Discussion

According to the key by Huang[2] the identification of *Ae. albopictus* larvae is carried out using the hair characteristics of segment VII: hair 1–VII is usually 4 branched (3–4 branches), hair 2–VII is single (1–2 hairs) and hairs 1–VII are less than 2 times as long as hairs 5–VII. The alternative features, which lead to identify *Ae. scutellaris*, in the dichotomous key are: hair 1–VII is usually 2 branched (2–3 branches), hair 2–VII is 3–4 branched and hairs 1–VII are at least 2.5 times as long as hairs 5–VII.

The investigation of hair numbers of segment VII of link bred *Ae. albopictus* larvae and larval skins revealed considerable variation. Hair branch 1–VII was found to have 3.25 ± 0.70 hairs, hair branch 2–VII revealed 1.19 ± 0.45 hairs, and hairs 1–VII were 2.05 ± 0.15 times as long as hairs 5–VII. The medians of two out of three parameters confirm the description given in Huang.[2] However, the variability is larger than described in Huang,[2] which is confirmed by the observation that the sides of one specimen differed in hair numbers in nearly 50 per cent of the specimens investigated. Eleven of the 24 larval specimens intercepted would not be accurately identified as *Ae. albopictus* and thus be misidentified as *Ae. scutellaris*.

Larval identification of *Aedes scutellaris* or *Ae. albopictus* from a single specimen using Huang's key[2] therefore poses a substantial potential of misidentification. This problem could be partly overcome by sampling a number of specimens. However, during an exotic interception, the number of larvae available is often small.

Identification keys are generally produced for zoogeographic regions covering the endemic species of a given area. Identification keys for larvae of *Aedes (Stegomyia)* of the Australasian region do not encompass all species of the *Aedes (Stegomyia) scutellaris group* of the nearby South-East Asian region.[15] Keys for the oriental and the South-East Asian regions do not include all of the Australasian species.[2,12] A comprehensive key of *Aedes (Stegomyia*) species is required for quarantine purposes in Australia.

A further difficulty is that larvae of some species already present in Australia such as *Ae. katherinensis* in the Northern Territory and *Ae. scutellaris* in Queensland (and also Indonesia and Papua New Guinea) can not be readily distinguished.[1,2,6,11] In addition, there is no description of larvae of *Ae. alorensis* and there is also the possibility of an undescribed species.[1] In an interception of larvae from overseas vessels, the knowledge of the last port of call or recent ports of call can often provide important information assisting problem identifications.

The world-wide problem of exotic importations of container breeding mosquito species via international transport has been discussed widely.[8,9,10,16,17,18] It is possible that distribution maps of the *Aedes (Stegomyia)* species of the Pacific region are out of date due to undetected species or the recent establishment of species from neighbouring or other countries. This potential increases the need for a comprehensive larval key for the *Aedes (Stegomyia)* species of the Pacific region as a whole.

The requirement to include the exotic or newly established species in identification keys has been successfully solved for *Ae. albopictus* larvae (and adults) in the Nearctic region.[18] However, in this example few *Aedes (Stegomyia)* species needed to be considered.[18] Additional taxonomic investigations with larger samples of specimens from different countries are essential to clarify larval identification of some of the *Aedes (Stegomyia)* species of the Pacific region. The application of molecular biological methods might provide supplementary information towards solving identification issues, but combined morphological and molecular biological studies remain to be done.[3,19]

AQIS routinely carries out inspections on overseas vessels in the Northern Territory and the finding of exotic mosquito larvae is not uncommon.[20] There have been 24 (in 2000/01) and 6 (in 2001/02) exotic mosquito interceptions found during routine inspections in Darwin. Port areas in Darwin are close to the city and therefore it is considered very important to react immediately on interceptions.

Elimination procedures in the above interception included chlorination, spraying and fumigation, and procedures were updated recently to include the rearing of larvae from South-East Asia, Papua New Guinea and the Pacific to adults.[20,21,22] This will assist in both the identification of intercepted specimens and the collection of specimens to establish comprehensive reference material towards the development of a comprehensive key as described above.

The elimination procedures above were successful in preventing the establishment of *Ae. albopictus* in Darwin. Darwin and the Northern Territory remain free of exotic dengue vectors. However, the container breeding survey carried out within the 400 m exclusion zone revealed a container index of 16.5. According to the assessment system of the World Health Organization, this container index is associated with significant risk for dengue transmission in countries with endemic dengue.[14] A container index between 1 and 2 is the objective to ensure that towns and seaports in a dengue endemic area are free from the danger of disease transmission.[14] If exotic *Aedes (Stegomyia)* species are imported into the Darwin port area, there are ample potential breeding places, increasing the potential of establishment of exotic vectors. Darwin is both vulnerable and receptive to

the introduction and establishment of exotic vectors of dengue.

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