Notes on the importance of sexing Gammaridae for identification: using *Dikerogammarus* as an example

Version 1

August 2014

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Published by:

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Cambridgeshire, PE28 4NE
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1. Introduction

The recent arrival of *Dikerogammarus villosus* and *haemobaphes* within the UK has drawn extra attention to the identification of individuals belonging to the Gammaridae family. Their relatively new appearance has required freshwater taxonomists to become familiar in using identification guides that highlight important and distinguishing morphological characters used in making a species determination. An important consideration that should be acknowledged, when identifying species of Gammaridae, is to know whether you have a male or female. This is because adult males have morphological features that are less variable than their female counterparts and are seen to be more diagnostic in leading to positive species identification. Hence the reason why morphological keys for Gammarids have been primarily developed for adult males, for e.g. Gledhill et al (1993), Müller et al (2002) and Özbeck and Özkan (2011).

The purpose of this note is to highlight the importance of sexing Gammarid specimens and to show that females, and indeed juveniles for that matter, can be more variable and/or may not show distinct features that have been highlighted in keys intended for adult males.

2. How to sex a Gammarid individual

Although sexual dimorphism is present in many Gammarid species, e.g. males having a longer body length, larger gnathopod size and hairier antennae, these external differences are not necessarily distinct or reliable enough to sex an individual. To sex an individual, this requires looking at the ventral surface of the body where the legs are attached (known as the pereon). Mature males have a minute pair of penial processes or genital papillae that are positioned between the last pair of legs on thoracic segment 7. This is shown in Figure 1 below. In order to see papillae the pleopods (swimmerets) may need to be folded back.

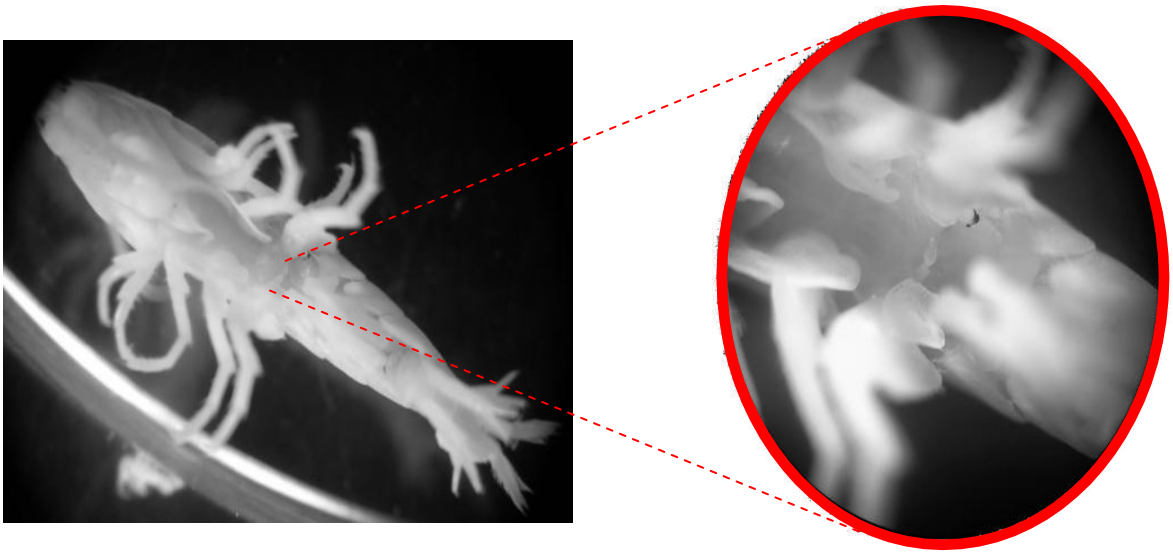

FIGURE 1 – Location of genital papillae on male gammaridae. Images: ©Drew Constable (2013).

In contrast breeding females possess brood plates (known as oostegites) which are located at the bases of coxal plates on thoracic segments 2-5. It is important that they are not confused with the gill plates, which are also located in the same area. The brood plates are positioned on the insides of the gills and are distinguished by their long fringing hairs (setae), which are used to hold the eggs, acting as a brood chamber (see Figure 2a-d).

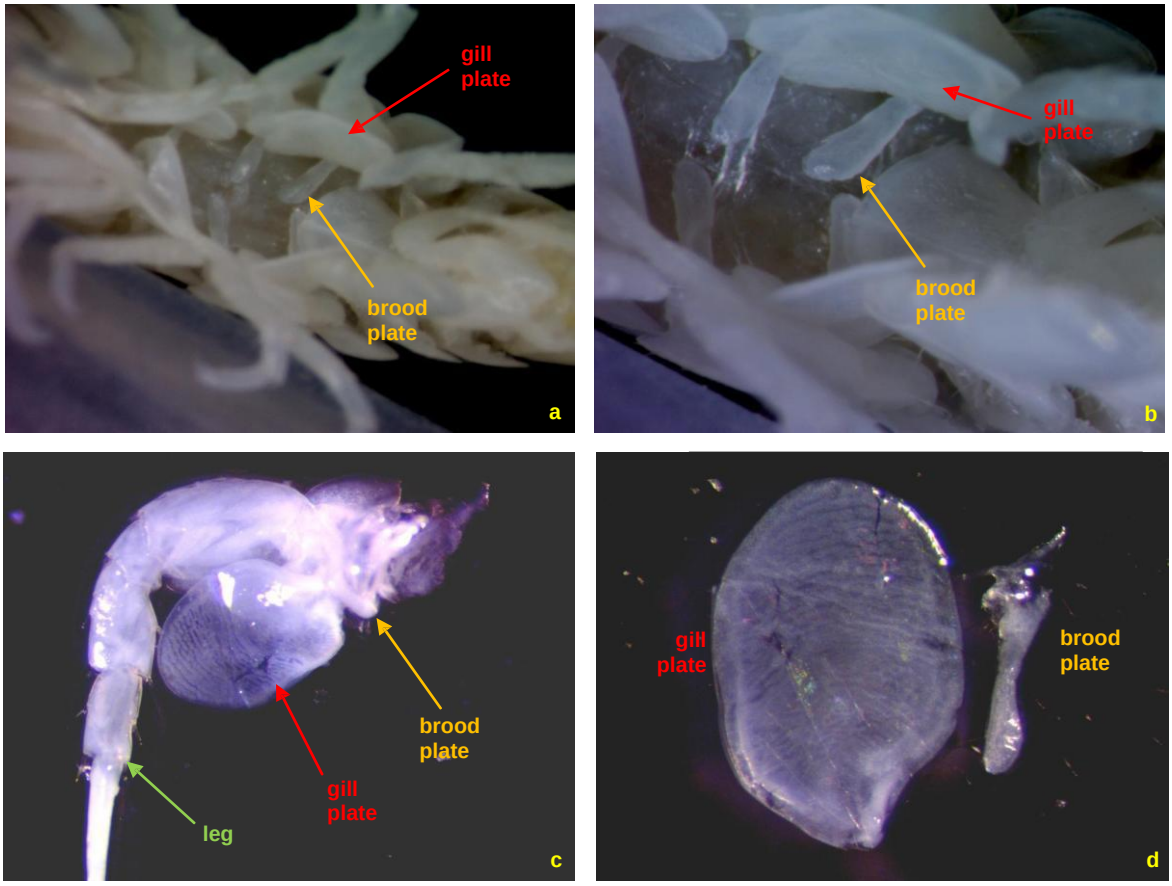

FIGURE 2a-d – (a) Location of brood plates (known as oostegites) on female gammaridae; (b) Close up of Figure 2a, note hairs on brood plate; (c) Removed section of female gammarid showing location of brood and gill plate in relation to the leg; (d) Removed gill and brood plate for size and shape comparison. Images: ©Drew Constable (2013).

According to the FBA crustacean key by Gledhill et al. (1993) the sexes of Gammarids are distinguishable when they are still quite small (3-5mm), when the female brood plates and the male penial processes are small but not fully formed. It still may however be difficult to discern this with such small individuals, specimens >6mm can be sexed with more ease.

3. Examples of female Gammarid variation using *Dikerogammarus*

Now being able to sex a Gammarid individual, this part of the technical note illustrates the importance of using mature male specimens for Gammarid identification using specimens of *D. villosus* and *D. haemobaphes*.

One possible reason why *Dikerogammarus* may be difficult to separate is because a female has been encountered that has morphological features which do not sufficiently fit the description given for the adult male of the species. This has been seen in some female individuals of *D. villosus* which have two spines on their urosome projections, and would therefore fit the description of *D. haemobaphes* or perhaps *D. bispinosus*, potentially leading to a misidentification if solely relying on this feature. This is portrayed in Figure 3a-d, which shows two female *D. villosus* having the same spine and setae arrangement as *D. haemobaphes*. A notable difference however between the species is the shape of the conical projections, which are distinctly high for *D. villosus*. The key point to note here is that it is not necessarily uncommon for female *D. villosus* to have less than 3 spines and a ring of setae on each urosome projection.

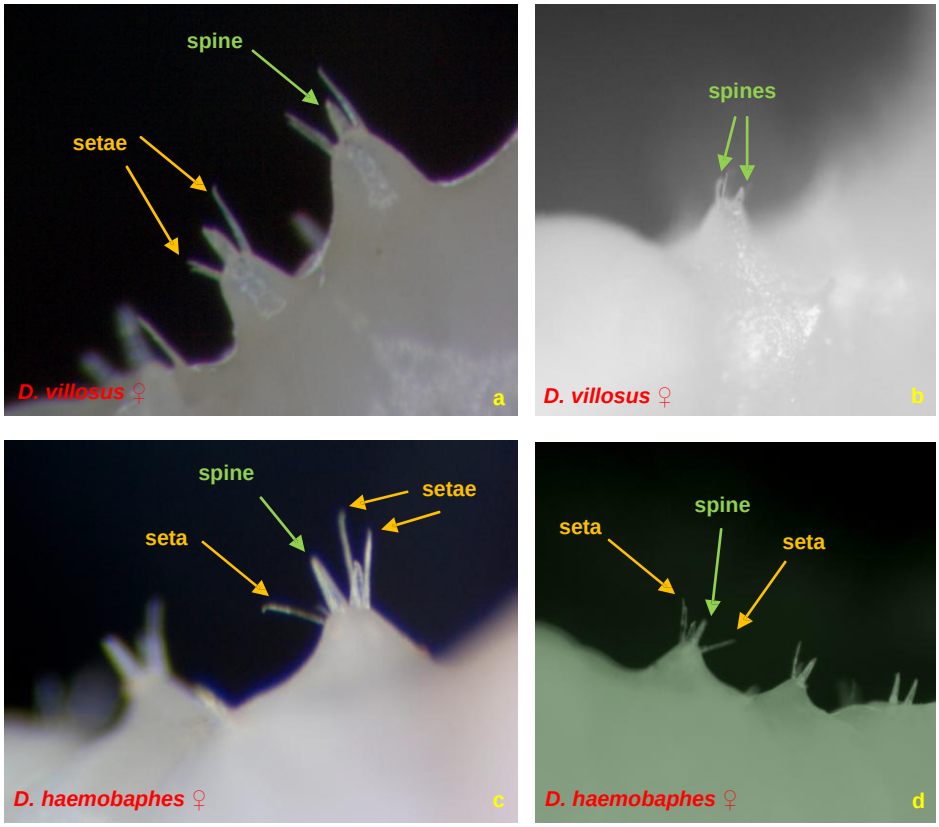

FIGURE 3a-d – (a) Urosome 1&2 of female *D. villosus* each having two spines and a ring of setae depicting the description of *D. haemobaphes*; (b) Another *D. villosus* female with urosome 1 having two spines; (c) A female *D. haemobaphes* showing two spines and ring of setae arrangement, urosome 1 in foreground; (d). Another example of Figure 3(c), urosome 1 with arrows. Images: ©Drew Constable (2013).

Another morphological female feature that may not fit the adult male description is the hairiness of the antennae. For differentiating between the *Dikerogammarus* species, *D. villosus*, *D. bispinosus* and *D. haemobaphes*, the setation (hairs) on antenna 2 can be used. *D. villosus* is described as having dense and long setation on the flagellum (a series of small segments of the antennae; see Figure 4a for location) of antenna 2. However, looking at some female *D. villosus*, this would not be considered the case. Figure 4a below shows a direct comparison of antenna 2 between a female *D. villosus* and *D. haemobaphes*, with the hairiness of the flagellum of the former looking like a typical sparsely hairy *D. haemobaphes* antenna, which is known to have short setation (hairs) on the flagellum. Also shown in Figure 4b is a male and female antenna 2 of *D. villosus* to show the difference that can be observed between the sexes, with the female flagellum being a lot less hairy than its male counterpart. Care should therefore be taken when using female antennae as an identification feature.

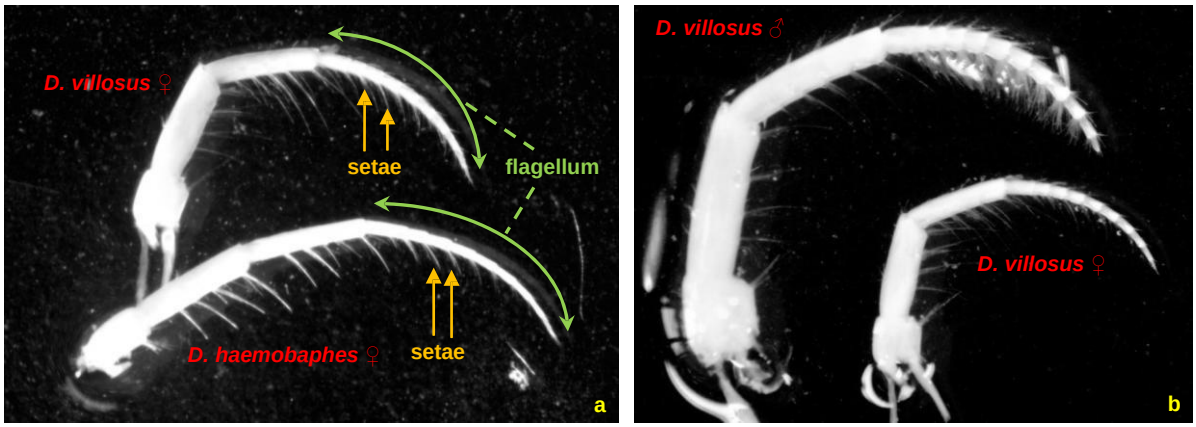

FIGURE 4a-b – (a) Antenna 2 of a female *D. villosus* and *D. haemobaphes*, notice how *D. villosus* has sparse tufts of setae like *D. haemobaphes*; (b) An antenna 2 of a male and female *D. villosus*, note the much hairier flagellum of the male. Images: ©Drew Constable (2013).

4. Additional points for identifying *Dikerogammarus*

Having highlighted the need to sometimes be careful in using female gammarids for species determination, there are also other additional points to be aware of when identifying *Dikerogammarus*, which do not necessarily relate to morphological differences between the sexes.

For taxonomists who may have not seen many *D. villosus* and/or *D. haemobaphes* individuals, there could be difficulty in determining the relative hairiness of the flagellum on antenna 2 of males. The identification terms used for this characteristic describe *D. villosus* as having long, dense hairiness and *D. haemobaphes* as having short, sparse hairiness. Figure 5a-b shows a male flagellum of antenna 2 for both species. Without viewing both together one may judge that the flagellum of Figure 5b (*D. haemobaphes*) is dense and long and potentially think that the feature resembles *D. villosus*. However, when comparing *D. haemobaphes* to Figure 5a (*D. villosus*) it puts the relative hairiness in to perspective. A further point to add with respect to this couplet is from the German amphipoda key by Eggers and Martens (2001), which describes that the setae of antenna 2 of a male *D. villosus* as being longer than the flagellum segments. In the case of Figure 5b below, the antenna 2 of *D. haemobaphes* could also be described as displaying this feature. It therefore might be worth considering that the setae are notably longer than the flagellum for *D. villosus* and that some *D. haemobaphes* may have setae that are at least or slightly longer than the flagellum segments.

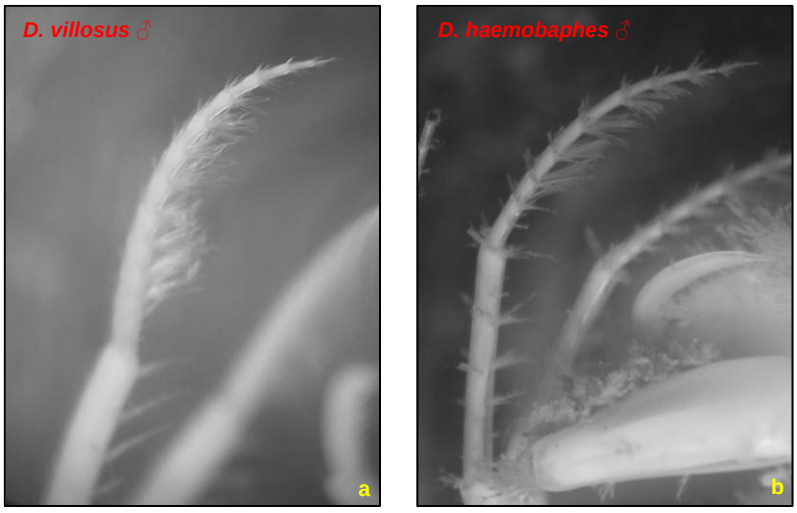

FIGURE 5a-b – (a) Flagellum of antenna 2 of a male *D. villosus*; (b) Flagellum of antenna 2 of a male *D. haemobaphes*, note the much hairier flagellum of *D. villosus*. Images: ©Drew Constable (2013).

Similarly to the hairiness of antenna 2, the relative shape of the urosome projections to an inexperienced eye may cause confusion. Figure 6a-b shows the urosome of a male *D. haemobaphes* and *D. villosus*. The conical projections for *D. haemobaphes* are longer than high, which conforms to the identification description for the species, but to an inexperienced analyst the projections could be mistakenly viewed as being markedly high, which is in fact the description given for *D. villosus* and *D. bispinosus*. However, when viewing the urosome projections of these different species side by side, as in Figure 6a-b, there is an immediate appreciation of their relative shapes.

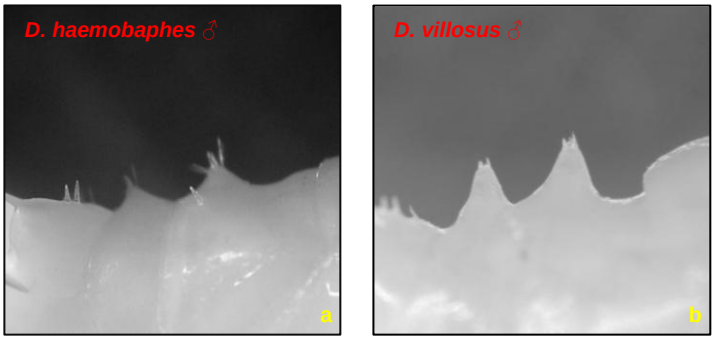

FIGURE 6a-b – (a) Urosome of a male *D. haemobaphes*; (b) Urosome of a male *D. villosus*, note the much more pointed urosome of *D. villosus*. Images: ©Drew Constable (2013).

Another identification feature to consider is being able to differentiate between what is a spine and a seta (hair) on the urosome projections. Spines are thickened setae that are less flexible and are easily broken when bent, whereas setae can be bent in the middle. It is therefore important to be aware that spines can break off and give the appearance of a projection having fewer spines than they otherwise should. Figure 7 shows a projection on urosome 1 of a male *D. haemobaphes* and shows the relative thickness of spines in relation to setae.

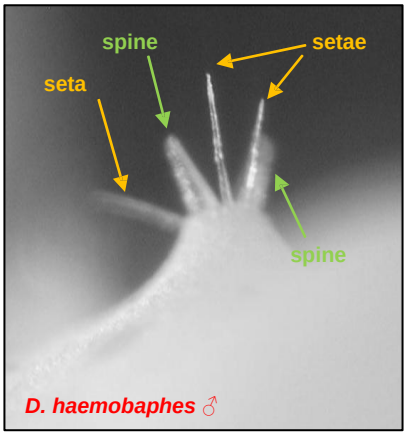

FIGURE 7 – Urosome 1 of a male *D. haemobaphes*. Image: ©Drew Constable (2013).

Juveniles are another potential issue when it comes to identification. Juvenile gammarids are more difficult to identify as the characterising traits are less apparent with them typically being poorly developed. Figure 8 shows three juvenile gammarids. The top specimen is a *Gammarus pulex*, the middle a *D. haemobaphes* and the bottom specimen is a *D. villosus*. From a distance they may be difficult to tell apart, but there are discernible features that can at least separate the two genera. For *G. pulex*, the short oval shaped eye, the lack of urosome projections and the location of urosome spines at the end of each segment should be enough to tell it apart from species of *Dikerogammarus*. However, separating the *Dikerogammarus* juveniles is likely to be more problematic and would come down to experience of having seen many before. *D. villosus* juveniles often appear to have more prominent urosome projections like the adults but obviously at a much reduced scale. They can also sometimes appear to be more robust and bigger built than *D. haemobaphes*, although in Figure 8 this is not obviously apparent.

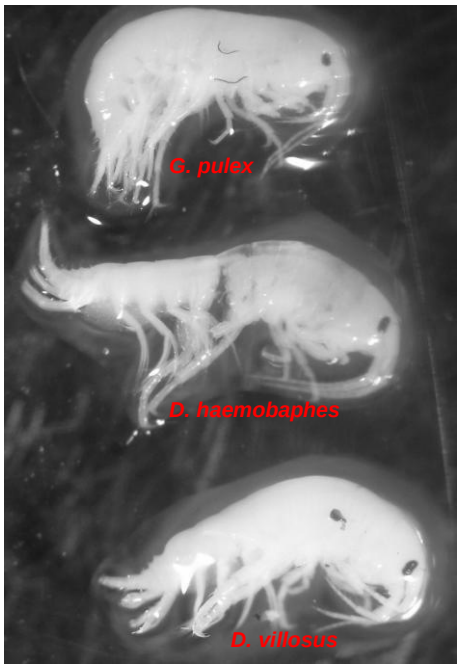

FIGURE 8 – (Top to bottom) a juvenile *Gammarus pulex*, *D. haemobaphes* and *D. villosus*. Image: ©Drew Constable (2013).

5. Concluding remarks

This technical note has highlighted the potential difficulties when not using adult male gammarids for species identification. In addition to knowing the sex of a gammarid, it is also important that when identifying unfamiliar specimens, in this example *Dikerogammarus*, that all morphological characteristics and descriptions are consulted to help determine a positive species identification.

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