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Morphological confirmation of the separate species status of *Culicoides (Avaritia) nudipalpis* Delfinado, 1961 and *C. (A.) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae)

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ABSTRACT

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Ten character states were used to compare five females and two males of two populations of *C. nudipalpis* from the Philippines and Timor in south-east Asia with 121 females and 167 males of 11 populations of *C. imicola* collected throughout its Afro-Asiatic range. It is concluded that they are closely related but good species, and together form a subgroup, or species-pair, in the Imicola group; currently this Old World group of the subgenus *Avaritia* comprises seven described species. *Culicoides nudipalpis* and *C. imicola* are most reliably separated on the ratio of the length of the proboscis to the height of the head (P/H ratio: 0,66–0,73 in *nudipalpis*, 0,82–1,02 in *imicola*). Compared to *C. imicola* very little is known of the life habits of *C. nudipalpis*. The latter's close taxonomic relationship to *C. imicola*, a proven vector of African horse sickness (AHS) and bluetongue (BT), indicates that the capacity of *C. nudipalpis* to vector these orbiviruses deserves to be investigated.

Keywords: African horse sickness, *Avaritia*, bluetongue, *Culicoides imicola*, *Culicoides nudipalpis*, Diptera: Ceratopogonidae, viruses

INTRODUCTION

Culicoides nudipalpis was described by Delfinado (1961) from four females collected on the island of Luzon, Philippines. The holotype has apparently been lost (Dyce 1979); Wirth & Hubert (1989) gave further notes on types and synonymy, and described the male for the first time from Sumbawa, Indonesia. To date, *C. nudipalpis* has been recorded only from various islands comprising the Philippines and Indonesia, i.e. from Luzon in the north-west to Timor in the south-east. These fall east of Huxley's modification of the Wallace line (Rosen 1988). Little is known about the biology of *C. nudipalpis*; by inference it is considered to be mammalophilic as the type series was taken in a carabao-baited trap (Delfinado 1961) and, more recently, low numbers were collected in light-traps '... sited adjacent to livestock, particularly penned cattle' (Sukarsih, Daniels, Sendow & Soleha 1994). *Culicoides imicola* on the other hand, is very abundant and widespread, being found through most of Africa into the Mediterranean, and thence across the Near and Middle East as far as southern China and Laos. It also appears to be catholic in its choice of hosts having been reported to feed on cattle, sheep, horses and even pigs (Venter, Meiswinkel, Nevill & Edwardes 1996). The known distributions of *C. imicola* and *C. nudipalpis* are not contiguous as neither has been recorded from peninsular Malaysia, Sumatra, Java, Borneo and Sulawesi.

Regarding their status as two species, the entire redescription of *C. nudipalpis* by Wirth & Hubert (1989) duplicates almost word-for-word that given by them for *C. imicola*. They conclude that *C. nudipalpis* '... is nearly identical with *C. imicola* ...' The single feature used by these authors to distinguish females of *C. nudipalpis* is '... the virtual absence of the palpal pit; the third palpal segment of *C. nudipalpis* has a round pitlike area on the surface, but it lacks depth'.

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For *C. imicola* the third segment is described as '. . . without definite sensory pit but with sensilla grouped in a circular area on surface of segment'. While the wording may differ, the meaning is similar. The same character is used to separate the males. Comparison of their descriptions suggests two clearer differences between *C. imicola* and *C. nudipalpis*: palpal ratio (PR) 2,3 vs 0,68 and proboscis/head ratio (P/H) 0,88 vs 0,68, respectively. However, the large difference between the palpal ratios is clearly a lapsus as measurement of Fig. 122b in Wirth & Hubert (1989) gives a PR of 2,25 which is close to the 2,4 they give in their Table 1, p. 54. They did not refer to the significant difference in P/H ratios or use it as a differentiating feature in their key. The taxonomic knowledge of *C. nudipalpis* is to be found in two publications only; the extant data are incomplete and conflicting, and are summarized in Table 1, along with the data from the current study.

This synopsis begs the question as to whether *C. imicola* and *C. nudipalpis* are indeed two good species. Doubt as to their separate status deepens when one considers that two Indian species, *C. minutus* Sen & Das Gupta, 1959 and *C. pseudoturgidus* Das Gupta, 1962, have already been found to be synonyms of *C. imicola* (Dyce & Wirth 1983). If *C. nudipalpis* is shown to be yet another synonym, it would mean a further extension in the already large geographic range of *C. imicola*, from the southern tip of Africa to the northern doorstep of Australia. This possibility of synonymy is important as *C. imicola* is probably the most important vector of the viruses of bluetongue (BT) and African horse sickness (AHS) known in the Old World.

These questions, and the recent acquisition of four females and two males of *C. nudipalpis* from Puerto Galera, Mindoro Island, Philippines, prompted its present comparison with *C. imicola*. Only key characters are compared as the sample size of *C. nudipalpis* was too small to attempt a full redescription.

MATERIALS AND METHODS

Initial examination, in alcohol, of the fresh unmounted Puerto Galera *C. nudipalpis* females indicated the species to be smaller than *C. imicola*, especially as regards the length of the proboscis. The taxonomic value of this apparent difference was evaluated in slide-mounted specimens, along with nine other character states.

To negate seasonal effects and geographic clines in the *C. imicola* data, 121 females representing eight populations from various ecological zones of Africa were randomly chosen, these augmented by three extralimital populations (Spain, Israel and Thailand). It was not possible to assess all ten character states in all 121 *C. imicola* because of damage to some specimens. Additional data on males and females were taken from Meiswinkel (1989). *Culicoides nudipalpis* was represented by four females and two males from Mindoro Island, Philippines, and one female from the island of Timor. Too few specimens of *C. nudipalpis* were available for a more detailed comparison.

Statistical analyses

For the purposes of statistical analysis, the 11 populations of *C. imicola* and five *C. nudipalpis* females were considered to be 12 separate populations. An objective of this study was then to see whether any of the ten character states allowed the *C. nudipalpis* to be distinguished from all of the *C. imicola* populations while, at the same time, not distinguishing among the different *C. imicola* populations. For four of the character states that were variable (proboscis length (P), wing length, head height (H) and P/H ratio), a one-way ANOVA was used to test for a significant effect of population on the mean. Where a significant effect of population was found, differences among pairs of means were investigated using a test for multiple comparison (Tukey's method). All statistical analyses were performed using the software Minitab™.

RESULTS

The findings of this study that relate directly to those of Delfinado (1961) and Wirth & Hubert (1989) are summarized in Table 1. Ten character states were examined; the taxonomic value of each is assessed separately.

Wing length

Fig. 1A shows the wing lengths of the five *C. nudipalpis* and 121 *C. imicola* from 11 populations. Although there is considerable overlap, the mean wing length of *C. nudipalpis* was shorter than that of any of the *C. imicola* populations. ANOVA found a significant effect of population on mean wing length ($F_{11,114}$ = 18,13, $P < 0,001$); however, using Tukey's method the mean wing length of *C. nudipalpis* was not significantly smaller than that of the Ndumu, Malawi, Mauritius, Egypt and Thailand populations of *C. imicola*. This suggests that *C. nudipalpis* has, in general, shorter wings than *C. imicola* but that wing length is not a good character for the differentiation of the two species. Of course, the sample size for *C. nudipalpis* was too small to allow reliable conclusions about its wing length to be drawn; future scrutiny of a longer series may confirm *C. nudipalpis* to have shorter wings than *C. imicola*. As regards *C. imicola*, it is notable that little to no overlap exists in wing length between three South African populations, i.e.

TABLE 1 Summary of data in published descriptions of *C. nudipalpis* and used to separate it from *C. imicola*; data according to Delfinado (1961), Wirth & Hubert (1989) and this study

Character	Author(s)	*C. nudipalpis*	X	*n*	*C. imicola*	X	*n*
Wing length (mm)	Delfinado Wirth & Hubert This study	0,77–1,02 0,80 0,66–0,91	0,85 – 0,82	9 – 5	– 0,79–0,86 0,92–1,17	– 0,82 1,06	– 5 150
Proboscis length (μm)	Delfinado Wirth & Hubert This study	– – 103,8–125,0	– – 116,5	– – 5	– – 117,5–182,5	– – 154,2	– – 121
Proboscis/head ratio (P/H)	Delfinado Wirth & Hubert This study	– 0,68 0,66–0,73	– – 0,70	– – 5	– 0,88 0,82–1,02	– – 0,90	– – 102
Antennal length (μm)	Delfinado Wirth & Hubert This study	– – 428,8–502,5	– – 477,5	– – 5	– – 435,0–494,5	– – 466,0	– – 25
Antennal ratio (AR)	Delfinado Wirth & Hubert This study	1,09–1,18 1,20 1,06–1,12	1,14 – 1,07	4 – 5	– 1,17–1,19 0,95–1,10	– 1,18 1,01	– 2 167
Sensilla coeloconica	Delfinado Wirth & Hubert This study 	3, 11–15 3, 11–15 3, 12–15 3, 11–15 3, 12–15	– – – – –	– – – 4 5	– 3, 11–15 3, 12–15 3, 11–15 3, 12–15	– – – – –	– – – 7 165

Ndumu, Upington and Stellenbosch (Fig. 1A). This result could be interpreted as representing three species, but as these same populations exhibit consistency in proboscis length, and all other characters, we do not hesitate to classify them as one species. This supports the earlier inference that wing length is an unreliable character state for the differentiation of species, being more variable under environmental influences, whether nutritional or seasonal. Hensleigh & Atchley (1977), in a study of natural and laboratory populations of the N. American *C. variipennis*, also found that there are '. . . highly significant differences among temperature groups in continuous body measurements, e.g. wing length and width . . .'

Proboscis length

The proboscis of *C. nudipalpis* is significantly shorter than that of *C. imicola*. Its usefulness as a distinguishing feature is graphically illustrated in Fig. 1B; only two specimens (1,6 %) of the 121 *C. imicola* have a proboscis length overlapping that of *C. nudipalpis*. The two *C. imicola* outliers are from the Ndumu and Thailand populations and on the basis of proboscis lengths could easily be identified as *C. nudipalpis*. ANOVA found a significant effect of population on mean proboscis length ($F_{11,114}$ = 15,93, $P < 0,001$) and, according to Tukey's method, the mean proboscis length of *C. nudipalpis* was significantly shorter than that of ten of the *C. imicola* populations; however, the mean proboscis length of *C. nudipalpis* was not significantly smaller than that of the Thailand population of *C. imicola*. This is suggestive of a geographic cline, but all of the Thailand specimens have the antennal ratio, better defined palpal pit and coeloconica distribution typical of *C. imicola*. Such results alert one to the danger of basing identifications and descriptions on single specimens.

Head height

This is defined as the distance from the tormae to the interocular seta. ANOVA found a significant effect of population on mean head height ($F_{11,95}$ = 6,45, $P < 0,001$) but, according to Tukey's method, the *C. nudipalpis* did not differ significantly from any of the 11 *C. imicola* populations. Instead, the Israel population of *C. imicola* had a larger head height than that of the Botswana, Egypt and Thailand populations (Fig. 1C) Head height is therefore not a good character for distinguishing *C. nudipalpis* and *C. imicola*.

P/H ratio

This is the ratio of proboscis length to head height. The P/H ratio of *C. nudipalpis* is significantly smaller than that of *C. imicola* (Fig. 1D). Indeed, there was no overlap in the ratios of the two species (*C. nudipalpis*, 0,66–0,73; *C. imicola*, 0,82–1,02; Table 1). ANOVA found a significant effect of population on P/H

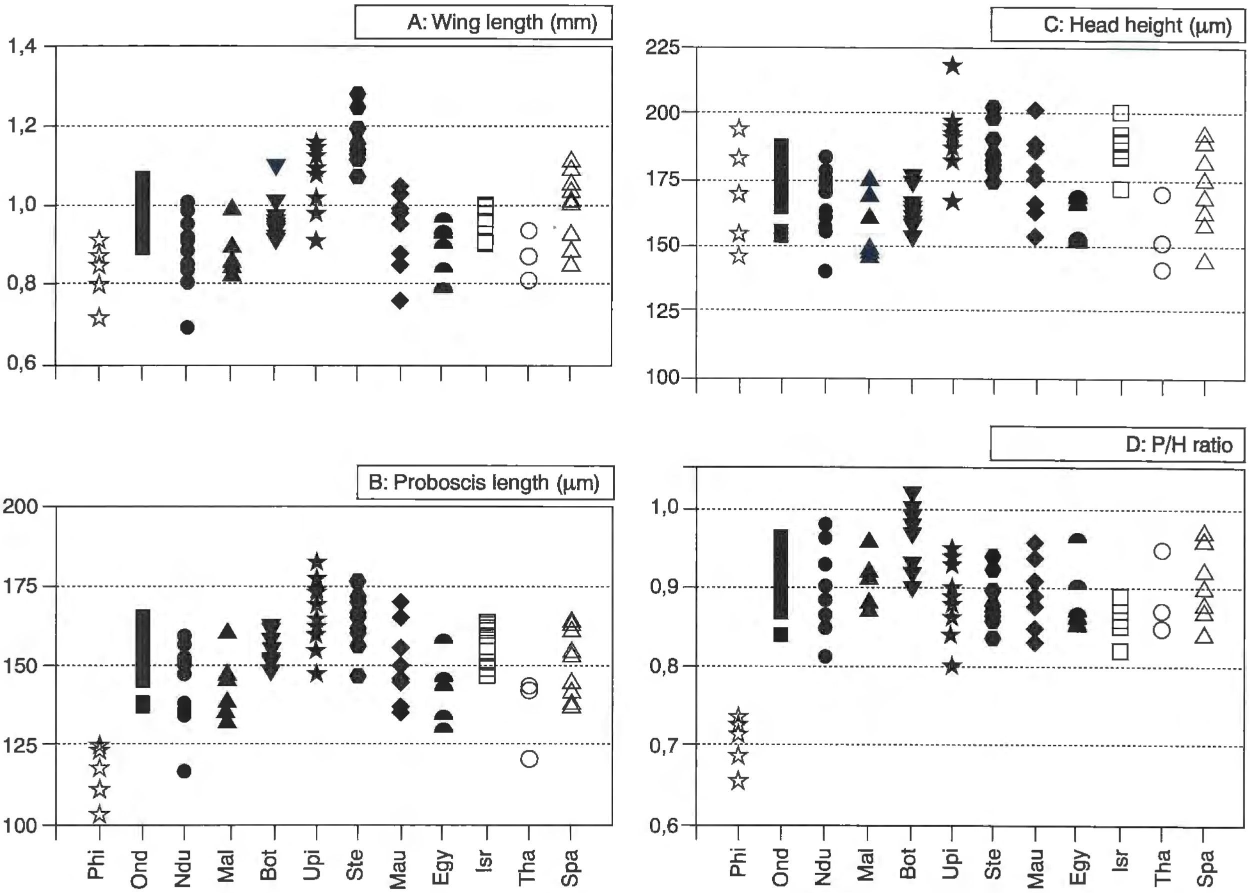

FIG. 1 Scatter diagrams of (A) wing length, (B) proboscis length, (C) head height and (D) the ratio of proboscis length to head height for five *C. nudipalpis* from the Philippines and Timor (Phi, *n* = 5) and 11 populations of *C. imicola* (*n* = 102–121) from Onderstepoort (Ond, *n* = 14–25), Ndumu (Ndu, *n* = 14–15), Malawi (Mal, *n* = 7), Botswana (Bot, *n* = 10), Upington (Upi, *n* = 11–12), Stellenbosch (Ste, *n* = 8–10), Mauritius (Mau, *n* = 10), Egypt (Egy, *n* = 6), Israel (Isr, *n* = 9–13), Thailand (Tha, *n* = 3) and Spain (Spa, *n* = 10)

ratio ($F_{11,95}$ = 15,11, P < 0,001) and, according to Tukey's method, the mean P/H ratio of *C. nudipalpis* was significantly smaller than that of all 11 of the *C. imicola* populations. However, the same analysis also found that the mean P/H ratio of the Botswana population of *C. imicola* was significantly larger than that of the Ndumu, Stellenbosch, Mauritius, Egypt, Israel, Thailand and Spain populations. Confirmation of the usefulness of the relationship between proboscis length and head height as a character to distinguish *C. nudipalpis* and *C. imicola* is provided by Fig. 2 where each species occurs as a distinct cluster, with no overlap between the two, or even in the 95 % confidence limits around each cluster. It is clear that for its head height *C. nudipalpis* has a significantly shorter proboscis than *C. imicola*. The two *C. imicola* specimens that have short proboscis lengths similar to those of *C. nudipalpis* also have, in fact, very small head heights (the smallest of all specimens examined; Fig. 2) and in all likelihood were simply very small individuals. We therefore consider the P/H ratio to be the most reliable way of separating these two species. These data support those provided by Wirth & Hubert (1989) for *C. nudipalpis*, and by Boorman (1991) for *C. imicola*.

Antennal length

Despite its small size the total antennal length of *C. nudipalpis* appears to be inseparable from that of *C. imicola* (Table 1). More data are needed to confirm this.

Antennal ratio (AR)

Culicoides nudipalpis has a significantly higher AR than *C. imicola* (*n* = 167; Onderstepoort population). Fig. 2 shows 15 Onderstepoort specimens to fall in the centre of all *C. imicola* populations scored, and thus to be reliably representative of the species. A higher antennal ratio indicates that the combined length of the distal five flagellar segments is longer than that of the basal eight segments. In *C. imicola*, a smaller difference between these two measurements results in a lower AR. The AR data provided by Delfinado (1961) and Wirth & Hubert (1989) for *C. nudipalpis* differ from those presented here (Table 1).

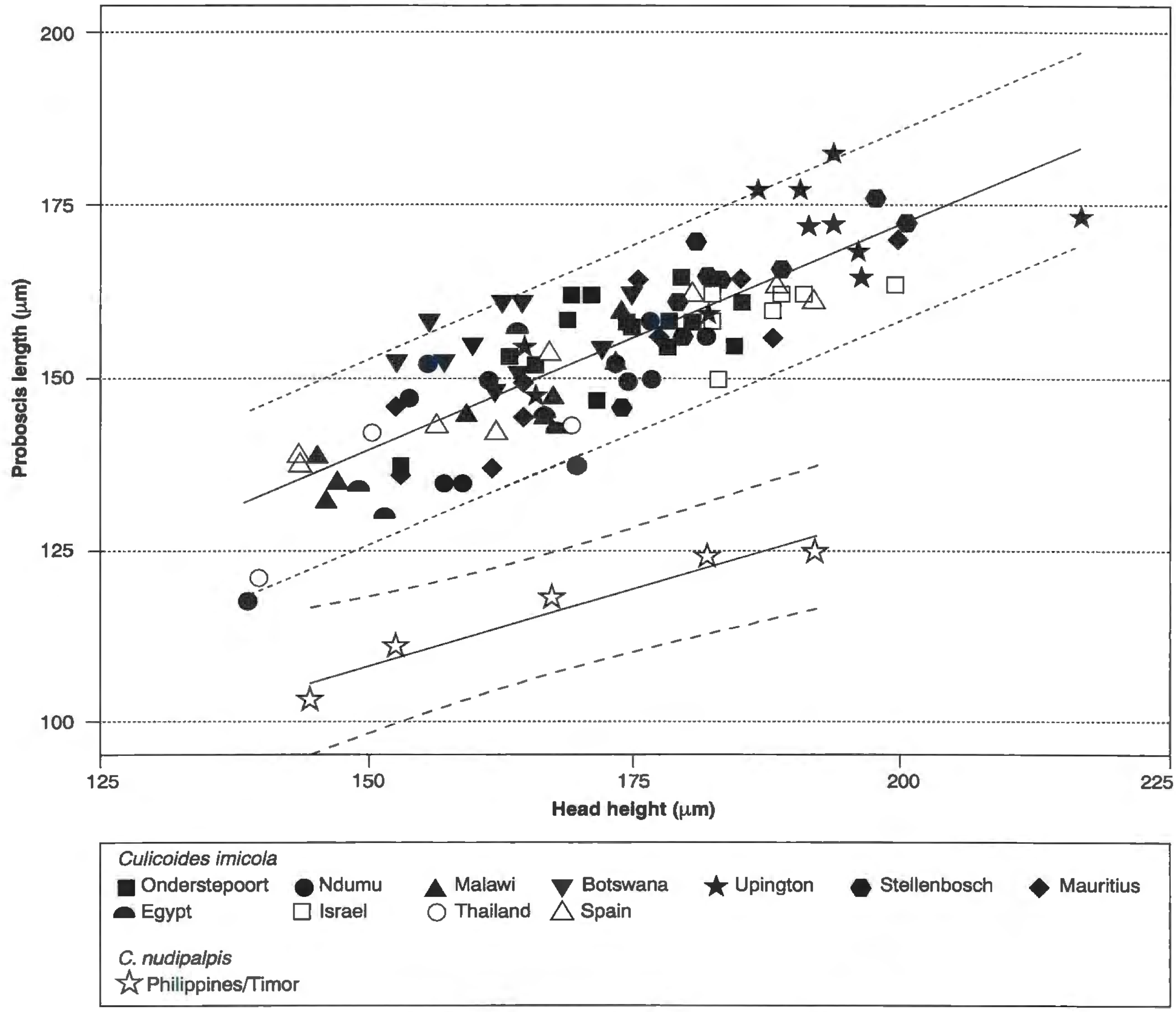

FIG. 2 The relationships between proboscis length and head height for 102 specimens of *C. imicola* from 11 populations and five specimens of *C. nudipalpis*. Solid lines are linear regression lines. Dashed lines show the 95 % confidence limits around the regression lines

Sensilla coeloconica distribution

Four of the nine *C. nudipalpis* female antennae examined have a coeloconica distribution of 3, 11–15; the remaining five (56 %) have 3, 12–15. In *C. imicola* only seven (4 %) of 172 antennae examined had a coeloconica on segment 11 (Onderstepoort population). The difference between the two species is significant but unreliable for identification, as more than half of the individuals examined of both species shared the coeloconica distribution of 3, 12–15.

Third palpal segment

In their key to the south-east Asian *Culicoides*, Wirth & Hubert (1989) separate *C. nudipalpis* and *C. imicola* in that the former has '... third palpal segment with indistinct pit, the margins not definite', and the latter to have the '... segment with distinct pit with well-defined sunken margins'. While this differentiation is more positive than the vague textual one made by Wirth & Hubert (1989) the key does pinpoint what appears to be an important difference between *C. nudipalpis* and *C. imicola*. Although few specimens of *C. nudipalpis* were examined, the palpal pit conformed with both the Delfinado (1961) and Wirth & Hubert (1989) descriptions. In most of the *C. imicola* material examined the palpal pit was better defined and deeper. However, the palpal pit may be a subjective character as its small size and variable angle of display can make accurate examination difficult. Furthermore, some specimens of *C. imicola* do have a shallow pit with poorly defined margins. To ensure accurate identification, the palpal pit is best used in combination with the P/H ratio.

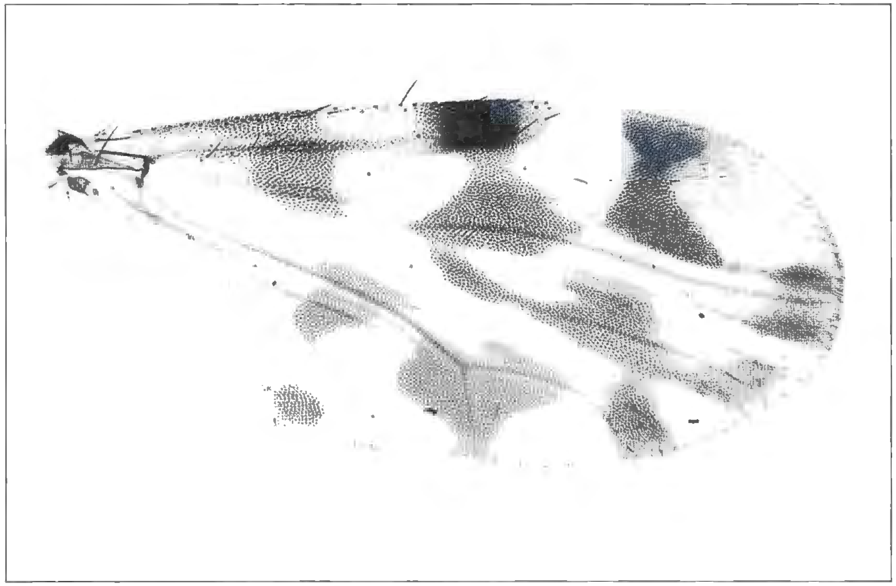

FIG. 3 *C. imicola*: Wing, female; Thailand (A.L. Dyce collection)

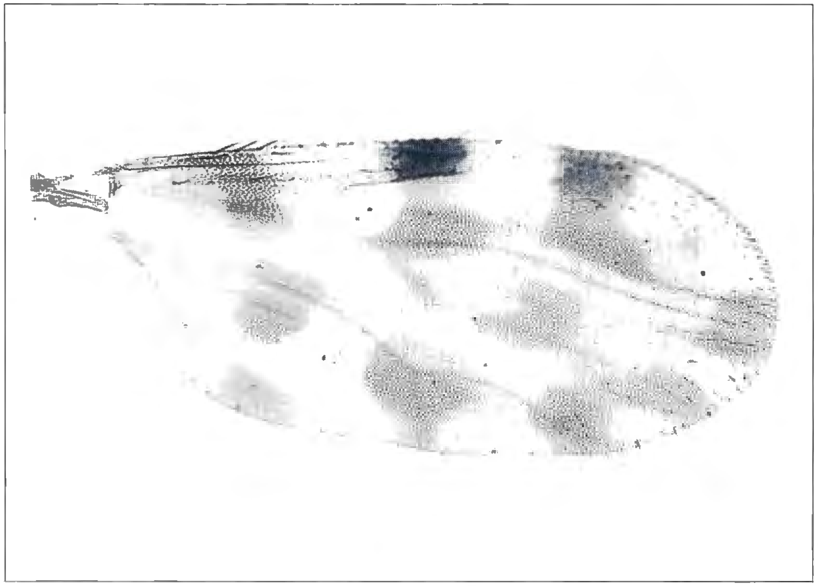

FIG. 5 *C. nudipalpis*: Wing, female; Timor (A.L. Dyce collection)

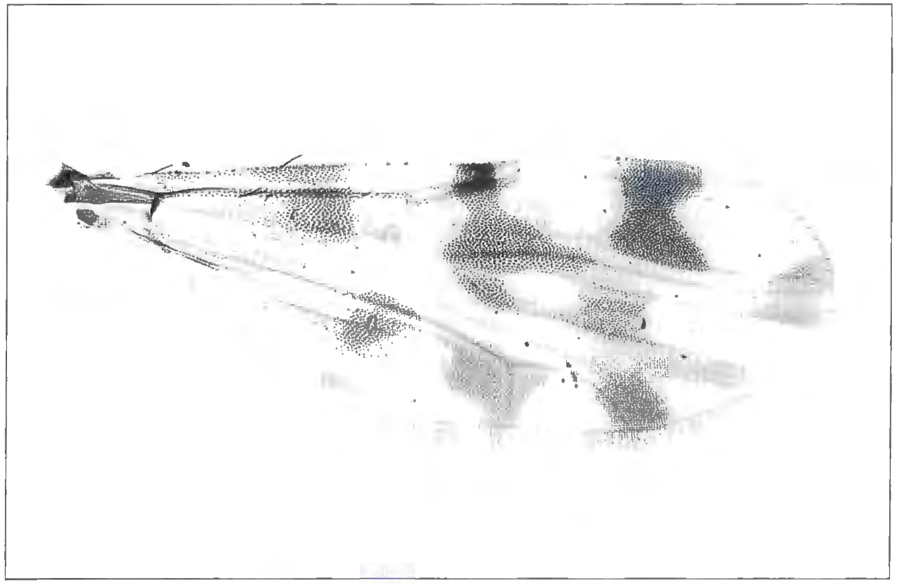

FIG. 4 *C. imicola*: Wing, male; Thailand (A.L. Dyce collection)

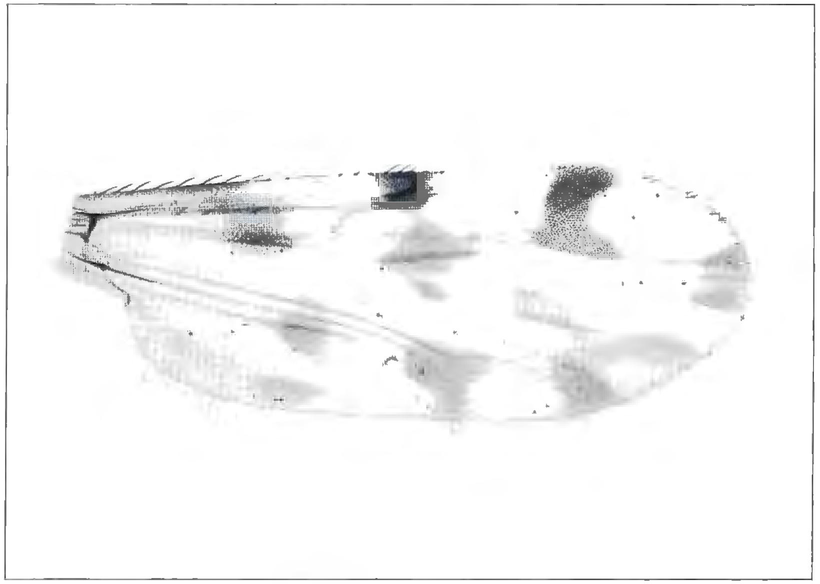

FIG. 6 *C. nudipalpis*: Wing, male; West Irian (A.L. Dyce collection)

Wing pattern

Comparison of Fig. 284 (*C. imicola*) with Fig. 287 (*C. nudipalpis*) in Wirth & Hubert (1989) shows the wing pattern of these two species to be virtually indistinguishable. This is confirmed by comparing two (& and %) wing photographs of *C. imicola* (Thailand: Fig. 3 and 4) and two (& and %) of *C. nudipalpis* (Timor: Fig. 5; West Irian: Fig. 6). The wing pattern feature that most reliably separates *C. imicola* from its known congeners *C. brevitarsis, C. pseudopallidipennis, C. bolitinos, C. miombo*, and *C. loxodontis*, is the preapical pale excision on the anterior margin of vein M_1 which leaves the apex of the vein broadly and squarely darkened (Meiswinkel 1989). The same feature exists in *C. nudipalpis*, even though the preapical excision is inclined to straddle vein M_1 more deeply and broadly, almost separating the dark spot at the apex of the vein from the dark area straddling the same vein medially. However, this is a nicety; there is no doubt that the wings of *C. nudipalpis* and *C. imicola* are more similar to one another than to any of the other five known members of the Imicola group worldwide. This strong similarity, if not representative of one species, at least confirms the two species to be closely related.

Male genitalia

The genitalia of *C. nudipalpis* are very similar to, if not inseparable from, those of *C. imicola*. The two males of *C. nudipalpis* had 84 and 112 spicules on the membrane of sternum 9, and so the membrane can be described as moderately densely spiculate. This is approximately double the average of 47 spicules found in 50 specimens of *C. imicola* examined (Meiswinkel 1989). However, the range in *C. imicola* is considerable: 8–145 spicules. This comfortably embraces the number found in *C. nudipalpis*. Furthermore, in the two specimens of *C. nudipalpis* examined, the spicules on sternum 9 occurred in a broad band (much as illustrated for *C. imicola* by Meiswinkel 1989), and not as a sparse central group as illustrated by Wirth & Hubert 1989. In some specimens of *C. imicola*, spicules can be absent medially, and results in the formation of two lateral groups of spicules. It is not known whether the same pattern of reduction occurs in *C. nudipalpis*.

CONCLUSION

Culicoides imicola and *C. nudipalpis* are good species, most easily distinguished by the P/H ratio. Proboscis length alone can be used to distinguish the two species, although very small specimens of *C. imicola* may be misclassified as *C. nudipalpis*. Characters of secondary significance are body size, antennal length, antennal ratio (AR), sensilla coeloconica distribution, and definition of sensillar pit on the third palpal segment. The two species are inseparable in wing pattern and male genitalia. Longer series of both sexes of *C. nudipalpis* need to be examined to more fully quantify species differences.

Current data show *C. imicola* and *C. nudipalpis* to occur allopatrically, i.e. west and east of the Wallace line respectively. This allopatry is a useful aid to identification, and suggests ancient vicariance involving the splitting of an ancestral species into two populations with subsequent speciation. Taxonomically there seems little doubt that *C. imicola* and *C. nudipalpis* are true sister species, and so form a subgroup within the Imicola group of the subgenus *Avaritia*. The Imicola group (sensu Meiswinkel 1995) comprises seven currently known species. Five are found in Africa, i.e. *C. imicola, C. pseudopallidipennis, C. bolitinos, C. miombo* and *C. loxodontis*, and the remaining two, *C. nudipalpis* and *C. brevitarsis*, in the Oriental/Australasian Region. *C. imicola* is common to the greater part of both regions.

In Africa, *C. imicola* predominates in the medium rainfall (300–750 mm/annum) savannas and savanna-woodland areas. It declines rapidly to virtually disappear in the higher-rainfall forested tropics (Itoua & Cornet 1986; Meiswinkel 1991) and frost-prone highland regions (Venter & Sweatman 1989; Venter & Meiswinkel 1994). There is now compelling evidence that *C. imicola* is also unable to persist in coastal areas where the soils are sandy and quick-draining (Meiswinkel 1997). Under optimal conditions, *C. imicola* can become superabundant where man husbands various kinds of livestock, especially if these are maintained on clayey, moisture-retentive soils. In such locales, *C. imicola* can comprise 99 % of very large numbers (> 100 000) of *Culicoides* collected (R. Meiswinkel, unpublished observations 1996).

As regards *C. nudipalpis* virtually nothing is known of its bionomics except that the type series was collected in a carabao-baited trap, and that Sukarsih *et al*. 1994 caught low numbers in the vicinity of penned cattle. In a recent study of *Culicoides* associated with poultry on the island of Luzon (the type locality of *C. nudipalpis*), not one specimen of *C. nudipalpis* was recorded among > 10 000 specimens of 17 species collected (Abella, Manuel, Cariaso & Kamiya 1994). This suggests that *C. nudipalpis* is mammalophilic like *C. imicola*.

Where known, the immature stages of *Avaritia* species worldwide thrive in semi-moist but organically rich habitats that include animal dung, rotting fruits, humus, leaf-litter and fungi. The pupae do not float at the water surface, and are thus prone to drowning in any situation that becomes waterlogged (e.g. ricefields). These realities may partially explain why both *C. imicola* and *C. nudipalpis* do not occur in abundance in the higher rainfall tropics of Africa and south-east Asia despite the presence of animal hosts. If it is correctly assumed that the larval habitat requirements of *C. nudipalpis* are similar to those of *C. imicola*, then the general sandiness of the islands comprising the Philippines archipelago may explain the low numbers of *C. nudipalpis*, and its limited distribution.

Culicoides imicola is recognized to be the most important vector of bluetongue and African horse sickness in the Old World. It remains to be established whether its congener, *C. nudipalpis*, also has the competence to vector these orbiviruses.

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