

**FEMALE GENITALIA AND COPULATION
OF THE PLANTHOPPER
HYALESTHES OBSOLETUS SIGNORET
(HEMIPTERA : FULGOROMORPHA : CIXIIDAE)**

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Résumé. – Accouplement et morphologie de l'appareil génital femelle de *Hyalesthes obsoletus* Signoret (Hemiptera : Fulgoromorpha : Cixiidae). – L'étude des genitalia femelles du genre *Hyalesthes* (Cixiidae) à ovipositeur court, montre que tant les structures gonocoxales que gonaphysiennes ont subi un phénomène de réduction, par rapport au genre *Cixius* à ovipositeur long. La paroi de la bourse copulatrice présente des ornements circulaires, connus chez de nombreux Fulgoromorphes. Ces différenciations cuticulaires sont vraisemblablement liées à des formations glandulaires. La dissection de spécimens in copula montre également que, durant la copulation, des différentes structures de l'appareil génital mâle sont introduites dans le vagin : l'édéage, le périandrium, ainsi que le processus latéral gauche du pygofer.

Abstract. – Female genitalia morphology in the cixiid genus *Hyalesthes* - with a short ovipositor - is described in comparison with another long ovipositor cixiid genus *Cixius*. In *Hyalesthes*, gonocoxal structures and not only gonapophysian ones are reduced. The circular sclerotized ornamentations of the bursa copulatrix wall, widely distributed in the Fulgoromorpha, seem to be linked to glandular units. Dissection of *Hyalesthes obsoletus* Signoret in copula shows that not only the aedeagus and the perianthrium are inserted into the vagina but also the left lateral process of the pygofer.

The Southwest Palaearctic cixiid genus *Hyalesthes* consists of more than thirty species the revision of which has been undertaken by HOCH & REMANE in 1985 and completed by HOCH (1986a, 1990) and REMANE & HOCH (1986). In these studies, monophyly of the genus was established and at least five monophyletic groups of species were recognized within it. *Hyalesthes obsoletus* Signoret, 1865, is the type species of the genus and belongs to the obsoletus group restricted mainly to the Northern Mediterranean countries (HOCH, 1986b).

H. obsoletus is known for several years as a major vector of the stolbur phytoplasma (previous MLO, mycoplasma-like organism), a wall-less bacteria located in phloem. This bacteria causes serious diseases in many crops : tomato, tobacco, grapevine, etc, both in European and Mediterranean countries (SUCHOV & VOVK, 1948 ; FOS et al., 1992 ; MAIXNER 1994 ; SFORZA et al., 1996, submitted). In France, adults of this monovoltine species is observed from May to August with an abundance peak around the end of June. In France, bindweed (*Convolvulus arvensis* Linné) and lavender (*Lavendula angustifolia* Mill.) were first

recognized as host-plants (LECLANT, 1968; Fos et al., 1992). Recently, hoary cress (*Lepidium draba* Linné) was also added to this list (SFORZA & BOURGOIN, 1996; SFORZA et al., submitted). Several other host-plants are known in other countries (GUCLU & OZBEK, 1988; HOCH & REMANE, 1985).

The engagement of male and female genitalia during copulation is poorly documented in Fulgoromorpha and concerns only few genera in the cixiids: *Cixius* (WEBER, 1930) and *Hyalesthes* (HOCH & REMANE, 1985), and in the delphacids: *Stenocranus* (ASCHE, 1985) and *Prokelisia* (HEADY & WILSON, 1990). These observations were summarized in BOURGOIN & HUANG (1991) who gave also information for the tettigometrid genus *Tettigometra* and provided a more complete description of the copulatory mechanism in the ditrysian trodudid genus *Trypetimorpha*.

The present study provides a description of the female genitalia of *Hyalesthes* which bears a short regressed ovipositor, with special attention paid to the sclerotized ornementations (BOURGOIN, 1993) of the bursa copulatrix in different species. The engagement of male and female genitalia during copulation is described for the species *H. obsoletus* to complete the description given by HOCH & REMANE (1985).

Materials and methods

Female genitalia were examined from dried or alcohol preserved specimens of *Hyalesthes obsoletus* Signoret and *H. luteipes* Fieber. This last species represents another monophyletic group of species recognized by HOCH & REMANE (1985).

Engagement of male and female genitalia was investigated by dissection of 5 pairs of *H. obsoletus* in copula according BOURGOIN & HUANG's materials and methods already published (1991). Two stains were used: chlorazol black E and Paragon Blue (BOURGOIN, 1993). Drawings were made using a camera lucida with specimens placed in glycerine. For morphological studies using scanning electron microscopy (SEM), insects were first cleaned using an ultrasonic cleaner by soaking specimens in a chloroform bath (3 min) and then cleaned twice in 70% alcohol or distilled water (2 min). Specimens were then dehydrated through increasing concentrations of alcohol, critical-point dried and coated with a 65-70 µm film of gold-palladium. They were examined using a Jeol JSM 840 scanning electron microscope. Terminology for male and female genitalia follows BOURGOIN & HUANG (1990) and BOURGOIN (1993).

OBSERVATIONS

Female genitalia

Segmental and appendicular structures (figs. 1-2)

In *H. obsoletus*, gonocoxae VIII (*Gx VIII*) are almost elliptical and feebly sclerotized plates. Each gonocoxa is linked ventrally to a more or less quadrangular plate pointing anteriorly, the endogonocoxal lobe (*GxL*). Each one bears the reduced and membranous gonapophysis VIII (*Gy VIII*). In its inner side, the anterior fibula of the gonapophysis VIII is short, feebly marked, and probably not functional.

Posteriorly, the gonocoxae IX are rod-like. Gonapophyses IX are short and membranous, not fused on their inner margins. Gonoplacs (*Gp*) are represented by two processes of rounded section not envelopping the gonapophyses. A wide wax gland area closes posteriorly the genital area. Internally (fig. 2) gonocoxae IX are divided by the fulcrum (*F*) into an anterior part bearing the gonapophyses IX and a posterior part developing into the gonoplacs. On each side, two strong apodems for attachment of the retractor muscle (*rM Gy IX*) and of the extensor muscle (*eM Gy IX*) of gonapophysis IX are observable. Dorsally to the anterior part of each gonocoxa IX, the gonangulum is limited to a thin rod-like sclerite. It joins anteriorly the gonapophysis VIII.

Complex of the ectodermal genital ducts (figs. 2-3)

H. obsoletus is monotrysic and the complex of the ectodermal genital ducts opens externally by the gonoporus via an enlarged vestibulum. The vestibulum (*Vst*) is limited anteriorly by a short triangular sclerotized sternal plate (fig. 1, *St VIII*) and posteriorly by a

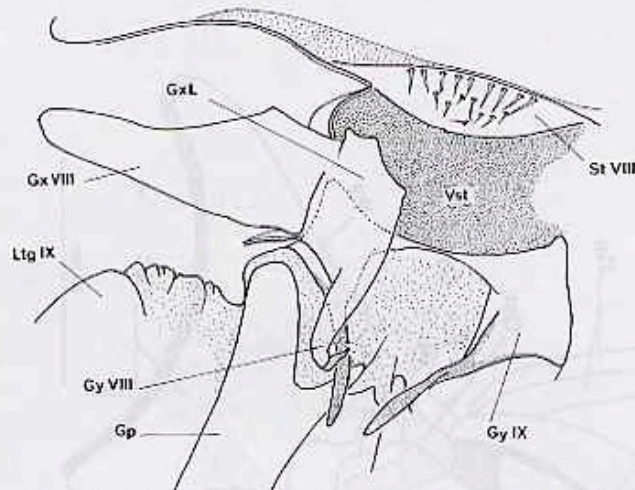


Fig. 1. *Hyalesthes obsoletus* Signoret, female genitalia ventral view. *Gp* : gonoplaques ; *Gx VIII* : gonocoxa VIII ; *GxL* : endogonocoxal lobe ; *Gy VIII* : gonapophysis VIII ; *Gy IX* : gonapophysis IX ; *Ltg IX* : laterotergite IX ; *St VIII* : Sternite VIII ; *Vst* : Vestibulum.

more or less quadrangular plate, the basal apodem (*BA*). The vestibulum opens widely into the posterior vagina (*VP*) which bears in its anterior part a wide bursa copulatrix ductus (*BCd*) opening into the bursa copulatrix (*BC*). Limits between the posterior and the anterior (*VA*) vagina are poorly marked. Anteriorly, the anterior vagina (*VA*) receives a long spermatheca ; and the common oviduct (*CO*). As in most fulgoromorphs, the spermatheca is divided into orificium receptaculi (placed ventro-laterally), ductus receptaculi (*Dr*), a wide and long diverticulum ductus (*Dd*), a short pars intermedialis (*Pi*) and the glandula apicalis (*GIA*) of which only the cuticular ducts are observable.

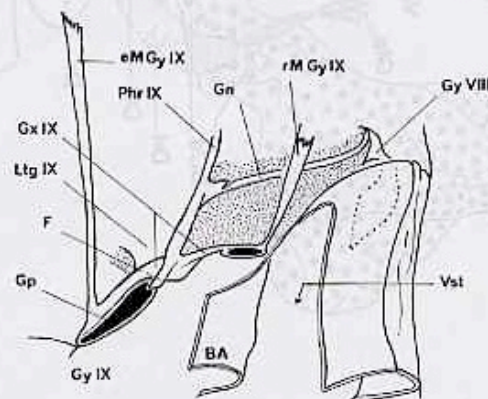


Fig. 2. *Hyalesthes obsoletus* Signoret, female genitalia internal semi-diagrammatic view (left side). *BA* : basal apodem ; *eMGy IX* : extensor muscle of gonapophysis IX ; *F* : fulcrum ; *Gn* : gonangulum ; *Gx IX* : gonocoxa IX ; *Phr IX* : phragme IX ; *rMGy IX* : retractor muscle of gonapophysis IX ; *VP* : posterior vagina. Other as in previous figure.

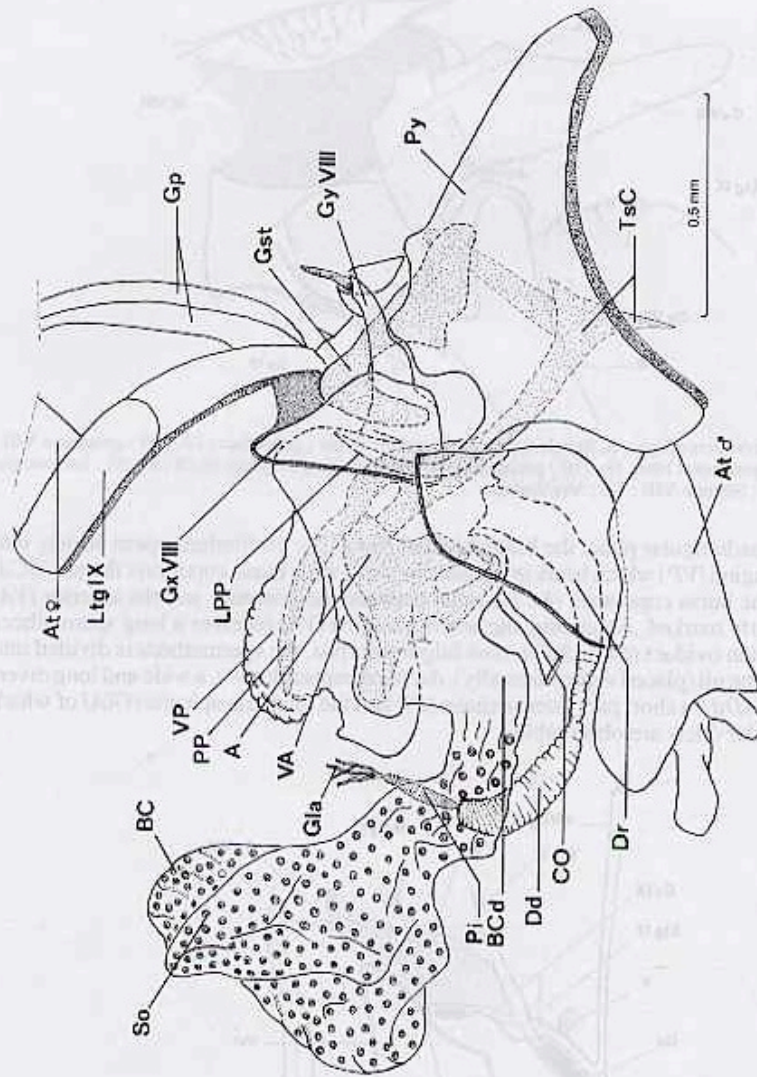


Fig. 3. *Hyalasthetes obsoletus* Signoret, in copula (male aedeagus, connective and gonostyli in grey). *A*: aedeagus s.l.; *At*: anal tube; *BC*: bursa copulatrix; *BCd*: bursa copulatrix ductus; *CO*: common oviduct; *Dd*: diverticulum ductus of the spermatheca; *Dr*: ductus receptaculi of the spermatheca; *Gla*: glandula apicalis of the spermatheca; *Gst*: male gonostyli; *LPP*: left lateral process of the pygote; *Pi*: pars intermedialis of the spermatheca; *PP*: periaandrium process; *Py*: pygote; *So*: sclerotized ornamentations; *TsC*: tectiform structure of the connective; *VA*: anterior vagina. Other as in previous figures.

Sclerotized ornamentations of the bursa copulatrix (fig. 4)

As in many fulgoromorphs small sclerotized ornamentations (*So*) are present in the wall of the bursa copulatrix. In *Hyalesthes obsoletus* these ornamentations are distributed on the whole wall of the bursa copulatrix. They are rounded structures of 15 μm in diameter limited externally by a sclerotized ring. The median part of the ring is a multimicro-punctulated cuticular area as shown by small white spots. In *H. luteipes* these sclerotized ornamentations were not observable.

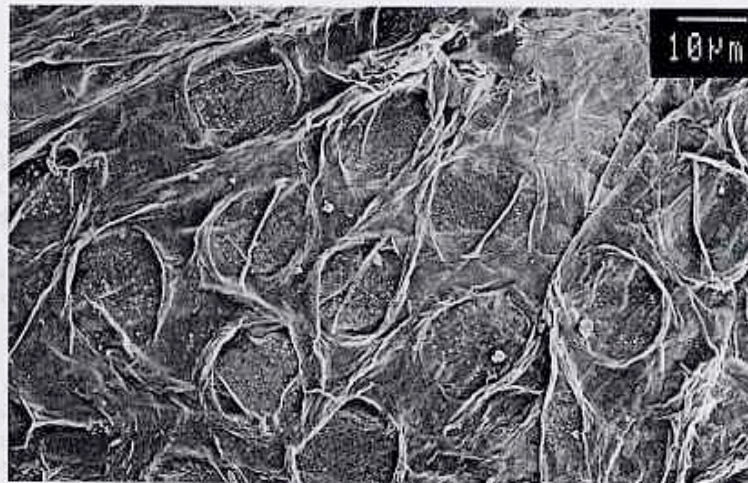


Fig. 4. *Hyalesthes obsoletus* Signoret. Sclerotized ornamentations of the bursa copulatrix, external view, x 1100.

Copulatory mechanism (fig. 3)

During copulation male and female are side by side but facing in opposite direction; male is slightly inclined in comparison with the female. If they have to move, it is the female which leads the pair during the displacement. This copulatory position involves the twist of the male genital segments. When specimens in copula are killed, the abdominal twist is lost and one of the specimens faces dorsal while the other remains facing ventral. Contractions of the abdomens were observed. For two copula observed, duration of the mating were about forty minutes.

During mating, the male anal tube (*AtM*) is turned up and applied on the abdominal sternal area of the female. The male genitalia in *Hyalesthes* are everted and both the aedeagal and perianthrium structures are inserted together into the female vagina. The perianthrium process (*PP*) is located in the dorsal part of the vagina (posterior vagina) and the aedeagus (*A*) into the anterior vagina but it does not enter into the bursa copulatrix. The male gonostyli (*Gst*) are inserted between the female gonapophysis. Together with the genitalic structures, the lateral processes of the pygofer are also inserted inside the vestibulum and the longer left process (*LPP*) reaches the posterodorsal part of the vagina.

DISCUSSION

Female genitalia in *Hyalesthes* show some slight differences from the description given by BOURGOIN (1993) for another cixiid genus, *Cixius*. These differences are related to the reduction of both gonocoxan and gonapophysian structures linked to an invagination of the sclerotized plates surrounding the gonoporus. For instance reduction of gonapophyses

IX is linked with the absence of their fusion on their inner sides, the absence of the intergonocoxal plate and of the postgonapophysian fold. The basal apodem which is considered as a synapomorphy for the fulgoromorphs with an orthopteroid-type ovipositor (BOURGOIN, *op. cit.*) is also strongly reduced, feebly sclerotized and shifted inside the body closing posteriorly the vestibulum. Internally, apodems of muscles are also comparatively strongly reduced. This is particularly the case for the extensor muscle of gonapophyses IX which apodem is rod-like while it is wide and fringed in *Cixius*. These morphological modifications have to be related to the fact that *Hyalesthes* lays the eggs on the soil surface or in the ground close to the plant crown but does not insert them into the host-plant.

- In the fulgoromorphs, the sclerotized ornamentations of the bursa copulatrix show a great diversity in their conformation. They are probably present in all the fulgoromorph families but not in all the species (BOURGOIN, *op. cit.*). They are present in *H. obsoletus* while they seem to be absent in *H. luteipes* or they could not have been observed with the technics used here. They have also been mentioned and illustrated in some Derbidae by YANG & WU (1993) (*Proustita* Kirkaldy, *Shizuka* Matsumura, *Neoproustita* Yang & Wu, *Pamendanga* Distant, *Helcita* Stal, *Zeugma* Westwood). In Tettigometridae they are generally present excepted in some Hildinae (BOURGOIN, 1985). In Meenoplidae their absence seems to be autapomorphic for the New-Caledonian genus *Suvanisia* (BOURGOIN, 1997).

The sclerotized ornamentations present in the wall of the bursa copulatrix correspond much probably to glandular units - but they may be associated also to a sensory function - as it has already been histologically described by BOULARD (1965) in cicadas (Cicadomorpha). The numerous microstructures of the median area are probably the place through which secretions are discharged. But what could be the role of such glandular units ?

In Fulgoromorpha spermatozoa are generally deposited at the bursa copulatrix basis and they must be transferred secondarily into the spermatheca (SOULIER-PERKINS & BOURGOIN, submitted). One possible role for these structures should be to allow the spermatozoa migration thanks to chemical secretions. Another role should be to destroy spermatozoa in excess as it has been already shown by BOULARD (1990) in cicadas where the dorso-vaginal pouch (= bursa copulatrix) has a glandular function and a destructive role of spermatozoa in excess. This should be also the case in the Fulgoromorpha even if this has never been studied. However the role of these structures remains unclear : what happens when they are absent ? A another possible role should be that they are connected to the presence of a spermatophore. While spermatophores are generally considered as absent in Hemiptera (TUZET, 1977 ; MAILLET, 1959) a spermatophore has been described by STRÜBING (1955) in the dictyopharid *Dictyophara*. It has also been observed in the derbid *Diostrombus* (BOURGOIN, unpublished). In this last genus the bursa copulatrix is divided into two successive rooms. The spermatophore is stored in the first basal one which shows sclerotized ornamentations in its wall while they are absent in the second room. One could make the hypothesis that these structures could inform the insect on the presence of the spermatophore and allow its destruction thanks to glandular secretions. Clearly an ultrastructural study of these glandular-sensory structures, their role after deposit and storage of spermatozoa and the occurrence of spermatophores in Fulgoromorpha would need special attention and investigation.

- The copulatory position observed here is close to the description given by HOCH & REMANE (1985) but no information was provided about the male and female engagement and the relative positions of genital structures. To maintain the copulatory position between male and female during copulation, these authors have observed that the paired asymmetric spine-like processes of the pygofer come to settle in the membranous sternal fold between

the sternite VIII and the gonocoxa VIII. However in the different pairs in copula studied, the pygofer processes are also inserted inside the female genitalia, with the genitalic structures. This difference is probably imputable to the moment when the pair has been killed during copulation. Engagement involving not only genitalic structures but also processes of the pygofer is important to note and will have to be analysed in the frame of the hypothesis of short cuts of mating behaviour suggested by SOULIER - PERKINS & BOURGOIN (submitted). Internally, the study shows that the bursa copulatrix is never reached by the aedeagus which is located only into the posterior vagina during copulation. Such behaviour seems to be the most common within the Fulgoromorpha (SOULIER-PERKINS & BOURGOIN, *op. cit.*).

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