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A KEY, BASED ON WING PATTERNS OF BITING MIDGES (GENUS *CULICOIDES* LATREILLE- DIPTERA: CERATOPOGONIDAE) IN THE IBERIAN PENINSULA, FOR USE IN EPIDEMIOLOGICAL STUDIES

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ABSTRACT

The identity of vectors of disease are often required speedily in epidemiological studies but with a precision which excludes as many other species as possible. Identification keys usually require the examination of many different parts of the suspected vector to pinpoint the species. This consumes considerable time and resources, so epidemiologists tend to ignore them. A simplified approach to identification is proposed, using the characteristics of a single part of the body (the wings) of biting midges of the genus *Culicoides*. The level of differentiation was epidemiologically valuable. The monoclave could not differentiate all the species from each other but more than one third (20/58) of identifications were for single species, and a further 12/58 identifications gave only two possibilities, making 55.2% of identifications to an accuracy of at most one of two species. The diagnosis of vector species was reached in a maximum of six decision points. The only notable exception to valuable differentiation was the four species in the *Culicoides obsoletus* group which had almost identical female wing patterns. The ready availability of simple keys, which can be used by anyone without formal training in taxonomy, for all the species of a group in a region should encourage greater standardisation of identifications in all studies, including those not primarily aimed at systematics. These monoclaves can also serve as the primary tools to build computerised image-recognition systems for genera, families and orders of insects.

Keywords: *Culicoides*, biting midges, vectors, epidemiology, identification, wing patterns, Iberian peninsula, monoclave.

RESUMEN

Clave de patrones alares de ceratopogónidos del género *Culicoides* Latreille (Diptera: Ceratopogonidae) en la Península Ibérica, para estudios epidemiológicos

Con frequencia en los estudios epidemiológicos hace falta conocer con rapidez, pero también con precisión, la identidad de los vectores. Por lo general los procedimientos de identificación y las claves exigen el examen de un elevado número de partes diferentes del vector sospechoso. Este enfoque consume mucho tiempo y recursos por lo que tiende a ser evitado por los epidemiólogos. Se propone un sistema simplificado para la identificación, el cual utiliza las caracteristicas de sólo una parte del cuerpo (las alas) de los ceratopogónidos del género *Culicoides*. El nivel de diferenciación es bueno (55.2 % de las 58 especies presentes) y abarca casi todas las especies implicadas en la transmisión de virus y otros agentes patógenos de animales en la Península Ibérica. El diagnóstico de una especie precisa un máximo de seis criterios. La única excepción importante la constituyen las cuatro especies del grupo *Culicoides obsoletus,* las hembras de las cuales muestran un diseño alar casi idéntico. La utilización de claves sencillas debería proporcionar una mejor estandarización de la identificación en todos los estudios, incluso los realizados por no sistemáticos. Estas claves pueden servir como el instrumento más importante para desarrollar sistemas informáticos de reconocimiento de géneros, familias y especies de insectos por medio de imágenes.

Palabras claves: *Culicoides,* ceratopogónidos, vectores, epidemiología, identificación, diseño alar, Península Ibérica, monoclave.

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Introduction

Identification of the vector is an important step in the epidemiology of vector-borne diseases. Information on the major vector species will give a clearer indication of the geographic distribution of the disease, or its potential distribution, the location of danger points of high risk contact between vector and host are likely to be and gives access to an alternative source of survey material to indicate the cycling of the parasite without need to sample the mammalian host. In most instances, where vector-borne diseases are endemic, the vector is frequently, perhaps always, one of the commonest species of that type (mosquito, tsetse, midge), especially at the time of, or immediately prior to, a rise in the incidence of disease. Suitable trapping strategies will usually yield a large sample of those types of insect sought and within this collection the vector often forms the majority of those captured. Keys may be available to identify most of the species present, but as the vector probably predominates, these keys, which are usually complex, are not often resorted to. In epidemic areas, the vector species may also predominate, especially at the time of outbreaks of disease when the super-abundance of a vector may be the primary cause of an epidemic occurring (Lord *et al*., 1996). At other times, and at adjacent localities or those at increasing distance from the outbreak sites, vector species may not be the majority and keys become an essential tool in the study of epidemics.

Identification keys usually are constructed in the form of a binary tree but presented as a series of couplets of text (Delecolle, 1985; Glukova, 1979; Potts, 1970). These couplets deal with alternative forms of a particular structure on the insect. Moving from couplet to couplet usually means moving around the body of the insect as well and so for a complete identification of any single specimen the investigator must have available, and appropriately displayed, all those parts required by the key. Rapid identification for an epidemiologist from such a key is very difficult. This has led to an anarchistic development of rough and ready keys using drawings, photographs and preserved specimens collected en route and from published articles to hand. Although these rough keys speed up the processing of samples (practical epidemiology often requires sorting large numbers of samples), there is a lack of consistency. Different epidemiologists working with the same spectrum of insects, some of which are vectors of disease, will develop different criteria to identify vectors, or groups inclusive of vectors. This will lead to incompatible data sets between workers and projects.

For the taxonomist a comprehensive key to all the species within a genus in any particular faunal region is a useful, primary tool. It allows new species to be identified and the movements of species into the area to be monitored as well as increasing the knowledge of the geographic range of those species known in the region. The time between field collection of the material and diagnosis by an expert taxonomist can be considerable. For field workers involved with disease prevention, control or cure then long delays are a burden or totally impractical. At the same time, there is usually no desire to know the identity of all the species in the sample, but just those species acknowledged by other experts to be implicated in the transmission of disease. Widely used, simple keys may avoid confusion about the true extent of potential geographical and seasonal distribution of epidemics by promoting data sharing. It seems necessary to develop for the epidemiologist tools derived from the detailed, whole animal diagnosis of taxonomy and systematics but which simplify identification and do not mislead.

As a first stage in the development of such a system of taxonomic diagnosis for vectors of animal diseases caused by viruses, midges of the genus *Culicoides* Latreille, 1809 have been examined. *Culicoides* are known to be vectors of a number of orbiviruses (Du Toit, 1944) which cause diseases such as African horse sickness (AHS), bluetongue (BT) and epizootic haemorrhagic disease of deer (Mellor & Boorman, 1995). In the Iberian peninsula, epidemics of AHS and BT have both occurred in the past 40 years (Campano López & Sánchez Botija, 1958; Rodríguez *et al*., 1987), with severe economic consequences for livestock owners and equestrian stables. Very few species within the genus *Culicoides* have been implicated in the transmission of these viruses (Mellor & Boorman, 1995) but the geographic and seasonal distribution of even these few species in southern Europe were poorly known. Gathering this information was complicated by the presence of about 60 species of *Culicoides* across Spain and Portugal and a tendency for species lists to be confined to a single country (Cambournac, 1970; Gil Collado &

Table I.— Species of *Culicoides* found in the Iberian peninsula.

Tabla I.— Especies de *Culicoides* encontradas en la Península Ibérica.

Specific name	Authority	Synonym	Source[1]
accraensis	Carter, Ingram & MacFie, 1920		1,6,11
achrayi	Kettle & Lawson, 1955		5
almeidae	Cambournac, 1970		2,3,6
atripennis	Shevchenko, 1972		3
bahrainensis	Boorman, 1989		10
begueti	Clastrier, 1957		1,3
brunnicans	Edwards, 1939		3,8
cataneii	Clastrier, 1957		1,3,6,9,10
chiopterus	(Meigen, 1830)		1,5
circumscriptus	Kieffer, 1918		1,2,3,6,8,9,10
corsicus	Kremer, LeBerre & Beaucournu-Saguez, 1971		1,5,6
derisor	Callot & Kremer, 1965		5
dewulfi	Goetghebuer, 1936	P. Rawlings & M.A. Abella (unpub) La Mortera de Palomar, Asturias	
duddingstoni	Kettle & Lawson, 1955		1,6,8
fagineus	Edwards, 1939		1,3,6,8,9,10
fascipennis	(Staeger, 1839)		3,6,9
festivipennis	Kieffer, 1914	*odibilis*	1,2,3,6,8,9,10
furcillatus	Callot, Kremer & Paradis, 1962		3
gejgelensis	Dzhafarov, 1964		3,8,9,10
haranti	Rioux, Descous & Pech, 1959		3,6
heliophilus	Edwards, 1921		2
helveticus	Callot, Kremer & Deduit, 1962		1
heteroclitus	Kremer & Callot, 1965		3,8,9
imicola	Kieffer, 1913	*pallidipennis*	1,3,4,7,8,9,10
impunctatus	Goetghebuer, 1920		3,6
jumineri	Callot & Kremer, 1970		3,6,9
kibunensis	Tokunaga, 1937	*cubitalis*	1,3,6,9,11
kurensis	Dzhafarov, 1960		3,8,10
longipennis	Khalaf, 1957		1,3,6,8,9,10
marcleti	Callot, Kremer & Basset, 1968		10
maritimus	Kieffer, 1924	*submaritimus*	1,3,6,9
minutissimus	(Zetterstedt, 1855)		6,11
newsteadi	Austen, 1921	*halophilus*	1,2,3,6,9,10,11
nubeculosus	(Meigen, 1830)		1,3,6
obsoletus	(Meigen, 1818)	*sintrensis*	1,2,3,6,8,9,10,11
odiatus	Austen, 1921	*indistinctus, lailae*	3,8,9,10
pallidicornis	Kieffer, 1919		3
parroti	Kieffer, 1922		1,3,6,8,9,10
pictipennis	(Staeger, 1839)		1,3,6
poperinghensis	Goetghebuer, 1953		1
pseudoheliophilus	Callot & Kremer, 1961	*albihalteratus*	3
pseudopallidus	Khalaf, 1961		3
pulicaris	(Linnaeus, 1758)		1,3,6,10,11
punctatus	(Meigen, 1804)		2,3,8,9,10
puncticollis	(Becker, 1903)	*algecirensis*	1,2,3,6,8,9,10
ribeiroi	Lemblé, Messadeq, Capela & Kremer, 1990		7
riethi	Kieffer, 1914		1,3,6,10
saevanicus	Dzhafarov, 1960		8
saevus	Kieffer, 1922		2,8
sahariensis	Kieffer, 1923	*coluzzii*	1,3,6,10
scoticus	Downes & Kettle, 1952		3
segnis	Campbell & Pelham-Clinton, 1960		1,6
shaklawensis	Khalaf, 1957		1,8,10
subfasciipennis	Kieffer, 1919		3,6,11
truncorum	Edwards, 1939	*sylvarum*	6,11
univittatus	Vimmer, 1932	*agathensis*	1,3,10
vexans	(Staeger, 1839)		5,6,11
vidourlensis	Callot, Kremer, Molet & Bach, 1968		8

[1] numbers refer to references listed in Table II.

Table II.— Sources of information on presence of *Culicoides* in the Iberian peninsula.

Tabla II.— Referencias bibliográficas sobre la presencia de *Culicoides* en la Península Ibérica.

1. Anon. 1992. Study on the geographical distribution and seasonal prevalence in Spain during 1990-1991 of different species of the genus *Culicoides* (Family Ceratopogonidae). Spanish Government Technical Committee Document VI/650/92 submitted to European Union. 57 pp.
2. Cambournac, F.J.C. 1970. Lista das especies do genero *Culicoides* (Nematocera, Ceratopogonidae) encontradas em Portugal. *Anais Escola Nacional Saude Publica e Medicina Tropical,* IV: 249-250.
3. Capela, R., Kremer, M., Messadeq, N., Lemble, C. & Waller, J. 1990. *Les Culicoides* (Diptera, Ceratoponidae) du Portugal continental et de Porto Santo. *Bulletin de la Société de Pathologie Exotique,* 83: 561-565.
4. Capela, R., Sousa, C., Pena, I. & Caeiro, V. 1993. Preliminary note on the distribution and ecology of *Culicoides imicola* in Portugal. *Medical and Veterinary Entomology,* 7: 23-26.
5. Capela, R., Pena, I. & Kremer, M. 1992. Contribucao para o conhecimento dos *Culicoides* (Diperta, Ceratopogonidae) existentes em Portugal. *Actas do V Congresso Iberico de Entomologia*: 381-386.
6. Gil Collado, J. & Sahuquillo Herráiz, C. 1983. Aportaciones al catálogo de *Culicoides* (Diptera, Ceratopogonidae) de España peninsular. *Revista Ibérica de Parasitología*, 43: 109-110.
7. Lemblé, C., Messadeq, N., Capela, R. & Kremer, M. 1990. Description de *Culicoides ribeiroi* n. sp. (Diptera, Ceratoponidae) du Portugal. *Annals Parasitologie Humaine et Comparative,* 65: 267-269.
8 Mellor, P.S., Boorman, J.P.T., Wilkinson, P.J. & Martinez-Gomez, F. 1983. Potential vectors of bluetongue and African horse sickness viruses in Spain. *Veterinary Record,* 112: 229-230.
9. Mellor, P.S., Jennings, D.M., Wilkinson, P.J. & Boorman, J.P.T. 1985. *Culicoides imicola*: A bluetongue virus vector in Spain and Portugal. *Veterinary Record,* 116: 589-590.
10. Ortega, M.D. 1993. Distribution and frequency of the *Culicoides* spp. (Diptera: Ceratopogonidae) in Andalucia, Spain. Livestock Insect Workers' Conference. Laramie, Wy, USA. 3 pp.
11. Sahuquillo-Herráiz, C. & Gil Collado, J. 1982. Ceratopogonidae (Diptera nematocera) de Navarra. *Revista de Sanidad e Higene Pública,* 56: 743-752.

Sahuquillo Herráiz, 1985). A reduced instruction set identification system, based solely on the wing markings of female *Culicoides*, was developed to allow the easy identification of the epidemiologically important species from all the others. This is presented here in full as a graphic binary decision tree, rather than the traditional couplets of text, for Iberian species of *Culicoides*. The development of this binary decision tree also serves as a template for other types of vectors or other diagnostic structures of *Culicoides*, such as antennae.

Materials, Methods and Results

No key exists for all the species of *Culicoides* known to occur in the Iberian peninsula, although a key for many (39) of the recognised species found in Spain has been published (Gil Collado & Sahuquillo Herráiz, 1985). In compiling the list to be included in the binary decision tree, only species acknowledged by Remm (1988) as true species, not synonyms or doubtful species, were included and his nomenclature was used throughout, with the single exception of *Culicoides sylvarum* Callot & Kremer, 1961, which should be known as *Culicoides truncorum* Edwards, 1939, following the notes of Boorman (1984). The less well known names, but required by the rule of precedence, of *Culicoides festivipennis* Kieffer, 1914 and *Culicoides kibunensis* Tokunaga, 1937, have been used instead of *Culicoides odibilis* Austen, 1921 and *Culicoides cubitalis* Edwards, 1939, respectively, to abide by Remm's palaearctic catalogue names, and *Culicoides halophilus* Kieffer, 1924 is maintained as a synonym of *Culicoides newsteadi*

Austen, 1921. The list for the identification decision tree for *Culicoides* in the Iberian peninsula included 58 species (Table I). The sources of information for their presence on the Iberian peninsula are included in Table II.

For the identification of *Culicoides* the wing pattern of light and dark areas have already been used for species identification. Additionally, details of the patterns on the dorsal side of the thorax (mesonotum), size and shape of the antennal segments, distribution of sensory pits along the antennae, relative position of the eyes, number of spermatheca in females and shape of the genital organs in males have been required to make a full identification. The wings of females are usually more profoundly marked than males of the same species, and are in general slightly shorter and broader.

Material to construct the decision tree for all the Iberian species of *Culicoides* was gathered from preserved material collected during a retrospective study of the causes of the 1987-1990 outbreaks of AHS in the Iberian peninsula, pinned and mounted specimens of *Culicoides* at the Institute for Animal Health, Pirbright, Surrey, UK, pinned specimens kept at the Natural History Museum, London and published descriptions and drawings of *Culicoides* (Callot *et al.,* 1968a, b; Campbell & Pelham-Clinton, 1960; Delecolle, 1985; Kremer *et al.*, 1973; Lemblé *et al.*, 1990). Wing patterns used in the decision tree were symbolised using Lotus Freelance Graphics version 4.2 software graphics programme whilst observing whole, preserved specimens under 30x magnification. All wings were presented to a standard size and shape as both these characteristics change with season and locality. The graphics symbols provided an opportunity to compare the observed specimen with the supposed end-point pattern after following a route through the decision tree.

In drawing up the decision tree discussions were held with a number of entomologist working on *Culicoides* identification to determine the pathways by which they initiated, continued and concluded a diagnosis. Discussions and visits to laboratories in Spain and Portugal, as well as UK, also established that an essential tool to species identification was a set of reference photographs, specimens and published articles giving full descriptions of wings and other diagnostic structures. These discussions and experience of sorting large numbers of *Culicoides* from many locations across Western Europe and

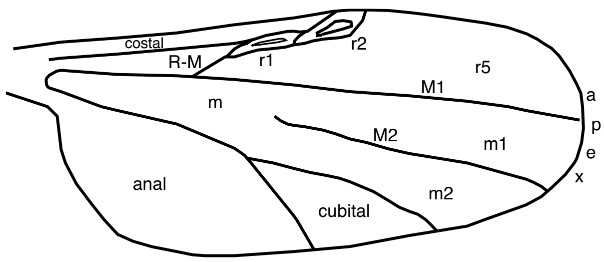


Fig. 1.— Wing terminology used in the decision tree.

Fig. 1.— Terminología alar utilizada en la clave.

West Africa indicated which wing features constituted the primary search area and which were used only for secondary, refining diagnosis.

The design of the decision tree reflected both the pathways of the visual search used by experienced entomologists and the aim of identifying potential vectors with the minimum of decision points. In the Iberian peninsula, isolations of AHS virus have been made from *Culicoides imicola* Kieffer, 1913, an Afro-Asian species first discovered in southern Spain in 1982 (Mellor *et al.*, 1983), but also from mixed pools of two traditionally palaearctic species, *Culicoides obsoletus s.l.* (Meigen, 1818) and *Culicoides pulicaris* (Linnaeus, 1758). In *Culicoides* only the females feed on blood and so are the only sex involved in the transmission of viruses. For females, the identification of *C. imicola* in the Iberian peninsula is currently unambiguous - no sibling species are reported from this region, although elsewhere *C. imicola* is one of a complex of six closely related species (Meiswinkel, 1992). *Culicoides pulicaris*, however, is very similar to *Culicoides almeidae* Cambournac, 1970 (Cambournac, 1970) in wing pattern and the name *C. obsoletus* often covers a group of 4 species, all commonly found throughout the palaearctic, whose wing patterns are almost identical. The males of these four species, though, are easily differentiated on the basis of the shape of the ninth sternite and the ædeagus (Campbell & Pelham-Clinton, 1960).

The lack of sibling species for the main vector of AHS and BT viruses allowed the construction of a decision tree which was relatively succinct and specific. No attempt was made to separate each of the species in the *C. obsoletus* group, although *Culicoides chiopterus* (Meigen, 1830) is usually smaller in size and can have fewer and less distinct

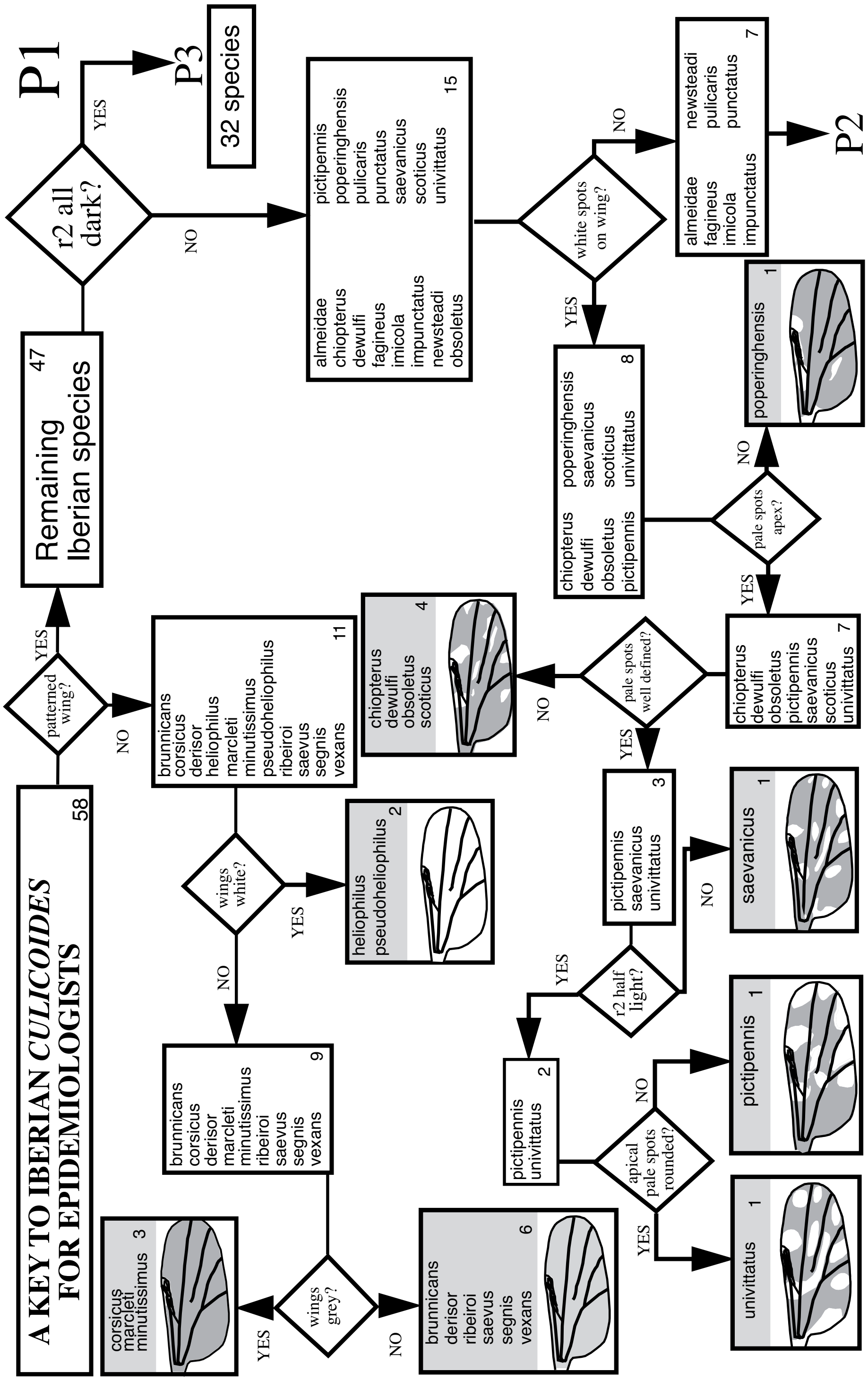


Fig. 2.— Opening page of the decision tree for identifying species of the genus *Culicoides* Latreille, 1809 in the Iberian peninsula, with pointers to further pages (Figures) to continue the search.

Fig. 2.— Página inicial de la clave de identificación de las especies del género *Culicoides* Latreille, 1809 de la Península Ibérica, que se continúa en las siguientes páginas (Figuras 3 a 6).

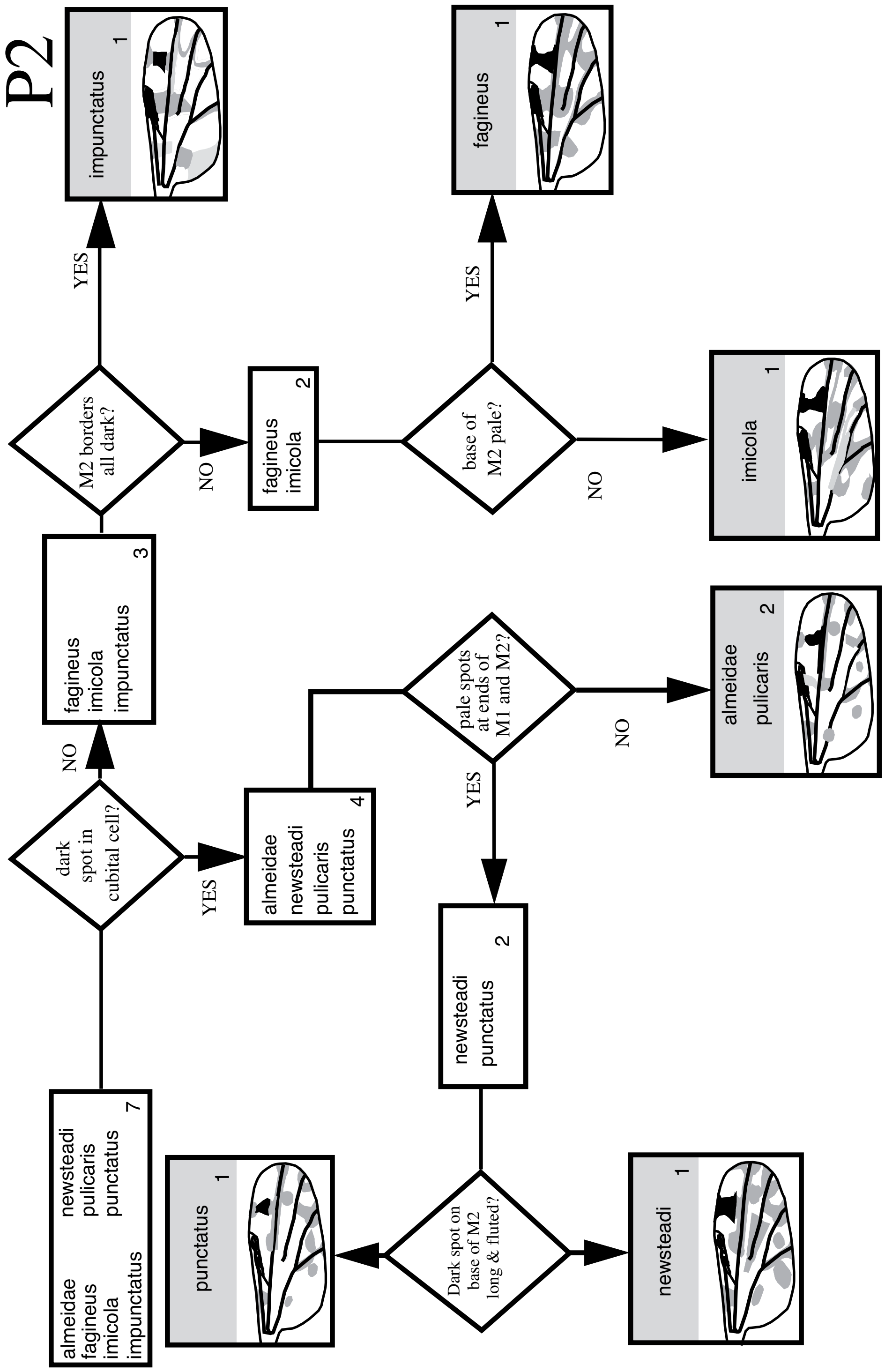


Fig. 3.— Second page of the decision tree for identifying species of the genus *Culicoides* Latreille, 1809 in the Iberian peninsula, showing the route to the most important vector of animal viruses, *Culicoides imicola* Kieffer, 1913.

Fig. 3.— Segunda página de la clave de identificación de las especies del género *Culicoides* Latreille, 1809 de la Península Ibérica; se muestra la ruta del vector más importante de virus animales, *Culicoides imicola* Kieffer, 1913.

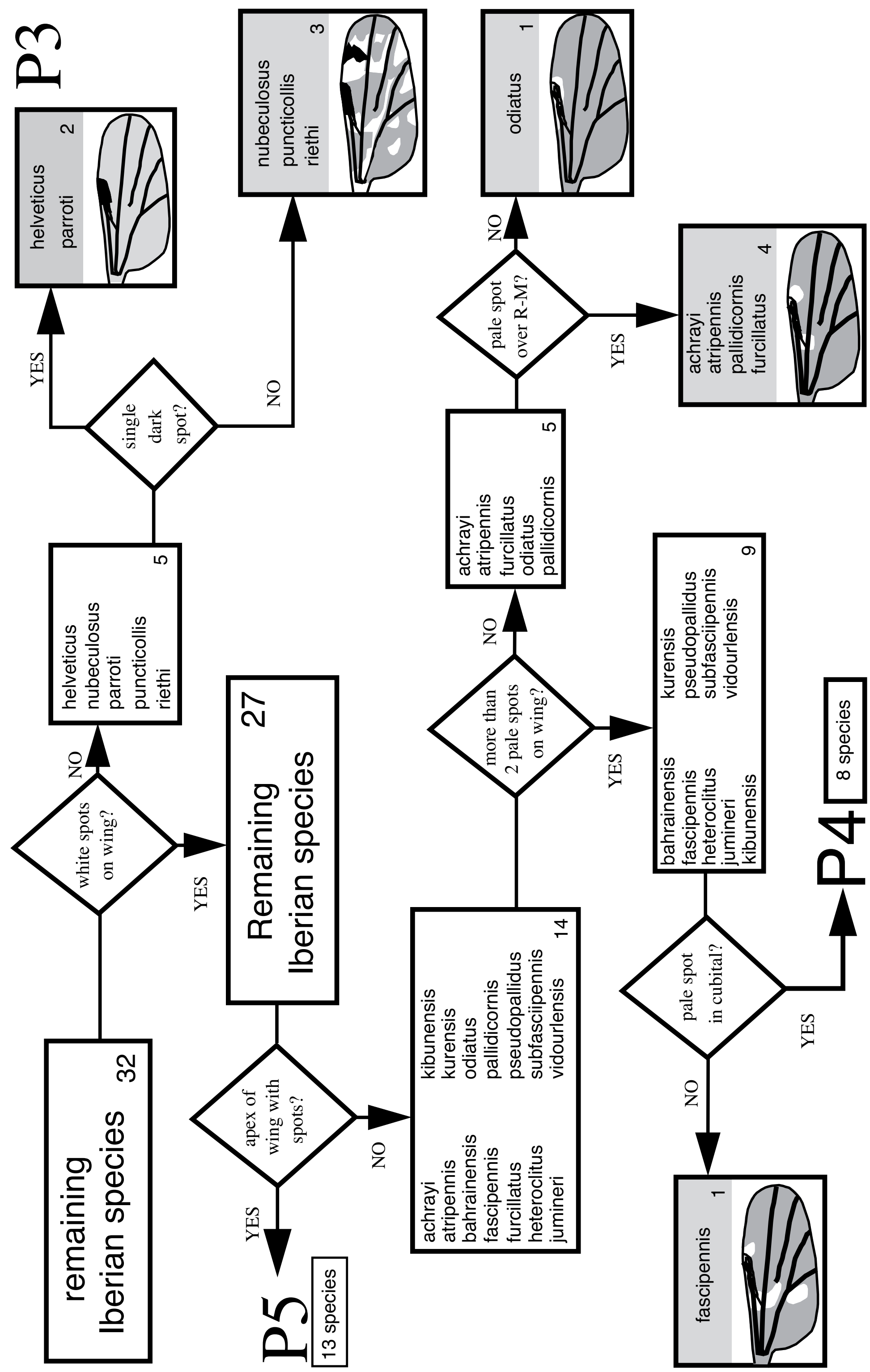


Fig. 4.— Third page of the decision tree for identifying species of the genus *Culicoides* Latreille, 1809 in the Iberian peninsula.

Fig. 4.— Tercera página de la clave de identificación de las especies del género *Culicoides* Latreille, 1809 de la Península Ibérica.

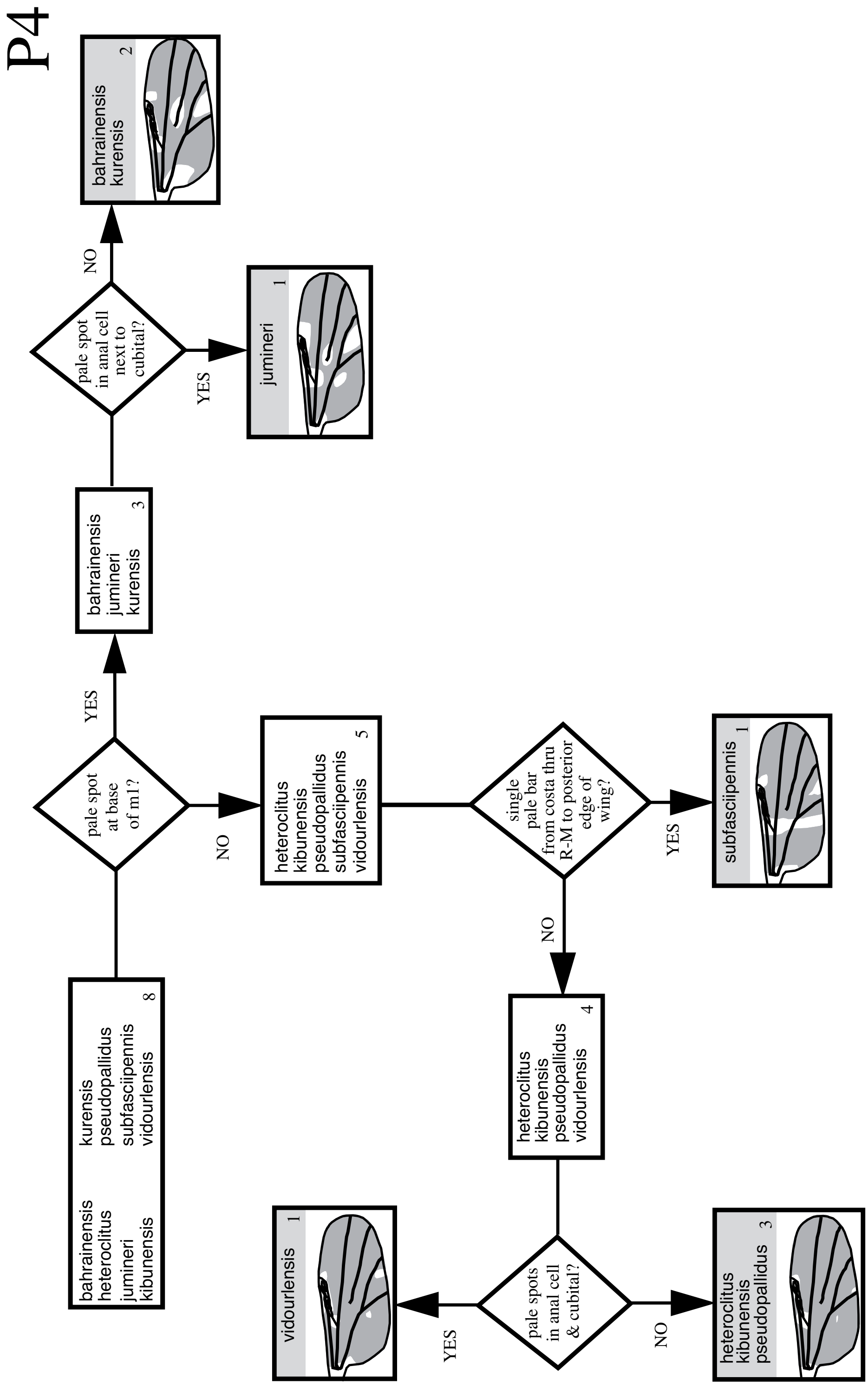


Fig. 5.— Fourth page of the decision tree for identifying species of the genus *Culicoides* Latreille, 1809 in the Iberian peninsula.

Fig. 5.— Cuarta página de la clave de identificación de las especies del género *Culicoides* Latreille, 1809 de la Península Ibérica.

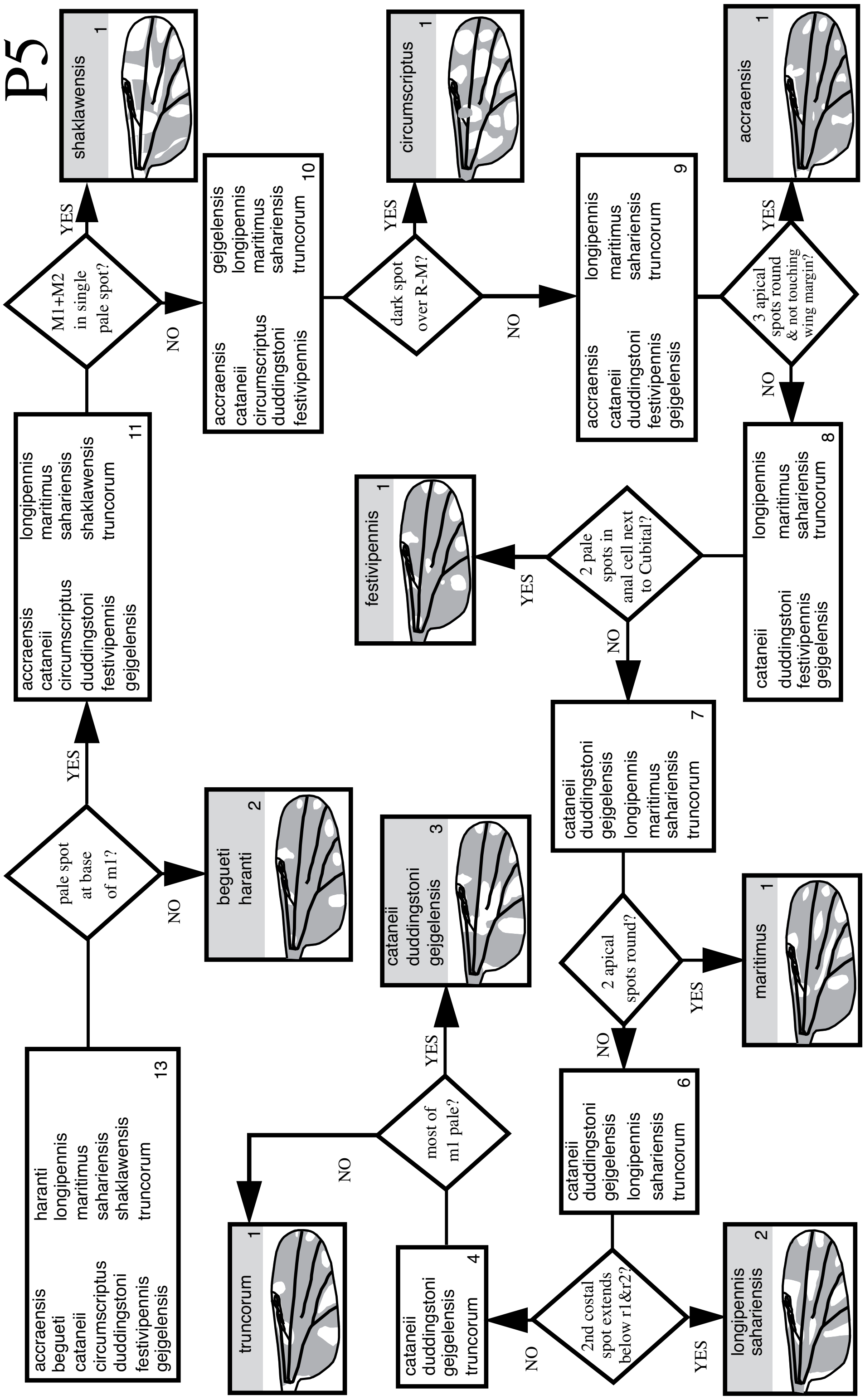


Fig. 6.— Fifth page of the decision tree for identifying species of the genus *Culicoides* Latreille, 1809 in the Iberian peninsula

Fig. 6.— Quinta página de la clave de identificación de las especies del género *Culicoides* Latreille, 1809 de la Península Ibérica.

Table III.— Analysis of the level of identification in the decision tree for biting midges of the genus *Culicoides* in the Iberian peninsula.

Tabla III.— Análisis del nivel de identificación de la clave de especies del género *Culicoides* en la Península Ibérica.

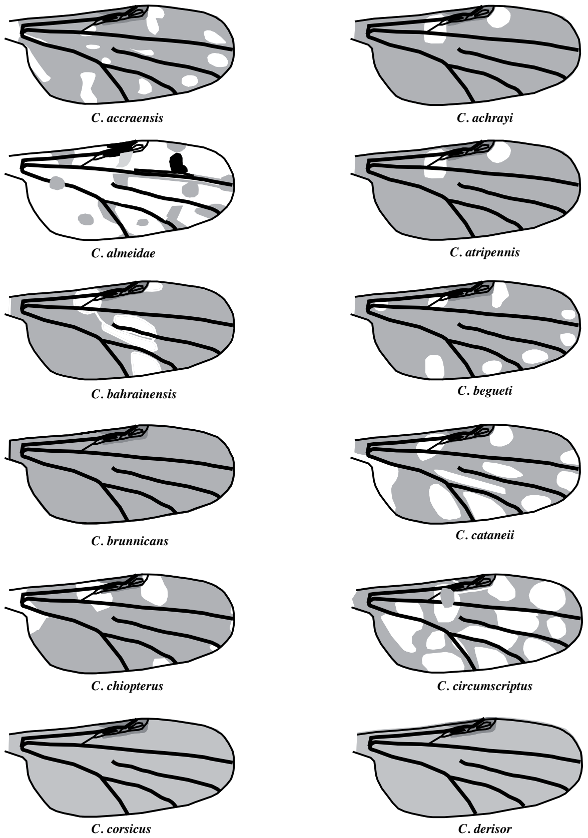
No. species at end point	1	2	3	4	5	6	Total
Frequency	20	6	4	2	0	1	33
% of total	34.5	20.7	20.7	13.8	0	10.3	100

spots on the wings than the other three species (as shown in Fig. 7). It is not known whether all of the species, some or none of them, in the *C. obsoletus* group are vectors of AHS virus. Similarly, *C. pulicaris* was not differentiated from *C. almeidae* in the decision tree.

The decision tree which was constructed used a standardised set of symbols and sizes. It was not possible to present the tree on a single page so pointers were used for continuation of the search to subsequent pages. On each page of the decision tree, listed as P1 through to P5 (Figs. 2-6), the point of entry to the pathway was at the top left corner. Species names were contained in rectangular boxes from which a single line led out to a diamond-shaped decision box. Arbitrarily, it was decided not to itemise any lists of more than 15 species. These long lists were simply referred to as 'Remaining Iberian species', with the number of species in the list at the bottom right corner of the rectangle, as for all other rectangles with itemised species. At each rectangle the species listed were those which would eventually be identified, if progress was continued down that route to the symbolic wing presented at the end-point. Passing along a pathway resulted either in the identification of a single species or a group of species with similar, inseparable wing patterns (with a maximum of six species in a group, this being all those clear wings without any pattern). End points to identification pathways, showed the species names in rectangles filled with light stippling with a symbolic wing pattern immediately below. Enlarged versions of each species' wing pattern is provided in an alphabetic list in Figs. 7-11. These provided clearer confirmation that the specimen had been allocated to the correct wing pattern (and hence, the correct species or one with a very similar wing pattern).

At each of the diamond-shaped decision boxes the answer to each question can only be 'Yes' or 'No'. Each question was designed to be as straight forward as possible, using as few technical terms as possible. The technical terms are all contained in a diagram of the wing nomenclature in Fig. 1. In the majority of questions the decision concerns the presence of spots or dark coloration in cells. For most specimens the presence of spots is obvious, but not in all cases and variation in the angle of the incident light or presentation of the wing can obscure feint markings. These problems have to be acknowledged, but also some account taken of their relative importance in species identification - for most species and most of the specimens examined there was not a problem with feint marking. Attempts have been made to avoid subjective decisions about wing patterns. Most questions require the observer to note the presence or absence of marking, with two notable exceptions. In the majority of cases the wings have either grey background with white spots or pale background with irregular dark patches. In some instances, like *Culicoides circumscriptus* Kieffer, 1918, some adjustment of perception is necessary before deciding if the wing is grey or pale and if the patches are the pale areas or the dark ones. Observation of the patterns in cell r5 is often indicative of which phase the wing should be viewed in.

The second subjective decision, whether spots are 'well defined' (Fig. 2), could cause some hesitation but observation of a few known specimens of *C. obsoletus* will clearly show the differences from *Culicoides univittatus* Vimmer, 1932 or *Culicoides pictipennis* (Staeger, 1839). The latter two have clear outlines on the spots around the apex of the wing. For the *C. obsoletus* group of species the spots melt into the grey of the wing, so become lar-



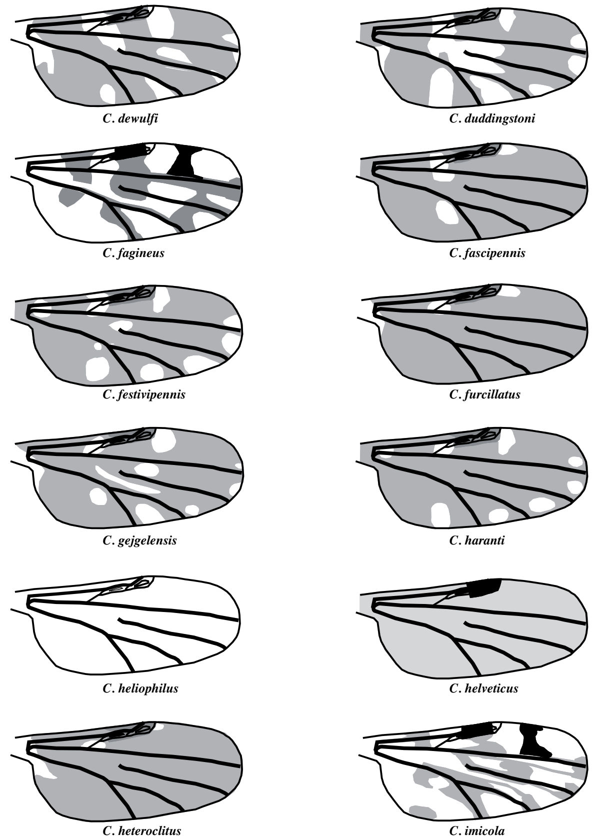


Fig. 7.— Symbolic wing patterns of Iberian *Culicoides* Latreille, 1809 listed alphabetically (*C. accraensis* Carter, Ingram & MacFie, 1920 to *C. derisor* Callot & Kremer, 1965).

Fig. 7.— Esquemas de los patrones alares de *Culicoides* ibéricos, por orden alfabético (*C. accraensis* Carter, Ingram & MacFie, 1920 hasta *C. derisor* Callot & Kremer, 1965).

Fig. 8.— Symbolic wing patterns of Iberian *Culicoides* Latreille, 1809 listed alphabetically (*C. dewulfi* Goetghebuer, 1936 to *C. imicola* Kieffer, 1913).

Fig. 8.— Esquemas de los patrones alares de *Culicoides* ibéricos, por orden alfabético (*C. dewulfi* Goetghebuer, 1936 hasta *C. imicola* Kieffer, 1913).

ger or smaller according to lighting and the wings appear suffused with lighter and darker greys rather than a series of identifiable spots.

Discussion

A key to all the *Culicoides* of the Iberian peninsula has been developed, which involved 58 species and used only the characteristic patterns of the wings for identification. From the binary decision tree which was developed, it can be seen that the use of a single body structure (the wings) produced a monoclave which cannot differentiate all the species from each other but more than one third (20/58) of identifications are for single species. As such the decision tree is taxonomically imperfect, although there were a further 12/58 identifications where there are only two possibilities, making 55.2% of identifications to an accuracy of at most one of two possibilities (Table III). The diagnosis of vector species is reached in a maximum of six decision points, including the initial one of whether the wing is patterned at all. From this primary function the decision tree can then be useful in allowing a rapid partition of the remaining species into consistent, named groups which can be stored for future identification. This level of identification using a monoclave could be highly useful in epidemiological investigations, where the major vectors, or sibling species groups, can be quickly and easily identified without time consuming preparation of captured specimens.

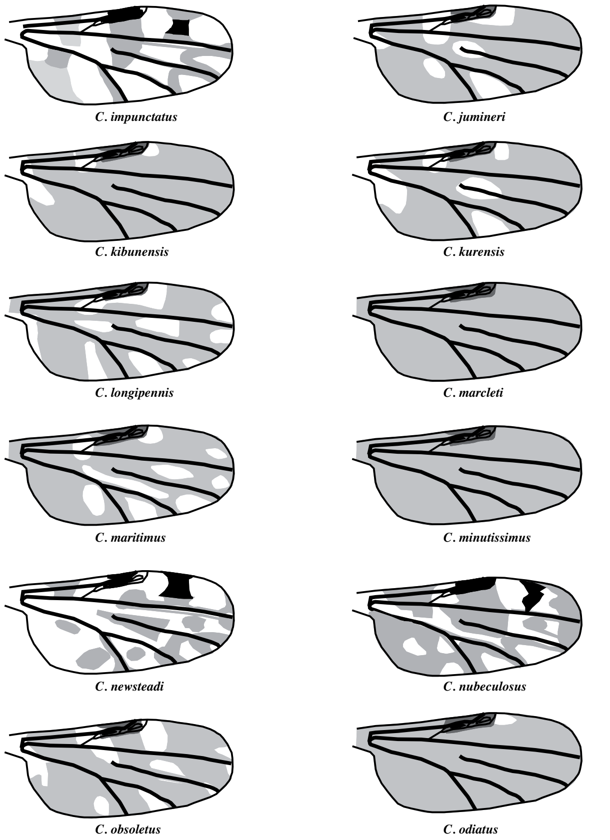


Fig. 9.— Symbolic wing patterns of Iberian *Culicoides* Latreille, 1809 listed alphabetically (*C. impunctatus* Goetghebuer, 1920 to *C. odiatus* Austen, 1921).

Fig. 9.— Esquemas de los patrones alares de *Culicoides* ibéricos, por orden alfabético (*C. impunctatus* Goetghebuer, 1920 hasta *C. odiatus* Austen, 1921).

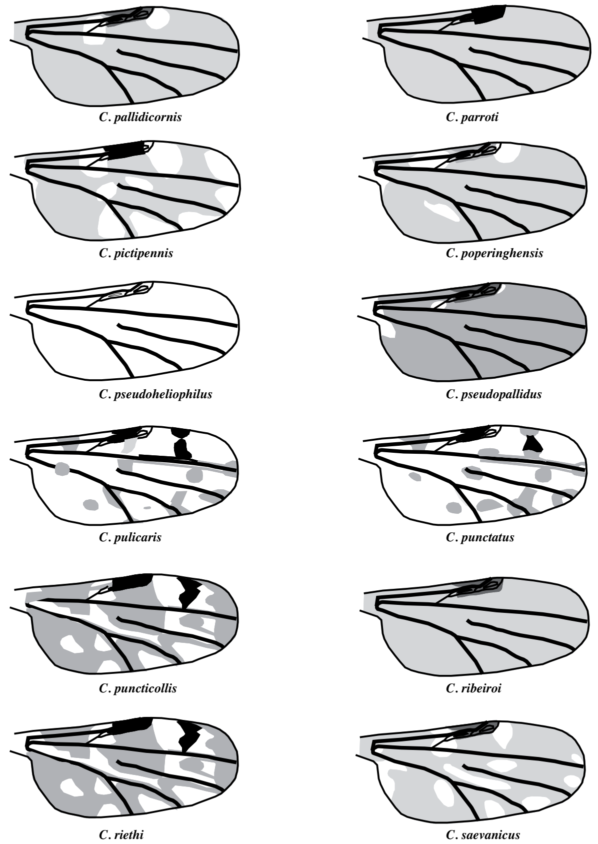


Fig. 10.— Symbolic wing patterns of Iberian *Culicoides* Latreille, 1809 listed alphabetically (*C. pallidicornis* Kieffer, 1919 to *C. saevanicus* Dzhafarov, 1960).

Fig. 10.— Esquemas de los patrones alares de *Culicoides* ibéricos, por orden alfabético (*C. pallidicornis* Kieffer, 1919 hasta *C. saevanicus* Dzhafarov, 1960).

Even though 44.8% of *Culicoides* species in the Iberian peninsula cannot be identified from their wing patterns alone from two or more other species these species constitute only 21.2% (7/33) of the 'leaves' (end-points) on the binary decision tree. Only two of these seven inseparable groups may be important in the transmission of any sort of disease to animals - the *C. obsoletus* and *C. nubeculosus* (Meigen, 1830) groups. *Culicoides obsoletus* group of four species (Fig. 2) has been implicated in the transmission of AHSV and BTV (Mellor & Pitzolis, 1979; Mellor *et al*, 1990) and *Culicoides* hyper-immunity or sweet itch (Mellor & McCaig, 1974) in Canada (Anderson *et al*., 1991) and Ireland (Townley *et al*., 1984). Markings on the costal side of female *C. chiopterus* wings are usually distinct but there is much variation in all other wing markings and are mostly very faint. The practical absence of spots adjacent to the mid-section of vein M2 can distinguish *C. chiopterus* from the other three members of the *C. obsoletus* group. Pointers to the composition of all the members of the *C. obsoletus* group present in any locality can be gained by observation of the male genitalia, which are diagnostic for this group (Campbell & Pelham-Clinton, 1960). Overnight light-suction trap collections near livestock (Rawlings *et al*., 1997) may contain few males, however. The trap may need to be sited nearer to *Culicoides* breeding sites to capture sufficient males. Nothing much is known about the vectorial capacity of three species in the *Culicoides nubeculosus* group (Fig. 4) for the

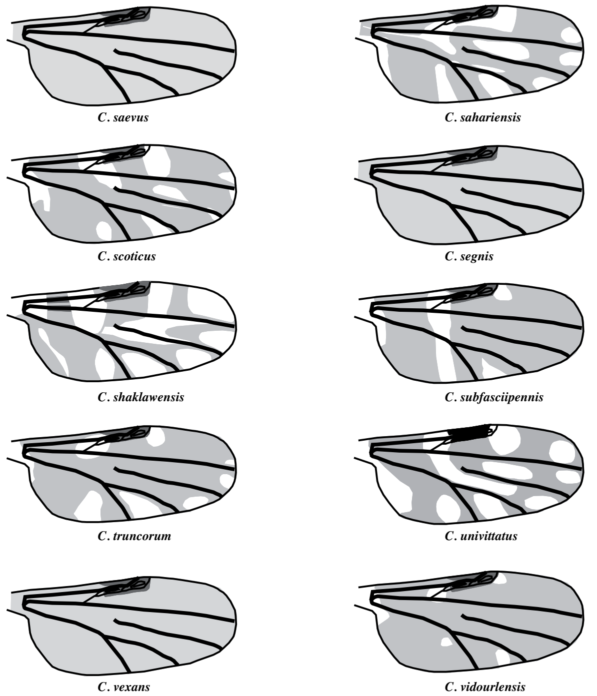


Fig. 10.— Symbolic wing patterns of Iberian *Culicoides* Latreille, 1809 listed alphabetically (*C. saevus* Kieffer, 1922 to *C. vidourlensis* Callot, Kremer, Molet & Bach, 1968).

Fig. 10.— Esquemas de los patrones alares de *Culicoides* ibéricos, por orden alfabético (*C. saevus* Kieffer, 1922 hasta *C. vidourlensis* Callot, Kremer, Molet & Bach, 1968).

transmission of *Onchocerca cervicalis* Raillet & Henry, 1910 microfilarial worms (Mellor, 1971). Their co-identity in the key currently therefore does not raise any epidemiological dilemmas.

The development of this single-structure analysis for species identification shows that storage of the details of diagnostic points on the wing and other structures can be used to construct relatively simple to follow decision trees. These could be adapted to computer assisted identification keys or image matching technology. Expert systems are now commonly in use in a wide range of commercial operations and are being developed for taxonomy. The problem for *Culicoides* taxonomy remains that few species have been studied to show how great is intra-specific variation in wing patterns or whether different wing patterns are true indicators of species separation. No judgements were made in this identification key as to whether the species listed were true species. The list merely includes all those recorded by entomologists in the literature and accepted as verified names in the catalogue of palaearctic Diptera, family Ceratopogonidae (Remm, 1988). The abundance of synonyms amongst *Culicoides* species from the Iberian peninsula (Table I) is a testimony to the confusion of the taxonomy of this genus. Simplified keys may open up the subject to more studies and reveal more clearly their important roles in the transmission of diseases of animals, and perhaps man.

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