A key to the larvae of Norwegian species of Dixidae (Diptera)

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This work consists of two parts. At first a short introduction to the study of Dixidae is given, especially the study of the larvae. Then a key is proposed for the larvae of most of the Norwegian and therefore most of the North European species of Dixidae. The larvae of *Dixella borealis* (Martini, 1928), *Dixella naevia* (Peus, 1934), *Dixella hyperborea* (Bergroth, 1889) and *Dixella dyari* (Garrett, 1924) has never been included in any keys before, and a description of the larva of *D. nigra* (Stæger, 1840) is only found in the old work of Martini (1929). The larva of *Dixella laeta* (Loew, 1849) as well as larvae of several more southern species has not yet been described or keyed. Some of the species included in the key have not been seen by the author.

Key words: Diptera, Dixidae, key, Norway.

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Introduction

It is a goal for anyone who studies nature to be able to identify the creatures he or she encounters, including larvae of midges. It is also important that the names give a correct sense of the evolutionary history of the creatures. Within the family Dixidae there is at least one genus that may be split into several parts, namely the genus *Dixella* Dyar & Shannon, 1924 (Belkin 1962). To do this it is necessary to describe the different species in all life stages more accurately and possibly also gather the similar species in species-groups until more species are better investigated. Hopefully this paper may contribute to this end,

The dipterous family Dixidae was in 1990 (Rozkošný, 1990) listed with 54 species in the palaearctic area. Fauna Europaea lists 18 species of *Dixella* from Europe, and 12 species of *Dixa*. In both these catalogues the species *borealis* has been placed in the genus *Dixa* while it certainly belongs in *Dixella*. Two species of *Dixella* has

been shown to be synonyms. These two species were described by Tarwid (1938) from Poland. They are mentioned in the catalogues of Rozkošný, 1990 and in Fauna europaea. Szadziewski (2007) synonymized the two species *Dixa intrudens* Tarwid 1938 = *Dixella serotina* (Meigen, 1818) and *Dixa campinosica* Tarwid 1938 = *Dixella amphibia* (De Geer, 1776). This means that there are only 28 species of Dixidae in Europe. Only 17 species have been recorded in Norway (Peus 1936, Håland 1967, 2013, 2017) but many parts of the country have been poorly investigated.

Dixidae is a small family of nematocerous mosquito-like insects that in the adult stage may superficially resemble a small Limoniid (Limoniidae) or maybe a fungus midge (Mycetophilidae s.l.). In English they are called Meniscus Midges, in German "Palpenmücken" and in Norwegian given the name "U-mygg" because of the habit of the larva to hang like an inverted U on the side of a leaf or stone just above the meniscus. They are most closely related to the Chaoboridae and the Culicidae (especially the larvae resemble the larvae of the Anophelinae), but as adults they can easily be separated from these by their naked wings, only very few hairs on their body, and the lack of sucking mouth parts. The antennae of the males are in Chaoboridae and Culicidae somewhat in the shape of a Christmas tree, while the antennae of the dixids are merely thin threads. The wings will also easily separate the dixids, especially the beautifully bent R_{2+3} from most other nematocereous flies.

This key is mostly an expansion of the key of Disney (1999), and it now covers most of the North European species, except *Dixella laeta* (Loew, 1849) and *Dixella bankowskae* (Vaillant, 1969). The larva of *Dixella goetghebueri* (Séguy, 1921) might also have been incorporated since the larva and pupa were described and figured together with the adult stage (Séguy 1923), but the descriptions give no good characters to separate this species from any of the others. It has been found in France and Poland (see Fauna Europaea). Peus (1934) described a few species from Germany that seems to have a very restricted distribution and whose larvae have not been described.

Morphological characters useful for separating larvae

The larvae of the Dixidae are easily separated from all other larvae of freshwater insects as they are folded up double like an inverted "U". They are fastened to a straw or a stone by stiff hairs and two or three ambulacral plates with series of crooks. In the frontal end the head there are several stiffer hairs and two "legs" with crooks. The respiratory dish lying flat just at the meniscus while the head is submerged and collecting food particles from the underside of the meniscus. The physics of the hydrophilic and hydrophobic structures of larvae in the genus *Dixa* has been studied by Suzuki *et al.* 2021.

The size of the larvae differs, and is dependent both of instar, food quality, the growth during the separate instars, and of species. Measurements of the head can be plotted and will separate the instars from each other (Håland 2009). This is also reflected in the size of the adults. Håland (2009) shows a table (Table 1) where body length and head width has been measured for the larvae of six species of the genus *Dixella*. More species could have been measured too, but the material is not great enough for all species.

The look of the hind end of the larvae is very special, but with some similarities to the larva of Anopheles (Diptera, Culicidae). There are two respiratory openings surrounded by a lot of stiff water repelling hairs, except just between and a little bit anterior of the openings where four different hairs and a small sclerotized plate (IP = interspiracular plate) are doing the job. The plate IP is raised almost perpendicular to the body axis and in some species have a bulge at the anterior/ dorsal edge. Differences in sclerotization and shape is sometimes helpful in determination of species. The basal plate is usually heavily sclerotized, but the borders towards the paddles are weakly sclerotized to allow movement. All the hairs and plates surrounding the respiratory openings can be raised and bent over the opening to hold on to a small air bubble, thus giving the larva some extra air as they dive under water when attacked from above. When attacked from below the larva climb up on the straw or stone and after a short time down to the water's edge again at another place, All the creeping is done with the middle part of the body in front of the movement. The larvae are always surrounded by a thin film of water. In the *Dixa* species there are also on the dorsal side of most of the abdominal segments a crown of hydrophobic hairs that give extra buoyancy, but this makes it more difficult to escape by diving underwater.

The Dixidae have four larval instars. The first instar lasts just a few days and are therefore seldom found, while the older ones become longer and longer, both in body length and duration of the instar. Newly hatched larvae of all instars can be recognized by the colourless, soft and bowed hairs that become stiff and black in a day or two. The last instar can be recognized by the caudal appendage being narrowed near the anterior end, while in the other instars this appendage becomes gradually narrower towards the posterior end. *Dixella nigra* does not have a separation between the distal and

Character/Species	D. aestivalis	D. borealis	D. naevia	D. hyperborea
Basal plate	Short, little sclerotized	Longer, more sclerotized, a darker wedge-shaped area along the middle of the plate	Longer, more sclerotized	As long as broad, more evenly sclerotized on the whole surface
IP	Y-shaped, quite dark all over, except a round area in the middle.	Round, darker on the lateral sides	Round, darker at the posterior edge	Quite like aestivalis
Length of paddles	Long and narrow (I.S.D. = 2.67), reaches far beyond the segmentation of the caudal appendage.	Long and narrow (I.S.D = 2.67), as in aestivalis	Shorter and broader $(I.S.D. = 2,5, does not reach the segmentation of the caudal appendage.$	Shorter and broad (I.S.D. ? 2.38), reaches the segmentation of the caudal appendage.
Dark patch under the lateral hairs on the basal lobe of the paddles.	Long, dark	Long, dark	Long, dark	Shorter than the other three species
The posterior edge of the anal plate	Quite short teeth only on the middle fourth of the posterior edge.	Teeth on a longer part of the posterior edge.	Thinner, longer, dark teeth on the middle of the posterior edge.	The whole posterior edge straight, teeth along all its length.
Caudal appendage	IPD = 2.42	IPD = 2.50	IPD = 2.0-2.2	IPD = 2.91
Subspiracular ventral setae	On a common plate	On a common plate	On a common plate, but this plate is very small	Not on a common plate, like obscura

TABLE 1. Key characters of *Dixella aestivalis* (Meigen, 1818), *D. naevia* (Peus, 1934), *D. borealis* (Martini, 1928) and *D. hyperborea* (Bergroth, 1889).

basal part however, and the distal part is parallelsided, somewhat similar to *D. naevia*.

The terminology of the different body parts is a little confusing, as Disney (1975, 1999) gave one set of names (partly based on Brindle (1959) and others, but different from the work of Sicart (1959), while Peters and Adamski (1982) used yet another set of names. The nomenclature of Disney is used in this work as his books is still a "must" for everyone who wants to study the Dixidae of northern Europe. The key proposed here will often refer to the figures of Disney (1999). Sicart (1959) formulated some indices for the descriptions and to separate some species (Figure 27). This author has not found them so useful, partly because they depend on a flat specimen and partly because the proximal limit for the length measurement of the paddles is not always easy to see, especially in the Dixella species.

The work of Savary (1992) has just recently come to the attention of the author. He mainly used the chaetotaxy of the larvae to identify them, a method that needs good equipment which has not been at the disposal of the author and therefore the method has not been employed here.

In the table the following species possibly but not yet found in Norway have been maintained, namely Dixella graeca Pandazis, Dixa submaculata Edwards and Dixa nubilipennis Curtis. The reason for this was that the more species represented in the key, the more useful it is, also outside the borders of Norway. The author has not seen specimens of these species nor specimens of attica or martinii (reported from Norway by Olsen (2008), and of course not the larva of Dixella laeta as this has not yet been described.

Species groups

The larvae of *Dixella aestivalis*, *D. borealis* and *D. naevia* are very similar, and the differences proposed in the key may not be useful for other populations. Because of this similarity these three species together with *Dixella hyperborea* that also resembles the three first mentioned they can be denoted av the *aestivalis* species group, named after the first described species.

The three species *amphibia*, *filicornis*, and *graeca* clearly belong together in a group that may be named the *amphibia* group.

The species *nigra* is different from all the other species in Norway and seems to be a group of its own, the *nigra* group. However, this author found two similar larvae (last instar) during a short trip to Japan in the autumn of 1988. These larvae were notably bigger than the Norwegian species and probably belong to a species not yet described. They were found in a pond near a hotel to the north of Kobe.

Material and methods

The larvae studied for this work all stems from the authors private collection. The larvae have been stored in 70-80% alcohol. A few of the larvae has been cut in two halves (dorsal/ventral or right/left) with a scalpel, and then put in 10% KOH until suitable for mounting in Euparal. In this way one avoids the disturbance of the picture from body parts from the other half of the larva.

The photographs were taken partly with a digital microscope, partly with a camera placed over the ocular of a microscope.

The insects were studied under a binocular microscope, the permanent mounts under a microscope.

Where possible larvae from localities where only one species was found were used. Since several species often occurs together, this is not easy to find.

How and where do the larvae live?

A good introduction to the biology of the larvae is given by Nowell (1951). The books of Disney (1975, 1999) also give good information on biology, collecting, preserving and making permanent slides etc.

The larvae filter nutritious particles from the meniscus and are thus small "cleaning stations". They are always on or very close to some emergent objects, living or dead vegetation or stones or man-made objects floating in the water. They pupate just a few centimeters above the water's edge, with the last larval pelt lying just beside the pupa (Dixella amphibia may pupate in or so close to the water's edge that the larval pelt can be found on the water surface). The Dixa species prefer running waters while the Dixella species prefer still waters, but they can be found together in slowly moving waters. The pupal stage lasts 2-5 days dependent on temperature. Most (possibly all?) the species of Dixella have 2 generations per year (Håland, 2009) in southern Norway. Dixella nigra will hatch in the end of May and in July/ August, while i.e., D. borealis will have adults late in June and in September/October in southern Norway. Dixella nigra and Dixella amphibia will spend the winter in last larval instar and therefore be ready to fly earlier in the spring than the others, and guite soon after the ice cover disappears. Both these two last mentioned species prefer or tolerate dystrophic waters.

The different species are also segregated by habitat characteristics. Dixella borealis is mostly found in shadowy habitats, ponds and puddles, D. aestivalis prefers more sun and is also found higher in the mountains, up to 1000 m asl in southern Norway. It can be found almost everywhere, and has even been found in a small water collection on a tarpaulin with one cm water on it. Dixella hyperborea seems to be found only in small ponds that dry out at least a few summers. D. dyari is found in high lying ponds or in slow flowing parts of streams, from 650 up to 1200 m. asl., sometimes together and almost as high up as D. obscura but this last species seems to prefer more nutrients in the waters. At the coast of Helgeland (southern part of Nordland County) obscura was

very common, even on small islands far out from the shore, in small ponds surely influenced by sea salt. *D. autumnalis* was also found in a small pond just 2 meters above sea level, in southern Norway. *D. amphibia* can be found in slightly dystrophic to eutrophic ponds, while *D. nigra* seems to prefer the dystrophic ones. The larvae of the *Dixa*-species live in running waters, *D. nebulosa* has been found in the slower flowing and bigger streams, while the larvae of *dilatata, puberula* and *maculata* are found in smaller and more turbulent streams.

How to capture and study the larvae

It is usually easy to find larvae of Dixidae if present. About 2-liter plastic boxes or smaller, square and light coloured, can be used to capture them. When pressed down into the emergent vegetation in a ditch or pond or along a stream it will be filled with water, debris and insects. The larvae of Dixidae will then swim in their characteristic fashion (Brackenbury 1999) to the walls of the box, and hang as an inverted "U" along the edge of the meniscus. The author has used a stamp collector's forceps with broad ends, and the larvae readily climbs up on it as unto the walls of the box. Sometimes egg laying females or newly hatched adults, and also pupae may follow the water into the box. All stages of the Dixidae are best preserved in 70-80% alcohol.

The larvae may also be taken back to the laboratory alive in a medium sized jam jar. They can endure several hours of driving, as long as the temperature in the car is not much over 20°C, Rearing is best done at about 16°C, to avoid fungi that may kill the larvae. Take also a jar or two of water from the larval habitat so that the water can be changed. Artificial feeding of the larvae has not been done by the author, so without change of water only later stages of the last instar will probably hatch, within a week or at most two weeks. If the adult is hatched from a larva/pupa it is imperative that the larval pelt, the pupal pelt and the adult lies in the same vial, and all vials must be labeled with the necessary information.

When a larva is killed in alcohol, the body will

straighten out and the head will be bent so that it's a bit difficult to see the dorsal side of the larva as it will usually tip over to one side. The use of a pin will put some weight on the larva enough to make it lie as wanted. It is then much easier to make measurements with an eyepiece-micrometer, i.e., to measure the head width and thereby to see which larval instar the specimen may be in.

Key to the larvae

Martini (1929) was the first to make an attempt to identify larvae of European dixids including 8 species but no key. Later Brindle (1959) made the first key for 9 British species in the same year as Sicart (1959) made one for 9 species and one "subspecies" living in southern France. The last one was Disney who made a new key for the 13 British species in 1975, adding one more species in a paper (Disney 1992) and incorporating it in a new key in 1999 now with 14 species. The present key is adding 5 more species from Northern Europe. It quotes Disney (1999) word for word except where it is deemed necessary to change it to insert one or more new species.

Three abdominal segments (5–7) with ventral combs (VC in Disney), or if the comb on segment seven is reduced (or even absent) then midline length of basal plate is approximately equal to width (Figure 1 and e.g. Figure 3 in Disney). 2
 Only two abdominal segments (5 and 6) with ventral comb (VC, Figure 4 in Disney). Midline length of the basal plate is less than half breadth (Figure 2) *Dixa puberula* Loew, 1849

2. Abdominal segments without crowns of hairs. *Dixella* Dyar and Shannon 1924.
Five or six abdominal segments with dorsal circular "crowns" of feathered hairs (Figure 3), but this can be reduced in *Dixa puberula*. *Dixa* Meigen 1818 (2)

 crowns of feathered setae 5

..... Dixa dilatata Strobl, 1900

5. Posterior paddles relatively narrow (Figure 5, Figures 6F and 7B of Disney). Microtrichia (the minute hairs) adjacent to the pairs of fine setae on ventral face of head (situated directly behind the bases of the maxillary palps) *relatively short*, dark and mostly evenly spaced (Figure 6C of Disney).

..... **Dixa maculata** Meigen, 1818 and **Dixa nubilipennis** Curtis, 1832 (Note: So far no reliable means of separating the larvae of these two species has been discovered,)

– Posterior paddles relatively broad (Figures 6E and 7B of Disney). Microtrichia adjacent to the pair of fine setae on ventral face of head *relatively long*, pale and unevenly spaced (Figure 6D of Disney) *Dixa submaculata* Edwards, 1920

 12. Each pair of sub-spiracular ventral setae are

15. The basal plate as in Figure 13, but quite variable in outline, usually with a dark zone on the middle of the hind edge, often narrower (as a band shorter in the animals length direction) than in *borealis* and *naevia*. ID more oblong, often sclerotized in a kind of V or Y-shape (Figures 13 and 21), in the other two species it is usually more circular. The antennae light, same colour or a little darker than the head, with a hair brush near the apical end. The body usually light yellow brown.

18. Antennae with fewer spinules along shaft (Figure 11L in Disney). Three anal setae (AS, Figure 4 in Disney) typically without any fusion of their basal platelets, but occasionally a shadowy platelet is discernible below the integument. Small hairs of ventral face of posterior portion of caudal appendage frequently absent. Tail end of larva as in Figure 25 and in Figure 12A in Disney. Dixella autumnalis (Meigen, 1838) - Antennae with more spinules along shaft (Figure 11M in Disney). Typically at least two of each set of anal setae arise from a common platelet (Figure 11J in Disney). Small hairs of ventral portion of caudal appendage typically from a continuous median band. Tail end of larva as in Figure 12B in Disney). Dixella attica (Pandazis, 1933)



FIGURES 1–7. 1. *Dixa puberula*. Ventral view showing combs. 2. *Dixa puberula*. Dorsal view of the terminal segments. 3. *Dixa nebulosa*. Dorsal view of the larva showing the crowns and the terminal segments. 4. *Dixa maculata*. Dorsal view of terminal segments. 5. *Dixa dilatata*. Dorsal view of terminal segments. 6. *Dixella nigra*. Dorsal view of terminal segments (without caudal lobe). 7. *Dixella amphibia*. Dorsal view of the terminal segments.



FIGURES 8–14. 8. *Dixella filicornis*. Dorsal view of central part of the basal plate. 9. *Dixella dyari*. Antenna. 10. *Dixella dyari*. Inter-spiracular plate (IP). 11. *Dixella dyari*. Lateral view of terminal end of body. 12. *Dixella aestivalis*. Lateral view of terminal part of larva. 13. *Dixella aestivalis*. Dorsal view of the terminal segments. 14. *Dixella aestivalis*. Subspiraculaire ventral hairs.



FIGURES 15–21. 15. *Dixella obscura*. Subspiracular ventral hairs. **16.** *Dixella obscura*. Dorsal view of terminal end of the body. **17.** *Dixella obscura*. Detail of interspirakular plate (IP). **18.** *Dixella naevia*. Basal plate. **19.** *Dixella hyperborea*. Basal plate. **20.** *Dixella borealis*. Basal plate. **21.** *Dixella aestivalis*. Interspiracular plate.



FIGURES 22–26. 22. *Dixella borealis*. Interspiracular plate. 23. *Dixella naevia*. Interspiracular plate. 24. *Dixella hyperborea*. Dorsal view of terminal end of body. 25. *Dixella borealis* Dorsal view of terminal end of body. 26. *Dixella autumnalis*. Dorsal view of terminal end of body.

FIGURE 27. Indices proposed by Sicart (1959). I.A.L. (1) for the spiracular appendage (SA); I.P.L. (2) for the caudal appendage (CA) and I.P.D. (3) for the paddles.



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Håland: A key to the larvae of Norwegian species of Dixidae

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