The taxonomy of *Culicoides* vector complexes – unfinished business

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Summary

The thirty species of *Culicoides* biting midges that play a greater or lesser role in the transmission of bluetongue (BT) disease in the pantropical regions of the world are listed. Where known, each species is assigned to its correct subgenus and species complex. In the Mediterranean region there are four species of *Culicoides* involved in the transmission of BT and belong in the subgenera *Avaritia* Fox, 1955 (three species) and *Culicoides* Latreille, 1809 (one species). Using both morphological and molecular second internal transcribed spacer (ITS2) sequence data, the authors reappraise the taxonomy of these four species and their congeners. A total of 56 populations of *Culicoides* collected from across Italy and representing 17 species (18 including the outgroup taxon *C. imicola* Kieffer, 1913) were analysed. The findings revealed the following:

- *C. imicola* is the only species of the Imicola Complex (subgenus *Avaritia*) to occur in the Mediterranean region

- in Europe the subgenera *Avaritia* and *Culicoides* (usually, but not quite correctly, equated with the *C. obsoletus* and *C. pulicaris* groups, respectively) are both polyphyletic, each comprising three or more species complexes (including a hitherto unknown complex)

- about half the species studied could not be identified with certainty; furthermore, the results indicate that at least three previously described species of Palaearctic *Culicoides* should be resurrected from synonymy

- finally, a high level of taxonomic congruence occurred between the morphological and the molecular data.

One of the 'new' vector species, *C. pulicaris*, was described by the father of taxonomy, Carl Linnaeus, in 1758, but today, almost 250 years later, no monograph has appeared that treats the *Culicoides* fauna of the northern hemisphere as a whole. At a time when such economically important livestock diseases as BT are affecting ever larger areas of Europe, it would seem appropriate to commence the production of such a monograph to aid in the field identification of vector *Culicoides*. This 'unfinished business' might best be achieved through a collaborative network embracing all ceratopogonid specialists currently active in both the Palaearctic and Nearctic faunal realms.

Introduction

The explosive outbreaks of bluetongue (BT) that cyclically decimate livestock in the Mediterranean Basin were understood, until recently, to be fuelled by the widely distributed Afro-Asian biting midge vector *C. imicola* only. In the past five years (1998-2003) the involvement of *C. imicola* has been reaffirmed following large outbreaks of this disease affecting sheep in the central Mediterranean, especially Italy. However, BT also penetrated areas devoid of *C. imicola* and, subsequently, multiple isolations of the causative virus were made from one or more *Culicoides* of the Obsoletus and Pulicaris

species complexes (9, 41). This would seem to confirm (finally and conclusively) their long-suspected (34, 36) involvement in the transmission of BT in southern Europe. Of significance is the fact that these two complexes contain species of *Culicoides* that are adapted to temperate climes with some extending as far north as the 70th parallel, and throughout this range occur in abundance, attacking both man and livestock. Whilst these 'new' vectors are widely referred to as *C. pulicaris*, *C. obsoletus* (Meigen), 1818, and *C. scoticus* Downes and Kettle, 1952, some doubt is still attached to these identifications as they fall within species complexes, the member taxa of which are notoriously difficult to identify. It is essential that their identity is clarified, given the spread of BT in Europe.

The authors re-evaluate the taxonomy of the *C. obsoletus* and *C. pulicaris* groups of earlier research workers and which equate, broadly, to the subgenera *Avaritia* and *Culicoides*, respectively. The principal aim is to clarify further the phylogenetic relationships that exist between the various taxa, and to discover 'new' morphological characteristics facilitating their more rapid and reliable identification in the field. The strategy used here was to appraise each taxon both morphologically and molecularly, and, at the populational level, to obtain data from multiple series of specimens and from more than one geographical location. Initial results are briefly presented here.

Materials and methods

Light-trap collections made throughout Italy and its islands were screened randomly (across an altitudinal and a latitudinal transect) for specimens of the member species of the two subgenera *Avaritia* and *Culicoides*. Selected species pools were split in two: one half was reserved for slide-mounting in Canada Balsam for detailed morphological studies, whilst the other half was used for the extraction of DNA for the sequencing of the ribosomal DNA second internal transcribed spacer (ITS2). A total of 56 populations were studied and included 17 species (18 with the outgroup species *C. imicola*). Phylogenetic analyses were performed using Kimura two-parameter genetic distances and the neighbour-joining (N-J) algorithm (40) in PAUP*4b10 (45). The reliability of the resultant tree topology was determined by 10 000 bootstrap replications (15). The dendrograms presented here are pared down versions of the larger trees originally generated (i.e. only one representative of each species is shown). The complete N-J trees will be published elsewhere. These results are compared with the morphological results obtained from the slide-

mounted adults. These adults, after preparation, were examined before a sometimes tentative identification was agreed upon. The formerly used 'species group' category was abandoned as it has been used mostly (and duplicatively) in lieu of the subgenus category. The neutral (and more modern) term 'species complex' is preferred and is employed in the strictest cladistic sense (i.e. to group closely related terminal taxa, presumably recently evolved, and united phylogenetically in that they share one or more synapomorphic features).

Results

Approximately 30 of the 1 254 species of *Culicoides* across the world have been incriminated to varying degrees in the transmission of BT disease. These are listed in Table I. The more clearly proven vectors (eight) are shown in bold. (Authors are cited only where isolations of BTV have been made from unidentified species.) These 30 species can be assigned to 8 of the 36 subgenera currently deemed to comprise the genus *Culicoides*; 14 of the vector species belong in the subgenus *Avaritia* alone, and can be subdivided further amongst seven species complexes (Table I). However, nearly all these species complexes are poorly defined (47); the situation is reviewed elsewhere in this volume (31). In regard to the four species linked to the transmission of BT disease in the Mediterranean region the most important is *C. imicola* and probably accounts for 90% of disease transmission. The three remaining vectors are *C. obsoletus* and *C. scoticus*, also of the subgenus *Avaritia*, but placed within the Obsoletus Complex, and *C. pulicaris* (subgenus *Culicoides*). The taxonomy of these four vectors, and of those species deemed most closely related to them, is appraised following the table of world vectors presented below.

Subgenus *Avaritia* Fox, 1955
Type-species: *Culicoides obsoletus* (Meigen), 1818: 76. Europe

The Imicola Complex

This complex is restricted to the Old World and comprises at least twelve species; the nine species that have been formally described (26, 27, 28, 29, 32, 33) are listed elsewhere (31), and include three important vectors of BT, namely: *C. imicola*, *C. brevitarsis* Kieffer, 1917 and *C. bolitinos* Meiswinkel, 1989.

Three morphological studies (3, 12, 32) indicate that *C. imicola* is still the only species of this complex to be found in the Mediterranean, a conclusion that has been recently confirmed also molecularly (10). However, constant vigilance must be maintained in

regard to the possible introduction of additional members of this species complex from either the east (Oriental region: *C. brevitarsis*) or from the south (Afrotropical region: *C. bolitinos*).

Table I
The 30 species of the genus *Culicoides* Latreille, 1809, that play a greater or lesser role in the transmission of bluetongue disease across the world
These are assigned to their correct subgenus and species complex (where known); the species given in bold are those more clearly implicated in the field transmission of BT virus

Subgenus	Species complex	Species
Avaritia Fox, 1955	Imicola	**C. imicola** **C. brevitarsis** **C. bolitinos**
	Obsoletus	**C. obsoletus** **C. scoticus**
	Orientalis	*C. fulvus* *C. dumdumi* *C. orientalis*
	Grahamii	*C. actoni*
	Pusillus	*C. pusillus*
	Suzukii	*C. wadai*
	Gulbenkiani	*C. brevipalpis* *C. gulbenkiani* *C. tororoensis*
Culicoides Latreille, 1809	Pulicaris	**C. pulicaris** *C. magnus*
Silvicola Mirzaeva and Isaev, 1990	Cockerellii	Species unknown (21)
Monoculicoides Khalaf, 1954	Variipennis	**C. sonorensis**
	Nubeculosus	*C. nubeculosus* *C. puncticollis*
Remmia Glukhova, 1977	Schultzei	*C. oxystoma* *C. nevilli* Species unknown (6)
Hoffmania Fox, 1948	Guttatus	**C. insignis** *C. filarifer*
	Peregrinus	*C. peregrinus*
	Milnei	*C. milnei*
Haematomyidium Goeldi, 1905	Complex unknown	*C. stellifer*
Oecacta Poey, 1853	Furens	*C. furens*
Subgenus unknown	Complex unknown	*C. trilineatus*

Culicoides imicola was first recorded from the Mediterranean region (Egypt) in 1943 (25) but only in the 1980s was it found to occur also in the north of the Mediterranean (35). Remarkably, it was only discovered in Italy and on the islands of Sardinia, Sicily, Corsica and the Balearics in 2000 (11, 16, 37) creating the impression that *C. imicola* is a recent invader, spreading rapidly northwards into Europe. This seems supported by a molecular study (10) showing *C. imicola* to be segregated into western,

central and eastern Mediterranean populations with little evidence for gene flow between them. However, further studies are required to more fully expose the haplotype structure of *C. imicola*, both in Africa and in south-west Asia, before it can be stated with greater certainty that this vector has only recently invaded Europe.

Despite the undoubted importance of *C. imicola* as a vector of livestock diseases, a comprehensive inventory of its distribution in southern Europe is far from complete. Since 2000, over 24 000 light-trap collections have been made across Italy and its distribution mapped in considerable detail (17). This work has shown the occurrence of *C. imicola* to be unexpectedly variable (and often across short distances) and, in many instances, has contradicted (7) the predictions made in risk maps utilising satellite imagery and climatic variables (1, 48). This implies that for the purposes of modelling, all the variables that determine the local prevalence of *C. imicola* have still not been identified. For example, differences in soil type might have a significant impact on its ability to breed successfully (30), and so deserve consideration in future modelling efforts. These efforts would be served well if more countries around the Mediterranean Basin were to initiate weekly or bi-weekly surveys across four seasons. The protocols for such surveys have been developed (18).

The accurate identification of *Culicoides* to the level of species is what plagues all epidemiological investigations, principally because intra- and interspecific variation is always being confused. For seven species of the Imicola Complex, their status as discrete genetic entities (good species) was supported in two molecular studies (23, 42) that confirmed each species to possess stable morphological markers that, once quantified, allowed for their reliable identification in the field. The capacity to identify species correctly is essential when species-linked virus isolations are being sought during outbreaks of disease. (Unfortunately species of the Obsoletus and Pulicaris Complexes cannot be identified with similar confidence, as discussed further below.)

Having stated that *C. imicola* is the only member of the Imicola Complex to occur in the Mediterranean region, it must be noted that another species, *C. pseudopallidipennis* Clastrier, 1958, was recently recorded in the Yemen (4). Although it would appear to have been misidentified (33), it nevertheless represents a second record of the Imicola Complex on the doorstep of Europe. However, until more is known about its ecology, it remains futile to speculate further on its possible northward penetration.

The Obsoletus Complex

In the Palaearctic region, the 30 or more described species of the subgenus *Avaritia* are usually referred to collectively as the *C. obsoletus* group (2, 8, 22, 24), or as the *C. chiopterus* group (20), i.e. these groups are used in the broad sense (and thus incorrectly) in lieu of *Avaritia*. An analysis of the ITS2 sequence (and of the morphological data) revealed that the subgenus *Avaritia* (at least as it occurs in Italy) is polyphyletic, and that the six species (excluding *C. imicola*) form three well-supported clades as follows (Fig. 1).
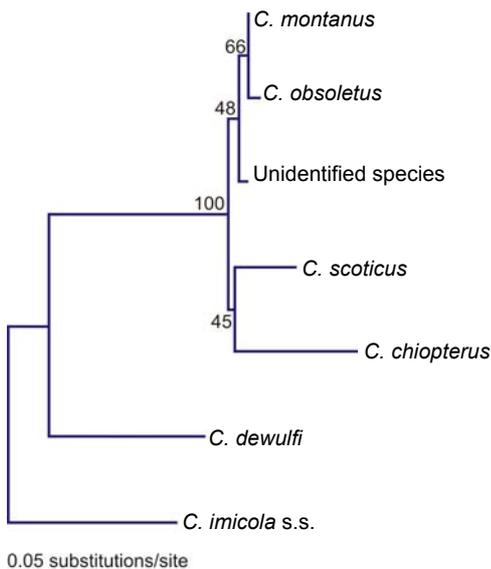

Figure 1
Phylogenetic tree of the subgenus *Avaritia* and related species in Italy, based on ITS2 sequences, using *Culicoides imicola* as an outgroup

The uppermost clade (Fig. 1) includes three tightly grouped species namely *C. montanus* Schakirzjanova, 1962, *C. obsoletus* and '*C.* species unidentified'. Together, these appear to represent the Obsoletus Complex *sensu stricto*; its monophyly is also strongly intimated morphologically. Figure 1 illustrates that *C. scoticus* falls a little outside the Obsoletus Complex clade; from a morphological point of view this is not entirely unexpected as its male genitalia (a reliable indicator of phylogenetic relationships) differ somewhat in both form and size from those of *C. obsoletus* (and its close congeners). Unfortunately, these differences are not reflected in the female sex and so it is close to impossible to separate these two vector taxa (based upon morphology) in the field. It would seem that their identification in the future may depend upon the development of species-specific molecular probes. Thus, of the 20 species of *Avaritia* known to occur throughout the Holarctic, it is considered that only seven fall within the Obsoletus species complex *sensu stricto*. These are the four species listed above, plus *C. sinanoensis*

Tokunaga, 1937, *C. gornostaevae* Mirzaeva, 1984 and *C. sanguisuga* Coquillett, 1901. Thirteen synonyms exist for *C. obsoletus* alone, illustrating how difficult it is to identify these species morphologically. Until the Obsoletus Complex is appraised over a wider geographic area, it will be impossible to judge whether any of these 13 names should be raised from synonymy.

Lower down the dendrogram (Fig. 1) appear two species: *C. chiopterus* (Meigen), 1830, and *C. dewulfi* Goetghebuer, 1936, which the authors prefer to keep separate from the above-mentioned Obsoletus Complex *sensu stricto*, and which, provisionally, are referred to as the Chiopterus Complex and the Dewulfi Complex. The species *C. chiopterus* is collected very rarely in Italy. Indeed, doubt exists in regard to its identity because specimens were too large to be *C. chiopterus* (considered to be one of the smallest *Culicoides* of Europe). This implies that the taxonomic status of the larger *C. dobyi* Callot and Kremer, 1969, currently considered a synonym of *C. chiopterus* by some authors (5) but not by others (38), must be re-evaluated. The second taxon, *C. dewulfi*, was more commonly encountered in the present study but was found to occur far less abundantly than either *C. obsoletus* or *C. scoticus*. The male genitalia of *C. dewulfi* somewhat resemble those of *C. imicola*, and consequently the two species may have been confused by some authors in the past. Whether this explains why two widely different 'forms' of *C. dewulfi* appear in the literature is debatable. One form has a strongly patterned wing (39), the other (as seen in material collected in this study) has a weakly patterned one (11).

Finally, *C. imicola* is a clearly separate outlier at the base of the dendrogram (Fig. 1), and demonstrates that the Palaearctic *Avaritia* fauna has an evolutionary history largely distinct and separate from that which has given rise to the *Avaritia* faunas of tropical Africa and Asia.

Subgenus *Culicoides* Latreille, 1809
Type species: *Culicoides punctatus* (Meigen), 1804: 29. Europe

The precise number of species that comprise the subgenus *Culicoides* in the Palaearctic region is unknown, as various authors lump an agglomeration of some 50 disparately related taxa into it (8, 11, 22). This includes representatives from at least four subgenera and/or species complexes, most of which are represented in Italy; the validity of these complexes (with minor adjustments) was borne out by the sequencing of the ITS2 region. Also included by most authors is the notorious pest species *C. impunctatus* Goetghebuer, 1920, a species whose true affinity has long confused taxonomists.

Unfortunately no material of this species became available to us for study.

Grouped at the top of the dendrogram are the six species *C. lupicaris* Downes and Kettle, 1952, *C. pulicaris*, *C. punctatus*, *C. newsteadi* Austen, 1921, *C. deltus* Edwards, 1939, and '*dark pulicaris*' (Fig. 2). These species comprise the subgenus *Culicoides sensu stricto*; all (except *C. deltus*) possess the distinctive dark spot in the cubital wing cell. Unfortunately this highly diagnostic character is homoplastic and is thus unreliable for the determination of monophyly. However, as shown by the distinct clade formed by *C. lupicaris* and *C. pulicaris* (Fig. 2), it is obvious that species complexes also exist within the subgenus *Culicoides*. However, their reality can only be assessed once more populations are studied from across a wider geographic range. The tantalising hint that *C. pulicaris* may comprise two species, a lowland (Grosseto) and a highland (Campotosto) form (Fig. 2) is also worthy of note.
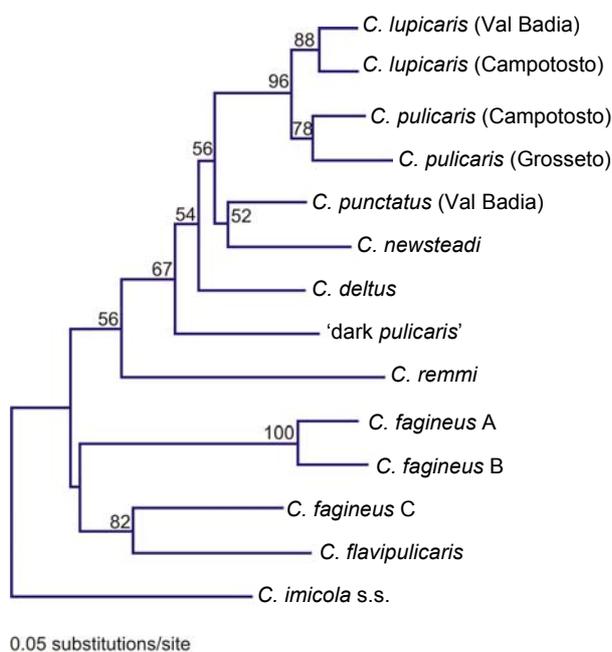

Figure 2
Phylogenetic tree of the subgenus *Culicoides* and related species in Italy, based on ITS2 sequences, using *Culicoides imicola* as an outgroup
Bootstrap values above 50% are indicated

The species identified to be *C. lupicaris* is currently considered a synonym of *C. deltus* (5). The dendrogram shows clearly, however, that they are separate taxa; this was confirmed following detailed morphological study of the female sex. This indicates that *C. lupicaris* should be raised from synonymy but can be done only once material of both species, collected in their respective type localities (in the British Isles), has also been molecularly analysed and the results compared. This is because the series used

for the current molecular study could not be identified as *C. lupicaris* when published keys (8) were employed. In addition, morphological similarity is insufficient cause for establishing synonymy; it could equally well transpire that the Italian material of supposed *C. lupicaris* represents a second, closely related, taxon. This inability to identify species using keys developed in earlier studies (8), and in those of high quality, is cause for concern. The authors have similar reservations about the identity of *C. newsteadi*; material from this study represents a species that is both smaller and darker than that deemed to be *C. newsteadi sensu* Delécolle (11). Furthermore, *C. newsteadi* material was captured at lower altitudes in warmer, frost-free areas only. This places into question the true status of the very similar *C. halophilus* Kieffer, 1924; it is currently deemed a synonym of *C. newsteadi* but appears to occur allopatrically being consistently reported only from the cooler climes of northern continental Europe.

The second clade in Fig. 2 is represented by a species identified to be *C. remmi* Damien-Georgescu, 1972, which is closely related to *C. grisescens* Edwards, 1939. The latter (not included in this study but which is known to occur in Italy) is the type species of the subgenus *Silvicola* Mirzaeva and Isaev, 1990. The ITS2 data from this study, together with the morphological data, strongly support the validity of *Silvicola*. Five species of this subgenus have been recorded from the Palaearctic and 12 from the Nearctic, where they are assembled in the Cockerellii group; this group is clearly synonymous with the Grisescens subgroup (22). Of potential relevance is that in North America, BTV has been isolated from a species of the Cockerellii group (21), and so introduces into the vectorial arena a subgenus of *Culicoides* that is adapted to cooler northerly climes. At least one species of *Silvicola* has penetrated into more southerly latitudes; this includes the central mountainous backbone of Italy that is favoured by local shepherds for their annual transhumance treks.

Near the base of the dendrogram occurs a trio of species of distinctive morphology for which the hitherto unrecognised Fagineus Complex is created. The adults can be distinguished from those of the subgenus *Culicoides sensu stricto* in that they lack a dark central spot in the cubital wing cell, and in that they usually have the cibarium armed with robust teeth (unfortunately both these characters are homoplastic). Species of the Fagineus Complex are niche specialists breeding in container habitats (phytotelmata) and consequently are captured more rarely than species of the subgenera *Silvicola* and *Culicoides*. Finally, and unexpectedly, the molecular data indicate that a new record for Italy, the enigmatic *C. flavipulicaris* Dzhafarov, 1964, should

perhaps be included also in the Fagineus Complex. Until now, this taxon, because it possesses the highly diagnostic (but homoplastic) dark spot in the centre of the cubital wing cell, has always been assigned to the subgenus *Culicoides*. However, various other morphological data would support its placement nearer to, or in, the Fagineus Complex.

Conclusions

Eight points emerged from this study, as follows:

1) *Culicoides imicola* remains the only member of the Imicola Complex to have penetrated into the Mediterranean region where a study on its matrilineal structure indicates it to be segregated into western, central and eastern populations, with little evidence of gene flow between them. Such findings suggest *C. imicola* to have recently invaded southern Europe, and that outbreaks of BT are mediated prinicipally by the dispersal of infected midges on the wind (43, 44). In this context, it is relevant to note that only two of 157 known species of Afrotropical *Culicoides* are found also in the Mediterranean region, suggesting that the migration (and subsequent establishment) of species outside their faunal 'homes' is the exception rather than the rule. (Whether this 'rule' will hold under the influence of global warming is but one of many questions still to be answered.) Nevertheless, it is now becoming clearer that further molecular studies are required on the haplotype structure of *C. imicola* across its incredibly vast Old World range to better understand the dispersal patterns of this important vector; this knowledge should, in turn, throw further light on the role played by livestock movement in the dissemination of BT into the Mediterranean Basin.

2) The subgenus *Avaritia* in the Palaearctic region is divisible into four species complexes, namely: the Obsoletus, Chiopterus, Dewulfi and Imicola Complexes. However, they remain to be resolved more fully through the study of additional species and populations from across a wider geographical area, including the Nearctic region.

3) In Italy, multiple isolations of BT virus (BTV) have been made from field populations of *Culicoides* that contained a mixture of the two species *C. obsoletus* and *C. scoticus* (41), and in areas devoid of *C. imicola*. Of particular concern is that field specimens of the former two could not be reliably separated into distinct species pools. It was only the presence of the vastly dissimilar males in the same light-trap collections that revealed the sympatric occurrence of these two species; this was confirmed subsequently by the molecular analysis of a subsample of females (data not shown). Thus, for the present, both species have to be considered to be vectors of BT in Europe. Of added concern is that the molecular data revealed both taxa to occur also widely in Italy, up to altitudes approaching 1 400 m. This places almost the entire country at risk to the transmission of BT disease; another implication is that if *C. obsoletus* is represented in the Palaearctic region by one and the same species across more than 50° of latitude, this would place a significant part of the region at potential risk to incursions by BT.

4) As noted above, the accurate morphological identification of species within the Obsoletus Complex *sensu stricto* has eluded taxonomists over a number of decades. Until a system for easy and rapid identification is developed, it will remain difficult to refine present knowledge at all investigative levels. It would seem inevitable that the future identification of *C. obsoletus* and *C. scoticus* (and their reliable separation from sister taxa) will depend upon the development and use of species-specific PCR assays.

5) In Italy, the mapping of the distribution and relative abundance of the Obsoletus Complex (excluding *C. dewulfi* and *C. chiopterus*), has commenced (17). Although the maps use conflated data, it is almost certain that 95% of the records (gleaned from 3 000 light-trap collections) are represented by *C. obsoletus* and *C. scoticus* alone. Howewer, the relative proportions in which these two taxa occur is the key question still to be answered. It is also imperative to establish whether only one, or both, of them are involved in the transmission of BT in southern Europe. The distribution of *C. imicola*, the principal vector of BT in Italy, has been mapped separately and in considerable detail (16, 17). Still requiring explanation is its highly anomalous distribution across Italy. Whether this is the result of recent invasion or is due to specific larval habitat preferences remains to be investigated.

6) The *C. pulicaris* group or the Pulicaris group *sensu lato* of earlier authors (8) is clearly a moderately disparate agglomeration of 11 species or more which, in the past, were subdivided into the *pulicaris*, *impunctatus* and *grisescens* subgroups (22). Although these subdivisions were neither adopted nor refined further, the 11 species in Italy were found to be similarly subdivisible; these subdivisions are referred to here as the subgenera *Culicoides sensu stricto*, the subgenus *Silvicola* and the Fagineus Complex (a hitherto unrecognised species assemblage). Whether or not

C. flavipulicaris should be included in the Fagineus Complex requires further study.

7) At the species level, the data suggest that *C. lupicaris* and *C. remmi* (and possibly also *C. halophilus*) should be raised from synonymy. The authors also have reservations regarding the identity of *C. newsteadi* in that it is suspected that a number of taxa are hidden under its wing. If the lowland and the highland 'forms' of *C. pulicaris* are eventually shown to be two species, which then should be taken to be *C. pulicaris sensu stricto*? This raises a further question: which of the two was involved in the transmission of BT in Sicily (9)? These are just some of the taxonomic issues still to be resolved but require our urgent attention as misidentifications in biology are worse than useless. All corrective nomenclatural decisions to be taken in future should, however, be based upon the study of fresh material collected from the respective type localities. A joint strategy in which each taxon is redescribed both morphologically and molecularly will strengthen greatly the taxonomic foundation of the genus *Culicoides* as it will provide the data needed to distinguish between intra- and interspecific variability.

8) The mapping of the geographic distribution and relative abundance of the subgenus *Culicoides sensu stricto* (i.e. excluding the subgenus *Silvicola* and the Fagineus Complex) in Italy has also been initiated (17). However, these maps (gleaned from 3 000 light-trap collections) conflate data representative of at least six species; it will require considerable effort to refine these maps to the single species level.

The very recent discovery of large and extensive populations of *C. imicola* in Italy, and the incrimination of two (perhaps three) additional BT vectors, illustrates the paucity of our knowledge on the biting midges of the Mediterranean region. Furthermore, nearly 250 years have passed since the father of taxonomy, Carl Linnaeus, described *C. pulicaris*, but yet no monograph has emerged that treats the Palaearctic *Culicoides* fauna as a whole. Although a number of very good studies exist (e.g. 3, 4, 8, 11, 12, 13, 14, 19, 24, 38, 39, 46, 47), they remain fragmentary, have been published in a number of languages, and vary greatly in style. This regional 'individuality' has introduced a subjective element into species identifications, and if taxonomic studies on the genus are not refined further, errors in identification are likely to persist. This small study illustrates this point as evidence has been found for undescribed species and an unrecognised species complex, and shows also that some previously described species should be resurrected from synonymy. A refined taxonomy is essential to modelling the distributions of known and potential vectors across the Palaearctic region, and more especially so for the Mediterranean Basin, the crucible in which three species of *Culicoides* are now known to circulate BT disease. Revisionary studies should embrace the entire Holarctic as some of the 40 Nearctic species of the subgenera *Avaritia*, *Culicoides* and *Silvicola*, could also display increased vectorial competence under the influence of global warming. This inclusion of the Nearctic would bring with it a host of competent Culicoidologists, and in so doing would harmonise further the large collaborative venture 'Unfinished business'.

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