Adult characters defining and separating the Imicola and Orientalis species complexes of the subgenus *Avaritia* Fox, 1955 (*Culicoides*, Diptera: Ceratopogonidae)

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Summary

Thirty-six subgenera comprise the biting midge genus *Culicoides* Latreille, 1809. One of these, the relatively small subgenus *Avaritia* Fox, 1955, is the most important as it contains nearly half of the 30 world species of *Culicoides* known to play a greater or lesser role in the transmission of orbiviral diseases to livestock pantropically. These diseases include bluetongue (BT), African horse sickness (AHS) and epizootic haemorrhagic disease of deer (EHD). The subgenus *Avaritia* is distributed globally and the 70 species described have been subdivided into seven species groups and four subgroups. These 11 informal categories, variously labelled as either 'subgroups', 'groups' or 'complexes', are reduced here to seven in number (six species complexes and one subgroup) and although they appear natural, they are nearly all poorly defined. In this study two of these, namely the Imicola and Orientalis species complexes, are re-evaluated to determine which morphological characters define them more precisely, and so may help to establish their monophyly in the future. The two complexes are separable on eight discrete adult characters (two in the female, six in the male); these characters, and three secondary ones, are discussed and illustrated. The Imicola and Orientalis Complexes together embrace 23 species; these species are assigned to their respective complexes and according to recent nomenclatural adjustments. The taxonomy of vector *Culicoides* worldwide remains superficial; to improve the situation it is recommended that the 'traditional' morphological method be integrated with the 'modern' molecular approach.

Keywords

Culicoides – Imicola Complex – *Orbivirus* vector – Orientalis Complex – Subgenus *Avaritia* – Taxonomy.

Introduction

The blood-sucking Dipteran genus *Culicoides* was established by Latreille in 1809. It has been divided since into 36 subgenera, which have been listed by Borkent and Wirth (4). One of these is the subgenus *Avaritia*. It was created by Fox in 1955 (8), and the Holarctic species *C. obsoletus* (Meigen) designated as subgenotype. Of the 109 names available for species of *Avaritia*, only 63 were considered valid a decade ago and 46 considered synonyms (3). The number of *Avaritia* species has grown since to about 70, approximately 5% of the total *Culicoides* world fauna of 1 254 species.

The employment of *Avaritia* by taxonomists worldwide has been chequered; most authors, until quite recently, preferred to assign species to groups and subgroups only, and often without reference to the subgenus (5, 9, 11, 12, 14). This dichotomous approach may have found cause in the fact that no other species of *Avaritia* outside of the Holarctic possess the complicated, almost flamboyantly aberrant, genitalia found in the subgenotype *C. obsoletus* and its true congeners *C. gornostaevae* Mirzaeva, 1984, *C. montanus* Shakirzjanova, 1962, *C. sanguisuga* (Coquillett), 1901, *C. scoticus* Downes and Kettle, 1952 and *C. sinanoensis* Tokunaga, 1937.

More recently, there has been a shift towards employing *Avaritia* more consistently and correctly (2, 6, 7, 10, 15, 18, 19, 20, 21, 22, 25, 27); others have gone a step further by acknowledging that distinct lineages comprise the subgenus (1, 3, 11, 22, 27, 28). Accordingly, seven species groups and four subgroups have been proposed. In the former category are listed the Obsoletus, Imicola (= Pallidipennis), Andicola, Actoni, Pusillus, Orientalis and Montanus groups (3, 5, 9, 14, 28, 29), and in the latter category the Grahamii, Trifasciellus, Imicola and Pseudopallidipennis subgroups (11, 23). In regard to the former category, all, except the Montanus group (3), appear to be valid. However, they are either not delineated (Andicola and Pusillus groups), have been established in the female only (Actoni and Orientalis groups), or are too broadly defined (Obsoletus, Imicola and Orientalis groups). As a result, their usage, especially regarding the last trio, is confused.

To address this, characters that define, and separate, the Imicola and Orientalis Complexes, are presented here. It is hoped that this will create the base for establishing their monophyly in future, and for defining the remaining species complexes.

Materials and methods

Approximately 1 000 slide-mounted males and females of 12 species (some undescribed) of the Imicola Complex were examined; these are the specimens listed in previous publications (19, 20, 21, 22, 23). Fifty males and females of 11 species of the Orientalis Complex (collected in Thailand and the Philippines) were examined; these were identified using either keys, illustrations or descriptions in the works cited. In the female, the two characters of most taxonomic weight are compared in Figure 1 (a-d), and those for the male compared in Figure 2 (a-h). The illustrations of the male genitalia shown in Figure 2 (a-d) are of *C. orientalis* Macfie, 1932, collected in Thailand, whilst Figure 2 (e-h) are of *C. imicola* (South Africa). Observations and illustrations were made from slide-mounted material in which the characters were symmetrically displayed, and not in any way distorted by compression during coverslipping. Three additional discriminatory characters (of secondary weight) are also considered because of their prominence in the literature: wing pattern, the sensilla coeloconica distribution on the female flagellomere and the spines on the hind tibial comb. As to what constitutes a taxonomic character, the methods outlined by Mayr *et al.* were followed (17), namely: 'A character in systematics may be defined as any feature which may be used to distinguish one taxon from another'. Recent nomenclatural adjustments (4) are adopted as reflected in Table I.
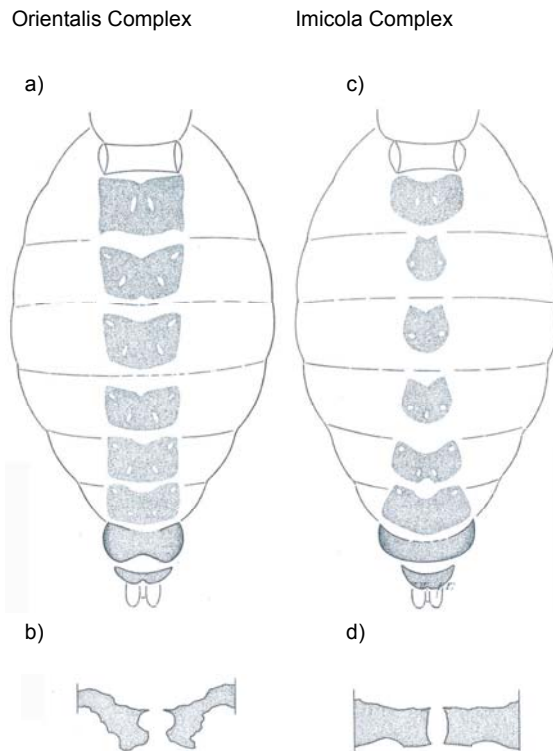
Orientalis Complex   Imicola Complex

Figure 1
Female abdomen
a) and c)   dorsal pigmented terga
b) and d)   sclerotised plates surrounding gonopore

Results

Principal characters defining and separating the Imicola and Orientalis Complexes

Abdomen♀: dorsal pigmented terga

As shown in Figure 1a, species of the Orientalis Complex always have large, rectangular, darkly pigmented terga dorsally on abdominal segments II-VII; in the Imicola Complex they are reduced in size especially on segments III-V where they appear almost round in shape (Fig. 1c). This feature is the most reliable for assigning a specimen to its respective complex when viewed under the dissecting microscope. Unfortunately, the terga are only clearly visible in nulliparous females as the burgundy pigmentation laid down in the abdomens of older parous and gravid females tends to obscure them. Similarly, during slide-mounting, these terga fade in material treated in potassium hydroxide (KOH), and are further obscured in those females whose abdomens are mounted ventral side up (for better examination of the spermathecae and of the sclerotised plates surrounding the gonopore).

Abdomen♀: sclerotised plates surrounding gonopore

As illustrated in Figure 1 (b and d), the precise form of the pigmented plates embracing the gonopore differs between the two complexes. They are simpler in the Imicola Complex, i.e. lack the forefinger and thumb-like projections that partially encircle the gonopore opening as seen in all Orientalis Complex species. Their precise delineation depends upon specimens being carefully prepared, and in which the distal segments of the abdomen have not been telescoped.

Antenna♀: sensilla coeloconica on flagellomeres

All species of the Orientalis Complex have sensilla coeloconica distributed on flagellomeres III, XI-XV. Nine of the 12 species of the Imicola Complex differ in having fewer coeloconica, i.e. on flagellomeres III, XII-XV only. However, the three species of the Pseudopallidipennis subgroup (within the Imicola Complex) have coeloconica on III, XI-XV, while a fourth species, *C. nudipalpis*, has this distribution in approximately 50% of specimens. It is fair to say that some 80% of the 70 species in the subgenus *Avaritia* found worldwide will share one of these two coeloconica distribution patterns, irrespective of species complex, and so they have limited value as a distinguishing character.

Legs♀: hind tibial comb

The Trifasciellus subgroup (= the Orientalis Complex) was erected on the fact that the first spine of the hind tibial comb is noticeably longer and thicker than the four adjoining spines (11). However, in this study, the first spine was found to be equally long for both species complexes. Whilst it does appear to be more darkly pigmented and robust in the Orientalis Complex, the degree of pigmentation can be affected by the particular clearing and slide-mounting protocol used.

Genitalia♂: aedeagus

In the Orientalis Complex, the infuscated 'peg', which is a prolongation of the distal process that projects anteriorly into the aedeagus, narrows to a sharp or slightly blunt point (Fig. 2b), and is rather smoothly pigmented. In the Imicola Complex, the anterior end of the 'peg' differs in that it usually expands into an irregular, almost amorphous shape (Fig. 2f), and is not smoothly pigmented, but granular. Whilst this seemingly insignificant character is highly diagnostic for the Imicola Complex, the form of the 'peg' seen in the Orientalis Complex is more likely to reappear in other species complexes yet to be defined.
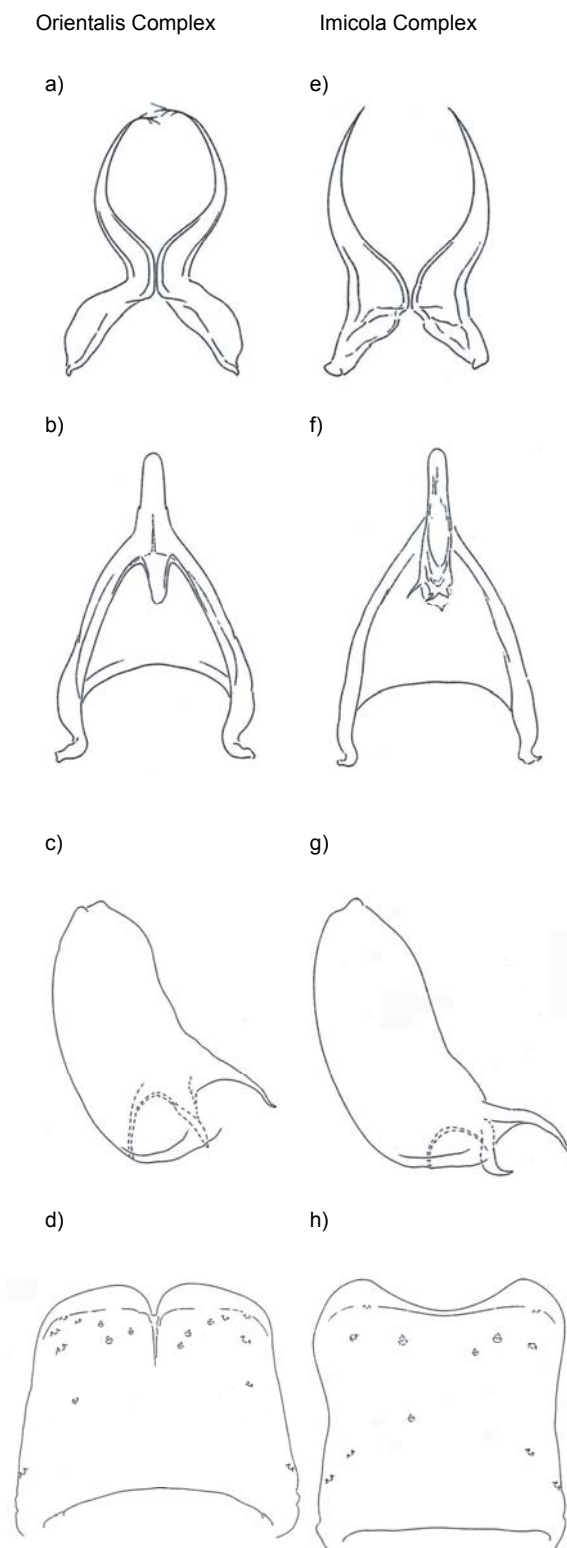
Orientalis Complex   Imicola Complex

Figure 2
Male genitalia
a) and e)   parameres
b) and f)   aedeagus
c) and g)   gonocoxite
d) and h)   tergum nine

Table I
Reassignment (by faunal region) of 23 Old World species of *Culicoides* of the subgenus *Avaritia* to the Orientalis and Imicola species complexes; nomenclatural adjustments follow Borkent and Wirth (4)

Orientalis Complex	Imicola Complex
Oriental/Australasian regions	**Oriental/Palaearctic/Australasian regions**
dumdumi Sen and Das Gupta, 1959: 628. India	**brevitarsis** Kieffer, 1917: 187. Australia
flavipunctatus Kitaoka, 1975: 199. Nansei Islands (Japan)	*robertsi* Lee and Reye, 1953: 386. Australia (Queensland)
fulvus Sen and Das Gupta, 1959: 628. India	*radicitus* Delfinado, 1961: 657. Philippines
hui Wirth and Hubert, 1961: 16. Taiwan	*superfulvus* Das Gupta, 1962: 253. India
jacobsoni Macfie, 1934: 215. Indonesia	**nudipalpis** Delfinado, 1961: 655. Philippines
buckleyi Macfie, 1937: 117. Malaysia	**imicola** Kieffer, 1913: 11. Kenya
kitaokai Tokunaga, 1955: 6. Japan	*iraqensis* Khalaf, 1957: 343. Iraq
unisetiferus Tokunaga, 1959: 236. Papua New Guinea	*minutus* Sen and Das Gupta, 1959: 622. India
obscurus Tokunaga and Murachi, 1959: 347. Belau (USA)	*pseudoturgidus* Das Gupta, 1962: 537. India
pungens de Meijere, 1909: 200. Sumatra	**Afrotropical region**
orientalis Macfie, 1932: 490. Malaysia	**bolitinos** Meiswinkel, 1989: 30. South Africa
nayabazari Das Gupta, 1963: 35. India	**imicola** Kieffer, 1913: 11. Kenya
tainanus Kieffer, 1916: 114. Taiwan	*pallidipennis* Carter, Ingram and Macfie, 1920: 265. Ghana
maculatus (Shiraki), 1913: 294. Taiwan	**kwagga** (R. Meiswinkel, 1995, unpublished MSc thesis)
kii Tokunaga, 1937: 284. Japan	**loxodontis** Meiswinkel, 1992: 147. South Africa
sigaensis Tokunaga, 1937: 322. Japan	**miombo** Meiswinkel, 1991: 161. Malaŵi
kyotoensis Tokunaga, 1937: 329. Japan	**pseudopallidipennis** Clastrier, 1958: 197. Senegal
suborientalis Tokunaga, 1951: 106. Indonesia	**tuttifrutti** Meiswinkel, Cornet and Dyce, 2003: 42. South Africa
Afrotropical region	
brosseti Vattier and Adam, 1966: 297. Gabon	
dubitatus Kremer, Rebholtz-Hirtzel and Delécolle, 1976: 233. Angola	
trifasciellus Goetghebuer, 1935: 175. Zaire	

Genitalia♂: aedeagus

In the Orientalis Complex, the infuscated 'peg' is connected to the lateral converging sclerotised arms of the aedeagus by a lightly pigmented membranous arch (Fig. 2b). This arch is better developed in some species than in others but is absent in all species of the Imicola Complex.

Genitalia♂: parameres

In the Imicola Complex, all species, without exception, have the tip of the parameres erect, sharp and simple (Fig. 2e). In the Orientalis Complex, the tips of the parameres are always sinuous, are most often limp and recurved, and are usually finely to conspicuously feathered (Fig. 2a). (In some species, this tip has been described as bare but this requires confirmation throughout the Orientalis Complex as its precise observation depends upon high magnification [800-1 000×], and upon material being well prepared.)

Genitalia♂: tergum nine

Figure 2d shows that in the Orientalis Complex the apicolateral processes or, more correctly, the 'flanged' processes, are broad, subtly triangular in shape, and most importantly, the apex of the process is positioned either laterally, sublaterally or submedianally. Furthermore, these 'flanges' are always narrowly and fairly abruptly separated medianally by a deep and clearly pigmented subtriangular excision (Fig. 2d). In the Imicola Complex, these 'flanged' processes are not as broadly developed, and arise always on the lateral corners of the tergum (Fig. 2h). The processes are thus always broadly separated by a gently concave posterior margin that is very saddle-like ('sway-backed') in shape and is not infuscated but can, on occasion, be longitudinally striated medially; the posterior margin is also never excised medianally (Fig. 2h).

Genitalia♂: tergum nine

In the Orientalis Complex, the tergum is trapezoidal in shape, gradually narrowing posteriorly (Fig. 2d); in the Imicola Complex, it is almost square as wide anteriorly as posteriorly, and noticeably waisted medially (Fig. 2h). This character is best observed in specimens where the genitalia have not been compressed out of their natural shape by

coverslipping. In compressed specimens, the anterior half of the tergum, in being three-dimensional where it is fused to the narrow strip that forms sternum 9, tends to splay outwards, i.e. will become artefactually trapezoidal, and so will give a false impression of its true shape.

Genitalia♂: gonocoxite

Figure 2c shows the basal half of the gonocoxite of most species of the Orientalis Complex to be broader than the apical half, and the ventral root to arise at a shallower angle (45°-75°); it shows too that the dorsal root is almost straight, i.e. it does not curl around the base of the paramere. In the Imicola Complex (Fig. 2g) the gonocoxite is nearly parallel-sided for its entire length, and the dorsal and ventral roots are distinctly bowed (almost like a curled forefinger and thumb). Furthermore, the ventral root arises almost at right angles (75°-90°) from the gonocoxite. In all species of the Imicola Complex the shorter dorsal root is rather broad and always curls noticeably around the base of the paramere.

Secondary characters

Wing pattern♀

Three features deserve mention, as follows:

1. As a general rule, the pale spot found at the tip of the second radial cell in all species of the Orientalis Complex combines with those found at the base of cell m1, and medianally in cells m2 and m4, to form a broadly pale and straight line that bisects the wing longitudinally. In the Imicola Complex, the small, but prominent, pale spot in the base of cell m1 is positioned more proximally and thus a subtle zigzag of pale areas bisects the wing.

2. In the Orientalis Complex, the short, slanting vein that forms the proximal boundary of the second radial cell is always thickened where it meets the costa; this thickening does not occur in the Imicola Complex.

3. Most species of the Orientalis Complex have the anal angle dark; in the Imicola Complex, the anal angle is pale in 10 of the 12 species.

Discussion

Five points emerge, as follows:

1. This study reveals that the earlier definitions (9, 29) of the Imicola and the Orientalis Complexes are too broad and will have to be amended in the future. This can be done only following the re-evaluation of other species complexes within the subgenus *Avaritia*. Nevertheless, it is now clear that the Imicola and the Orientalis Complexes are restricted to the Old World. The distribution of the latter is more tropical, whilst the former has radiated outside of the equatorial forest block in the adjoining lower rainfall subtropical woodlands, savannah grasslands and semi-desert regions. The added revelation that closely related species (e.g. *imicola*/*nudipalpis*; *bolitinos*/*brevitarsis*) occur in separate biogeographic faunas does indicate that discrete lineages have developed within *Avaritia* over millions of years, but that current species complex definitions have been too weak to expose these patterns of radiation and vicariance.

2. Twenty three species of *Avaritia* are now assigned to either the Imicola or the Orientalis Complex. Only eight of the 19 species previously assigned by Wirth and Hubert to the Orientalis Complex are retained (29); but this number is now expanded to 11 by the inclusion of the three afrotropical species formerly placed in the Trifasciellus subgroup. Of the remaining 11 species of Wirth and Hubert, seven belong to as yet undefined complexes, and four to the Imicola Complex. Of the 12 species comprising the predominantly afrotropical Imicola Complex, three await description, and so only nine species are listed in Table I; the systematics of the Orientalis Complex is also fluid as at least 11 species occur in South-East Asia, three more than the number currently recognised (R. Meiswinkel, personal observation).

3. The 'subgroup' category is being employed at different hierarchical levels. For example the Pseudopallidipennis subgroup was created for a trio of allied species that reside within the Imicola Complex (23), whereas other workers have employed the 'subgroup' category at the higher 'group' or 'complex' level (11). Furthermore, they used also the Imicola group in lieu of *Avaritia*, and subdivided it into the Grahamii, Trifasciellus and Imicola subgroups (which were not defined) (11). This hierarchical discordance obscures the fact that their first two subgroups are synonymous with the South-East Asian Actoni and Orientalis groups of Wirth and Hubert (29). To align their studies with those being conducted in other faunal regions, the three subgroups of Itoua and Cornet (11) are raised here to the 'species complex' (or 'Complex') level.

4. This realignment and synonymy lowers to seven (six species complexes and one subgroup) the number of categories currently comprising the subgenus *Avaritia* worldwide: these are the Obsoletus, Imicola, Orientalis, Grahamii, Andicola and Pusillus Complexes, and the Pseudopallidipennis subgroup. The existence of a

further two species complexes, i.e. the Gulbenkiani and Suzukii Complexes, has recently been mooted (24).

5. It is proposed that in future studies the term 'Complex' or 'species complex' should be used in preference to 'group'. The term 'Complex' is not employed here as defined earlier (13) wherein 'complexes' were applied at a higher hierarchical level, i.e. as an additional category between 'species groups' and subgenera. The term 'species complex' is employed here to group closely related terminal taxa (presumably recently evolved) and united phylogenetically in sharing one or more synapomorphic features. However, if one were to apply this definition strictly, then the recently created Pseudopallidipennis subgroup (23), a distinct clade within the Imicola Complex, merits also consideration as a 'species complex'; the rational splitting of subgenera into species complexes clearly requires further phylogenetic study.

Conclusion

A future cladistic analysis will probably confirm the monophyly of the Imicola and the Orientalis Complexes within the subgenus *Avaritia*. However, a complete redefinition of these two complexes, and of the subgenus *Avaritia*, must await the further evaluation of the distinguishing characters presented here across the entire world fauna.

The admission that '…due to poor preparation…' of slide-mounted specimens certain characters were '…not determined…' (29) to a great extent sums up problems surrounding the systematics of world *Culicoides*. The capture of insufficient numbers of specimens of both sexes, the lack of attention to detail in regard to their preparation, description and illustration, and the employment of a descriptive format that is too superficial and stylised, is hampering taxonomic progress. As *Culicoides* transmit a number of orbiviral diseases that threaten the livestock industry pantropically there is a constant need to identify species with precision. A firm taxonomic foundation is also essential to advancing knowledge on other fronts ranging from basic biology to the development of predictive risk maps using satellite imagery.

Due to this lack of depth, many taxonomic decisions are today still being taken at the species complex, and not at the species, level (e.g. the Obsoletus Complex). In other instances, the existence of vector complexes is only being revealed gradually (e.g. the Imicola Complex); alternatively, where such complexes have been recognised to exist for some

time, their taxonomy has long remained in a state of flux (e.g. the Variipennis Complex). From these examples, it is abundantly clear that taxonomic studies on the genus *Culicoides* need to be refined. As some vector complexes have eluded the best taxonomic efforts (e.g. the Obsoletus Complex), it would seem the way forward is to now integrate 'traditional' morphological systematics with a 'modern' molecular approach.

This integrated approach has recently been applied effectively on the Imicola Complex (16, 26), and so promises to resolve the kinds of difficult taxonomic issues that continue to plague vector systematics. One of the most pressing is the resolution of the taxonomy of the Obsoletus and Pulicaris Complexes following their recent incrimination in the transmission of bluetongue in south-eastern Europe, and in areas hitherto unaffected by this economically devastating disease. At this moment these vectors are assumed to be *C. obsoletus sensu stricto*, *C. scoticus sensu stricto* (both subgenus *Avaritia*) and *C. pulicaris sensu stricto*, (subgenus *Culicoides*) but these identifications remain to be unequivocally proven.

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