Biting midges (Diptera: Ceratopogonidae: Culicoides Latr.) associated with livestock farms in the Faroe Islands

Mitter (Diptera: Ceratopogonidae: *Culicoides* Latr.) indsamlet i og omkring kostalde på Færøerne

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Sammendrag

I 2007 blev der, med henblik på registrering af blodsugende insekter, foretaget indsamlinger med lysfælder samtidig udenfor og indenfor i 25 færøske stalde. Fangsterne bestod hovedsagelig af et stort antal stald-stikfluer (*Stomoxys calcitrans*) og mitter (*Culicoides* spp.). Mitterne var repræsenteret med to arter, hvor *Culicoides impunctatus* Goetghebuer, 1920 udgjorde > 98% af det totalt indsamlede mitte-materiale og derudover *Culicoides pseudoheliophilus* Callot & Kremer, 1961, der er ny for den færøske fauna. *C. impunctatus* der er kendt fra Færøerne fra en tidligere undersøgelse, var udbredt på alle øerne, hvorimod *C. pseudoheliophilus* havde en mere begrænset udbredelse, hvor arten kun blev registreret på ni gårde. Der var forskel på den relative fordeling af *C. impunctatus* hanner samt af de fysiologiske hun-typer i lysfælderne udenfor og indenfor staldene, hvor andelen af blodfyldte hunner var størst inde i staldene, hvorimod andelen af hanner samt nullipare hunner var størst udenfor staldene. På Færøerne har *C. impunctatus* en unimodal og univoltin fordeling med kun én generation per år. *Culicoides* spp. er kendt som vektorer for et antal vira der overføres til kvæg - for eksempel Bluetongue virus (BTV), der hvis virussen blev spredt til Færøerne ville have katastrofale følger især for fåreavlerne. *C. impunctatus* er mistænkt for at kunne være en potentiel vektor for BTV, men risikoen for at dette sker vurderes at være minimal på grund af artens reproduktionsforhold på Færøerne.

Abstract

A large number of biting midges (*Culicoides*) were collected in light traps operating simultaneously outside and inside 25 Faroese byres. The catch comprised two species: *C. (Culicoides) impunctatus* Goetghebuer, 1920, accounting for >98% of all biting midges trapped, and *C. (Oecacta) pseudoheliophilus* Callot & Kremer, 1961, new to the Faroese fauna. *C. impunctatus* was common at most of the farms, females contributing >95% of the catch. Apparently *C. pseudoheliophilus* has a more limited distribution in the Faroe Islands and was only recorded from nine byres. The relative distribution of female *C. impunctatus* on physiological types differs in light trap catches inside and outside the byres, e.g. the proportion of engorged females was higher inside the cowshed, whereas the proportion of nulliparous females was higher outdoors. In the Faroes *C. impunctatus* appears to be unimodal and univoltine with only one brood per year.

Introduction

Until now two species of *Culicoides* have been reported from the Faroes, viz. *C. duddingstoni* Kettle & Lawson, 1955 (Pedersen, 1971) and *C. impunctatus* Goetghebuer, 1920 (Nielsen & Nielsen, 2002). However, the Faroese specimens of the former species deposited in the Zoological Museum, Natural History Museum of Denmark, Copenhagen actually proved to be *C.* (*Oecacta*) *pseudoheliophilus* Callot & Kremer, 1961. The record of the stable fly (*Stomoxys calcitrans* (Linnaeus, 1758)) from byres in the vicinity of Thorshavn (Nielsen & Bloch, 2005) started a comprehensive sampling program in byres on the larger Faroese islands with the purpose of evaluating the incidence and prevalence of bloodsucking flies in livestock farms in the Faroes (Holm, 2010). A large number of stable flies and biting midges (*Culicoides*)

representing two species were caught, viz. *C. pseudoheliophilus*, new to the Faroese fauna, and *C. impunctatus*. In this paper the two species are described based on specimens from the Faroes and the distribution and relative abundance of biting midges within and outside Faroese byres are presented.

Material and methods

Research sites and livestock

In 2007 adult biting midges were trapped in 25 livestock farms across all the larger Faroese islands (Fig. 1). All farms were surrounded by mountains covered with low grass sparsely mixed with low herbs (grass moorland). In many places water percolating from the mountains created humid areas overgrown with various moss species, including *Sphagnum* spp. Most farms were rather isolated owing to the surrounding mountains.

The livestock was Norwegian Fjord cattle mixed with other races. With one exception, the cattle were stalled all year round. Only on fine summer days some of the herds were pastured, but always stalled during the night. The herds ranged from 30 to 60 dairy cows, age distribution 2-8 years, and in addition 10-15 calves were present. Animal husbandry was mainly based on conventional milk production. With one exception, the byres were relatively low-ceilinged stanchion byres, area 200-300 m² with traditional manual mocking. In the calf boxes the bottom layer consisting of hay mixed with sawdust was only purged periodically. The cattle fodder was composed of hay, silage (acidic hay) and concentrate.

In all byres insecticide (Stable-shock fly spray D, pyrethrin I and II, 0.40%, piperonylbutoxyd 2.00% Company: Aeropak) and adhesive strips were used, primarily to control stable flies. In the summer, when the flies seemed to multiply, the insecticide was frequently used, sometimes at intervals of a few days. The strips were replaced as needed, i.e. when covered with flies.

Trapping methods and protocol

The collections were performed by means of modified Ento-Tech light weight light traps, 15Watt fluorescent tube (Sylvania (BL QUANTUM – 15W/T8/BL Made in Germany) (Olsen et al., 1984). The traps were connected to 230 V power supply via a choke coil. The insects were collected in 2% formalin (detergent added), removed by filtration and transferred to 70% ethanol. Subsequently, the insects were sorted under a stereo microscope (magnification 4-80 x) and specimens of *Culicoides* were selected for slide preparation.

At each site two light traps operated simultaneously: one inside and one outside the byre. In the byres the traps were mounted at about 2 m height in the darkest part of the building. Outside the byres the traps were placed on the ground close to the wall of a cow shed. From June 10 to September 1 2007 light trap collections were made at each site (duration: one day or two consecutive days). Sampling was carried out 130 times within and outside the byres, each site being represented by 2-7 collections. As far as possible sampling was performed in June/early July and August in every site, trapping early as well as late hatching species. However, at the sites St. 1, St. 2, St. 9, and Vi 1 (Table 1, Fig. 1) sampling was only implemented in the period June 12 to June 22.

Simultaneously with the individual trap catches the air temperature was recorded using data loggers (HOBO® (Onset Computer Corporation, 470 MacArthur Blvd., Bourne, MA 02532, PO Box 3450, Pocasset, MA 02559-3450)), attached to the traps. Measurements were made every 10 min. Subsequently, the average temperatures in the period from 18.00 to 6.00 were calculated.



Fig. 1. The location of the sampling sites in the Faroe Islands.

Tot nos.	6°42'26.08"W	6°48'51.33"W	6°39'58.97"W	6°48'54.42"W	6°49'52.88"W	6°53'10.82"W	6°57'06.76"W	6°57'53.40"W	6°47'52.05"W	6°51'21.93"W	6°50'11.77"W	6°50'41.82"W	6°50'34.10"W	6°57'13.20"W	6°49'37.07"W	6°49'28.31"W	7°12'22.96"W	7°15'08.08"W	7°24'02.44"W	6°51'13.44"W	6°39'54.01"W	6°45'57.23"W	6°51'42.93"W	7° 5'25.06"W	6°32'17.55"W	Coordinate
	Suduroy	Suduroy	Sandoy	Sandoy	Sandoy	Sandoy	Streymoy	Vádoy	Vádoy	Vádoy	Eysturoy	Eysturoy	Eysturoy	Eysturoy	Eysturoy	Vidoy	Locality									
	SU 3	SU 1	SAN 6	SAN 4	SAN 2	SAN 1	St. 14	St. 13	St. 11	St. 10	St. 9	St. 8	St. 7	St. 3	St. 2	St. 1	VA 5	VA 4	VA 2	Ey 6	Ey 5	Ey 4	Ey 2	Ey 1	Vi 1	Location
5168	0	+	ŧ	ŧ	ŧ	ŧ	+	ŧ	+	0	0	0	0	ŧ	ŧ	ŧ	+	+	ŧ	‡ ‡	‡ ‡	ŧ	+++++++++++++++++++++++++++++++++++++++	‡	+	C.imindoor
380	0	0	0	+	‡	0	0	‡	+	0	0	0	+	0	0	0	0	+	0	+	+	0	++++	0	ŧ	C.imoutdoor
83	0	0	+	+	0	+	0	0	+	0	+	0	0	0	0	0	0	0	0	+	0	+	‡	0	0	C.phindoor
1	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C.phoutdoor Tot-C.im.
5548	0	1	255	66	161	328	2	315	13	0	0	0	_	170	4	20	26	36	131	938	1396	200	1266	52	134	Tot-C.im.
84	0	0	_	4	0	4	0	_	-	0	_	0	0	0	0	0	0	0	0	13	0	12	47	0	0	Tot.C.ph.
5632	0	1	256	103	161	332	2	316	14	0	-	0	1	170	4	20	26	36	131	951	1396	212	1313	52	134	Tot-Culic.

++++ 30,1-150. C. im. : Culicoides impunctatus, C. ph: Culicoides pseudoheliophilus. Table 1. Culicoides species captured out- and indoor at the sampling sites in the Faroe Islands. Average number of individuals per day: + 0,1-2, ++ 2,1-5, +++ 5,1-30,

Identification

Under a stereo microscope (magnification 12-15x) head, wing and tip of abdomen were placed in a drop of Euparal (Carl RothGmbH + Co, 76185 Karlsruhe, Germany) on a slide. Both antennae were removed from the head with microneedles and all body parts were covered with coverslips. Subsequently the length of wings (from arculus to tip), and of every palpal segment and flagellomere were measured, and spermathecae and male hypopygium were studied under an Olympus CX41 microscope equipped with an Olympus SC30 digital camera. Measurements of the body parts were performed using the CellA analyzing software. Antennal ratio (AR: flagellomeres 9 -13 / flagellomeres 1- 8) and palpal ratio (PR: length of segment 3 divided by greatest width of the segment) were calculated (Campbell & Pelham-Clinton, 1960). Morphological identification according to Campbell & Pelham-Clinton (1960), Kremer (1965), Dzhafarov (1964, 1976), Országh (1976), Delécolle (1985) and Gluchova (2005).

Results

A total of 5632 male and female *Culicoides* were trapped, viz. 5251 inside and 381 outside the byres (Table 1). The two *Culicoides* species present in the light trap catches are easily distinguished on wing characters (Figs. 2a-d, 3a-d) and other morphological details (Figs. 2 & 3, Table 2).



Fig. 2. *Culicoides pseudoheliophilus*. a: female wing, brightfield photo; b: female wing, darkfield photo; c: male wing, brightfield photo; d: male wing, darkfield photo; e: right palp dorsal view; f: female vertex; g: female spermathecae; h: male hypopygium. Scales: a, b, c, d = 500 μ m; e, f, g, h = 50 μ m.



Fig. 3. *Culicoides impunctatus.* a: female wing, brightfield photo ; b: female wing, darkfield photo; c: male wing, brightfield photo; d: male wing, darkfield photo; e: right palp dorsal view; f: female vertex; g: female spermathecae; h: male hypopygium. Scales: a, b, c, d = 500 μ m; e, f, g, h = 50 μ m.

C. pseudoheliophilus Callot & Kremer, 1961 (syn.: *C. albihalteratus* Goetghebuer, 1935, preocc.)

Females: Eyes well-separated by more than 1-1.5 facets, no pubescence (Fig. 2f). Antennae with sensilla coeloconica on flagellomere 1 and 11-13 (Table 2). Third maxillary palpal segment slender, with a single sensory pit (Fig. 2e). Wings with an indistinct pattern of blurred pale spots (Fig. 2a-d). Spermathecae spherical with long necks (Fig. 2g). *C. pseudoheliophilus* is one of the few *Culicoides* species lacking teeth on the maxillae. A crucial difference between female *C. duddingstoni* and *C. pseudoheliophilus* is that *C. duddingstoni* has teeth on both mandibles and maxillae, whereas *C. pseudoheliophilus* has teeth only on the mandibles.

Males: Ninth tergite with two well-developed apicolateral processes (Fig. 2h). A notch in ninth tergite absent. Caudomedian excavation of ninth sternite concave with bare membrane. Apodemes (ventral roots) elongate, sharp and weakly curved. Body of aedeagus trapezoid, parameres short, stout and thick (Fig. 2h).

All but a single *C. pseudoheliophilus* were captured within the byres, > 84% were females all of which were nullipararous. The species has a limited distribution in the Faroe Islands and was only found in nine byres on Eysturoy, Streymoy and Sandoy (Fig. 1, Table 1).

Table 2. Morphometric measurements of the two species of *Culicoides* registered from the Faroe Islands. 1 - Species (C. i.: *Culicoides impunctatus* ($\stackrel{\circ}{\rightarrow}$); C. p.: *Culicoides pseudoheliophilus* ($\stackrel{\circ}{\rightarrow}$); 2 - Flagellum, length (µm); 3 - Antennal ratio (AR: length of flagellomeres 9–13 divided by length of flagellomeres 1–8); 4 - Maxillary palp, length (µm); 5 - Maxillary palpal ratio PR (length/width of third palp segment); 6 -Maxillary palpal ratio P3/P2 (length of third maxillary palpal segment divided by length of second); 7 -Length of wing (µm); 8 - Spermathecal ratio S/R (Length of the larger spermathecae/ length of the smaller spermathecae); 9 - Head/proboscis ratio; 10 - Maxillary teeth; 11 - Mandibulary teeth; 12 - Ratio M/M (maxillary teeth/mandibulary teeth); 13 - Antennal sensilla coeloconica, left column: Mean number (\pm SD) of sensilla on individual flagellomeres, right column: Mean number (\pm SD) of total number of sensilla on flagellum. Brackets indicate min-max values (min-max).

1	2	3	4	5	6	7	
C.i.(N=10)	595±55 (418- 666)	1.04±0.03 (0.98-1.11)	191±24 (131-216)	3.11±0.30 (2.63-3.59)	0.95±0.11 (0.79-1.19)	1419±99 (1256- 1561)	
C.p.(N=10)	516±40 (456-589)	1.12±0,04 (1.05-1.18)	146±16 (182-123)	2.62±0.23 (2.23-2.98)	0.94±0.11 (1.01-1.41)	1164±93 (1045- 1328)	

1	8	9	10	11	12	13		
C.i.(N=10)	1.17±0.15 (1.01- 1.41)	1.33±0.07 (1.20-1.42)	16±1.17 (14-18)	14.2±0.77 (13-16)	1.15±0.08 (1.0-1.29)	1:2.3±0.5 9:0.6±0.5 10:0.3±0.5 11:0.9±0.4 12:1.8±0.4 13:2.4±0.6	Tot: 8.3±1.4 (6-11)	
C.p.(N=10)	1.07±0.06 (1.00- 1.21)	1.51±0.20 (1.25-1.82)	0	7.7±1.06 (6-9)	-	1:2.0±0.0 11:0.8±0.4 12:1.6±0.5 13:1.3±0.6	5.7±1.1 (3-7)	

C. impunctatus Goetghebuer, 1920

Females: Eyes contiguous by more than 1 facet (Fig. 3f), no pubescence. Antennae with sensilla coeloconica on flagellomere 1 and 9-13 (Table 2). Third maxillary palpal segment rhomboid and with palpal sensorium dispersed among multiple shallow excavations (Fig. 3e). Wings with dark spots on a light ground (Figs. 3a-d). Spermathecae ovoid with a short neck (Fig. 3g).

Males: Ninth tergite with apicolateral processes small and inconspicuous (Fig. 3h). Ninth tergite concave. Hypopygium is very similar to that of other species of the subgenus *Culicoides* and the identification of the species is therefore primarily based on the wing pattern (Fig. 3c-d).

C. impunctatus was predominant, accounting for >98% (N= 5548) of all trapped biting midges. It was frequent and widespread on all the islands visited and was recorded from all farms excepting three on Streymoy (St. 8-10) and one on Suderoy (SU 3) (Fig. 1, Table 1). Females accounted for > 97% and > 99% of all *C. impunctatus* trapped outdoors and indoors, respectively. *C. impunctatus* was abundant (5-150 individuals per day) at northern locations on Eysturoy (Ey 2,4-6) and Vidoy (Vi 1), at locations on Vágar (VA 2), Streymoy (St. 3 and 13) and on Sandoy (SAN, 1,2,6), while very few individuals were registered from the locations St. 7 on Streymoy (Table 1).

Table 3. The percentage distribution of *C. impunctatus* females on physiological types inside and outside the byres.

C. impunctatus	Inside	Outside
	nos./%	nos./%
Nulliparous	3858/74.7	318/83.7
Bloodfed	315/6.1	1/0.3
Gravid	81/1.6	3/0.9
Parous	878/17.0	48/12.6
Males	36/0.7	10/2.6

The relative distribution of *C. impunctatus* females on physiological types differed in light trap catches inside and outside the byres. For instance, a greater proportion of nulliparous females was recorded outside the byres, whereas the proportion of engorged females was larger indoors (Table 3).

C. impunctatus was recorded in low numbers in the byres in week 24 (first half of June) and peaked during week 25 (late June) (Fig. 4).



Fig. 4. Relative numbers of *C. impunctatus* indoor and outdoor during the season.

Discussion

Apparently *C. pseudoheliophilus* has not been associated with livestock farms (e.g. Gonzales et al., 2013; Zimmer et al., 2013) and none of the females trapped were blood engorged, all of them being nulliparous. However, the absence of teeth on the maxillae does not mean that the species is unable to feed on vertebrate blood. For instance, *C. albicans* Winnertz, 1852 lacking teeth on the maxillae as well as the mandibles has been reported as anthropophilic (Kremer, 1965; Gonzales, 2013). The biology and ecology of *C. pseudoheliophilus* are unknown. In Belgium it has been found in habitats corresponding to a peaty moor,

characterized by acidic and oxygen-free substrates (Zimmer et al., 2013). The species is recorded from Spain, Portugal, France, Italy, Germany, Poland, Czech Republic, Belgium and Estonia (Mathieu et al., 2010).

Nearly all *C. pseudoheliophilus* have been collected inside the byres, however, the larval habitat of the species is unknown and it is as yet uncertain whether it has been attracted by the odour of the cattle.

C. impunctatus is widespread in the Faroe Islands (Table 1). It is described as a cold-adapted northern Palaearctic species, the limit of its southern distribution being found in the Iberian Peninsula (Rawlings, 1996). A preference for cooler zones may arise since breeding habitats are less likely to dry out in cooler sites and further, lower temperatures favour adult survival (Carpenter et al., 2006). The species is widespread, occurring in sites of boggy and acidic soil in mainland Europe and Asia (Remm, 1988). In northern Europe it is recorded from e.g. Britain (including Orkney) (Campbell and Pelham 1960; Blackwell et al., 1994b, 1999), Denmark (Nielsen, 1964; Nielsen et al., 2014), Norway (Hagan et al., 2000) and Sweden (Nielsen et al., 2010). In southern Scotland the activity period of C. *impunctatus* is May-September, viz. considerably longer than in the Faroes. *C. impunctatus* attacks a broad range of hosts, evidently feeding on a variety of livestock and wildlife in addition to humans (Blackwell et al., 1994a, 1995; Logan et al., 2009). Attacks on man may occur, when alternative livestock or wildlife hosts are rare or absent (Carpenter et al., 2013).

C. impunctatus is described as autogenous, only requiring blood meals after the first batch of eggs is produced (Blackwell et al., 1992). Apparently, the first, autogenous egg-batch is the largest. This is considered to be a selectively advantageous trait in areas of low density of available hosts, and where sites for the development of *Culicoides* larvae are consistently available (Boorman & Goddard, 1970; Linley, 1983). Autogenous Culicoides spp. are known to have short gonotrophic cycles (Linley et al., 1970), in some species as short as two days (Holmes & Birley, 1987). After mating and production of the first egg batches, parous females fly are in search of blood meals (Blackwell et al., 1992). Autogeny would still play an essential role in the maintenance of the species, and in the case of a second (smaller) generation of C. *impunctatus* this would originate primarily from eggs laid by the first generation of nulliparous females. In the Faroes C. impunctatus is considered to be unimodal and univoltine with only one brood per year (Fig. 4). Also in other studies adult C. impunctatus has a relatively short seasonal appearance with peak activity during May and June (light-suction trap surveys: Hill, 1947; Blackwell et al., 1992; Holmes & Boorman, 1987; Takken et al., 2008). Males are only trapped in small numbers compared to the numbers of females, which may reflect that the samplings are carried out at a certain distance from the breeding habitat of the species, or that the males only to a lesser degree are attracted to the byres. Such differences in femalemale ratio are unusual in nature and may be explained by the higher flight activity of females, particularly of light-sensitive parous females (Anderson & Linhares, 1989). In populations of C. impunctatus males apparently emerge before females, which minimizes the time lag before mating and extends the time available for egg laving (Fagerstrom & Wiklund, 1982). In the present study, however, the males were first caught about a week after the females, possibly reflecting a low number of males.

It is noteworthy that the majority of *C. impunctatus* were caught in the byres. This may be attributed to attraction by the odours of livestock, or perhaps owing to the warm and calm conditions in the byres. Already from week 24 blood-filled individuals of *C. impunctatus* are registered in the byres. This suggests that *C. impunctatus* may hatch and complete the autogenous cycle before week 24. It is assumed that the biting midges hatch in the

environment around the courtyard areas and subsequently attracted to the byres. The percentage distribution of the physiological types between traps inside and outside byres differs, the percentage of bloodfed and gravid females being higher within the byres (Table 3). Outside the byres *C. impunctatus* was not observed in light-traps until week 25 (Fig. 4), primarily reflecting a strong attraction to the byres for blood feeding and a low population density in the surroundings. Males are more often caught outside the byres (2,6%) than within (0,7%), which supports the assumption that the male to a lesser extent are attracted by the byres (Table 3).

Worldwide, biting midges are vectors of a number of serious livestock viruses for example Bluetongue (BTV). If BTV is spread to the Faroe Islands, it would be disastrous in particular for the sheep-breeding. *C. impunctatus* is considered a potential vector of BTV, since it is able to support virus multiplication in the laboratory after ingestion (Jennings & Mellor, 1988). However, the risk of BTV dissemination via *C. impunctatus* in the Faroe Islands is presumably minimal. Due to the expression of autogeny in this species, successful transmission of BTV between hosts would require females surviving at least three reproductive cycles for successful transmission between hosts rather than two cycles required in anautogenous species (Carpenter et al., 2013).

Litteratur

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