

Identification and biological traits of a planthopper from the genus *Pentastiridius* (Hemiptera: Cixiidae) adapted to an annual cropping rotation

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Abstract. Cixiid planthoppers have been shown to vector phloem-limited prokaryotes associated with plant diseases world-wide. In eastern France, an emerging disease of sugar beet called syndrome “basses richesses” has been associated with phloem-restricted bacteria transmitted by a cixiid planthopper within the genus *Pentastiridius*. Early investigation suggested the species being *Pentastiridius beieri*. On the basis of a morphological and phylogenetic study we report the identification of the planthopper as *Pentastiridius leporinus*. Furthermore we report some biological traits of the species, which shows a surprising ecological adaptation to an annual cropping rotation sugar beet-winter cereals.

INTRODUCTION

The family Cixiidae Spinola, 1839 (Hemiptera: Fulgoromorpha) includes about 160 genera comprising almost 2000 phytophagous planthopper species distributed world-wide (Holzinger et al., 2002; Ceotto & Bourgoin, 2008). Generally, cixiid planthoppers spend a significant part of their life cycle underground as immature stadia feeding on host plant roots; whereas adults feed and reproduce on the aerial parts of their host plants. In temperate regions, most of cixiid planthoppers have been reported as univoltine, overwintering as nymphal stages underground. However, detailed information on their ecology and biology is restricted to a few pests such as *Hylalesthes obsoletus* Signoret (Leclant, 1968; Sforza et al., 1999; Sharon et al., 2005; Johannesen et al., 2008) that transmits stolbur phytoplasma to a number of cultivated plant species (Fos et al., 1992; Maixner, 1994; Sforza, 1998). However the emergence and re-emergence of vector-transmitted diseases associated with plant pathogenic prokaryotes have recently promoted investigation on these fulgoroids that include an increasing number of potential vector species (Gatineau et al., 2001, 2002; Bogoutdinov, 2003; Danet et al., 2003; Jovic et al., 2007).

In eastern France, an unidentified cixiid planthopper within the genus *Pentastiridius* has been shown to transmit both a stolbur phytoplasma and a phloem-restricted γ -3 proteobacterium (called SBR bacterium in

this paper) to sugar beet (Gatineau et al., 2001, 2002). The disease has been termed syndrome “basses richesses” for the reduced sugar content in infected beet tap roots (Richard-Molard et al., 1995). Previous research has shown that SBR bacterium is the most important pathogen causing syndrome “basses richesses” disease, while stolbur phytoplasma has a marginal etiological role (Sémétey et al., 2007a). *Pentastiridius* sp. has consistently shown to be the major vector of SBR bacterium as populations can reach very high densities in sugar beet fields (Gatineau, 2002; Sémétey, 2006; Bressan et al., 2008) with high rates of SBR bacterium infection (Sémétey et al., 2007a; Bressan et al., 2008).

The planthopper species was tentatively identified as *Pentastiridius beieri* Wagner, 1970 (Gatineau, 2002). However, further morphological examination rejected this identification (unpubl.), and we have referred to this species as *Pentastiridius* sp. (Gatineau et al., 2001, 2002; Sémétey et al., 2007a, b). On the basis of recent investigations we have assigned and used the name *Pentastiridius leporinus* (Linnaeus, 1761) for the cixiid planthopper spreading SBR bacterium in eastern France (Arneodo et al., 2008; Bressan et al., 2008; Bressan, in press; Bressan et al., in press). However, demonstration for this taxonomic assignment has not yet been published.

To date, three *Pentastiridius* species of the subgenus *Pentastiridius* s. str. are known from Europe: *P. beieri*, *P. leporinus* and *P. spinicoronatus* Dlabola, 1988 (Emel-

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TABLE 1. Collection sites, host plants, and accession numbers of cytochrome oxidase gene I and II sequences from cixiid planthoppers examined.

Planthopper species	Collection site, region (Country)	Host plant	Sequence accession numbers
<i>Hyalesthes obsoletus</i>	Roncá, Veneto (Italy)	<i>Urtica dioica</i>	FN179291
¹ <i>Pentastiridius leporinus</i>	Russia	Unknown	FN179288
<i>Pentastiridius beieri</i>	Valbelle, Upper Savoy (France)	<i>Salix</i> sp.	FN179290
<i>Pentastiridius</i> sp.	Fenay, Burgundy (France)	<i>Beta vulgaris</i>	FN179289
<i>Reptalus cuspidatus</i>	Roncá, Veneto (Italy)	<i>Artemisia vulgaris</i>	FN179292

¹Kindly provided by D.Z. Bogoutdinov.

janov, 1995). *Pentastiridius leporinus* was described from Sweden and is distributed throughout Europe, near East, Middle and East Asia, and Northern Africa (Anufriev & Emeljanov, 1988). *Pentastiridius beieri* was described by Wagner (1970) from Italy and Austria. Further records originate from France, Germany, Poland, Slovenia, Switzerland and Ukraine (Günthart, 1987; Holzinger & Seljak 2001; Holzinger et al., 2003; Nast, 1977; Nickel et al., 2002; Nickel, 2003; Remane & Fröhlich, 1994). *Pentastiridius beieri* is distinguishable from *P. leporinus* only by the shape of the aedeagus (Wagner, 1970; Holzinger et al., 2003). *Pentastiridius leporinus* has been recorded to feed mainly on *Phragmites australis* (Nickel, 2003; Holzinger et al., 2003; Anufriev & Emeljanov, 1988) while *P. beieri* feeds on various shrubs and tall herbs (*Salix*, *Alnus*, *Myricaria*, *Tripleurospermum*) (Wagner, 1970; Holzinger et al., 2003). The third species, *P. spinicoronatus* is known from Italy (Emilia province) only. According to Dlabola (1988), the shape of the male gonostyles is unique in the genus *Pentastiridius*. Dlabola's (l.c.) drawings indicate similarities on the shape of the aedeagus between *P. spinicoronatus* and *P. beieri*, but they showed clear differences with *P. leporinus*.

In this work we analyzed the morphology of genitalia from *Pentastiridius* sp., the major vector of bacteria associated with syndrome "basses richesses" disease (Gatineau et al., 2001, 2002; Séméty et al., 2007a, b). Because literature has reported detailed studies only on male genitalia of planthoppers within the genus *Pentastiridius*, here we have exclusively studied male genitalia. Furthermore, based on mitochondrial DNA sequences, we have conducted a phylogenetic analysis to examine the evolutionary relationship of *Pentastiridius* sp. from eastern France with reference specimens of *P. leporinus*, *P. beieri*, and related cixiid planthoppers from the tribe Pentastirini.

We also report a number of observations on the biology of this insect including adults and nymphs distribution and abundance across wheat and sugar beet crops, showing that *Pentastiridius* in eastern France displays a surprising life cycle adapted to the cropping rotation, sugar beet-winter cereals.

MATERIAL AND METHODS

Morphological and phylogenetic study

We collected several specimens of *Pentastiridius* sp. from numerous sugar beet and winter cereal (wheat or barley) fields in Burgundy and Franche-Comté regions of France between 1999 and 2005. Representative specimens have been deposited

at the museum of Natural History (Paris, France) under reference numbers: MNHN(EH)-3573 through MNHN(EH)-3632. Additional *P. leporinus* and *P. beieri* males from the collections of the Natural History museums in Vienna and Linz (Austria), and from the Oekoteam collection (Graz) were studied morphologically. These specimens originated from Marismas near Santona, Santander (Spain), Holstein, Oldesloe (Germany), Feldkirch/Vorarlberg (Austria), Rödschitzer Moor near Mitterndorf (Austria), Gutenstein (Austria), Grado (Italy), Skyros (Greece), Sari-Tzelek (Kirghizia).

Male genitalia were isolated from individual males after immersion and dissection of the anal segment in 10% KOH solution for about 10 h at room temperature. Male genitalia were used for morphological identification by comparison with taxonomic keys available for the genus *Pentastiridius* (Wagner, 1970; Dlabola, 1988; Holzinger et al., 2003). Drawings of male genitalia from specimens of *Pentastiridius* sp. were made by using a stereoscopic microscope (Nikon SMZ-U) and a binocular (Wild and Olympus SZH10) with a camera lucida.

For phylogenetic analysis, in addition to specimens of *Pentastiridius* sp. collected from Burgundy and Franche-Comté regions, we included specimens of *H. obsoletus*, *P. beieri*, *P. leporinus*, and *Reptalus cuspidatus* (Fieber 1876). Sites for insect collection, and host plants are reported in Table 1. To reconstruct the phylogeny of these specimens, we sequenced cytochrome oxidase subunits I and II (COI and COII). For DNA isolation insects were individually processed with a cetyltrimethyl ammonium bromide (CTAB) procedure according to Gatineau et al. (2001). For each species considered, we obtained independent sequences from three individual insects.

To obtain sequences from COI and COII, we first amplified in PCR assays a fragment of about 800 bp from gene COI with primer pair C1J 2183-TL2N 3014 (Simon et al., 2006). A longer fragment that included a portion of COI, a tRNA for leucine, and a portion of about 550 bp from gene COII was obtained with PCR amplification with primer pair C1J 2441-C2N 3661 (Simon et al., 2006). Reactions were performed in a final volume of 20 µl, containing 2 µl of DNA template, 0.375 µM of each primer, 1 unit of *Taq* DNA Polymerase (BioRad, Hercules, Ca, USA) and 1 mM MgCl₂. PCR conditions for C1J 2183-TL2N 3014 primer pair were a predenaturation step at 94°C for 1 min, followed by 35 cycles at 94°C for 60 s, 50°C for 60 s, and 72°C for 90 s. The final extension step was at 72°C for 300 s. For C1J 2441-C2N 3661 primer pair, conditions were similar except for annealing at 55°C for 90 s. PCR products were visualized under UV light after electrophoresis of 5 µl of the amplified reaction on 1.2% agarose gel stained in ethidium bromide. Sequencing from both 5' and 3' end of purified PCR products were performed by MWG GmbH (Ebersberg, Germany). For host phylogeny analysis, overlapping sequences C1J 2183-TL2N 3014 and C1J 2441-C2N 3661 were combined to obtain a unique sequence of 1311 bp for each insect analyzed, which included 717 bp for COI, 64 bp for a tRNA for leucine, and 528 bp for COII. Obtained sequences were deposited in GenBank

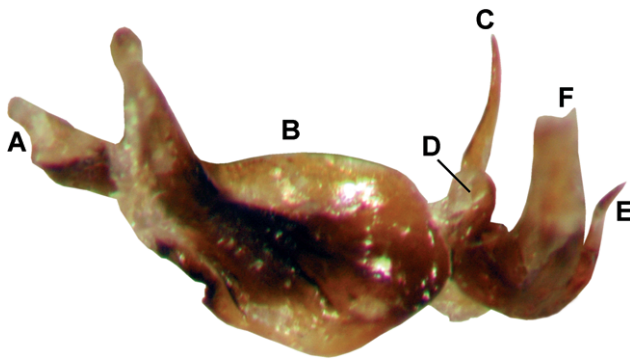


Fig. 1. Aedeagus of *Pentastiridius leporinus* on left dorso-lateral view. Specimen from Fenay, Bugundy-France. A – base of aedeagus, B – sclerotized plate of phallosome, C – right basolateral spine of flagellum, D – bulbous process, E – basomedian spine of flagellum, F – Flagellum. Note: The left basolateral spine of the flagellum is in front of F, but out of focus. The long spine at the base of the phallosome is on the opposite side and thus not visible.

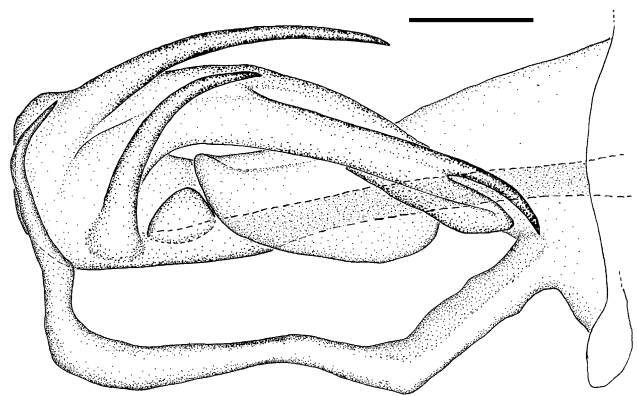


Fig. 2. Aedeagus of *Pentastiridius leporinus* on dorsal view. Specimen from Verjux, Bugundy-France. Scale bar: 0.1 mm.

(Table 1). As an out-group species for the phylogenetic reconstruction, we used sequences obtained from *H. obsoletus*. Multiple sequence alignments were generated by using CLUSTAL X (Thompson et al., 1997) and the alignments were then inspected and edited manually.

A tree representing the similarity relationships among sequences was obtained by using the Neighbor-Joining method (Saitou & Nei, 1987) with Kimura two-parameters corrections. For the clustering analysis we used MEGA software, version 4.1.

Insect biology

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In order to study temporal distribution of adults, we selected two sampling sites in the Burgundy region (France). Each sampling site included a wheat field next to a sugar beet field. Furthermore in the year before the sampling, both wheat fields had been sown following sugar beets that had been affected by syndrome “basses richesses” disease, suggesting *Pentastiridius* sp. planthoppers had colonized the crop. One site was located nearby Saint Aubin village, while a second site was nearby Agencourt village; they were sampled in 1999 and 2000 respectively. Planthoppers were sampled weekly from April through October on both wheat and nearby sugar beet fields by using a D-vac apparatus. Each sampling comprised aspirations of 5 min along field transects, repeated three times in randomly selected locations within the field sites. The number of *Pentastiridius* sp. planthoppers caught into the mesh was recorded for each aspiration.

In 2005 we tested whether adults could emerge from cereal fields following sugar beets planted in the previous year. Therefore we sampled from the Burgundy and Franche-Comté regions 21 cereal fields (either wheat or barley) that followed sugar beet crops planted in the previous year, and additionally as control, we sampled 20 cereal fields (either wheat or barley) that followed any other crop than sugar beet. Selection of fields was facilitated using maps produced yearly by ITB (Institut Technique de la Betterave industrielle), providing information on the spatial distribution of sugar beet fields across the two regions. All fields were sampled in a temporal window of two weeks starting on the last week of June. During this temporal window, a high number of adults can emerge from cereal fields (Gatineau

et al., 2001; Bressan et al., in press). Samplings were restricted to a single date and were carried out as described above, except that D-vac aspirations were replicated four times.

Fecundity

We analyzed the reproductive status of planthopper females colonizing a sugar beet field located nearby Chissey village (Franche-Comté region). Starting June 13, 2005, we sampled weekly adults on sugar beets using a D-vac apparatus. Captures were carried out as previously described. Females were segregated from D-vac meshes and placed on a caged potted sugar beet seedling and transported to the laboratory. Females were then transferred to 1.5 ml tubes and frozen at -80°C until they were dissected to analyze ovaries.

Abdomens from females were immersed in a 0.85% sodium chloride solution and dissected under a stereomicroscope with forceps and pins. Dissected ovaries were divided into 3 categories: previtellogenic, vitellogenic, and postvitellogenic. Previtellogenic insects were defined as bearing ovarioles with no evidence for growing oocytes, vitellogenic females as bearing ovarioles with growing oocytes, and postvitellogenic females had fully developed and chorionated eggs, or lacked any egg, suggesting that they had oviposited and the vitellogenic process was completed. To analyze the potential fecundity, we counted the number of forming eggs within ovarioles in a sub-sample of 30 vitellogenic individuals.

Nymphs

Sugar beet field that had been sampled for females in spring and summer were resampled for nymphs in September before sugar beet harvesting. At that time nymph size is enough to allow their visual localization (Gatineau, 2002; Séméty, 2006). We randomly selected either isolated sugar beet plants (without neighbor sugar beets either on the rows or on the inter-rows) or non isolated plants (with neighbor sugar beets). Roots from selected plants were carefully removed with their surrounding soil using a spade and the interface between root and soil was carefully inspected for nymphs. For each plant analyzed the number of nymphs, their feeding sites and their depth below the ground level, expressed in centimeters, were recorded. Sampling for nymphs was carried out in the same field during the following spring (May 2006) at which time it was cultivated with wheat sown in October 2005, after sugar beet harvest. We counted nymphs in randomly selected soil removal of about 1250 cm^3 (50 cm^2 -surface and 25 cm -depth) that included wheat roots. The soil was carefully dug out with a spade and put into a basket, where it was gently disaggregated and the number of nymphs and their depth below ground level were recorded.

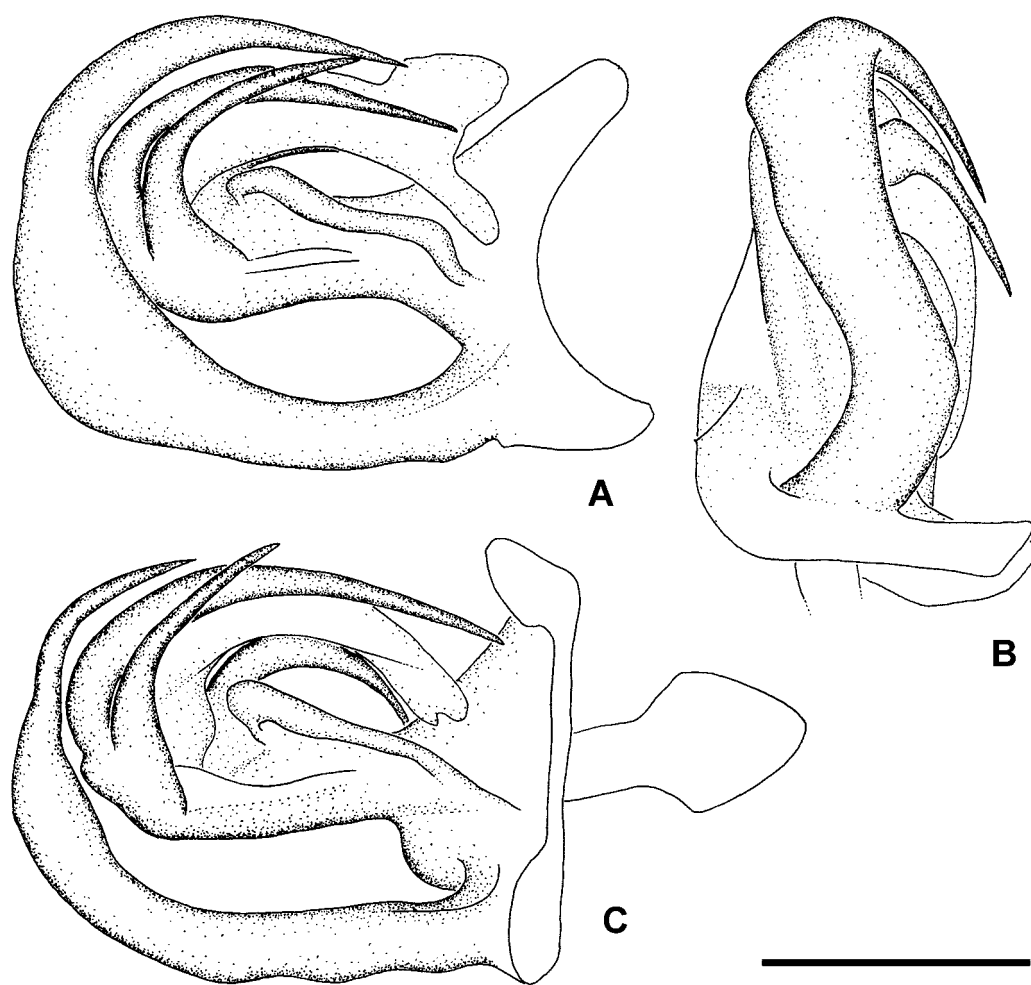


Fig. 3. Aedeagus of *Pentastiridius beieri* on dorsal (A, C) and right lateral views (B). Specimen from Friuli, Italy (A), and from Kalsdorf near Graz-Austria (B, C). Scale bar: 0.25 mm.

Nymphs from both sugar beet roots and wheat roots were collected and transferred to small mesh-sealed containers with pieces of sugar beet tap root obtained from greenhouse-maintained seedlings. Nymphs were maintained in a growth chamber ($23 \pm 2^\circ\text{C}$ and 16L : 8D) and allowed to develop and emerge by supplying them periodically with fresh sugar beet tap root pieces. Emerging males were identified as described above.

Statistic analysis

Number of emerging adults across cereal fields, eggs inside vitellogenic females, and nymphs on sugar beet roots or on soil removals were reported as means with their standard deviation (SD). Despite several data transformations, some datasets were not normally distributed owing to high frequencies of zeros in certain treatments. Therefore differences between mean number of emerging adults across cereal fields, and mean number of nymphs from isolated versus non-isolated sugar beet roots, were tested using a Mann-Whitney U nonparametric test (SigmaStat version 3.5, Systat Software, Inc.).

RESULTS

Morphological and phylogenetic study

A morphological analysis of genitalia dissected from 40 *Pentastiridius* males collected from 1999 through 2005 from the Burgundy and Franche-Comté regions revealed that all of them had genitalia structures very similar to

those described in previous literature for *P. leporinus* (Wagner, 1970; Vilbaste, 1971; Logvinenko, 1975; Ossianilsson, 1978; Holzinger et al., 2003) and distinct from the genitalia of *P. beieri*.

The aedeagal complex of *Pentastiridius leporinus* consists, as in many cixiid planthoppers, of a rigid basal part called phallosoma (periandrium) and a movable apical part called flagellum Fig. 1 (see also Fig. 15b in Holzinger et al., 2003). The phallosoma is tubular and approximates in length 5 times its diameter. Laterally to the base of the phallosoma, a long, slender, rigid spine originates. This spine is ovoid in cross section and directed caudad, parallel to the phallosoma, until the end of the latter, undulating. On the level of the end of the phallosoma the spine is rectangularly bent towards the phallosoma and then peaked upwards (dorsad), pointing to the anal segment (Fig. 1).

The tubular phallosoma consists of a strongly sclerotized plate situated dorsolaterally, opposite to the spine described above, and its tissue is less sclerotized completing the tube around the (inner) basal part of the aedeagus s. str.. This plate ("B" in Fig. 1) forms the two prominent keels of the phallosoma: The smaller one emerges dorsally and is bent to the right, towards the

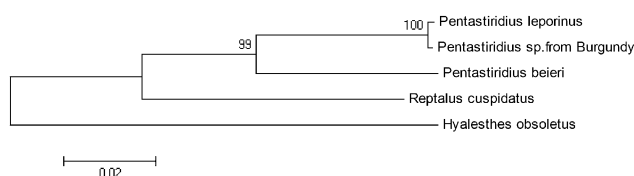


Fig. 4. Neighbor-Joining tree of cytochrome oxidase subunits I and II from cixiid planthoppers within the Tribe Pentastirini. Phylogram was constructed upon the alignment of a common portion of 1311 nucleotides. Nodes report bootstrap values after 10,000 replications.

basal spine; it reaches from the base of the phallosome to half its length. The second is a larger ventrolateral ridge and bears a little hook directed cranial at its ventrobasal margin.

Three movable spines are situated at the base of the flagellum: A large, almost straight one caudad, and two distinctively smaller, curved ones on both sides, respectively (Fig. 2). In addition, a sclerotized bulge leads from the left lateral spine to the right one, ending in a bulbous process situated cranial of the right lateral spine. The flagellum is slightly flattened dorsoventrally. A small, almost straight spine emerges subapically. The gonoporus is large and ovoid (Fig. 1).

The basal spine of *P. beieri* is about four to five times thicker than the spine of *P. leporinus*, and it is twice as thick in lateral view as in dorsal view (Fig. 3). It is never

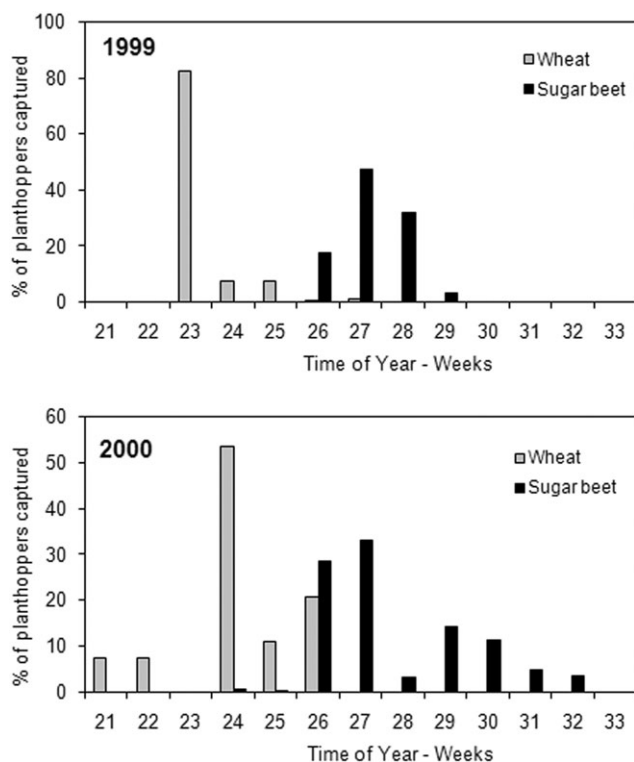


Fig. 5. Weekly abundance of *Pentastiridius leporinus* in wheat and sugar beet fields from Burgundy region. Captures were carried out by using a D-vac aspirator and are expressed as percentage of insects captured in wheat and sugar beet fields, respectively. Time has been expressed as week number from the beginning of each year.

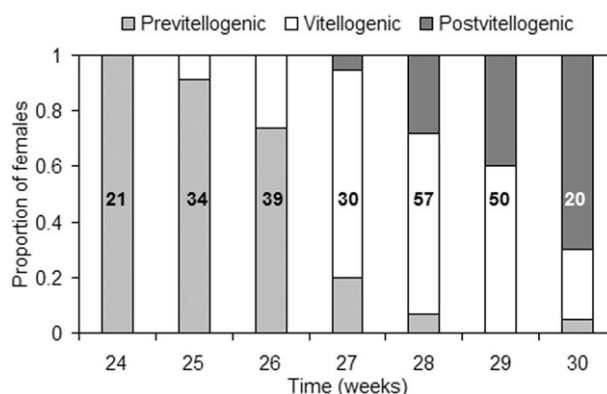


Fig. 6. Bars representing proportion of previtellogenic, vitellogenic, and postvitellogenic females of *Pentastiridius leporinus* collected from sugar beets over time. Within each bar the number of females analyzed are reported. Time has been expressed as number of weeks from the beginning of the year.

bent rectangularly, but more or less evenly curved, its tip also pointing upwards (Fig. 3). Among the 40 *Pentastiridius leporinus* specimens examined from the Burgundy and Franche-Comté regions and other sources, both length and shape of the basal spine and of the spines of the flagellum showed distinct variability (see Wagner, 1970). Also, the size of the dorsolateral plate of the phallosome and thus the size and shape of the two ridges of the phallosome are variable. Anyhow, this morphological variability seems gradual and within the range of intra-specific variability. Thus we suggest the diagnostic characters presented by Wagner (1970) for separation of *P. beieri* from *P. leporinus* are still appropriate.

For clustering analysis we obtained mitochondrial DNA sequences of 1311 bp from all 15 individuals analyzed. Sequences obtained from individuals belonging to the same species were identical and they were merged. Fig. 4 shows a neighbor joining tree constructed with the mitochondrial sequences obtained. It shows that *Pentastiridius* sp. from Burgundy and Franche-Comté regions is very similar to *P. leporinus* specimens from Russia. The genetic distance between these specimens (0.2) is within the range of intraspecific variation (Roe & Sperling, 2006), while mutational differences with *Pentastiridius beieri* (7.8) are at levels of interspecific DNA divergence for COI and COII genes in insects (Roe & Sperling, 2006).

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Fig. 5 reports insect captures on wheat and nearby sugar beet fields. For both surveyed years peaks of planthopper captures on wheat preceded the peaks on sugar beet fields. Furthermore the appearance of planthoppers in sugar beets was associated with a decrease in populations from wheat fields, suggesting that adults migrated from wheat to the neighbouring sugar beet fields. D-vac sampling of emerging adults from cereal fields revealed higher densities on crops that followed a sugar beet crop than on those that did not follow sugar beet. Captures

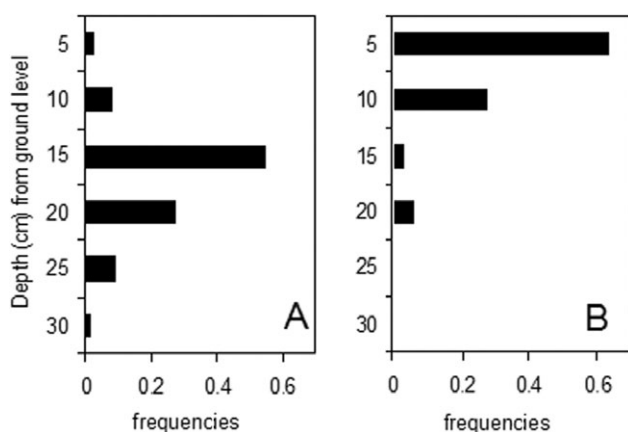


Fig. 7. Underground distribution of *Pentastiridius leporinus* nymphs on sugar beet tap roots in September before harvesting (A) and wheat roots in May of the following year before adult emergence (B). Frequencies were calculated from a total of 89 and 33 nymphs from sugar beet tap roots and wheat roots, respectively.

averaged at 8 ± 15.80 (SD) and 0.4 ± 0.82 (SD) for each of these field categories, respectively. These captures were statistically different ($P = 0.013$, Mann-Whitney test).

Fecundity

Up to 24 ovarioles were observed for each of the two ovaries from dissected females. From a sample of 30 vitellogenic females we counted an average of 46.6 ± 1.9 (SD) eggs. We analyzed ovaries from a total of 278 females captured on sugar beets during seven successive sampling dates. Patterns of previtellogenic, vitellogenic, and postvitellogenic females are reported in Fig. 6 as proportions of females belonging to each of the three categories. The proportion of females that were vitellogenic increased over the sampling period whereas that of previtellogenic females decreased. Postvitellogenic females became the most frequent category late in the sampling period.

Nymphs

We sampled nymphs from roots on both sugar beets and wheat. On sugar beet roots sampled in September, we could identify mostly second- and third-instar nymphs that were consistently found aggregated at the intersection of the taproot with secondary roots. Nymphs were concentrated at a depth of 10–25 cm below the ground level (Fig. 7A). Furthermore, they were strongly aggregated on isolated sugar beets rather than non-isolated ones, with averages of 3.9 ± 3.55 (SD) and 0.2 ± 0.53 (SD) nymphs per root from 33 and 87 sampled plants respectively. Differences were highly significant ($P < 0.001$, Mann-Whitney test).

At spring time, in the same plot, cultivated with wheat at that time, we identified third through fifth instar nymphs. By exploring soil samples we counted mean densities of 3.4 ± 2.36 (SD) nymphs on 16 sampled soil volumes. Based on the sample mean the number of nymphs per hectare can be projected to be about 134,800.

In some cases we observed newly emerging adults next to the ground surface on wheat roots. In contrast to the autumn distribution, older nymphs were concentrated near the ground surface (Fig. 7B). All adults that emerged from field-collected nymphs had typical external morphology of planthoppers from the genus *Pentastiridius*, and the structure of male genitalia reported in Fig. 2.

DISCUSSION

Our results provide evidence on the identity and biology of a cixiid planthopper within the genus *Pentastiridius* that completes its life cycle between sugar beet and winter cereal crops. Morphological analysis showed that male genitalia are very similar to those described in the literature for *P. leporinus* collected in common reeds from Central and Northern Europe (Wagner, 1970; Ossianilsson, 1978; Holzinger et al., 2003), clearly distinct from the genitalia of *P. beieri* (Wagner, 1970; Holzinger et al., 2003).

Tree inferences based on sequence similarity for two mitochondrial genes supported the findings of the morphological study. Sequences obtained from *Pentastiridius* sp. and from individuals of a *P. leporinus* vector of tomato stolbur in Russia (Bogoutdinov, 2003) did not exceed the range considered to indicate intraspecific variation. However a higher divergence, consistent with interspecific variation, was obtained comparing sequences of *Pentastiridius* sp. with those from *P. beieri*.

Morphological and genetic data obtained in this work strongly suggest that the cixiid planthopper from sugar beet in eastern France belongs to *P. leporinus* as species; we will therefore refer hereafter as to *P. leporinus*.

We suggest that *P. leporinus* in eastern France, similar to most cixiid planthoppers from temperate regions, is univoltine and that it overwinters as nymphs underground. From the middle of June to the beginning of July, adults migrate from winter wheat fields (cultivated with sugar beet in the previous year) to fields newly sown with sugar beets. Females lay eggs nearby sugar beet tap roots and hatching nymphs develop thereafter by sucking sugar beet root sap. In autumn (October–November) sugar beet are harvested and because of the declining temperature regime, nymphs are probably induced to diapause. Post-diapausing nymphs complete their development at spring time, presumably by feeding on cereal roots that are sown after sugar beet harvesting. To complete the life cycle, adults that emerge from cereals migrate to newly sown sugar beet fields.

Such life cycle requires that nymphs can develop by feeding both on sugar beet roots and after the winter diapause on wheat roots. Furthermore, newly emerged adults at spring time migrate from cereal fields (cultivated to sugar beet in the previous year) to nearby newly sown sugar beet fields. We suggest migration takes place when cereal plants senesce and gradually become unsuitable to insect feeding.

In this study we have reported evidences that strongly suggest *P. leporinus* completes the afore-mentioned life cycle. Actually we have shown that adults peak on cereals

earlier in the season than on nearby sugar beet fields, and that appearance of planthoppers in sugar beets is associated with their decrease from nearby wheat fields, suggesting insect migration. We have also shown that adults emerge only from wheat fields that were cultivated to sugar beets in the previous year. Furthermore, migrant females colonizing sugar beets develop ovaries and bear mature eggs, showing that sugar beet was suitable as a food plant to support ovarian development. We have quantified and localized young instar nymphs in autumn on sugar beet roots and late instar nymphs at spring time on wheat roots. Identification of immature stadia showed that females actually laid eggs nearby sugar beets and suggested that nymphs could develop by feeding both on sugar beet tap roots and on wheat roots. We observed that young nymphs were aggregated on "isolated" sugar beets. Such distribution may depend on a specific behavior of gravid females that may prefer to lay eggs nearby isolated plants. An alternative assumes that underground nymphal movement is limited on isolated sugar beets and therefore nymphs tend to remain aggregated. In contrast, the high density of aggregated sugar beets would allow spreading of nymphs from original oviposition sites, resulting in a lower number of nymphs per plant than on isolated tap roots.

In other surveys (Bressan et al., in press), we specifically analyzed the migratory behavior of *P. leporinus* by using sticky traps posted on sugar beet and other surrounding crops. We found that planthoppers migrated more abundantly and colonized sugar beets for longer periods than on any other crop available, i.e. corn, soya bean or wheat. Flight activity was very high during the migratory phase and planthoppers colonized primarily the centre of sugar beet fields.

In general the shifting mosaics of habitats that vary through time provide a discontinuity in host suitability for insect development and reproduction (Kennedy & Storer, 2000). Thus, annual cropping rotation may promote the establishment of species whose biological traits are adapted to exploit temporary habitats (Kennedy & Storer, 2000). Young brown fields are other habitats that, similarly to annual cropping rotations, undergo rapid succession (Strauss & Biedermann, 2008). In such habitats, leafhopper species with pioneer biological traits (i.e., feeding on annual herbs, polyphagy, egg overwintering, and polyvoltinism) occur with much higher frequency than slow coloniser species (Nickel, 2003; Strauss & Biedermann, 2008). Although planthoppers in the genus *Pentastiridius* have been found in disturbed and ruderal habitats (Nickel, 1999, 2003), having juvenile instars confined underground for long periods of the year, nymphs overwintering, and being univoltine they do not display biological traits common with pioneer species (Strauss & Biedermann, 2008).

However, *P. leporinus* can survive to a crop change and harvesting, allowing them to establish large populations in a situation of crop rotation (Bressan et al., in press), whereas the majority of insect species that cannot survive

in the same field after harvesting will inevitably become extinct in agricultural systems.

Although we failed to find alternative host plants for *P. leporinus* in eastern France, we propose that the planthopper shifted to sugar beet from an ancestral host plant. Research on cixiid planthoppers have documented their potential to select different plant species for development and reproduction. The best studied case concerns *H. obsoletus*. Recently this species has been increasing its host plant range across several countries. For instance, in Germany, *H. obsoletus* could accomplish its life cycle mostly on bindweed (*Convolvulus arvensis* L.) (Darimont & Maixner, 2001), but recently nettle (*Urtica dioica* L.) became widely used (e.g., Johannesen et al., 2008).

Overall, our findings strongly suggest the emerging disease syndrome "basses richesses" is a consequence of the increased populations of *P. leporinus* in sugar beet crops as a result of its adaptation to the cropping system sugar beet-winter cereals. Since no information is available on the presence of *P. leporinus* on sugar beet from areas others than eastern France, further studies need to be conducted to understand the distribution of the planthopper vector for the risk assessment of disease outbreaks in other areas in France and western Europe where sugar beet is cultivated extensively.

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