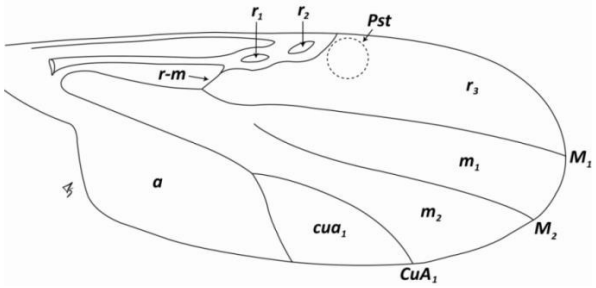
Identification key for Portuguese *Culicoides* female specimens

Source: Ramilo D. (2016). Phenotypic and genetic characterization of *Culicoides* (Diptera: Ceratopogonidae) in Portugal and comparison of the effect of pyrethroid insecticides in their control. PhD Thesis in Veterinary Sciences, Animal Health. Faculty of Veterinary Medicine, University of Lisbon. http://www.repository.utl.pt/handle/10400.5/11331.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (1/19)

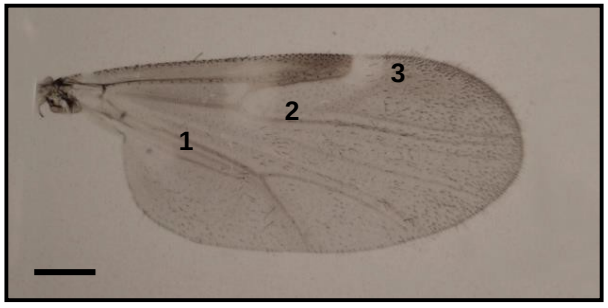


Cells and veins of a *Culicoides* biting midge wing: r-m – Radio-medial crossvein; r_1 – First radial cell; r_2 – Second radial cell; r_3 – Third radial cell; m_1 – First medial cell; m_2 – Second medial cell; cua_1 – Anterior cubital vein; a – Anal cell; Pst – Poststigmatic pale spot; M_1 – First medial vein; M_2 – Second medial vein; CuA_1 – First branch of anterior cubital vein (reproduced with kindly permission of M.Sc. Marcos Santos).

A – Species with characteristic wing pattern (do not require insect mounting):

Culicoides imicola – The presence of a characteristic dark spot in the r_3 cell (1), a white almond shaped pattern in the proximal part of m_1 cell (2) and a white vertical hourglass shaped pattern in the anal cell (3) is typical of this species. Scale bar: 100 µm.

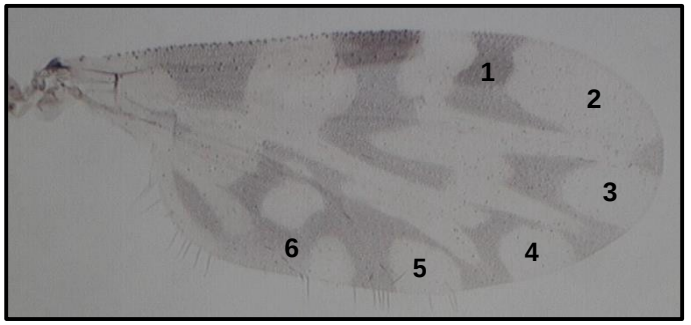
Culicoides circumscriptus – The presence of a dark circle in the r-m crossvein (1) is typical of this species. Scale bar: 200 µm.



Culicoides achrayi – This species has a pronounced dark wing pattern with three characteristic white spots: in the wing proximal region (1), in the r-m crossvein (2) and a poststigmatic pale spot (3). Scale bar: 200 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (2/19)

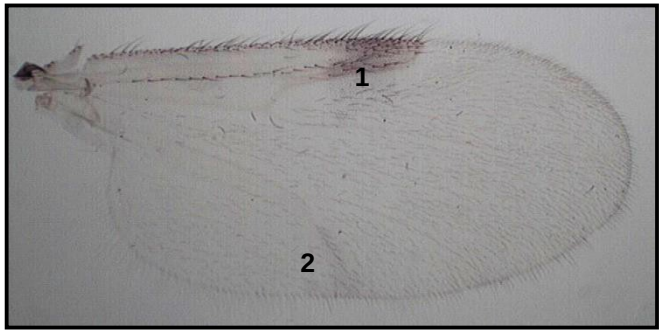
Culicoides fascipennis – This species has a pronounced dark wing pattern with a very small white spot in the wing posterior region (1) and four additional white spots: in the r-m crossvein (2), in the m cell (3), in the anal cell (4) and a poststigmatic pale spot (5). Sometimes, the white spot in m cell can be fused with that of the r-m crossvein. Scale bar: 200 µm.



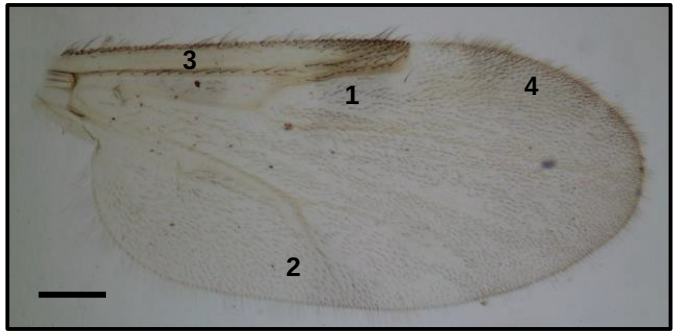
Culicoides shaklawensis – This species has a characteristic wing pattern with a dark spot in the r_3 cell (1), white spots at the apex of the r_3, m_1, m_2 and cua_1 cells (2, 3, 4 and 5, respectively) and two isolated white spots in the anal cell (6) (Mathieu *et al*., 2012).

B – Species with similar wing pattern (do not require insect mounting):

1. Light wing pattern with a dark spot covering the two radial cells (1) and another in the left branch of the CuA vein (2):



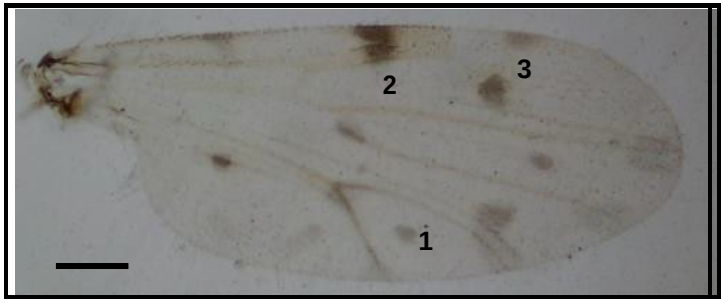
Culicoides brunnicans (Mathieu *et al*., 2012)



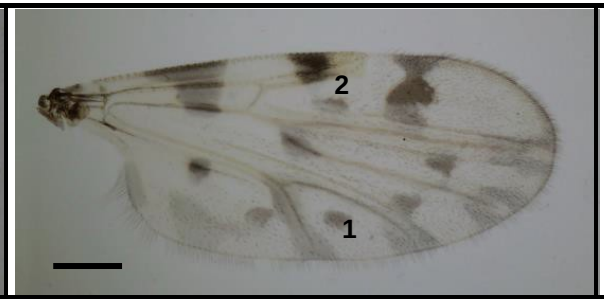
Culicoides santonicus – Dark spots in the r-m crossvein proximal region (3) and in the anterior part of the r_3 cell (4). Scale bar: 200 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (3/19)

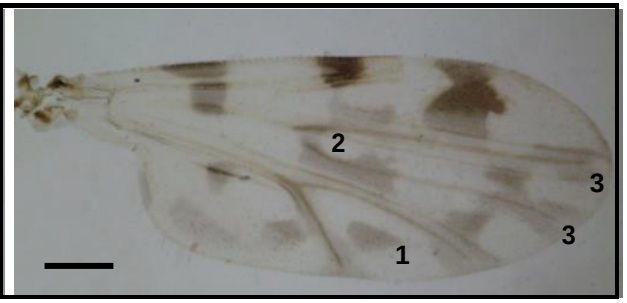
2. Wings with a dark spot in the cua_1 cell (1):



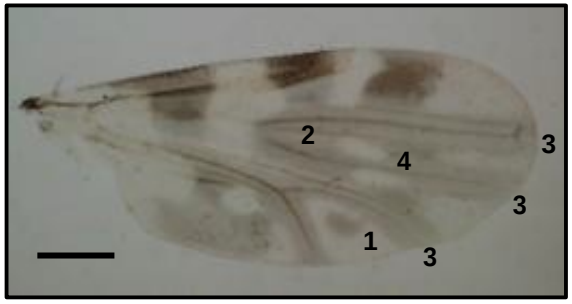
Culicoides pulicaris – This wing pattern lacks a dark spot below the r_2 cell (2). The dark spots at the r_3 cell (3) can be united like in *Culicoides punctatus*. Scale bar: 200 µm.



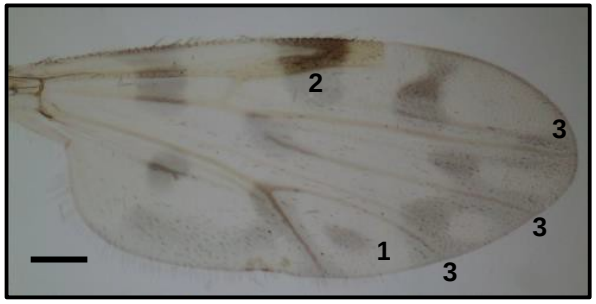
Culicoides punctatus – This wing pattern has a dark spot below the r_2 cell (2), unlike *Culicoides pulicaris*. Scale bar: 200 µm.



Culicoides newsteadi – This wing pattern has a dark spot in the proximal part of the M_2 vein (bigger than that of *Culicoides punctatus*), surrounding a white spot in the proximal part of m_1 cell (2). There is always, at least, one white spot at the apex of the M_1, M_2 and CuA_1 veins (3). Scale bar: 200 µm.



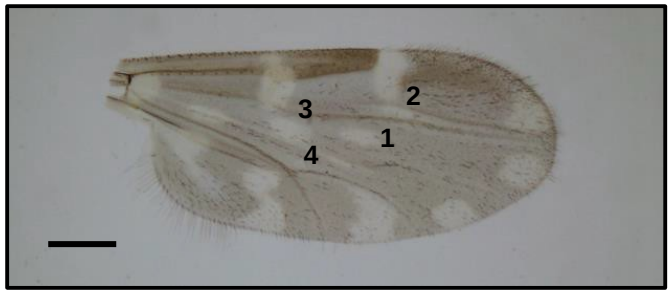
Culicoides paradoxalis – The white spot above the proximal part of M_2 vein is not present like in *Culicoides newsteadi* (2). There are no white spots in the apex of the M_1, M_2 and CuA_1 veins (3). The white spots in the middle of the m_1 and m_2 cells are not fused (4). The dark spot in cua_1 cell may be linked with the black spot of the CuA_1 vein (1). Scale bar: 200 µm.



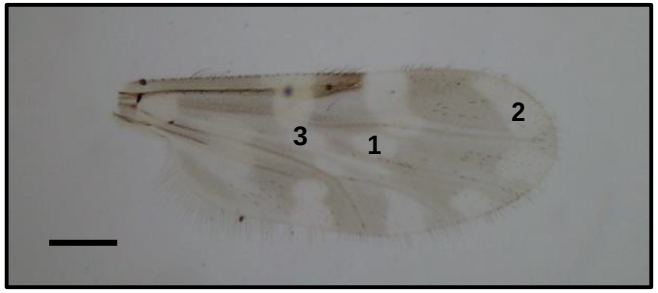
Culicoides lupicaris – A continuous dark spot begins in the wing anterior margin and goes through the two radial cells (2). White spots in the apex of the M_1, M_2 and CuA_1 veins can be absent (3). Scale bar: 200 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (4/19)

3. Wings with one isolated white spot in the proximal/middle part of the m_1 cell (1):



Culicoides festivipennis – A white spot is present in the middle part of the M_1 vein, below the poststigmatic pale spot (2). The white spot in the m cell is not fused with any white spot around it and does not extend itself into the m_2 cell (3). Sometimes, two white spots can be observed in the basal part of m_2 cell (4). Scale bar: 200µm.



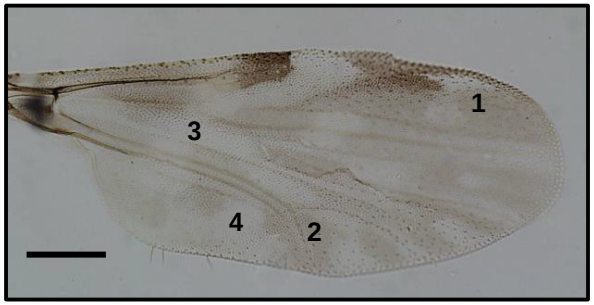
Culicoides clastrieri – The r_3 white spot is bigger than that observed in *Culicoides festivipennis* (2). The m cell white spot is connected with the r-m crossvein white spot and expands itself into the m_2 cell (3). Scale bar: 200µm.

Culicoides longipennis – There is no white spot in the middle of the M_1 or M_2 veins (2). Scale bar: 200µm.

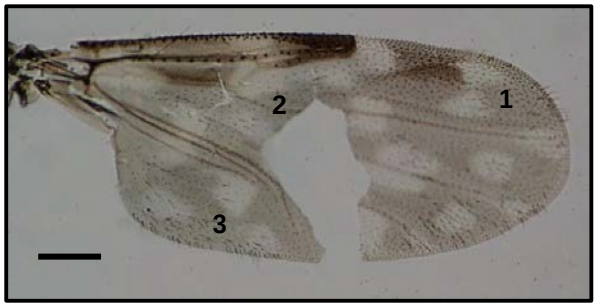
Culicoides sahariensis – The white spot in the middle of the m_1 cell reaches the M_1 and M_2 veins (1). The post-stigmatic spot does not reach the M_1 vein (2). Scale bar: 200µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (5/19)

4. Wings with two isolated white spots in the r_3 cell (1):



Culicoides kingi – The presence of two isolated white spots in cua_1 cell, surrounded by a dark area, is typical of this species (2). m cell white spot fused with the r-m crossvein white spot (3). Two fused white spots in anal cell (4). Scale bar: 100 µm.



Culicoides paolae – Wing pattern similar to *Culicoides kingi* but with two fused white spots in cua_1 cell, surrounded by a black area (not visible); m cell white spot not fused with the r-m crossvein white spot (2). Anal cell with two isolated white spots (3). (Incomplete wing obtained from a single observed specimen). Scale bar: 200 µm.

5. Wings with absence of white spots in the middle of the m_1 (1) and m_2 (2) cells:

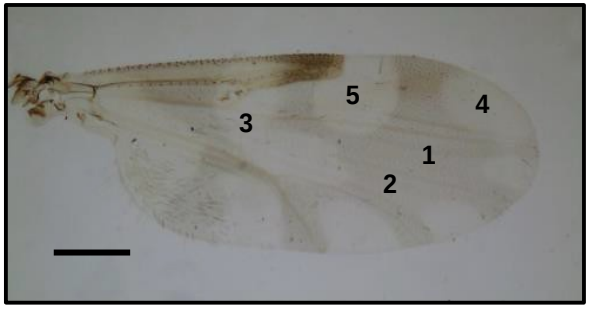
Culicoides alazanicus – Presence of an isolated m cell white spot (3). Pronounced black lines between M_1, M_2 and CuA_1 veins (4). Scale bar: 200 µm.

Culicoides haranti – Absence of the m cell white spot (3). Scale bar: 200 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (6/19)

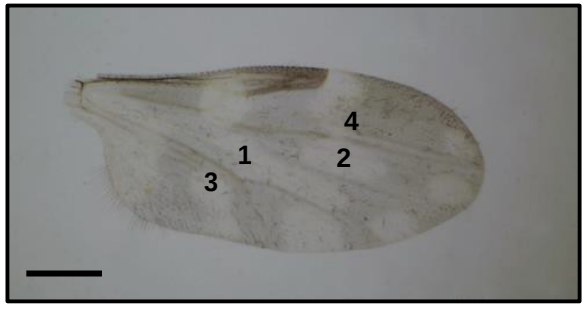
6. Wings with absence of white spots in the middle of the m_1 (1) and m_2 (2) cells and m cell white spot fused with r-m crossvein white spot (3):

Culicoides picturatus – White spots in the distal part of the r_3 and m_1 cells are bad defined (4). Scale bar: 200 µm.



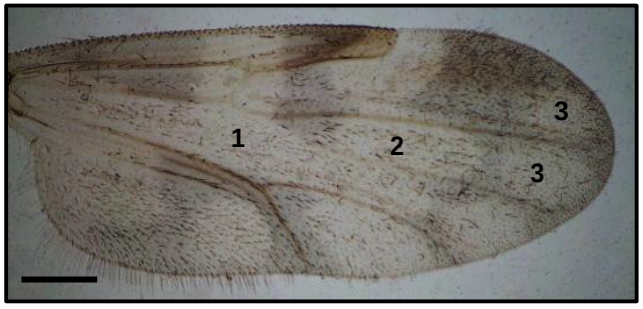
Culicoides simulator – Presence of a white spot in the distal part of r_3 cell, bigger than the distal m_1 cell white spot (4). Poststigmatic pale spot starts in the wing margin and crosses the M_1 vein (5). m cell white spot expands slightly into the m_1 and m_2 cells (3). Scale bar: 200 µm.

7. Wings where the m cell white spot expands itself into the m_2 cell (1) and presence of a white spot in the middle of m_1 cell (2):



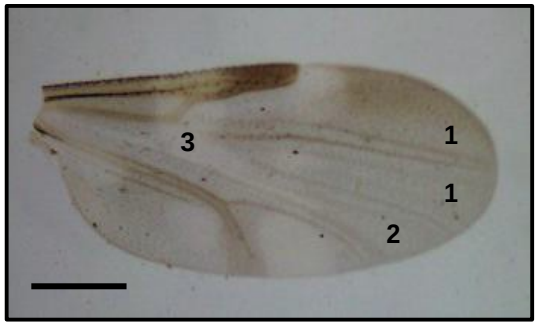
Culicoides maritimus – Presence of two white spots in anal cell (fused or separated) (3). Presence of a small white spot in the middle of the M_1 vein (4). Scale bar: 200 µm.

Culicoides univittatus – White spots of the distal part of the r_3 and m_1 cells close before they reach wing margin (3). When this feature is difficult to observe, it can be confounded with *C. gejgelensis*, *C. cataneii* and *C. pictipennis*. Scale bar: 200 µm.

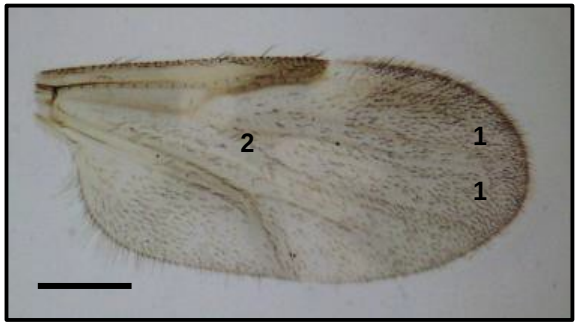


Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (7/19)

8. Wings without pale spots in the distal part of r_3 and m_1 cells (1):



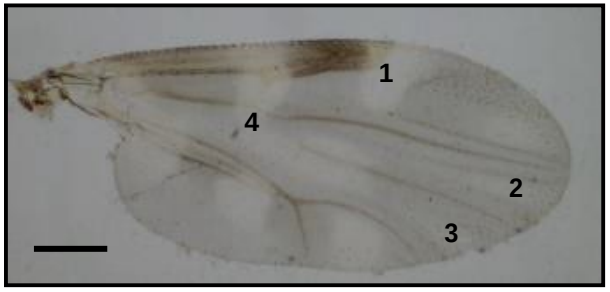
Culicoides jumineri near *Culicoides bahrainensis* – This wing does not possess the white spot in the distal part of m_2 cell like in *Culicoides jumineri* (2). The white spot in the m cell extends itself into the m_2 cell (3). Scale bar: 200 µm.



Culicoides kurensis – Although not easy to observe, a smooth grey patch isolates the white spot in the m_2 cell from the white spot of the r-m crossvein (2). Scale bar: 200 µm.

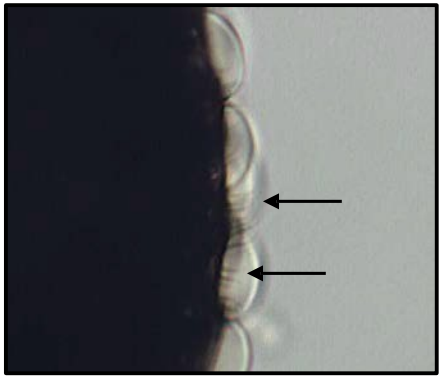
C – Undistinguishable wing patterns (requires insect mounting):

1. Wings with a characteristic poststigmatic pale spot (1) and presence of two isolated white spots which can cross the entire m_1 (2) and m_2 (3) cells, being this last one fused with the m cell white spot (4):

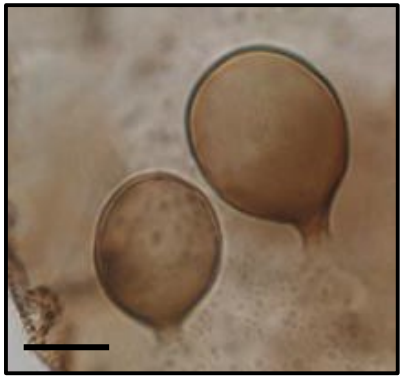


Includes species from Obsoletus group: *Culicoides chiopterus*, *Culicoides dewulfi, Culicoides montanus, Culicoides obsoletus* (left image) and *Culicoides scoticus*. Scale bar: 200 µm.

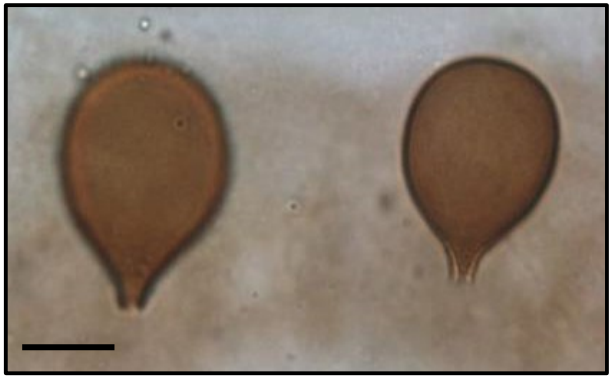
Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (8/19)



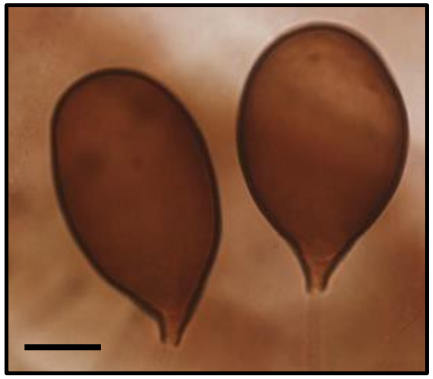
Culicoides chiopterus – This species has scattered and short interfacetal hairs (black arrows) (Mathieu *et al*., 2012).



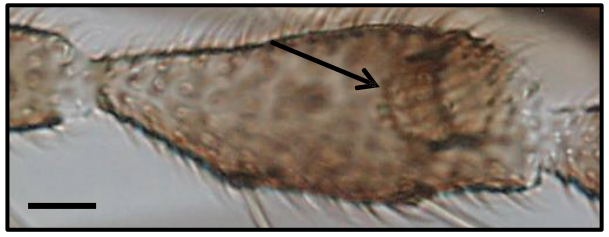
Culicoides dewulfi – This species has a two different sized spermathecae. Scale bar: 20 µm.



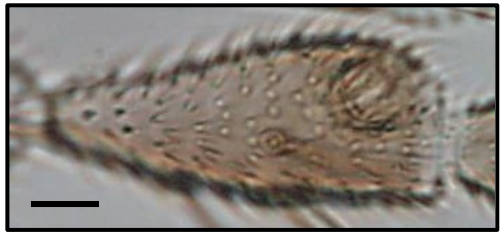
Culicoides obsoletus and *C. montanus* – Both species have an equal sized spermathecae with ≤ 62.5 µm. Scale bar: 20 µm.



Culicoides scoticus – This species has equal sized spermathecae with ≥ 57 µm. Scale bar: 20 µm.



Culicoides montanus – This species has a deeper sensorial pit (black arrow) when compared with *C. obsoletus* and *C. scoticus*. Scale bar: 10 µm.

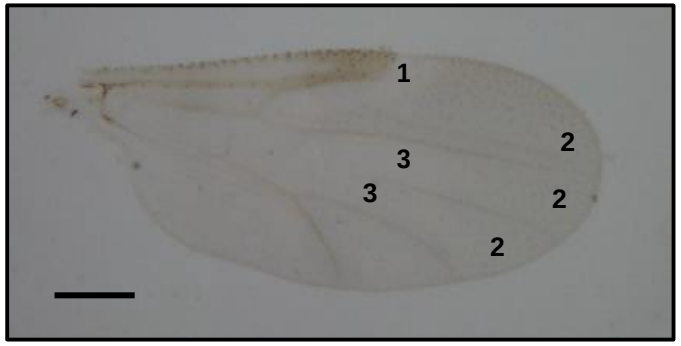


Culicoides obsoletus – The 3rd palpus segment of this species has a ratio length/width ≤ 2.7. Scale bar: 10 µm.

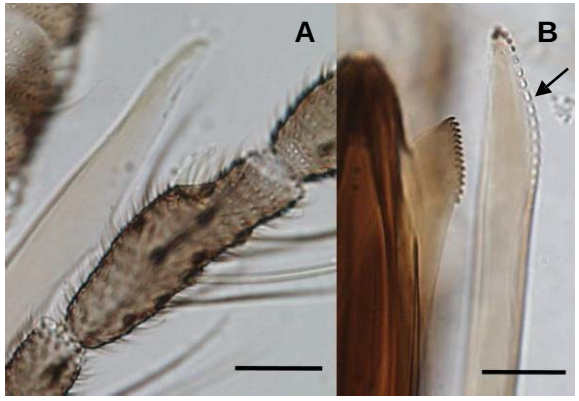
Culicoides scoticus – The 3rd palpus segment of this species has a ratio length/width ≥ 2.7. Scale bar: 10 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (9/19)

Note: *Culicoides albihalteratus* wing pattern, although different, can be very similar to that of Obsoletus group species and is easily confounded as belonging to this group. For this reason it is included here.



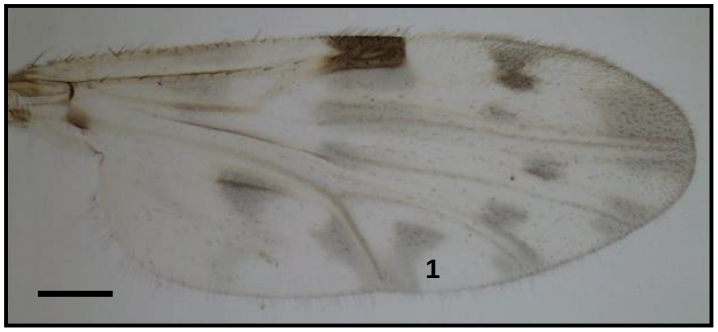
Culicoides albihalteratus – the poststigmatic pale spot has a different shape from that observed in species from Obsoletus group (1). There are no white spots in the distal part of the r_3, m_1 and m_2 cells (2) and the white spots are very difficult to observe (3) (aspect that can also occur in Obsoletus group species). Scale bar: 200 µm.



Culicoides albihalteratus (A) does not have teeth in the mandible while species from Obsoletus group have. B – *Culicoides scoticus*. Black arrow – teeth. Scale bars: 20 µm

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (10/19)

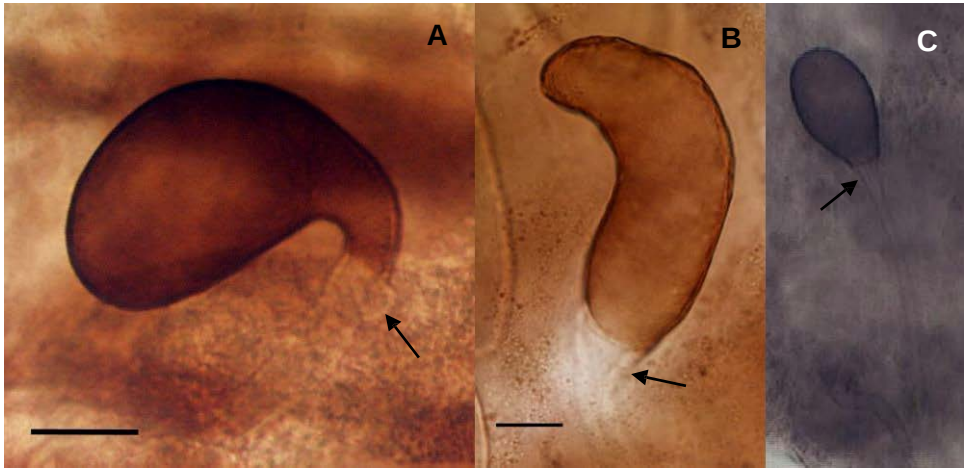
2. Wings with a dark spot in the cua_1 cell connected with the left branch of the CuA vein (1):



Includes: *Culicoides nubeculosus* (left image), *Culicoides puncticollis* and *C. riethi*. Scale bar: 200 µm.

Culicoides nubeculosus (A) – Almost all spermatheca is curved on itself. Presence of an enlarged ring in the beginning of the spermathecal duct (black arrow). Spermathecal duct longer than spermatheca. Scale bar: 20 µm.

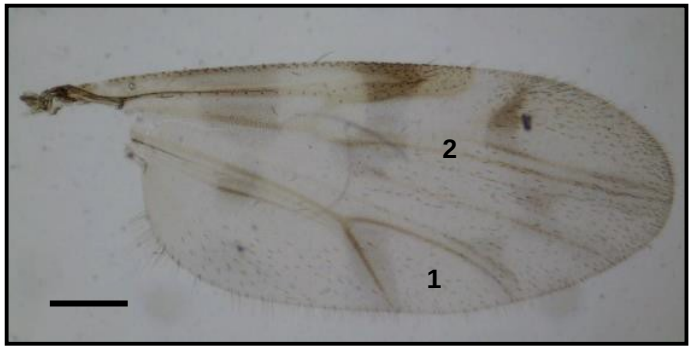
Culicoides puncticollis (B) – Spermatheca can be straight or its anterior portion slightly curved. Absence of an enlarged ring in the beginning of the spermathecal duct (black arrow). Spermathecal duct in the length of spermatheca. Scale bar: 20 µm.



Culicoides riethi (C) - Spermatheca can be straight or its anterior portion slightly curved. Absence of an enlarged ring in the beginning of the spermathecal duct (black arrow). Spermathecal duct longer than spermatheca (Mathieu *et al*., 2012).

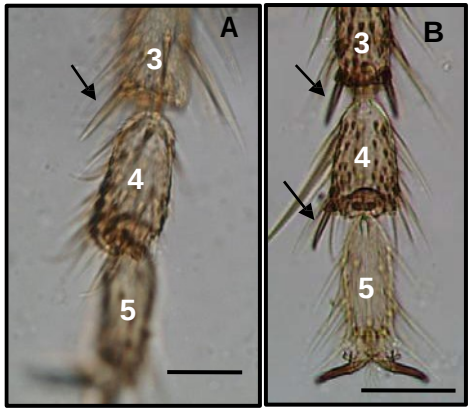
Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (11/19)

3. Wing pattern similar to *Culicoides lupicaris* but without a dark circle in the cua_1 cell (1):

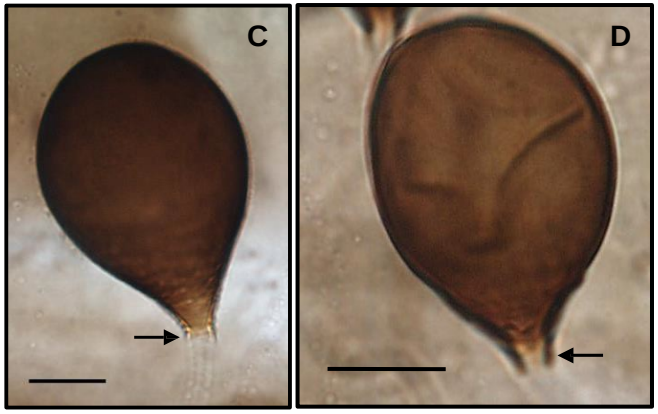


Absence of a dark spot in the middle of the M_1 vein (2). Includes: *Culicoides deltus* (upper image), *Culicoides impunctatus* and *Culicoides remmi*. Scale bar: 200 µm

Presence of a dark spot in the middle of the M_1 vein (2). Includes: *Culicoides fagineus* and *Culicoides subfagineus* (upper image). Scale bar: 200 µm



Culicoides impunctatus (A) has spines from the 1st to the 3rd tarsomere of middle legs, while *Culicoides deltus* (B) and *Culicoides remmi* have spines from the 1st to the 4th tarsomere of middle legs. Black arrows – tarsomere spines; 3 to 5 – 3rd to 5th tarsomere. Scale bars: 20 µm.



Culicoides deltus (C) has a shorter spermathecae pigmented neck than *Culicoides remmi* (D). Black arrow: spermathaecae pigmented neck. Scale bars: 20 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (12/19)

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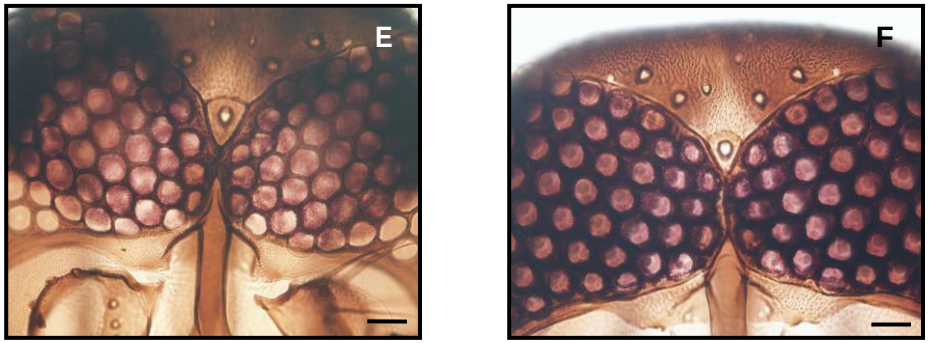
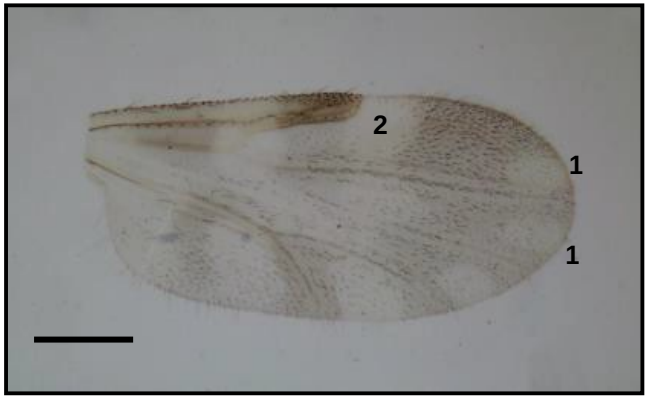


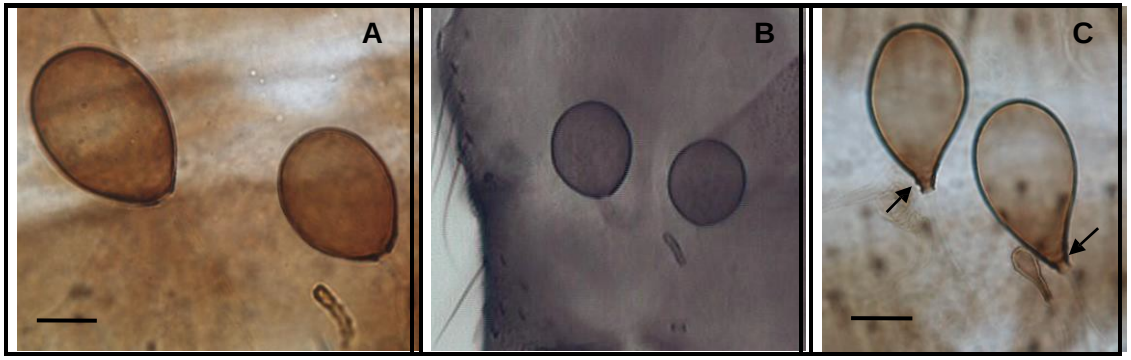
The eyes are joined for a shorter distance in *Culicoides subfagineus* (E) than in *Culicoides fagineus* (F). Scale bars: 20 µm.

4. Wing pattern similar to *Culicoides univittatus* but the white spots in the distal part of the r_3 and m_1 cells do not close before wing margin (1):

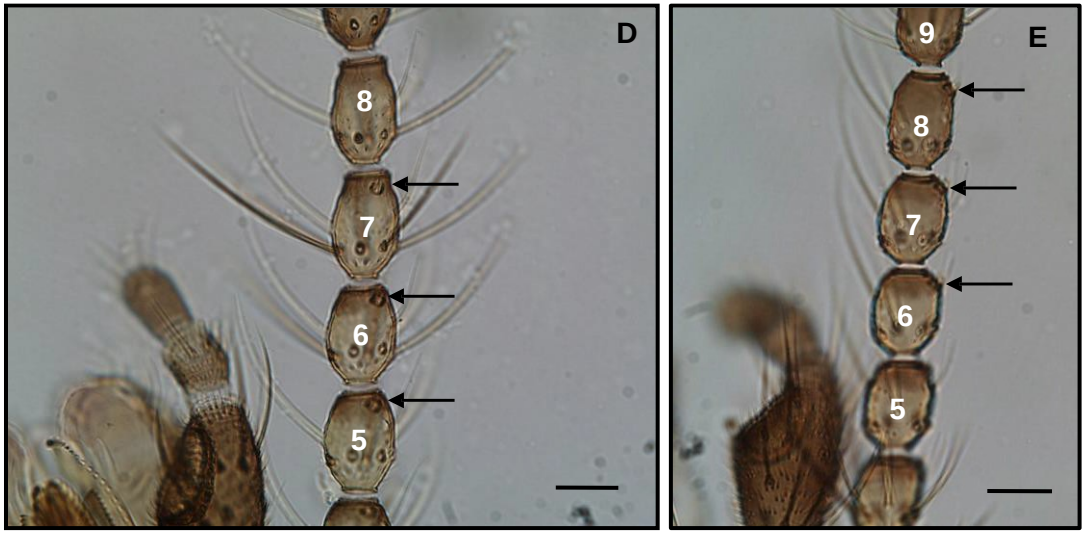


Includes: *Culicoides cataneii*, *Culicoides gejgelensis* (left image), *Culicoides pictipennis* and *Culicoides univitattus* (when its wing pattern characteristics are difficult to observe). In *Culicoides pictipennis*, the poststigmatic pale spot (2) covers more than 1/3 of the r_2 cell. Scale bar: 200 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (13/19)



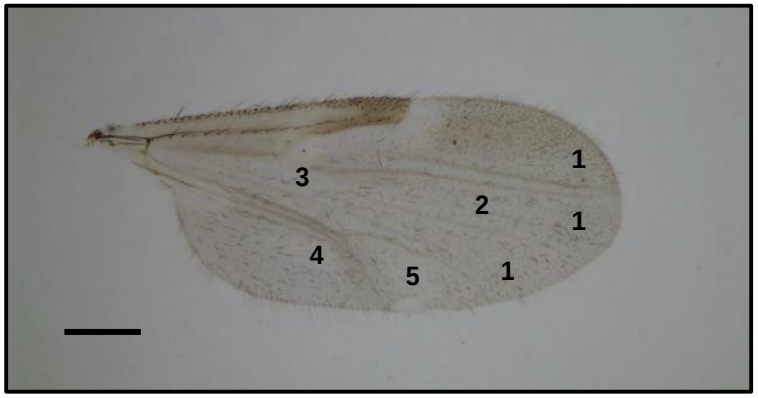
Culicoides univittatus (A) has unequal spermathecae length, while *Culicoides pictipennis* (B), *Culicoides cataneii* and *Culicoides gejgelensis* (C) have equal spermathecae length. This last species also have a small sclerotic ring in their spermathecae pigmented neck (black arrows). *Culicoides pictipennis* and *Culicoides univittatus* do not possess a pigmented neck, while *Culicoides cataneii* and *Culicoides gejgelensis* have a pigmented neck. Scale bars: 20 µm. Image B: Mathieu *et al*., 2012.



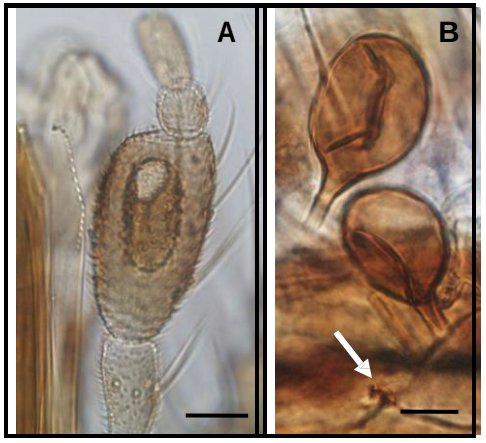
Culicoides cataneii (D) has sensilla coeloconica from the 5th to the 7th antennal flagellomere and *Culicoides gejgelensis* (E) from the 5th to the 8th antennal flagellomere. Black arrows – sensilla coeloconica; 5 to 9 – 5th to 9th antennal flagellomere. Scale bars: 20 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (14/19)

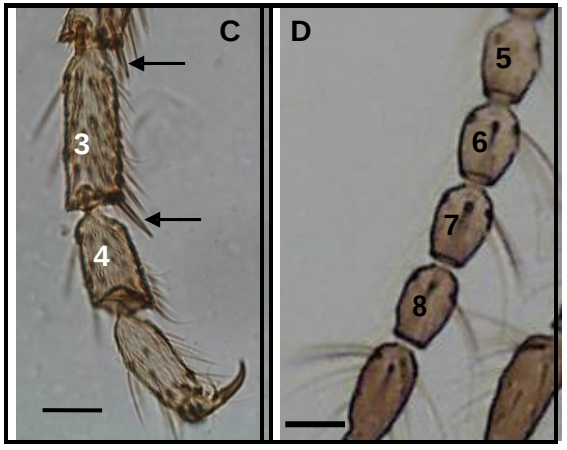
5. Dark wings with no white spots in the distal part of the r_3, m_1 and m_2 cells (1) and no white spot in the middle of m_1 cell (2):



Includes: *Culicoides heteroclitus* (left image), *Culicoides pseudopallidus*, *Culicoides semimaculatus* and *Culicoides subfasciipennis*. These species have a high level of wing pattern intraspecific variation. Concerning the m cell white spot (3), in *C. semimaculatus* it can be absent, present or present and fused with the r-m crossvein white spot. The anal cell white spot (4) can be absent in *C. heteroclitus*, *C. pallidicornis* and *C. subfasciipennis*. Finally, cua_1 white spot (5) can be absent in *C. heteroclitus* and *C. pallidicornis*. *C. heteroclitus* and *C. pseudopallidus* species are very difficult to distinguish between them. Scale bar: 200 µm.

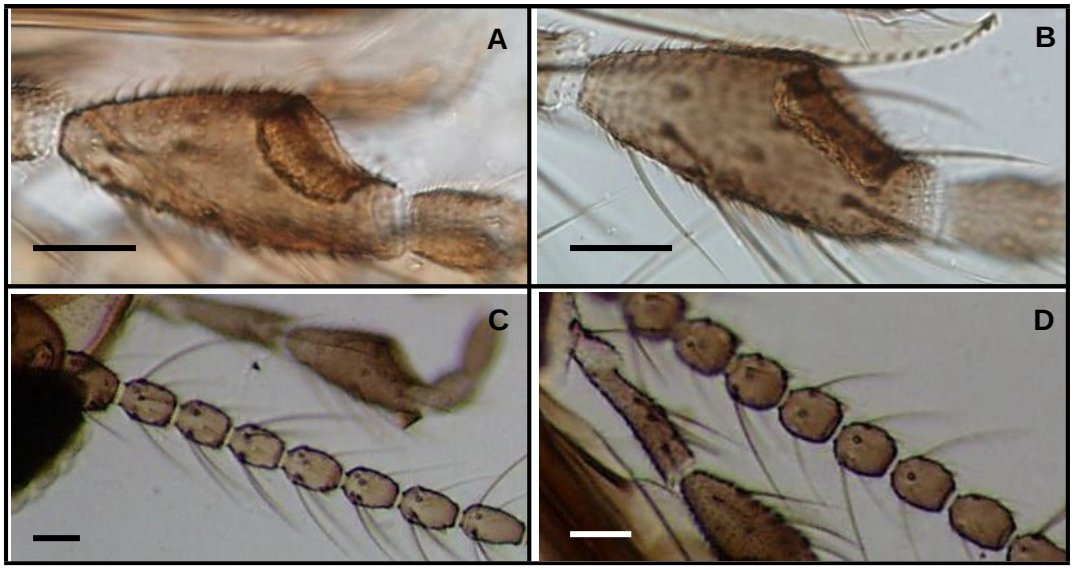


Culicoides semimaculatus has a strongly swollen 3rd palpus segment (A), spermathecae with a long pigmented neck and a donut shaped sclerotic ring (B) (white arrow). Scale bars: 20 µm.



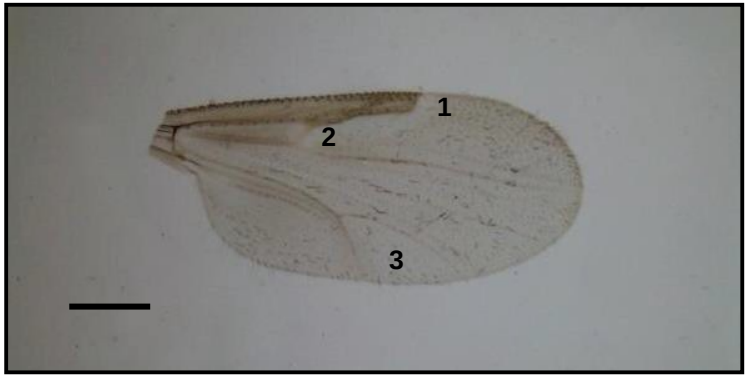
Culicoides subfasciipennis does not have a spine in the 4th tarsomere (C) and lacks sensilla coeloconica from the 5th to the 8th antennal flagellomere (D). Black arrows – tarsomere spines; 3 and 4 – 3rd and 4th tarsomere; 5 to 8 – 5th to 8th antennal flagellomeres. Scale bars: 20 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (15/19)



Culicoides heteroclitus and *Culicoides pseudopallidus* are very difficult to distinguish between them. 3rd palpus sensorial pit is shorter in *Culicoides heteroclitus* (A) than in *Culicoides pseudopallidus* (B). 2nd to 8th antennal flagellomeres may have an inflated shape in *Culicoides pseudopallidus* (D), although it can also have a flask shape like in *Culicoides heteroclitus* (C). *Culicoides heteroclitus* does not have a sclerotized ring in abdomen while in *Culicoides pseudopallidus* the sclerotized ring is present (not shown). Scale bars: 20 µm.

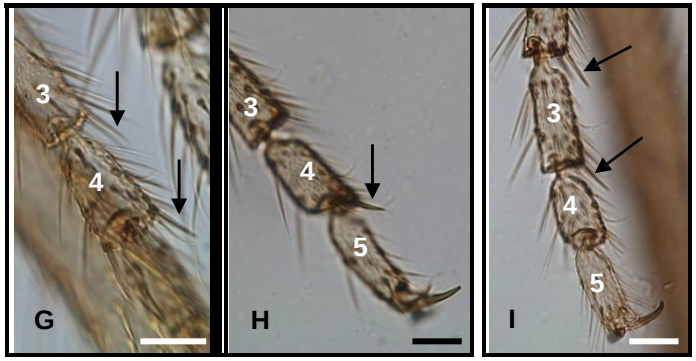
6. Dark wings with a very small poststigmatic pale spot (1) and a white spot in the r-m crossvein (2):



Includes: *Culicoides atripennis*, *Culicoides begueti* (left image), *Culicoides furcillatus*, *Culicoides indistinctus*, *Culicoides kibunensis*, *Culicoides odiatus* and *Culicoides pallidicornis*. *Culicoides kibunensis* can have an additional white spot in the cua_1 cell (3). Scale bar: 200 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (16/19)

Third palpus segment of *Culicoides begueti* (A), *Culicoides indistinctus* (B), *Culicoides odiatus* (C), *Culicoides kibunensis* (D), *Culicoides pallidicornis* (E) and *Culicoides furcillatus* (F). All five species have different 3rd palpus segment shapes, being D, E and F more similar between them. Image F: Mathieu *et al*., 2012. Scale bars: 20 µm.



Both *Culicoides odiatus* (G), *Culicoides kibunensis* (H) and *Culicoides furcillatus* (not shown) have spines from the 1st to the 4th tarsomere, while *Culicoides pallidicornis* (I) have spines from the 1st to the 3rd tarsomere. Black arrows: tarsomere spines; 3 to 5: 3rd to 5th tarsomeres. Scale bars: 20 µm.

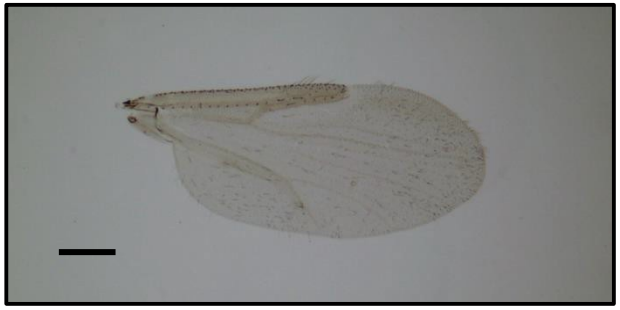
Culicoides kibunensis (J) has sensilla coeloconica from the 5th to the 8th antennal flagellomere (8th not shown), while *Culicoides pallidicornis* (K) does not have. Furthermore, *Culicoides furcillatus* has sensilla coeloconica on the 1st antennal flagellomere (not shown) while *Culicoides kibunensis* has sensilla coeloconica from the 1st to the 4th antennal flagellomere (not shown). Black arrows: sensilla coeloconica; 5 to 8: 5th to 8th antennal flagellomeres. Scale bars: 20 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (17/19)

Note: *C. atripennis* and *C. kibunensis* differ only in body size, being the first species bigger than the second. The maximum length of some *C. kibunensis* body structures are as follows (Delécolle, 1985):

Body structure	Length (µm)
Wing	1206
Palpus	200
Antenna	622

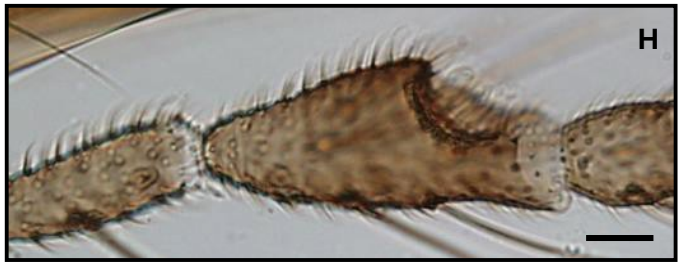
7. Dark wing pattern without white spots:



Includes: *Culicoides corsicus* (left image), *Culicoides derisor*, *Culicoides jurensis*, *Culicoides malevillei*, *Culicoides riebi* and *Culicoides tbilisicus*. Scale bar: 200 µm.

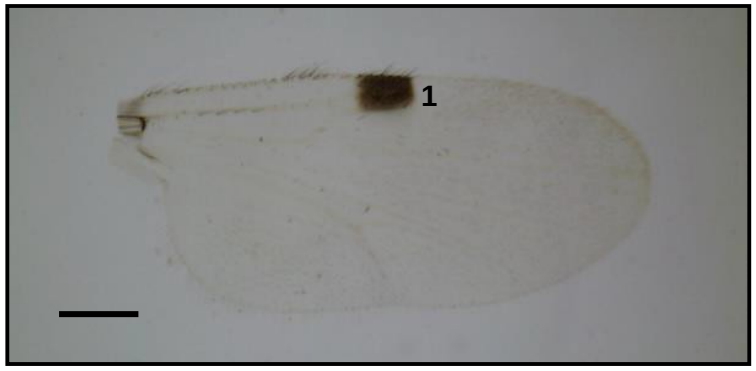
Culicoides jurensis (A) has a strongly swollen 3rd palpus segment. *Culicoides corsicus* (B and C) has a long spermatheca pigmented neck and its sclerotic ring has a donut shape. *Culicoides derisor* (D) does not have a spermatheca pigmented neck. *Culicoides malevillei* does not have a spine in the 4th tarsomere of middle legs (E), while *Culicoides riebi* (F) and *Culicoides tbilisicus* (G) have a spine in the 4th tarsomere of middle legs. Scale bars: 20 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (18/19)

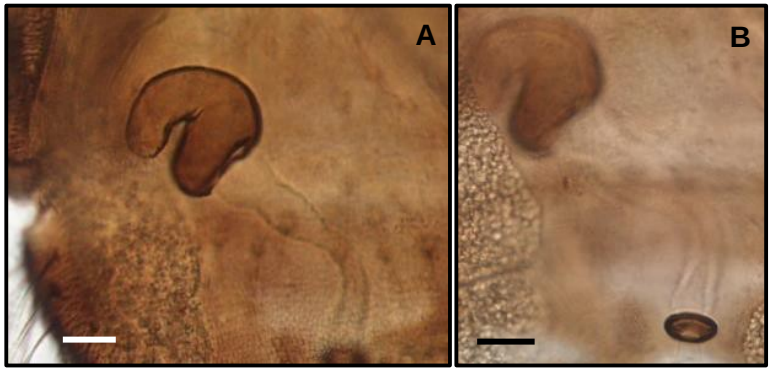


Culicoides riebi (H) has a single sensorial pit, while *Culicoides tbilisicus* (I) has multiple sensorial pits. Scale bars: 10 µm.

8. Light wing pattern with only one dark spot in the radial cells (1):



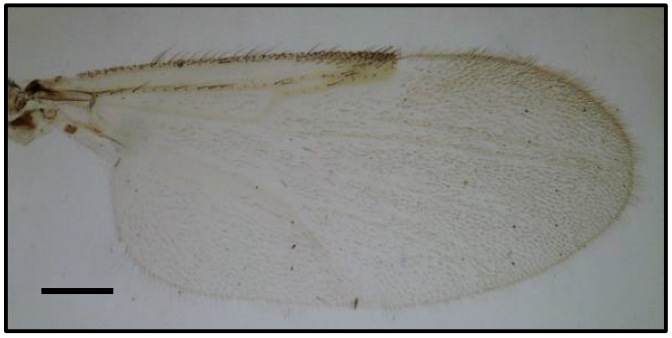
Although there are, at least, three species with this wing pattern in Palearctic ecozone (*Culicoides helveticus*, *Culicoides parroti* and *Culicoides stigma*), only *Culicoides parroti* (left image) was referred in Portugal and, thus, only that species is referred in this identification key. Scale bar: 200 µm.



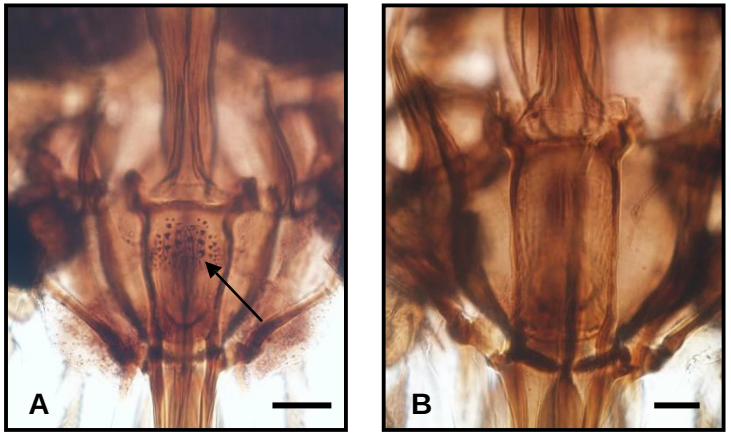
Culicoides parroti – This species has an "U" shaped spermathecae (A) and a donut shaped sclerotic ring (B). Scale bars: 20 µm.

Identification key for Portuguese *Culicoides* female specimens (original photos, except where noted) (19/19)

9. Light wing pattern with no dark spots:



Includes: *Culicoides heliophilus* and *Culicoides vexans* (left image). Scale bar: 200 µm.



Culicoides heliophilus (A) has an ornamented cibarium (black arrow), while *Culicoides vexans* (B) presents a simple cibarium. Scale bars: 20 µm.